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## Foreword

In the present global situation, agriculture plays a major role in the interaction between socio-economic and biophysical processes. In addition to its principal and fundamental role of providing food, it now also needs to consider other ecosystem services provided by agriculture and to explore the new frontiers for the future. In the 50's of the 20<sup>th</sup> century the major topic was the introduction of inorganic fertilizers, in the 60's the use of synthetic compounds for plant protection (insecticides, herbicides, fungicides), in the 70's industrial crops, in the 80's organic farming and the environmental impact of agronomic practices, and in the 90's genetically modified crops (herbicide tolerance, insect resistance). In the current decade the themes are: land and water degradation, the production of agricultural biomass for bio-energy, and the increased expression of functional compounds in crops.

The Bologna X Congress of ESA “Multi-functional Agriculture - Agriculture as a Resource for Energy and Environmental Preservation”, will meet the needs of finding tools to deal with environmental problems coupled with the increasing demand for food, and filling the knowledge gap on the physiological relationships between functional compound bio-synthesis and agricultural practices.

Members of the European Society for Agronomy already have a deep knowledge of these issues, and the Bologna ESA Congress will provide an opportunity to develop them further particularly in regard to innovative agricultural techniques, new energy sources and better environmental monitoring.

The goals of the this congress are to:

- identify new perspectives and frontiers for European agriculture in the third millennium, collecting together the major inputs from the public and private agri-food sector (Commercial R&D, Universities, Research Institutes).
- encourage cross-cutting studies of monitoring, prediction and evaluation of the effects of climate change and variability on the rural environment, and on the related social aspects.



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## **SESSION 1**

# **AGRICULTURE TO PRESERVE ENVIRONMENT**



**SUB SESSION 1.1**  
**LAND AND WATER CONSERVATION**

1.1a - EROSION CONTROL; POINT AND DIFFUSE POLLUTION CONTROL  
(PHYTODEPURATION, PHYTOREMEDIATION AND OTHER TECHNIQUES)

Chairman: Pier Paolo Roggero



# Soil and its Importance for Human Civilizations

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Soils are the foundation of human societies. Without soils, human societies cannot exist. Humans rely on soils primarily for food production, but also for clean and sufficient water supplies. The first advanced human civilizations developed in regions where soil and water resources were abundant, allowing people to advance agriculture and to produce surplus food. The availability of surplus food freed up time and other resources for the development of arts, science, and technology.

As fertile and abundant soils allowed societies to thrive, societies declined and disappeared when soil resources deteriorated, as history has repetitively witnessed. Fall and rise of past civilization is often tightly connected with soil resources: abundant and rich soils allowed societies to blossom; degraded or destroyed soils caused societies to collapse (Hillel, 1992; Diamond, 2005).

## Methodology

This paper reviews past events and accounts on societal collapses. The relation of the societal collapses to the natural resources available to the societies is highlighted. Particularly, the role of soils as foundation for forestry and agriculture is demonstrated with several case studies from various locations around the world. Based on historical accounts, it will be shown why soil degradation and destruction often go unnoticed until it is too late for remedy. Challenges of today's modern agriculture to provide food, fuel, and fiber, and at the same time, maintain sustainable use of soil resources are discussed.

## Results

Historical evidence for this reliance on soils for success and collapse of civilizations has been provided for the early civilizations in Mesopotamia, and societies the Pacific, the Americas, and Northern and Southern Europe (Hillel, 1992; Diamond, 2005; Montgomery, 2007a). Advanced societies in Mesopotamia declined and eventually disappeared because soils degraded, via erosion and salinization, societies in Greece and Italy suffered severe soil erosion due to overgrazing, resulting in loss of agricultural productivity and eventually collapse of the societies. Similar examples of societal collapses can be provided from societies in the Pacific and the Americas (Diamond, 2005). Soil degradation is only one of the factors leading to societal collapse, but it is an important factor (Figure 1).

A major, yet unresolved, question is why societies do destroy their soil, destroy the very foundation of their existence? Why did former civilizations continue with soil management practices that led to soil degradation and ultimately their own downfall? This question has puzzled archeologists, historians, and scientists alike, and no good answers have been offered and found yet. Other major reasons for societal downfall and collapse are declining trade, war, disease, and climate change. While these reasons often cannot be controlled well, it seems that soils and natural resources could be managed in such a way to ensure long-term survival. But apparently, former societies did not take sufficient care of their soils.

Soils form very slowly, over periods of hundreds and thousands of years, through the process of weathering from parent material. As such, soils are continuously forming, but soil formation is too slow for being a viable remediation option for an existing society (Montgomery, 2007b). Consequently, if soils are destroyed or degraded, civilizations are doomed to collapse.



Figure 1: The mysterious stone status on Easter Island. Their presence gives testimony of a civilization that has once thrived, but then disappeared due to environmental destruction and soil degradation.

Today, the most productive agricultural regions in the Americas, Europe, and Asia still have fertile soils. However, the soils have been, and are being, degraded at an alarming rate. Erosion due to wind and water is a widespread, and well-recognized problem. Degradation of soil quality, as indicated for instance by loss of organic matter and deterioration of soil structure, is clearly evident.

Changes in farming systems under conservation practices have helped to reduce soil degradation in the past 30 years, and remarkable successes have been achieved in erosion control. Nevertheless, erosion is still a major problem. In addition, we are now faced with another change of farming practices, which will likely put more pressure on soil resources and sustainability: the shift of food production to generation of renewable energy and non-food biomass. At the same time, soils are considered to offer opportunities for carbon sequestration to reduce atmospheric CO<sub>2</sub>. These shifts in agricultural production and the new demands on soils will not only lead to competition between food and non-food-based production, but will also cause increased mining of soil resources. Can food, renewable energy, and non-food bioproducts be produced without degradation of soil resources? Can soil resources be managed sustainably under this increased pressure, or will we just be mining soil as we have mined oil?

## Conclusions

A common feature in historical accounts of rise and fall of civilizations is that the societies, initially thriving on abundant soil resources, did not use their soil resources in a sustainable manner. Soils have been overused and exploited in the short-term. There are a few peculiarities that make soils unique, and these offer some explanation why societies often care little about their soils. A major reason for soil mismanagement is that the soil is beneath our feet. Hence, we do not detect soil degradation readily, and its temporal evolution is so slow and gradual such that adverse effects on plant growth and yield are often masked by random fluctuations and remain unnoticed for years. The effects of salinization and erosion are often likewise gradual, and go unnoticed as only little salt is accumulated and little soil is removed per year. When adverse effects are finally becoming clearly visible and noticeable, however, it is often too late for remediation.

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# Application of a Crop-Farm-Indicators modelling chain to assess the impact of the EU nitrate directive in the Midi-Pyrenees region

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**Key-words :** crop model, farm model, indicators, sustainability, nitrate, integrated assessment.

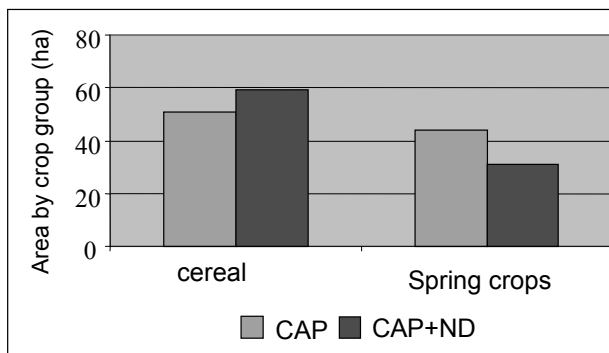
The rapid evolution of economic and environmental constraints on farming systems require cropping systems design or assessment to be conducted in the context of market instability as well as economic (e.g. CAP in EU) and environmental policy changes. The Nitrate Directive (91/676/EC) is one of the oldest EU environmental policy, designed to reduce water pollution by nitrate from agricultural sources, through a set of measures, defined at regional level, and mandatory for farmers of vulnerable zones.

## Methodology

On the basis of methodologies developed in the SEAMLESS Integrated Project (van Ittersum et al., 2007), we have combined a crop model (CropSyst), a farm model (FSSIM), a farm typology and a set of indicators. The objective was to simulate how these “virtual” farmers react to the external constraint (price changes, CAP reform, Nitrate Directive) and what is the impact on environmental and economic indicators at farm and regional levels. This study was conducted in a French region (Midi Pyrenees) and we have compared, for the 2003-2013 period, a business as usual scenario (CAP, 2003 CAP reform until 2013) and an alternative scenario adding to the previous one the Nitrate Directive (CAP+ND). The latter combined alternative N management (based on target yield and soil type) proposed as a ND measure in the region, and a range of penalties applied to the CAP subsidies if this measure was not selected by the virtual farmer. CropSyst was previously calibrated on field experiments conducted in the region and FSSIM was calibrated on crops area per farm type using FADN data (Louhichi et al., 2008). For this application the model chain was applied to a set of 3 farm types representing the arable farming systems in Midi-Pyrenees, defined in term of size, intensity, land use and specialisation. Biophysical data, policy parameters and exogenous assumptions were combined to define each scenario (Belhoucette et al., 2007).

## Results

With a 3% penalty none of the farm types adopted entirely the alternative N management but they changed their cropping systems in comparison with the CAP scenario (Table 1). The CAP+ND scenario induced minor reductions of farm income compared to CAP and between -6% and +5% on N leaching, depending on the farm type. For both scenarios, the average nitrogen leaching was almost the same (45 kg N/ha) for the two dominant soil types in the region. The major changes occurred on soil erosion which was reduced by 16 to 29%, depending on farm



**Figure 1.** Farm type 1: Area by crop group and soil type for the CAP and the CAP+ND scenarios.

type, mainly because of the reduction of spring crops (sunflower, soybean, maize) to the benefit of winter wheat, thereby reducing the bare soil area during winter (fig 1). A sensitivity analysis showed that 13 to 17% of penalty, according to farm type, was required to force the farmer to adopt the alternative N management. It allowed to maintain farm income (-6% on average), despite a 28% reduction of N fertilisers compared to the CAP scenario, nitrate leaching was reduced by only 9%.

**Table 1.** Economic and environmental impacts of the CAP+ND scenarios compared to the CAP scenario at farm scale.

Economic and environmental indicators	ND + CAP (% change to CAP scenario)		
	Farm type 1 (cereal)	Farm type 2 (cereal/fallow)	Farm type 3 (mixed)
Farm income	-1.0 %	0.0 %	0.0 %
Premium	-3.0 %	-3.0 %	-3.0 %
Nitrate leaching	5.0 %	1.0 %	-6.0 %
Soil Erosion	-16.0 %	-21.0 %	-29.0 %

The analysis of the two scenarios shows that the irrigable area was partially used e.g. 70%, 50% and 20% respectively for farm types 1, 2 and 3. This result can be explained by the fact that the gross margin of the irrigated maize (the major irrigated crop) was decreased by almost 10% with the CAP reform, mainly because of the reduction of the premium for irrigated crops. For the CAP scenario, the main rotation with irrigated crops selected by the model was maize-soybean. This rotation was substituted in the CAP+ND scenarios by the irrigated crop rotation maize-maize which becomes more profitable with the adoption of the better N management. Thanks to this kind of substitutions, the loss of farm income due to the implementation of 3% of penalty was entirely compensated. Despite some differences between farm types, the trend obtained at the farm level was conserved after aggregation at the regional level: (i) no modification of farm income and a marginal decrease of premium (-3 %) due to penalty and a modification in the crop patterns ; (ii) a very low decline of nitrate leaching attributed to the modification in the crop pattern, and iii) a significant reduction of soil erosion (- 22 %).

## Conclusions

The results presented in this study represent a first application of the model chain CropSyst-FSSIM-indicators in real application conducted in interaction with users and stakeholders for the definition and the analysis of the scenarios in the Midi-Pyrenees region. The results show that this modeling chain can be functional for complex scenarios combining economic and environmental drivers and provides sound results, with regards to current knowledge and when discussed with local experts. This work provides insights in some key methodological aspects for future improvements and further uses of the meso backbone modeling chain of SEAMLESS-IF. The main aspects are the need for: i) several interactions with local experts and further methodological development for a better models calibration and validation at farm and regional levels, ii) a sensitivity analysis for each application before defining the final scenario to be used for communication with the users.

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# Quantitative Parameters for the Evaluation of Cover Crop Performance in Relation to Soil Cover and Rooting Dynamics

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## Introduction

Cover cropping is a common practice for erosion control and soil structure stabilization. The dynamics of canopy coverage and rooting of the soil are essential to achieve these agro-environmental benefits of cover crops. An adequate management thus requires a detailed quantitative knowledge about the performance of different species under variable climatic conditions. In this study, the use of empirical growth model parameters to quantify the performance of different cover crop species and their sensitivity towards intra-annual variability in weather conditions is analysed.

## Methodology

Data of canopy coverage and root distribution of four cover crops (phacelia, hairy vetch, rye, mustard) were measured in a field experiment in the pannonic region of Eastern Austria. The experimental layout was a randomized complete block design. The two years of study differed substantially in rainfall distribution. 2004 was characterized by dry conditions during and after cover crop seeding, while there was evenly distributed rainfall during the later vegetation period (1<sup>st</sup> October to 31<sup>st</sup> December: 118 mm). In 2005, after high rainfall in August (154 mm), a particularly dry autumn occurred with only 41 mm rainfall compared to the same period in the previous year. Canopy coverage was assessed four times during cover crop growth by image analysis following Richardson et al. (2001). Root length density was measured using WinRhizo software according to the method given in Himmelbauer et al. (2004). Canopy coverage dynamics were described using a Gompertz growth model. In case of significant reduction in measured ground cover towards the later growing season, a modified growth model including a decay term (Werker and Jaggard, 1997) was fit to the data. Root distribution was characterized by the two parameters of the exponential model of Gerwitz and Pages (1974). The growth and root models were fit to the measurement data using the non-linear regression procedure NLIN of the SAS software and functional parameters were evaluated by common ANOVA and comparison of means following Scheuermann et al. (2003).

## Results

Table 1 shows the parameters describing the canopy cover dynamics of the crops for the two years.

**Table 1.** Parameters of canopy cover growth (In case of significant differences in the ANOVA at  $p < 0.05$ , comparison of means is indicated by lower-case letters.)

	2004			2005			
	$y_{max,G}$ <sup>1</sup>	$t_{max,G}$	$k_G$	$y_{max,WJ}$ <sup>2</sup>	$t_{max,WJ}$	$\mu_{min}$	$k_{WJ}$
Phacelia	79.9	36.4a	0.098	47.0a	66.1	-0.197a	0.006
Vetch	64.1	47.9b	0.091	93.5b	62.8	-0.011c	0.052
Rye	60.6	31.3a	0.073	49.6a	54.0	-0.061ab	0.023
Mustard	72.1	32.5a	0.161	76.8b	45.9	-0.034bc	0.131
	p=0.1656	p=0.0301	p=0.6101	p=0.0034	p=0.1380	p=0.0151	p=0.0771

<sup>1</sup>Gompertz parameters:  $y_{max}$ =maximum cover (%),  $t_{max}$ =time (days) to maximum growth rate,  $k$ =growth rate ( $day^{-1}$ ).

<sup>2</sup>Werker and Jaggard parameters:  $y_{max}$ =maximum cover (%),  $t_{max}$ = time (days) to reach  $y_{max}$ ,  $\mu_{min}$ =decay rate ( $day^{-1}$ ),  $k$ =rate constant ( $day^{-1}$ ) how fast initial growth rate approaches  $\mu_{min}$

While in 2004 canopy growth was described best by the Gompertz growth model, in 2005, after a prolonged dry period in autumn, all species showed a decrease in canopy coverage due to leaf wilting and thus characterization of their performance required inclusion of a decay term. In 2004, the cover crop species differed mainly in the time to reach maximum canopy growth rate ( $t_{max,G}$ ) with vetch showing a substantially delay in early growth. This reflects susceptibility of vetch to initially dry conditions which is probably related to the high water requirements of large vetch seeds for germination. In 2005 on the contrary, it was the maximum canopy coverage ( $y_{max,WJ}$ ) of the crops as well as the final decrease ( $\mu_{min}$ ) where most distinctive differences were found, reflecting the crop specific sensitivity to dry conditions in autumn. Vetch and mustard developed a significantly higher canopy coverage compared to phacelia and rye. Vetch also showed the lowest final cover reduction after the dry autumn while phacelia and rye were strongly affected by the lack of rainfall. This is probably related to the concentration of roots and thus water uptake near the soil surface (Fig. 1) while water availability in autumn 2005 largely depended on deeper soil water resources after drying of the surface near layer.

Root distribution was significantly affected by year (Tab. 2), with a higher tendency of root proliferation to depth in the dry autumn 2005. Among species, phacelia had the highest root concentration near the soil surface as expressed in the  $L_0$  parameter, while vetch showed the lowest value. The decrease parameter did not differ significantly between species. However, phacelia showed the lowest intra-annual variability with  $a$  being only -33% lower in the 2005 compared to 2004, while vetch declined by as much as 85 % in its tendency of root growth to depth in the dry year 2005.

**Table 2.** Root distribution parameters (*In case of significant differences in the ANOVA at  $p < 0.05$ , comparison of means is indicated by lower-case letters.*)

$\bar{O}$ 2004/2005	$L_0^1$	$a$
Phacelia	9.7a	0.056
Vetch	4.5c	0.080
Rye	8.6ab	0.054
Mustard	6.3b	0.065
	$p=0.0296$	$p=0.8317$
$\bar{O}$ Species		
2004	7.0	0.097a
2005	7.5	0.031b
	$p=0.9251$	$p=0.0134$

<sup>1</sup>Gerwitz and Page parameters:  $L_0$ =root length density ( $\text{cm cm}^{-3}$ ) at the soil surface,  $a$ =decrease constant ( $\text{cm}^{-1}$ )

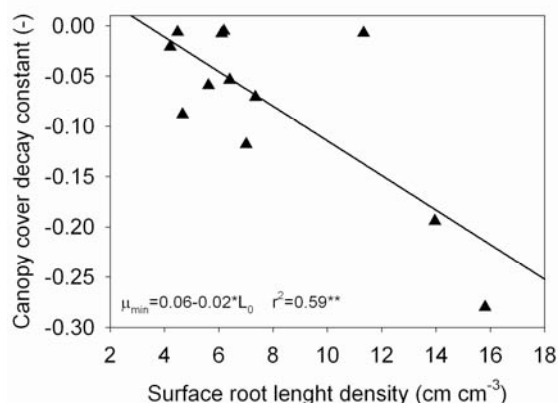
## Conclusions

Growth and root distribution models can be used to quantitatively describe differences in the performance of canopy cover and rooting intensity of cover crops and their relation to intra-annual climatic differences. It was shown that vetch was most susceptible to drought after seeding, while otherwise it provides a high soil cover even in case of low rainfall during autumn. On the contrary, phacelia was most sensitive to soil moisture deficit in autumn due to a high concentration of roots near the soil surface.

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**Fig. 1.** Relation between decrease in canopy coverage after a dry autumn and surface near concentration of root density.



# Water Infiltration and Soil Losses in a Permanent Bed Irrigated System in Southern Spain

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## Introduction

Soil erosion is one of the major problems in Mediterranean area (Singer and Le Bissonnais, 1998; Romero-Díaz et al., 1999). In south Spain, soil loss rates by water erosion can be very high (Gómez et al. 2005; De Santisteban et al., 2006). Erosion is a natural process; however, human alterations in land use have caused erosion rates to increase in many areas of the world. Controlling erosion is not always easy and it may require new management strategies. Reducing the intensity of tillage and leaving some residues on the surface slow down soil losses and improve soil infiltration rate (Franzluebbers, 2002; Smith et al., 2007). Similar results were obtained in a permanent bed planting system with crop residue retention (Govaert et al., 2007). The objective of this research was to compare a permanent bed planting system with the traditional conventional tillage in terms of water infiltration rate and soil loss in a commercial farm.

## Methodology

This study was carried out on a commercial farm (37°44' N, 5°09' O) in Fuente Palmera (Córdoba, southern Spain). Two bed planting systems established in adjacent fields were compared: permanent bed planting (PB) and conventional bed planting (CB). The PB implied no tillage and maintenance of residues. PB had 210 g m<sup>-2</sup> of dry residues covering the surface after 5 years of no tillage whereas CB has none. The CB consisted of tilling the soil and forming the beds every year. Rainfall simulation experiments were conducted on December 2007. Four microplots measuring 0.9 m x 0.9 m were identified in each field in homogeneous zones of 9% slope. Each rainfall simulation lasted 60 min starting with runoff initiation. Runoff rate was measured every 2 minutes. Every 6 minutes a runoff sample was collected for determination of sediment concentration. The infiltration rate was calculated as the difference between measured rain intensity and the corresponding runoff rate. Saturated hydraulic conductivity (K<sub>s</sub>) and sorptivity (S) were determined from the relationship between accumulated infiltration and time. Topsoil samples were collected at 0-20 cm before each rainfall event to determine initial water content (θ<sub>i</sub>) and water stable of macroaggregate (WSA). Surface roughness ratio (RI) was measured using the chain method (Saleh, 1993). Surface soil samples of 0-30 cm depth were collected for the analysis of soil physical and chemical properties and organic matter. Bulk density (ρ) was measured by the excavation method adapted from Grossman and Reinsch (2002).

## Results

In comparison with CB, PB had significantly higher θ<sub>i</sub>; electrical conductivity (EC), soil organic matter (SOM), ρ and WSA (Table 1). Differences in pH, ρ and the RI were not statistically significant. The high values of θ<sub>i</sub> in PB is attributed to maintaining residues in PB that reduced soil evaporation and increased soil water content. After 5 years of PB, the residues retention also contributed to increasing SOM and improving WSA. In Mexico, Govaert et al. (2007) have also demonstrated the potential of PB to maintaining higher level of SOM.

No significant effect of tillage system on sorptivity was measured (Table 1). Results show that in PB K<sub>s</sub> was 60% higher than in CB. This indicated that the modification in WSA and SOM after 5 years under PB system had more pronounced effect on K<sub>s</sub>.

Table 1. Selected physical, chemical, biological and hydraulic soil properties in PB and CB systems

Tillage system	$\theta_i$ (%)	pH (H <sub>2</sub> O)	EC (dS / m)	SOM (%)	$\rho$ (g / cm <sup>3</sup> )	WSA (%)	RI (%)	S (mm.min <sup>-1/2</sup> )	Ks (mm.min <sup>-1</sup> )
PB	21.70b	8.54a	0.35b	2.17b	1.31a	64.30b	3.18a	2.63a	0.25b
CB	12.60a	8.40a	0.25a	1.00a	1.22a	42.91a	4.23a	2.21a	0.15a

Values followed by different letter in the same column differ significantly at  $P < 0.05$ .

Figure 1a present the infiltration rate for the two bed planting systems (PB and CB). Already after 6 min of rainfall initiation, the infiltration rate in PB was higher than in CB. The presence of residues in PB increases rainfall infiltration rates either by protecting the surface from rain drop impact or by improving the soil structure and aggregates (Blevins and Fry, 1993). The lower infiltration rate in CB is probably the result of aggregate destruction and surface sealing. The lower value of WSA in CB supports this hypothesis. Soil loss increases more dramatically with time in CB than in PB (Figure 1b). After 1 h of runoff, soil loss was 12.9 t ha<sup>-1</sup> under CB and 2.7 t ha<sup>-1</sup> under PB. Soil in CB with lower level of SOM and without residues is exposed directly to rain drops and this contributes to the higher level of soil loss in this system.

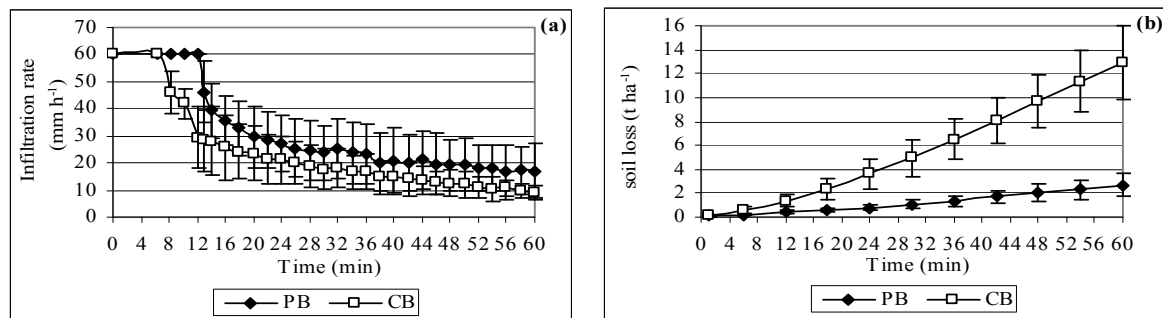


Figure 1. Evolution of soil infiltration rate (a) and soil loss (b) in PB and CB

## Conclusions

Our results show that the PB system improved the saturated hydraulic conductivity, the soil infiltration rate and reduced significantly soil loss in our conditions. These improvements are attributed to the higher organic matter and increased stability of soil macroaggregates in water.

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# Sod Seeding and Soil Erosion in a Semi-arid Mediterranean Environment of South of Italy

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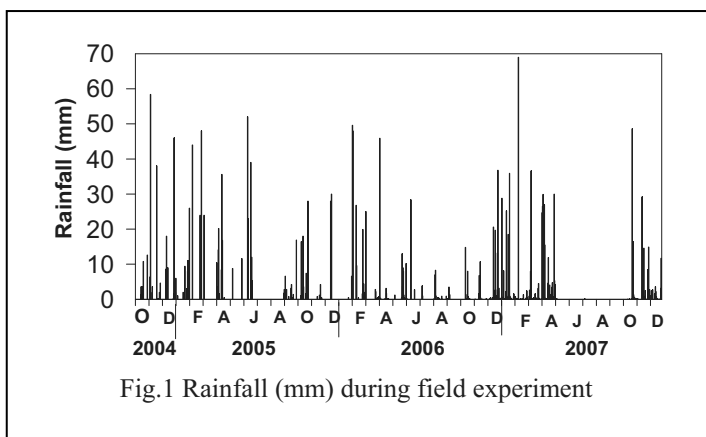
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Experimental plots equipped with devices to measure runoff and soil erosion were established since 1996 in a Sicilian hilly area subjected to water erosion (Foti et al., 2000). Researches showed that cropping systems with perennial crops resulted to be suitable in minimizing soil erosion and surface runoff while cropping systems with annual crops did not (Foti et al., 2001, Cosentino et al., 2004). Since 2004 the efficiency of sod seeding technique in controlling soil erosion in slopes subjected to water erosion have been studied on the above mentioned experimental plots (Cosentino et al., 2006).

## Methodology

The research was carried out in 2004-2007 years in an area of the internal hill of Sicily region (Enna, Geracello, 550 m a. s. l. 37° 23' N Lat, 14°21' E Long), mainly devoted to durum wheat cultivation, using the experimental plots established since 1996 on a hill with a 26-28% slope. Twelve bounded plots, having 40 m length and 8 m width, were equipped with a series of devices, in order to collect surface runoff (Foti et al., 2000). In order to study the effect of sod seeding technique two plots were not tilled since summer 2004 and plant residues were left on soil surface. In these two plots, annual crops were cultivated (wheat - rapeseed - rapeseed; wheat - wheat - faba bean). The two plots were compared to several perennial crops (*Medicago arborea*; alfalfa; *Lolium multiflorum* and subterranean clover intercropped) and annual crop rotations conventionally sown (wheat - faba bean - wheat, wheat - set aside - wheat; rapeseed – wheat - wheat; faba bean – wheat - *Brassica carinata*; wheat - fallow - wheat; wheat - wheat - wheat; *Lolium multiflorum*). In the plots devoted to sod seeding, weeds were controlled applying ‘glyphosate’ (2004-2005) and ‘paraquat’ (2006) before sowing. A modified seeding drill was adopted. At each erosive rainfall, the total amount of runoff was measured in each container and samples of runoff were oven dried at 105°C in order to determine the amount of runoff sediments.

## Results



During the research period, (October 2004 - November 2007), 19 rainfalls causing runoff and soil erosion occurred (fig. 1). Erosive rainfalls ranged between 9 and 59 mm. Cropping systems affected soil erosion; highest values of soil erosion were recorded in plot 4 with “wheat - not tilled - wheat” (45.8 t ha<sup>-1</sup>) (tab.1). On the other side, the lowest values of soil erosion were recorded in plot 1, 6, 11, where perennial crops respectively ensured permanent cover of the soil

(0.2 t ha<sup>-1</sup>, 0.5 t ha<sup>-1</sup>, 0.6 t ha<sup>-1</sup>). Among the annual cropping systems the highest amount of soil losses were observed in plot conventionally tilled: 17.5 t ha<sup>-1</sup> in plot 2 “wheat - fallow - wheat”, 20.6 t ha<sup>-1</sup> in plots 3 “wheat - wheat - wheat” and 20.5 t ha<sup>-1</sup> in plot 5 “wheat - faba bean - wheat”.

On the contrary, in the plot 10, where sod seeding was applied, good erosion control was observed (2.5 t ha<sup>-1</sup> of soil losses).

In plot 9 bad seed germination in rapeseed in sod seeded soil determined two years of bare soil and then the plants did not cover the plots (19.3 t ha<sup>-1</sup>).

Tab. 2 - Soil erosion (t ha<sup>-1</sup>) in relation to cropping systems

Plot no.	(October 2004 – September 2005)	Soil loss (t ha <sup>-1</sup> )	(October 2005 – September 2006)	Soil loss (t ha <sup>-1</sup> )	(October 2006 – November 2007)	Soil loss (t ha <sup>-1</sup> )	Total soil loss (t ha <sup>-1</sup> )
1	<i>Medicago arborea</i>	0.1	<i>Medicago arborea</i>	0.1	<i>Medicago arborea</i>	0.0	0.2
2	Wheat	3.9	fallow	13.5	wheat	0.1	17.5
3	Continuous wheat	3.2	Continuous wheat	17.2	Continuous wheat	0.1	20.5
4	Wheat	4.3	Not tilled	40.1	Wheat	1.3	45.8
5	Wheat	2.7	Faba bean	17.8	Wheat	0.04	20.6
6	Alfalfa	0.2	Alfalfa	0.2	Alfalfa	0.01	0.5
7	Rapeseed	9.8	Wheat	13.8	Faba bean	1.1	24.7
8	<i>Lolium multiflorum</i> ,	2.6	<i>Lolium multiflorum</i> ,	27.5	<i>Lolium multiflorum</i> ,	4.9	35.1
9	Wheat (sod seeding)	3.3	Rapeseed (sod seeding)	5.4	Rapeseed (sod seeding)	10.6	19.3
10	Wheat (sod seeding)	1.0	Wheat (sod seeding)	1.3	Faba bean (sod seeding)	0.1	2.5
11	<i>Lolium multiflorum</i> subterranean clover	0.2	<i>L. multiflorum</i> and subterranean clover	0.4	<i>L. multiflorum</i>	0.01	0.6
12	Faba bean	8.2	Wheat	19.4	<i>Brassica carinata</i>	1.0	28.7
	<b>Average</b>	<b>3.3</b>		<b>13.1</b>		<b>1.8</b>	<b>18.0</b>

## Conclusions

The results pointed out that cropping systems with perennial crops allowed keeping low the soil loss, while annual crop rotation determined a high amount of soil loss. Among the annual cropping systems sod seeding determined soil erosion comparable to the erosion collected in the plots with permanent crops. However, sod seeding technique appeared to be not suitable for crops with small seeds like rapeseed. This promising technique needs more observations on the long run in order to verifying the effectiveness in terms of productivity and soil fertility and to experimenting it for many crops.

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# Irrigated Agriculture and Urban Waste Water: the Cases of Bean and Tomato

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## Introduction

Semi-arid regions suffer from shortages in water supply for agricultural purpose. Treated effluents can be used for irrigation, however the use of treated urban waste waters (UWW) must be probed in agriculture (Qadir et al., 2007).

This study addressed the use of UWW. The following factors were evaluated: a) the level of production in the bean and tomato crops; b) the variations in the circulating solution along the soil profile; and c) the composition of the drained waters.

## Methodology

The trial was carried out in aboveground lysimeters filled with two soil types (clay and sandy) and equipped with porous cups at 4 depths. The lysimeter set-up was placed at the CRA-SCA experimental farm in Rutigliano (Southern Italy). The treatments (4-time replicated) were arranged in a split-plot experimental design on bean 'Lingua di fuoco' (2005) and tomato 'HF<sub>1</sub> 2776' (2006). The soil type (sandy vs clay) was split on the quality of the irrigation water (T0=100% groundwater; T50=50% UWW and 50% groundwater; T75=75% UWW and 25% groundwater; T100=100% UWW). Usual agro-techniques were adopted for both crops. In particular, irrigation was scheduled whenever plants completely used the easily available water in the soil. Crop water-use was calculated according the FAO-56 approach.

## Results

The treatments with different types of water influenced neither the vegetative response nor the productive response of the bean. These results are consistent with the analysis of the Cl<sup>-</sup> concentration and electrical conductivity (EC) measured in the circulating water in the soil profile (Figure 1). The sensitivity thresholds (250 mg of Cl<sup>-</sup> per litre and EC of 1.0 dS m<sup>-1</sup>) for the bean were not reached in the soil layer colonized by the root system.

On the contrary, the production of seeds in the

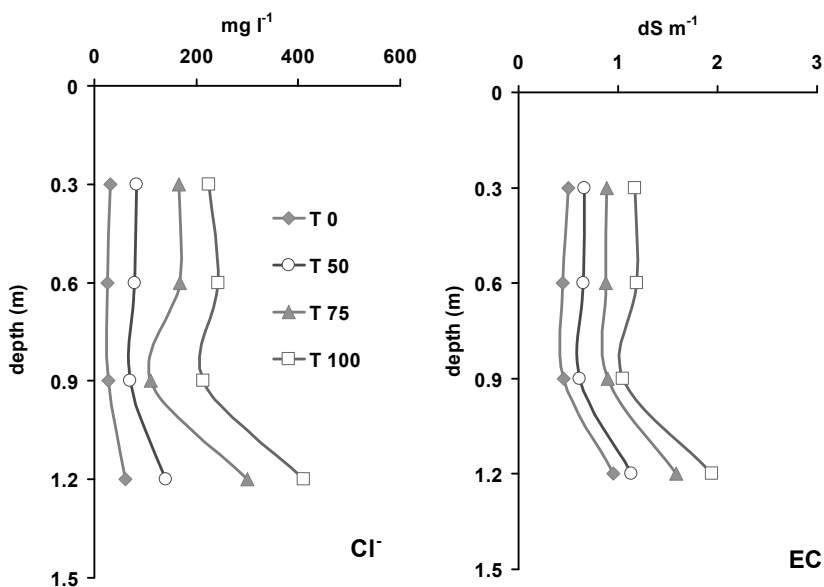


Fig. 1- Bean in sandy soil: chlorides concentration (Cl<sup>-</sup>) and electrical conductivity (EC) measured at harvest in the soil solution circulating at different depths

sandy soil was less than that of the clay soil (177 vs 298g/plant).

In the drained water, the pH and the nitrates were more or less constant. In contrast, variations were found in the chlorides (from 70 to 680 in clay soil and from 60 to 410 mg l<sup>-1</sup> in sandy soil) and the EC (from 0.9 to 2.4 in clay soil and from 1.0 to 2.0 dS m<sup>-1</sup> in sandy soil), and proportionally to the increase of UWW in the irrigation water.

The production of mature tomato fruit varied significantly in the two types of soil (4.5 kg m<sup>-2</sup> in the sandy soil vs 5.6 kg m<sup>-2</sup> in the clay soil) but not as a result of the composition of the irrigation water. These results are in agreement with Aiello et al. (2007) who reported that tomato productivity did not decrease by irrigating with reclaimed wastewaters. Instead, the waste water influenced the total soluble solids (4.8 °Brix in the T0 treatment vs 5.3 °Brix in the T50-T75-T100 treatments, on average). These results are not in agreement with Al-Lahham et al. (2003) who reported that the highest values of total soluble solids were obtained in the control treatment without UWW.

As in the case of the bean (in the previous year), the pH and the nitrates in the drainage water remained unchanged. On the contrary, variations were found in the chlorides (from 170 to 590 in clay soil and from 140 to 910 mg l<sup>-1</sup> in sandy soil) and in the electrical conductivity (from 0.9 to 2.8 in clay soil and from 0.6 to 2.8 dS m<sup>-1</sup> in sandy soil), and proportionally to the increase of UWW in the irrigation water (Figure 2).

As shown for the bean, the chlorides accumulated below the layer explored by the roots (>0.60 m).

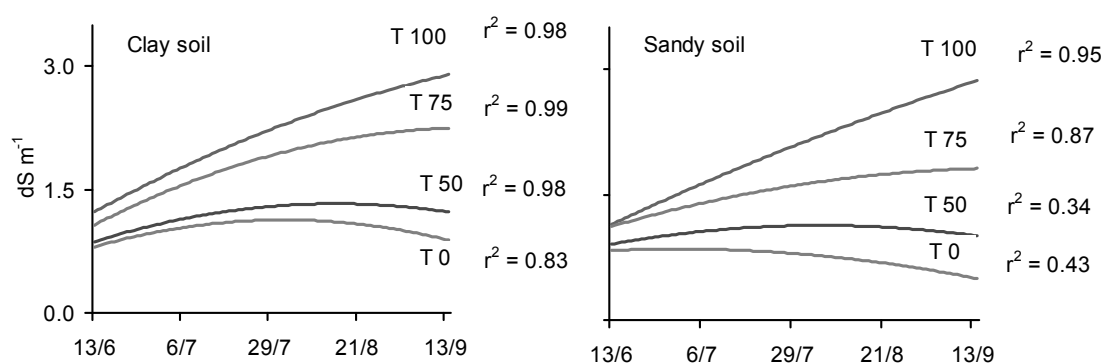


Fig. 2 – Tomato: time evolution of EC (dS m<sup>-1</sup>) measured in the drainage waters from the clay and sandy soils

## Conclusions

From the results obtained, UWW can be used in irrigation without compromising the production of bean and tomato and without causing important variations in the layer of soil affecting the roots, in part thanks to the leaching caused by rains falling in autumn-winter season, after the cultivation cycles. The qualitative composition of the drainage waters does not cause concern for the pH and nitrates, while a threshold level must be carefully considered for the EC and chlorides, especially when UWW is used without the integration of other types of irrigation water.

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# Responses to Ozone Pollution of Alfalfa Grown with Increasing Levels of Salt Stress

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It's well known that ozone is the most dangerous atmospheric pollutant for vegetation and reduces yield of several crops (Ferretti et al., 2007). Considering that ozone enters into plant tissues through the stomata, all the factors that reduce stomatal conductance also reduce ozone injuries to crops (Fagnano and Merola, 2007). Among these factors, in Mediterranean area increasing problems of watertable and soil salification are widespread reported. Salt stress reduced ozone sensitivity of some crop (Maggio et al., 2007), thanks to its effect on stomatal conductance and antioxidants production. The aim of this paper was to verify if irrigation with saline water can modify alfalfa responses to ozone pollution.

## Methodology

With the aim to analyze the interaction between ozone pollution and salt stress, an experiment in a coastal plain of Southern Italy was made with alfalfa grown in filtered (AF OTC) and not filtered (NF OTC) open top chambers and irrigated with water at increasing salinity levels. A split plot design was used with presence (NF OTC) vs. absence (AF OTC) of ozone as main factor and 4 salinity levels as sub-factor (0, 2.0, 3.2 e 4.4 g L<sup>-1</sup> di NaCl). During spring-summer 2006 several measurements of stomatal conductance were made during the day on a 2 year alfalfa meadow. Harvests were made cutting alfalfa plants at the start of flowering. Yield losses due to zone were calculated by the ratio between NF OTC /OTC AF alfalfa yield. Ozone uptake by plants was estimated by multiplying stomatal conductance per 0.613 (gas diffusivity ratio between water and ozone) per the hourly ozone concentrations. Harvests were made on May 4, June 1, June 27, July 25, August 24.

## Results

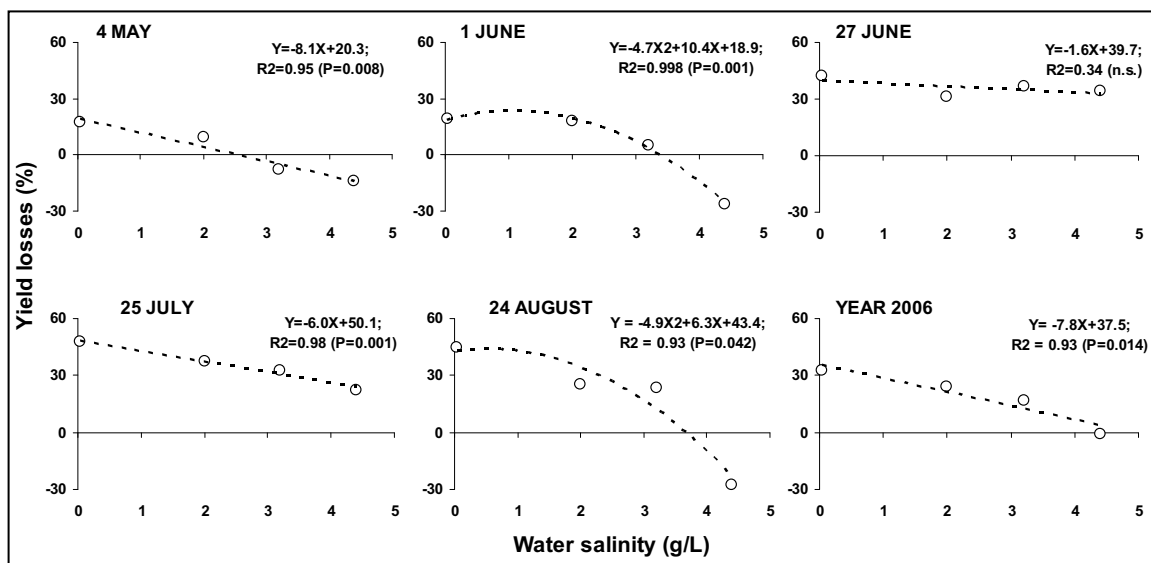
During 2006, ozone levels were lower than during 2005, because of the frequent summer rainfalls, as already noticed in previous trials made in similar environments (Fagnano et al., 2004). AOT40 from May to July was 10.1 ppm h in 2006 vs. 13.1 recorded in the same period of 2005. Therefore, in Mediterranean Countries, this pollutant reaches levels higher than the protection threshold for protection of vegetation (9 ppm h, Directive 2002/03/CE), also in years with not-favourable conditions for ozone accumulation.

AOT40 values in the not-filtered chambers were 68% in 2006 on the average, as compared with AOT40 values recorded in ambient air. The daily maximum values were very high on June 19 (92 ppb), on July 13 (87 ppb), 15 (89 ppb), 20 (90 ppb) and 27 (80 ppb).

From the analysis of variance, the interaction between ozone and salinity was significant in all the harvests: alfalfa grown in presence of ozone showed lower biomass yield, as compared to plants grown in purified air, only when irrigated with the lower salinity levels. On the whole 2006 cropping period, yield losses due to ozone decreased as increased salinity levels ranging from 37.5% (without salt) to 0 (4.4 g L<sup>-1</sup> di sale), showing a decreasing trend = -7.8% per g L<sup>-1</sup>. Besides, in presence of ozone alfalfa yield was not influenced by the salinity levels used in this experiment (Fig. 1).

Of course the increase of salinity (S) in the irrigation water reduced alfalfa stomatal conductance (SC): SC=0.55-0.0204S (R<sup>2</sup>= 0.93, P=0.05) in NF OTC and SC=0.60-0.0299S (R<sup>2</sup>=0.81, P=0.10) in AF OTC. It is noteworthy that ozone too reduced stomatal conductance sensitivity to salt stress: from -29.9 mmol m<sup>-1</sup>s<sup>-1</sup> per g L<sup>-1</sup> of water salinity in filtered air to -20.4 mmol m<sup>-1</sup>s<sup>-1</sup> per g L<sup>-1</sup> in not filtered air.

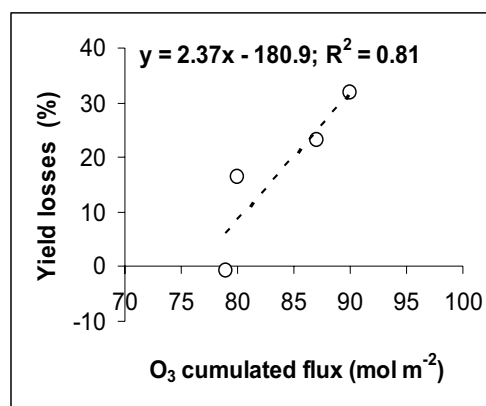
Therefore salt stress also reduced ozone uptake by plants: ozone uptake cumulated throughout the alfalfa growth period was correlated with yield losses due to ozone (Figure 2).



**Figure 1. Linear regressions between yield losses due to ozone and water salinity.**

## Conclusions

From this experiment, it was confirmed that environmental conditions can strongly modify the response of plants to ozone pollution. Therefore, the thresholds for the protection of vegetation actually based on plant exposure to ozone tropospheric concentration are not suitable for the Mediterranean cropping systems, because these environments are characterized by multiple stresses (i.e. drought, salinity and ozone) that can modify the response of plants to the single stresses. For these reasons a ozone flux approach that takes into account other pedoclimatic stressors, therefore based on the real ozone dose absorbed by plants, will be more suitable for estimating ozone damages to crops in the different agro-environmental conditions of Europe.



**Figure 2. Linear regression between yield losses and Ozone flux into the plant**

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# Effect of Cover Crops on Water Balance, Nitrate Leaching and Crop Productivity

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## Introduction and objective

Reducing nitrogen (N) fertilizer application is a main concern in EU, as a mean to diminish environmental problems related to water contamination and gaseous losses from agricultural systems (Addiscott et al., 1991). Irrigated agricultural areas have considerable potential for contaminating groundwater because irrigated crops are abundantly fertilized and it is usual to find large quantities of residual N in the soil after harvest (Vazquez et al., 2005). Sustainability of these cropping systems depends on developing agricultural techniques that minimize N fertilizer application while improving N use efficiency. One strategy for reducing the amount of nitrate leached, is using winter cover crops that absorb residual nitrate and N mineralized during non cropping periods (Dinnes et al., 2002). However, when water is scarce at planting the effect of cover crops on water availability might have an impact on crop productivity. In Mediterranean areas there is limited information and research is needed to study if cover crops can increase sustainability of cropping systems.

The objective of this work was to study the effect of cover crops on the water balance, nitrate leaching and crop productivity of irrigated maize grown in Mediterranean climate.

## Methodology

A four factor (bare soil and three cover crops) experiment was carried out on a silty clay loam soil in Aranjuez (Madrid, Spain). A maize field (3000 m<sup>2</sup>) was split in sixteen plots (144 m<sup>2</sup>) after harvest, and distributed in four treatments with for replications: barley (*Hordeum vulgare* L., cv. Vanessa), vetch (*Vicia villosa* L., cv. Vereda), rapeseed (*Brassica napus* L., cv. Licapo) and bare soil. Cover crops were killed in late winter, allowing seeding of maize of the entire trial in early spring. Soil inorganic N content was measured along the soil profile before sowing, and after harvesting maize and cover crops. Plant N uptake, biomass production and yield were recorded at harvest for maize and each cover crop. During the whole experiment, daily soil water content measurements based on capacitance probes were used to calculate drainage at 1,2 m depth, by applying the water balance equation. Nitrate leached at 1,2 m depth was calculated as the drainage volume multiplied by the nitrate concentration of the soil solution extracted in ceramic cups.

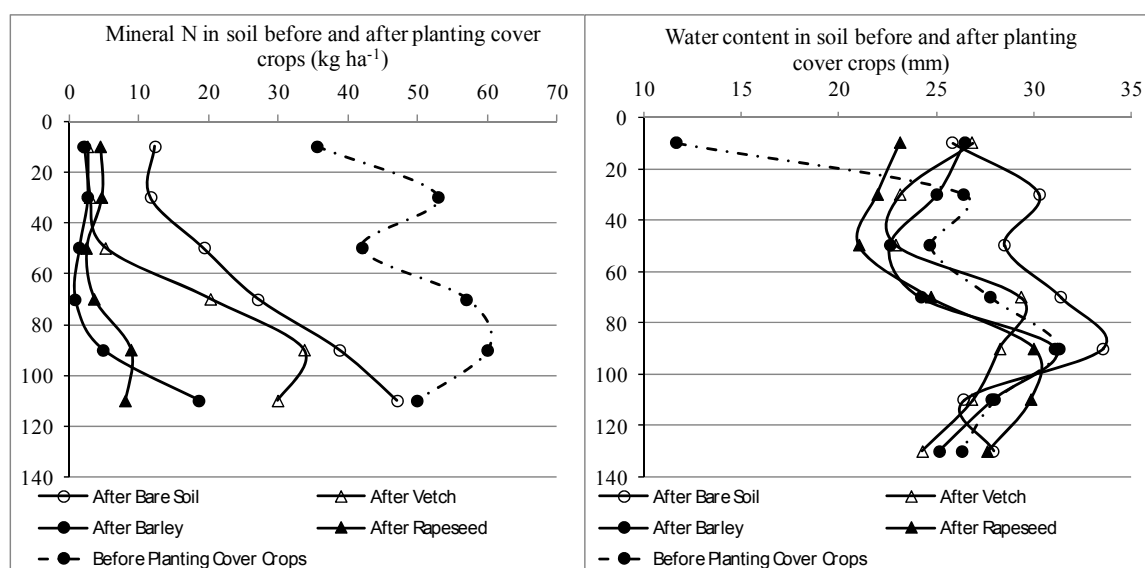
## Results

Measured variables showed differences between cover crop treatments and bare soil, but not between cover crop treatments. Cover crops uptake on average 160 kg N ha<sup>-1</sup>, while in the bare soil weeds only uptake 36 kg N ha<sup>-1</sup> (Table 1). All cover crops reduced nitrate leaching with respect to bare soil, but no difference was observed between cover crops (around 70 kg N-NO<sub>3</sub><sup>-</sup> ha<sup>-1</sup>). Drainage during the irrigated period was nule and most of the nitrate leaching took place during the autumn-winter period, between the two maize crops. In treatments sown with a cover crop the drainage period last for 80 d while in the bare soil last for 150 d. Soil water and N content at maize seeding were larger for the bare soil than for the cover crops (Fig.1). Maize production (biomass and yield) and plant N uptake was higher after the bare soil treatment (10-15%) than after the cover crop (Table 1).

**Table 1:** Yield of maize and N extracted by cover crops (\* the N extracted in the bare soil was absorbed by the weed).

Treatment	Yield (kg ha <sup>-1</sup> ) of maize (wet of grains: 14%)	N extracted by cover crops (kg ha <sup>-1</sup> )
Bare soil	15,264.3      a	36.0*      b
Vetch	13,382.3      b	179.3      a
Barley	12,796.6      b	156.9      a
Rapeseed	12,988.4      b	161.6      a

**Figure 1:** Conditions in the soil before and after planting cover crops (mineral N and water contents).



## Conclusions

The use of cover crops in Mediterranean conditions can minimize the risk of environmental problems due to the nitrate leaching. Nevertheless, a reduction in corn yield after cover crops might occur due to the combine effect water and N. Probably, this negative impact on crop yield could be avoided adapting irrigation and N fertilization management on the first stages of maize.

## Acknowledgements

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# Effect of Salinity Stress on Germination Indices in Six Rapeseed (*Brassica Napus*) Cultivar

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## Introduction

Current estimates indicate that 10-35% of the worlds agricultural lands is now affected by the salinity stress (Shaton 1997). It can be said that it is one of the major devastating stresses of the arid and semi arid regions. Salinity stress, can play an important role in reduction of the plant growth stages, specially germination stage. More than 90 percent of Iranian domestic need for oil is imported. Rapeseed, is valuable oil seeds in Iran, for its tolerance to salinity stress. Its seeds contain 40% high-quality oil. Germination is a critical stage of the plant life and resistance against salinity during the germination is important for stability. One of the commonest experiments in germination of the seeds is uses NaCl. Many experiments have been done and the results have showed that plumule has higher sensitivity to salinity stress than other traits. Ali Darjani (2006) in the study of four varieties of Rapeseed expresses that Hayola 401 had the highest germination percentage and radical length . E. Mogheisheh in the study of the effect of salinity and temperature on seed germination of two variety Brassica napus expresses that in germination stage PF cultivar is more tolerant than Hayola against salinity because had high germination in 30C in  $EC=16dSm^{-1}$ .

The physiological attributes of salinity tolerance in *Alyosia albicans* (C. albicans) and the  $F_1$  hybrids include Na and Cl retention in roots and limited translocation to the shoots , and maintenance of transpiration rate under saline condition. (sub barao et al 1990).

Because of salinity increase , germination percentage decreased and germination time increased . (Jajarmi. 2007)

In the study of the safflower reaction to the salinity of the water and soil, (Elias S.Basil 2001) expresses that with increases in the level of the salinity from 1.8 to 2.7 ds/m length of radical decreases. In the study of Influences of light, temperature and salinity on seed germination of *Reaumuria trigyna* (Xue Yan 2007) expresses that Seed germination trial and pot trial were conducted to investigate the effect of increasing concentrations (0, 0.1, 0.5, 1.0, 5.0, 10.0 g/litre) of salts (NaCl) with cv. Shandan 16. Seed germination rate, the growth of root and sprout, the number of roots increased with NaCL at less than or equal to 0.5 g/litre and decreased significantly as the concentration increased.

Mehmet Demir (2003) in the study of 3 varieties of safflower , showed that with the increase of salinity, length of radical and plumule dried weight of radical and plumule decrease.

## Methodology

In order to study the effects of salinity stress on germination indices in safflower cultivars, an experiment was conducted in factorial form, using a completely randomized design with three replications. In this experiment, seven safflower cultivars (A factor: Okp, Colvert, Orient, SLm, Fornax, Cobra )were evaluated in six levels of salinity treatment (B factor: distilled water, -3, -6, -9 bar) of NaCl.

NaCl (gr/lit)	Stress level (bar)
3.54	-3
7.8	-6
10.62	-9

After 4 days germination percentage war recorded every day. Seeds were considered germinated the emergent radical reached 2 mm length rate of germination and coefficient of velocity of germination were calculated as follow

Rate of germination = dg/dt

Coefficient of velocity of germination =  $100 \times \frac{A_1 + A_2 + \dots A_x}{A_1 T_1 + A_2 T_2 + \dots A_x T_x}$  (Pollock and Ross 1972)

Mean of day germination =  $\sum (Nt / \sum N)$

## Results

Increased salinity levels has deleterious effect on germination and radical length and plumule length. The recorded values are presented in table 1 .

A) Radical length and Pulmus length.

There are significant differences among varieties and salinity levels. The highest radical belonged to Colvert with 44.773 mm and the least radical belonged to Orient with 20.498 mm. Interaction between variety and salinity levels was not significant. With the increase of salinity stress levels radical length decreased.

The highest pulmus belonged to Colvert variety with 31.263 mm , while Orient with 18.381 mm had the shortest pulmus. There is no significant differences between -6 , -9 bar for pulmus length. The highest pulmus appeared in the water distilled. (table 1)

B) Germination percentage

The highest germination percentage took place in -3 bar with 91% and the least belonged to -9 bar with 66%. Okp had the highest germination percentage and the least belonged to Cobra (Table1).

C) Average Velocity of Germination

The highest (AVG) belong to Okp and the least belong to Cobra. The highest AVG belong to -3 bar stress and the least belong to -3 bar stress.

## Conclusions

In the studying the tolerance of varieties to salinity stress, germination percentage cannot be a good index in screening varieties (Germination percentage trait was affected by salinity stress less than the other traits). To find the best one tolerant variety to such condition, taking all traits, into account in this study , we found that Okp and Colvert varieties are the most resistant and Cobra and Orient are the most sensitive

Table 1 . Mean comparison of different levels of salinity

Average velocity of germination	Germination Percentage	Pulmus length (mm)	Radical length (mm)	Variety
5.87 a	95.668 a	41.3 ab	23.58 b	Okp
4.906 b	75 c	20.498 d	18.381 c	Orient
5.17 b	87 b	44.773 a	31.263 a	Colvert
5.19 b	85.32 b	28.328 cd	22.85 bc	Fornex
3.82 c	66 d	27.983 cd	24.82 b	Cobra
4.9 b	80.332 bc	38.992 ab	19.77 bc	SLm

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# Growth of Hemp and Lupin in Chromium, Arsenic and Copper Contaminated Soil

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CCA (chromated copper arsenate) is a wood preservative and thus soil around wood processing areas is often polluted with arsenic, chromium and copper. Complicated and expensive physico-chemical cleaning methods are sometimes used where the pollution is a mixture of arsenic and copper. There are various methods for the copper but options for arsenic are very limited (Lombi et al. 2004) and chromium is relatively little studied. Amendments may result in increased mobility and bioavailability of arsenic (Lombi et al. 2004) which then must be managed to ensure uptake rather than runoff. Plants can be utilised to solve (remediate) the soil contamination problem in various ways (McGrath & Zhao 2003), e.g. to extract the metals and metalloids from the soil and then be harvested for biomass (Lasat 2002). It is not only an ecologically and economically sustainable method but it also improves the fertility, biological and physical properties of the soil and biodiversity in the area (Cunningham et al. 1995). The heavy metal hyperaccumulator species that have been identified to date are slow-growing plants producing little biomass (Khan et al. 2000) and thus, fast-growing, high-biomass, moderate accumulators, generally removing more heavy metals per hectare should be identified (Ebbs et al. 1997). The studies concerning the CCA phytoremediation are scarce and many focus on *Pteris vittata*, a tropical fern hyperaccumulator. Hemp (*Cannabis sativa*) grows rapidly, producing high biomass, adapts easily to different climates and has a deep root system (Small & Marcus 2002). It is also an excellent companion and rotational crop, having soil restoration capacity (Citterio et al. 2005), and known to accumulate copper, cadmium, lead and zinc (Angelova et al. 2004). White lupins (*Lupinus albus*) grow in soil with low pH and low nutrient availability, increasing the soil pH and accumulating arsenic and cadmium in roots and root nodules (Vazquez et al. 2006). The development of proteoid roots may also increase the uptake of arsenate (Esteban et al. 2003). Nonetheless, the mechanism of CCA tolerance, when arsenic, copper and chromium are acting simultaneously, is still not known. Our objective is to utilize phytoremediation processes to remove the contaminants from the soil. The aim of the experiment was to determine the phytoremediation potential and heavy metal tolerance of crops [blue (*L. angustifolius*), white and yellow (*L. luteus*) lupins and hemp] in soils contaminated with As, Cr and Cu and to explore the possibility to utilize the biomass for bioenergy production.

## Methodology

In the greenhouse, fertilizers were mixed in sandy soil as follows: hemp 150 mg N kg<sup>-1</sup> soil and lupins 70 mg N kg<sup>-1</sup> soil, for all plants 40 mg P kg<sup>-1</sup> soil and 100 mg K kg<sup>-1</sup> soil (KS and Puutarhan täyslannos, Kemira Grow-How, Finland). Soil was added in 7.5 L pot and in each pot 10 seeds were sown. After emergence, plants were thinned to 5 per pot. Pollutants were mixed in the soil in four ratios: Cu:Cr:As 50:50:15, 100:100:5, 200:300:100 and 1000:1500:500 mg kg<sup>-1</sup> soil. The controls did not contain any pollutants. The experiment was arranged in a completely randomised design with eight replicates. Growing conditions were: day/night 16/8h, 21/16°C, PPFD 400 µmol m<sup>-2</sup> s<sup>-1</sup>. Net photosynthesis and leaf water potential were measured twice, during the estimated intensive growth phase. Samples for biomass accumulation were collected every second week, one plant per pot. The

development of plants was monitored from emergence to maturity. The concentration of contaminants in different plants parts was analysed at MTT Agrifood Research, Finland. Elemental concentrations were analysed with high resolution ICP (Thermo Jarrel Ash, IRIS Advantage). Dried sample was digested in nitric acid (p.a.), diluted with water and filtered. Cr and Cu were determined by ICP-OES. As was determined by ICP-MS.

## Results

Seeds did not germinate or plantlets were instantly killed in soil containing the highest concentrations of contaminants. The second highest concentration of contaminants applied resulted in death or stunted growth three weeks after germination. The second lowest concentration decreased the leaf area, whereas the lowest concentration (Table 1) did not seem to affect markedly the leaf area or shoot dry weight of fibre hemp and white lupin. Roots did not grow directly to deeper soil, rather they mainly grew in the upper part of the contaminated soil. Roots were also short, thickened and malformed. Leaf water potential decreased ca. 15% in fibre hemp and in white lupin, whereas net photosynthesis was not affected markedly by contaminants (Table 1). In the Cu-Cr-As:50-50-15 treatment, roots of fibre hemp contained Cu 14, Cr 6.7, As 3.8; stems Cu 4.8, Cr 1.3, As 2.1; leaves Cu 13, Cr 10, As 2.8 mg kg<sup>-1</sup> dw. Roots of white lupin contained Cu 21, Cr 9.8, As 1.0; stems Cu 7.2, Cr 0.5, As 1.2; leaves Cu 21, Cr 4.5, As 3.7; seeds Cu 12, Cr 0.1, As 0.4 mg kg<sup>-1</sup> dw. In both, fibre hemp and white lupin, the Cr and As concentrations were markedly higher in stressed plants than in controls and in white lupin the Cu concentration was also elevated. Thus, fibre hemp apparently excluded Cu.

Table 1. Dry weight of shoot and root in mature plants, maximal leaf area, net photosynthesis (Pn) and leaf water potential ( $\Psi_w$ ) in the middle of the growing period in fibre hemp and white lupins grown in soil containing, Cu 50, Cr 50, and As 15 mg kg<sup>-1</sup>. Data are means  $\pm$  SE, n=4-8.

Treatment	Crop	Dry weight, g plant <sup>-1</sup>		Leaf area, cm <sup>2</sup>	Pn, $\mu\text{mol m}^{-2} \text{s}^{-1}$	$\Psi_w$ , MPa
		Shoot	Root			
Control	Fibre Hemp	6.73 $\pm$ 0.27	0.34 $\pm$ 0.03	516 $\pm$ 32	19.0 $\pm$ 1.9	-0.90 $\pm$ 0.1
	White Lupin	14.28 $\pm$ 1.49	0.94 $\pm$ 0.20	418 $\pm$ 64	10.5 $\pm$ 0.7	-0.65 $\pm$ 0.1
Stress	Fibre Hemp	6.16 $\pm$ 0.47	0.58 $\pm$ 0.08	508 $\pm$ 96	19.4 $\pm$ 2.0	-1.05 $\pm$ 0.1
	White Lupin	12.94 $\pm$ 2.16	0.69 $\pm$ 0.11	471 $\pm$ 96	12.2 $\pm$ 0.6	-0.71 $\pm$ 0.1

## Conclusions

It seems that hemp and lupins have the potential for use in phytoremediation in e.g. landfills, where the contaminant concentrations are restricted to less than 100 mg kg<sup>-1</sup> soil.

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# Nitrate Concentration of Runoff in Two Rural Micro-Catchments of Central Italy: Results From a Ten-Years Survey

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## Introduction and objective

Aquatic pollution of agricultural origin is an issue that is receiving increasing attention in the EU (Macgregor and Warren, 2005). An understanding of the impact of cropping systems and management practices on N balance, storage, and loss is important in designing management strategies that will both increase agricultural productivity and minimize the risk of environmental pollution (Arregui et al., 2006). These impacts can be observed through long-term field experiments to examine crop yield and soil processes that affect the fate of soil N.

The objective of this study was to provide new field-based scientific evidence on the relationships between agronomic practices and the dynamic of N-NO<sub>3</sub> in the silty-clay hills of central Italy at micro-catchment scale.

## Methodology

Cropping systems and water runoff were continuously monitored in the decade 1998-2008 in two micro-catchments of the hilly rural area of Serra de' Conti (Ancona): Spescia (S; 80 ha, 69.4 ha of arable land; 43° 33' N; 13° 04' E) and Bottiglie (B; 60 ha, 48.0 of arable land; 43° 31' N; 13°02'E). The two micro-catchments were characterised by over 70% of arable land but different average field size (B = 1.2 ha; S = 6.0 ha), total number of fields (B = 40; S = 10), and crop space diversification, S being cropped with just one or two crops per year (durum wheat in rotation with sunflower, maize or sugar beet) for most of the time, while a number of different crops were grown in Bottiglie every year (winter wheat, durum wheat, durum barley, emmer, sunflower, sugar beet, alfalfa, vetchling, chick pea, lucerne, maize, olive grove, vineyards, other tree crops). From 1998 to 2002, the EU reg. 2078/92 agro-environment scheme were applied over the municipality area, including both micro-catchments. Prescriptions were focused on maximum fertilizer rates (N90 for wheat) and ploughing depth (< 25 cm). In the subsequent years, more than 50% of the arable land of B was managed with an organic farming scheme.

Surface runoff was monitored with an "area velocity" flow meter coupled with an automatic water sampling unit, installed at the bottom of a concrete pipe (1.2 m in diameter) positioned downstream the main ditch. Water samples were analysed for nitrate concentration, which will be discussed in relation to the agricultural practices and micro-catchment land use. In the two micro-catchments the soil permeability below rooting depth was almost null (Corti et al., 2006), so that almost all water surplus was discharged as runoff.

## Results

In the decade, the runoff coefficient was on average 25% in S and 7% in B. Average nitrate nitrogen losses associated to surface runoff were also significantly higher in S than in B (table 1). Fertilizers input and surplus were significantly lower in B than in S (table 2) and the average annual nitrate concentration in runoff water was significantly higher than 50 mg L<sup>-1</sup> for six years out of nine in S, reaching occasionally up to 328 mg L<sup>-1</sup>, while average annual nitrate concentration in runoff water at B was never higher than 50 mg L<sup>-1</sup>, reaching occasionally a maximum of 114 mg L<sup>-1</sup>.

The nitrate concentration dynamics of the two micro-catchments were associated to soil cover, spatial distribution of crops and field size and soil characteristics of the two watersheds. Nitrogen fertilization was recognised as the main cause of nitrate pollution in runoff water just in the runoff events that occurred after applications in the autumn or within 4 weeks after the fertilizer distribution and when nitrogen was applied before wheat seeding in the autumn, too early for the wheat plants to be able to absorb it.

Table 1 – Average rainfall depth and intensity, runoff, nitrate concentration and nitrate-nitrogen losses ( $\pm$  standard error) in "Spescia" and "Bottiglie" watersheds during the 10-years period, during (1998-02) and after (2003-08) the implementation of the EU reg. 2078/92 agro-environment scheme.

Period	Rainfall	$I_{60\max}$	Spescia			Bottiglie		
			runoff	$\text{NO}_3^-$ $\text{mg L}^{-1}$	$\text{N-NO}_3^-$ $\text{kg ha}^{-1}$	runoff	$\text{NO}_3^-$ $\text{mg L}^{-1}$	$\text{N-NO}_3^-$ $\text{kg ha}^{-1}$
1998-2002	$873 \pm 72$	$27 \pm 7$	$141 \pm 42$	$51 \pm 6$	$17 \pm 7$	$66 \pm 27$	$27 \pm 5$	$5 \pm 2$
2003-2008	$843 \pm 85$	$24 \pm 8$	$276 \pm 60$	$82 \pm 14$	$47 \pm 14$	$74^* \pm 21$	$27^* \pm 6$	$9^* \pm 6$
Average	$857 \pm 54$	$25 \pm 5$	$216 \pm 43$	$68 \pm 10$	$34 \pm 9$	$70^* \pm 15$	$27^* \pm 4$	$7^* \pm 3$

\*: excluding 2004 because sampler was out of order

Table 2 – Average N input and surplus ( $\pm$  standard error) in the Spescia and Bottiglie watersheds during the study period

Period	Input N ( $\text{kg ha}^{-1}$ arable land)		Surplus N ( $\text{kg ha}^{-1}$ arable land)	
	Spescia	Bottiglie	Spescia	Bottiglie
1998-2002	$86 \pm 17$	$60 \pm 4$	$21 \pm 15$	$14 \pm 4$
2003-2008	$96 \pm 23$	$46 \pm 6$	$57 \pm 9$	$7 \pm 11$
Average	$91 \pm 12$	$53 \pm 4$	$39 \pm 10$	$11 \pm 6$

## Conclusions

Results show that, under the context of central Italy hill cropping systems, soil cover and land use spatial diversification represents key factors to keep nitrate leaching within sustainable limits. Even low input hill cropping systems are not free of nitrate pollution of freshwaters if bare soil periods occur during water surplus in the autumn-winter period. For this reason, while the nitrogen surplus of the two micro-catchments was greatly improved by the application of the EU agro-environment scheme, this was not sufficient to preserve surface water from nitrate pollution. However, available incentives were provided to grow grass covers in winter were not used by farmers as these would hamper the yield performance of the subsequent spring-summer crop (Iezzi et al., 2002; Roggero and Toderi, 2002). Autumn nitrogen applications before wheat seeding, was also recognised as one of the most relevant polluting agricultural practice.

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# Efficacy of Narrow Buffer Strips in Removing Agricultural Pollutants in the Shallow Subsurface Water Flux from a Cultivated Field in North-East Italy

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Buffer strips are landscape features composed of a combination of trees, shrubs and native grasses, which, if interposed between cropland and surface water bodies, remove pollutants deriving from intensive agriculture before they reach the receiving water body (Peterjohn and Correll, 1984). In some cases overland storm flows entering the riparian buffer zone, infiltrate the soils and become groundwater. Many studies have shown >90% reductions in nitrate concentrations in subsurface flows as water passes through riparian areas (e.g. Fennessy and Cronk, 1997). Although there are other significant water quality effects: i.e. pH and dissolved phosphorus concentrations are modified (Borin et al., 2004), few studies have evaluated the differences in pollutant abatement efficiency of different buffer features. This paper assesses the cleaning performances of 4 different narrow buffer strips (BS) on the concentrations of nitrate-N, soluble P, electrical conductivity and pH in subsurface water coming from cropland.

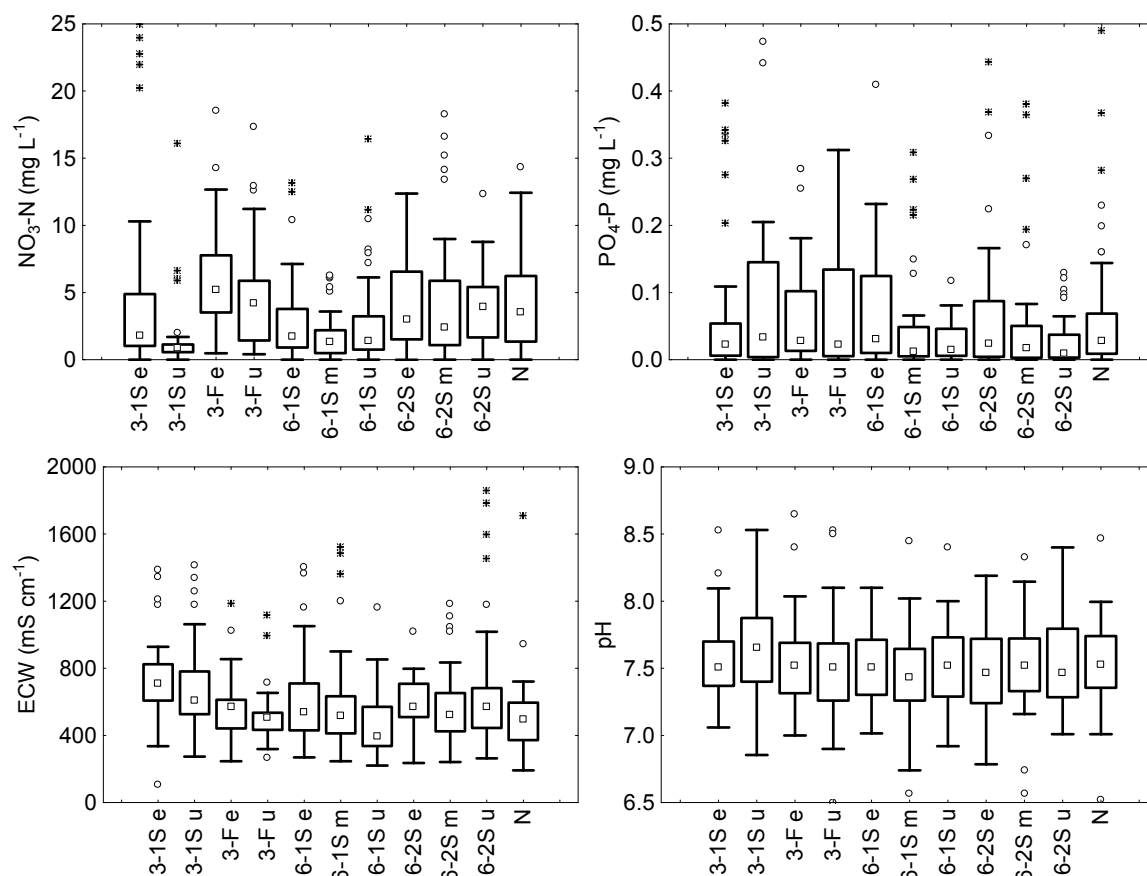
## Methodology

The experiment was conducted from January 1998 to August 2007 on the low-lying plains of the Veneto Region (NE Italy). The experimental site is a rectangular field, with a 35 m long 1.8% slope downwards to a ditch. The local climate is sub-humid, with average annual rainfall of about 830 mm. According to the FAO-UNESCO (1990) classification the soil is fulvi-calcaric Cambisol, with a loamy texture in the upper 80 cm. The following four types of BS, interposed between field and ditch, were studied in comparison with absence of BS (N): 3 m wide, with only grass cover (*Festuca arundinacea* L.) (3-F); 3 m wide, with one row of regularly alternating trees (*Platanus hybrida* Brot.) and shrubs (*Viburnum opulus* L.) (3-1S); 6 m wide, with a 3 m strip of grass and a tree/shrub row (6-1S), 6 m wide, with two tree/shrub rows (6-2S). Each treatment had two replicates. The field was cropped with winter wheat, maize and soybean, following standard agronomic practices. Subsurface water quality was tested entering and exiting the BSs. Data refer to the period from when the buffers were newly planted to vegetation maturity. Water table depth was measured and sampled with 22 phreatimeters. There were 2 phreatimeters in each treatment, at the entering and exiting points of the BS, aligned with the water flow direction. In the 6 m wide BS, there was also a phreatimeter in the middle of the buffer. There were 40 samplings during the monitoring period, taking 0.2 L water table surface samples from each phreatimeter, to give a total of approx. 830 water samples.

## Results

The buffer cleaning effect was heterogeneous, changing, for each parameter analysed, with the buffer strip types (Fig.1). The nitrate-N values showed a decrease in each treatment, particularly in the 3 m wide buffers. Only in 6-2S the median of exit concentrations was higher than entry, with a slight increase after the middle of the buffer (between the hedgerows). Nevertheless the BS cleaning efficiency is evident in each treatment, with a reduction of the highest values (3<sup>rd</sup> quartile). The distribution of soluble P concentrations showed a marked cleaning effect of the 6 m wide buffer strips.

Both median value and 2<sup>nd</sup> and 3<sup>rd</sup> quartile values were reduced at the exit point compared with the entry. The 3 m wide BS showed a general increase of soluble P release, compared with the entry concentrations. The ECW values showed decreases between BS entry and exit points in each treatment, except for 6-2S. The effect of BS on pH was confused, with no evident variations between BS entry and exit points in each treatment.



**Fig.1** - Box and whiskers of dissolved N, P concentrations and ECW, pH, in subsurface water sampled at the entry (e), exit (u) and middle (m) of the buffers and in reference (N) in the period 1997-2007.

## Conclusions

In numerous events the cleaning efficiency of the BS was evident. Nevertheless, the buffer effect was heterogeneous, changing, for each parameter analysed, with BS type. For example, the 6 m wide buffers were more efficient in abating phosphorus than nitrogen, while the 3 m wide abated only nitrogen. This is probably because the narrower strip of soil in the 3 m wide buffers was saturated faster by soluble P releases.

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# Bulk Atmospheric Deposition In An Urban And Rural Sites In The Southern Po Valley (Northern Italy)

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The atmosphere deposition influences the cycling of nutrients in eco and agroecosystems, contributing to the chemistry of plants, soils and surface waters.

The present work reports the pH and chemical composition of the rainfall bulk deposition at two sites in Italy, one in Bologna downtown and one in a rural site away from Bologna.

Efforts have been made to differentiate the degree of neutralisation and the seasonal trend of some ions. In addition, the two sampling points, located in two areas at different land use, have given us the possibility to determine the ions origins.

## Methodology

The climate of the experimental sites is temperate-continental, with a mean temperature of 13°C and precipitation annual amount of 700 mm, rained mostly in spring and autumn.

The Site 1 (40 masl; 11° 28'; 44° 30' N) is located in the center city of Bologna (373,000 inhabitants), 80 km far from the Adriatic Sea. It is 50 m far from the nearest busy road (traffic density  $\approx 10^3$  vehicles per day) and the two main sources of domestic energy are gas oil (approximately 60%) and gas methane (approximately 40%).

The Site 2 (190 masl; 11° 29' E; 44° 25' N) is located in a rural area on the hills (calcareous soil) surrounding the town of Ozzano (5300 inhabitants). It is situated at a distance of 25 km from Bologna monitoring station. Agricultural fields and natural vegetation surround the Ozzano monitoring station. Wheat and barley are the main crops during the winter, while maize, sunflower and soybean are the main crops during the summer. The nearest industrial area is 6 km away towards the Northeast but two busy roads "Via Emilia" ( $10^3$ - $10^4$  vehicles per day) and "A14 highway" ( $10^4$ - $10^5$  vehicles per day) are in plain 2 km and 5 km.

Sampling of bulk deposition was carried out at the two sites by means of standard rain gauge. During the study period a number of 163 and 123 samples were collected at the Site 1 and 2, respectively.

On the day after collection, conductivity and pH (at 20°C) of samples were measured. Then a capillary electrophoresis system (Beckman P/ACE 5500) was used for the quantification of major anions ( $\text{SO}_4$ , Cl,  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{PO}_4$ ,  $\text{HCO}_3$ ) and cations (Ca, K, Mg, Na,  $\text{NH}_4$ ), by the external standard calibration method with linear regression, according to the methods proposed by Dinelli et al. (1998).

## Results

The weighted mean pH in the period of the three years was 5.1 in Bologna and 5.3 in Ozzano, with restricted fluctuation, ranging from 4.5 to 6.0 in Bologna and from 4.4 to 5.5 in Ozzano. The mean values fall within the pH range of the clean atmosphere, so this rainfall can not be define acid.

As it is evident in the table, the bulk deposition chemistry was dominated by three anions:  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and Cl<sup>-</sup>.

Site	Cl	$\text{HCO}_3$	$\text{NO}_2$	$\text{NO}_3$	$\text{PO}_4$	$\text{SO}_4$	K	$\text{NH}_4$	Na	Mg	Ca	H
<b>Bologna</b>												
1997	22.26	7.04	1.52	27.32	0.73	30.12	8.03	7.43	11.09	0.17	12.71	0.05
1998	16.74	12.07	1.75	25.2	1.15	39.16	9.03	5.85	11.95	0.73	15.81	0.04
1999	35.66	9.52	2.51	31.03	2.21	44.34	9.52	7.77	21.02	2.14	20.27	0.06
MEAN	24.9	9.5	1.9	27.8	1.4	37.9	8.9	7.0	12.2	1.4	17.5	0.04
<b>Ozzano</b>												
1997	22.04	23.71	1.07	33.15	1.66	32.84	9.29	11.47	13.59	1.44	15.18	0.04
1998	17.93	23.20	1.10	36.23	0.58	32.31	4.21	9.29	12.19	1.36	20.25	0.04
1999	19.37	18.78	1.04	35.69	0.99	30.15	3.99	7.69	10.75	1.55	16.97	0.04
MEAN	19.8	21.9	1.1	35.0	1.1	31.8	5.8	9.5	14.7	1.0	16.3	0.05

Annual amount of anions ( $\text{kg ha}^{-1}\text{y}^{-1}$ ) at the Bologna and Ozzano monitoring stations.

In Bologna and Ozzano the sum of  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and  $\text{Cl}^-$  volume weighted mean of their concentrations accounted respectively for 89% and 81% of the total determined anions, while  $\text{Ca}^{2+}$  and  $\text{Na}^+$  were the two most abundant cations.

In the Bologna town the sulphates deposition increases significantly during the autumn-winter period. Probably, this is due to the residential heating, which constitutes, during the cold period, a local sulphure dioxide source, with elevate emission of that pollutant in the air.

Nevertheless in Ozzano  $\text{SO}_4$  doesn't present a seasonal trend and its value remain similar to that one in Bologna during the spring-summer period all over the year.

Since, the rain pH depends on the chemical species present in the air and their relationships, the rainfall pH is maintained to normal value, despite the high concentration in the atmosphere of oxides of nitrogen and sulphates, thanks to the calcium presence in the air. This element arrives at our environment transported by the dust-rich wind of the Sahara desert during all the year. The other anions, taking part at the neutralisation of the main acidity, are hydrogenous and the ammonium.

Starting from the ions concentration in the rainwater, an index determines the acidity of rain with the following equation (Flues et al., 2002):

$$\text{Index} = \frac{S + N}{Ca}$$

where S, N and Ca are the sulphates, nitrates and calcium concentrations respectively, expressed in  $\mu\text{eq l}^{-1}$ . When this value is close to 1, the rainfall is not acid, despite the high concentration of acidic pollutant in the air.

### Conclusions

Despite of the fact that our study sides are located in one of the most industrialized country in the world, pH values range from slightly acid to slightly alkaline. The chemistry composition of the bulk deposition can help to understand this: results show concentration of sulphates and nitrates, which are the main responsible for rainfall acidification, very elevate and in several cases higher then that ones surveyed in typical acid rain regions. The explanation of this apparent contradiction is in the study of the chemical reaction that takes part in the atmosphere. In particular, the presence of alkaline elements, principally the calcium, derived by the dust-wind from Sahara desert, neutralize the acid pollutants, keeping the pH to value typical of the clean atmosphere. That means that the rainfall pH can not be the only parameter to describe the atmosphere and rainfall quality. To obtain more specific and complete information, it is important an analysis of the contained ions. The presented index  $(S+N)/Ca$  can be used effectively to forecast the acidity of the rainfall. All the countries of the Mediterranean basin beneficiate of this phenomenon.

Annual mean deposition on soil of anions and cations can be ordered in a descending way as follows:  $\text{SO}_4 > \text{NO}_3 > \text{Cl}$  and  $\text{Ca} > \text{Na}$  in Bologna;  $\text{NO}_3 > \text{SO}_4 > \text{Cl}$  and  $\text{Ca} > \text{Na}$  in Ozzano.

The different locations of the two sides, one in the Bologna downtown and the other in an urban area, help to understand the source of these ions. Thus, the anions differences can be ascribed to the different environmental and land use of the two sides: the higher amount of  $\text{SO}_4$  in the town is due to the residential heating during the winter. In fact, the  $\text{SO}_4$  has a predominant anthropogenic origin; the high nitrates deposition in both the sites is due to the proximity to trafficked roads and the prevalent wind direction in the case of the site in the rural area. In Ozzano we found also an elevate concentration of ammonium due to fertilizers applications in agricultural fields. This ion is present in lower concentration in Bologna town.

These results indicate that the principle sources of pollution are local: fossil fuel combustions, heating and fertilizers.

The other ions present in the atmosphere derive principally from seawater or are crustal element.

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# Ability Of Different Cover Crops To Reduce N Leaching In Irrigated Maize Under Mediterranean Conditions

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## Introduction and Objectives

Maize grown in monoculture is a common practice in the irrigated areas of the Ebro Valley (Spain). Nitrate leaching in this area is associated to excessive N fertilization to maize and residual mineral N ( $N_{inorg}$ ) left in the soil after crop harvest (Caveró et al., 2003). Winter cover crops could be sown after maize harvest. Deep rooted cover crops would scavenge residual  $N_{inorg}$  in the soil profile and incorporation of the cover crop biomass into the soil in early spring would provide N to the next spring maize crop (Isse et al, 1999). However, little is known about cover crop management and effects on irrigated maize in Mediterranean conditions. The aim of the study was (1) to quantify the effect of winter cover crops on N leaching and soil  $N_{inorg}$  in a maize monoculture; and (2) to quantify the effect of cover crops on maize grain yields.

## Methodology

The experiment was conducted in 12 drainage lysimeters (5.2 m<sup>2</sup> x 1.5 m deep) filled up with a silt loam soil, located in the CITA experimental farm station (Zaragoza, Spain). Maize was planted on 28 April 2006 at a plant density of 84,600 plants ha<sup>-1</sup> and fertilized with 300 kg N ha<sup>-1</sup>. After maize harvest, different cover crops were seeded.. Four treatments were tested: winter barley (*cv.*Hispanic ), common vetch (*cv.* Armantes), winter rape (*cv.* Perko) and a control (bare soil). Cover crops were incorporated into the soil on 11 March 2007 and a new maize crop was sown on 15 April 2007. Maize was fertilized with 300 kg N ha<sup>-1</sup> in the control treatment, and this amount was reduced in the treatments with winter cover crops (Table 1). N fertilization was split in three applications. Irrigation was scheduled considering a leaching fraction of 25%. The volume and NO<sub>3</sub>-N concentration in the drainage water from the lysimeters was obtained on a weekly basis. Cover crop and maize biomass and N content was measured. Soil was sampled and soil  $N_{inorg}$  was determined at each maize harvest, before incorporating cover crops and before the first sidedress N application.

## Results

The amount of N in the above ground biomass of cover crops before its incorporation into the soil was similar for barley and winter rape. However, it was only 48 kg ha<sup>-1</sup> in common vetch due to winter frost and high soil salinity (Table 1). No drainage was observed during autumn and winter time. Drainage started in April with maize irrigation. The drainage observed in the control (340 mm) was reduced by 13 – 19 % when maize was preceded by a winter cover crop.

Treatment	Nitrogen applied		Maize		
	Cover crop kg ha <sup>-1</sup>	Fertilizer kg ha <sup>-1</sup>	Grain yield t ha <sup>-1</sup>	N uptake kg ha <sup>-1</sup>	N leached kg ha <sup>-1</sup>
Control	0	300	16.8 ab	307 a	17.5 a
Barley	173	154	13.9 b	210 ab	3.7 b
Winter rape	139	152	13.3 b	185 b	3.5 b
Common vetch	48	266	18.6 a	326 a	21.0 a

Table 1. Cover crop N, N fertilizer applied, maize grain yield, maize N uptake and N-NO<sub>3</sub><sup>-</sup> leached in the different treatments

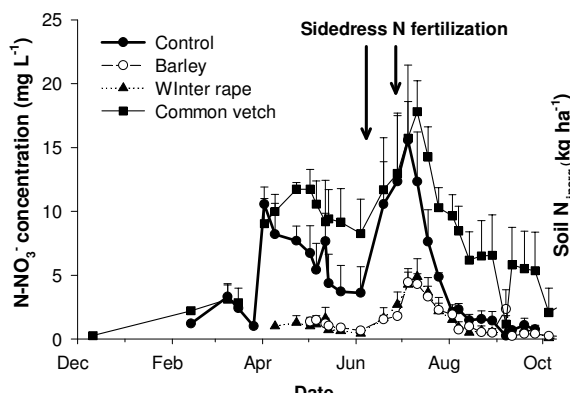


Figure 1.  $\text{NO}_3\text{-N}$  concentration in drainage water during maize crop in 2007. Bars indicate standard error.

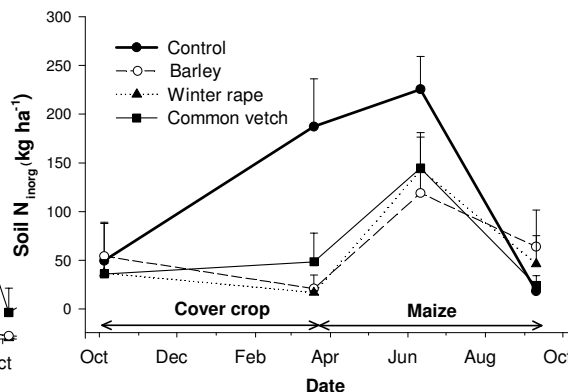


Figure 2. Soil  $\text{N}_{\text{inorg}}$  in the 0 to 0.3 m soil layer at different sampling dates during the growing season. Bars indicate standard error.

The  $\text{NO}_3\text{-N}$  concentration in the drainage water was significantly lower in the treatments where maize was grown after barley or winter rape compared to the control treatment, whereas it was higher after common vetch (Figure 1). Two peaks in  $\text{NO}_3\text{-N}$  concentration were observed (1) at the start of the irrigation after maize planting and (2) after the first sidedress N fertilization. The total mass of  $\text{NO}_3\text{-N}$  leached in the drainage was reduced in the barley and winter rape treatments by 78-80 % compared to the control treatment, whereas common vetch had no effect on  $\text{NO}_3\text{-N}$  leached (Table 1). Although the incorporation of the cover crop increased the soil  $\text{N}_{\text{inorg}}$  content due to mineralization, the soil  $\text{N}_{\text{inorg}}$  in the 0-0.3 m soil layer was significantly higher for the control treatment during the maize growing season, except at harvest time (Figure 2). The highest maize yield was observed in the common vetch treatment, being higher than the other cover crops treatments. The barley and winter rape treatments resulted in lower maize yield, although the differences with the control treatment were not statistically significant ( $P > 0.05$ ) (Table 1).

## Conclusions

The use of cover crops reduced  $\text{NO}_3\text{-N}$  leaching at the start of the following maize crop due to low precipitation during the autumn-winter period. The reduction of  $\text{NO}_3\text{-N}$  mass leached was related to the reduction of  $\text{NO}_3\text{-N}$  concentration and volume of drainage water. Maize yield was reduced, although not significantly, when barley and winter rape were grown during the maize intercrop period. However, common vetch resulted in the highest maize yield. Although soil  $\text{N}_{\text{inorg}}$  in the upper layer increased after cover crops incorporation, it was lower than in the control treatment. The reduction observed in  $\text{NO}_3\text{-N}$  leaching and the possibility to reduce N fertilizer inputs, gives scope for the use of cover crops in irrigated maize to reduce N leaching risks. However, cover crop management and maize fertilization should be further studied for these conditions to avoid yield reductions in maize.

## Acknowledgements

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# Performance of a Constructed Surface Flow Wetland in Abating Nutrients in Agricultural Drainage Waters

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Constructed surface flow wetlands (SFW) are vegetated basins capable of removing many contaminants from polluted waters (Maine et al., 2007). Scattered on agricultural land, they can intercept and treat the drainage flow coming from fields and abate the non point source pollution.

In order to collect data on the performance in abating nutrients, an experimental SFW was constructed in 1996 at the Experimental Farm of the University of Padova in Legnaro (Italy). During the period 1998-2002 the wetland received nearly 2,000 kg ha<sup>-1</sup> and discharged 206 kg ha<sup>-1</sup> of nitrogen, with an abatement close to 90% (Borin and Tocchetto, 2007).

In this paper, data regarding the period 2004-2007 are taken into account to continue the monitoring of NO<sub>3</sub>-N abatement and to gather the first results on soluble P treatment.

## Methodology

The wetland, vegetated with *Phragmites australis* (Cav.) Trin, is a single treatment that covers an almost square area of 3,200 m<sup>2</sup> and 0.4 m in depth. It receives, via a pump, the drainage waters from an agricultural basin of about 6 hectares. The water is collected by subsurface pipes and diverted to a manhole. Here, a pump discharges the water into the SFW before final delivery into a stream. Arable crops are cultivated in the agricultural basin following standard agricultural practices. Fertilization is applied to crops according to plant requirements, using only chemical fertilizers; further specifications are given in Borin et al. (2001; 2002). Within the wetland, three small banks (25 cm high) force the water to follow the longest route from inlet (pump) to outlet (stream). At the outlet, an upward-curving pipe allows space for other pipes of varying lengths to be inserted to regulate the desired depth of water inside the wetland.

All water volumes: rainfall, entering the wetland from the pump, and exiting into the stream were recorded. The depth of flooding or water table depth were also measured. Ninety-nine samples of input and 36 samples of output water were taken when water entered or was discharged from the wetland; the samples were analyzed for NO<sub>3</sub>-N using the Cataldo method (Cataldo et al., 1975) and for soluble P using the Olsen method (Olsen et.al., 1954) to assess the wetland performance in reducing nitrogen and phosphorus loads.

## Results

Taking the whole period, the median NO<sub>3</sub>-N concentration was 3.73 ppm in the inflow water and 2.38 ppm in the outflow (Figure 1), with a statistical difference at P<0.05. On the contrary, no statistical differences were found on the median values of soluble P (0.008 ppm in the inflow and 0.026 ppm in the outflow) (Figure 1). No correlations were found between pollutants concentration and water volumes.

The wetland received a total amount of about 25,000 mm of water from the drainage (on average, about 7 times the environmental rainfall); its water regimen was discontinuous and flooding occurred on a variable number of days per year (4 to 27). The wetland discharged only 1,625 mm of water. (Table 1).

The total NO<sub>3</sub>-N load entering the wetland was 1,047 kg ha<sup>-1</sup> and the total discharged was about 94 kg ha<sup>-1</sup>, with an abatement higher than 90%. The total load of soluble P was around 10 kg ha<sup>-1</sup> on entry and the total discharged was 0.7 kg ha<sup>-1</sup>, with an abatement higher than 92%. In both cases the abatement is strictly connected with the reduction of the water volumes flowing through the wetland.

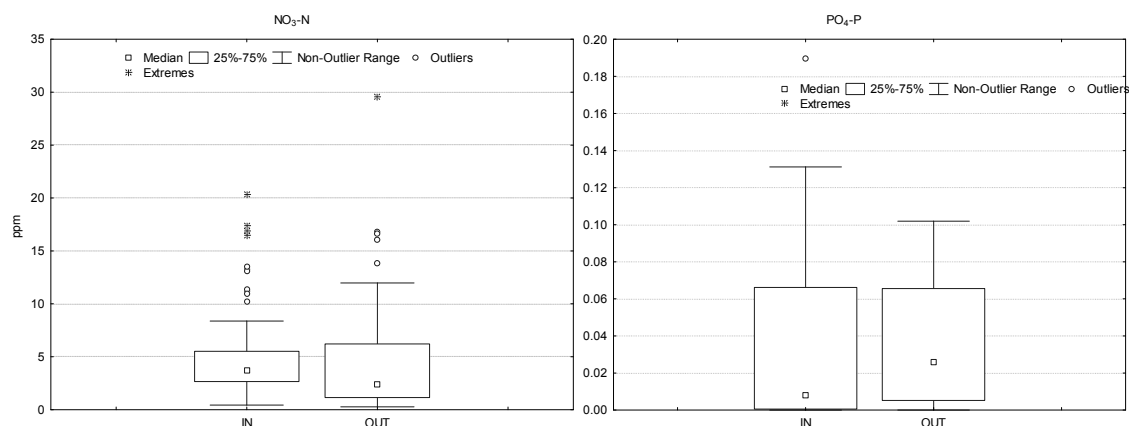


Figure 1 Box and whiskers of NO<sub>3</sub>-N and soluble P in the water entering and exiting the wetland

years	water (mm)		flooding days		NO3-N (kg/ha)		soluble P(kg/ha)		
	in from drainage	rainfall	total in	out	number	in	out	in	out
2004	6,995	1,054	8,049	537	23	593	69	3.85	0.33
2005	10,488	1,03	11,518	573	26	196	6	4.17	0.25
2006	4,755	699	5,454	42	4	108	0.7	1.62	0.00
2007	2,831	640	3,471	473	27	150	18	0	0.02
Total	25,069	3,423	28,492	1,625	80	1,047	93,7	9.64	0.7

Table 1 Water and nutrients balance of the wetland

## Conclusions

These data confirm the findings of the monitoring during the first five years of wetland performance on nitrogen abatement and add original data showing similar behaviour on phosphorus removal. They are an astonishing example of how, dedicating a small percentage of agricultural land to constructed wetlands, it is possible to greatly enhance the sustainability of the farming practices.

## Acknowledgment

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# The Use of Potassium Humate for Regulation of Heavy Metal Uptake by Spring Wheat and White Mustard

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## Introduction

Contamination of soils with heavy metals is an important problem in several agricultural areas. Crops grown on polluted soils can accumulate considerable quantities of these toxic elements, which may become toxic for the plant and dangerous in the food chain. Several possible methods exist, how to solve this problem, but they mostly are expensive and demand long time in range of ten years. Soil treatment with substances affecting heavy metal availability could offer one possibility how to influence the transfer of heavy metals from soil to plants. Humic substances e.g., can affect the hygiene of the soil due to absorption of pesticide residues and of further elements of risk. Richter and Hlušek (1991) e.g. found a significant decrease of Cd and Pb, and a lesser decline of Zn in potato shoots when solid soda humates were added into the soil. Important, however, is the quality of the added humic substance, especially the ratio between fulvic and humic acids, as fulvic acids possess a considerable capacity to create water-soluble chelate complexes and in this way to mobilize elements of risk in the soil. On the other hand, humic acids dispose of a great capacity to adsorb most of these elements. Kolář a Pezlarová (1990) recommend using specially prepared humic acids for immobilization of Cd. The importance of humic acids for sorption of Cd confirmed also Bolton et al. (1996). In addition, humic acids in soils affect positively the soil fertility.

## Methodology

The effects of solid and liquid potassium humates on heavy metal uptake (Cd, Pb, Zn) by spring wheat (variety Munk) and white mustard (variety Veronika) were studied in pot experiments. Both types of humates were applied to the contaminated soil in final concentrations from 0.015% to 0.3% (w:w, v:w). The used growing substrate was a contaminated soil sampled near a lead smelter. In this soil the concentrations of elements exceed marginal permitted values (4.15 mg Cd kg<sup>-1</sup> soil, 991 mg Pb kg<sup>-1</sup> soil, 480 mg Zn kg<sup>-1</sup>). The spring wheat plants were harvested in three stages – early stem elongation (pot experiment in a climatic box under regulated temperature and light/ dark conditions) and at stage of plant flowering and maturity (in a greenhouse), white mustard was harvested at stage of its maturity in a greenhouse. Plants were regularly fertilized with a 1M NH<sub>4</sub>NO<sub>3</sub> solution in order to allow a regular growth. After harvesting, the shoots were dried at 60° C and weighed. Roots were taken out from the soil, washed, dried and weighed. Thereafter, pulverized shoots and roots were digested using microwave apparatus Milestone 1200. Heavy metal concentrations in plant extracts were determined by means of ICP spectrometer Thermo Jarrel Ash.

## Results

1) Both types of humates decreased significantly the Cd and Zn concentrations in plant shoots in spring wheat grown in a climate-box and harvested at stage of early stem elongation. Higher concentrations of humates decreased significantly the Zn-content also in wheat roots. The content of Pb in plants was not significantly affected by treatments with potassium humates (Fig. 1). All treatments with the solid humate and the highest dose of liquid humate increased the shoot-dry matter, the root growth was positively affected only by higher concentrations of the solid humate.

2) In a greenhouse, the one half of the spring wheat was harvested at stage of flowering, the second part at stage of maturity. The white mustard was harvested at stage of maturity. The heavy metal content in wheat shoots increased during development from plant flowering to maturity. So the concentration of Cd increased in wheat shoots 16 times, whereas the concentrations of Pb 4.5 times and of Zn only 1.5

times. In contrast to Pb and Zn, the Cd-content in the spring wheat and the white mustard at stage of maturity was higher in shoots than in roots. Similar results described Angelova et al. (2008) in the oil seed rape. A greater transfer of Cd from roots to shoots in comparison with Pb and Zn was observed. However, the concentrations of metals in seeds of both, spring wheat and white mustard, usually did not exceed the natural heavy metal contents. In contrary to the spring wheat harvested in the stage of early stem elongation, the uptake of the studied elements by plants in advanced stages of growth was not significantly affected by the two used kinds of humates. A certain decrease of the Zn- concentration was observed in plant shoots and partly in roots when the 0.3% concentration of humates was used. Treatment with humates positively affected mostly the growth of white mustard as both types of humates (concentrations 0.075%, 0.3% respectively) increased dry matter of shoots and seeds (Fig. 2).

Fig 1: Changes of heavy metal concentrations (Cd, Zn, Pb) in shoots and roots of the spring wheat (variety Munk) following the treatment with solid and liquid humates at stage of early stem elongation.

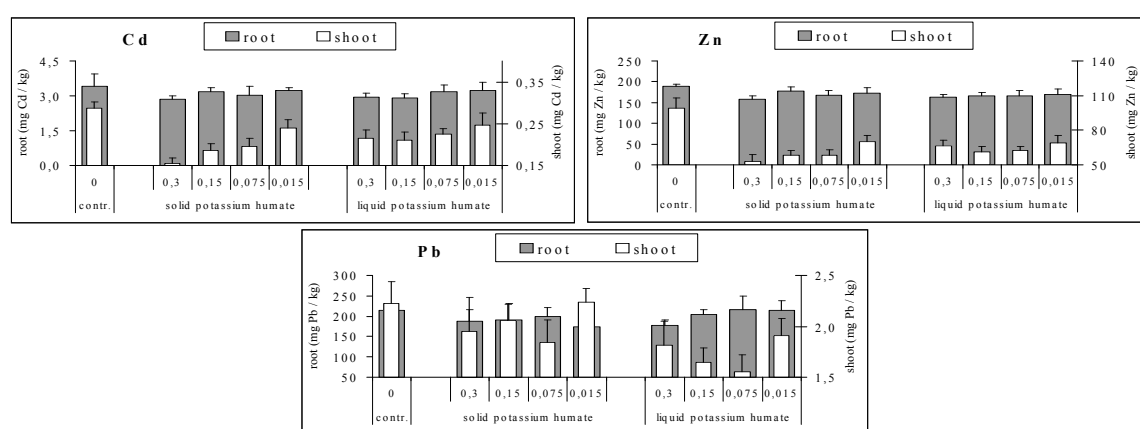
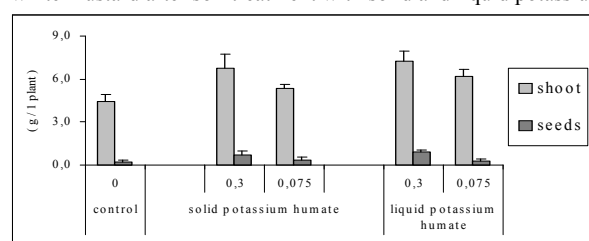


Fig. 2 : Dry matter and seeds of white mustard after soil treatment with solid and liquid potassium humate at maturity plant



## Conclusions

The solid and liquid potassium-humate applied into the contaminated soil decreased heavy metal uptake, particularly the Cd- and Zn-uptake, in the spring wheat at the stage of early stem elongation. At stage of maturity this effect could be observed only in the Zn-content with the two highest used concentrations of humates. The most effective concentration was 0.3%. Both kinds of humates improved root growth and increased dry matter of shoots.

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# Phytoremediation of Arsenic by Woody Species in Pluri-Contaminated Wastes

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Environmental pollution with trace metals is a world-wide problem, and the development of phytoremediation technologies for plant-based clean-up of contaminated soils is of great interest. Green technologies are currently available for only a small subset of pollution problems. For arsenic, a poisonous semi-metal occurring naturally in certain rocks and commonly used in pesticides, there is great concern due to its extensive contamination and carcinogenic effects. In several countries, common soil concentrations are  $10 \text{ mg kg}^{-1}$ , with even higher values in the vicinity of steel industries. Unlike metals, As bioavailability and plant uptake increase with soil alkalinity, thus making the remediation of pluri-contaminated sites more difficult. An arsenic-hyperaccumulating fern (*Pteris vittata* L.) has recently been discovered, allowing reliable *in situ* phytoremediation of contaminated soils or water (Kertulis-Tartar et al., 2006), mainly in tropical and warm environments. In temperate climates, study of the phytoremediation potential of various woody species may be more interesting, partly in view of their ability to take up various pollutants and also their landscape-enhancing role. In this framework, the present study focuses on the phytoremediation capabilities of various *Salicaceae* species grown in arsenic- and metal-contaminated wastes.

## Methodology

A two-year pot trial was carried out at the experimental farm of the University of Padova at Legnaro (Italy): 4 species, white poplar (*Populus alba* L.), black poplar (*Populus nigra* L.), European aspen (*Populus tremula* L.) and white willow (*Salix alba* L.) were grown in severely polluted pyrite cinders. The wastes had been discharged in past decades at Torviscosa (Udine, NE Italy) after pyrite ore roasting, as the by-product of sulphur extraction, and covered with a less polluted layer of gravelly soil. Uncultivated cinders were subalkaline (pH 7.3), lacking in organic matter, and had  $0.3 \text{ S m}^{-1}$  electrical conductivity. Arsenic content was  $886 \text{ mg kg}^{-1}$ , i.e., 44 times over the maximum Italian permitted limit for agricultural uses ( $20 \text{ mg kg}^{-1}$ ). The wastes were also markedly contaminated by Co, Cu, Pb and Zn. Three replicates of one-year-old bare rooted cuttings with naked roots (clones), obtained from plants naturally established at S. Giustina in Colle (Padova, Italy), were transplanted at the end of June 2004 into large (inner diameter 0.25 m, 0.45 m height) opaque PVC pots, filled with pyrite waste and covered with a 0.15-m deep layer of unpolluted silty-loam soil, to mimic the polluted site stratigraphy as closely as possible. The dynamics of root growth were monitored weekly during the second year (2005) through the non-destructive method of electrical capacitance (EC) (Preston et al., 2004). Growth (weight) and As accumulation (ICP-OES analysis) were evaluated at the end of the experiment (October 2005) in wood, leaves, and coarse and fine roots (2-mm diameter threshold), in comparison with plants grown in an uncontaminated reference soil (the above-mentioned silty-loam), with  $18 \text{ mg As kg}^{-1}$ .

## Results

In comparison with plants grown in the uncontaminated reference soil, plant growth was markedly reduced in cinders (all species), especially at above-ground level (Table 1). Although growth reduction involved the whole plant, roots were generally less affected than shoots, thus leading to an increase in the root-to-shoot ratio (RSR), a result also found by Ballach (1995) in poplars grown in platinum-polluted soils. On average, wood decreased by 45%, leaves by 30% and roots by 36%. Arsenic was not found in wood (below the Limit Of Quantification, i.e.,  $\text{LOQ}=1.4 \text{ mg kg}^{-1}$ ). Low concentrations were

also detected in leaves, with values ranging between 2 mg kg<sup>-1</sup> (*Populus nigra*) and 5 mg kg<sup>-1</sup> (*Salix alba*) (Table 1). Instead, at root level, As reached appreciable concentrations, with greater values in fine roots than in coarse ones (averages: 86 vs. 31 mg kg<sup>-1</sup>).

Table 1. Plant biomass and As concentration in various organs in four *Salicaceae* species. Letters: differences among species for same organ (LSD test; P≤0.05). \* below LOQ.

Organ	Species	Uncontaminated reference		Polluted wastes	
		Biomass (g)	As (mg kg <sup>-1</sup> )	Biomass (g)	As (mg kg <sup>-1</sup> )
Leaves	<i>P. alba</i>	32.3	a	17.0	a
	<i>P. nigra</i>	20.6	ab	16.8	a
	<i>P. tremula</i>	21.4	ab	17.9	a
	<i>S. alba</i>	10.4	b	6.47	b
Coarse roots	<i>P. alba</i>	102	a	71.0	a
	<i>P. nigra</i>	85.2	ab	57.6	a
	<i>P. tremula</i>	65.1	b	35.4	b
	<i>S. alba</i>	68.5	ab	36.3	b
Fine roots	<i>P. alba</i>	67.9	a	58.9	a
	<i>P. nigra</i>	50.5	a	32.4	b
	<i>P. tremula</i>	61.1	a	39.2	b
	<i>S. alba</i>	65.3	a	34.3	b

*Populus nigra* had As concentrations 4.3 times higher than the other species in coarse roots (Table 1); whereas there were no differences among species in fine roots. Correlation analysis showed that leaf As concentration was significantly and positively correlated with root weight within the layer of cinders ( $R^2=0.56$ ;  $P\leq0.01$ ) and with the seasonal root EC (integral) standardised (divided) by the shoot biomass ( $R^2=0.71$ ;  $P\leq0.001$ ). Arsenic uptake and translocation are therefore closely correlated with root colonisation of the polluted layer and with root activity. Arsenic offtake, result of biomass×concentration, was negligible in leaves (0.04 mg per plant, maximum), but appreciable values were calculated at below-ground level, particularly in fine roots. In two years of cultivation, the whole root system of *P. nigra* accumulated 8.4 mg As per plant, a value very similar to that of *P. Alba* (6.3 mg), but more than double that of the other species (Fig. 1).

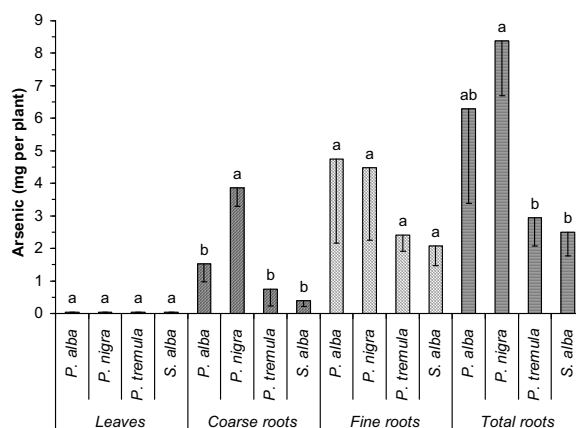


Fig. 1. Arsenic offtake in various organs and species grown in wastes. Letters: differences among species for same organ (LSD test; P≤0.05). Vertical bars: standard error.

## Conclusions

Arsenic phytoextraction by our woody species was completely inefficient because of poor translocation to the aerial biomass. However, appreciable immobilisation of this metalloid within roots suggests the possibility of efficient phytostabilisation through adequate species selection. In this regard, studies of root turnover would be necessary to establish the exact contribution of fine roots in long-term metal immobilisation.

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# Distribution of Some Heavy Metals in the System: Irrigation Water-Soil-Fresh Potatoes

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## Introduction

A number of investigations (Dokmen, 2004; Nazif et al., 2006) have been carried out on the determination of heavy metals in irrigation waters. The effect of elevated concentrations of these metals in irrigation waters on their distribution in soils and plants was also studied (Kalavrouzitis and Drakatos, 2002, Sharma et al., 2007). The results obtained indicate that waters polluted with heavy metals can have significant influence on their content in soils and plants. Various methods for the removal of heavy metals from contaminated waters have been studied (Erdem et al., 2004; Jochova et al., 2004). The purpose of the investigation was to determine:

- efficiency of a filter used for removal of heavy metals (As, Cd, Cr, Cu and Pb) from irrigation water
- the effect of irrigation water on the content of the aforementioned elements in the soil and fresh potatoes.

## Methodology

The experiment used in this investigation was set up in April 2007 on a humogley soil located near Belgrade. It consisted of the following water treatments and irrigation systems: 1. channel water (furrow), 2. channel water with sand filter (subsurface drip) and 3. channel water with sand filter + heavy metal dosing + heavy metal removal (subsurface drip). The following heavy metals were added to the irrigation water: As, Cd, Cr, Cu and Pb. Water samples for the analysis were taken on the 11<sup>th</sup> of July and 28<sup>th</sup> of August 2007. Soil samples (0-20 and 20-60 cm) were taken on the 20<sup>th</sup> of August, whilst potatoes were collected at the harvest (6<sup>th</sup> of September). The content of the investigated heavy metals in water, soil and potato samples was determined by ICP method. The results obtained were subjected to statistical analysis (analysis of variance and lsd test).

## Results

Average concentrations of the investigated heavy metals in irrigation water before and after passing through the filter for their removal are presented in Table 1. They were below maximum permissible values for their concentration in irrigation water.

Table 1. – Average concentrations of the heavy metals (in µg/l) in irrigation water

Element	Before filter (a)	After filter (b)	Ratio a/b
As	3.9	2.6	1.5
Cd	10.8	1.6	6.8
Cr	17	2.4	7.1
Cu	154	39.5	3.9
Pb	21.9	3.1	7.1

It can be seen from the results presented in Table 1 that the investigated filter was least efficient in removal of arsenic from the irrigation water. The concentration of this element was reduced by factor 1.5. Highest reduction in concentration was obtained in the case of chromium and lead.

Table 2 shows total contents of the metals in soil samples taken from two depths. Total contents of As, Cd, Cu and Pb are below maximum permissible values for their contents in agricultural soils. Treatment 3 had higher content of total Cr and Cu in both soil layers in relation to treatment 2. However, these increases were not statistically significant at the 0.05 probability level.

Table 2. – Total contents of the investigated metals (mg/kg) in two soil layers (0-20 and 20-60 cm)

Treatment	As		Cd		Cr		Cu		Pb	
	0-20	20-60	0-20	20-60	0-20	20-60	0-20	20-60	0-20	20-60
2	<20	<20	2.3	2	174	171	22	21.7	25.7	22.7
3	<20	<20	2.0	2.3	183	179	24	23.3	25.3	27.0

It can be seen from the results presented in Table 3 that fresh potatoes from treatment 3 had higher content of Cd and Cr in relation to potatoes from treatment 2. The differences are statistically significant.

Table 3. – Concentrations of the heavy metals (in mg/kg) in fresh potatoes

Treatment	As	Cd	Cr	Cu	Pb
1	<0.05	0.07	2.81	0.08	0.13
2	<0.05	0.03	1.14	0.10	0.20
3	<0.05	0.06	2.51	0.02	0.11

## Conclusions

On the basis of the results obtained, the following important conclusions can be drawn.

1. The investigated filter was not very efficient in removing As from the irrigation water. The concentrations of other metals decreased considerably after passing through the filter.
2. Irrigation with water to which heavy metals were added and which passed through a filter for their removal caused a slight but statistically insignificant increase in total Cr and Cu soil content. This is valid for both soil layers.
3. Content of Cd and Cr in fresh potatoes increased as a result of applying irrigation water to which heavy metals had been added (treatment 3). Concentrations of the investigated heavy metals in the potatoes are mainly below proposed threshold values for trace metals in tuber crops.

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# Rhizospheric Microbial Inoculum Addition to Soil in Maize Cultivation to Reduce the Environment Impact of Mineral Fertilisation and Improve Yield and Product Quality

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Rhizospheric microorganisms can significantly influence plant growth and productivity. In particular, arbuscular mycorrhizal fungi are considered important components in sustainable agroecosystems. They form mutualistic symbiosis with roots of most plants, improving the uptake of water and mineral nutrients; moreover, they confer bioprotection against pathogens. The association of Plant Growth Promoting Rhizobacteria (PGPR) to mycorrhizal fungi in microbial consortia used as inocula added to soil have also proved to be effective in improvement of crop productivity. In this work we checked the use of a rhizospheric microbial consortium in maize cultivation with the aim to verify if it can be useful to reduce the addition of mineral nutrients to soil, to reach lower costs, minor risks of groundwater pollution and minor impact to soil structure. Moreover the effect of the rhizospheric microbial inoculum on crop yield from the quantitative and qualitative point of view was examined.

A three-years project was financed by Regione Piemonte, Direzione Sviluppo dell'Agricoltura (Turin, Italy). The results of the first year of assays are presented.

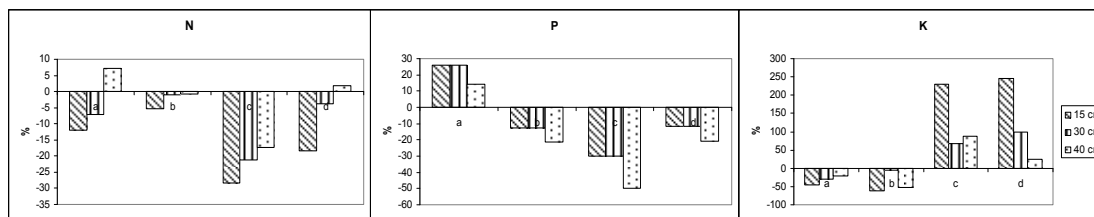
## Methodology

The assays were carried out in field trials with a local maize variety for food (Pignoletto giallo). Three different fertilisation modalities: a: 40% mineral fertilisation associated to rhizospheric microbial inoculum, b: 100% mineral fertilisation associated to rhizospheric microbial inoculum, c: 100% mineral fertilisation without rhizospheric microbial inoculum, and a blank: d: without any addition, were compared. The commercial microbial consortium contained rhizospheric microorganisms (*Glomus caledonium* GM24, *Glomus intraradices* GG31, *Glomus coronatum* GU53, *Pseudomonas fluorescens* PA28, *Pseudomonas borealis* PA29, *Bacillus subtilis* BA41). The assays were executed in triplicate (12 plots). Soil samples were withdrawn at different depth (15 cm, 30 cm and 40 cm) before seeding and after harvest; chemical analysis, to determine N, P and K content, and microbiological analysis, to determine heterotrophic microbial populations by CFU analysis (anaerobic and aerobic bacteria, yeasts and molds), were carried out. Morphometric determinations were carried out on plants: dry weight of roots and shoots, fresh and dry weight of ears, ear diameter and length; roots mycorrhizal colonization was estimated according to Trouvelot et al. (1986). Grain yield was determined in t/ha; grain quality was determined by analysis of proteins, lipids, starch, ashes and carotenoids.

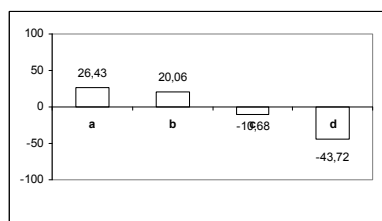
## Results

The analysis of soil N, P and K content revealed that the amount of % variations from the start (before seeding) to the end (after harvesting) of the assays were differently affected for each element and for different soil layers by the amount of mineral fertilisation and by the rhizospheric microbial inoculum (Figure 1). N and P strongly diminished where fertilisation was not associated to rhizospheric microbial inoculum (c), while P content increased where reduced mineral fertilisation was associated to rhizospheric microbial inoculum (a), probably due to the known effect of phosphates solubilisation by arbuscular mycorrhizal fungi. Rhizospheric microbial inoculum also induced an evident decrease of K. These results confirm the positive effect of soil microorganism, in particular of arbuscular mycorrhizal fungi, on mineral nutrition of plants.

Figure 1 - % variation of N, P and K soil content from pre-seeding to post-harvesting at different depth.



Soil microbial population analysis did not show any significant difference among the different treatments, probably due to the high variability checked throughout the assays in the field on the whole.



The % variation of roots mycorrhizal colonization from one to three months after seeding showed that symbiotic relationship was successfully obtained where rhizospheric microbial inoculum was added, while the colonization of roots from arbuscular mycorrhizal fungi naturally present in field soil decreased at the end of the assays (Figure 2).

Figure 2 - % variation of roots mycorrhizal colonization from one to three months after seeding.

The total plant biomass produced (dry weight) was significantly higher in b (100% mineral fertilisation + rhizospheric microbial inoculum); the same result was obtained considering each plant fraction (shoots, roots, ears). The water content of ears was also a little, but not significantly, higher in b. In b was also found the highest number of ears with the highest length range (17-22 cm), while there were no significant differences in ears diameters.

The grain yield was highest in b, reaching a mean production of 35,4 q/ha with a 14 % water content, while it was very low (5,6 q/ha) in d (neither fertilization nor rhizospheric microbial inoculum) (Figure 3).

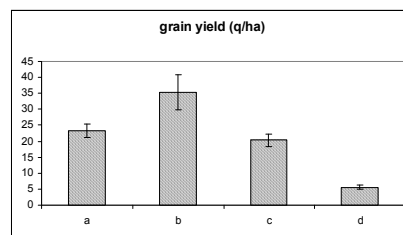
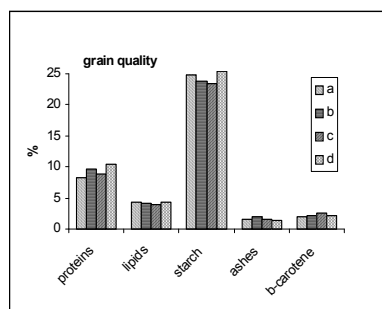


Figure 3 – Grain yield (q/ha)



Grain quality parameters showed a highest % content in proteins, lipids and starch in d (neither fertilization nor rhizospheric microbial inoculum). A significantly highest  $\beta$ -carotene content was found in c (mineral fertilisation without rhizospheric microbial inoculum).

Figure 4 – Grain quality

## Conclusions

A significant increase in grain yield can be reached if a rhizospheric microbial inoculum is added to soil and associated to mineral fertilisation. The grain quality was not significantly improved by the rhizospheric microbial inoculum, but was inversely related to the grain yield; the  $\beta$ -carotene content was highest when mineral fertilisation was not associated to rhizospheric microbial inoculum. Finally, these preliminary data suggest that rhizospheric microbial inoculum seems not to be useful to reduce the risk of groundwater pollution.

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# Agronomy for Sustainable Development

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## Renovation

ASD Journal has undergone a deep renovation from 2003 to 2005, involving major changes such as the set-up of a pre-selection committee at submission stage; novel topics better focussed on sustainable agriculture; novel format instructions for more concise articles; novel title (formerly *Agronomie*); a switch from harcopy to fully electronic managing; 100% of articles in English and a novel journal cover.

Major article topics currently include Agriculture and global changes; Agricultural production of renewable energies; Ecological pest control and biopesticides; Organic farming; Genetically modified organisms (GMOs) in cropping systems; Environmental impact on soil, water, air and biodiversity; Risk assessment for food, ecotoxicology; Decision support systems and companion modelling; Social and economical issues of agricultural changes; Innovation in farming systems; Pollutants in agrosystems.

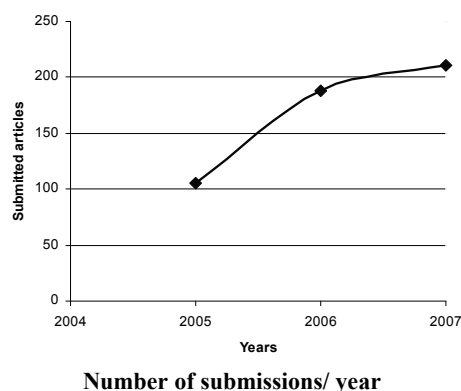
## Articles submission

The journal publishes both *research articles* and *review articles*.

Submitted articles are first pre-selected within 15 days by Pre-Selection Committee including Editor-in-Chief and Associate Editors. Submissions number has strikingly increased +77% between 2005 and 2006, and +12.24% more between 2006 and 2007.

Criteria used to decline manuscripts at Pre-Selection stage are: outside of ASD topics, clearly low innovation degree, and poor English writing style. Selected articles, about 50%, are sent for in-depth review to Field Editors, helped by two peer-reviewers.

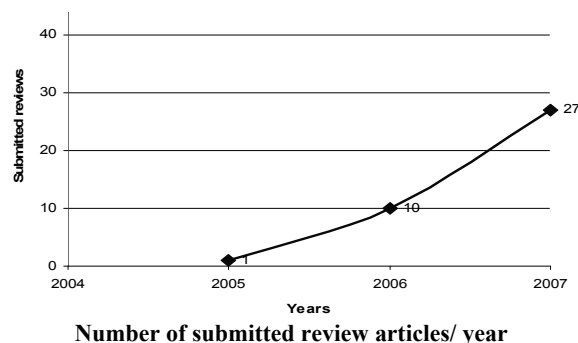
If the article is acceptable, Editor-in-Chief sends to author the three anonymous reports together with his own editorial corrections.



### Review articles

Since the change of name of our journal, formerly *Agronomie*, review articles number has increased from 1 in 2005 to 27 in 2007, with only 3 review articles rejected in 2007.

Review articles will be gathered in a new book series entitled Sustainable Agriculture. The first volume is scheduled to be issued end 2008.



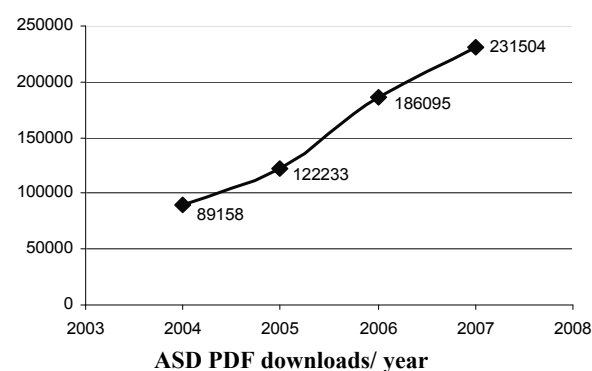
### Rejection rate

Global rejection rate has increased from 44% in 2003 to 78% in 2005. This rate is maintained in 2006 (77%) and 2007 (72.7% on between articles with ended review process).

### Electronic services

New electronic services allow better visibility of articles, with on-line first publication with a DOI (allowing earlier citation); electronic only subscription; and Open Choice option for authors wishing to make their article immediately available in Open Access by paying a fee.

A great increase of downloaded articles number from 2004 (89,158 downloaded PDF) to 2007 (231,504 downloaded PDF) would be noticed.

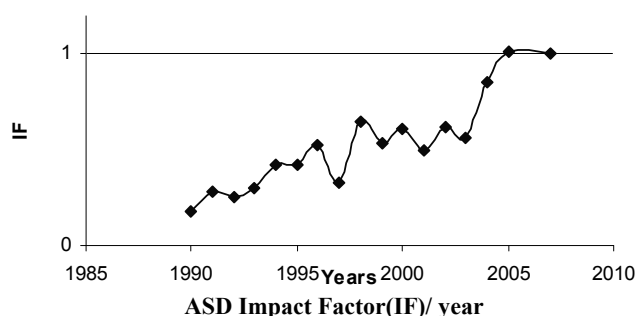


### Impact Factor

Since 2005, our journal has recorded an encouraging increase of its impact factor reaching 1.008 in 2005 with a rank of 16/48 in Agronomy category (2005 values referring to citations in 2005 of articles published in 2003 and 2004). This increase would be explained at least partly by the higher quality of published articles, following the various changes undergone during the 2003-2005 renovation period.

*Journal of Citation Report* published two 2006 impact factors in June 2007 due to the journal title change from *Agronomie* in 2004 to *Agronomy for Sustainable Development* in 2005.

2007 Impact Factor is maintained at 1.000 (*Journal of Citation Report*, June 2008), with a rank of 25/49 in Agronomy category.



# Phytoremediation of Metal-Polluted Wastes Through Auxin-Enhanced Root Growth in Radish (*Raphanus sativus* L.)

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Phytoextraction exploits the ability of plants to take up inorganic pollutants from contaminated substrates and transport them to stems and leaves (harvestable biomass). Since the root system is the key structure for metal absorption, the use of plant growth promoters such as auxins may potentially improve phytoremediation of trace elements, because more roots are available for their uptake. Despite this, very few studies regarding the use of auxins in phytoremediation are available in the literature. Among these, Lopez et al. (2005) found increased Pb concentrations in *Medicago sativa* treated with EDTA when plants received indol-3-acetic acid (IAA) in an aqueous solution. Liphadzi et al. (2006) also found improvements in metal uptake in *Helianthus annuus* when IAA was given to leaves and soil, regardless of IAA concentrations of 3 or 6 mg L<sup>-1</sup>. In non EDTA-amended soil only, these authors observed increases in root growth that were associated with higher leaf Mn and Ni contents. Because of species and metal specificity, we should expect a different plant response to the application of auxins. In this study, the effects of application of indol-butyric acid (IBA), a powerful root growth promoter, were investigated at different doses and methods of application in *Raphanus sativus* in controlled conditions on pluri-contaminated wastes.

## Methodology

The experiment involved radish (*Raphanus sativus* L. cv. Siletta nova), previously identified in a site experiment at Torviscosa (Udine, NE Italy) as the most efficient species in phytoextraction among various herbaceous species (Vamerali et al., 2006). Plants were cultivated for 75 days, from 25 May to 8 August 2007, in pots (490-mm height, 57-mm diameter) filled with metal-polluted pyrite cinders mixed with sand (1:1 w/w) to improve water drainage. The cinders had been dumped in the past as the by-product of pyrite ore roasting for sulphur extraction at Torviscosa within an area which is part of the site of national interest for reclamation called 'Lagoon of Grado and Marano and adjacent rivers'. The cinders were severely contaminated by As (886 mg kg<sup>-1</sup>) and trace metals, i.e., Co, Cu, Pb and Zn (100, 1,735, 493 and 2,404 mg kg<sup>-1</sup>, respectively), exceeding the Italian tolerance limits for agricultural uses several times.

The experiment was carried out in greenhouses at the experimental farm of the University of Padova at Legnaro (Italy). Treatments consisted of 5 applications (ten-day interval) of indol-butyric acid at different doses and methods of application, starting from 28 days after sowing (DAS). Treatments were: foliar spraying (F, 10 mg IBA L<sup>-1</sup>), 0.1 and 1 mg IBA kg<sup>-1</sup> of soil DW applied through irrigation (called 0.1ppm and 1ppm, respectively), and the same two doses in association with foliar spraying (called 0.1ppm+F and 1ppm+F, respectively), in comparison with untreated controls (C). During the growth period, leaf expansion, leaf chlorophyll content (measured on a Minolta SPAD-502 Chlorophyll Meter) and electrical capacitance of roots were monitored at weekly intervals, starting from 11 July (47 DAS). At harvest, root length and diameter were assessed by automatic analysis of root digital images acquired through a flatbed scanner, and concentrations of metals were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), in both shoots and roots separately.

## Results

With the exception of foliar spraying, IBA unexpectedly reduced the whole plant biomass: the higher, the dose the lower the growth. Reduction was maximum for 1ppm treatment, both with and without

foliar application (-71% and -63% (vs. controls) for shoots and roots, respectively). This result may be due to the growth-inhibition effect of auxins at high rates. At root level, the dynamics of root electrical capacitance showed that differences among treatments emerged only in the last part of the experiment (i.e., from the beginning of August). Root length did not follow the same trend as root weight, as foliar treatment showed higher values with respect to C (+16%) (Fig. 1). For the other treatments, length was reduced by a minimum of 5% (0.1ppm) and a maximum of 27% (1ppm+F).

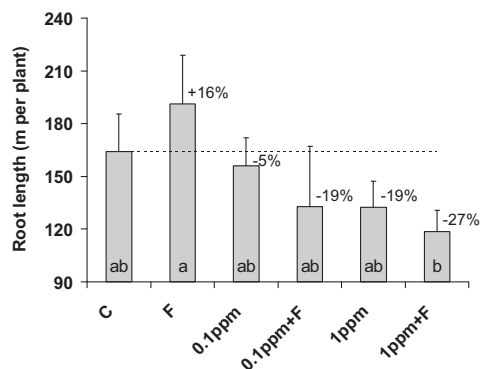


Fig. 1. Total root length (percentages: variations with respect to controls). Letters: significant differences among treatments (LSD test,  $P \leq 0.05$ ). Vertical bars: standard error.

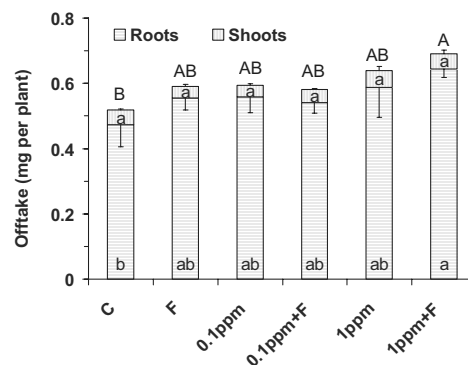


Fig. 2. Total offtake of As+Co+Cu+Pb+Zn in shoot and root biomass. Different letters: significant differences among treatments (LSD test,  $P \leq 0.05$ ).

Concentrations of trace metals in shoots were enhanced only at 1 mg IBA  $\text{kg}^{-1}$  of soil for Co (0.55 vs. 0.28  $\text{mg kg}^{-1}$ ) and Pb (4.33 vs. 2.24  $\text{mg kg}^{-1}$ ). In roots, Zn concentration was maximum for 1ppm and 1ppm+F (853 and 996 vs. 596  $\text{mg kg}^{-1}$ , i.e., +43% and +67% vs. controls, respectively). As a result of 'metal concentration  $\times$  biomass', the contents of the above-limit pollutants did not differ among treatments at above-ground level. Instead, in roots greater amounts of Zn as well as of the overall contents of As+Co+Cu+Pb+Zn were found in 1ppm+F (Fig. 2). The overall concentration (summation of  $\mu\text{Moles}$ ) of the 5 metals in the percolation water was maximum at 1ppm+F and minimum in F. As water percolation, as expected, was negatively correlated with both the growth of shoots (weight and leaf area) and roots, the increased metal leaching found in IBA treatments (excluding foliar) was explained. The root-to-shoot ratios of overall metal contents were generally high, ranging from 11 (C) to 17 (1ppm).

## Conclusions

At the tested doses, IBA did not induce modifications in shoot metal offtake, although the highest dose (1ppm+F) increased the accumulation of various metals at root level. There was a generally low translocation of metals from roots to shoots and a tendency toward greater removal (whole plant) with IBA, mainly due to improved concentrations rather than biomass. This aspect should be examined, as reduction in growth may be responsible for significant metal leaching in groundwater. Only IBA foliar application was able to increase the soil-root exchange surface through greater root length, but further investigations are needed to establish the most suitable dosage.

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# Evaluation of Ammonia Emission Reduction by Nitrogen Fertilizer Use in Italy

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Ammonia emissions are one of the main causes of acidification and eutrophication processes, and one of the most important contributors to the formation of secondary PM. Recent studies identified ammonia emissions as a major air quality concern whose reduction represents an important goal for atmospheric pollution control. Volatilized ammonia can travel for miles from the site of origin and in Europe Nitrogen compound depositions are higher in areas with intensive agriculture. In this paper the ammonia emission reduction by Nitrogen fertilizer use and in particular by urea consumption has been investigated. Urea is widely used in Italy for its chemical and physical characteristics; at the year 2005 it represented the 44% of the total simple nitrogen fertilizers (ISTAT, 2006). To reduce the Nitrogen fertilizer use three different options, urea substitution, increasing urea use efficiency and decrease volatilization have been compared and evaluated in order to estimate their effects as potential options to mitigate emissions.

The European NEC directive 2001/81/CE introduced compulsory national emission ceiling for different pollutants and fixed for Italy a national ceiling for ammonia emissions of 419 kt per year to be reached in 2010. Agriculture plays a crucial role by emitting, in the year 2000, more than 95% of the total ammonia emissions in Italy while transport and waste sectors are responsible of only 5% of emissions (APAT, 2000). The main emission causes are livestock and Nitrogen fertilizer consumption. The predominance of the agricultural sector is confirmed by the analysis of the ammonia Current LEgislation (CLE) emission scenario (see fig. 1) calculated by ENEA from the year 2000 to the year 2020 with the RAINS-Italy integrated assessment model (D'Elia et al, 2007).

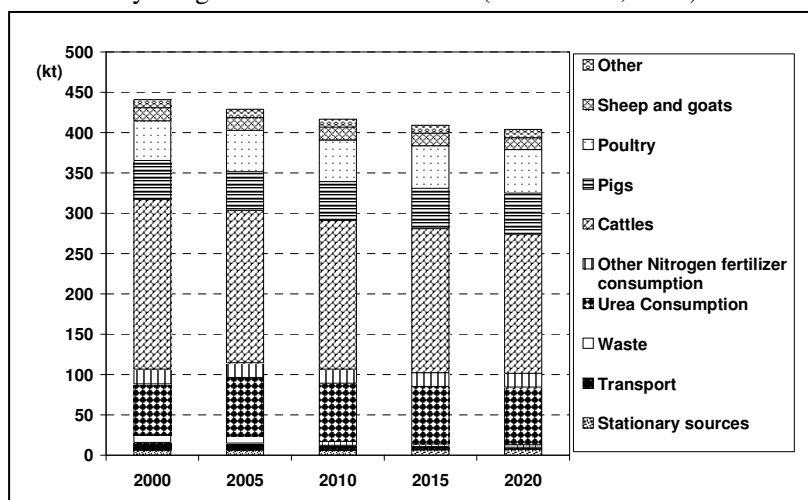


Figure 1 – CLE ammonia emission scenario by sectors calculated by the RAINS-Italy model at a 5-year interval.

Ammonia emissions from livestock operations have recently received significant attention, while fertilizer role has been less investigated; therefore in the present analysis we have focused our attention on the effects of different potential strategies to reduce Nitrogen fertilizer use and specifically the use of urea in Italy.

## Methodology

Measures and techniques evaluated in the present work are fertirrigation, use of N controlled release fertilizers and biological agriculture.

Fertirrigation represents an innovative agronomical technique based on fertilizers distribution with irrigation water. This technique is encountering a huge expansion because it allows a reduction of the amount of nutrient to be used. To estimate the fertilizer saved, an estimation of the total surface using fertirrigation has been carried out and a hypothesis of 20% fertilizer reduction has been adopted.

The use of N controlled release fertilizers increases the N use efficiency, saving labour and application costs and reducing nutrient losses by the use of innovative agronomical technique. Some studies have demonstrated (Balsari P. and Gioielli F., 2002) a reduction of 70% of ammonia emissions. To assess the reduction of Nitrogen losses using these innovative products, the hypothesis of 40% reduction volatilization losses has been made (Nastri et al., 2002).

Biological agriculture (i.e. the substitution of nitrogen fertilizers with organic fertilizers) is in large expansion in Italy, so a hypothesis of its future diffusion has been made.

## Results

The hypotheses adopted for each one of the three techniques are summarized as following:

1. Fertirrigation: in 2020 a diffusion of 80% in farm with drip irrigation (a method which minimizes the use of water by allowing water to drip slowly to the roots of plants, either onto the soil surface or directly onto the root zone);
2. Controlled release of N fertilizers: N fertilizers substitution of 2.5% and 5.0% in the year 2010 and 2020 respectively;
3. Biological agriculture: increase of 3% of the biological agriculture surfaces every 5 years from 2005 until 2020, with reduction of the use of N fertilizers by 1%.

The results of this study are presented in table 1.

Table 1 – NH<sub>3</sub> emission reductions by fertirrigation, controlled released fertilizer and biological agriculture hypothesis.

NH <sub>3</sub> emission reduction (t)	2005	2010	2020
Scenario 1: Fertirrigation	Reference year	-1,154	-2,309
Scenario 2: Controlled release of N fertilizers	Reference year	-1,857	-3,724
Scenario 3: Biological agriculture	Reference year	-4,718	-4,762

## Conclusions

Nitrogen fertilizer consumption is one of the main ammonia emission causes whose reduction faces many different obstacles. New innovative techniques have been encountering a wide diffusion and the results of our study show that their introduction could improve the atmospheric fertilizer impact reducing ammonia air emissions. Strategies and policies to increase the diffusion of those techniques and options could be easily implemented at regional level using measures and incentives of the Rural Development Plan while more researches are needed to evaluate the impact of other techniques and calculate more appropriate emission factors.

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# Adaptation of N Catch Crop Species to Simplified Management

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The use of catch crop in between maize crops on an irrigated maize monoculture system has been proved as an efficient way to minimize N leaching during winter (Domingo *et al.*, 2004). Nevertheless, there is a need to look for a way to establish those catch crops at a reduced cost to be worth in practice. At the Empordà area (NE Iberian peninsula; Mediterranean conditions), maize is irrigated using a furrow system which do not allow direct sowing after maize crop. The aim of this work was look for a sowing system for catch crops able to reduce implantation costs without reduction of the efficiency of them.

## Methodology

A two years trial (2007 and 2008) was carried out at La Tallada d'Empordà (NE of Iberian peninsula) under Mediterranean conditions (mild winters) using different catch crop species and two simplified sowing management to minimize field work and cost. The experimental design was a split-plot where the main factor was the soil tillage before sowing (minimum tillage and no tillage at all) and secondary factor was the catch crop specie (oat -*Avena sativa*-, rapeseed -*Brassica sp.*-, fenugreek -*Trigonella foeniculum graecum*- and a control without any catch crop). Catch crops were sown after maize was harvested (October 2006 and October 2007) and they lasted till beginning of March 2007 and 2008 respectively. Dry matter yield and N uptake of the catch crops were measured at harvest, both years, although in this paper some 2008 data are not shown as they are not available yet.

A maize crop was established after 2007 catch crops were finalized and incorporated into the soil. In order to be able to observe the influence of the winter management on the following maize crop, only 150 kg N ha<sup>-1</sup> (at dressing) were applied to this crop. Nitrate content in the soil solution at 1 m depth was measured periodically during maize crop through suction cups sampling.

## Results

Among catch crops, in 2007 the highest N uptake was obtained for rapeseed (105-145 kg N ha<sup>-1</sup>) with a biomass yield of 3,500 kg DM ha<sup>-1</sup> for no tillage treatments and 2,300 kg DM ha<sup>-1</sup> for the tilled ones (Table 1). In 2007-08, a severe punctual winter frost (-6,8 °C) killed the small plants of rapeseed at early emergence and no crop developed on those plots. Oat and fenugreek developed all right both years of study, oats being more biomass productive than fenugreek in both years, although total N content was higher for fenugreek than oats. As fenugreek is a leguminous crop, not all the N in the plant can be considered as uptaken from mineral N in the soil. N-uptake by oats and fenugreek (70-120 kg N ha<sup>-1</sup>) were lower than for rapeseed (yr 2007).

Productivity of subsequent maize crop did not show significant differences among different catch crop treatments (Table 1). Nitrate-N content in soil solution sampled with suction cups during maize crop was lower during maize crop (Figure 1) after oat and rapeseed than for control plots without any catch crop during winter. At the end of the measurement period the 3 treatments reached the same level of nitrate content in the soil solution (13 mg L<sup>-1</sup> at the end of the period). Contrarily, after fenugreek crop

this content was significantly higher than those mentioned above along all the maize development period (190-240 mg L<sup>-1</sup> through all the period). So, thi leguminous crop can induce a higher level of nitrate leaching during maize crop than the other catch crops (oat and rapeseed).

Soil tillage	Winter management	Catch crop biomass (kg DM ha <sup>-1</sup> ) 2007	Catch crop biomass (kg DM ha <sup>-1</sup> ) 2008	N plant content (%) 2007	N uptake (kgN ha <sup>-1</sup> ) 2007	2007 maize yield (kg ha <sup>-1</sup> )
No till	Rapeseed	3,498	-	4.53	145	13,030
	Oat	3,486	4,242	2.05	68	10,978
	Fenugreek	2,550	1,450	4.78	114	14,883
	No crop	-	-	-	-	13,629
Till	Rapeseed	2,319	-	4.90	105	12,335
	Oat	3,974	3,484,821	2.36	93	12,282
	Fenugreek	2,722	1,472,03	4.69	121	14,399
	No crop	-	-	-	-	13,464

Table 1.- Catch crop biomass production, N uptake and N plant content and Yield of the subsequent maize crop (year 2007) for the different tillage treatments and catch crop species. La Tallada d'Empordà.

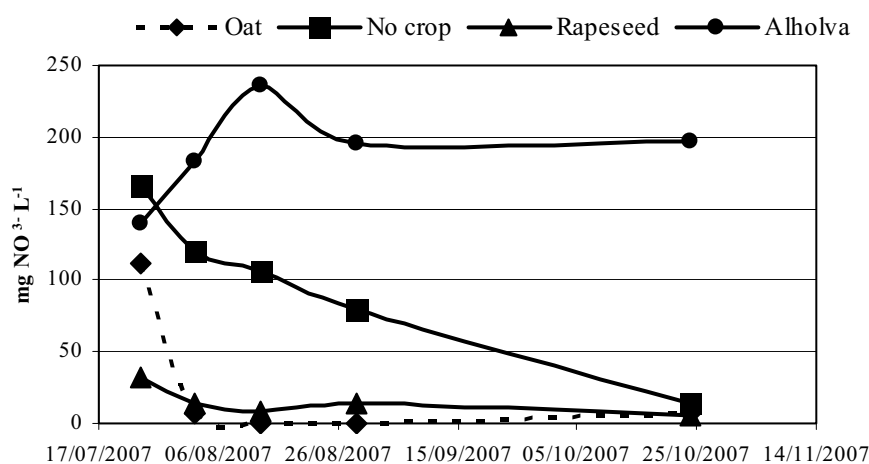


Figure 1.- Evolution of nitrate content in the soil solution after different catch crop species during the maize crops. La Tallada d'Empordà.

## Conclusions

Catch crop establishment, development and N uptake during autumn-winter was not different between different sowing management. Therefore, catch crop can effectively work with a simplified management and at a very low cost of implantation.

Leguminous crops can produce higher N leaching on subsequent maize crop.

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# Ni Requirement of the Hyperaccumulator *Alyssum murale*

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Among the hyperaccumulators, *Alyssum murale* grown in serpentine soils can accumulate more than 20 g kg<sup>-1</sup> Ni in its dry shoots at flowering (Reeves and Baker, 2000) while normal plants suffer from phytotoxicity when shoot Ni exceeds 25-50 mg kg<sup>-1</sup>. Ni is a proven micronutrient for higher plants in the activation of urease which is used by the plants to hydrolyze urea and ureides to release NH<sub>3</sub>. For this reason, Ni deficiency causes toxicity symptoms due to the accumulation of urea within plant tissues. Ni hyperaccumulators such as *Alyssum murale* are extremely interesting as plants that may allow commercial phytomining of Ni the value of which has increased in recent years.

Because some *Alyssum* spp. are capable of accumulating such higher Ni concentration than normal plants, we questioned whether their Ni requirement might also be higher compared to non-hyperaccumulators. We compared the behavior of two hyperaccumulators species of the *Alyssum* genus (*A. murale* and *A. corsicum*) and two non-hyperaccumulators (*A. montanum* and tomato) when exposed to different levels of Ni<sup>2+</sup> activity in chelator-buffered nutrient solutions containing urea as nitrogen source to define the Ni requirement.

## Methodology

The experiment aimed to define the Ni requirement and to study the response of *Alyssum* spp. by growing plants in hydroponics at six different Ni<sup>2+</sup> activities under controlled conditions (temperature: 24°<sub>day</sub> /20°<sub>night</sub>; humidity: 70%<sub>day</sub>, 50%<sub>night</sub>; PAR: 300 μmol s<sup>-1</sup>m<sup>-2</sup>, 16h day<sup>-1</sup>). 4-weeks old seedlings were transferred into the hydroponics solution without Ni or urea to allow the root system to recover from possible transplant damages. After two weeks, 4 replicates for each species were collected, separated into roots and shoots and prepared for analysis to measure the starting Ni levels. The nutrient solution was then replaced with the test nutrient solutions to expose the plants to varied Ni supply for one month. The pH of the nutrient solution was checked every two days and adjusted to 7.0 by KOH addition when needed while the nutrient solution was changed after two weeks.

Based on preliminary experiments with severe Ni deficiency on urea media using hydroxyethylthylenediaminetriacetate (HEDTA), in which the requirement was found to be near 10<sup>-11.9</sup> free Ni<sup>2+</sup>, we used cyclohexaneethylenediaminetetraacetate (CDTA) and 6 levels of Ni around the expected requirement. Two Ni levels (0.01 μM and 0.10 μM) were set below the expected Ni requirement, two Ni levels (2.00 μM and 5.00 μM) were close to the expected required level, and two (10.0 μM and 31.6 μM) were set to provide a higher activity to assure adequacy and see the response of the plants when exposed to adequate Ni. The 10 μM Ni provided 10<sup>-11.3</sup> M free Ni<sup>2+</sup>. Also, we included a treatment without Ni and without urea but with 1.00 mM Ca(NO<sub>3</sub>)<sub>2</sub> and 2.50 mM KNO<sub>3</sub> as source of nitrogen. The nutrient solution contained 2 mM Mg and 1 mM Ca to simulate serpentine soil fertility conditions; the Ni hyperaccumulators are serpentine endemic species. After 15 days of treatment the plants did not show any clear urea toxicity symptoms. In order to reproduce the results previously observed, we decided to maintain three of the six treatments (0.01 μM, 5.00 μM and 31.6 μM) and to replicate the previous experiments by replacing the other three treatments with three new Ni levels buffered by HEDTA as follows: 1 μM Ni, 10 μM Ni and 130 μM Ni. In the latter case, 30 μM Ni out of 130 were added without the HEDTA to increase free Ni<sup>2+</sup> considerably as in the earlier test. The highest Ni treatment, both for CDTA and HEDTA, was expected to provide a higher activity to assure adequacy and see the response (symptoms and accumulation) of the plants to adequate Ni.

After 30 additional days of treatment, the plants were harvested. The roots were separated from the aboveground biomass, rinsed and oven dried together with the aboveground biomasses at 100°C for 24h. The dried samples, including the samples collected for the background Ni level, were ashed at 480°C for 16h and then digested with concentrated HNO<sub>3</sub> dissolved in 3M HCl, filtered and diluted to 25ml with 0.1M HCl. The Ni concentrations were determined using AAS and ICP-AES. Samples of *Alyssum* seeds underwent the same procedure to define the Ni seed endowment. An internal standard (Y) was used to improve analysis reliability, and standard samples analyzed for QA.

## Results

Table 1 shows the average Ni concentration found in the shoots and roots for *A.murale* Kotodesh, *A.corsicum*, *A. montanum* and tomato for the seven treatments. For all the treatments and for *A.murale*

Table 1 treatment	fraction	mg kg <sup>-1</sup> Ni			
		<i>A.corsicum</i>	<i>A.murale</i>	<i>A.montanum</i>	Tomato
Control	Roots	0.62 c	1.50 b	1.06 b	0.81 a
Control	Shoots	3.49 d	1.89 c	0.05 d	<dl a
0.01 µM Ni CDTA	Roots	0.83 c	1.25 b	0.79 b	<dl a
5 µM Ni CDTA	Roots	2.25 c	1.91 b	1.78 ab	2.55 a
31.6 µM Ni CDTA	Roots	7.08 b	7.85 a	8.10 ab	1.90 a
1 µM Ni HEDTA	Roots	1.51 c	1.52 b	1.56 b	<dl a
10 µM Ni HEDTA	Roots	1.93 c	3.13 b	3.37 ab	0.53 a
130 µM Ni HEDTA	Roots	10.7 a	9.42 a	10.5 a	9.26 a
0.01 µM Ni CDTA	Shoots	7.29 cd	3.99 bc	0.06 d	0.19 b
5 µM Ni CDTA	Shoots	10.2 bc	5.59 bc	0.67 cd	0.24 b
31.6 µM Ni CDTA	Shoots	13.9 b	16.3 a	6.02 b	1.10 b
1 µM Ni HEDTA	Shoots	9.55 bc	4.19 bc	0.32 cd	0.08 b
10 µM Ni HEDTA	Shoots	11.5 bc	9.16 b	2.12 c	0.51 b
130 µM Ni HEDTA	Shoots	24.9 a	22.1 a	9.91 a	9.63 a

Means within tissue followed by the same letter are not significantly different (Waller-Duncan test).

and *A.corsicum*, the average Ni concentration of the aboveground biomass is always greater than the corresponding root Ni concentration confirming the natural ability of the two species to translocate and accumulate the element into their shoots. On the other hand, *A.murale* and tomato show a higher Ni concentration in their roots compared to the shoots as expected, with the exception of treatment 10 µM Ni HEDTA for *A. montanum* and treatment 130 µM Ni HEDTA for tomato. In contrast with the expectations, no clear urea toxicity symptoms were observed; yield at the lowest Ni levels were significantly lower than the higher Ni level suggesting deficiency had occurred. This result may be explained by the seed Ni supply and assuming that the Ni requirement level for the two hyperaccumulators may likely be below the concentrations measured after 10 weeks of growing. The analysis of the plants harvested before the beginning of the treatments to define the background Ni content of the plants and the analysis of the seeds showed that the average Ni content in mg are very similar for the hyperaccumulators and the corresponding seeds (respectively: 9.15 µg and 9.81 µg for *A. corsicum*; 3.10 µg and 1.83 µg for *A. murale*). On the other hand, *A. montanum* showed a higher Ni content in the seeds (0.11 µg vs 1.72 10<sup>-3</sup> µg).

The seed Ni content was determined by analysing the Ni concentration in seeds samples of the three species. Seeds of *A. corsicum* had Ni=9,090 mg kg<sup>-1</sup> while *A. murale* had Ni= 7,480 mg kg<sup>-1</sup>; *A. montanum* had a seed Ni=0.97 ppm which shows its inability to hyperaccumulate the element.

## Conclusions

The results of the experiment highlight the crucial role of the seeds Ni endowment. In fact, the Ni provided by the seeds was enough to cover the Ni requirements of the plants preventing severe urea toxicity symptoms throughout the whole growth period, from germination to harvesting. This important outcome allowed us to better define a new experiment which is currently ongoing, in order to induce Ni deficiency and so finally define the Ni requirements of the species considered in this paper.

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# Combining Field and Laboratory methods to calculate Soil Water Content at Field Capacity and Permanent Wilting Point

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Field Capacity (FC) and Permanent Wilting Point (PWP) are typical terms that are used to define soil water content and its availability to plants. Related concepts such as Total Available Water (TAW) capacity and the Readily Available Water (RAW) capacity are also used in many agronomic applications where soil water balance calculations are done, such as, irrigation scheduling, soil conservation, etc. Therefore, obtaining realistic values of the volumetric water content at FC and PWP is crucial. It is a general practice to determine the water content at FC and PWP using pressure plates at -33kPa and -1500kPa, respectively. Recently, the soils scientific community has been questioning this approach. One of the problems is associated with sample equilibration at low potentials (-1000 and -1500 kPa), (Cresswell et al., 2008; Gee et al., 2002) and the effect of the osmotic potential in saline soils (also, PWP related). As remarked by Ratliff et al. (1983) and Gebregiorgis and Savage (2006), another important aspect is that Field Capacity should be determined in the field, since there are many errors associated with laboratory determinations done with pressure plate apparatus. The objective of this study is to compare pressure plate laboratory determinations of water content at FC and PWP with combined field and laboratory techniques.

## Methodology

To do this experiment, two separate profiles from a loam texture soil from Raimat (Lleida, NE Spain) were used (Table 1). The Electrical Conductivity ( $EC_{1:1}$ ) ranged between 1 and 4 dS m<sup>-1</sup>, depending on the soil layer. Disturbed air dried soil samples were sieved at 2mm and wetted. Three subsamples were brought to a target pressure of -1500kPa using Pressure Plate apparatus (Soilmoisture Equipment Corp., USA), while in three other subsamples, total soil water potential near PWP was measured with a WP4T chilled mirror hygrometer (Decagon Devices, USA). Water content at FC was done in the laboratory with the Pressure Plates at -33kPa, and in the field by saturating the soil and taking soil cores after drainage ceased (Pla, 1983). After the measurements were completed, all samples were weighted and oven dried at 105°C for determination of gravimetric soil water content (w/w). Volumetric water content (v/v) was then calculated by multiplying the gravimetric soil water content by the bulk density and corrected for the stone content.

**Table 1.** Main characteristics of the two profiles used in the study

Soil Type	Depth (m)	Texture class	Stone content (% w/w)	Bulk density <sup>(1)</sup> (Kg m <sup>-3</sup> )	EC <sub>1:1</sub> (dS m <sup>-1</sup> )
Loma	0-40	Silt Loam	8.5	1500	1.6
	40-90	Loam	30.8	1800	4.1
	90-130	Loam	39.8	1800	4.1
Bajo	0-40	Silt Loam	17.4	1500	0.8
	40-70	Silt Loam	37.9	1800	0.8
	70-100	Silt Loam	13.1	1600	1.2
	100-130	Silt Loam	17.6	1800	1.2

(1) Determined in the field according to Pla (1983)

## Results

Table 2 shows the results of the measured soil water contents and the derived values of Total Available Water (TAW).

Table 2. Values of Total Available Water (TAW) and soil water content (v/v) at Saturation, Field Capacity and Permanent Wilting Point obtained with pressure plates, field condition and the chilled mirror hygrometer.

Soil Type	Depth (m)	$\theta_{\text{sat}}$	$\theta_{\text{fc}}$ (m <sup>3</sup> m <sup>-3</sup> )		$\theta_{\text{pwp}}$ (m <sup>3</sup> m <sup>-3</sup> )		$\text{TAW}_{\text{pp}}^{(2)}$	$\text{TAW}_{\text{comb}}^{(3)}$
		(m <sup>3</sup> m <sup>-3</sup> )	Pres.plate <sup>(1)</sup>	Field	Pres.plate <sup>(1)</sup>	WP4T	(m <sup>3</sup> m <sup>-3</sup> )	(m <sup>3</sup> m <sup>-3</sup> )
Loma	0-40	40.9	36.5	33.3	17.0	17.2	19.5	16.1
	40-90	37.4	42.2	35.4	19.3	26.5	22.9	9.0
	90-130	49.1	43.6	37.3	19.4	29.2	24.1	8.1
Bajo	0-40	41.6	36.7	35.6	20.0	17.3	16.7	18.4
	40-70	42.6	45.1	35.9	22.2	19.8	22.9	16.0
	70-100	38.0	38.2	31.9	20.4	17.3	17.7	14.6
	100-130	41.9	44.3	35.0	21.4	20.1	22.9	14.8

<sup>(1)</sup> Pres.plate: values obtained using Pressure Plate apparatus at -33kPa (Field Capacity) and -1500kPa (Permanent Wilting Point). <sup>(2)</sup>  $\text{TAW}_{\text{pp}}$ : Total Available Water calculated from the pressure plate values ( $\theta_{\text{fc}} - \theta_{\text{pwp}}$ ). <sup>(3)</sup>  $\text{TAW}_{\text{comb}}$ : Total Available Water calculated from  $\theta_{\text{fc}}$  obtained in the field and  $\theta_{\text{pwp}}$  obtained with the chilled mirror hygrometer WP4T (combined method).

At Filed Capacity, discrepancies were observed between pressure plate at -33 kPa and the field method. With the pressure plates, the values are higher and sometimes fall beyond saturation, which is not realistic. Considering that a suction of -33kPa corresponds to field capacity, may not be adequate for this soil (loam and silt loam) resulting on a higher retention of water. At Permanent Wilting Point, discrepancies are observed specially in the two saline layers from the Loma soil profile. The hygrometer measures two of the main soil water potential components, the matric and the osmotic, while with the pressure plates only the matric component is determined. That explains the higher water contents in the deeper horizons of the Loma profile. In the other hand, the retention of water measured with the pressure plates at -1500kPa seems lower than the values obtained with the hygrometer. That could be explained by the lack of equilibrium as suggested by Gee et al. (2002). If the values of the TAW are compared, using only the pressure plates for determining  $\theta_{\text{fc}}$  and  $\theta_{\text{pwp}}$ , results in an overestimation of the water holding capacity of the soil. This can be of importance if the TAW value is used as an input value for water balance studies and modelling. Further studies are required (different soil types) to get a wider picture of the discrepancies and get a better understanding of the reasons behind them.

## Conclusions

Using only pressure plate apparatus for determining  $\theta_{\text{fc}}$  and  $\theta_{\text{pwp}}$ , may lead to important estimation errors. Care should be taken when salts are present and when the reference value of -33kPa is taken for Field Capacity. The work strongly recommends to use the field method for the structure and process dependent values ( $\theta_{\text{sat}}$  and  $\theta_{\text{fc}}$ ) and to use the hygrometer for the Permanent Wilting Point determination ( $\theta_{\text{pwp}}$ ).

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# Sustainable Use Of Legume Cover Crops In Citrus Orchards

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Cover crops are a sustainable tool to preserve soil fertility and to control weeds in agroecosystems due to their ability to fix nitrogen, prevent soil erosion, improve soil organic matter and enhance nutrient availability, especially in drought environments (Shennan, 1992, Caporali et al., 1997). Moreover, in tree orchards, cover crops may play a major role in the management of weeds (Teasdale, 1996; Teasdale and Daughtry, 1993). Weed control in tree systems represents, in fact, a major problem both for the relevant demand of work and energy to tackle them and for the negative effects on tree plants development and production (Hanna et al. 1995). In evergreen citrus, adversely to what happen in other deciduous orchards, cover crops need to possess an adaptation to shaded environments. The present research has the aim of evaluating the effect of soil cover and weeds control capability of two self-reseeding legumes in intensive citrus orchards cultivation.

## Methodology

The trial was carried out in 2005 in two orange orchards cultivated with Tarocco ‘Gallo’ of Eastern Sicily – Italy (Mineo – 510 m a.s.l. and Scordia – 150 m a.s.l.), using two self-reseeding legume as cover crops, *Medicago scutellata* cv Kelson and *Trifolium subterraneum* cv Mount Barker, and compared with a control in which weeds grow spontaneously. In Scordia the experiment was carried out also for a second year in order to evaluate the long term establishing capability. A randomised block design with great plots (96 m<sup>2</sup> each) three times replicated was adopted. The sowing was executed on the 18<sup>th</sup> January in both locations with 1.500 germinable seeds m<sup>-2</sup>, but in Scordia, due to unfavorable climatic conditions, the sowing need to be repeated on the 25<sup>th</sup> March. The soil coverage percentage at regular interval and final biomass of cover crops and weeds were evaluated.

## Results

In the first year, *Medicago* showed in both environments a better establishment, higher plants (data not reported), a greater soil coverage and a lower weeds coverage compared to the *Trifolium* (Fig. 1 and 2). Since from few weeks after sowing, in *Medicago* were always recorded higher values of soil coverage till reaching 100% in the late spring and weeds cover below 15%. In both environments, *Medicago*

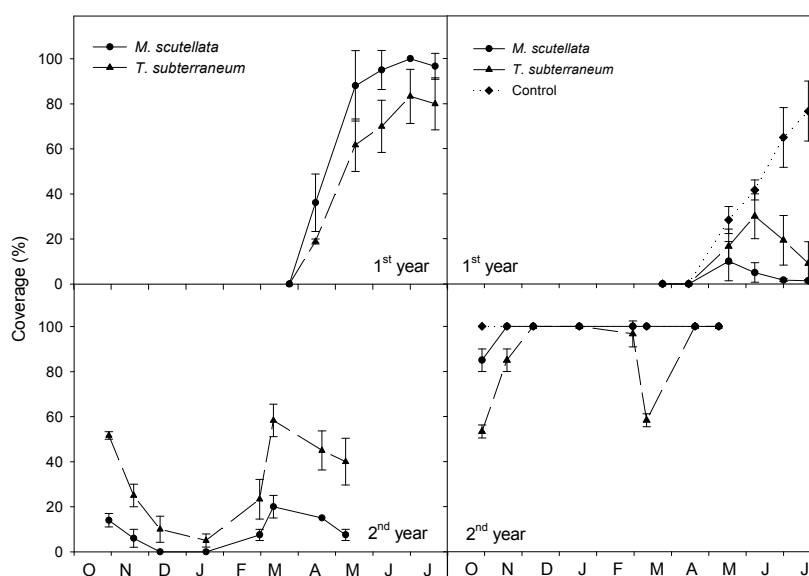


Fig. 1. Percent coverage of cover crops and weeds in Scordia

also reached around 1,800 g m<sup>-2</sup> of dry biomass, while *Trifolium* did not exceeded 1,200 g m<sup>-2</sup> in Scordia and 600 g m<sup>-2</sup> in Mineo (Fig. 3a and 3b).

In the second year in Scordia, weeds biomass greatly overtakes cover crops. The cover crops coverage of both species showed a reduction during the winter due to the low temperature.

Contrarily to what happened in previous year, *Trifolium* prevailed on *Medicago* both in term of soil coverage (60% vs 20% in the sample of March) and biomass (624 vs 194 g m<sup>-2</sup>). This is certainly the result of a lesser hardseededness of *Trifolium* that allowed a prompt germination in the season after sowing.

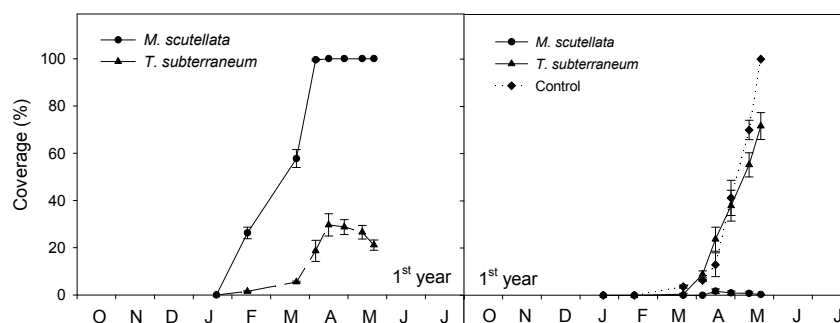


Fig. 2. Percent coverage of cover crops and weeds in Mineo

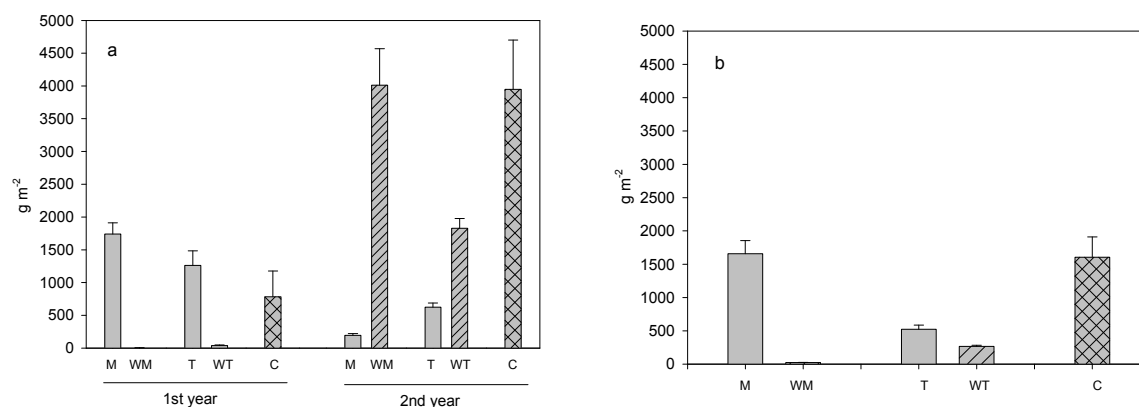


Fig. 3. Biomass production of cover crops and weeds in Scordia (a) and Mineo (b) for *M. scutellata* (M), *T. subterraneum* (T), weeds in *M. scutellata* (WM) and in *T. subterraneum* (WT) and Control (C).

## Conclusions

The two studied annual legumes were able to control spring weed infestation, due to a satisfactory soil cover showing a good adaptability to the shaded environment of citrus intra-rows. *M. scutellata* showed, in the first year, a better establishment compared to *T. subterraneum*, that turned in a faster cover development, a wider soil cover, a higher dry biomass and a better competition capability against weeds. On the contrary, in second year *T. subterraneum* showed higher soil coverage and biomass due to the greater selfseeding capability. The late sowing (January-march) greatly reduced the infestation degree, allowing the cover crops to compete with the spring weeds, while in the second year weeds prevailed on cover crops.

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# Effect of Salinity Stress on Germination Indices in Seven Safflower Cultivar (*Cartamus Tinctorius*, L.)

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## Introduction

Current estimates indicate that 10-35% of the worlds agricultural lands is now affected by the salinity stress (shaton 1997). It can be said that it is one of the major devastating stresses of the arid and semi arid regions. Environmental stress, especially salinity stress, can play an important role in the reduction of the plant growth stage especially germination stage. Germination is a critical stage of the plant life and resistance against salinity during the germination is important for stability. More than 90 percent of Iranian domestic need for oil is imported. Safflower, one of the native and valuable oil seeds in Iran, is tolerant to salinity. Its seeds contain 35% high-quality oil and 15% protein.

In the study of the safflower reaction to the salinity of the water and soil , (Elias S. Basil 2001) expresses that with increase in the level of the salinity from 1.8 to 2.7 ds/m length of radical decreases.

Mehmet Demir (2003) in the study of 3 varieties of safflower , showed that with the increase of salinity , length of radical , plumule dried weight of radical plumule decreased.

The physiological attributes of salinity tolerance in *Atylosia albicans* (C. albicans) and the F<sub>1</sub> hybrids include Na and Cl retention in roots and limited translocation to the shoots , and maintenance of transpiration rate under saline condition. (Subbarao et al 1990).

Because of salinity increase , germination percentage decreases and germination time increases . (Jajarmi. 2007)

## Methodology

In order to study the effects of salinity stress on germination indices in safflower cultivars, an experiment was conducted in factorial form, using a completely randomized design with three replications. In this experiment, seven safflower cultivars (A factor: CH353 ,CH65 ,Asteria ,CH697 ,Rinconada ,iranian variety Zarghan and Isfahan )were evaluated in six levels of salinity treatment (B factor: distilled water,-3,-6,-9,-12,-15 bar) of NaCl.

Nacl (gr/lit)	Stress level (bar)
3.54	-3
7.8	-6
10.62	-9
14.17	-12
17.71	-15

Seeds were considered germinated the emergent radical reached 2 mm length, rate of germination and coefficient of velocity of germination were calculated as follow

Rate of germination =  $dg/dt$

Coefficient of velocity of germination =  $100 \times \frac{A_1 + A_2 + \dots A_x}{A_1 T_1 + A_2 T_2 + \dots A_x T_x}$  (Pollock and Ross 1972)

Mean of day germination =  $\sum (Nt / \sum N)$

## Results

Increased salinity levels has deleterious effect on germination and radical length and plumule length. The recorded values are presented in table 1 .

A) Radical length .

There are significant differences among varieties and salinity levels. The highest radical belonged to Rinconad with 22.783 mm and the least radical belonged to CH353 with 16.875 mm .

B) Length of Pulmus

The highest pulmus belonged to Isfahan variety with 9.87 mm , while Rinconad had the least pulmus, howere this variety had the highest radical (table1) . There is no significant different between -12 , -15 bar for pulmus length. The highest pulmus appeared in the Zarghan variety and distilled water.

C) Germination percentage

The highest germination percentage took place in distilled water with 92% and the least belong to -12 bar with 71.8. Rinconad , Isfaham, CH353 had the highest germination percentage and the least belonged to Zarghan (Table1).

In study the effect of drought stress in 7 varieties in safflower express that safflower is resistant against drought stress in -15 bar. (Jajarmi 2007)

D) Mean of day Germination & Average Velocity of Germination:

The highest mean of day germination belonged to CH697 with 2.26 day. It showed that average velocity of germination in this variety was very slow, Although Isfahan variety had the least with 1.29 day.

## Conclusions

In the studying the tolerance of varieties to salinity stress, germination percentage cannot be a good index in screening varieties (Germination percentage trait was affected by salinity stress less than the other traits). To find the best one tolerant variety to such condition, taking all traits, into account in this study , we found that Isfahan variety is the most resistant and CH697 is the most sensitive

Table 1 . Mean comparison of varieties safflower

Average velocity of germination	Mean of Day germination	Germination Percentage	Pulmus length (mm)	Radical length (mm)	Variety
18.14b	1.62 b	9288a	7.016cd	16.875d	CH 353
16.46c	1.53 bc	80.22b	8.3 b	19.86bc	CH 65
18.28b	1.49 bc	94a	5.64d	22.78a	Rinconada
20.79a	1.29d	89.08a	9.87a	20.2bc	Isfahan
19.25b	1.39dc	93.32 a	6.63d	21.75ab	Asteria
11.57d	2.26a	66d	6.17d	10.894cd	CH 697
15.26c	1.45dc	72.22c	9.48ab	19.75bc	Zarghan

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# Effect of Water Stress on Germination Indices in Seven Safflower Cultivar (*Carthamus Tinctorius*, L.)

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## Introduction

Current estimates indicate that 25% of the worlds agricultural lands is now affected by water stress. Environmental stress, especially drought stress, can play an important role in the reduction of the plant growth stages, typically germination stage in arid and semi arid regions in Iran. More than 90 percent of Iranian domestic need for oil is imported. Safflower, one of the native and valuable oil seeds in Iran, is tolerant to drought and salinity. Its seeds contain 35% high-quality oil and 15% protein.

One of the commonest experiments in germination of the seeds is the application of PEG. Many experiments have been done and the results have showed that plumule is more likely to be affected by water stress than other traits.

Maliwal and Paliwal(1982) showed that under water stress, in grain legumes, mung bean and black gram and cluster bean both germination and height of seedlings were significantly decreased. Seeds of 10 cultivars of bean were exposed to six levels of water stress (0 , -0.2 , -0.4 , -0.6 , -0.8 and -1.0 MPa) induced by polyethylene glycol. Results showed that germination percentage decreased with increasing water stress (de Queiroz et .al., 1997). Cultivar Jalo, Precoce, and Onix were the most tolerant to water stress.

Alfalfa (*Medicago sativa* L.) germplasm for seedling tolerance was screened in the field as well as in the laboratory tests. The accessions that can emerge at -6.5 bars of soil water potential in the laboratory was adjusted to provide -6.5 bars of soil water potential have better field emergence and survival under drought stress than accessions that can not emerge in the laboratory (Rumbaugh and Johnson, 1981).

the wheat varieties reaction to the drought stress (concentration of PEG – 6000), (Domandan 2002) expresses with increase in the level of the drought -0.1 to 1.5 (MP) length of radical decreased. In the study of effects of drought stresses induced by polyethylene glycol on germination of *Pinus sylvestris* var mongolica seeds from natural and plantation forests on sandy land , the results indicated that the seeds from both provenances did not germinate when PEG concentration was more than 25% (Zhu JiaoJun 2006).

## Methodology

In order to study the effects of water stress on germination indices in safflower cultivars, an experiment was conducted in factorial form, using a completely randomized design with four replications. In this experiment, seven safflower cultivars (A factor: CH353 ,CH65 ,Asteria ,CH697 ,Rinconada ,iranian variety Zarghan and Isfahan )were evaluated in six levels of drought treatment (B factor: distilled water,-3,-6,-9,-12,-15 bar) of PEG 6000. The experiment took ten days.

Germination percentage was recorded everyday after soaking . Seeds were considered germinated when day the emergent radical reached 2 mm length. Rate of germination and cofficent of velocity of germination were calculated as follow

Rate of germination =  $dg/dt$

Coefficient of velocity of germination =  $100 \times \frac{A_1 + A_2 + \dots A_x}{A_1 T_1 + A_2 T_2 + \dots A_x T_x}$  (Pollock and Ross 1972)

Mean of day germination =  $\sum (Nt / \sum N)$

## Results

Increased salinity levels have deleterious effect on germination and radical length and plumule length. The recorded values are presented in table 1.

#### A) Radical length and Length of Pulmus.

There are significant difference among varieties and salinity levels. The highest radical belonged to CH65 with 20.73 mm and the least radical belonged to CH697 with 10.896 mm . The least radical belonged to -15 bar. Results showed that with the increase of drought stress levels radical length decreased.

The highest pulmus belonged to CH65 , CH353 and Rinconada. Rinconada variety with 70.066 mm had the highest pulmus. Ch697 , Asteria variety Zarghan in -9 , -12 , -15 had no length pulmus. (Table 1)

#### B) Germination percentage

The highest germination percentage was observed in distilled water with 98% in -6 level and the least belonged to -15 bar with 24.144%. Among varieties the least germination belonged to CH697 ,and the highest belong to Rinconad with 98% .

#### C) Mean of day Germination :

The highest mean of day germination belonged to CH697 with 6.22 day. It showed that average velocity of germination in this variety was very slow, Although Asteria variety had the least with 5.21 day.

#### D) Average Velocity of Germination

The highest (AVG) belong to Rinconada and the least belong to CH697 , Zarghan .

### Conclusions

In the studying the tolerance of varieties to drought stress , germination percentage cannot be a good index in screening varieties (Germination percentage trait was affected by salinity stress less than the other traits). To find the best one tolerant variety to such condition, taking all traits into account in this study , we found that Isfahan variety is the most resistant and CH697 is the most sensitive

Table 1 . Mean comparison of varieties safflower

Average velocity of germination	Index Average velocity of germination	Mean of Day germination	Germination Percentage	Pulmus length (mm)	Radical length (mm)	Variety
3.74ab	15.22a	5.22c	77.168a	7.066a	18.462a	CH 353
3.63ab	15.03a	5.34c	67.5a	6.98a	20.273a	CH 65
3.85a	15.14a	5.32c	77.168a	7.067a	18.519a	Rinconada
3.46bc	12.76b	5.699b	71.332a	6.23ab	13.638b	Isfahan
1.91d	11.59c	6.22a	35.668a	5.414b	10.896c	CH 697
3.793ab	15.25a	5.21c	76.168a	5.413b	19.344a	Asteria
3.2c	15.16a	5.28c	78.168a	6.55ab	18.828a	Zarghan

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# Soil Nitrogen, Growth, Yield Parameters in Oil Pumpkins (*Cucurbita pepo* L. convar. *citrullina* (L.) Greb. var. *Styriaca* Greb.) Affected by Cover Crops

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## Introduction

Oil pumpkins are a traditional field crop in Slovenia, whose edible seeds are used mostly for producing oil for salad dressing (Bavec et al., 2007). Production as well as organic production of oil pumpkins are increasing. Organic farming needs to be in accordance with the necessary organic farming guidelines, particularly by nitrogen management. Crops grown in fields after legume crops can uptake at least 60 percent of the nitrogen that the legume produced (Clark et al., 2007; Cherr et al., 2006). Inclusion of different opportunities such as the use of seedlings instead of seed sowing in the field (Bavec et al., 2002) and various cover crops offer suitable oil pumpkin production systems. These production systems decrease chemical inputs into the soil and also have positive effect on production such as quality and yield parameters.

## Methodology

The field experiments with oil pumpkins including two cover crops and two different sowing systems with control plots (seed sowing and seedling planting) were carried out at the University Agriculture Centre Pohorski dvor, Maribor, Slovenia (46° 39'N, 15° 41'E) on loamy sand soil in 2004 and 2005. The region belongs to climate with 9,8 °C mean long-term temperature and 1047 mm of rainfall. The average month temperature during the two-year trial period was higher than the long-term average value. The year 2004 was characterized by high concentration of rainfall (stormy weather and hail at the end of May) and extensive drought accompanied with high temperatures. The cumulative annual rainfall and the average annual temperature during two years were similar. The experimental design was the split-plot with four replications. Crimson clover (*Trifolium incarnatum* L.) cv. 'Inkarnatka' and non-winter berseem clover (*Trifolium alexandrinum* L.) cv. 'Winner' were used as cover crops. They were sown in August in both years in ploughed and stubbly field. Control plots included bare soil with no cover crop as it is usual in traditionally oil pumpkin production. In spring only the test sowing strips for oil pumpkins were ploughed in plots with cover crops (two strips 1,2 m wide per plot). The ratio between the ploughed and unploughed production land area was 0,78 : 1. The control plots were ploughed in autumn and then cultivated shortly before sowing of oil pumpkins in May. The seedlings were planted on 12th May in both years three weeks before they reached 8 to 9 cm height and developed first true leaf. The cultivation practice corresponded with the national guidelines for organic field crop production. The ploughed area with oil pumpkins was cultivated in May and in the beginning of June. Unploughed production land was mulched at the end of the May. In beginning of BBCH 701 phase (beginning of August) the length of main stem (LSt) and fruit yield per plant (FY) were measured. On the harvest in the first week of October number of fruits (FN), total fruit weight (FW), number of decayed (FD) and immature fruits (FI) and seed yield per plot (SY) were determined. The mineral soil nitrogen (N<sub>min</sub>) was analysed four times (at sowing of cover crops in August, at sowing of

oil pumpkins in May, at the beginning of flowering stage in June and at harvest in October).

## Results

All measuring parameters differ between years. Statistical analysis showed significant effect of the sowing system (seeds vs. planting) and cover crop on main stem length, fruit weight and yield of seeds (Table 1). Sowing system in interaction with seedlings had also significant effect on the seed yield at harvest. Interaction between year and sowing system had significant effect only on seed yield. Cover crops had no significant effect on number of immature and decayed fruits. The length of main stem and seed yield at harvest were significantly higher in plots with crimson and berseem clover compared with control plot. Higher fruit yield per plant in August was obtained in plots with crimson clover.

Table 1: Effect of years, different cover crops and sowing system on the length of main stem (LSt), fruit yield per plant (FY), number of fruits (FN), total fruit weight (FW), number of decayed (FD), immature fruits (FI) and seed yield per plot (SY).

	BBCH 701			At harvest			
	LSt (m)	(FY) (kg)	SY (t ha <sup>-1</sup> )	FN	FW (kg)	FI	FD
A: Year	**	**	**	**	**	**	*
B: Cover crop	*	**	*	n.s.	n.s.	n.s.	n.s.
C: Sowing system	n.s.	n.s.	**	**	**	n.s.	n.s.
Interaction			A×C				
<b>Year</b>							
2004	2.65b	1.17b	0.510b	9.2b	12.5b	2.0b	1.3a
2005	4.91a	3.79a	0.905a	12.5a	15.7a	1.5a	1.7b
<b>Cover crop</b>							
Control	3.39b	1.87b	0.631b	10.2	13.7	1.8	1.7
Berseem clover	4.02a	2.42b	0.737a	11.4	14.8	2.0	1.5
Crimson clover	3.93a	3.15a	0.753a	10.9	13.8	1.5	1.4
<b>Sowing system</b>							
Seeds	3.76	2.85	0.624b	9.3b	12.4b	1.8	1.4
Seedlings	3.80	2.86	0.790a	12.4a	15.8a	1.8	1.6

n.s. No significant differences.

\* Significance indicated at p = 0.05, \*\* at p = 0.01.

a,b,c Means within column followed by the same letter are not significantly different at the 95% confidence level (Duncan test).

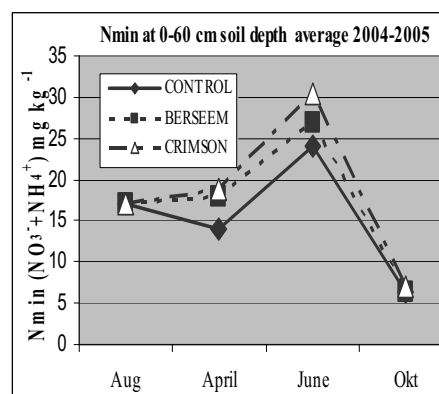


Figure 1: Average of two years  $N_{min}$  values ( $NH_4^+$  and  $NO_3^-$ ) up to 60 cm depth during the vegetation period.

The soil  $N_{min}$  up to depth 0,6 m ( $N_{min}$ ) reached the highest value in first half of June and lowest one after the harvest. There was no significant effect of cover crop treatment on soil mineral nitrogen content except in April, although results showed trend of increasing  $N_{min}$  from control plots toward to berseem clover and crimson clover treatments in all sampling dates (Figure 1).

## Conclusions

Cover crops are an important alternative for better nitrogen supply and increase growth and yield in oil pumpkins cultivated according to organic guidelines.

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# Some consequences of carbon sequestration in Luvisol, loam soil owing to long-term conservation tillage use

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Contemporary farming practices deplete soil carbon, which in turn degrades soil quality, reduces productivity, and results in the need for more fertilization, irrigation, and pesticides. Conservation tillage farming with residue mulching, cover cropping, and other means of organic matter management would reverse these harmful effects (Franzluebbers, 2002).

## Methodology

A thirteen-year field experiment has been running in a temperate semiarid climate, 338 m above sea level, with an mean annual air temperature of 8.2°C, and mean annual precipitation of 477 mm. The field site has a soil of clay-loam texture (Orthic Luvisol, FAO Taxonomy). In this field trial, winter wheat, spring barely, and pea are grown in short crop rotation. The stands of these crops are established by classic plough technology (PL), shallow tillage with chopped straw incorporation (ST) and direct drilling into non-tilled soil (NT), as well as direct drilling with mulch soil covering (NTM). After the harvest in the winter wheat plots, soil samples were taken for analyses. Before analysing the soil, these samples were adjusted by sieving and air-drying. Total carbon (TC) and total nitrogen (TN) in the air-dried soil samples were determined on a VARIO MAX CNS analyzer. The aggregate stability was determined using the Kemper & Koch method (1966).

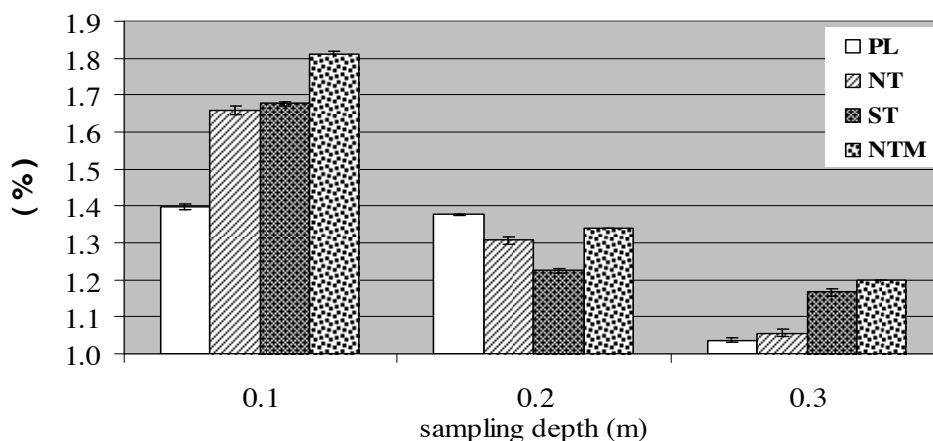
## Results

Results of the soil analyses demonstrated that differences of TC in soil samples among tillage treatments depended upon depth of the sampling. In the upper layer (Fig.1) the highest TC content was found with the NTM treatment; while the lowest content was found with the variant under conventional tillage. Differences between conventional tillage and all the variants of conservation tillage are statistically significant. A similar result was reached by Chan et al. (2001), when they detected the largest (significant) differences of TC content between PT and NT in the upper 0.05 m. In the lower layer (0.1 - 0.2 m) of topsoil the situation was quite the opposite. Compared with PT, the C content in conservation treatments was lower, but differences were insignificant (as well as in the lowest sampling layer). The results of TN analysing paralleled the TC data relatively closely. The significance of these differences, between ploughing and conservation treatments, was the same as in the case of total carbon. Gal et al. 2007 mention having found the similar results.

Soil sequestration of carbon and nitrogen by use of conservation technologies is indicated by growth of the humus content in the topsoil. The data, shown as an average of the last 5 years, are in Table 1. It is possible to find statistically significant differences between plough tillage and no tillage, especially with mulch in the upper and middle layers of topsoil. This corresponds closely with the C/N analysis data mentioned above.

A positive correlation exists between the aggregate stability and total C content in the soil (Seybold, Herrick 2001). The long-term results of our study of soil aggregate stability confirm this, as well. Aggregate stability measurements showed that in a horizon depth, down to 20cm in NT, there is double the amount of water resistant aggregates, than in the PT treatment. These differences between PT and NT treatments were statistically significant (Table 2).

**Fig. 1: The impact of soil tillage method on total C content**



**Table 1: The impact of soil tillage methods on humus content**

Sampling profile /m/	Percentage of humus content (average last 5 years)				LSD 0.05
	Plough tillage	No tillage	Shallow tillage	No tillage and mulch	
0 – 0.1	2.79	3.47	3.31	3.82 <sup>+</sup>	0.726
0.1 – 0.2	2.70	3.37 <sup>+</sup>	3.10	3.44 <sup>+</sup>	0.612
0.2 – 0.3	2.60	3.10	2.64	3.04	0.598

**Table 2: The impact of soil tillage methods on soil aggregate stability**

Crop	Percentage of water resistant aggregate (average last 5 years)				LSD 0.05
	Plough tillage	No tillage	Shallow tillage	No tillage and mulch	
Winter wheat	23.5	47.1 <sup>+</sup>	34.2	56.3 <sup>+</sup>	11.81
Spring barley	32.4	45.6 <sup>+</sup>	40.7	51.8 <sup>+</sup>	9.74
Pea	27.6	44.9 <sup>+</sup>	36.5	49.8 <sup>+</sup>	10.31

## Conclusions

This research demonstrated that the long-term use of conservation tillage methods caused larger C and N sequestration (predominantly in the upper layer of topsoil) with the conditions defined above, when compared with plough tillage. This fact influences positively on both the humus content and soil aggregate stability, thus improving the overall soil quality and decreasing its erodibility.

## Acknowledgement

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# Rapid Screening for Salinity Tolerance of Rice Varieties

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Salinity is considered among the major environmental limitations for rice growth in many areas of the world. Salt presence negatively affects various growth parameters of rice plant and degrades the quality of the product. In a saline environment, germination can fail or may be delayed, crop establishment, dry matter production and leaf area development may be decreased, seed set may drop and sterility levels raise (Asch and Wopereis, 2001). Therefore, breeding for salinity tolerance is one of the major objectives in rice breeding programs for areas with high salt concentrations in the soil (e.g. deltas of rivers). Although rice is considered to be moderately susceptible to salinity, variation between varieties and growth stages have been reported (Yoshida, 1981). Several studies have shown that rice is tolerant during germination and becomes very sensitive during early seedling growth. It again becomes more tolerant during vegetative growth with the salt sensitivity becoming critical during pollination and fertilization phases (Fageria, 1985). The current study was conducted to rapidly and efficiently assess, at seedling stage, the variability to salt stress existing in a European rice core collection to identify the most salt resistant rice accessions.

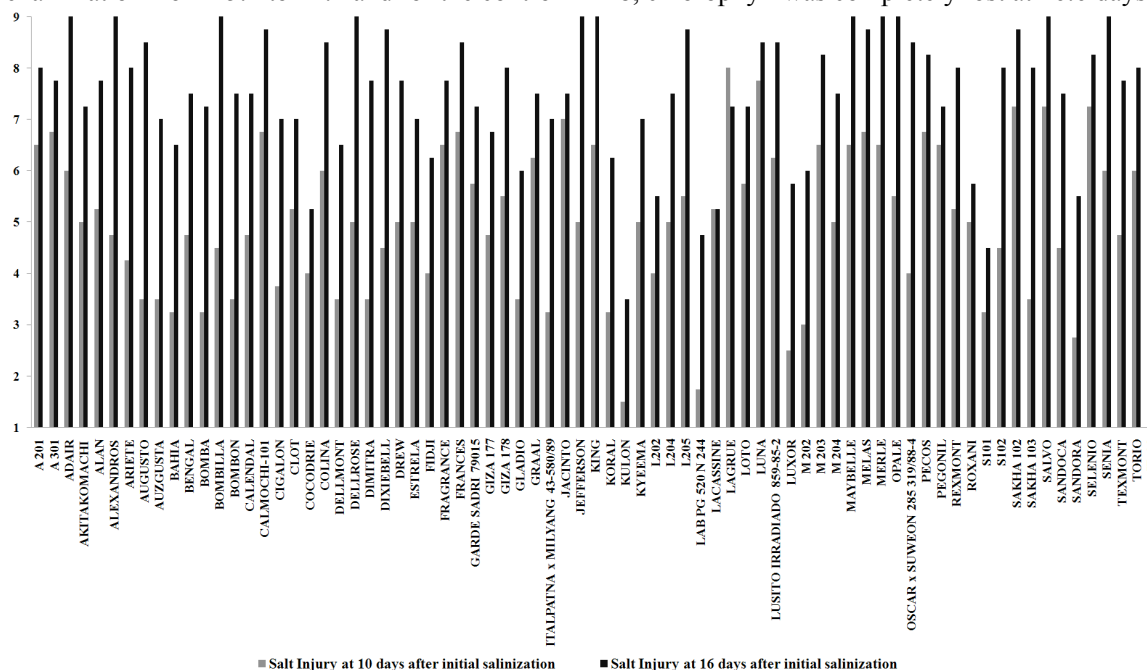
## Methodology

The screening test was performed hydroponically at the Cereal Institute of Thessaloniki, Greece. A total of 75 rice European accessions were tested for salt tolerance, using IR-28 variety as highly susceptible control. Seedling floats made of a Styrofoam tray consisted of 96 holes (with 2.5 cm diameter). A nylon net was attached to the bottom of the tray to keep the seedlings in place. Seeds were heat-treated for 5 days at 50°C to break seed dormancy and then they were surface sterilized and pre-germinated before transferring them to the trays. The experiment was conducted in a glasshouse maintained at 29°/22°C day/night and 70% relative humidity. Two germinated seeds were placed in each hole of the Styrofoam tray and each tested rice accession was replicated 8 times. The Styrofoam tray was placed in a rectangular plastic container and nutrient solution was added according to Gregorio et al. (1997). On the fourth day 3 g NaCl / L (electrical conductivity, EC 6 dS m<sup>-1</sup>) was added in the nutrient solution, while 3 days later the amount of NaCl was doubled. The pH was constantly kept at 5 and calibrated three times a day, while the NaCl solution was renewed every 8 days. The salt injury of the seedlings was assessed at 10 and 16 days after the initial salinization (AIS) treatment using a 1-9 scale: (1)=normal growth, no leaf symptoms (highly tolerant), (3)=nearly normal growth, but leaf tips or few leaves bleaching and rolled (tolerant), (5)=growth severely retarded, most leaves rolled, only a few elongating (moderately tolerant), (7)=complete cessation of growth, most leaves dried, some plants dying (salt susceptible), (9)=almost all plants dead or dying (highly salt susceptible). Additionally, the number of days to reach the complete loss of chlorophyll was also recorded.

## Results

This study revealed that there was a great variability in response to salt injury within the 75 rice tested accessions in both phenotypic assessments (Figure 1). The salinity injury recorded at 10 days AIS, had a mean value of 5.0 and ranged from 1.5 to 8.0. According to their response to salt, the rice accessions were divided into four groups: 5.3% were classified as “high salt tolerant to salt tolerant”, 37.3% as “salt tolerant to moderately salt tolerant”, 49.3% as “moderately salt tolerant to susceptible and 8% as

“salt susceptible to salt highly susceptible”. The most tolerant genotypes were Kulon (1.5) Lab PG 520 N 244 (1.8), Luxor (2.5) and Sandora (2.8), while the susceptible control IR-28 expressed salt damage symptoms rated as 6.8. Salinity injury recorded at 16 days AIS, had a mean value of 7.5 and ranged from 3.5 to 9.0. The genotypes were divided into three groups, where 4.0% of them were classified as “salt tolerant to salt moderately tolerant”, 17.3% as “salt moderately tolerant to salt susceptible”, while 79.0% of them expressed great salt injury and they were grouped as “salt susceptible to salt highly susceptible”. The most tolerant genotypes were Kulon (3.5) S 101 (4.5) and Lab PG 520 N 244 (4.8). The susceptible control variety IR-28 expressed salt injury as high as 8.5 in the scale employed. The number of days until the complete loss of chlorophyll ranged in the 75 rice accessions under examination from 15.4 to 22.1 and for the control IR-28, chlorophyll was completely lost at 16.6 days.



**Figure 1.** Salt injury of 75 rice European accessions assessed at 10 and 16 days AIS.

## Conclusions

Screening for salinity tolerance protocol in a panel of rice accessions proved to be efficient for the rapid and efficient identification of highly salt resistant rice genotypes. The most salt tolerant rice accessions, expressing a reproducible behavior in all evaluations, were found to be Kulon, LAB PG 520 N 244 and Luxor. Further investigations, will be assessing the salt tolerance at adult plant stage and will be focused on the characterisation of the molecular bases of salt tolerance in European rice accessions by identifying the genes and/or loci and QTLs underlying the mechanisms of resistance to salt injury. The integration of phenotypic and genotypic data will be used to develop novel strategies to guide rice breeding programs at European level for enhanced salt tolerance traits.

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# Management of Water Quantity and Quality in Agricultural Landscape

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## Introduction

Water problems are very important for Polish economy but they are the most visible in Wielkopolska region. Water shortages during vegetation season as well as pollution of ground and surface water bodies are ones of the most important barriers for sustainable development of Polish agriculture. These problems are caused by natural climatic conditions (high evapotranspiration, low precipitation), many errors in water management that have been committed in the past, abuse the artificial fertilizers and simplification structure of agricultural landscape.

The point sources pollution can be controlled much easier than non-point pollution. The purification stations cleaning the sewage and special filters can reduce to a very high degree the amount of pollutants flowing into environment. It is very difficult to control the non point pollution. It can not be done by technical methods. It needs special ecological means.

The tools for increasing water retention in the small post glacial ponds, in local depressions, and the increase of soil water retention are discussed, as well as the creating proper land use structure as one of the most effective ways of improving water conditions in agricultural landscape. The shelterbelts occur to be the best tool for melioration of heat, and the same way the water balance structure, as well as the tools for controlling dispersion of nutrients within agricultural landscape and reduction their concentration in ground water and surface water bodies. By reducing wind speed, they reduce the potential and real evapotranspiration of fields, but on the other hand they intensify the water cycling by taking the water from the deeper layer of soil while cultivated fields use the water stored in the thin upper layer of soil. By taking the nutrients from the ground water flowing from crop fields to the water courses shelterbelts reduce concentration of nutrients in the water.

The guidelines for water management for sustainable agriculture development in Wielkopolska are finally formulated.

## Methodology

The investigations on the impact of landscape structure on quantity and quality of water in an agricultural landscape are carried out since 1970's. The investigations are carried out in experimental catchment located 50 km south from Poznań in Wielkopolska region. The laboratory and field measurement methods as well as modelling were used in this investigation. The meteorological data are obtained by the use the standard, automatic station, the data of hydrological parameters are obtained by the use of the net of hydrological observations.

## Results

The shelterbelts introduced into grain monoculture landscape change the microclimatic conditions of the field as well as aerodynamic characteristics of an active surface. Shelterbelts by reducing wind speed, stomatal resistance and increasing the humidity, turbulence and net radiation cause a little increase of actual evapotranspiration of landscape taken as a whole, but decrease it from the cultivated field lying between shelterbelts. Thanks to reducing wind speed and increasing humidity, shelterbelts reduce significantly potential evapotranspiration, especially when convection of dry and hot air occurs. During plant growth season, the introduction of shelterbelts can save as much as 40 mm of water in non irrigated field, and as much as 200 mm in strongly irrigated field surrounded by dry and hot areas.

The content of biogens in ground water is reduced to a high degree when water flows under shelterbelts or meadow strips. A shelterbelt or meadow strip a dozen or so meter wide reduce nitrates concentration by 50 to 90% and phosphorus up to more than 90%.

The content of organic matter is one of the most important factors creating hydropedological properties of the soil. Organic matter can absorb ten times more water than mineral soils can do. Additionally, organic matter improves the structure of the soil; increases the amount of mezopores which results in large amount of water available for plants. The increase of organic matter content in upper layer of soil by 1% causes the increase of available water by 30 mm which in the scale of the country gives the increase of water supply equal to the volume of all artificial reservoirs in Poland. The better structure of upper layer also results in increasing of infiltration rates which allows to catch more water from precipitation and decrease surface runoff.

### Conclusions

Improvement of water cycling in the landscape needs:

1. Developing of landscape complexity by introduction shelterbelts, strips of meadows and restoration of midfield ponds,
2. Increasing of organic matter content in the soil,
3. Keeping as much water as possible in the landscape for as long as possible, taking care that it is properly allocated,
4. Ensuring that as much water as possible flows from the soil into the atmosphere via plant transpiration, but not as evaporation from the soil to the atmosphere.

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# Pathogens in Environment and Bacterial Contamination of Vegetables

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Microbiological criteria of food quality are considered of great importance due to the significance of public health and safety. It is well-documented that there is very big potential risk for the health of consumers of crops irrigated with contaminated water, especially fresh vegetables which will not be cooked prior to consumption (Steele and Odumeru, 2004). Most of the reported outbreaks have been associated with bacterial contamination. Pathogenic bacteria often found on vegetables and fruits are: *Salmonella spp.*, *Shigella spp.*, *E. coli*, *Escherichia coli* O157:H7, *Campylobacter sp.*, *Listeria spp.* Many of human illness are caused by pathogens as: *Campylobacter enteritis*, *Hemorrhagic colitis*, *salmonellosis*, *diarrhoea syndrome*, *hemolytic uremic syndrome (HUS)* etc. Microbial contamination depends on different factors including soil conditions that could be the reservoir of foodborne pathogens as *Bacillus cereus* (Lund, 1986) or water for irrigation. Water may be source of different pathogenic as *Salmonella spp.*, or *E. coli* O157:H7. They could be transferred from water to the fruits or vegetables (NACMCF, 1999).

The coliform groups of bacteria are frequently used to determine faecal contamination of water (Greenberg et al., 1992). Although a wide range of pathogens can be transmitted from water to soil and irrigated crops, the results of total and fecal coliform bacteria (as indicators of bacterial pathogen contamination of water) will be presented.

## Methodology

The channel used for investigations is in the area of Eastern Serbia. Water samples were taken from different part of channel and they were marked as: beginning of the channel (U1), sewage flows into the channel (2K), water from pig farm enter the channel (3K), position after all pollutants (U6), channel empties into Danube River (U7).

The number of coliform bacteria in the water was determined by using MPN method. The number of positive tubes at each dilution was compared to MPN tables to give number of bacteria present in the original sample (Wiggins, 1996; Grant, 1997). For identification of *E. coli*, *Salmonella sp.* it was used API 20E kit and API-WEB (Biomerieux, France). Several vegetable species, which were grown near channel, were tested for contamination with waste water. The number of total and faecal coliforms on vegetable species was determined by using Petri-film method (3M, USA).

## Results

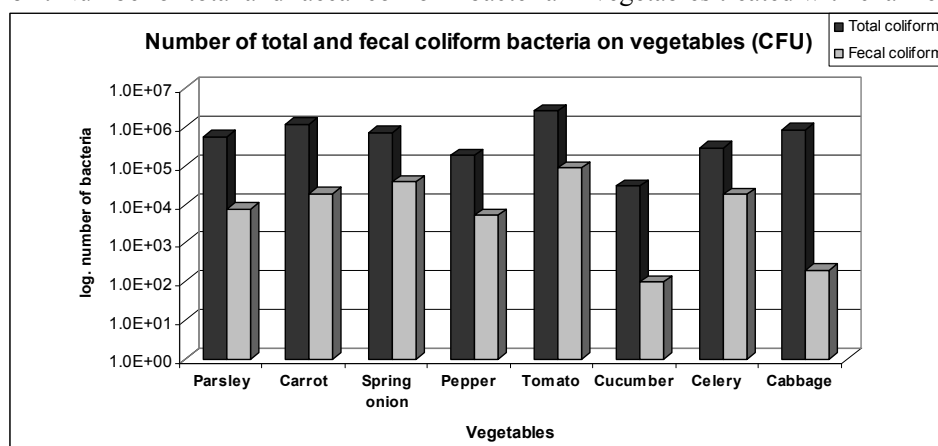
The results show a high degree of contamination of water during whole growing season. Also, these results showed significant contamination on the position where channel empties into Danube River (Tab. 1).

**Table 1.** Number of coliform bacteria in channel water samples

Water Samples	Number of coliform bacteria (per 100 ml MPN)					
	Date of sampling					
	27.07.2006.	22.03.2007.	25.07.2007.	11.10.2007.	27.11.2007.	27.12.2007.
U1	/	/	/	22	> 2,400	380
2K	> 240,000	> 240,000	> 240,000	96,000	> 240,000	240,000
3K	/	/	/	2,000	4,000	2,100
U6	/	/	/	3,800	> 24,000	1,500
U7	/	/	/	500	24,000	3,800

Microbiological results indicated on big potential risk if water from investigated channel is used for irrigation. *E. coli* and *Salmonella spp.* were identified in all water samples during the investigation period.

**Figure 1.** Number of total and faecal coliform bacteria in vegetables treated with channel water



Pathogenic bacteria results also showed a significant increase and differences between investigated vegetables. The highest number was founded in tomato fruit and the lowest in cucumber. *E. coli* was identified on: carrot, spring onion, pepper and tomato.

**Table 2.** Presence of pathogenic bacteria in vegetable treated with channel water

Vegetable	Presence of pathogenic bacteria		
	<i>E. coli</i>	<i>E. coli</i> O157:H7	<i>Salmonella sp.</i>
Parsley	-	+	+
Carrot	+	+	-
Spring onion	+	-	+
Pepper	+	-	+
Tomato	+	+	+
Cucumber	-	-	-
Celery	-	-	-
Cabbage	-	-	-

*E. coli* O157:H7 was identified on: parsley, carrot and, tomato although *Salmonella sp.* was founded on: parsley, spring onion, pepper, tomato (Tab. 2). Pathogenic bacteria results, as well as total and number of coliform bacteria, showed that the most susceptible to contamination was tomato and most resistant cucumber (Fig. 1).

## Conclusions

Irrigation water quality plays a significant role in produce food safety, but currently in the Serbia there are no irrigation water quality regulations. Microbiological results indirectly pointed out on potential risk of using channel with contaminated water for irrigation of crops. Generally, as EU (2002) recommended, the most efficient way to improve safety of fruits and vegetable is to rely on a proactive system reducing risk factors during production and handling.

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# Groundwater Quality Risk due to Conventional Irrigated Agriculture in the “Apulian Tavoliere”

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High-value and intensively managed crops usually take advantage of large amounts of irrigation water and nitrogen fertilizers, therefore greatly contributing to the risk of groundwater contamination due to drainage water and nitrate leaching. The problem is much more difficult to manage in case of brackish irrigation water, due to saline groundwater and sea-water intrusion in coastal areas. An real accounting of this risk is a priority knowledge at the field as well as at the land scale, in order to start up effective agro-environmental measures to control or mitigate those impacts. This study was carried out in order to assess the main composition and quality of the drainage water resulting from the current cultivation practice (irrigation and fertilization) performed by an ordinary farmer with respect to an area of the Apulian Tavoliere, close to the coast of the Manfredonia gulf (Adriatic sea).

## Methodology

A permanent experimental field-unit was established in autumn 2006; three plots of 100 m<sup>2</sup> each (6.4 x 15.6 m) were delimited; at the center of each plot an artificial draining basin was arranged digging the soil out of a trench, 3.2 m wide, along the entire plot length; the bottom of each trench was covered with a plastic sheet in order to prevent water percolation; a set of drains (two groups per trench, three drains per group) were displaced over the plastic cover to collect the percolating water and conveying it into tanks placed at the edge of each plot (two tanks per plot). The trenches were then filled with the same soil obtained by the excavating procedure, trying to correctly reproduce the original soil stratification. Two subsequent crop cycles (tomato and spinach respectively) and an interposed fallow period were considered, from spring to winter 2007. Tomato was transplanted on 20 April and harvested on 26 July; spinach was sowed on 18 October and harvested on 27 December. In the course of the trial, the current cultivation practices (irrigation and fertilization) were performed by an ordinary farmer. During every experimental periods (tomato crop cycle, fallow and spinach crop cycle), salts and nitrate were considered, periodically measuring the electrical conductivity (EC<sub>DW</sub> - dS m<sup>-1</sup>), the sodium adsorption ratio (S.A.R.) and the NO<sub>3</sub>-N concentration (mg l<sup>-1</sup>) of the drainage water. The electrical conductivity (EC<sub>IW</sub> - dS m<sup>-1</sup>) and S.A.R. of the irrigation water were also measured at every crop watering. Furthermore, the volumes of irrigation, rain and drainage were regularly recorded.

## Results

While tomato was regularly irrigated, autumn rainfalls completely satisfied the spinach water requirements and no irrigations were needed. The seasonal water supply (irrigation and rain), the

**Table 1** Water supply and drainage, nitrogen fertilization applied, N concentration of the percolating water and N leached

	Seasonal irrigation (m <sup>3</sup> ha <sup>-1</sup> )	Rain amount (m <sup>3</sup> ha <sup>-1</sup> )	Drainage volume (m <sup>3</sup> ha <sup>-1</sup> )	N fertilization (kg ha <sup>-1</sup> )	NO <sub>3</sub> -N concentration (mg l <sup>-1</sup> )	NO <sub>3</sub> -N Leached (kg ha <sup>-1</sup> )
Tomato crop cycle	5,440	1,170	379	100	34.7	13.0
Fallow period	--	320	164	--	65.5	10.6
Spinach crop cycle	--	1,480	379	250	110.4	41.5

drainage volume, the farmer applied N fertilization, the NO<sub>3</sub>-N concentration of the drainage water and the NO<sub>3</sub>-N leached in the course of the trial are given in *Table 1*. The

$EC_{WI}$  values ranged from 4.7 to 5.3  $dS\ m^{-1}$  and progressively increased along the summer season; the S.A.R. of the irrigation water was on average equal to 9.1. A flow-weighted average of  $NO_3-N$  concentration over multiple drainage water samplings allowed to calculate a higher overall loss of  $NO_3-N$  (41.5  $kg\ ha^{-1}$ ) during the spinach cultivation, as a consequence of a higher N fertilization rate. The N fertilization broadcasted by the farmer along the spinach crop cycle exceeded the amount actually absorbed by the plants thus determining  $NO_3-N$  leaching with the percolating water (379  $m^3\ ha^{-1}$ ). The splitting of N fertilization along the tomato crop cycle and the lower amount of N actually distributed determined a corresponding lower N loss by leaching as compared with spinach (13.0  $kg\ ha^{-1}$ ) even if the same volume of drainage water was registered. A considerable  $NO_3-N$  leaching (10.6  $kg\ ha^{-1}$ ) was also measured during the fallow period. Relevant  $NO_3-N$  losses (Fig. 1a) were observed at the end of

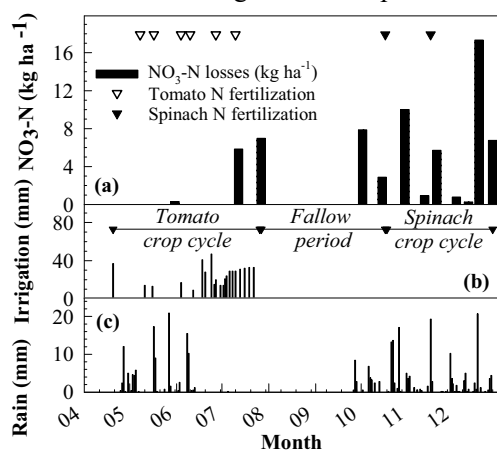


Figure 1 - Nitrogen losses (a), irrigation (b) and rain (c) during the two crop cycles and the fallow period.

Starting from the initial value of 1.8  $dS\ m^{-1}$  the  $EC_{DW}$  reached a value of 14.8  $dS\ m^{-1}$ , close to the end of spinach crop cycle, thus indicating a high concentration of dissolved salt in the soil circulating water and, consequently, an effective salt leaching process.

## Conclusions

Significant and worrying concentration values of salts and  $NO_3-N$  were detected in the drainage water coming out from a cultivated field, according to the conventional practices employed by farmers to grow tomato and spinach in the considered area. Strong signs of soil salification and alkalisation were also detected. This first investigation, in the frame of a long-term experiment, confirmed the dangerous conditions of the local agricultural practices and the need of a drastic and quick technical adjustments. The application of optimized amounts of water and nitrogen to meet realistic yield goals, as well as the timely application of N fertilizers, can be a viable measures to minimize nitrate and salts leaching.

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of the tomato crop cycle, when the irrigations performed by the farmer increased their frequency (Fig. 1b). The detected  $NO_3-N$  losses along the fallow period were strictly in time with the first autumn rains (Fig. 1c), just before the spinach sowing. In the course of the spinach cropping season, higher values of  $NO_3-N$  losses were registered, particularly near the end of the cycle (Fig. 1a) as consequence of the large amount of N fertilizer applied and of the seasonal rains (Fig. 1c). The S.A.R. values of the drainage water (Fig. 2a) were always lower than the corresponding values of the irrigation water and mostly similar in the different experimental periods, on average equal to 6.5. This remark lead to suppose that the sodium load due to brackish irrigation was mainly adsorbed by the soil, thus increasing the risk of its alkalisation. An increasing  $EC_{DW}$  was also observed along the whole examined period (Fig. 2b).

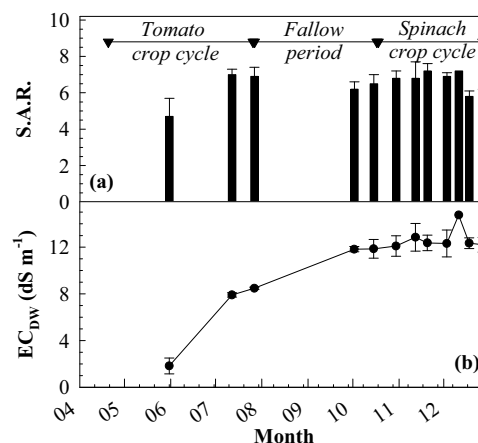


Figure 2 - S.A.R. (a) and EC (b) of the drainage water, during the two crop cycles and the fallow period.



# Salt Concentration and Movement along the Soil Profile under Irrigated Agriculture with Brackish Water

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The technical management of irrigation when brackish water is used must be aimed to keep soil salification below a maximum threshold, still compatible with a rationale agriculture, matching water quality, soil characteristics and sensibility of the crops with the expected productivity and the economical farm return. Leaching is the unique way to control salt accumulation in the soil root layers and is generally accepted that the leaching requirement could be satisfied not necessarily during the irrigated cropping cycle but whenever it is believed useful, successful and effective, in the course of the year. In the Mediterranean type of climate, with drought summer months, the autumn-winter rainfalls could play a very important role in promoting salt leaching from the soil.

The aim of the study was therefore to evaluate the effect of summer brackish irrigation and subsequent autumn rains on the salt soil profile in order to check the opportunity to perform leaching without intentional extra-irrigation volumes.

## Methodology

A permanent experimental field-unit was established in autumn 2006; three plots of 100 m<sup>2</sup> each (6.4 x 15.6 m) were delimited; at the center of each plot an artificial draining basin was arranged digging the soil out of a trench, 3.2 m wide, along the entire plot length; the bottom of each trench was covered with a plastic sheet in order to prevent water percolation; a set of drains (two groups per trench, three drains per group) were displaced over the plastic cover to collect the percolating water and conveying it into thanks placed at the edge of each plot (two thanks per plot). The trenches were then filled with the same soil obtained by the excavating procedure, trying to correctly reproduce the original soil stratification. The experimental field is placed in an area of the Apulian Tavoliere, close to the coast of the Manfredonia gulf (Adriatic sea).

Two subsequent crop cycles were considered, from spring to winter 2007: tomato and spinach, respectively. Tomato was transplanted on 20 April and harvested on 26 July; spinach was sowed on 18 October and harvested on 27 December. In the course of the trial, the current cultivation practices (irrigation and fertilization) were performed by an ordinary farmer. In the last part of the fallow period a soil watering with brackish water was applied to the bare soil, in order to create the proper soil moisture conditions to facilitate salt leaching by autumn rains. The electrical conductivity (EC<sub>w</sub> - dS m<sup>-1</sup>) of the irrigation water was regularly measured. During every experimental periods (tomato crop cycle, fallow and spinach crop cycle), also the electrical conductivity of the saturated soil water extract (EC<sub>e</sub> - dS m<sup>-1</sup>) was measured, approximately every 10 days, samplings the soil at the following depths: 0.20, 0.40, 0.60 m.

## Results

While tomato was regularly irrigated, autumn rainfalls completely satisfied the spinach water requirements and no irrigations were needed. During the tomato crop cycle, the EC<sub>e</sub> progressively increased from 4.7 to 5.3 dS m<sup>-1</sup>. The seasonal irrigation applied to tomato crop was 544 mm (*Fig. 1a*), while rain amount was equal to 117 mm (*Fig. 1b*); thus the total water supply was 661 mm. During the

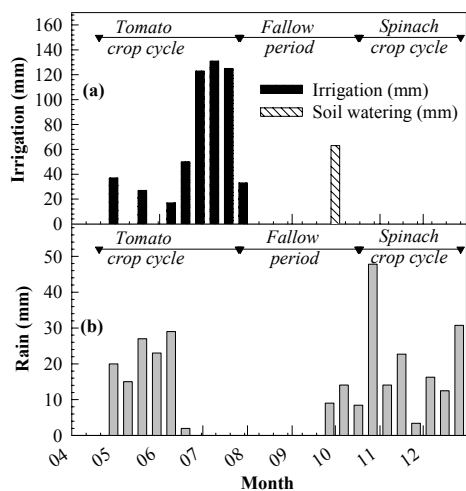


Figure 1 - Irrigation and soil watering (a), rain amount (b) during the two crop cycles and the fallow period.

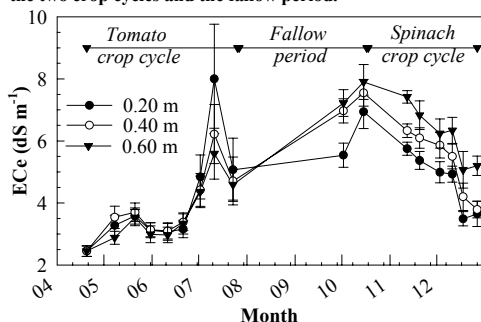


Figure 2 - ECe (dS m<sup>-1</sup>) at the soil depth of 0.20, 0.40, 0.60 m. during the two crop cycles and the fallow period.

increased (2.3 dS m<sup>-1</sup>) at 0.60 m. Along the spinach cropping season (Fig. 3c), the winter rains leached out the salts from the soil profile; at every examined depths, ECe decreased significantly (- 3.3, - 3.8, - 2.7 dS m<sup>-1</sup>, respectively from the top to the bottom).

## Conclusions

In this first trial, in the frame of a long-term experiment, a sequence of a marked soil salification due to brackish irrigation followed by salt leaching due to autumn rainfalls was observed. No leaching fraction was currently applied to irrigation volumes but an extra-watering of the soil with brackish water at the end of the fallow period significantly promoted salt leaching. The salt soil profile was greatly influenced by the water flows in input and output, clearly displaying a salt accumulation, redistribution and discharge phases. Further and continuous monitoring is however needed to follow the soil salinity evolution and dynamically modulate the necessary leaching interventions.

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spinach cropping season, rain amount was equal to 148 mm (Fig. 1b). The soil watering applied at the end of the fallow period was 60 mm of brackish water (Fig. 1a). The time-course of soil salinity at the three soil depths is shown in Figure 2. Along the tomato cropping season, brackish irrigation induced a remarkable ECe increase, higher at the soil surface than in the lower layers, particularly close to harvest (8.0, 6.0 and 5.5 dS m<sup>-1</sup> from the top to the bottom). The brackish water applied during the fallow period didn't produce a significant salt leaching (due to a low rain amount) but determined a completely reversed ECe profile, displacing the salts from the topsoil down towards the deeper soil layers; at the end of this period, the ECe reached values of 6.9, 7.5 and 7.9 dS m<sup>-1</sup>, respectively. Along the spinach crop cycle, rains succeeded to leach out the salts from the soil, determining a significant ECe decreasing, still confirming a higher salt concentration at the bottom layers than at the soil surface (3.5, 4.0, 5.0 dS m<sup>-1</sup>, respectively).

Salt movement along the soil profile, expressed as ECe variation (increases or decreases) at each examined soil depths, during the two crop cycles and the fallow period, is given in Figure 3. In the tomato crop cycle (Fig. 3a), ECe increased along the soil profile, particularly at the 0.20 m soil depths (5.5 dS m<sup>-1</sup>). During the fallow period (Fig. 3b), the salts moved from the upper to the lower layer; ECe decreased (- 1.3 dS m<sup>-1</sup>) at 0.20 m soil depths but

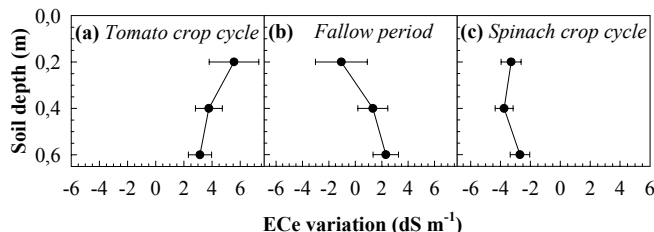


Figure 3 - ECe (dS m<sup>-1</sup>) variation along the soil profile, during the tomato crop cycle (a), the fallow period (b) and the spinach crop cycle (c).

# Inorganic Nitrogen Dynamics in Soil During Sugar Beet Crop Growth

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Nitrogen (N) is the crop nutrient which more directly influences the production and quality of sugar beet (*Beta vulgaris* subsp. *vulgaris* L., SB) root. It is therefore important to accurately estimate the amount of N fertilizer to be supplied to the crop. Soil N excess, besides being detrimental for SB technological quality, may also increase the environmental pollution risk. The aim of this work was to improve our general knowledge of N availability for SB, by monitoring the inorganic N content in the cultivated soil layer during the crop growth season. The N dynamics in soil under SB was compared with that under winter wheat (*Triticum aestivum* L., WW), in the same kind of soil.

## Materials and Methods

The experiment was carried out in 2006, at the experimental farm of the CRA-CIN of Rovigo, Eastern Po Valley, Italy (Lat.: 45°04'; Long.: 11°47'), on a clay loam soil. The experimental design was a strip-plot with 2 replicates. Crops (SB, WW) were assigned to the main plots and 3 N fertilizer rates (0, 60 and 120 kg N ha<sup>-1</sup>) to the sub-plots (sub-plot area: 402 m<sup>2</sup>). The preceding crop was WW, for both SB and WW. Wheat straw was removed from the field. Winter wheat (cv. Abusson) was seeded on 20 Oct 2005 and harvested on 3 July 2006. Sugar beet (cv. Dorotea) was seeded on 15 March 2006 and harvested on Aug 15. Nitrogen fertilizer was distributed as urea on March 10, for both crops. The WW and SB cultivation techniques were those typical of the Padana plain (Po Valley). Crop biomass dry matter (DM) and N removal (as Kjeldahl N) were measured on 4 dates for WW (shoots and ears) and on 6 dates for SB (tops, leaves and roots), from mid April to the crop-harvest date. Nitrogen removal was calculated by multiplying each plant part DM by its N concentration. For WW, root N removal was estimated, by assuming the WW root dry weight as equal to 20% of total crop biomass (shoot + root), and its N concentration as equal to the shoot N concentration. Soil samples were collected at 0-0.25 and 0.25-0.50-m soil depth on 6 dates, from mid April to mid August. The soil inorganic N (nitrate + ammonium N) content was measured colorimetrically. Statistical analysis was performed using a mixed model for measurements repeated in time. In a preliminary analysis N removal by WW and SB was compared at common sampling dates. A second analysis was then performed separately, on each crop data set. Soil inorganic N data was log-transformed before statistical treatment.

## Results

In the analysis comparing crop N removal at common sampling dates (from April 19 until July 3), N removal by SB was on average higher than N removal by WW ( $P < 0.05$ , data not shown). Highly significant effects of Date of sampling ( $P < 0.001$ ) and of Crop x Date of sampling interaction ( $P < 0.01$ ) were also detected. In fact, in the April-May sampling dates, N removal by SB was on average lower than that by WW. However, starting from June, N removal by SB was always much higher than that of WW (Tab. 1). Nitrogen fertilizer significantly increased crop N removal only for WW.

Statistical analysis for soil inorganic N content depending on crop, date and depth of sampling, showed highly significant effects for all the main sources of variation and for some first and second order interactions (Data not shown). Despite the SB having removed more N than WW had, throughout the growth period the inorganic N content in soil under SB was on average twice (14.1 mg N kg<sup>-1</sup> dry soil)

that of soil under WW (7.5 mg N kg<sup>-1</sup> dry soil) (Tab. 2). As the soil was the same, we concluded that the observed differences in the inorganic soil N content could be due to the crop type.

Table 1. Total N removal (shoot + root) by sugar beet (SB) and winter wheat (WW), in spring-summer 2006, at Rovigo (Italy). Treatments: N0, control; N1, 60 kg N ha<sup>-1</sup>; N2: 120 kg N ha<sup>-1</sup>.

Crop	Treatment	Total crop N removal, kg ha <sup>-1</sup>						Means
		19/04	16/05	01/06	03/07	18/07	14/08	
SB	N0	0.3	55	127	200	175	224	130A
	N1	0.2	48	152	176	208	206	132A
	N2	0.2	52	145	247	287	281	169A
	Means	0.2C	52BC	141AB	208A	223A	237A	
WW	N0	31.9a	43b	27a	70a			43B
	N1	30.7a	73ab	41a	74a			55B
	N2	57.7a	123a	86a	122a			97A
	Means	40.1B	80A	51B	89A			

<sup>1</sup> For each crop, with reference to the significant sources of variation (see text), upper-case letters were used for comparisons of the mean effects, lower-case letters for the comparison of first order interaction effects. The interaction Date x Treatment, when significant, was estimated at each date (comparison between treatments within each column). For each source of variation, means followed by the same letters are not significantly different for P<0.05, according to the Tukey-Kramer test for mean comparisons.

Table 2. Inorganic N content in soil cropped with sugar beet (SB) and winter wheat (WW), in spring-summer 2006, at Rovigo (Italy). Treatments: N0, control; N1, 60 kg N ha<sup>-1</sup>; N2: 120 kg N ha<sup>-1</sup>.

Crop	Soil depth (m)	Treatment	Inorganic N content (mg N kg <sup>-1</sup> dry soil) at the dates						Means
			10/04	16/05	01/06	03/07	18/07	14/08	
SB	0-0.25	N0	11.5	7.3	4.8	8.9	8.8	11.9	8.9
		N1	26.9	18.7	12.0	15.1	13.7	23.1	18.3
		N2	29.7	26.7	29.4	22.3	17.2	25.9	25.2
		Means	22.7	17.6	15.4	15.4	13.2	20.3	17.4
	0.25-0.5	N0	10.0	8.1	4.9	5.2	7.2	8.1	7.3
		N1	18.3	13.9	7.1	6.5	8.9	10.5	10.9
		N2	17.8	19.9	17.2	8.3	8.5	13.3	14.2
		Means	15.4	14.0	9.7	6.7	8.2	10.6	10.8
	Means of date for SB		19.0	15.8	12.6	11.1	10.7	15.5	<b>14.1</b>
WW	0-0.25	N0	3.8	2.8	3.1	5.7	7.2	6.7	4.9
		N1	16.0	3.5	3.7	7.7	8.2	11.9	8.5
		N2	24.8	4.2	3.9	7.0	7.0	11.6	9.7
		Means	14.9	3.5	3.6	6.8	7.5	10.0	7.7
	0.25-0.5	N0	5.0	2.5	3.4	5.0	5.9	6.5	4.7
		N1	13.3	3.4	3.5	5.9	7.6	9.6	7.2
		N2	26.7	4.3	3.8	6.4	7.1	9.6	9.7
		Means	15.0	3.4	3.6	5.8	6.9	8.6	7.2
	Means of date for WW		14.9	3.4	3.6	6.3	7.2	9.3	<b>7.5</b>

## Conclusions

Our results suggest the need to estimate the amount of N which may become available to crops during the crop growth season, by taking into account not only the soil potential mineralization but also the crop type.

## Acknowledgements

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# Phytostabilization of Pb/Zn Mine Tailings and a Polymetallic Soil with *Thlaspi caerulescens*

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Hyperaccumulators are a small group of plants that can tolerate and accumulate in their tissues high amounts of metals; for these reason they were proposed for phytostabilization of (i) heavily polluted soils and (ii) mine tailings. Such plants are specialized for no more than two metals; on the other hand, in soil polluted from anthropogenic sources and in industrial slags they are always present several metals that exert a strong pressure against the establishment of native plants. For this reason the effectiveness of metal tolerant or hyperaccumulators must be tested in polymetallic soils.

The fertility of heavily polluted soils and mine tailings is always very low, therefore properly designed agronomic practices are expected to support plant growth and biomass yield of hyperaccumulators. Establishment of a vegetative cover on polluted sites can retain contaminants in place, by reducing soil erosion and percolation of metals through the soil profile. When revegetation of contaminated soil is combined with addition of soil amendments such organic matter, the mobility of contaminants in the soil can be further reduced.

The objective of this study was to determine the effects of compost amendment and/or mineral fertilization on plants of *Thlaspi caerulescens* grown in presence of several heavy metals.

## Methodology

Two substrates polluted by several heavy metals were considered for a pot experiment; the substrates were respectively: (i) Pb/Zn mine tailings collected at the former mining site of Cave del Predil, Italy) and (ii) a soil contaminated by pyrite cinders collected in an industrial area (Torviscosa, Italy).

The concentrations of heavy metals in the soil (pH=7.88) were the followings: As 188 mg kg<sup>-1</sup>, Cd 3.77 mg kg<sup>-1</sup>, Cu 1527 mg kg<sup>-1</sup> and Zn 980 mg kg<sup>-1</sup>. The mine tailings are characterized by an alkaline reaction (pH=8), and the concentrations of the metals are the followings: Cd 26.9 mg kg<sup>-1</sup>, Pb 6020 mg kg<sup>-1</sup>, Tl 287 mg kg<sup>-1</sup> and Zn 13,894 mg kg<sup>-1</sup>. Both the substrates, very poor in nutrients, were amended with compost at two different ratios (4% and 12%, w/w); also were considered two levels of N fertilization (70 and 140 kg N ha<sup>-1</sup>).

The experiment was organized following a factorial design with 2 substrates, 9 treatments (2 controls and 7 combination of organic amendment and mineral fertilization), 5 replicates and 3 plants per pot (Tab. 1). Seeds of *Thlaspi caerulescens* (Ganges population, Montpellier, F) were germinated in a controlled environment (16 h/8 h day/night cycle, PAR 500 µmol m<sup>-2</sup> s<sup>-1</sup>, 20-25 °C); 70 d old seedlings were transplanted in 1 l pots containing the Pb/Zn tailings and the polluted soil and growth for 90 d in a greenhouse. Currently the experiment is in progress (Fig. 1).

At the end of the growth cycle, plants will be carefully removed from each pot and the roots will be washed. Plants will divided into roots and shoots and dried for 24 h at 105 °C in a forces air oven. Samples of plant fractions will then acid-digested in a microwave oven according to EPA method 3052 (USEPA, 1995). After the mineralization the samples were filtered (0.45 µm PTFE) and diluted. The content of As, Cd, Co, Cu, Pb and Zn in plant and soil samples was determined with an ICP-OES instrument (Varian Inc., Vista MPX). The measurement of As was done separately with a continuous-flow vapour generation (VGA-77, Varian Inc.).

Table 1 – Experimental design for the greenhouse pot experiment.

Treatment	
T	100 % Mine tailings
TC <sub>1</sub>	Mine tailings + 4% Compost (w/w)
TC <sub>2</sub>	Mine tailings + 12% Compost (w/w)
TF <sub>1</sub>	Mine tailings + NPK Fertilization 70 kg N ha <sup>-1</sup>
TF <sub>2</sub>	Mine tailings + NPK Fertilization 140 kg N ha <sup>-1</sup>
TC <sub>1</sub> F <sub>1</sub>	Mine tailings + 4% Compost (w/w) + 70 kg N ha <sup>-1</sup>
TC <sub>1</sub> F <sub>2</sub>	Mine tailings + 4% Compost (w/w) + 140 kg N ha <sup>-1</sup>
TC <sub>2</sub> F <sub>1</sub>	Mine tailings + 12% Compost (w/w) + 70 kg N ha <sup>-1</sup>
TC <sub>2</sub> F <sub>2</sub>	Mine tailings + 12% Compost (w/w) + 140 kg N ha <sup>-1</sup>
P	100% Pyrite
PC <sub>1</sub>	Pyrite + 4% Compost (w/w)
PC <sub>2</sub>	Pyrite + 12% Compost (w/w)
PF <sub>1</sub>	Pyrite + NPK Fertilization 70 kg N ha <sup>-1</sup>
PF <sub>2</sub>	Pyrite + NPK Fertilization 140 kg N ha <sup>-1</sup>
PC <sub>1</sub> F <sub>1</sub>	Pyrite + 4% Compost (w/w) + 70 kg N ha <sup>-1</sup>
PC <sub>1</sub> F <sub>2</sub>	Pyrite + 4% Compost (w/w) + 140 kg N ha <sup>-1</sup>
PC <sub>2</sub> F <sub>1</sub>	Pyrite + 12% Compost (w/w) + 70 kg N ha <sup>-1</sup>
PC <sub>2</sub> F <sub>2</sub>	Pyrite + 12% Compost (w/w) + 140 kg N ha <sup>-1</sup>

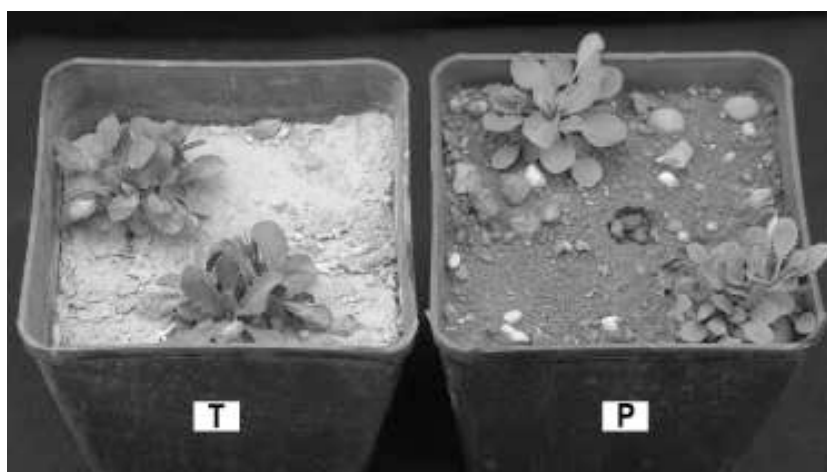


Figure 2 - Plants of *Thlaspi caerulescens* growing on (T) Pb/Zn mine tailings and (P) a soil contaminated by pyrite cinders, respectively.

## Results

The effects of the treatments will be evaluated by observing (i) the plant biomass yield, (ii) the accumulation of the metals in the aboveground and belowground tissues of *Thlaspi caerulescens*. The metal hyperaccumulation – formerly defined simply considering the metal concentration in the plant biomass – will be evaluated taking into account the bioconcentration factor and the translocation factor ((McGrath and Zhao, 2003; Yanqun et al., 2007).

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# Nitrogen Balance in a Hilly Agricultural Watershed in Northern Italy

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The soil surface nitrogen balance indicator measures the difference between the nitrogen available to an agricultural system (inputs, mainly from livestock manure and chemical fertilizers) and the uptake of nitrogen by agriculture (outputs, largely crops and forage). Even if it is only an estimation of the potential environmental damage, it can be useful to understand the possible effect of a certain agricultural and environmental management and policy. Agricultural surely influences the natural N cycling attending both to the input and output components, that's why the eradication of N contamination of the water systems require a comprehensive understanding of how N processed in agricultural systems. Many factors are involved in this phenomenon, contributing to the input and output components. High nitrate accumulation and the flow of water into the soil profile are pre-conditions for nitrate leaching in plain area, while in hillside the soil management is relevant. A soil with vegetation coverage reduces the raindrop impact, favouring the water infiltration into the soil and limiting the superficial runoff, most important nitrogen conveyance in sloping area.

Objective of this research was to determine the nitrogen balance in a hilly semi-agricultural watershed in seven years, to emphasize the potential environmental pollution risk. Then by the analyses of the input and output components we wanted to stress how the management and the land use can influence them.

## Methodology

The Centonara basin is located in the Bologna province (44°28' N, 11°28'E), in Northern Italy, and it can be considered representative of the hillysites of the Emilia Romagna region.

The elevation is 200 m a.s.l, with main slopes of 25-35%. This area is characterized by a continental climate, where the highest and lowest temperatures occur in July–August and December–January respectively and the annual average precipitation is 750 mm (over a 15-year period), concentrated in two rainy seasons: spring and autumn. Despite this, during the summer, some intensive thunderstorms can occur (Roggero et al., 2002).

The trial carried out in a period of 7 years. The basin was occupied by badlands and spontaneous vegetation for about 60% of the surface. The rest of the watershed was used for agricultural activities, prevalently herbaceous crops such as alfalfa and cereals, especially wheat, barley and sorghum (table 1). During the trial two managements were tested: a conventional tillage and an organic tillage.

In the first two testing years, 40 ha were used for cattle. The watershed is hydrologically isolated and the Centonara stream collects all its drainage water. Because no industrial and urban wastes are present in the basin, the Centonara stream chemical composition derives mainly from the agricultural activity.

Over the trial duration (1997-2005), we monitored the basin, recording all the information regarding land use, tillage and management. In particular we had all the information about sown, tillage operations, fertilizations, herbicide applications and yields.

The drainage water was continuously monitored thanks to sensors, which provides the discharges, level and velocity of the Centonara stream. An automatic sampler collected daily a water sample from the stream.

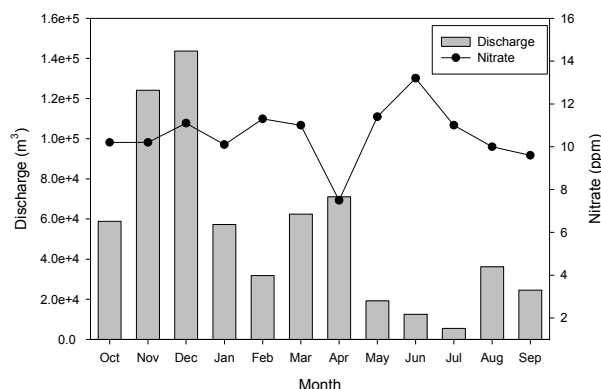
Between the various ways of calculating a nutrient budget, we followed the simplest deterministic approach of Howarth (1996), which provides a static accounting of the input and output component:

Nitrogen balance = input components (fertilizer + manure + atmospheric deposition + symbiotic and non-symbiotic N fixation + N from crop residue combustion + N from excrement of animals) – output components (crop harvests + losses in drainage water + denitrification losses + NH<sub>3</sub> volatilization).

CROPS	1998-99 ha	1999-00 ha	2000-01 ha	2001-02 ha	2002-03 ha	2003-04 ha	2004-05 ha
Wheat	15.3	14.5	14.8	12.1		9.1	9.3
Organic wheat	4.2	2.9	0.6	4.1	2.2	1.7	
Corn	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alfalfa	12.3	12.3	16.2	16.9	11.3	19.7	19.7
Sorghum	4.2						
Organic sorghum			1.4	3.3			
Sunflower		9.3			12.1		2.7
Barley						3.0	
Organic barley			1.4	3.0	0.8		
Italian millet				5.4	5.4	5.4	5.5
Switch grass				1.4	1.7		
TOTAL	36.4	39.5	34.9	46.7	34.0	39.4	39.4
Percentage*	18	20	18	24	17	20	20

## Results

In our watershed the biological fixation constituted the most relevant input, with a mean of 3.1 Tg per year. It is especially the alfalfa crop with its 180 kg ha<sup>-1</sup> of nitrogen annually fixed (Vance, 1998) that provides the highest N contribution. The percentage on the total input grew from 24% in 1998-99, when the leguminous occupied 12 ha, to about 40% in the last years, when the legume area doubled.



The application of synthetic N fertilizers is the second largest N source in Centonara watershed, even if its contribution varies year by year.

The plant removal is the most efficient method of removing N from agricultural fields. It was calculated for the part of the basin occupied by agricultural activities and its value depends on the land use, the kind of cultivated crop and its yield. From literature, the values of nitrogen removed vary from 29 kg per Tg of yield of the wheat to 3 kg of N per Tg of yield for the sugar beet. We constructed the N balance by

calculating the differences between total inputs and total outputs. N budget for Centonara watershed from 1998-99 to 2004-05 (table), put in evidence that the budget results are all positive, with input exceeding output ranging from 11.3 kg ha<sup>-1</sup> in 1999-00 and 1.8 kg ha<sup>-1</sup> in 2002-03; the only exception is the 2001-02 with a balance almost close (-0.6 kg ha<sup>-1</sup>).

The variability of the nitrogen budget year by year is probably due to the different land use and meteorological-environmental conditions. In the first two years, 1998-99 and 1999-00, the discrepancy is the highest; this result reflects the land use diversity in this period: in these two years, part of the basin was dedicated to pastures that contributed to 1.1 Tg of nitrogen per year, constituting about the 10 % of the total input. The following years most of these fields, about 18 ha, were cultivated with alfalfa and remained almost constant till 2004/05.

YEAR	1998-99	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05
<b>INPUT</b>							
Fertilizer	11.6	15.4	6.6	5.2	8.8	8.3	8.2
Manure	2.4	3.1	2.7	3.4	1.1	1.5	1.7
Cattle	6.0	6.0					
Symbiotic-fixation	11.5	11.5	18.0	16.9	17.1	18.5	18.5
Non-symbiotic fixation	9.4	9.4	9.0	9.0	9.0	9.0	9.0
Atmospheric deposition	6.4	7.2	7.0	8.5	5.4	8.9	8.4
<b>TOTAL</b>	<b>47.3</b>	<b>52.5</b>	<b>43.2</b>	<b>43.1</b>	<b>41.4</b>	<b>46.2</b>	<b>45.8</b>
<b>OUTPUT</b>							
Plant removal	20.0	20.0	22.7	27.1	19.8	24.3	22.8
Discharge	3.0	6.4	5.3	7.1	9.8	8.2	9.7
Denitrification	9.2	10.3	8.4	8.4	8.1	9.0	8.9
NH <sub>3</sub> volatilization	2.6	3.4	1.5	1.2	1.9	1.8	1.8
NH <sub>3</sub> volatilization*	1.2	1.2					
<b>TOTAL</b>	<b>36.1</b>	<b>41.2</b>	<b>37.9</b>	<b>43.7</b>	<b>39.6</b>	<b>43.4</b>	<b>43.2</b>
<b>BALANCE</b>	<b>11.2</b>	<b>11.3</b>	<b>5.3</b>	<b>-0.6</b>	<b>1.8</b>	<b>2.8</b>	<b>2.6</b>

The contribution of the biological fixation in the basin budget is very high: starting from 2.2 Tg of N fixed in the first two years, when 12 ha were cultivated with alfalfa, it arrives to 3.5 Tg of N in the last year with 19 ha of alfalfa. The presence of leguminous influences the final nitrogen budget, considerably.

## Conclusions

Our watershed results sustainable all over the trial, but some differences can be appreciated depending on the surface dedicated to leguminous and the application of organic techniques, which reduce the input. Surely because the plant removal constitute the most important output, the species cultivated can influence the final result. The research shows also the importance to choose crop and fertilizer management that show the best N-use efficiency, computed by and index which take in account the nitrogen fertilization dose and the yield.

## Acknowledgments

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# Microbial Characterization During Aerobic Composting of Tobacco Industry Solid Waste

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For the experiments, the homogenized solid tobacco waste (70%) and the compost generated from the communal waste (30%) were mixed and the piles formed. The nicotine content in tobacco waste was from 1.66 to 0.58%, and after 15 days nicotine content was 0.020 to 0.010%. In this phase of composting, the high count of mezophilic ( $9,6 \times 10^9 \text{CFUg}^{-1}\text{DW}$ ) and thermophilic bacteria ( $2,4 \times 10^9 \text{CFUg}^{-1}\text{DW}$ ), mezophilic ( $2,63 \times 10^5 \text{CFUg}^{-1}\text{DW}$ ) and thermophilic fungi ( $5,45 \times 10^5 \text{CFUg}^{-1}\text{DW}$ ) and mezophilic actinomycetes ( $3,5 \times 10^4 \text{CFUg}^{-1}\text{DW}$ ) founded. However, after 25 days of composting the count of thermophilic bacteria is still high ( $7,2 \times 10^8 \text{CFUg}^{-1}\text{DW}$ ), while the count of thermophilic and mezophilic fungi are reduced, but the nicotine content was only 0.0016%. The reaction of this compost is alkaline with pH 9.09 and high content of salt and relationship C/N was 14.5:1.

**Key words:** *composting, nicotine degradation, mezophilic bacteria, termophilic bacteria, fungi, actinomycetes*

## Introduction

Solid waste from tobacco industry qualifies as agroindustrial waste and its disposal represents a big national problem in Serbia. The ecological and economically acceptable ways of solving this problem is to make compost. Composting represents a controlled process of transforming organic matter, in which microorganisms perform a significant part. In this way, waste represents a valuable resource, and environmental pollution considerably reduced. Large quantities of waste containing high concentrations of nicotine are generating during the tobacco manufacturing process as well as in other activities that use tobacco (Novotny and Zhao, 1999). Due the nicotine is harmful to human health and environment (Holmstedt, 1988); the United States Environmental Protection Agency (EPA) designated it as a Toxics Release Inventory chemical since 1994. This waste also is classified as “toxic and hazardous” by European Union Regulations when its nicotine content exceeds 0.05% (w/w) (Novotny and Zhao 1999). Nicotine is not readily degradable and is very toxic to most population of microorganisms. However, several microorganisms that are able to degrade nicotine have been isolated from the environment (Yuan et al., 2007). “Denicotinization” of materials can be done with chemical and physical treatments, but alternatives are possible involving biological methods, which exploit the microbial ability to grow on and destroy nicotine.

The aim of these experiments is the investigation of change in microbial population during nicotine composting in aerobic conditions.

## Material and methods

Viable plate count had determined by the decimal dilution method and the results expressed as colony-forming units (CFU) of mesophilic or thermophilic bacteria, actinomycetes and fungi per gram of the composting mass. The incubation temperatures were 30°C for mesophiles and 55°C for thermophiles. Plates of thermophiles were incubated for 3 days and those of mesophiles for 7 days

For composting material, the wet tobacco, leaf tobacco, cut tobacco and tobacco dust used Such homogenized and prepared tobacco wastes mixed than with compost generated from the communal waste (proportion 70:30percentage) and the piles had formed. The nicotine content had measured by standard methods used in tobacco industry.

## Results

Composting is the aerobic process through which biodegradable organic materials undergo a partial mineralization and profound transformations due to the metabolism of a complex microbial population. The result of such a process is a biologically stable and humified product, the compost, which can be applied in agriculture. For composting the wet tobacco, leaf tobacco, cut tobacco and tobacco dust used in which nicotine content varied from 0.58 to 1.66 percent. From such homogenized and prepared tobacco wastes and municipal waste and compost generated from the communal waste (proportion 70:30percentage) the piles have been formed. In the beginning of experiments the highest microbial populations belongs mezophilic and thermophilic bacteria (Table 1.).

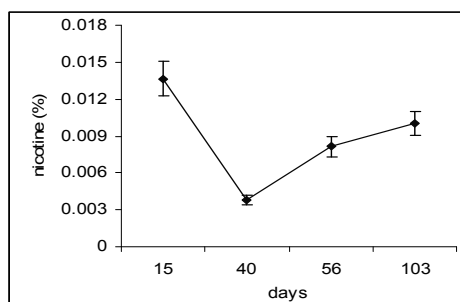
Table 1. Number of microbial population during composting.

Number of microorganisms (CFUg <sup>-1</sup> DW)		
	Start	25 days
Mezophilic bacteria	9,6 x 10 <sup>9</sup>	4,8x10 <sup>7</sup>
Thermophilic bacteria	2,4 x 10 <sup>9</sup>	7,2x10 <sup>8</sup>
Mezophilic fungi	2,63 x 10 <sup>5</sup>	8,1x10 <sup>3</sup>
Thermophilic fungi	5,45 x 10 <sup>5</sup>	2,3x10 <sup>3</sup>
Mezophilic actinomycetes.	3,5 x 10 <sup>4</sup>	5,7x10 <sup>3</sup>
Thermophilic actinomycetes	7,8x10 <sup>4</sup>	6,9x10 <sup>3</sup>
Azotobacter	2,7x10 <sup>5</sup>	4,3x10 <sup>3</sup>

Thermophilic bacteria are of great importance in organic matter decomposition during composting process. After 25 days of composting the count of thermophilic bacteria is still high (7,2x10<sup>8</sup>CFUg<sup>-1</sup>DW.), while the count of thermophilic and mezophilic fungi had reduced.

The nicotine content in pile during composting process (Fig. 1) decreased and at the end of experiment was 0.01. This value is much lower compare with mixture of different tabacco parts prepared for composting (dust, leaves etc. ranged from 0.58 to 1.66 %).

Figure 1. The decrease in nicotine content in piles during composting process.



As a conclusion, the microbial population used in compost process is successfully reduced nicotine content to environmentally accepted levels.

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# Integrated Soil and Water Management for Vineyards in Southern Italy: a Case Study

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The accelerated erosion of soil by water from hill-slopes under viticulture is a major problem in the Mediterranean basin (Garcia-Torres and Martinez-Vilela, 2002; Martinez-Casanovas and Sanchez-Bosch, 2000 and Tropeano, 1983). It is largely a result of the land management techniques employed and their influence on soil protection, structure and stability.

Vineyard is one type of land use that incurs in large amounts of soil loss in the Mediterranean hillside environment; this is particularly evident in vineyard where the soil is managed with an intensive tillage. With the time this has led to the degradation of soil structure, soil compaction, decreased levels of soil organic matter which in turn has caused a wide range of environmental issues including: soil degradation, water and wind erosion, increased carbon emissions from the soil and an overall reduction in soil organisms. Vegetative cover has long been accepted as one of the most effective means of conserving soil and improving soil condition (Lal, 1994). However, for successful integration with an established cropping system, the type of cover and management require careful consideration.

The objective of the present study is to investigate on the suitability of some agronomic practices for the conservation of the soil quality and the minimization of soil erosion process within viticulture of the centre and south of Italy.

## Methodology

The trial is being carried out at the experimental field of the Agronomy and Crop Sciences Research and Education Center, Department of Food Sciences, University of Teramo over a three year period (2007- 2009). The site is located 42°45'N, 13° 50' E, 140 m above sea level on a typical hill side of the Adriatic belt with an average slope of about 21% and with a clay-loam soil with a depth ranging from 0,50 to 1 meter. The experimental vineyards consists of 10 years old Montepulciano d'Abruzzo vines, bilateral Guyot trained, spacing 2,7 x 1,5 m. The vine rows are north-south oriented and parallel to the maximum slope gradient. The vineyard has been regularly cultivated within the inter row spaces for a number of years before the experimentation.

On a randomised block design with two replicates, conventional tillage (CT) and soil conservation management with cover crops (CC) are being compared. The experimental plots consist of three consecutive inter-rows and have a total area of 810 m<sup>2</sup> (100 m x 2,7 m x 3). Conventional tillage consists in two-three cultivations each year with a hoeing in order to keep permanently weed-free the inter-rows. The cover crop, a mixture of 60% barley (*Hordeum vulgare*) and 40% field bean (*Vicia faba minor*), is mechanically mowed two-three times during spring-early summer. In all the plots the floor strip under vines is treated with a wide spectrum herbicide.

Runoff, soil losses, quality of water (size and distribution of eroded particles, organic matter and nutrient content) are monitored at the plot scale with a suitable trapped system installed at the end of the central inter-row in each of the four plots. The material captured by the funnel is then channelled through a length of 200 mm diameter plastic pipe to a series of plastic tanks, which has been adapted to serve as a collection system.

Sampling is made once a tank is registered full, or runoff has occurred and further rainfall is not expected for several weeks. The sampling regime is restricted to volumetric measurements of runoff and sediment, and textural composition of the sediment. Chemical analysis of sediment and runoff samples is also undertaken. Air temperature, soil temperature, relative humidity, rainfall, sunshine, wind speed and wind direction are all monitored by an automatic weather station.

Data were subjected to ANOVA; an F test, in its simplest form, was used to assess the significance of the differences among thesis.

## Results

The preliminary results of monitoring programme demonstrate that conservative soil managements, soil managed with cover cropping, generates substantially less sediment than the conventional treatment (Tab.1).

Table 1. Rainfall, runoff and sediment for some events recorded in March, April and May 2008 in vineyard with soil managed with cover cropping and conventional tillage.

Event (period)	Cover crop			Conventional tillage	
	Rainfall (mm)	Sediment (mm)	Runoff (mm)	Sediment (mm)	Runoff (mm)
March	155.3	0.2	3.7	1.9	10.7
April	65.4	0.1	0.5	0.9	1.8
May	32.8	0.1	0.4	0.8	1.7
F-test					
sediment				**	
runoff				**	

With regards to runoff and soil loss, conservation management thesis shows significant lower values than conventional one when both extreme and light events happen. Differences emerge also in term of chemical characteristics of water: high amount of nitrogen, phosphorus and organic matter are found in samples coming from conventional tillage thesis (data not reported).

## Conclusions

The main purpose of this research is to contribute to the development of a soil conservation strategy, suitable for the Mediterranean viticulture, and to demonstrate that strategy's effectiveness at conserving soil. Preliminary results confirm that permanent cover has a considerable potential to lower rates of soil erosion and, subsequently, to increase the soil-water infiltration.

Care must be taken in selecting suitable grass species and in developing a rational management of the cover in order to minimise risk of competition for water and nutrients and to fit site-specific environmental condition. Thus, to get a better indication of treatment performance and sustainability over time, the monitoring programme should be extended over several years and locations.

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# The Waste Production of Fresh Tomato Packinghouses

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For one of the first links of the food-supply chain, the fresh fruit and vegetables packinghouses, abundant in Spain, Italy and Holland, the information on the amounts of waste generated and on their seasonal fluctuation are scarce. Nevertheless, the industrial approach of these farms and their narrower geographic dispersion, when compared to growers, represent an advantage for a waste valorization system planning. The knowledge achieved on this matter could represent an useful tool for waste management decision-makers, which, nowadays, have to take into account the principle of minimizing landfilling by maximizing resource recovery and reuse (Hogg et al., 2003). With this in mind, we evaluated the waste stream of tomato packinghouses operating in SE Sicily, one of the greatest protected cultivation areas in Italy, aiming at characterizing this unstudied aspect of an important agroindustrial sector and at obtaining preliminary data for a bio-waste management system planning.

## Methodology

The study was conducted during 2007 in south-eastern Sicily (Italy), since almost one-third of Italy's national production of greenhouse tomatoes ( $510,000 \text{ Mg year}^{-1}$ ) is harvested in this area (ISTAT, 2001-2005), and almost entirely collected by packinghouses for selection and packaging activities. The packinghouses were selected in order to obtain a sample mass reaching at least the 20% of the total amount of collected tomatoes in south-eastern Sicily, and the bulked sample resulted constituted by 10 packinghouses. Throughout the year, we carried out weekly interviews, according to the applied 3-7 day collection period, and monitored the collected tomato and the wasted material. We cumulated the weekly values on a monthly basis to simplify the presentation of the results and to improve their practical use. We applied a statistical elaboration approach, involving the calculation of percentiles, as the description of a phenomena characterized by skewed data variability, such as the waste production, needs data elaboration methods mainly addressed to the representation of the distribution.

## Results and Discussion

The observed interval between waste collection has to be emphasized as a preliminary result especially useful in the context of waste management system. The putrescible nature of this waste material, as a matter of fact, avoids storing periods which exceed 6-7 days. Nevertheless, this widely applied collecting schedule represents a positive logistic aspect that assures enough flexibility for the waste management activities. Total fresh tomato processed in the packinghouses (fig. 1A) rapidly grew from September to March and then a plateau phase during the spring months was observed ( $436 \text{ Mg month}^{-1}$  for months from March to June combined); after that, total fresh tomato amount dramatically declined during the summer months ( $145$  and  $36 \text{ Mg month}^{-1}$  for July and August, respectively). According to the different dimensions of the studied packinghouses, the variations observed in each month in relation to farms appeared quite asymmetric. Large differences between the maximum value and the 90<sup>th</sup> percentile were observed from October to March. From July to October very low 10<sup>th</sup> percentile values have been calculated as at least two farms with no packaging activities were reported. Wasted material (fig. 1B) presented a quite stable trend from autumn to the first half of spring, ranging from  $17.0 \text{ Mg month}^{-1}$  (October) to  $17.5 \text{ Mg month}^{-1}$  (April). Then we observed a drastic increase in the late spring and finally a severe reduction during the summer months. A highly asymmetric distribution was recorded within each month in relation to farms, and the medians, in most of the cases, appeared closer to the bottom of the boxes, emphasizing the presence of at least the 50% of studied farms with very low waste production. In particular, during the spring months, the relative length of the lines extending from the boxes emphasizes the skewness of the data distribution. From July to October, the first

quartile reported values lower than  $4.0 \text{ Mg month}^{-1}$  and for the 10<sup>th</sup> percentile of studied bulk, no waste production was detected. The waste percentage (waste production / total fresh tomato packed ratio),

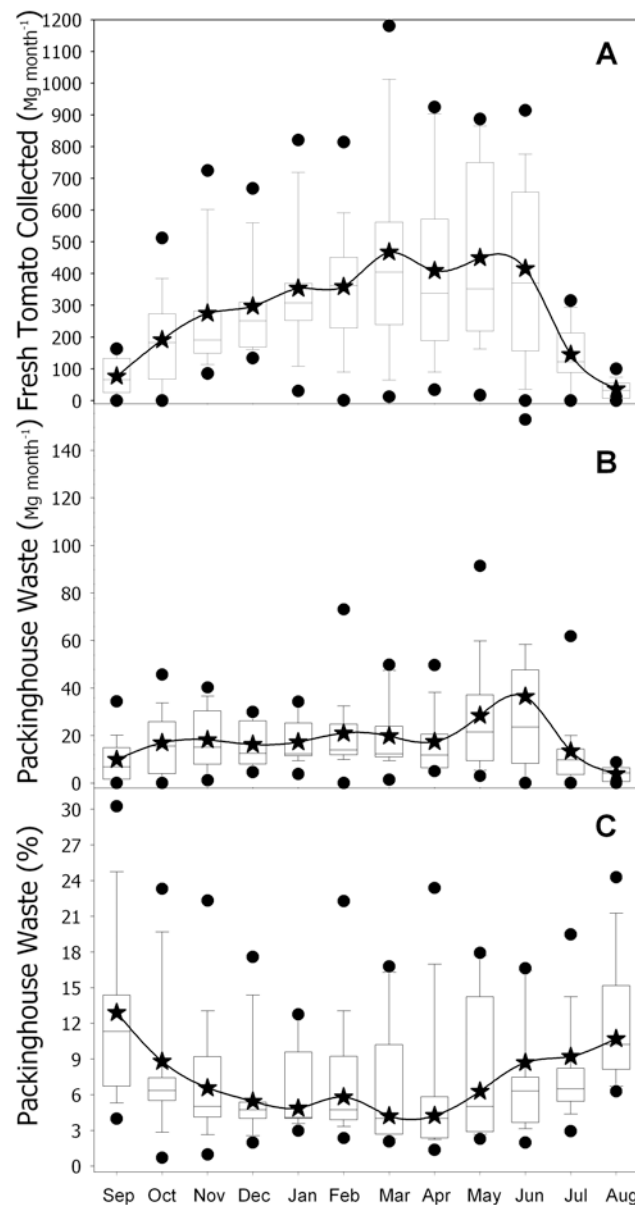


Figure 1 – Distribution of the amount of fresh tomato collected for packaging (A), of packinghouse waste (B) and of waste/collected tomato ratio (C). In the boxes, each representing the distribution of 10 farms data, the lowest and highest boundaries indicate the 25<sup>th</sup> and 75<sup>th</sup> percentile, respectively, and the black line within the box marks the median. Whiskers above and below the box indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles, respectively. Dark circles represent the extreme values. The stars represent the monthly mean values of 10 farms data combined.

Dumas Y. et al., 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Review. J. Sci. Food Agric. 83, 369-382

showed a growing trend on the summer months, from June (8.8%) to September (13.0%), then declining in autumn and winter down to the 4.2% revealed on March (fig. 1C). The high value observed on summer could be explained by the environmental conditions experienced by plant in greenhouses which usually, in the studied area, have limited climate control systems. In these conditions, high temperature and air humidity levels exert a negative influence on the quality attributes of tomato fruit (Dumas et al., 2003), and consequently affect the amount of discharged fruits. The distribution ascertained within months pointed out that the medians appeared constantly close to the lower whiskers (10<sup>th</sup> percentile), whilst the lengths of the lines exceeding the 75<sup>th</sup> percentile emphasized a wider range characterizing the distribution of the higher values. The variability of the waste amount, strongly affected by both “time” and “source”, results from the nature of the studied waste and from working schedule of the studied industry. This variability is due to a multiplicity of factors (biological, environmental, agronomical, economical etc.) that influence directly fruit and vegetables production and quality and just indirectly waste amount. Its residual nature, limits the possibility of acting on the above reported factors and should lead the waste management planner to a adopt flexible approach.

## Conclusions

The availability of treatable waste all year round and its quite stable amount in the great part of the year, confirms the technical and economical relevance of this specific waste management sector. Nevertheless, a flexible approach for the waste management planning should be applied.

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# Impact of Cropping System Management on Groundwater Pollution in a “Nitrate Vulnerable Zone” of Spain

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The use of crop simulation models coupled to geographic information systems (GIS) might be a powerful tool to compare management strategies to mitigate groundwater pollution in Nitrate Vulnerable Zones (NVZ). In this study, the crop simulation model STICS was used to evaluate impacts of water quality and organic residue application on the total nitrate leaching in a NVZ of *ca.* 850 ha located in La Rioja (North Spain). Being irrigated vineyard the main cropping system, STICS-vigne was previously calibrated and adapted from García de Cortázar (2006). The objective was to identify management strategies of the cropping systems that could minimize the impact on nitrate leaching.

## Methodology

The methodology consisted on the definition of simulation units defined by soil type and land use in which the crop model STICS was applied (Brisson et al., 2003). The use of a GIS made possible spatial analysis of simulation inputs and outputs. The soil input data was a regional soil map at 1: 20000, involving 3 soil groups and 8 families. Simulations included the combination of three levels of organic manure application, and two levels of nitrate concentration in irrigation water. Levels of organic manure application were null (R0 treatments), 10 Mg ha<sup>-1</sup> every two years (R1, observed management), and 50 Mg ha<sup>-1</sup> every year (RZ, total amount of residues produced in the NVZ). Levels of nitrate in irrigation water depend on the water source, when coming from the Ebro river the average concentration was 6 mg NO<sub>3</sub> l<sup>-1</sup> (E treatments), while water pump from the aquifer contained 145 mg NO<sub>3</sub> l<sup>-1</sup> (P treatments). Therefore six treatments resulted from the combination of both factors: R0E, R1E, RZE, R0P, R1P and RZP. Several indicators were selected among the simulated variables: crop yield, evapotranspiration, drainage, N leaching, soil nitrate content, and crop nitrogen uptake. After simulating all treatments for a period of 20 years, maps and tables were constructed with the indicator values to detect trends. Total amount of nitrate leaching per year across the NVZ was calculated from these simulations for every treatment. The values obtained were compared to the amount of nitrate delivered to the Ebro river from the aquifer each year, estimated as 91 Mg NO<sub>3</sub> following the hydrological studies of the NVZ (Zeta Amaltea, 2005).

## Results

The results showed that nitrate concentration of irrigation water was the most important factor in soil and water pollution in the NVZ. All E treatments showed initial values of nitrate leaching ranging from 0 to 50 kg NO<sub>3</sub> ha<sup>-1</sup> per year, while for P treatments the values varied between 0 and 250 kg NO<sub>3</sub> ha<sup>-1</sup>. Final values after 20 years of simulation were between 0 and 5 kg NO<sub>3</sub> ha<sup>-1</sup> for E treatments, and between 0 and 100 kg NO<sub>3</sub> ha<sup>-1</sup> for P treatments. Therefore, all treatments applied with low level of nitrate in irrigation water lead to a reduction on N leaching when comparing with the actual situation (Figure 1, maps a, b and c). Even at high application rate of organic N, 50% of the NVZ showed a decrease on N leaching (Figure 1, map c). All treatments irrigated with water from the aquifer increased nitrate leaching (Figure 1, maps d, e and f). The calculation of total nitrate leaching per year

for each treatment over the whole NVZ, confirmed these results: nitrate leaching from R0E, R1E and RZE treatments was 14, 16 and 36 Mg NO<sub>3</sub> per year, respectively; far from the 91 Mg NO<sub>3</sub> delivered to the river from the aquifer. However, R0P, R1P and RZP treatments exceeded this threshold, being nitrate leaching 137, 140 and 165 Mg NO<sub>3</sub>, respectively.

When water from the aquifer was used in combination with high organic manure applications, nitrate leaching and soil nitrate content rapidly increased in the whole area, leading to a large impact on water quality. On the contrary, when water from the river was used, the descendent trend on nitrate leaching shown by R0E and R1E treatments offers opportunity for soils and underground water recovery. In addition, soil type was an important factor controlling nitrate leaching. Even for the treatment with higher organic manure (RZE) a selective application of the residues depending on the soil type could be conducted without dramatically increasing nitrate leaching; therefore, organic manure management could be environmentally sustainable without exporting them out of the NVZ.

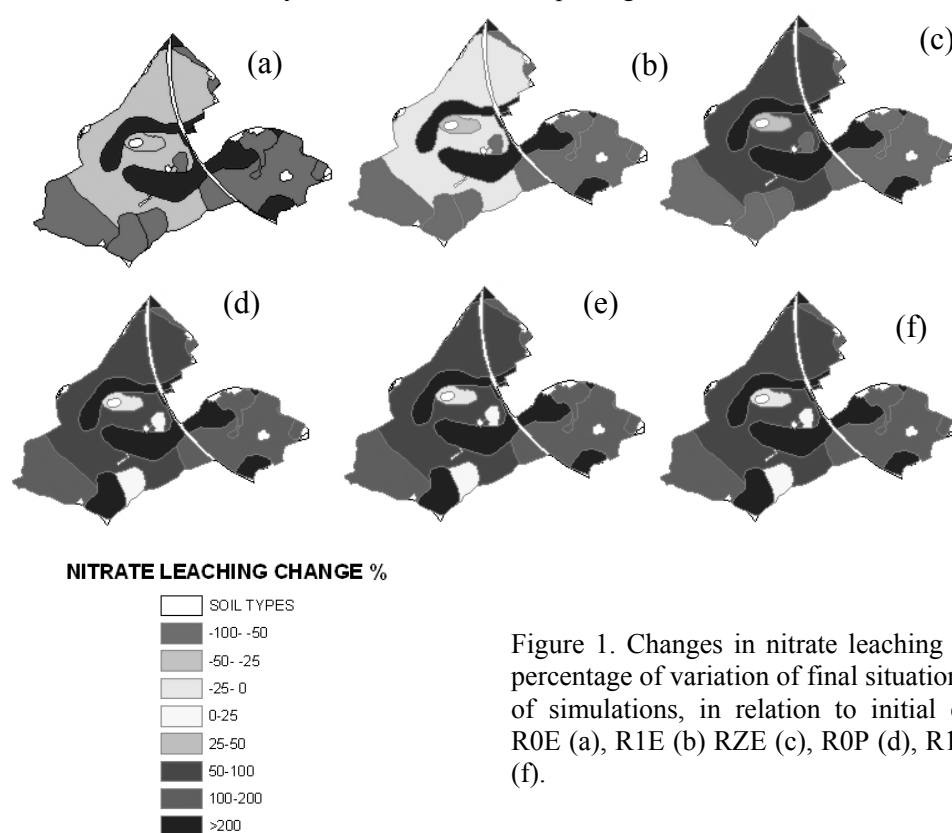


Figure 1. Changes in nitrate leaching expressed as a percentage of variation of final situation after 20 years of simulations, in relation to initial conditions, for R0E (a), R1E (b) RZE (c), R0P (d), R1P (e) and RZP (f).

## Conclusions

Management strategies proposed were to: i) use only water from the Ebro river for irrigation, ii) limit the application of organic manure to 10 Mg ha<sup>-1</sup> per year for non-selective application; iii) limit the application of organic manure based on soil type if the total amount of manures produced have to be applied in the NVZ. Therefore, this work allowed for identifying agricultural strategies of pollution mitigation in the NVZ.

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# Wheat response to a soil previously irrigated with saline water

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## Introduction

The worldwide concern for the insufficient freshwater resources induces to explore solutions for the sustainable use of non-conventional waters in agriculture; the present research was aimed at assessing wheat response, in terms biomass production, chemical composition and of sucrose accumulation as well as of SPS (sucrose phosphate synthase) and SS (sucrose synthase) activation, to a variously saline substrate. Sucrose accumulation in fact is acknowledged as a plant feedback response in terms of osmoregulation and crioprotection (Wang et al., 2000), regulating plant photosynthesis and development (Paul and Pellny, 2003), while SPS and SS are the enzymes regulating its biosynthesis. In detail, SPS influences the glucids metabolism (Worrell et al, 1991) as demonstrated by the variations in the sucrose/starch ratio in leaves; SS (EC 2.4.1.13) is a revealer of the processes of sucrose demolition (Heim et al., 1993).

## Methodology

In December, twenty wheat plants (*Triticum aestivum*, cv. Ofanto) was planted per lysimeters and irrigated with variously diluted seawater with two different leaching fractions (approximately 10 and 30%, labelled as LF1 and LF2, respectively, corresponding to about 550 and 660 mm of applied water and 110 and 290 mm of drained water; S1 and S2 indicate seawater concentrations of 1/6 and 1/3, respectively). Three replications were used for each treatment and two samplings were done, one at the heading phase (67 days after emergence, DAE) and another at the milky-waxy ripeness phase (127 DAE), with three plants sampled from every lysimeter. On biomass fresh and dry weight was determined: ions ( $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $PO_4^{3-}$ ,  $Cl^-$ ), SPS (EC 2.3.1.14) according to the method of Tognetti et al. (1990), permitting to assess the highest (-P) and lowest (+P) enzyme activity: the addition of phosphoric acid to the system inhibits the synthesis of sucrose and represents directly the minimal enzyme activity (+P) and indirectly the SS activity. Results were statistically analyzed for two-ways variance, with the Tukey test.

## Results

In table 1 the dry weights and ions content of the wheat aboveground biomass are reported, showing the limited influence of the initial soil salinity on the final plant development, thanks also to the sufficient (although less than average) precipitations occurred during the winter. In table 2 the organic acids, sugars and SPS activity are reported.

## Conclusions

Plant tolerance is evidenced by the relatively high content in  $Ca^{2+}$  and  $K^+$ , indicating a plant ability to buffer  $Na^+$  with an efficient  $Ca^{2+}/Na^+$  exchange system, in a homeostasis mechanism typical of salt tolerating plants (Yokoi et al., 2002). Also the trend of glucose and sorbitol percentages, rising with soil salinity, indicates the plant ability to contrast lower osmotic values in the soil (Mahn et al., 2002). The reaction of SPS to soil salinity values concurs to show an adaptive plant response to salts, obtained through the different utilization of the monosaccharide component by this enzyme, as remarked in other stress conditions (Yoshida et al., 1998). The sharply decreasing SPS activity in response to soil salinity suggests in fact a larger production of hydrolyzed molecules, in accordance with increasing sorbitol, glucose and organic acids: such increase in osmo-compatible components can be seen as a defence mechanism enacted by plants to determine the osmotic adjustment required to

contrast salt stress depending on the enhanced translocation of Na<sup>+</sup> from roots to leaves (Kerepesi and Galiba, 2000).

Table 1- dry weight (g plant<sup>-1</sup>) and ions (% dry matter)

Treatment	Control LF1	Control LF2	S1 LF1	S2 LF1	S1 LF2	S2 LF2
Dry weight	18	13	13	12	16	14
K <sup>+</sup>	2.46	0.21	3.17	1.23	1.19	1.17
Na <sup>+</sup>	0.15	0.15	0.46	0.50	0.26	0.50
Ca <sup>2+</sup>	0.49	0.50	0.50	0.52	0.53	0.68
Cl <sup>-</sup>	0.35	0.23	0.86	0.92	0.67	0.69
NO <sub>3</sub> <sup>-</sup>	2.41	3.47	2.06	2.18	2.91	2.27
PO <sub>4</sub> <sup>3-</sup>	0.57	0.52	0.46	0.46	0.41	0.42

Table 2- Content in citric and malic acid (mg 100 g<sup>-1</sup> dry matter), carbohydrates and sorbitol (mg 100 g<sup>-1</sup> dry matter) and range of activity of SS and SPS (μmol\*h<sup>-1</sup> g fresh matter)

Treatment	Control LF1	Control LF2	S1 LF1	S2 LF1	S1 LF2	S2 LF2
citric acid	29.07	30.38	42.26	41.45	42.65	55.65
malic acid	14.90	14.25	14.97	16.19	19.53	20.94
fructose	129.32	118.35	107.29	129.32	122.97	203.43
glucose	71.36	71.24	76.92	90.95	83.45	143.25
sucrose	140.40	229.74	121.24	121.06	164.74	157.83
sorbitol	0.44	0.58	0.68	0.76	0.73	1.19
SS (min)	2.10	0.87	2.73	2.79	1.65	3.55
SPS (max)	4.08	4.13	2.65	1.79	1.76	1.45

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# Impact of irrigation with unconventional waters on the soil-plant system: some experiences with sunflower

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## Introduction

Since salinity depending on seawater addition determines effects different from those of other salinity sources, while seawater is a widely available resource, it was selected for the research and the effects of two different concentrations were observed. The present research investigated the effects of the use of highly saline water for the irrigation of two cycles of sunflower (*Helianthus annuus*, cv Katharina) with an intermediate cycle of rainfed durum wheat (*Triticum durum*, cv Ofanto) with the aim of exploring the response of the plants and the soil to the various saline treatments and therefore refers to plant response to irrigation with saline water and salt balance in the soil as affected by precipitations during three consecutive growth cycles. The purpose of the research was twofold: 1) analyzing in some depth plant response in terms of biomass and yield production and of chemical components variation as an adaptation to salinity, and 2) following salinity fluctuations in the soil as affected by leaching fractions and rainfall.

## Methodology

A research was conducted on lysimeter-grown sunflower plants (*Helianthus annuus* L.), irrigated with seawater diluted to 1/6 and 1/3; two crop cycles were grown in two successive years while in the intermediate season rainfed winter wheat was grown. Treatments included two salinity levels plus a control with freshwater and two leaching fractions approximately 20 % (LF1) and 33 % (LF2). Sunflower plants (cv Katharina) on lysimeter were planted in the month of April and were harvested in July; a second cycle was planted in June and harvested in September. In November between the two sunflower cycles, durum wheat of the cultivar Ofanto was planted and was harvested in May.

On the plants were monitored biometric parameters, mineral and organic contents.

At the end of every cycle, the electrical conductivity of soil saturation extract was analyzed.

## Results

Soil salinity showed its fluctuations in dependence of the irrigation with saline water during the two cropping cycles and the influence of rainwater leaching between them (Tab. 1), causing the reduction in dry biomass in the saline treatments as compared to the control (Tab. 2).

**Table 1-** fluctuations in soil EC (dS m<sup>-1</sup>)

treatment	LF1fw	LF1 1/6	LF1 1/3	LF2 fw	LF2 1/6	LF2 1/3
Initial				0.08		
End 1 <sup>st</sup> cycle	1.14	8.98	10.48	1.10	12.19	19.85
Beginning 2 <sup>nd</sup> cycle	0.68	1.34	2.63	0.79	2.31	3.08
End 2 <sup>nd</sup> cycle	1.2	22.13	23.15	1.14	25.18	28.20

Inorganic components followed increase of Na and Cl in treatments 1/6 and 1/3; Ca and K too showed the usual opposite pattern, as a consequence also the K/Na ratio was considerably decreasing (Hamdy et al., 2004). Phosphorus final content in both cycles showed a close inverse relationship to soil salinity in all the treatments while, contrary to it, the nitrogen percentage was hardly affected (Tab. 3).

Carbohydrates and sorbitol (Tab. 3) showed a remarkable increase with salinity. Proline response was also consistent, thus confirming the results of earlier researches (Belligno et al., 2005).

**Table 2-** reduction in biomass production relative to the control (%)

crop	treatment					
	LF1fw	LF1 1/6	LF1 1/3	LF2 fw	LF2 1/6	LF2 1/3
sunflower 1	--	27	38	--	14	25
sunflower 2	--	51	61	--	14	44

**Table 3** Mineral element ( % dry matter) and sorbitol, carbohydrates (mg 100 g<sup>-1</sup> dm) and proline (μ mol g<sup>-1</sup> fm) content

parameters	treatment					
	LF1fw	LF1 1/6	LF1 1/3	LF2 fw	LF2 1/6	LF2 1/3
N	0.92	0.85	0.86	0.93	0.89	0.77
P	0.62	0.40	0.32	0.86	0.33	0.29
K	3.49	3.17	2.06	3.61	3.42	2.62
Ca	3.92	4.66	5.63	3.71	4.79	4.90
Na	0.33	0.54	0.69	0.16	0.34	0.75
Cl	0.26	0.81	1.27	0.29	0.66	1.14
K/Na	10.57	5.87	2.98	22.56	10.06	4.03
sorbitol	1.20	2.00	2.35	2.35	2.85	4.25
fructose	79.50	146.00	186.10	90.10	152.40	150.40
glucose	135.55	136.55	238.90	134.25	163.65	167.15
sucrose	19.05	37.15	50.10	31.25	39.25	56.80
proline	0.46	3.13	3.03	0.49	1.10	3.37

## Conclusions

The results largely confirmed earlier data on mechanisms enacted by plants to contrast salt stress, such as the changes in relative content of inorganic and organic components, all aimed at balancing the osmotic potential and the nutritional disorders. Also the possibility of assuming some parameters including the K/Na ratio, the proline and sorbitol relative content as indicators of plant stress level was confirmed. The results from treatments with different leaching fractions showed the inadequacy of the approach generally adopted in determining leaching requirement, since higher volumes of applied saline water resulted in a higher saline content in soil saturated extract due to the soil colloid adsorption. Moreover winter precipitations is a potential for sustainably using brackish waters for irrigation, provided that an efficient drainage system is available.

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# *In vitro* Grown Plants as a Tool for a Rapid Screening in Phytoremediation Studies

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## Introduction and objective

Most experiments used to establish phytoremediation techniques were done with normal soil-grown or hydroponically grown plants. Plant tissue culture can be an alternative tool for phytoremediation studies with some advantages such as the faster growing of the *in vitro* plants and the standard laboratory conditions.

Several hyperaccumulators have been identified in the *Brassicaceae* family. Among these, *Brassica carinata* Braum and *Brassica napus* L. var. *oleifera* D.C are species known for their metal tolerance and accumulators useful for phytoremediation. *In vitro* cultivated plants techniques could allow researchers to rapidly screen a large numbers of species and within them many interesting lines for their ability to absorb contaminants and store them in the biomass. The aim of the present research was to study the response to Zn and Pb in *in vitro* cultivated plants of two selected lines of *B. carinata* (L194252 and BRK35) and *B. napus* (Betty and PR46W31).

## Methodology

Seeds of *B. carinata* and *B. napus* were surface sterilized for 15 min in 0.1% mercuric chloride and then placed on germination medium comprising of macronutrients (Murashige and Skoog, 1962), micronutrients (Nitsch and Nitsch, 1972), Fe-EDTA (25 mg l<sup>-1</sup>), thiamine HCl (0.4 mg l<sup>-1</sup>), myoinositol (100 mg l<sup>-1</sup>), sucrose (20 g l<sup>-1</sup>) and agar (7 g l<sup>-1</sup>). After 12 days from germination, the sterile plantlets were transferred into glass vessels containing the same nutritive medium and 1 mg l<sup>-1</sup> 6-benzylaminopurine to induce proliferation. Separating shoots, obtained from 20 days shoots, were subcultured on fresh medium every three weeks for two times. For metal treatments, media were prepared without growth regulators and 30 mg l<sup>-1</sup> of Fe-citrate as iron source. Instead of Fe-EDTA, to minimize the formation of metal ion-EDTA complexes, which could interfere with metal availability. Well-developed shoots (1.5-3 cm tall, depending on the species) were exposed to treatments with various concentration of Zn or Pb (0.1, 0.5 or 1 mM of toxic metal per liter) which were compared to the control. For each experiment there were four glass vessels per treatment containing 6 shoots each.

The pH of the media was adjusted to 5.6–5.8 before autoclaving. Plantlets were maintained in a growth chamber at 22±1°C with a photoperiod of 16 h light under a light intensity of 50 mE s<sup>-1</sup> m<sup>-2</sup>.

The morphological changes were recorded on the basis of visual observations.

After 10 and 20 days from the beginning of the treatments in each medium fresh and dry weights of the plantlets were recorded. Relative growth rate in percent (%RGR) were measured using the formula:

$$\%RGR = \frac{\ln Dw_{1or2} - \ln Dw_0}{t_{1or2} - t_0}$$

where Dw<sub>1</sub> are the dry weight at 10 days (t<sub>1</sub>) and Dw<sub>2</sub> are the dry weight at 20 days (t<sub>2</sub>).

At the end of the trial, on all the plantlets from each treatment the concentration of the metal was determined.

## Results

The methodology evaluated for plant multiplication on medium containing 1 mg l<sup>-1</sup> BAP showed that all the *brassica* selected lines gave a good multiplication rate with means of new shoots derived from each explant of 4.6 for L194252, 3.8 for BRK35, 3.0 for Betty and 3.5 for PR46W31.

During the trials the plantlets grown *in vitro* did not show any visible signs of metal toxicity.

Relative growth rate of dry weight was not significantly modified by Pb or Zn treatments, compared to the control (Tabs. 1 and 2), even if relative growth rates (%) recorded after 20 days decrease in

compared to relative growth rates recorded after 10 days. Moreover, unless BRK35 at 10 days, relative growth rates obtained on Pb treatments is always higher than relative growth rates obtained on Zn. The results of the metal concentration are still working out.

Table 1 - Relative growth rates (in %) at 10 and 20 days with respect to dry weights of *B. carinata* plantlets grown in vitro on different concentrations of Pb and Zn

		culture days	Pb (mM)				Zn (mM)			
			1	0.5	0.1	0	1	0.5	0.1	0
<i>B. carinata</i>	L194252	10	5.6 a	6.3 a	5.5 a	6.8 a	5.7 a	4.5 a	4.2 a	4.4 a
		20	5.3 a	7.2 a	6.8 a	7.5 a	4.8 a	6.2 a	5.3 a	5.6 a
	BRK35	10	11.4 a	9.65 ab	9.8 ab	4.8 b	11.3 a	10 a	11.2 a	7.7 a
		20	8.7 a	9.1 a	8.5 a	9.2 a	7.4 a	7.1 a	7.5 a	6 a

Significant differences (P=0.05) are indicated by different letters in each row for each toxic metal

Table 2 - Relative growth rates (in %) at 10 and 20 days with respect to dry weights of *B. napus* plantlets grown in vitro on different concentrations of Pb and Zn

		culture days	Pb (mM)				Zn (mM)			
			1	0.5	0.1	0	1	0.5	0.1	0
<i>B. napus</i>	Betty	10	9.7 a	13.9 a	12.9 a	7.9 a	6.2 a	6.2 a	7 a	7.1 a
		20	7.5 ab	8.3 ab	9.3 a	7.1 b	6.3 a	5.9 a	5.7 a	5.2 a
	PR46W31	10	10.8 a	11.7 a	11.7 a	9.8 a	7.6 a	8.0 a	9.6 a	10.5 a
		20	6.6 a	7.9 a	7 a	7.2 a	7.5 ab	6.2 b	8.4 a	7 ab

Significant differences (P=0.05) are indicated by different letters in each row for each toxic metal

## Conclusions

The objective of this work was to study the response to Zn and Pb in *in vitro* cultivated plants of two selected lines of *B. carinata* (L194252 and BRK35) and *B. napus* (Betty and PR46W31).

The results obtained show that plant tissue culture can be an alternative tool for phytoremediation studies.

All the *brassica* selected lines tested grow *in vitro* fast and without visible signs of metal toxicity.

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# Optimisation of Water and Nitrogen in Irrigated Permanent Meadows in the Province of Reggio Emilia

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## Introduction

Permanent meadows located in the provinces of Parma and Reggio Emilia (Northern Italy) are extremely important for the production of Parmigiano – Reggiano cheese. Traditionally, this crop is irrigated by means of flood irrigation. In recent years, the reduced availability of irrigation water has meant that it has become necessary to optimise water use for crops with high irrigation requirements like permanent meadows.

The purpose of this research was to evaluate the possibility of using the sprinkling method to optimise irrigation. The influence of nitrogen fertilisation has also been studied over a three year period, to verify the environmental effects of the different irrigation methods.

## Methodology

A split-plot experimental design with four replicates was set up on a permanent meadow. The soil was a loamy-skeletal, mixed, mesic, Typic Ustochrepts according to Soil Taxonomy. The main factor was the irrigation system (sprinkling vs. flood irrigation) while the secondary factor was the fertilisation rate (0, 100 and 150 kg N ha<sup>-1</sup>) for a total of 24 plots. Each of the plots were 5 m wide and 25 m long.

Water from the Enza river was used for flood irrigation while water from the farm well was used for the sprinkling method. The quantity of water used for each irrigation was calculated by the software “IRRIPRATO V2T” developed by Canale Emiliano Romagnolo (CER, 2005). The seasonal volumes employed for the irrigation are shown in table 1.

Table 1: Seasonal volumes used for irrigation

Irrigation method	Seasonal irrigation volume (m <sup>3</sup> ha <sup>-1</sup> )		
	2005	2006	2007
Sprinkling method	2400	3362	4734
Flood method	21060	17885	18157

The crops were harvested five times over the first two years but only 4 harvests were possible in 2007 because of adverse weather conditions. At each harvest measurements were made of dry-matter yield and forage nutritional composition and soil samples were taken at two depths (0-25 cm and 25-50 cm) to measure nitrates content by ionic chromatography.

## Results

Forage dry matter yield (table 2) was influenced by the fertilisation rate in each year. In contrast there were no significant differences deriving from the irrigation method used except in the first year partially due to initial difficulties in the set up of the sprinkling method. In the last two years the sprinkling method produced a slightly higher dry matter yield but this was not statistically significant. N fertilisation significantly increased forage yield in all three years, with average increments of 18%, 17% and 45% respectively for 2005, 2006 and 2007. N<sub>2</sub> rate was slightly more productive than N<sub>1</sub>, but the extra 50 kg ha<sup>-1</sup> did not have a statistically significant effect.

The irrigation method did not seem to influence the nutritional composition in any of the three years as can be seen in table 3. On the other hand, the nitrogen fertilisation significantly modified the forage composition: crude protein content was higher and NDF lower when no nitrogen was applied: in fact on the fertilised plots nitrogen selectively promoted the development of graminaceous species to the detriment of legumes, lowering the protein content of the forage.

Table 2: Forage dry matter yield (Mg ha<sup>-1</sup>)

<b>Irrigation method</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>
Sprinkling method	11.69 b	12.49	9.29
Flood method	12.60 a	11.39	9.05
<b>Significance</b>	<b>**</b>	<b>n.s.</b>	<b>n.s.</b>
<b>Fertilisation rate</b>			
N0: 0 kg N.ha <sup>-1</sup>	10.84 b	10.71 b	7.07 b
N1: 100 kg N.ha <sup>-1</sup>	12.77 a	12.21 a	9.97 a
N2: 150 kg N.ha <sup>-1</sup>	12.83 a	12.90 a	10.48 a
<b>Significance</b>	<b>***</b>	<b>**</b>	<b>***</b>
<b>Interaction Irrigation x Fertilisation</b>			
Significance	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>
Field mean	<b>12.15</b>	<b>11.94</b>	<b>9.17</b>

Table 3: Forage crude protein (% DM) and NDF (%DM) content

<b>Irrigation method</b>	<b>2005</b>		<b>2006</b>		<b>2007</b>	
	Crude Protein	NDF	Crude Protein	NDF	Protein	NDF
Sprinkling method	13.19	51.48	11.96	55.30	10.15	57.84
Flood method	12.80	52.10	12.22	54.70	9.85	57.64
<b>Significance</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>
<b>Fertilisation rate</b>						
N0: 0 kg N.ha <sup>-1</sup>	13.40	49.42a	12.82a	52.47a	10.24	56.21a
N1: 100 kg N.ha <sup>-1</sup>	12.68	52.55b	11.52b	56.09b	9.56	58.40b
N2: 150 kg N.ha <sup>-1</sup>	12.90	53.40b	11.94b	54.44b	10.20	58.61b
<b>Significance</b>	<b>n.s</b>	<b>**</b>	<b>***</b>	<b>***</b>	<b>n.s</b>	<b>*</b>
<b>Interaction Irrigation x Fertilisation</b>						
<b>Significance</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>

Throughout the three years of the study the level of nitrates in soils always remained below 20 mg kg<sup>-1</sup> for the upper soil layer (0-25cm) and below 12 mg kg<sup>-1</sup> for the 25-50 cm layer. No significant differences were found either for the irrigation method or the fertilisation rate.

## Conclusions

There is no evidence from the above results that the use of the sprinkling irrigation method has negative effects on permanent meadows if irrigation is carried out at the proper time, supplying the right water quantity. The economic and technical aspects of the use of this irrigation method on permanent meadows requires further study as well as continuing observations on the botanical composition of the sward to confirm there are no observable effects over a longer period (Paris et al.,1992).

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# Nitrogen Balance and Land Use in Three Different Watersheds of the Po Valley (Italy)

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Sustainable agriculture requires assessments of nitrogen (N) fluxes and monitoring of potential nitrate losses. Watershed studies are particularly valuable to calculate nitrogen balances and quantify the relative importance of different sources of inputs and outputs (Leach et al., 2003).

In Emilia-Romagna region, Northern Italy, the problem is very complex because of various issues related to environmental, economic and social aspects. Adriatic sea is in fact one of the regions in Europe suffering most from eutrophication (deWit and Bendoricchio, 2001). Being agriculture a diffuse source of pollution, watershed studies are particularly valuable to calculate nitrogen balances, quantify the relative importance of different sources of N inputs and outputs (Leach et al., 2003) and evaluate the influence of soil use on the quality of surface water (Osborne and Wiley, 1988; Rossi Pisa et al., 1996; Gardi, 2001).

Aim of this project is to stress the different impact on the environment (particularly on water quality) of three small watersheds, hydrologically isolated, with typical agricultural and naturalistic scenarios, evaluating the nitrogen and phosphorus balance at watershed level, as well as ecological health of the water bodies.

## Methodology

Water quantity (rainfall, inflow and outflow) and quality (nitrate inflow and losses) were monitored in three different watersheds. Site 1, Ozzano dell'Emilia (Bologna, Italy), 300 ha, is a hilly site, 40% cultivated, in part organic, and drained by a creek; site 2, Argelato (Bologna, Italy), 750 ha, is in the intensively cultivated plain near Bologna; site 3, Valle Volta (Ferrara, Italy), 2000 ha, intensively cultivated, is in the Ferrara plain, below the sea level. Site 2 and 3 are artificially drained by ditches. Influx and efflux water volumes are directly measured in site 1 and 2. In Valle Volta they are obtained from the local water board (Secondo Circondario Polesine-San Giorgio; Ventura et al., 2008).

Meteorological data comes from agrometeorological stations located into the watersheds.

Surface water are sampled automatically in Ozzano and Argelato, and manually in Valle Volta, in both cases twice per month. Precipitations are sampled periodically, depending on events frequency. Starting from November 2007 groundwater in site 2 is sampled and analyzed. All water samples are analysed with an automatic analyser (AA3, Bran+Luebbe GmbH, Norderstedt, D).

Nitrogen balance was performed, for each basin, considering not only water quality but also fertilizers inputs and crop uptake by means of land use surveys and farmers interviews.

## Results

In Valle Volta and Argelato watersheds nitrate-N concentrations showed a large seasonal fluctuation with the lowest values in Summer ( $< 2 \text{ mg L}^{-1}$ ) and highest from late Autumn to late Spring (Fig 1a).

In the Centonara watershed, the water quality is not affected by the agricultural activity: nitrates concentrations have a low variability during the whole year, always under  $6 \text{ mg L}^{-1}$  (Fig 1b).

In both situations most of the data are below the U.E. limit for drinking water.

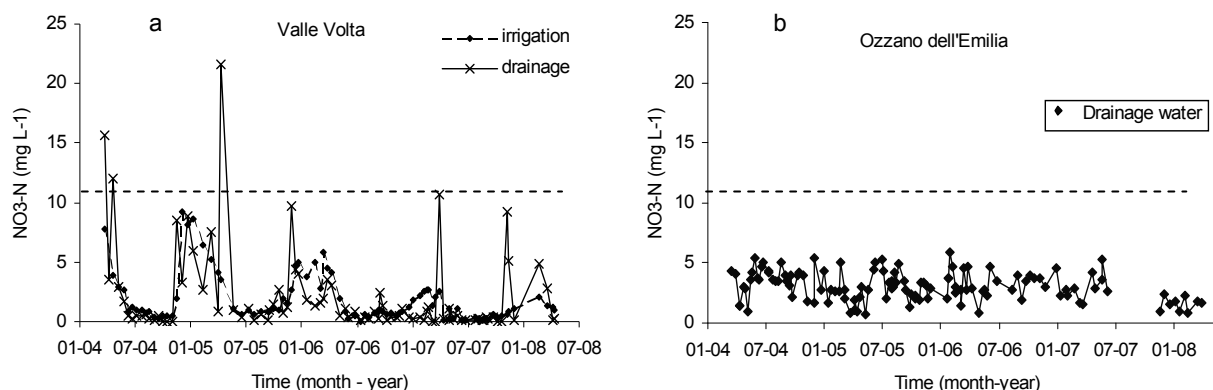


Fig 1: Nitrate concentration in: a) Valle Volta basin, input (irrigation) and Output (drainage) water; b) Ozzano dell'Emilia, drainage water. Missing data in the second part of 2007 are due to samples taken but not yet analysed. Broken line represents The UE limit for drinking water.

First result from groundwatertable in site 2 showed high variability, both in time and space, with values ranging from 2 to 30 mg L<sup>-1</sup>. More results are needed to draw any conclusion.

In the Valle Volta and Argelato watersheds, total N outputs and inputs are of similar magnitude, indicating that crop management, especially N fertilization techniques, has reached good levels of ecological sustainability. This could be the effect of high N removals with high value crops, or of the absence of relevant fertilizer applications in autumn and of crop rotations. In Ozzano the agroecosystem is well balanced and only very little chemicals are detected in the drained creek, probably thanks to organic techniques application.

## Conclusions

In this research work nitrate concentration data from three different agricultural watershed are presented. In any case data are quite good, showing low concentrations in surface water, but very variable concentrations in groundwater.

In the future the aim will be the extension of the study monitoring to biological indicators of the trophic status and ecotoxicological indexes, and relating ecological indicators of water bodies with the landscape characteristics. This will allow to obtain a more precise definition of the factors affecting agricultural impact on aquatic environments (Edwards et al., 2000).

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# Comparison Between Aquatic Plants in Small Artificial Wetlands: First Results on Nitrate Uptake

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Wastewater conventional treatments in the last few years have been paired by constructed wetland systems based on natural process. Aquatic macrophyta and rhizosphere micro-organisms create a synergic ecosystem able to remove pollutants from wastewater and restore a pretty good water quality. Constructed wetlands typically have been used downstream of a primary conventional system to treat polluted water from many different source like domestic and urban sewage, industrial wastewater and agricultural drainage water. Constructed wetlands represent natural habitats that positively affect the environment, are low cost systems with fairly easy maintenance and usually well accepted by public opinion

The aim of this research is to evaluate the different attitude to clean up wastewater of three hydrophyte species, considered particularly interesting for their ornamental and functional value. The species taken in to consideration are: *Phragmites australis*, *Iris pseudacorus* and *Lythrum salicaria*.

## Methodology

A pilot scale tank system has been established in S.Lazzaro di Savena-Bologna (Azienda Agricola Ninfea), in Northern Italy (44° 27' N, 11° 23' E). The system consists in twelve round plastic tanks (height 0.8 m, diameter 0.9 m), four tanks for each species. The bottom of each tank is filled with a gravel bed (10 cm height) for roots growing. The water level is maintained constant and it is adjusted by means of a plumbing. Temperature, humidity and rainfall input have been monitored by a pluviometer and a thermo-hygrometer. The three species have been planted with different density: *Phragmites australis*, 50 plants m<sup>-2</sup>, *Iris pseudacorus*, 13 plants m<sup>-2</sup>, *Lythrum salicaria*, 13 plants m<sup>-2</sup>. The plants were transplanted in july and the experiment started in the early september. The first step of this study focused on the capacity of the system to uptake nitrate. KNO<sub>3</sub> fertilizer was added in two different periods, in summer and in winter, to evaluate the capacity of the system to work also during the cold season. Samples of water have been taken for one month after the nitrate input and were analyzed by the ion chromatography technique.

For the trial in the summer period for each block of plants were decided, two different level of NO<sub>3</sub><sup>-</sup> concentration, 50 ppm and 150 ppm (for each species two tanks at 50 ppm and two at 150 ppm). In winter period only six tanks were used, two for each species, with 150 ppm concentration level.

## Results

These first series of results show in the summer period a good performance in nitrate removal for all the three species, even if *Lythrum* demonstrated a less capacity and less quickness then the others two. *Phragmites* shows a considerable rapidity in removing nitrate since the initial phase, the lower dosage was almost completely removed in the first week and also at the higher dosage the concentration reduction is comparable. *Iris* has a slower reaction to the nitrate input, but the final result for both the concentration level is similar to *Phragmites*. In the best case of *Phragmites* the removal percentage varies between the 100% for the lower dosage to the 60% for the highest. These value accord essentially with the values reported by other authors, which show removal percentages ranging from 78% to 95% for constructed wetlands (Masi et al., 2002) and 76% in the case of an experimental trial (Sim et al., 2007).

*Lythrum* appears less efficient, particularly the tanks with the upper dosage maintained high value up to a month after the nitrate input. In *tab.1* are the mean concentrations for samples taken in summer period

In the cold period, from early january to the end of march, appears evident the effect of the low temperature and vegetative stasis on nitrate reduction. With a mean value of 6.6°C, and many days in january and february with minimum temperature below zero, the mean value of concentration maintain high until the end of february, even if a slight decreasing trend is visible. The nitrate concentrations show an important downward tend only when temperature resolutely increase in march. The graph in *fig.1*, shows the trend of nitrate concentration during the cold period and in *tab.2* are the mean concentrations for samples taken in winter.

Species	[NO <sub>3</sub> <sup>-</sup> ]ppm 08/09/2007	[NO <sub>3</sub> <sup>-</sup> ] ppm 15/09/2007	[NO <sub>3</sub> <sup>-</sup> ] ppm 29/09/2007
<i>Phragmites a.</i>	40.3	1.7	0
<i>Phragmites a.</i>	150.0	102.7	66.9
<i>Iris p.</i>	52.6	32.8	8.6
<i>Iris p.</i>	160.0	131.5	75.9
<i>Lythrum s.</i>	59.6	44.2	17.6
<i>Lythrum s.</i>	163.0	156.5	135.5

Tab.1

Species	[NO <sub>3</sub> <sup>-</sup> ]ppm 19/01/2008	[NO <sub>3</sub> <sup>-</sup> ] ppm 26/01/2008	[NO <sub>3</sub> <sup>-</sup> ] ppm 02/02/2008	[NO <sub>3</sub> <sup>-</sup> ] ppm 09/02/2008	[NO <sub>3</sub> <sup>-</sup> ] ppm 23/02/2008	[NO <sub>3</sub> <sup>-</sup> ] ppm 25/03/2008
<i>Phragmites a.</i>	148.36	156.85	152.03	149.02	136.20	87.32
<i>Iris p.</i>	146.03	155.18	148.79	139.90	124.92	97.73
<i>Lythrum s.</i>	163.54	167.18	152.88	140.95	142.33	104.01

Tab.2

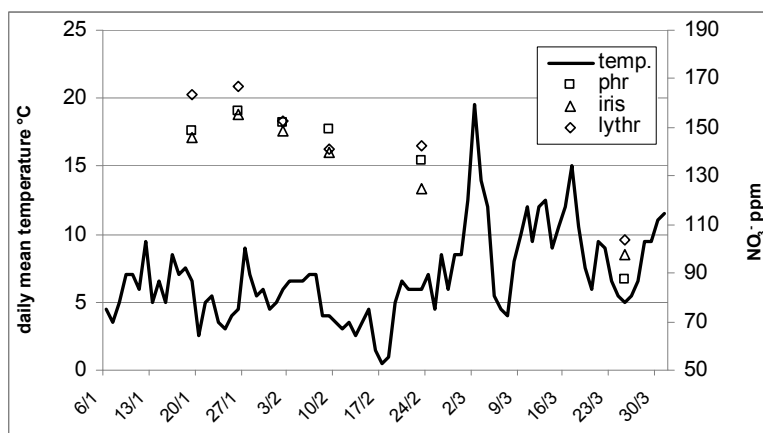


Fig.1

considerably decreased, depending on the low temperature, even if not completely inhibited.

### Acknowledgments

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### Conclusions

The first results on nitrate shows that in the summer period (september), with minimum temperature always above 10°C and maximum over 20°C, the three species taken in to consideration have a good performance in reducing the amount of NO<sub>3</sub><sup>-</sup>, particularly *Phragmites australis* was quick and efficient (as expected). In winter period the removal efficiency appears to be

# Monitoring of Inorganic Pollutant from a Waste Incineration Plant in Rainfall, Groundwater and Washed-Leaves Water

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## Introduction

Incineration destroys hazardous waste, reduces mass and volume of residues and recovers energy content from un-recyclable materials. However, when compared with other methods, the combustion process has also the disadvantages of creating harmful solid wastes and large volume of toxic gaseous emissions. The concentrations of inorganic (heavy metals) and organic (dioxins and furans) pollutants in atmosphere increase. The airborne pollutants originating from various diffuse and point emission sources (namely: industry, farming practices, waste incineration, domestic heating systems and vehicular traffic) deposit on soil, vegetables and are also present in rainfall.

The aim of this work is the identification of heavy metals in rainfall, in groundwater and in washed-leaves water, which describes the airborne particles fallen from the trees.

## Methodology

The waste treatment plant, that incinerates urban wastes from the metropolitan area of Bologna, is in Granarolo dell'Emilia (44°33'15"N, 11°26'38"E). The monitoring regarded 12 different stations in an area of 28.3 km<sup>2</sup> (Figure 1), started in April 2005. A network of 6 rainfall gauges and 5 phreatimeters were installed around the area, in the direction of preferential winds.

Rainfall and groundwater samples were taken twice per month, depending on rainfall events and water table depth. Rainfall samples were taken with the same time step in the agrometeorological station of the experimental farm of the University of Bologna, in Cadriano, about 5 km NW from the plant and in that of Agriculture Faculty (CAAB). Groundwater was sampled in the selected stations 2, 3, 5, 10 and 12. The rainfall and groundwater samples were filtered and acidified with nitric acid suprapure.

Washing-leaf water were carried out in Spring and Winter 2005/2006 on samples of coniferous plants, Summer and Autumn 2005 on samples of deciduous leaves. The sample of leaves (100g) was washed with 500 ml of HCl 0.001 M solution to simulate a possible acid rain which could wash away any particulate deposited on the leaves of coniferous and deciduous trees. The washing water was reduced in volume and then filtered and acidified with nitric acid suprapure to maintain the chemical elements to be analysed in solution form.

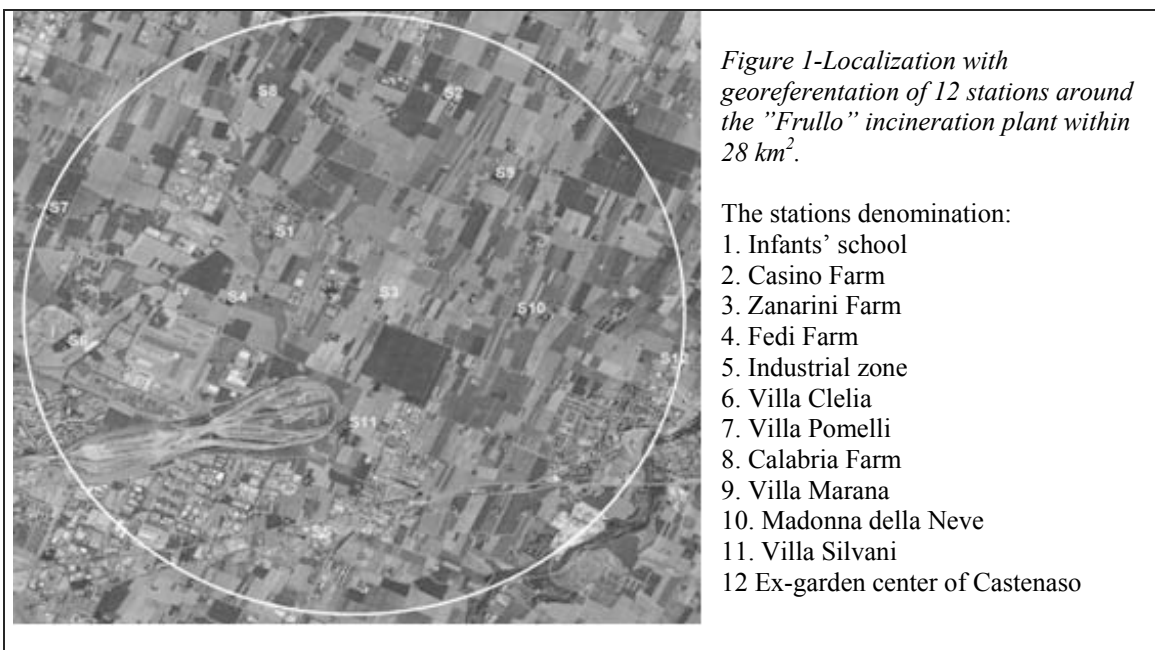
The macro and microelements present in the water samples were quantified using a Spectro Inductively Coupled Plasma Optical Emission Spectrometer (Circular Optical System CIR.O.S<sup>CCD</sup>). Certified multi-standard solutions for the construction of calibration lines were used (CPI-international-Amsterdam). The accuracy of the analytical method was verified by analysing certified (BCR 606).

## Results

**Rainfall waters.** Precipitation resulted similar between the sites, with quantities close to the annual rainfall of the area. The elements concentration in rainfall were higher in Cadriano than in the Agricultural Faculty (CAAB). The reasons could be related at the presence of industrial areas near to the Cadriano Farm. In particular Cu was higher than 0.1 mg/L, above the limit of Italian Law reported in Table 4 of D.Lgs 152/2006; Ni and Zn also exceeded, in some rainfall periods the law limit of 0.02 and 0.5 mg/L. The rainfall waters analysed in 5 different stations did not exceed the law limits, but the rainfall water in the Industrial areas (Station 5) near Villanova di Castenaso showed the presence of Cd and Cr, whereas Ni, V, Zn and Mn exceeded the law limits.

**Groundwater.** Water table depth decreased but did not reach the bottom of the phreatimeters (2.80m), allowing the sampling during all the monitored period. The elements concentration in groundwater in 5 stations showed a good quality in relation to the Italian Law (D. Lgs 152/2006).

**Washed –leaves water.** In Spring time Cd and Zn was higher in 2 and 8 stations, whereas in winter time Pb was higher in 3, 5 and 11 stations respect to the Italian Law. The micro elements in washed-leaves water showed higher concentrations in Winter time samples, probably due the domestic heating systems.



### Conclusions

The determinations of heavy metals in 1) rainfall, 2) groundwater and 3) washing-leaf water showed the following. All the samples of water analysed highlight that the mainly polluted areas were near to industrial zones. The concentrations of Cd and Zn were higher in rainfall and washed-leaves water in the same stations. The groundwater did not show phenomena of pollutions in any station.

### Acknowledgement

This work was developed with the financial support of FEA srl, Provincia di Bologna, Assessorato Ambiente, Comuni di Castenaso and Granarolo.

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**SUB SESSION 1.1**  
**LAND AND WATER CONSERVATION**

1.1b - AGRONOMIC TECHNIQUES FOR PRESERVING ECOSYSTEM SERVICES

Chairman: Sven Eric Jacobsen





# A context for agronomic techniques to preserve ecosystem services

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Agricultural research generally, and agronomic research in particular, are back in the world agenda. Agriculture is now being asked to produce more while still saving enough land for nature and its other values, ie maintaining ecosystems services. Agriculture is now expected to provide energy for internal combustion engines as well as food and fibre, new industrial materials, and 'pharmaceuticals'. The total demand is made even greater by an increasing world population that is also seeking to improve lifestyle and diet.

The presentation will address the context for agronomic techniques for preserving ecosystems services, presenting a possible framework for increasing the impact of research and technology transfer.

It will acknowledge and build on the following facts:

- World population is now 6.7 billion and predicted to rise to 8.2 billion by 2030. 800 million are seriously undernourished. Not only is population growing but inhabitants of developing countries are seeking a better diet.
- Arable land is ca. 1500 Mha with few opportunities for expansion or change. Europe is intensively farmed on 100 Mha of cropping land (EU25) and will use set aside land.
- Until a recent quiet announcement, EC had maintained a mandate for 5.75% biofuel use in transport by 2010, increasing to 10% by 2020. No country was anywhere near that goal and EU would have had to allocate a large proportion of land area for biofuel (OECD, 2005). The new target is likely to be 4% and future emphasis will be on agricultural and forestry residues and dedicated energy crops but the supply of biomass and processing are both incompletely explored fields (EEA, 2007a, b).
- Food grain prices have risen due to complex factors; greater demand for grains, higher petroleum prices, speculation for continuing price rises, and competition from biofuels. Farmers benefit from higher prices provided additional returns exceed additional costs. Consumers are losers, especially the landless poor of developing countries.
- There is confusion about what constitutes sustainable agriculture. Until last December, FAO helped to promote organic agriculture (OA) as a solution to many challenges facing agriculture, even though the smaller yield of OA than conventional agriculture was already well established (Smil 2001, 2004).
- Funding for agricultural research has declined in recent decades (Nature, 2008) and an increasing proportion is dedicated to biotechnology to the disadvantage of agronomy (crop and cropping system management).
- The spectre of climate change confuses the population and policy makers. How often does one read that climate change has already had this and that deleterious effect on production, disease, water supply etc., when the real concern is what might happen in the future.

## **Defining the resource base. Mapping: large scale.**

Low resolution mapping does not generally provide data for decision making. It does provide information for ranges. What better example than the issues about factors that define productivity now applied to residue availability for biofuel. General analyses are not adequate to evaluate sustainable

removal levels. It is not enough to know that there are 3-4 Gt stubble produced year and that perhaps 30% are burnt. Sustainable removal is determined by local factors of productivity and demand for other purposes, e.g. animal fodder, soil conservation etc.

### **Linking farmers to consumers**

Good farming practices and provision of safe food need to be addressed both ways, not only from fork to farm (EC's FP7). The need to expand certification to include payments for ecosystems services (PES) and involvement of informed consumers in choosing certified products can be reinforcement steps.

### **Linking farmers to their land. Mapping: farm scale**

Measurements to assist farmers manage better, includes precision agriculture. Irrigation and N management, soil recovery from pollution, catch and cover crops, etc. as shown in this session on 'Available techniques for conservation of agro-ecosystems productivity' represent a crucial and silent on-going research. We are now at a point where these technologies have to be presented as 'tool boxes' for optimum management of water, nitrogen for specific cases, i.e. farms or farm types within a region. Further specification of what research techniques do agronomists have to assist in the establishment of the agricultural systems that can meet the goals of sustainability (sufficient produce, environmentally sensitive, economically feasible) is needed.

### **Models for many purposes**

The examples of poor policy shifts in OA and biofuel reveal what happens when the performance of entire systems are ignored. Models linked with long-term field experiments and observations are the only way to make those analyses, more work is needed here. Cost-benefit analyses have long been shown to be insufficient and a more appropriate paradigm lies within ecological economics.

### **The meaning of ecosystem services**

The conservation of productive capacity of agricultural land is indisputable. Preservation of soil and water is essential as is also the economic and social structure of farming. The question is what changes are compatible with the demand for greater productivity - efficiency requires timely operations for which mechanization is essential. These changes will impact on field size, farm size, how close a mixture of agriculture and nature: i.e. landscape-scale (local or regional) management and landscape design to improve aspects that provide for the ecosystems services (ES). The social vision of agricultural landscapes is important, and can help to internalise the concept of ecosystems services.

Questions such as: How does one best spare land for nature (Waggoner, 1994)? Intimate mixtures of agriculture and nature or separation? Ensuring that agriculture is located on only the most robust land? will need to be addressed at global and local scale.

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# The Effects of the Increasing Share of Cereals in Crop Rotations on the Selected Soil Properties

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The structure of the crop rotation has a special meaning in the farming. The single crops leave in the soil different amounts of root and harvested rests which are the primary source of humus-making material. The grown crops influenced physical properties. The intensity of crop rotation influence depends on the share of crops in crop rotation and it can strengthened by organic fertilization or the growing intercrops. The aim of our research was to determine the influence of crop rotations with higher share of cereals (up to continuous cropping) on the changes of the physical soil properties and on the grain yield of winter wheat and spring barley in a long-time period.

## Methodology

The stationary trial was established in 1974 in the experimental station Borovce of the Research Institute of Plant Production in Piešťany, Slovakia. The field experiment was located on Luvi-Haplic Chernozem on loess. In the depth of 0.50–0.85 m mollic horizon proceeded into calciferous loess. The trial occurs in the area of continental climate; the average annual temperature is 9.2 °C; the sum of the annual precipitation is 593 mm (30 years average). The trial consisted of two parts. In the first part, crop rotations were used with 40, 60 and 80 % share of the cereals. In crop rotation there was used the mineral fertilization. In the second part of the trial, winter wheat and spring barley were grown in continuous cropping (without any other crops). To decrease the negative impacts of continuous cropping, various measures were taken, e.g. incorporation of organic matter into the soil (cereal straw and/or cereal straw and green manure in the next variant) and the introduction of compensating crops (silage maize, grain maize, oats). Physical soil parameters (bulk density, total porosity, maximum capillary capacity, soil structure coefficient) and the chemical soil parameters (the content of humus) were in the years 1988 and 2007 observed and consequently evaluated. This was done on the selected soil samples. There was used variance analysis. The selected variants: A – winter wheat in crop rotation with 40 % share of cereals [with mineral fertilization (MF)]; B - winter wheat in crop rotation with 80 % share of cereals (MF); C - the continuous cropping of winter wheat is interrupted by maize on silage in every second year (MF); D - the continuous cropping of winter wheat is interrupted by oats in every second year (MF); E – the continuous cropping of winter wheat (MF); F - the continuous cropping of winter wheat (MF + incorporation of straw and green manure); G – the continuous cropping of spring barley (MF); H - the continuous cropping of spring barley (MF + incorporation of straw and green manure).

## Results

The selected physical soil properties and the grain yield of winter wheat were influenced by the different share of cereals in crop rotations and the different fertilization in a statistically significant degree. The average dry bulk density (see Table 1) by continuous cropping of winter wheat ( $1.40 \text{ t/m}^3$ – $1.42 \text{ t/m}^3$ ) was statistically lower than by continuous cropping of spring barley ( $1.50 \text{ t/m}^3$ ). The highest average total porosity was reached by continuous cropping of winter wheat with straw and green manure incorporation (44.99 %) and in the variant where continuous cropping of winter wheat was interrupted by oats in every second year (44.96 %). The lowest average total porosity was found by continuous cropping of spring barley in the G and H variants (42.24 %; 42.82 %). The incorporation of straw and green manure influenced the maximum capillary capacity. The lowest average maximum capillary capacity was found in the crop rotation with 80 % of cereals (32.77 %). The maximum capillary capacity was statistically lower by the continuous cropping of spring barley (34.20 %; 33.56

%) than by continuous cropping of winter wheat (37.17 %; 36.07 %). The highest average value of soil structure coefficient was reached in F variant (5.58). The soil structure coefficient was statistically higher by the continuous cropping of winter wheat (5.35 a 5.83) than by continuous cropping of spring barley (3.39 a 3.92). Within the years 1988 to 2007 the humus content was continuously decreasing in all the variants except for variants C and D (variants where continuous cropping of winter wheat and spring barley was interrupted by maize on silage or oats). In 2007 the humus content remained on the same level than in 1988 in the variant with straw and green manure (variant F). The highest average grain yield of winter wheat was in crop rotation with 40 % (6.99 t/ha) and 80 % of cereals (6.75 t/ha). In crop rotation with 40 % of cereals the average grain yield was by 2.69 t/ha higher than in the continuous cropping of winter wheat. Introducing maize on silage and oats (variants C and D) as interrupting crops the grain yield of winter wheat was higher by 1.38 t/ha than in continuous cropping (E and F variants).

## Conclusions

Comparing the physical soil properties in 1988 with those in 2007 was observed the improvement of soil structure in crop rotation with 40 % cereals and in short sequence with maize on silage (winter wheat – winter wheat – maize on silage) was observed. The degradation of soil properties (bulk density, maximum capillary capacity, total porosity) was markedly higher by continuous cropping of winter wheat than by continuous cropping of spring barley. The incorporation of organic matter (straw and green manure) positively contributed to by the improvement of total porosity by growing both crops (winter wheat and spring barley). The soil structure coefficient by winter wheat was higher in the variant with organic fertilization by continuous cropping of winter wheat. The incorporation of straw and green manure helps to keep the organic matter in the soil throughout a long period by winter wheat growing.

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**Table 1 The selected soil properties and grain yield of winter wheat and sprig barley**

Sequence of crops	year	DBD <sup>(1)</sup> t/m <sup>3</sup>	TP <sup>(2)</sup> (%)	MCC <sup>(3)</sup> (%)	CSS <sup>(4)</sup>	content of humus (%)	grain yield (t/ha)
A	1988	1.51	41.85	32.53	3.96	1.899	8.02
	2007	1.43	44.48	37.28	5.58	1.583	5.95
B	1988	1.51	41.28	32.38	4.35	1.948	8.01
	2007	1.39	47.81	33.16	4.30	1.628	5.48
C	1988	1.49	41.28	32.98	4.66	1.856	7.87
	2007	1.39	46.95	36.62	5.08	2.026	3.99
D	1988	1.50	43.25	31.98	4.74	1.656	7.54
	2007	1.41	46.56	36.12	4.11	1.794	4.12
E	1988	1.48	41.83	32.65	5.83	1.937	6.88
	2007	1.32	47.94	41.70	4.86	1.761	2.51
F	1988	1.47	41.75	32.50	5.91	2.005	5.92
	2007	1.37	48.22	39.64	5.75	2.002	2.68
G	1988	1.51	41.25	32.63	3.50	1.937	5.91
	2007	1.50	43.23	35.77	3.28	1.728	1.39
H	1988	1.50	41.60	32.55	4.12	2.005	5.98
	2007	1.49	44.03	34.57	3.72	1.633	1.56

<sup>(1)</sup> dry bulk density; <sup>(2)</sup> total porosity; <sup>(3)</sup> maximum capillary capacity; <sup>(4)</sup> coefficient of soil structure

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# Myrosinase Immobilized on a Highly Demethylated Pectin: Effect of Soil Water and Organic Matter Content on the Enzyme Efficiency at Soil-Root Interface

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Among the root mucilage constituents, pectines are one of the most active compounds not only in binding soil particles and microorganisms to the root surface, but also in retaining water, due to the activity of their oxydrilic and carboxylic groups. In addition, pectins in root mucilage make easy the accumulation of nutrition elements and the defence against toxic metals (1, 2). The Ca-polygalacturonate (Ca-PGA) hydrogel, a model for pectines with a high demethylation degree, has been found to bind several plant and microbial enzymes (e.g. isoperoxidases and urease (3, 4)) and play an important role in the protection of enzymes against the activity of proteases (4). Myrosinase (MYR), widespread in seeds and tissues of *Cruciferae*, is an enzyme which catalyzes the hydrolysis of glucosinolates to form several byproducts with biocidal activity (mainly isothiocyanates and nitriles). To date, although the presence of the MYR-glucosinolates system in *Brassicaceae* makes these plants a natural alternative to soil fumigation with methyl bromide (5, 6), no information concerning the behaviour of MYR at the soil-root interface is available. In this study, we investigated the activity of MYR immobilized on a network of Ca-PGA hydrogel, which shows a composition and morphology similar to the mucigel present at the soil-root interface, as a function of pH, temperature, storage time and type of substrate. Finally, MYR activity, in free or immobilized forms, was evaluated during the time in soils differently characterized by water and organic matter (OM) content.

## Methodology

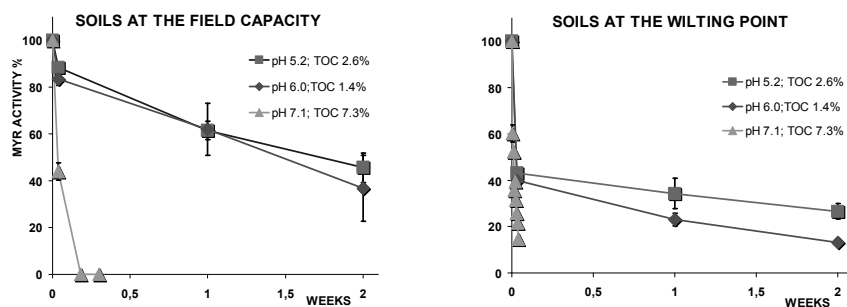
MYR from *Sinapis alba* (EC 3.2.1.147), isolated as already reported (7), was immobilized on PGA hydrogel in the presence of Ca ions at pH 5. The activity of immobilized MYR as a function of pH, temperature and storage time was tested by measuring the pH changes by a pH-stat instrument as already reported (8), using sinigrin glucosinolate as substrate. The pH-stat method was used also for the measurement of the activity of immobilized MYR as a function of different substrates: sinigrin, gluconasturtin, sinalbin, and *epi*-progroitrin. The activity of immobilized MYR in soils was tested by a spectrophotometric assay at 227 nm and 37°C using sinigrin as substrate.

## Results

The immobilized MYR shows an activity which is 40% lower than the free enzyme and, similarly to the free enzyme, an optimum temperature at 55 °C and an optimum pH in the range 5.0-7.0. We observed that Ca-PGA preserved the activity of the immobilized enzyme for more than two months.

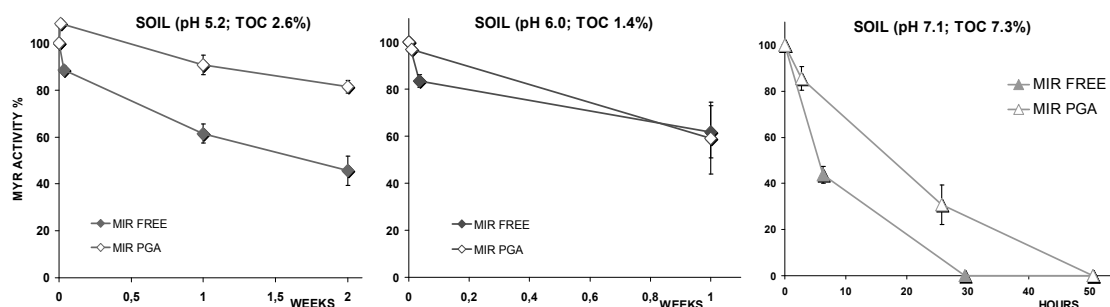
The Michaelis-Menten parameters,  $V_{\max}$  and  $K_m$ , were determined for the free and immobilized MYR. Both kinetic parameters notably increased in the immobilized form of the enzyme:  $V_{\max}$  from 60 up to 127 U mg<sup>-1</sup> protein and  $K_m$  from 0.17 up to 1.55 mM.

When the MYR activity was studied as a function of different substrates, the highest activity of enzyme immobilized on the mucigel network was recorded using gluconasturtin as a substrate, the glucosinolate found in roots of *Brassicaceae*. The activity of immobilized enzyme tested as a function of soil water potential in three soils differently characterized in OM content is shown in Figure 1. At the field capacity (2.0 pF), the activity of immobilized MYR was preserved in soils poor of OM for two weeks, whereas, at the wilting point (4.2 pF), the activity was very quickly lost in all soils.



**Figure 1.** Evolution of immobilized MYR activity during the time in soils at different water and organic matter content (TOC: total organic carbon)

The immobilization of MYR on Ca-PGA hydrogel preserved the enzymatic activity with time only in soils at the field capacity with low OM content as shown in Figure 2.



**Figure 2.** Activity of Myrosinase (free or immobilized on Ca-PGA) with time in soils with different organic matter content at the field capacity (TOC: total organic carbon)

## Conclusions

According to the behaviour of other immobilized enzymes, the kinetic parameters of MYR immobilized into Ca-PGA induces a higher specific activity together with a lower substrate affinity. The preservation of the activity of immobilized MYR in soils with low OM content and at the field capacity indicates not only that the soil water status is an important factor for MYR protection, but also that mucigel maintains the enzyme in the hydrated form at the soil-root interface. On the other hand, the soil organic matter, owing to its hydrophilic properties, strongly competes with the mucigel for soil water content thus reducing the maintenance of the enzymatic activity.

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# Evolution of the Soil Nitrogen Mineral Content in a Wheat-Rapeseed Crop Rotation in Response to Nitrogen and Sulphur Fertilization

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## Introduction

More than 50 % of the crop surface of the province of Alava (northern Spain) is sown with winter wheat and nowadays rapeseed is getting importance inside the rotation with wheat due to the interest of rape oil for biodiesel production.

The aim of the present study is to determine the influence of nitrogen and sulphur fertilization on soil Nmin and its evolution along the rotation.

## Methodology

The response of soil Nmin to nitrogen and sulphur fertilization rates was evaluated in a crop rotation of winter wheat (*Triticum aestivum* L., Cezanne cultivar) and winter rapeseed (*Brassica napus* L., Standing cultivar) during 2005-2006 and 2006-2007 years respectively. Trials were conducted in Gauna, Alava, 823 mm year<sup>-1</sup> and 11.4 °C average annual rain and temperature. Experimental design was a randomized complete block with two factors and four replications. Before wheat sowing, in October 2005 soils samples were collected (0-60 cm depth) to determine the soil Nmin: 63 kg N ha<sup>-1</sup>, and before first nitrogen application, soil Nmin was calculate again (73 kg N ha<sup>-1</sup>). Five nitrogen treatments (0, 140, 180, 220 kg N ha<sup>-1</sup>) were applied at wheat in two or three (only for 180 kg N ha<sup>-1</sup>) applications at Z21, Z30 and Z37 and three sulphur treatments (0, 16, 32 kg S ha<sup>-1</sup>) in one application at Z30. In rapeseed, five nitrogen treatments (0, 100, 140, 180, 220 kg N ha<sup>-1</sup>) were applied in two applications, BBCH 31 and BBCH 50, and three levels of sulphur (0, 30, 60 kg S ha<sup>-1</sup>) at BBCH 31. Soil samples to determinate soil Nmin were taken after harvesting for wheat, and at planting, before first N application and after harvesting for rapeseed in every treatment-block combination.

## Results

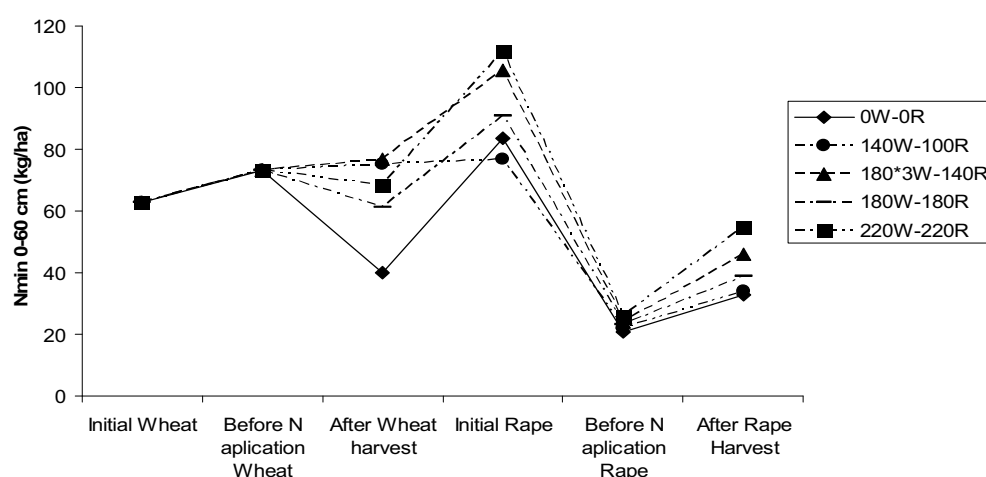


Figure1. Nmin content in the first 60 cm of the soil profile, 0W-0R: treatment with 0 kgN/ha applied to wheat and 0 kgN/ha applied to rapeseed, 180\*3W-140R: treatment with 180 kgN/ha applied to wheat in three applications and 140 kgN/ha applied to rapeseed.

No significant interaction was observed between N and S application rate in any case in Nmin values. On the other hand, no effect of S rate application was registered. After wheat harvest, soil Nmin decreases significantly in plots without N fertilization to 40 kg N ha<sup>-1</sup> respect to the treatments with N application (61 to 75 kg N ha<sup>-1</sup>, figure 1). Treatments with a third N application showed significantly higher soil Nmin (77 kg N ha<sup>-1</sup>) than the same N rate applied in two amendments (61 kg N ha<sup>-1</sup>), confirming the results of Ortúzar (2007). When rapeseed was planted, soil Nmin increased in all treatments, especially when N applied to previous wheat was up to 180 kg N ha<sup>-1</sup> where values exceeded 90 kg N ha<sup>-1</sup>, figure 1. Before first N application to rapeseed, soil Nmin values decreased in all treatments (21 to 26 kg N ha<sup>-1</sup>), with no significant differences between treatments (figure 1). This decrease can be originated by the great autumnal growth of the rapeseed, biomass measured at this moment was 3,381 kg ha<sup>-1</sup> while wheat biomass measured before N application was 151 kg ha<sup>-1</sup>. After harvesting the rapeseed, soil Nmin increased again in every treatment (figure 1), being the highest value for the treatment where 220 kg N ha<sup>-1</sup> were applied both to wheat and rapeseed, (55 kg N ha<sup>-1</sup>) and the lowest one for the treatment without N (33 kg N ha<sup>-1</sup>).

### Conclusions

Different rates of S applied had no effect in soil Nmin.

Soil Nmin differences only can be observed between treatments without N fertilization and treatments with more than 180 kg N ha<sup>-1</sup> applied along the rotation.

The high autumnal growth and the subsequent N uptake by rapeseed caused a better use of soil Nmin, limiting the risk of being lixiviate during this period.

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# A Combined Approach to Delineating Management Zones for Precision Agriculture

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Fundamental to the philosophy of precision agriculture is the concept of matching inputs to needs. Recent research in precision agriculture has focused on use of Management Zones which are field areas possessing homogeneous attributes in landscape and soil conditions. There are several methods for delineating management zones, depending on the available resources and the characteristics of the field being mapped. Existing traditional clustering techniques do not account for the spatial correlation between observations and take little account of gradual change, either from one class to another or within any one class. Differently, geostatistics treats variables as continua in a joint attribute and geographic space. Therefore, in geostatistical applications clusters are unnecessary, nevertheless in precision farming it may be sensible to divide the field into a restricted number of practical management zones. It then needs to develop an algorithm of clustering that is also spatially constrained, in order to ensure spatial contiguity. The methods based on nonparametric density estimation are the ones which allow clusters of unequal size and dispersion and with highly irregular shapes to be detected (Castrignanò et al., 2006). The objectives of this work are to propose a combined approach to aggregate soil and crop properties into contiguous management zones, based on multivariate geostatistics and a non-parametric density algorithm of clustering, and to use visualization for displaying data and statistical analysis.

## Methodology

One hundred georeferenced measurements of soil (coarse and fine sand, silt and clay contents, organic matter), vegetation radiometric (thermic infrared [TIR] temperature) and crop (LAI, aboveground biomass at harvest, gluten content of grain) properties were collected on a 12-ha field cropped to durum wheat in two seasons (2005-2006, 2006-2007). The trial was carried out in the experimental farm of CRA-CER (Foggia, south-east Italy) and the locations of the samples were chosen so that they were evenly distributed on the field. The multivariate spatial structure of data was investigated through a linear model of coregionalization (LMC) fitted to all direct and cross-variograms, by expressing each variogram as a linear combination of the same basic structures (Goovaerts, 1997). The whole data set was split into different subsets of homogeneous variables to which a LMC or a direct variogram model was separately fitted. Finally, each subset of variables was interpolated on a 1 by 1 m-grid using the geostatistical techniques of (co)kriging. To divide the fields into a number of clusters an algorithm, based on nonparametric density estimate and using a smoothing parameter (Scott, 1992), was applied. The variables were standardised to the same mean 0 and to the same variance 1. Moreover to obtain spatially contiguous clusters, the clustering algorithm was applied to the data set of the interpolated variables including also the spatial coordinates. The combined approach was implemented by using the geostatistical ISATIS software package (Geovariances, 2008) and MODECLUS procedure of the SAS/STAT software package (SAS, 2008).

## Results

An isotropic LMC was fitted to all variograms of the soil and crop attribute data set including the following basic spatial structures: (1) nugget effect; (2) spherical model with range of 87 m and (3) spherical model with range of 500 m; for the data set of LAI: (1) nugget effect; (2) spherical model with range of 87 m; (3) Bessel-k model with range of 300 m and parameter = 2; for TIR temperature data an isotropic variogram model including: (1) nugget effect; (2) exponential model with range of 300 m. In any case spatial variance is dominated by random and short-range components.

The cokriged maps for the variables show that the field can be roughly divided into two main zones along the N-S direction with the southern part characterised by higher values of LAI and aboveground biomass in both seasons and more rich in organic matter and clay. The maps of gluten contents look to be more variable, with a large northern zone characterised by the higher values during the drier and less productive 2006-07 season. To synthesise the complex multivariate variation of the field in a restricted number of zones to be submitted to the same management, the clustering approach was applied to the all, co- and kriged variables. After several trials, the smoothing parameter was chosen equal to 2.2, because it produced the subdivision of the field into 2 distinct classes of suitable size for uniform management.

The clusters 1 and 2 differ in the textural components, as the maximum values of clay are associated with the cluster 1 and the highest values of fine and coarse sand with cluster 2. The clusters 1 was more luxuriant and productive in the 2005-06 season, whereas during the drier 2006-07 season the two clusters did not differ sensibly. Quite likely the plants in the northern part of the field were suffering some sort of water stress in 2007, as confirmed by TIR temperature that was higher in cluster 2.

One of the main advantages of the proposed approach, compared with the other traditional methods of clustering, is that it gives information also on the intrinsic spatial structure of the cluster and on the distribution of the residual variation within each class. The main statistics of each cluster (the number of observations, the maximum estimated density, the number of observations in the cluster with neighbours belonging to a different cluster, the estimated saddle density, the number of observations within the neighbourhood of the modal observation, the number of observations within the neighbourhood of the saddle observation, the number of observations within the overlap of the two previous neighbourhoods and the approximate p-value for the cluster) help to interpret the results: the cluster number 1 looks better defined, with the higher values of maximum estimated density and mode count and the lower values of boundary frequency and estimated saddle density. The fuzzy characters of the cluster boundaries are defined by the ratio between saddle count and mode count and by overlap count. Again, it results that the cluster 1 is the most clearly defined realising the higher values of the ratio, on the contrary, the cluster 2 looks to be more muddled with a fuzzy character of the boundary, also verified by the presence of points in the overlap zone.

However, the 3D visualization of the density function shows that a high residual variation still occurs within both clusters.

## Conclusions

The proposed approach has proved to be able to identify spatially contiguous zones which are more homogeneous in soil and vegetation properties than the whole field. One of its greater advantages is the possibility to describe the residual variation within each management zone, which can be a very useful piece of information in precision agriculture for variable-rate application of agronomic inputs.

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# Survey and Optimization of Nitrogen Management in Farming and Cropping Systems

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In the last decades, the demand for an integrated evaluation of agricultural systems has increased. In our project, that we have started in order to survey and optimise representative farming and cropping systems, and to provide recommendations for improved nitrogen (N) management, we calculated a set of indicators about nitrogen and energy use, economic performance and weed control.

## Methodology

We have first identified, in Lombardy region (northern Italy), six farms (arable and dairy), representative of different soil and climate types, different crop and animal production systems, and different management types. We conducted a survey in each farm with periodic visits to collect data about average management, using a structured questionnaire.

Data included annual input and output N flows at the farm level (purchase of mineral fertilisers, feed and bedding materials; sales of animals and vegetable products), and the average crop management at the scale of each single field: tillage (date and type), sowing (date, dose, species, variety and cost) fertiliser and manure application (date, dose, nutrients concentration and cost), pesticide application (date, dose, active ingredients concentration and cost), irrigation (date, type and volume of water application), harvest (date, yield, humidity, type of harvested product and selling price). A list of machinery available on farm was compiled (indicating size and expected workrate), and the several tractor-implement combinations were detected and recorded for each individual crop operations. We also monitored livestock management by collecting data about distribution of the animals in different age categories, their average weight and daily fodder ration.

To simplify data management, fields were aggregated into homogeneous areas. Soil type and agronomic practices were equal within each homogeneous area. Using these data, we have calculated a number of indicators at field scale. As nitrogen indicator, we have calculated the soil surface balance (the difference between N that enters the soil via surface and N that leaves the soil via crop uptake, Fumagalli et al., 2008). To evaluate energy use we have considered only the consumption of non-renewable fossil energy resources, using specific coefficients to convert mass fluxes into energy fluxes. The energy use efficiency was calculated as energy output / energy input (Fumagalli et al., 2008). As economic indicators, we calculated the total costs (variable + fixed). The sum of variable costs includes the costs for plant protection agents (fungicides, herbicides, insecticides, pesticides, adjuvants), fertilisers, seeds, fuel and lubricants. To describe weed management and control, we have defined a weed control indicator that evaluates the impact of cultivation system on weed development probability (WDP). It provides a value from 1 (worst case) to 5 (best case). A value of 3 represents the achievement of a minimum level. The potential toxicity of plant protection agents was described using the Load Index (LI) (OECD, 2005; application rate of active ingredient / LD50 or LC50). All the

indicators were calculated for each homogeneous area and will be preliminarily presented here for one farm only (a non-irrigated dairy farm).

## Results

The farm is representative of an area with silt-clay soil types and elevated annual precipitation (1150 mm). The cultivated crops are silage maize, Italian ryegrass and permanent meadows. Due to the lack of irrigation water, yields of silage maize are moderate: ~16 t DM/ha for early sowing maize (April), and ~12 t DM/ha for sowing after a winter forage crop (May). They correspond to crop uptake of less than 200 kg N/ha. Yields of meadows are approximately of 9 t DM/ha, with N uptake of 172 kg N/ha.

The calculated indicators are presented in Table 1. For all maize crops there is an important N surplus, ranging from 162 to 255 kg N/ha. This is due to excessive use of mineral fertilisers (211 and 156 kg N/ha for first and second-sowing maize, respectively). The nutrient value of manure (200 and 110 kg N/ha are applied with solid and liquid manure, respectively on area #1, 2 and 3, 4, 5) is not fully taken into account. Double-crop systems (areas #4 and 5) have lower surpluses. N balance for meadows is extremely diversified, ranging from -81 to 58 kg N/ha. This is due to the heterogeneity of the application rate of animal manure. The farmer uses large amounts of solid manure on fields close to the village (to minimise odour problems: 200 kg N/ha; area #7), while liquid manure, being preferentially employed on maize, is scarce for meadows (only 61 kg N/ha; area #6). Finally, on area #8 meadows are fertilised with inorganic N only, with a moderate deficit. Maize-based systems have higher energy inputs compared to meadows, due to tillage operations and pesticide application. Double-crop systems (areas #4 and 5) have much higher inputs compared to continuous maize (areas #1, 2 and 3). Despite being at a similar distance from farm compared to area #3, area #2 has higher energy consumptions due to the use of a small solid manure spreader, that requires many displacements.

There is a higher probability of weed development for continuous maize compared to the double-crop system (silage maize 2<sup>nd</sup> sowing + Italian ryegrass). Best results in reducing weed development probability should be achieved in meadows.

Economic costs are much lower for meadows compared to maize. For maize-based systems, the addition of the winter fodder crop (Italian ryegrass) does not increase much the total costs.

Table 1. Values of all indicators for each homogeneous area.

Hom. area	Crop	Fertilisers applied	Distance from farm (km)	Surplus (kg N/ha)	Energy inputs (GJ/ha)	Total costs (€/ha)	WDP
1	csm1	s + mn	2.1	255	36.7	1,259	1
2	csm1	s + mn	1.6	255	37.1	1,215	1
3	csm1	l + mn	1.5	165	29.2	785	1
4	sm2 + irg	l + mn	1.0	162	45.3	1,375	2
5	sm2 + irg	l + mn	0.4	162	43.6	1,222	2
6	m	l	1.0	-81	8.9	582	5
7	m	s	1.0	58	14.3	822	5
8	m	mn	1.0	-33	12.0	554	5

irg = Italian ryegrass, csm1 = continuous silage maize, sowing in April, sm2 = silage maize, sowing in May, m = meadows; s = solid manure, l = liquid manure, mn = mineral fertilizers.

## Conclusions

This methodology made it possible to provide an integrated assessment of various aspects of agriculture sustainability. A similar analysis is on going in six other dairy and arable farms.

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# Small Farmers' Access to Market: The Case of Quinoa, a Seed Crop from the Andes Region

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## **Quinoa, main advantages and problems in relation to the market**

One of the most important Andean crops is quinoa (*Chenopodium quinoa* Willd.), which is a very nutritious seed crop (Repo-Carrasco et al., 2003) of high tolerance to drought, frost, saline soils and other adverse factors (Garcia et al., 2003; Jacobsen et al., 2001; 2004; 2005; 2007). The production of quinoa is traditionally done for food security reasons, but now the global market demand of quinoa is rapidly increasing. The main problems for satisfying this increasing market demand are related to an intensified production, which may cause soil fertility and depletion of water to increasingly constrain agricultural production (Bois et al., 2006; Jacobsen et al., 2003).

As the focus on the export market is likely to increase production risks, it raises the question as to whether a choice must be made between maintaining a diverse production system for food security at home or a certified organic quinoa production for Europe and the USA. The role of quinoa for either production for sale or subsistence is fundamentally different, so development efforts should not be limited to deal with only increases in production and yield, but also in an increasing commercial competitiveness of the sector, and quality of the produce (Alarcón and Ordinola, 2002). The two factors of importance are food security (variety diversity stabilises yield), and market sale (specific quality demanded by market).

A case study performed in Peru 2003-2006 considered it important to have a local as well as an international market for quinoa in order to sustain production and development. From estimations on the local market opportunities, it became clear that quinoa was regarded as low status food by the Peruvian consumer in favour of rice and pasta. A part of the explanation was that quinoa is often sold with impurities and not washed adequately, making it less attractive and the preparation more time-consuming. Secondly, it was surprising to discover how hidden away the quinoa was for the tourists. In total 1 mill. tourists visit Peru each year, which represents a large domestic potential market. Furthermore, once the tourists have been exposed to quinoa in Peru, they may look for it in their home countries after returning.

## **Case study in Peru**

The objective was to optimize the farming system, and to perform a market study for identification of actual and potential markets for quinoa. To overcome the problems mentioned in the commercialization of quinoa we must secure the supply, that is volume, stability and quality. Production should be secured by improving soil fertility, optimizing the use of water for crop production, introducing natural pest control by use of biocide plants without health risk to the farmers, utilizing the diversity of quinoa to obtain plants resistant to pests and adverse environmental conditions, and using seed of good quality.

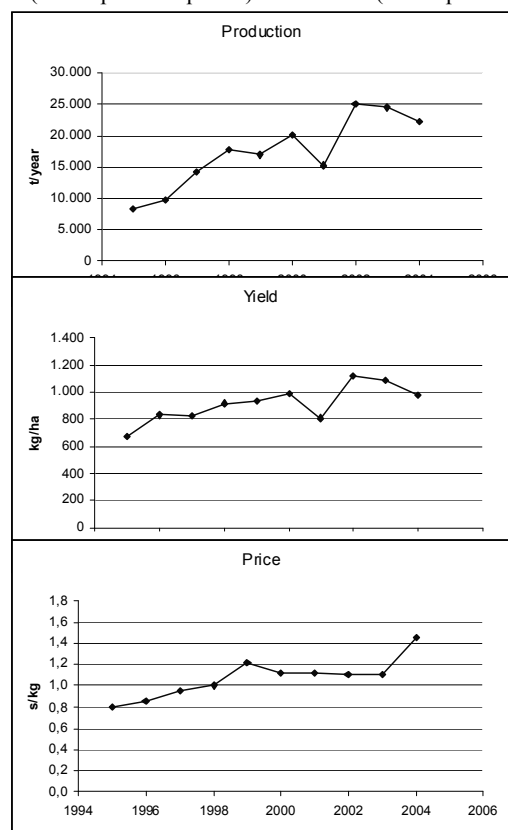
The philosophy was to establish work with farmers' leaders in farmers' fields and at research stations of the national research institution of Peru, INIA, both serving to test new technologies and acting as training centers for the participating farmers and the nearby communities. The success would depend greatly on the interest shown by the local population and on the strategies used to incorporate all stakeholders from the initial stages.

Governmental entities involved in production of quinoa, such as the regional offices of the Ministry of Agriculture, responsible for the Production Chain of Quinoa in Puno, 3800 masl., were collaborating with the project. The ministry is situated in Puno at the shore of the Titicaca lake, close to the Bolivian border. The Ministry is playing an important role in training, organization and generation of market information for the farmers involved in the production of quinoa, being present in farmers' communities. Hence we

took advantage of the contact between the institutions and the quinoa producers in the department of Puno, already existing. A collaboration was established with the international NGO CARE, with much experience in the organization of farmers, especially for production and marketing of quinoa, and with the National University of the Altiplano, Puno, with research experience in farming systems and uses of quinoa. The development of production and prices is seen in fig. 1, demonstrating the increase in production, yield and price, over the duration of the project.

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# Selection of Grain Legumes Species for Intercropping System with Maize – Polish Experience

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## Introduction

Intercropping has been practised for 10 thousand years and is common in many parts of the world. Purpose of this sowing method was to acquire of food, fibre and other products (Francis 1989). Intercropping is typical for tropical regions with high amount of precipitation, high temperature and a long vegetation period. In temperate climate these sowing methods are troublesome because of mechanization in sowing, fertilization and harvesting (Anil et al. 1998).

Polish farmers have widely been using intercrops of cereals species (cereals spring mixtures) quite often with legumes. Cereals mixtures harvested for forage occupied approximately 1 million ha.

In the last decades maize became a main forage crop in our country and the area of its cultivation has steadily increased. At the same time, however, grain legumes production decreased. It constitutes now only 2% of total area of arable land. A system of intercropping maize with different legumes can improve quality of forage and has positive effects on the environment. Selection of species for these sowing methods must focus on development synchronism. Plants should reach similar growing stage during harvesting and as well as dry matter content.

Intercropping of maize with different legumes has to be tested in Polish conditions.

## Methodology

From 2003 onwards, at the Research Station Pawlowice which belongs to the Crop Production Department of the Wrocław University of Environmental and Life Sciences (WUELS), carried out a few series of experiments of intercropping maize with grain legumes (for ensiling). On first series (2003-2005) the main task was to estimate quantity and quality of maize-beans intercrops. Maize was sown with two bean species: common bean – runner varieties (*Phaseolus vulgaris* L.) and scarlet runner bean (*Phaseolus multiflorus* Lam.). Plants were harvested during the maize dough maturity stage. On second series carried out in the years 2005-2007 we evaluated the effect of various sowing rate of field beans (*Vicia faba* L.) with maize intercrops on yielding and quality of forage. Maize and field bean was sown in alternating rows. On the third series, which started in 2006 and continue yellow lupine (*Lupinus luteus* L.) and scarlet bean (*Phaseolus multiflorus* L.) – short stem variety have been tested as an intercrops with maize. From 2007 (and continue) forage soybean has also been tested in this system. Maize and legumes were sown at the same time in alternating rows (apart common bean, and runner scarlet bean). Growing stages were noted using the BBCH scale (Meier 2001). Before maize harvesting (the optimal stage for silage) plants samples were taken for dry matter estimation (maize separately from legumes).

Purpose of this study was to analyze growth synchronism between maize and legumes, sown at the same time and growth in the same environmental conditions.

## Results

Maize was harvested at the same growing stage during different series of experiment. Approximately after 120 days after sowing on average maize hybrid (medium hybrid type FAO 250) reached 85 on the BBCH scale (tab. 1). Common bean (runner variety) as well as field bean has developed faster than

maize and during harvesting reached 92-99 on the BBCH scale. Soybean (American, late forage type) developed slower than maize, and reached 72 on the BBCH

Development of yellow lupine, scarlet bean (short and runner type) was best synchronized with maize growth.

Tab. 1 Growing stages for maize, lupine, common and scarlet bean, soybean in BBCH-scale

Days after sowing	Maize	Common bean (runner)	Scarlet runner bean	Field bean	Yellow lupine	Scarlet bean (short stem)	Soybean
0	00	00	00	00	00	00	00
35	16	16	16	14	19	19	16
64	55	55	51	63	65	69	51
73	65	65	60	75	70	75	60
120	85	92	81	99	87	87	72

Concentration of dry matter in forage use for ensiling should range from 30 to 35% (Wiersma et al. 1993). Percent of dry matter in maize plants during harvest was on optimal level – 33.2% on average, and was very stable – standard deviation was very low only 1.8 (tab. 2). Scarlet bean (short stem variety), common bean as well as soybean have dry matter concentration very close to optimal level (from 28.1 to 38.7%). Common bean (runner variety) characterizes high differences between min and max levels and SD was highest among tested legumes species. During harvesting time the yellow lupine was still green and had the least dry matter content in whole plants (18.9%). Scarlet runner bean variety, concentration of dry matter was too low (24%) for ensilage. Field bean has the shortest vegetation period and reached full maturity stage during harvest. Dry matter content for whole plants was too high for ensiling processes (84.4%).

Tab. 2 Percent of dry matter in whole plants of maize and legumes before harvesting

	Maize	Common bean (runner)	Scarlet runner bean	Field bean	Yellow lupine	Scarlet bean (short stem)	Soybean
Average	33,2	38,7	24,0	84,4	18,9	34,2	28,1
Min	31,2	30,8	20,0	82,0	17,0	29,9	26,5
Max	36,7	55,0	27,2	90,0	23,0	36,6	31,0
SD	1,8	11,1	3,1	2,5	2,4	2,6	1,6

## Conclusions

Among six tested forage legumes species best synchronised development with maize has scarlet bean (short variety), common bean and soybean. Dry matter content for these species was near optimal for using forage for ensiling processes. In intercropping maize with yellow lupine or scarlet runner bean later forage harvesting should be recommended because dry matter content in legumes was too small. Field bean are not recommend for maize intercropping system. In the Polish conditions these species have too short vegetation season and reach full maturity much earlier than maize.

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# Effect of Farmyard Manure and Mineral Fertiliser on the Growth Parameters of Maize ( *Zea mays* L.)

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The long-term experiment on continuous maize was set up in 1958 with various nutrient levels, provided as farmyard manure (FYM), FYM+mineral fertiliser (NPK) or NPK on the active agent equivalence principle. The original aim of the experiment was to determine whether the NPK nutrients in FYM could be replaced partially or entirely by inorganic NPK fertiliser. Good nitrogen supplies promote a rapid initial growth in the leaf area index of maize, allowing optimum LAI values to be maintained with good biomass duration. This is favourable for the flow of assimilates into the grain (Berzsenyi et al., 2007). The effect of farmyard manure and mineral fertiliser on the dynamics of dry matter production and leaf area index was studied for three years (2005–2007).

## Methodology

The long-term experiment was set up in a Latin square design on eroded chernozem soil with forest residues in Martonvásár, Hungary. The treatments were as follows: 1. Control; 2. 35 t ha<sup>-1</sup> FYM; 3. 17.5 t ha<sup>-1</sup> FYM + N<sub>1/2</sub>P<sub>1/2</sub>K<sub>1/2</sub>; 4. N<sub>1</sub>P<sub>1</sub>K<sub>1</sub>; 5. 70 t ha<sup>-1</sup> FYM; 6. 35 t ha<sup>-1</sup> FYM + N<sub>1</sub>P<sub>1</sub>K<sub>1</sub>; 7. N<sub>2</sub>P<sub>2</sub>K<sub>2</sub>. The total quantities of active agents (kg ha<sup>-1</sup>) applied each year were N: 66, P<sub>2</sub>O<sub>5</sub>: 38, K<sub>2</sub>O: 75 in Treatments 2–4 and N: 132, P<sub>2</sub>O<sub>5</sub>: 76, K<sub>2</sub>O: 150 in Treatments 5–7. Of the three experimental years the weather in 2005 was very favourable for maize as regards both rainfall and temperature. In 2006 only half the normal quantity of rainfall was received during the sowing period, while 2007 was an extremely hot year with 58 heat days and uneven distribution of precipitation, resulting in poor fertilisation and severe yield losses. The destructive (direct) and indirect methods of growth analysis were applied from the 3-4-leaf growth stage until physiological maturity. From the agronomic point of view dry matter accumulation is of the greatest importance, so this is generally used as an indicator of growth dynamics.

## Results

**Yield data.** In all three years the yield was significantly the lowest in the control treatment (Table 1). Similar tendencies were observed in 2005 and 2006, with the significantly highest yields (9.8 and 7.9 t ha<sup>-1</sup>) in Treatment 7, while in 2007 the highest yield (3.4 t ha<sup>-1</sup>) was obtained in Treatment 5, indicating the positive effect of FYM in dry years. In 2007 the yield achieved with high rates of

Table 1. Effect of fertilisation treatments on maize grain yield over years (2005-2007)

Treatments	Grain yield tha <sup>-1</sup>		
	2005	2006	2007
1. Control	4.30	3.99	2.49
2. 35 tha <sup>-1</sup> farmyard manure (FYM)	6.00	4.88	3.01
3. 17.5 tha <sup>-1</sup> FYM + N <sub>1/2</sub> P <sub>1/2</sub> K <sub>1/2</sub>	7.70	6.01	3.18
4. N <sub>1</sub> P <sub>1</sub> K <sub>1</sub>	8.00	6.28	3.11
5. 70 tha <sup>-1</sup> FYM	6.81	5.17	3.35
6. 35 tha <sup>-1</sup> FYM + N <sub>1</sub> P <sub>1</sub> K <sub>1</sub>	9.22	6.25	2.44
7. N <sub>2</sub> P <sub>2</sub> K <sub>2</sub>	9.82	7.69	2.32
LSD 5%	0.82	0.95	0.51

mineral fertiliser ( $2.4 \text{ t ha}^{-1}$ ) did not differ significantly from the control and the experimental treatments had little or no measurable effect on the yield, demonstrating the yield-limiting effect of severe rainfall deficiency.

**Dynamics of dry matter production.** The dynamics of dry matter incorporation could be described with a sigmoid curve, the course of which was similar in 2005 and 2006, but differed greatly in 2007 (Fig. 1). The effects of the various treatments were quite distinct in 2005 and 2006, allowing three groups to be distinguished. The greatest dry matter production was achieved in Treatments 6 and 7 and the lowest in the control and after the application of  $35 \text{ t ha}^{-1}$  FYM. In 2007 no further dry matter incorporation was recorded after the 8<sup>th</sup> sampling date in Treatments 1, 2 and 6, while accumulation continued in Treatments 3, 4, 5 and 7. A long period of linear growth was observed in 2005 and 2006, while due to

the high mean temperature the growth curve reached its maximum value within a short time in 2007.

**Dynamics of leaf area index.** The effects of the seven treatments on the seasonal dynamics of the leaf area index were clearly distinct in 2005 and 2006, while in 2007 only Treatments 4, 6 and 7 had characteristic dynamics (Fig. 1). In 2006 the differences between the LAI values of the treatments were smaller than in 2005. In 2005 the LAI values remained constant in all the treatments between sampling dates 4 and 7, ensuring ideal conditions for yield formation. In 2006 the  $\text{LAI}_{\text{max}}$  values were only maintained for two weeks in the control and the FYM treatments, while in the other treatments this period was six weeks. In 2007 the greatest leaf area index values were obtained in Treatments 1–3, but this had very short duration, dropping sharply after the maximum was reached.

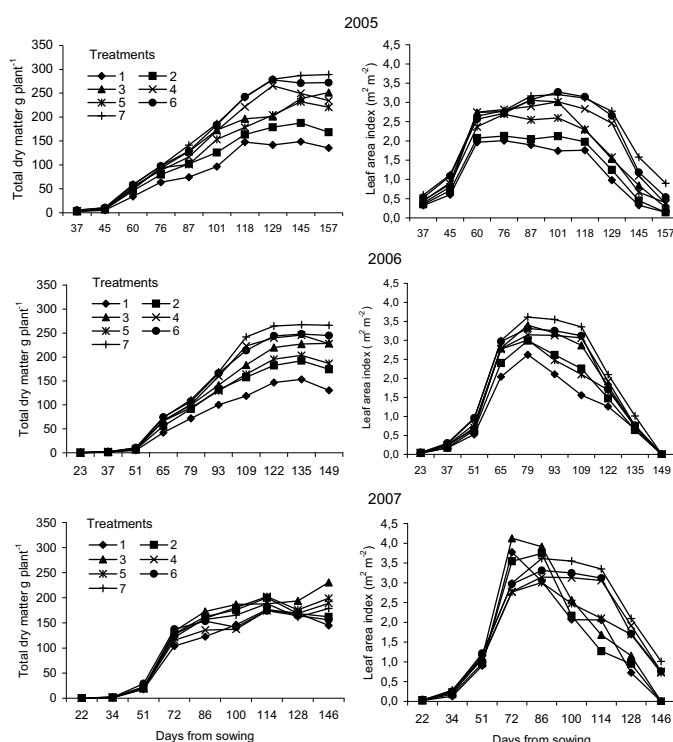


Figure 1. Effect of farmyard manure and mineral fertiliser on the growth dynamics of total dry matter and leaf area index of Martonvásár maize hybrids (Mv) in different years (2005-2007).

## Conclusions

The results of analysis of variance demonstrated significant differences between the treatments in all three years as regards total dry matter production and leaf area index. It could be proved by growth analysis that different levels of FYM and NPK application had a substantial influence on the dynamics of dry matter accumulation and on the growth of the leaf area. An analysis of the year effect showed that rainfall deficiency was an important yield-limiting factor (yield declines of  $4.57$  and  $2.91 \text{ t ha}^{-1}$  in 2007 compared with 2005 and 2006, respectively), as a consequence of which the experimental treatments had little or no effect on the yields, while the effect of the year could be described well by means of the diverse dynamics of dry matter production and the leaf area index.

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# Direct and Residual Effects of Organic Fertilisation on Crop Yields and Soil Organic Matter Content

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Raising the organic matter in soil is useful both from agricultural and environmental standpoint. Manure recycling seems particularly suitable for this task. However it is difficult to find the optimal rate because manuring is expensive and environmentally risky (Campbell et al., 2001). Manure benefits should be long lasting for its high content of humic substances. Direct and residual effects of manure supplied to various rotations have been studied for 36 years in the fertile Po Valley, near Bologna.

## Methodology

A long-term trial, which started in 1966 and is still in progress at Cadriano (Bologna), tests rainfed rotations with different levels of cattle manure supply. A 9-year rotation (3 cycles of corn-wheat alternated with a 3-years alfalfa meadow), a 2-year rotation (corn-wheat), continuous corn and continuous wheat, are compared at three intensities of manuring (M0, M1, M2), corresponding to 0, 20 and 40 t ha<sup>-1</sup> year<sup>-1</sup> of fresh material, respectively, without inorganic fertilizers. Plots are arranged according to a split-plot design replicated twice (56 m<sup>2</sup> sub-plots). In 1984 (after two cycles of the 9-year rotation) M2 supply was interrupted to study manure residual influence. In the trial crop yields are annually assessed and the soil (silty loam, low in organic matter) is sampled to 0.4 m depth for organic matter analysis (Walkley-Black acid digestion method).

## Results

Until 1984, when M2 was actually applied, manure effects varied with rotations (Table 1). In particular, the lower rate (M1) was most beneficial to continuous crops; a little less to those inserted in 2- and 9-year rotations. Doubling the rate further increased yields (+ 16% for wheat and corn; +12% for alfalfa), and masked any influence of rotation. This confirms how manure improves fertility of medium textured soil, even when applied at high rates, and the greater nutritional requirements of simplified rotations.

Wheat grain (t ha <sup>-1</sup> at 13% moisture)			
<i>Rotations</i>	<i>9-years</i>	<i>Corn-Wheat</i>	<i>Wheat-Wheat</i>
Control	2.87	2.21	1.83
Manure1	3.44	2.86	2.58
Manure2	4.00	3.38	2.98
Corn grain (t ha <sup>-1</sup> at 15.5% moisture)			
<i>Rotations</i>	<i>9-years</i>	<i>Corn-Wheat</i>	<i>Corn-Corn</i>
Control	5.49	3.63	3.35
Manure1	6.82	5.37	5.21
Manure2	7.94	6.48	5.83
Alfalfa hay (t ha <sup>-1</sup> at 20% moisture)			
Control		7.25	
Manure1		10.10	
Manure2		11.32	

Table 1. Direct effects of manure supplied at two rates on wheat and corn yields in various rotations and on alfalfa in 9-year rotation. Averages of the first 18 years (1967-1984)

M2 interruption caused a progressive yield reduction over the years (figure 1). In 1993, nine years after the interruption, both cereals that had received the double rate gave yields not different from those in the unstopped M1 treatment.

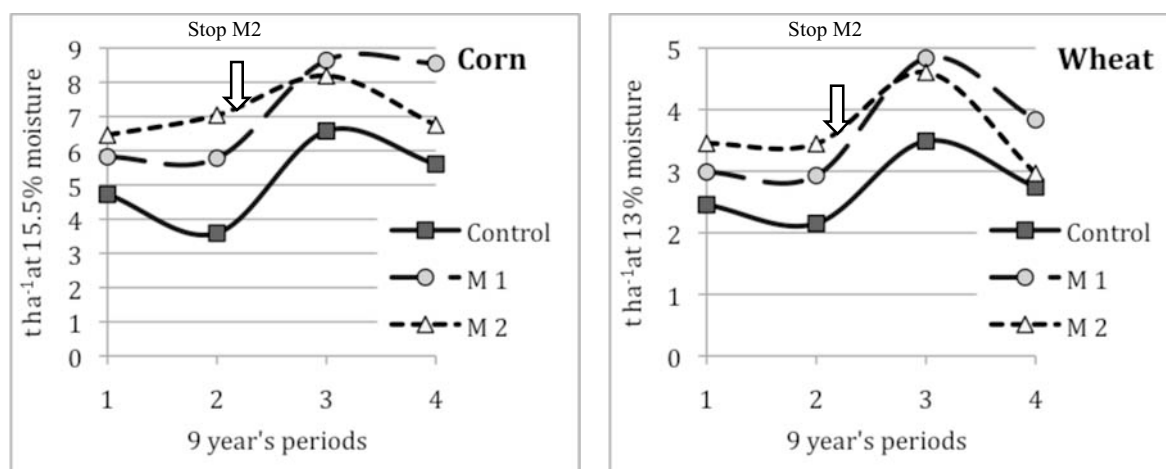


Figure 1. Influence of manure supply on grain yield of corn and wheat. Averages of 9 years. The last two M2 data of both crops reflect the residual effect of manuring that was interrupted in 1984.

On average, from 1984 to 1993, M2 yields decreased by 72% and 28% in corn and wheat, respectively. In the following 9 years (1994-02) residual effects almost disappeared in wheat, while in corn they still resulted in an average +20% yield compared to the control. Those effects varied according to rotation only in corn, for which alfalfa presence reduced M2 difference from the still manured M1 (-0.2, -1.4 and -1.7 t ha<sup>-1</sup> in 9-year, 2-year rotation, and continuous corn, respectively). M2 interruption scarcely affected alfalfa hay production, probably for the high N availability during the leguminous meadow. The residual effects of manure on yields can be mainly ascribed to an enrichment of organic matter in the soil (table 2). Indeed, even after 18 years since manuring interruption, M2 plots showed +14% soil organic matter content than the control.

Periods	Control	Manure 1	Manure 2
1973-1975	1.14	1.22	1.33
1982-1984	0.99	1.09	1.21
1991-1993	1.02	1.17	(1.26)
2000-2002	1.10	1.36	(1.13)

Table 2. Direct and residual (in brackets) effects of manuring on the soil organic carbon concentration (%) in 0-0.4 m soil layer. Averages of 9-years periods of all rotations and crops.

## Conclusion

Manure can reduce the negative effects of continuous crops, even when used at high rates (40 t ha<sup>-1</sup> year<sup>-1</sup>). Its benefits to yields lasted at least for 18 years, particularly in corn and without alfalfa. They can be ascribed to an enrichment of soil organic matter. Thus this by-product revealed particularly precious. Unfortunately in Italy it is scarcely available now, and its spreading costs are high.

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# Classification of Winter Triticale Cultivars Based on Nitrogen Status Measurements

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## Introduction

Strong relationships between chlorophyll, nitrogen (N) content and SPAD readings have been well documented in the literature for different plant species. However, cultivar and growth stage influence chlorophyll meter readings, obtained by non destructive measurements, making the calibration of this tool difficult.

The paper is aimed at investigating genotypic variability of N status indicators among (dwarf and traditional) winter triticale cultivars at subsequent growth stages.

## Methodology

In the years 2001-2003 a field experiment localized on a good rye complex at the Chylice Experimental Station (52° 05' N , 20°33' E) was performed. Data coming from one-year measurements were used for statistical analyses. Sixteen winter triticale (x *Triticosecale* Wittm.) genotypes were grown in a field at three different nitrogen rates, i.e., 0, 80 and 170 kg ha<sup>-1</sup>.

A split plot treatment design was used. Triticale cultivars were assigned as the main plots, nitrogen rates as the subplots. A detailed description of growing conditions is presented in a paper of Kozak et al. 2007.

Twenty, the newest fully expanded leaves were used at stages GS 31 and GS 49 (Zadoks et al., 1974) for SPAD-502 measurements, chlorophyll and N content analyses. SPAD values were also obtained for stage GS 65. The mean values of observed traits were used for multivariate classification of cultivars. Cluster analysis was performed with Ward's method using Statgraphics v.4.1. The correlation coefficients were calculated between values of the same traits in subsequent measurements.

## Results

The cluster analysis allowed to identify 3 groups of cultivars (table 1). Group 1 had the highest N content while group 3 the lowest. Only 7 cultivars belonged to the same group at the two growth stages. This means that the remaining genotypes exhibited different N status in time. Correlation coefficients calculated to measure the relationship between the same traits at two growth stages indicated that N content characterizes by the highest genotypic variability in between measurements (0,35 compared to 0,55 and 0,52 for SPAD and chlorophyll content (CC), respectively).

As among the last two traits only SPAD measurements have a practical use for plants N status assessment, further relationships between SPAD values for the same genotype at three growth stages were investigated. SPAD values at three N rates were treated as variables in Ward's cluster analysis. The results showed that only 3 cultivars (Disco, Magnat, Ugo) showed no variability of SPAD readings in the course of time (table 2).

Variability of SPAD values for the other cultivars indicates that genotypes were classified into different groups at subsequent growth stages. Cultivars Woltario, Alzo, Janko and Lasko, belonged to group 3 with high SPAD values at GS 31, than group 2 (medium SPAD values) at GS 49 and finally group 1 at GS 65 with low SPAD readings. Not always, like in this case, a clear decreasing trend of SPAD values during plant vegetation was observed. Cultivars Lamberto, Prado and Marko revealed non directional SPAD readings changes in course of their growth.

Table 1. Multivariate classification (number of groups) of triticale cultivars based on chlorophyll, nitrogen (N) content and SPAD readings

cultivar	GS 31	GS 49	cultivar	GS 31	GS 49
<b>Lamberto</b>	<b>1</b>	<b>1</b>	Bogo	3	2
Kitaro	3	2	<b>Tornado</b>	<b>3</b>	<b>3</b>
Disco	<b>3</b>	<b>3</b>	<b>Prado</b>	<b>3</b>	<b>3</b>
Fidelio	1	3	Marko	2	3
Pronto	1	2	Ugo	1	2
<b>Magnat</b>	<b>3</b>	<b>3</b>	Alzo	2	1
<b>Woltario</b>	<b>3</b>	<b>3</b>	<b>Janko</b>	<b>3</b>	<b>3</b>
DED798	2	1	Lasko	2	3

Table 2. Multivariate classification (number of groups) of triticale cultivars based on SPAD readings obtained with three N rates

cultivar	GS 31	GS 49	GS 65	cultivar	GS 31	GS 49	GS 65
Lamberto	1	2	1	Bogo	3	1	1
Kitaro	3	1	1	Tornado	3	2	2
<b>Disco</b>	<b>3</b>	<b>3</b>	<b>3</b>	Prado	3	2	3
Fidelio	2	2	1	Marko	3	2	3
Pronto	2	1	1	<b>Ugo</b>	<b>1</b>	<b>1</b>	<b>1</b>
<b>Magnat</b>	<b>3</b>	<b>3</b>	<b>3</b>	Alzo	3	2	1
Woltario	3	2	1	Janko	3	2	1
DED798	3	1	1	Lasko	3	2	1

## Conclusions

Plant nitrogen status measured with 3 methods showed high cultivar variability and a relatively weak relationship between subsequent measurements. Grouping of triticale cultivars only in terms of SPAD readings even increased the variability. Therefore establishment of SPAD threshold values which would indicate optimal plant N status of a specific cultivar and would be reliable at several growth stages is rather impossible.

Location of research sites in various growing conditions to create SPAD threshold values seems only to increase the SPAD values variability as there are numerous factors, besides nitrogen effecting plant greenness. The use of existing cultivar correction tables to guide the timing of N fertilizers seems to be questionable. A method based on nitrogen sufficiency index (NSI), as proposed by Peterson (1993), indicates the N deficiency onset for a particular cultivar in the site which is going to be fertilized. Such an approach may more correctly indicate the need for in-season N fertilization in farm conditions.

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# Evaluation of Slow Release Nitrogen Fertilisers for Tall Cabbage Grown in Autumn

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Tall cabbage (*Brassica oleracea*, var. *costata*, cv. Penca de Mirandela) is very popular in Portugal. It forms part of the traditional Christmas Eve dinner every year. The nurseries are prepared in the middle of summer and the young plants transplanted late in August. The highest growth rates of this vegetable as well as the highest demands for nitrogen (N) occur in October and early November, a typical rainy period where there is a high risk of nitrate leaching. Therefore, the use of slow release N fertilisers may be a viable option as a sound N fertilization strategy for this production system. The objectives of the research were to examine the effect on crop yield of three materials that delay N availability to plants and also to monitor when N becomes available to the crop. Results of a pot experiment are presented where tall cabbage was grown in the autumn as a main crop and Italian ryegrass (*Lolium multiflorum* L.) was sown in spring in order to evaluate the residual effect of fertilisers.

## Methodology

Young plants of tall cabbage were planted on 21 August 2007 in pots with 15 kg of screened (6 mm mesh) and air dried soil mixed with the respective fertilisers. The fertiliser treatments were: Urea, applied at rates of 1.47 g/pot (Urea1/2) and 2.94 g/pot (Urea1); Floranid Permanent 16-7-15 (slow-release, IBDU/Isodur fertiliser); Basacote plus 9M 16-8-12 (controlled-release fertiliser, copolymer ethylene acrylic); and Entec 26 (stabilized-fertiliser, DMPP as nitrification inhibitor), applied all at rates of 2.94 g/pot. A zero N control was also included. Phosphorus and potassium rates were balanced by using singular-granular superphosphate and potassium chloride. Five replicates per treatments were provided. The plants were irrigated with distilled water and the pots protected from rain to avoid nitrate leaching. The plants were cut at the ground level on 14 Dec 2007. Thereafter the soil was kept moist to stimulate microbial activity. On 4 Apr 2008, 1 g/pot of seed of Italian ryegrass was sown to recover the residual N released from December. Italian ryegrass was cut on 17 May 2008. Collected data at harvest of tall cabbage included above-ground plant dry matter (DM), tissue N concentration (TNC) and petiole nitrate concentration (PNO<sub>3</sub>C). Nitrogen uptake and apparent N recovery (ANR) were estimated from collected data. Dry matter yield and tissue N concentration were also recorded from Italian ryegrass. Nitrogen released from soil and fertilisers was monitored by recovering the NO<sub>3</sub><sup>-</sup> in 1x2 cm strips of an anion exchange membrane (AEM) inserted directly into the soil and kept there for a week. Soil NO<sub>3</sub><sup>-</sup> concentrations were determined on 29 Sep 2007, 24 Oct 07, 5 Dec 07 and 4 Apr 2008. N concentration in tissues was determined in a Kjeltac Autoanalyser 1030. Nitrate concentration in extracts, prepared from petioles and from AEM, was determined by UV-Vis. spectrophotometry.

## Results

The dry matter yields of tall cabbage differed significantly among treatments. Maximum (82.9 g/pot) and minimum (32.8 g/pot) values were recorded in Urea1 and Control treatments, respectively (Table 1). TNC and PNO<sub>3</sub>C differed also among treatments. However, Entec and Floranid yielded similar results than Urea1. Values of N uptake and ANR were also very similar among Entec, Floranid and Urea1. Tall cabbage plants recovered 80 to 90% of N applied as Urea, Entec and Floranid, whereas the ANR from Basacote was only 32.9%. Soil nitrate concentration 31 days after fertiliser application was higher in Floranid and Urea1 treatments (Fig. 1). The results of Entec and Urea1/2 were similar and the result of Basacote significantly higher than the Control. Nitrogen uptake from Italian ryegrass was significantly higher for Basacote (0.47 g/pot) than for any of the other treatments (Table 1). Floranid,

Entec and Urea1 showed similar N uptake values. Nitrogen uptake from Urea1/2 was not significantly different than N uptake from Control. Total ANR for both the crops were in the range of 85.7 and 95.7% from Urea, Floranid and Entec. Total ANR from Basacote was 41.6%. Nitrate concentration in AEM extracts 220 days after planting was higher in Floranid, Basacote and Urea1 treatments (Fig. 1).

Table 1. Crop performance, N nutritional indices and N recovered as a function of fertiliser treatment

Fertiliser treatment	-----Tall cabbage-----					I. ryegrass	Both crops
	DM yield (g/pot)	TNC (g kg <sup>-1</sup> )	PN <sub>2</sub> O <sub>3</sub> C (g kg <sup>-1</sup> )	N uptake (g/pot)	ANR <sup>1</sup> (%)	N uptake (g/pot)	ANR <sup>1</sup> (%)
Control	32.8 b	19.7 c	22.9 b	0.64 c	---	0.21 d	---
Urea1/2	62.5 ab	29.3 bc	47.0 b	1.83 b	81.4	0.29 cd	85.7
Urea1	82.9 a	40.0 ab	107.0 a	3.21 a	87.2	0.36 bc	92.3
Entec	68.5 a	47.0 a	111.8 a	3.18 a	86.2	0.34 bc	90.8
Basacote	66.5 a	24.5 c	36.7 b	1.61 bc	32.9	0.47 a	41.6
Floranid	78.5 a	42.1 a	102.7 a	3.29 a	90.0	0.36 b	95.1

<sup>1</sup>(N uptake, from fertiliser treatments – N uptake, from control) / (N applied as fertiliser) x 100

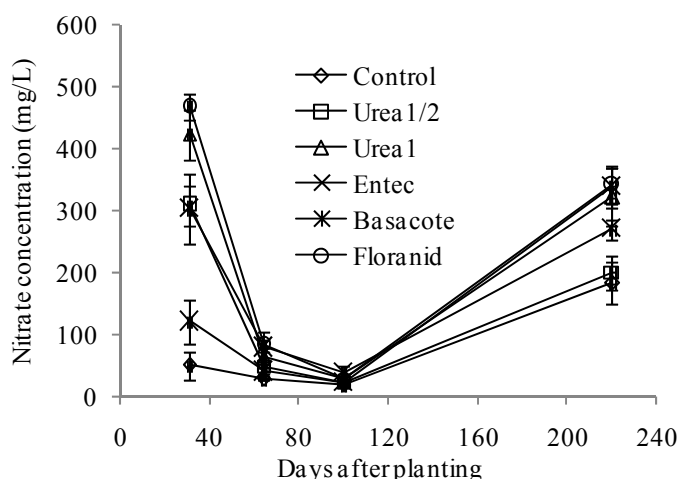


Figure 1. Nitrate concentration in AEM extracts

## Conclusions

Basacote showed a marked slow release pattern. According to the marketing specifications of the product it is intended to release nutrients over 9 months. In this experiment 9M after fertiliser application only 41.6% of N was recovered by the crops. Basacote continued to actively release N 220d after planting. On 4 Apr NO<sub>3</sub><sup>-</sup> concentration in AEM extracts from Basacote was 339 mg/L. Floranid yielded similar NO<sub>3</sub><sup>-</sup> concentration in AEM extracts as Urea1 at 31d after fertiliser application. Nitrogen of urea is available to plants after hydrolysis to

NH<sub>4</sub><sup>+</sup> and nitrification to NO<sub>3</sub><sup>-</sup>. In moist and warm soils these processes occur in a few weeks (Rodrigues, 2004). The N in Floranid is 2.1% NO<sub>3</sub><sup>-</sup>, 7.9% NH<sub>4</sub><sup>+</sup> and 6.0% IBDU. Nitrogen released from IBDU is mainly affected by soil moisture and temperature (Trenkel, 1997). Thus, the apparent rapid hydrolysis of IBDU would be due to the favourable conditions in terms of soil moisture and temperature. Entec contains 7.5% N-NO<sub>3</sub><sup>-</sup> and 18.5% N-NH<sub>4</sub><sup>+</sup> and also 0.8% DMPP. Nitrate concentration in AEM extracts 31d after planting was lower in Entec than in Urea1 and Floranid. It seems that DMPP effectively delayed NH<sub>4</sub><sup>+</sup> nitrification. Further studies under field conditions that allow for N (urea, NO<sub>3</sub><sup>-</sup>, ...) leaching are needed to clarify the potential of Entec and Floranid for use in the autumn growing cycle of tall cabbage. Even not considering the high cost of Basacote plus 9M, it was clear that it would not be advisable to use it in a crop of a growing cycle of 4 months.

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# Sustainable N Management in Oilseed Rape

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## Introduction and objective

During the last decades the acreage of winter oilseed rape (OSR) has been increased considerably in Europe. Winter wheat is often grown subsequently using OSR as favorable preceding crop. However, large N losses via leaching are frequently observed during winter, mainly due to large amounts of soil mineral N available after the OSR harvest and the small N uptake of wheat in autumn (Sieling and Kage, 2006). In order to minimize the environmental impact of OSR cropping, several approaches are discussed and confirmed by results of own field trials, mainly carried out at the Hohenschulen Experimental Farm which is located in NW Germany near Kiel.

## N fertilization in autumn

Applying autumn nitrogen to oilseed rape is common practice in NW Europe, especially if the sowing date is delayed (e.g. if following wheat) and/or under minimum tillage. To compensate for these worsening of establishment conditions, farmers additionally apply about 30-50 kg N/ha in autumn, often directly upon the stubble of the preceding crop, to ensure crop N supply and adequate crop growth before winter.

In general it has to be distinguished if OSR is actually able to take up autumn applied N before winter. If not, and this is mainly the case if low temperature limits OSR growth due to delayed sowing dates, applied N increases the soil mineral N ( $\text{NO}_3\text{-N}$  plus  $\text{NH}_4\text{-N}$ : SMN) pool and, in consequence, the potential of N leaching during the subsequent percolation period (Sieling and Kage, 2007). If autumn N leads to a better growth and enhances N accumulation before winter, it is a moot question whether the seed yield increases as well. Ogilvy and Bastiman (1992) reported that, although plots receiving nitrogen in the seedbed or at the two leaf stage appeared more vigorous before winter compared with unfertilized plants, neither the number of plants established, the survival over winter nor the seed yield appeared to be affected by this treatment. Sieling and Kage (2007) observed after an autumn N supply of 40 kg N/ha a yield increase of about 0.2 t/ha which was related to an additional N offtake by the seeds of about 4 kg N/ha (Tab. 1). In maximum only 10% of the applied N amount was removed from the system, whereas 36 kg N/ha remained in the soil and increased the N surpluses. However, in other experiments autumn nitrogen gave a small yield response where the preceding cereal straw was baled or incorporated instead of burning (Chalmers and Darby, 1992). No N should be applied in autumn since the effects on seed yield are small (Sieling et al. 1997).

Table 1: Effect of 40 kg N/ha applied in autumn on above-ground dry matter before winter, N uptake before winter, seed yield and simple N balance of the autumn applied fertilizer N of OSR (2003/04-2005/06, cv. Talent, minimum tillage following wheat)

	Above-ground dry matter ( $\text{g/m}^2$ )	N uptake (kg N/ha)	Seed yield (t/ha)	N balance (kg N/ha)
Unfertilized control	48.2 <sup>b‡</sup>	18.2 <sup>b</sup>	4.59 <sup>b</sup>	-
40 kg N/ha on the wheat stubble	68.1 <sup>ab</sup>	24.7 <sup>b</sup>	4.71 <sup>ab</sup>	+36
40 kg N/ha directly after drilling	86.6 <sup>a</sup>	36.3 <sup>a</sup>	4.69 <sup>ab</sup>	+37
40 kg N/ha in the 2-4 leave stage of OSR	87.0 <sup>a</sup>	36.7 <sup>a</sup>	4.81 <sup>a</sup>	+33

<sup>‡</sup> Within a column, means followed by the same letter are not significantly different at  $P < 0.05$ .

### **N fertilization in spring**

An exact estimation of fertilizer N demand becomes increasingly important to minimize the environmental impact, but also to achieve high seed yields and maximum economic returns. A balance sheet methods regarding the amounts of mineralization of soil borne N and the amount N in OSR canopies at the end of autumn and the end of winter have been developed in France (Makowski et al., 2005) resulting in an optimized N fertilization of OSR with on average lower doses of N fertilizer. In a series of field trials consisting of 6 sites and 2 years, Henke (2008) tried to verify this approach under different climatic conditions in order to derive site specific N fertilization rates. In his experiments, canopy N in autumn was the most suitable parameter to optimize the N supply in spring. The regression showed a significant negative relationship between canopy N in autumn and optimal N amount, indicating that these N amounts should be partly considered when calculating N fertilization rates. Canopy N in autumn can be an indicator for the mineralization potential of the site. The implementation of this approach into the practical use by farmers is based on an average OSR canopy with 50 kg N/ha accumulated in autumn which will be fertilized in spring according to the official recommendation (e.g. 200 kg N/ha). Each kg N/ha exceeding the threshold of 50 kg N/ha reduces the N fertilization in spring by 0.7 kg N/ha. However, it is not possible to give absolute recommendations. In future, autumn canopy N can be site-specifically estimated using remote sensing, resulting in an optimized N distribution within the field.

### **N dynamic after OSR harvest**

According to Di and Cameron (2002) the prevention of SMN accumulation in the soil after harvest is the key to reduce N leaching. First of all reducing tillage depth and delaying tillage after harvest diminish soil disturbance and, consequently soil N release, since intensive tillage operations after harvest stimulate net mineralization of soil born N due to soil disturbance (Lickfett, 1993). Secondly, Changing the crop rotation by growing a catch crop which prevent N from leaching followed by a spring crop is very effective to reduce N losses from the system, but the acceptance by the farmers remains quite low due to economic losses.

### **Conclusions**

Despite the above addressed problems, OSR is indispensable as a favorable preceding crop for cereals, especially wheat. Growing legumes will raise similar problems as in OSR, whereas the area of sugar beets is limited. Increasing the percentage of wheat within the rotation, up to monoculture, is no sustainable alternative, because wheat yield decreases while the amount of fertilizer N will increase to achieve optimal yields.

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# Highlights of an Experimental Study on the Vulnerability of a Soil-Crop System to Drought and Saline Water

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Climate change, if it occurs as projected, might have significant implications for agricultural and water resources. Among them soil moisture changes, which reflect changes in agricultural water availability, are of special concern due to their direct impact on crop growth. In this situation drought and salinity can be considered as related stressors and influence the productivity of agricultural systems. The present work is a review of the results of 7 years experiments in areas where the sea water intrusion affects the soil hydrological physical properties and the crop production.

## Methodology

On two sites, Torre Lama (TL) and Vitulazio (VT), at each spring soil samples were taken at different depths to determine the ECe and ESP, moreover from the top soil layer samples were taken to determine the index of aggregates water stability in water (IASW). Undisturbed soil samples were taken too in different years on each sites to determine the hydrological parameters  $h(\theta)$ , and the soil porosity by image analysis. The impact of the different soil hydrological properties on the temporal variability of soil water content was evaluated for a range of meteorological conditions, in particular for a dry, wet and normal climatic year. At VT the yield response of sunflower, sweet pepper and eggplant growth under saline water irrigation was tested. Treatments consisted in a factorial combination of 3 treatments (T025, T05, T1) irrigated with water to a concentration of 0.25, 0.5 and 1% of NaCl and a control (T0) irrigated with fresh water.

## Results

Soil properties and hydrology. At both sites, for the 20 cm depth, the ECe measured each year in spring shows, only for the treatments with 10 g l<sup>-1</sup> in the irrigation water determined a progressive soil salinization. ECe measured each year in spring increased significantly for all the treatments evaluated in both sites (VT and TL). The ESP (VT) increases with the saline concentration of the irrigation water in the layer of 0-20 cm and gradually decreases with depth where the ESP is higher than in the other layers.. Same trend was observed for TL. Irrigation with saline water led to an increase of ESP and a degradation of the soil physical properties that were estimated indirectly by measuring IASW. For the layer 0-0.15 m layer IASW was inversely correlated to the ESP values at VT and TL sites. In the latter site soil structure degradation occurred also in the deepest layers.

At TL the  $h(\theta)$  curve of the 1% saline water treatments had lower values of  $\theta$  than the 0% treatments at the same pressure head, while at VT, the total porosity for the  $I_0$  is around 0.55 against 0.58 of the  $I_1$ , in contrast with what was observed for the TL (Fig.1). Such difference could be due to the shorter duration of saline water application at VT (2-yr) than at TL (5-yr). Moreover, the difference between TL and VT might depend from the type of clay minerals: at VT there is an abundance of smectite-type clay, while at TL illite-type. Despite these differences in total porosity of the two sites, the soil water retention capacity (WRC), calculated between 100 cm and 10<sup>4</sup> cm pressure head, showed a similar trend at the two sites. At TL for all the irrigation treatments, the control had lower WRC-values than the saline treatments (e.g 0% T<sub>2</sub> 20-30 cm WRC = 30.8 mm vs. 1% T<sub>2</sub> 20-30 cm WRC = 74 mm). At VT, even if the total porosity was different than TL, the calculated AW showed the similar trend ( $I_0$  at VT had WRC = 128 mm vs. 178 mm of  $I_1$ ). The total porosity at TL changed from about 29% ( $I_0$ ) to 20% for the  $I_1$ , while at VT site values ranged from 21 to 15% for  $I_0$  and  $I_1$ , respectively.

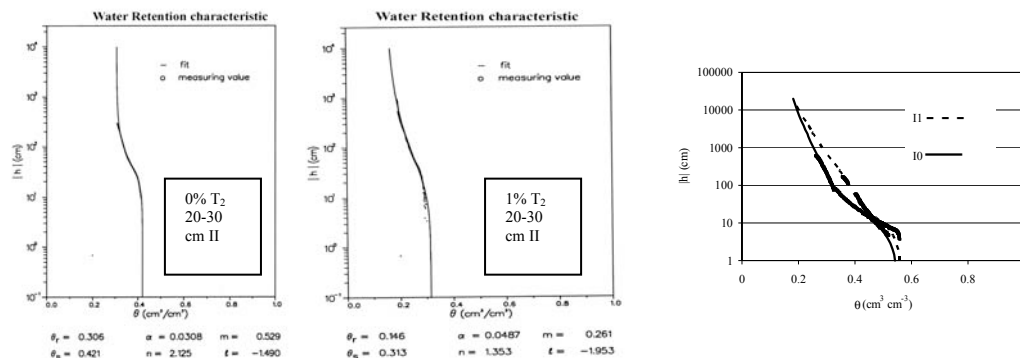


Figure 2 Water retention characteristics: (on left) TL site treatments 0% and 1% with 2-day frequencies in layer 20-30 cm.; (on right) Water retention characteristics VT site for treatment I<sub>0</sub> and I<sub>1</sub> in the layer 0-10 cm.

The numerical experiments showed that the differences in  $h(\theta)$  and  $K(h)$  (unsaturated capillary conductivity) of I<sub>0</sub> and I<sub>1</sub> led to higher available water (AW) for I<sub>1</sub> against the I<sub>0</sub> treatment through the year: In the dry year fewer days with  $AW < 124$  mm respectively 119 mm were obtained for I<sub>1</sub>, i.e. 105, than for I<sub>0</sub>, i.e. 152. In the wet year the number of days for the same cases was 46 for I<sub>1</sub> and 67 for I<sub>0</sub>. Crop. The Maas and Hoffman analysis was applied to sunflower yield to give a slope of 5.88 (m dS<sup>-1</sup>), while the 50% yield reduction was reached at  $E_{ce} = 9.2$  dS m<sup>-1</sup> level. These results indicate that this species is moderately tolerant to salinity; therefore it could be cropped in environments affected by salinity problems. Sweet pepper yield decreased with  $E_{ce} > 1.23$  dS m<sup>-1</sup> while the 50% of yield loss was reached at  $E_{ce} = 5.11$  dS m<sup>-1</sup> and it was zero at  $E_{ce} 8.99$  dS m<sup>-1</sup>, thus characterizing this species as moderately sensitive to salinity. Eggplant was also moderately sensitive to salinity since the relative yield decreased with  $E_{ce} > 1.11$  dS m<sup>-1</sup> with a slope of 9.84 ((m dS<sup>-1</sup>)).

Table 1 Absolute and relative difference in crop yield, Y (t ha<sup>-1</sup>) observed between a drier and a wetter year, ( $Y_{dry} - Y_{wet}$ ); relative differences are given as % of mean yield over the drier and wetter year.

Irrigation level	NaCl content	Sunflower '96 - '95		Sweet pepper '97 - '98		Eggplant '01 - '00	
		t ha <sup>-1</sup>	%	t ha <sup>-1</sup>	%	t ha <sup>-1</sup>	%
100%	0%	0.56	12	0.70	3	4.92	7
100%	0.25%	0.57	13	7.49	45	-6.68	-11
100%	0.50%	0.47	11	8.03	58	-17.12	-40
100%	1%	0.39	10	1.75	50	-7.52	-41
40%	0%	0.47	11	5.18	71	18.89	59
40%	0.25%	0.54	13	4.12	72	16.41	59
40%	0.50%	0.66	15	3.35	76	18.96	66
not irrig.	-	0.38	9	0.37	19	6.07	54

1997, ( $P - ET_0$ ) = - 432.6 mm, and 1998, ( $P - ET_0$ ) = - 425.7 mm. Eggplant in 2000, ( $P - ET_0$ ) = - 431.6 mm, and 2001, ( $P - ET_0$ ) = - 485.6 mm. For almost all irrigation and saline treatments yield was higher in the drier than in the wetter year (Table 1).

On the one hand these results call for caution: differences in net water demand ( $P - ET_0$ ) between 1997 and 1998, when sweet pepper was grown, were negligible. Overall, however, yield was higher in the drier year of each pair. Exceptions were the saline treatments of the eggplant crop: here full irrigation implies a very high salt load, especially in a dry year, which may explain the results.

## Conclusions

The question of vulnerability of a soil – crop system requires taking into account the positive effects of saline water on the water retention capacity of soils and of the yield response to relatively small changes in water availability.

# Effect of Cadmium on Plant Growth, Dry Matter Accumulation, Cd, and Zn Uptake of Different Tobacco Cultivars (*Nicotiana tabacum* L.)

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Cadmium (Cd) is a heavy metal that normally occurs in low concentrations in soils (Kirkham, 2006). However, there are several areas in many countries where the concentration in the soil solution can reach up to 100–600 mg kg<sup>-1</sup> dry weight (Ernst and Neilssen, 2000). Anthropogenic activities such as the non-ferrous metal industry, mining and energy production from coal, application of pesticides and especially phosphate fertilisers are important sources of Cd contamination in many areas of the world (Kirkham, 2006). When Cd is taken up in excess by plants can inhibit directly or indirectly many physiological processes such as respiration, photosynthesis, cell elongation, plant–water relationships, N metabolism and mineral nutrition, enzymatic activities, membrane function and hormone balance resulting in poor growth and low biomass (Sanita di Toppi and Gabrielli, 1999; Foy, et al., 1978). The aim of the present study was to investigate: (1) the effect of Cd on the growth and development of different tobacco cultivars, (2) the differences among tobacco cultivars in Cd concentration and uptake, (3) the interactions between Cd and Zn with respect to concentration and uptake in tobacco plants.

## Methodology

In the present study 17 tobacco cultivars were used in a pot experiment. The soil type that was used in this study was a sandy clay loam (sand 493 g kg<sup>-1</sup>, silt 240 g kg<sup>-1</sup>, clay 267 g kg<sup>-1</sup>) and contained all the necessary nutrients for plant growth (NO<sub>3</sub><sup>-</sup> 84 mg kg<sup>-1</sup>, P (Olsen) 205 mg kg<sup>-1</sup>, K (exchangeable) 408 mg kg<sup>-1</sup>). The soil pH was 7.6. After the transplantation the plants were watered with Cd solution containing four different Cd concentrations 0, 100, 200, and 300 mg l<sup>-1</sup> and three replicates for each Cd level were used. The form of Cd that was used was CdCl<sub>2</sub>. Following after 45 days from transplantation the entire shoot of the plants was cut, dried in an oven, weighed and analyzed for Cd and Zn content using dry ashing and atomic absorption spectroscopy.

## Results

The Cd levels affected dry matter accumulation, Cd concentration, Cd uptake, Zn concentration, and Zn uptake (Table 1). Also cultivars affected dry matter accumulation, Cd concentration, Cd uptake, Zn concentration, and Zn uptake. In addition, the interaction between cultivars and Cd level affected only the Cd concentration and Cd uptake indicating that the effect of Cd on Cd concentration and Cd uptake differed with cultivars (Table 1). The difference in the response of Cd among the tobacco cultivars could be because lower Cd was transported from the roots to the shoots, lower amount of Cd can be present in the cytosol and higher amount can be bound to the cell wall (Sanita di Toppi and Gabrielli, 1999).

Zinc concentration was affected by the cultivar and also by the Cd level as in some cultivars the Zn concentration was lower and in others it was higher (Table 1). As Cd level increased there was a decrease at the Zn concentration and it was higher at the higher Cd levels. Zn uptake was affected by the cultivar and the Cd level much less than the Zn concentration. The Zn uptake was higher in the control and there were small changes in the different Cd concentrations.

The interaction between Cd and essential elements on uptake and distribution in crop plants is quite important and especially this one with Zn. There are many similarities between Cd and Zn uptake in the uptake mechanisms and long distance transport and distribution (Kabat-Pendias and Pendias, 1984). Cd can be transported most likely by transporters in the form of ions or chelate complexes. In addition, organic acids and other root exudates such as phytosiderophores can solubilize Cd, Zn, Mn, Cu, and Fe (Rohmeld, 1991).

**Table 1.** Analysis of variance of dry matter, Cd concentration and Cd uptake, Zn concentration and Zn uptake of the four levels of Cd in 17 tobacco cultivars.

	df	Dry matter	Cd concentration	Cd uptake	Zn concentration	Zn uptake
Cd levels (Cd)	3	15.37***	12613.8***	0.655***	551.4***	0.13***
Cultivars (C)	16	12.96***	858.5***	0.11***	240.46***	0.052***
Cd x C	48	0.44	21.9*	0.0034**	26.03	0.02
Error		0.42	17.62	0.02	23.86	0.02
CV %		7.5	11	8	9	12

\* Significant at the 0.05 level of probability.

\*\* Significant at the 0.01 level of probability.

\*\*\* Significant at the 0.001 level of probability.

There was negative correlation between the leaf number and Cd concentration and Cd uptake. In addition, there was a negative correlation between Cd concentration and Cd uptake and the number of leaves, dry matter, Zn concentration, and Zn uptake (Table 2). There were also negative correlations between Cd and Zn as Cd accumulation increased, Zn accumulation decreased showing that the two heavy metals had antagonistic behaviour between them. These data agree with others who found similar responses (Cataldo et al., 1983).

**Table 2.** Pearson correlation coefficients among the different characteristics that were studied.

	Dry matter	Cd concentration	Cd uptake	Zn concentration
Dry matter	-			
Cd concentration	-0.524***	-		
Cd uptake	-0.324***	0.96***	-	
Zn concentration	0.706***	-0.542***	-0.380***	-
Zn uptake	0.264***	-0.422***	-0.471***	0.817***

\* Significant at the 0.05 level of probability.

\*\* Significant at the 0.01 level of probability.

\*\*\* Significant at the 0.001 level of probability.

## Conclusions

Cadmium level affected dry matter accumulation and there were differences among the different cultivars that were used. Significant differences were found among the cultivars for Cd concentration and uptake. Also there were negative correlations between Cd concentration and Zn concentration, as Cd accumulation increased, Zn accumulation decreased, showing that the two heavy metals were antagonistic. Tobacco cultivars that accumulate high amount of Cd can be used for phytoremediation of Cd-contaminated sites.

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# Nitrogen and Zeolite Application Effect on the Yield of Canola, Nitrogen Leaching Loss and Nitrogen Efficiency in a Sandy Soil

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## Introduction

Canola (*Brassica napus* L.) production has undergone a rapid increase in recent decades as a dominant oilseed crop in the world (Ozer, 2003). Generally, it requires high amounts of N but is characterized by low N efficiency (Rathke et al., 2006). Nitrogen is the most important fertilizer for crop yield, and sustainability of the environment. So the efficient use of N fertilizers in crop production is of major importance. The wide use of N fertilizer is unfortunately not entirely free from risk. Grant and Bailey (1993) summarized the most important information and strategies on N-fertilizer management in canola production, such as cultivars, previous crop and the corresponding N rate, form, time and method of application. In recent years, application of natural Zeolite in soil known as a soil treatment for reducing N leaching and increase N efficiency especially in sandy soils (Leggo, 2000; Rehakova et al., 2004).

Amendment of clinoptilolite Zeolite to sandy soil has been reported to increase moisture and nutrients in the soil due to increase soil surface area and cation exchange capacity (CEC) (He et al., 2002). Two main objective of present study were determining response of canola to different nitrogen rates and finding the role of natural zeolite in reducing N leaching and improving NUE for canola under field circumstances in a sandy soil.

## Methodology

Experiment was conducted at a sandy loam soil with relatively low water holding capacity (and low CEC (approximately 6 meq per 100 g) with a pH of 7.7 in Tehran, (35° 44' N, 51° 16' E, 1352 masl, Iran on 2006-2007 growing season. Treatments were arranged in randomized complete blocks with three replications and consisted of a factorial combination of three N levels (90, 180 and 270 kg N/ha) and four Zeolite rates (0, 3, 6 and 9 t Zeolite/ha). N-fertilizer was applied as urea [Co (NH<sub>2</sub>)<sub>2</sub>] forms. In this work, a zeolitised volcanic tuff containing K-Ca clinoptilolite with an exchange capacity in the order of 200 meq per 100g has been used. Canola (cv. Okapi) was sown on 4th October 2006. Each plots consisted of 10 rows (30 cm apart) and 4m long. Plots were over seeded and subsequently thinned (4cm between seedlings) to final plant density of about 83 plant/m<sup>2</sup>. Grains harvested on 15th June 2007. All data were analyzed statistically using GLM procedure in SAS (SAS Institute, 2000). Least significant difference (LSD0.05) was used for means comparison.

## Results

The result showed that Similar to biological yield the highest grain yield (2452.3 kg/ha) obtained from N270Z9 treatment and control treatment (N0Z0) produce the lowest grain yield (1038.3 kg/ha). Moreover using 270 kg N/ha without Zeolite (N270Z0) has the highest amount of N leaching loss (144.23 kg/ha). Zeolite application has a clear reducing effect on N leaching loss for all N rates. It found that N leaching loss is a main reason for low nitrogen use efficiency in high nitrogen application (Table 1). It seem the

profitability of N<sub>270</sub>Z<sub>9</sub> treatment in N uptake (N concentration × DM.) is related to more biological yield and its high N concentration that resulted from more N rate and extended availability of N by Zeolite. Despite of nitrogen levels, treatment including 9 t Zeolite/ha showed higher NUE than others. For instance a comparison of N<sub>90</sub>Z<sub>9</sub> with N<sub>90</sub>Z<sub>0</sub>, N<sub>180</sub>Z<sub>9</sub> with N<sub>180</sub>Z<sub>0</sub> and N<sub>270</sub>Z<sub>9</sub> with N<sub>270</sub>Z<sub>0</sub> illustrated 4, 23 and 29 % increase in NUE.

**Table 1- Effect of N and Zeolite on biological and grain yield, nitrogen concentration and uptake in dry matter and grain and nitrogen leaching loss**

N	Z <sup>•</sup>	Biological	Seed	N con <sup>•</sup>	N con	Uptake N in	Uptake N in	N leaching
Rate		Yield	Yield	in dry matter	in seed	dry matter	seed	loss
	kg/ha t/ha	(kg/ha)	(kg/ha)	(g/kg)	(g/kg)	(kg/ha)	(kg/ha)	(kg/ha)
0	0	5274.9	1038.3	8.38	21.84	44.23	22.65	13.09
90	0	6575.6	1569.3	10.49	27.31	68.51	42.56	28.29
180	0	7162.8	1811.5	12.02	30.55	84.62	54.91	65.63
270	0	7550.5	1896.2	12.80	32.37	95.37	60.97	144.23
90	3	6523.0	1531.9	10.75	27.48	69.68	41.84	25.55
180	3	7181.7	1856.7	12.36	30.44	87.12	55.86	59.17
270	3	7700.5	1951.5	13.36	32.07	100.85	61.79	128.81
90	6	6473.3	1502.8	11.04	27.61	70.46	40.66	22.72
180	6	7384.8	1903.1	13.36	31.19	97.28	58.32	50.72
270	6	8239.3	2055.3	15.02	35.42	124.54	72.71	107.72
90	9	6958.8	1637.6	11.42	28.48	87.63	48.53	20.26
180	9	8003.9	2228.2	14.16	32.58	111.56	71.91	43.17
270	9	8828.7	2452.3	16.77	36.10	146.36	88.42	91.93
Mean		<b>7219.11</b>	<b>1802.66</b>	<b>12.46</b>	<b>30.26</b>	<b>90.70</b>	<b>55.26</b>	<b>61.64</b>
P-value		<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
LSD		<b>656.01</b>	<b>208.89</b>	<b>2.10</b>	<b>2.88</b>	<b>21.9</b>	<b>10.14</b>	<b>11.29</b>
SE		<b>182.68</b>	<b>64.54</b>	<b>0.56</b>	<b>0.84</b>	<b>5.08</b>	<b>2.81</b>	<b>6.81</b>

• concentration

## Conclusions

Summarizing results indicate that combine application of Zeolite and chemical nitrogen to canola production in a sandy soil, in addition to ensure us to reach an acceptable grain yield (agronomic importance), protect the soil from high N leaching loss (economical and environmental importance). So application of Zeolite in canola at light soils is scientifically recommended. However for determining more precise of Zeolite performance, more research is needed.

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# Maize Response to Repeated Compost Additions in Two Contrasting Environments in Italy

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The increase in the soil organic carbon (SOC) content has well-acknowledged positive effects on the soil chemical, biological and physical fertility. Recent global warming concerns have recommended maintaining and restoring soil carbon as a measure to mitigate rising atmospheric carbon dioxide (CO<sub>2</sub>) concentration (Follett, 2001; Lal, 2008). Organic fertilization is one of the most diffused practices that increase the SOC content (Smith, 2004), and composting park or municipal solid wastes has become an important way to recover organic matter from wastes (Gigliotti et al., 1996; Centemero, 2007).

The objective of this paper was to assess the potential of compost fertilizer addition to influence the maize crop yield and the soil carbon sequestration, compared to green manuring and minimum tillage. Preliminary results are here discussed.

## Methodology

A field trial was established in 2006 in two contrasting environments in Italy: Torino (TO) and Napoli (NA), in the framework of the MESCOSAGR FISIR project.

The climate was temperate sub-continental at the TO site and mediterranean at the NA site, the soil was loamy sand at TO, with a bulk density of 1.4 g cm<sup>-3</sup>, and clay loam at NA, with a bulk density of 1.3 g cm<sup>-3</sup>. The average SOC content was 1.14 at TO and 1.06 at NA, and the C/N was 12.1 and 9.7, respectively.

A compost (mixture of park, garden and separately collected urban waste) was distributed at c. 10 and 20 t ha<sup>-1</sup> before ploughing (COM1 and COM2), for two years. The same compost was used at the two sites. Its N content was 2.3 % (on a DM basis), C/N was 13 and the DM was 59%, on average.

Other treatments were vetch (*Vicia villosa*, Roth.) green manuring (SOV) and minimum tillage (MIN). Controls included no N fertilizer (0N) and 130 kg ha<sup>-1</sup> of urea-N (TRA). MIN plots were also fertilized with 130 kg urea-N ha<sup>-1</sup>. SOV plots were supplied with 130 kg ha<sup>-1</sup> of urea-N in 2006 at both sites, owing to the poor growth of the vetch, while in 2007 the net N supply, calculated considering N-fixation as 73% of the uptake (Borreani et al., 2003), was 125 and 50 kg ha<sup>-1</sup> at TO and NA, respectively, therefore an extra-amount of N as urea was supplied at NA, up to a total of 130 kg ha<sup>-1</sup>.

P and K were supplied via mineral fertilizer (100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 200 kg ha<sup>-1</sup> of K<sub>2</sub>O) to all treatments. Maize for silage was sown on the same day the plots were fertilized and ploughed. Plots were 30-48 m<sup>2</sup> at NA and TO, respectively, replicated 4 times and completely randomized.

Soil mineral nitrogen (SMN) extractable in 1M KCl was measured in the 0-60 cm soil horizon in all plots, 4 times per year (only a subset of data will be here discussed).

## Results

The crop response to compost addition was evident at the NA site in both years: the total crop yield and N uptake were heavily reduced by the compost addition, at both levels of supply. While no effect was noticed at the TO site in the first year, N uptake was reduced in the COM2 plots in the second year, and yield was slightly lower than that of the urea treatment (Table 1).

Table 1. Maize total DM production and N uptake. Letters refer to the SNK test.

	Total DM production (t ha <sup>-1</sup> )					Total N uptake (kg ha <sup>-1</sup> )				
	TO		NA		avg.	TO		NA		avg.
	2006	2007	2006	2007		2006	2007	2006	2007	
COM1	23.6	23.8 a	7.0 b	6.7 b	15.3	246	224 a	48 c	41 b	140
COM2	24.1	22.8 ab	9.5 b	8.6 b	16.3	261	203 ab	63 c	56 b	147
MIN	21.9	24.4 a	22.0 a	20.2 a	22.2	257	249 a	243 ab	184 a	233
SOV	26.6	24.4 a	21.5 a	17.1 a	22.4	301	222 a	254 a	180 a	240
TRA	25.4	23.9 a	21.9 a	18.7 a	22.5	287	252 a	253 a	186 a	245
0N	28.4	21.1 b	21.6 a	9.3 b	20.2	291	182 b	179 b	58 b	177

The negative effect of compost could be interpreted as an immobilization of N that was subtracted from the soil solution. To support this hypothesis, an indicator of potential mineralization of the native SOM was calculated in the 0N treatment, as N uptake + SMN(harvest) – SMN(sowing). At NA, it was 173 and 50 kg ha<sup>-1</sup> in the two years, respectively. This suggests that the mineralization of native SOM was sufficient to feed the crop, but the amount of N available to maize was reduced in the treatments fertilized with compost, where the N uptake ranged between 41 and 63 kg ha<sup>-1</sup>. While N released from the unfertilized soil decreased with time, no year effect was detected in COM1 and COM2, thus suggesting that N immobilization by the compost was persistent. In contrast, the same indicator in TO, where N mineralization was higher, showed that the compost contribution to maize nutrition was positive: the SOM provided in fact 235 and 151 kg ha<sup>-1</sup> of N in the two years respectively, that is less than the observed N uptake in COM1 and COM2. Another possible interpretation of the yield reduction due to compost could be that the crop suffered for phytotoxicity, but in that case the negative effect was expected to increase with increasing amounts supplied (while the yield of COM2 was slightly, although non significantly, higher than that of COM1 in both years).

Green manure could be instead a good source of N to the crop in both sites, as the crop yield and N uptake were the same as those observed in plots fertilized with urea (TRA and MIN), but the effectiveness of this practice depends on the growth and amount of N fixed by the legume used as green manure.

The SMN at harvest (Table 2) showed high mineral N contents in the two treatments with urea, and low in those supplied with compost, thus suggesting N immobilization by the compost. Fresh organic matter (SOV) seemed to be rapidly decomposed in the well-aerated TO soil and more slowly in the anoxic NA soil, as the residual SMN after harvest was higher than those measured in all the other treatments. A support to the hypothesis that NA soil suffered for anoxic conditions is the NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratio, that was 7:10 at NA, and 6:1000 at TO.

At the end of the first year the compost addition had not increased the SOC content of the 0-30 cm layer in none of the two soils. Preliminary data show no evidence of the systems capability of increasing SOC content in the short term.

Table 2. Average SMN in the 0-60 cm layer at harvest (kg ha<sup>-1</sup>)

Treatment	TO		NA	
	2006	2007	2006	2007
COM1	14	30	82	87
COM2	13	21	81	71
MIN	38	174	120	99
SOV	23	33	176	127
TRA	27	137	108	90
0N	10	18	121	78

## Conclusions

Preliminary results suggest that compost supplied at low-medium rates may exert a negative effect on maize growth, especially in poorly-aerated soils, probably owing to the immobilization of N. An increase in the SOM content could not be detected after the first applications to the soil.

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# The Efficiency Of Durum Wheat And Winter Pea Intercropping To Increase Wheat Grain Protein Content Depends On Nitrogen Availability And Wheat Cultivar

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Grain protein concentration (GPC) of durum wheat is often a major concern particularly in low input systems where nitrogen acquisition is low due to limited resource of soil mineral N. By consequence, intercropping (IC) which can improve the use of light, nutrients and water resources (Willey, 1979) could be an alternative to the use of mineral fertilizer (Hauggaard-Nielsen, 2003 and Corre-Hellou, 2006). In this paper, we assessed the hypothesis that the performances of durum wheat and winter pea grown in intercrop (IC) to valorise natural resources are better than in sole crops (SC) in low-input systems. Besides, they would however depend on both nitrogen availability and wheat cultivar.

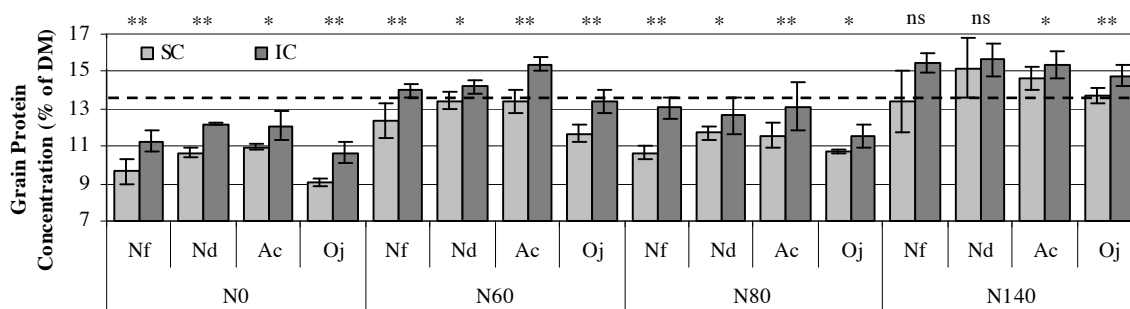
## Methodology

An experiment was carried out in Auzeville (SW France) in a clayed loamy soil in 2006-2007. Three main treatments were compared: *i*) durum wheat sown at 280 plants.m<sup>-2</sup> (W-SC), *ii*) winter pea (cv. Lucy) sown at 60 plants.m<sup>-2</sup> (P-SC), *iii*) durum wheat-winter pea IC, each specie sown at half of normal density (IC). Four wheat cultivars named Acalou (Ac), Nefer (Nf), Neodur (Nd) and Orjaune (Oj) were evaluated as SC or IC. Four fertiliser-N sub-treatments were applied on W-SC and IC as following: *i*) no fertilizer (N0), *ii*) 60 kg N.ha<sup>-1</sup> (N60) at stage 'flag leaf visible' to increase wheat grain protein concentration, *iii*) 80 kg N.ha<sup>-1</sup> (N80) at wheat tillering to increase wheat yield and *iv*) moderate fertilization splitted in 2 applications corresponding to N80 and N60. P-SC was only evaluated without fertilization. The two species were sown in row-intercropping on Nov. 9, 2006. The experiment was a three replicates split-split-plot design with N treatments as main plot, crops as subplot and wheat cultivars as sub-subplot. Each sub-subplot (21 m<sup>2</sup>) consisted of 11 rows of length 12 m spaced 14.5 cm. Grain yield and grain protein concentration (GPC) were measured at harvest. Analyses of variance were performed and means compared using the least significant difference test (LSD) at a risk of 0.05.

## Results

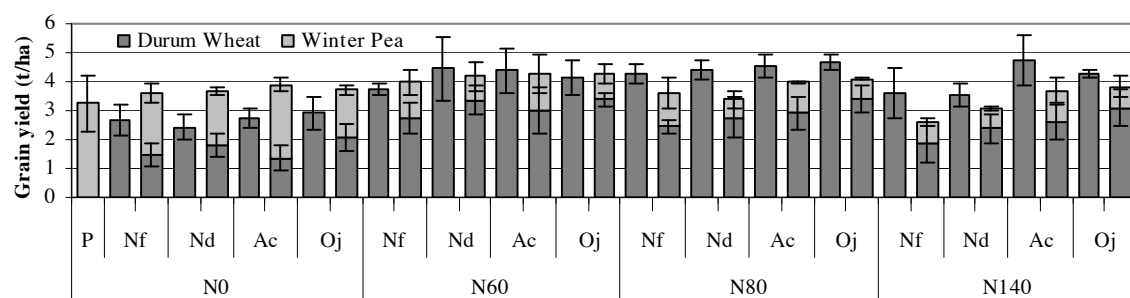
As hypothesised, for all treatments the wheat GPC was significantly higher in IC than SC (Figure 1) excepted for Nf and Nd in N140. Wheat GPC was affected by fertilization for both sole cropping and intercropping and highest values were obtained with N60 and N180. In SC and for same N levels, the cultivars Ac and Nd had significant greater GPC than Nf and Oj. In intercropping the same results were obtained excepted for N80 and N140 where no difference was observed between Nf, Nd and Ac. The increase of GPC between SC and IC was of 8, 11, 12 and 17% for Nd, Ac, Oj and Nf respectively and of 15%, 12%, 13% and 8% for N0, N60, N80 and N140 respectively.

Figure 1: Durum wheat grain protein concentration (% of dry matter) in sole (SC) and intercrop (IC). The dotted line indicates a wheat grain protein concentration of 13.5 % which is the quality required. \*\* and \* indicate a significant difference between IC and SC at a risk of 0.05 and 0.10 respectively and 'ns' indicates that means are not significantly different. Values are the mean (n=3) ± S.E.



For all N treatments and wheat cultivars, grain yield of durum wheat was significantly greater in SC than in IC (Figure 2). However wheat yield in SC was lower than the whole IC yield in N0, but was similar in N60 and greater in N80 and N140. Pea yield in IC was lower when fertilizer-N was applied while wheat grain production was increased by N. Wheat yields in N140 were lower than those of N60 and N80 due to more diseases and flatten. Focusing on wheat cultivars, Nf and Ac were more affected by IC compared to Nd and Oj. Indeed, yield losses in IC (sown at half density) compared to SC were of 42 % for Nf and Ac but only of 28 % for Nd and Oj.

Figure 2: Grain yield ( $\text{t} \cdot \text{ha}^{-1}$  at 0 % of humidity) of durum wheat and winter pea in sole cropping or intercropping for the four wheat cultivars and the four N treatments. Values are the mean ( $n=3$ )  $\pm$  S.E.



## Conclusions

The greatest GPC of wheat in IC was mainly due to a greater N uptake of soil mineral N per plant and depended on the wheat cultivar. Moreover these differences were partially due to wheat yield reduction in IC compared to SC and depended on the fertilisation-N (amount and splitting up). The behaviour of wheat cultivar can be related to their genetical characteristics and particularly their height. Indeed we observed that Oj was the tallest cultivar (116 cm at flowering) and the less affected by IC while Acalou was the smallest (89 cm) and the most affected by IC. However, Nf and Nd had intermediate height (98 cm) but were differently affected by IC. By consequence it can be suggested that tillering, growth dynamics or aerial structure could also explain such differences. Moreover, N fertilization modified the complementarities between cereal and legume by increasing wheat growth and then competition against pea. In conclusion, durum wheat-winter pea intercropping seems well adapted to the conditions of Southern France in particular for the unfertilized treatment, confirming the interest of IC for low nitrogen input systems. Moreover, the choice of wheat cultivar is of particular interest to optimise the cropping combinations according to the objective of such IC (yield, GPC, specie proportion,...).

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# Why and how design monitoring processes of farmer practices at local/regional level? Agronomists endorsing Community Information Systems on farmer practices

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## Introduction

Sixty years after its launching through the «Marshall Plan», the European agriculture revolution is up again, but with some strong contradictions: water pollution, biodiversity erosion, landscape uniformization, ethical crisis (Fresco, 2000). These harmful side-effects of agriculture could be aggravated if the evolution of agricultural practices continues following the current trends towards greater concentration, intensification and technicality.

We focus our communication on agricultural practices, from their choice by farmers decisions to their effects, as they continuously remodel our agricultural landscapes. The approach of farming systems as landscape “builders” is a new one, but its background is the vision of land as resource for agriculture (de Wit, 1992; Lardon et al, 1990). These farmer practices changes appear to be strongly linked to all the severe environmental questions in Europe (Benoît et al, 2005).

But there is a large lack of data and information systems helping stakeholders in their negotiations. The object of this paper is to propose a research procedure of farmer practices monitoring at local/regional scale and to illustrate it for some european situations: München watershed protection, the Seine basin, four specific regional zones in France. We name Community Information Systems on Agricultural Practices (CISAP), those monitoring processes.

## Methodology

We jointly develop a social and technical organizational arrangement on agricultural production, faced to landscape transformations, and other processes effected by individuals whose actions both take place within, and shape a recognized and bounded agriculture-dominated area (“*territoire and terroir*”). Gaining a better information on these processes makes it possible to address them via analysis, modeling and recommendations.

We consider those Community Information Systems as social and technical organizational arrangements which connect information and knowledge, practices and landscapes.

## Results

CISAP as a tool to produce changes in local farming systems:

The design of monitoring of farmer practices is a central challenge for stakeholders and researchers on farming system changes if we want to protect natural resources. For us, the main questions are:

- How to build a generic framework for those data ? This question refers to collective building of facts and argues studied as situated and distributed knowledge (Amin et Cohendet, 2004; Brassac et Le Ber, 2005).
- How can we organize numerical data bases linking cropping system, farming system, soil, communes, environmental zones? We propose to use the UML modelling framework to organize the levels and their relationships.
- What should be the rules for data use? This central question will lead to improve the status of information uses during farming system changes
- How can we take into account the “situated and distributed” dimension of those data? This question helps us to identify all the stakeholders involved in farmer changes for natural resources management (Le Ber et Brassac, to appear 2008).
- For those monitoring, what kind of complementarity have we to build between remote sensing, statistical data, local surveys?
- How to help stakeholders build and manage CISAP? (Nogry et al. 2007)

To illustrate answers to these questions we use a survey on 32 european CISAP to evaluate their effectiveness (Nogry et al, 2007).

To conclude, we propose a generic framework for future CISPA and a list of main key points to help agronomists involved in their development.

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# Effect of N Fertilisation on the Growth Characteristics of Maize (*Zea Mays* L.) Hybrids in a Long-Term Experiment

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There is a strong relationship between relative growth rate (RGR) and plant productivity. A major cause of differences in growth rate is the variation in nitrogen (N) availability. Plant growth analysis can be used to analyse the causes of variation in growth rate. The aim of the research was (i) to use growth analysis to study the effect of N fertilisation on the growth and growth parameters of maize hybrids and (ii) to reveal correlations between RGR (and its components) and the grain yield.

## Materials and methods

The effect of N fertilisation on the growth and growth parameters of maize hybrids was studied in a long-term experiment set up on chernozem soil with forest residues in the institute nursery in 1961. The main plot in the split-plot design, set up in four replications, was the N treatment, with the maize hybrid in the subplots. The N fertiliser rates were as follows: 0, 80, 160, 240 kg ha<sup>-1</sup> (denoted by N<sub>0</sub>, N<sub>80</sub>, N<sub>160</sub>, N<sub>240</sub>). The P and K fertiliser rates were the same in all the treatments (160 kg ha<sup>-1</sup>). The investigations were made in 2001 and 2002 on three maize hybrids with different vegetation periods: *Mv 272* (FAO 280), *Mv 355* (FAO 390) and *Maraton* (FAO 450). Plant samples for growth analysis were taken at 13–15-day intervals. The effect of N fertilisation on the growth of maize plants was characterised using the RGR parameter and its components (NAR, LAR, LWR, SLA), the duration of leaf area and biomass (LAD and BMD). The growth parameters were calculating using the growth analysis program of Hunt et al. (2002), which also carries out statistical analysis.

## Results and discussion

The effect of N fertilisation on the dynamics of dry matter accumulation in the whole maize plant and in the grain yield, and on the seasonal dynamics of the leaf area is illustrated in Figure 1. It is clear that the maximum value of biomass production was highest in the N<sub>160</sub> and N<sub>240</sub> treatments and lowest in the N<sub>0</sub> treatment. Hybrids with longer vegetation periods had higher BMD values (*Mv 272*: 10.67, *Mv 355*: 13.93, *Maraton*: 12.06 10<sup>3</sup> g day) and higher grain yield (*Mv 272*: 6.95, *Mv 355*: 7.93, *Maraton*: 7.97 t ha<sup>-1</sup>). The following cumulated BMD values (10<sup>3</sup> g day) were recorded in the various treatments: N<sub>0</sub>: 8.99, N<sub>80</sub>: 12.25, N<sub>160</sub>: 14.23, N<sub>240</sub>: 13.41. Averaged over hybrids and years the grain yield increased significantly up to an N<sub>160</sub> rate : N<sub>0</sub>: 4.91c, N<sub>80</sub>: 7.87b, N<sub>160</sub>: 8.91a, N<sub>240</sub>: 8.77a (t ha<sup>-1</sup>). Table 1 contains the mean values of the RGR, NAR and LAR parameters in 2001 for each N treatment and hybrid during the vegetative and generative growth phases. There was a tendency for the RGR and NAR values to rise with improvements in the N supply, while that of LAR declined. It is also clear that these parameters indicate greater growth during the vegetative period, when the mean values of RGR and NAR were highest in the N<sub>80</sub> treatment. By contrast, the value of LAR gradually dropped. In the generative phase the RGR and NAR values, characteristic of grain yield development, consistently increased in response to N fertilisation, while that of LAR exhibited a slight decrease. Hybrids with longer vegetation periods had higher LAD values (m<sup>2</sup> day): *Mv 272*: 28.5, *Mv 355*: 32.2, *Maraton*: 37.6. The cumulated LAD values in the various N treatments were as follows (m<sup>2</sup> day): N<sub>0</sub>: 23.8, N<sub>80</sub>: 32.8, N<sub>160</sub>: 37.5, N<sub>240</sub>: 36.9. The values of both BMD and LAD increased up to the N<sub>160</sub> level, after which there was no further significant change.

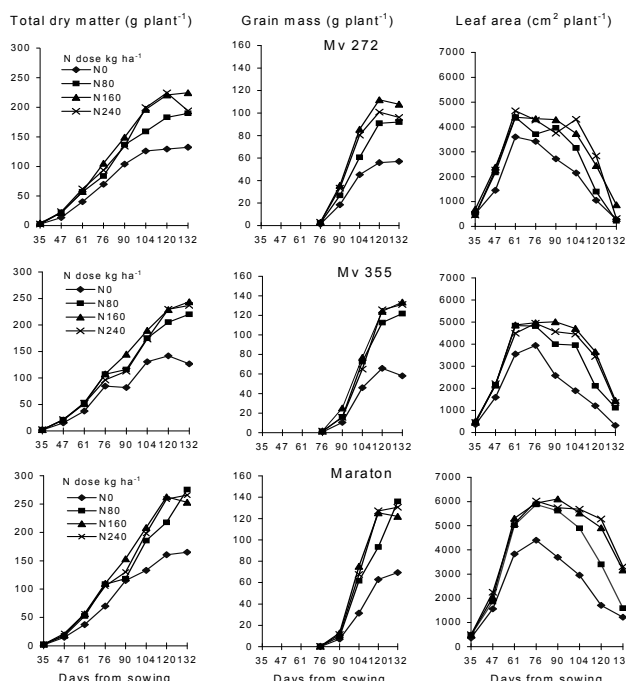


Figure 1. Effect of N fertilisation on the growth dynamics of total dry matter, grain mass and leaf area in three maize hybrids from different maturity groups

During the vegetative phase of growth RGR was positively correlated with LAR ( $r = 0.417^*$ ) and SLA ( $r = 0.439^*$ ), while there were negative correlations between NAR and LAR ( $r = -0.765^{**}$ ) and NAR and LWR ( $r = -0.866^{**}$ ). A positive correlation was found between LAR and its components, i.e. between LAR and LWR ( $r = 0.919^{**}$ ) and between LAR and SLA ( $r = 0.418^{**}$ ). During the generative phase of growth the RGR parameter of the grain yield was in positive correlation with NAR ( $r = 0.714^{**}$ ) and SLA ( $r = 0.412^*$ ), but was negatively correlated with LAR ( $r = -0.624^{**}$ ) and LWR ( $r = -0.746^{**}$ ). There was a significant correlation between the grain yield and the cumulated LAD ( $r = 0.790^{**}$ ) and between the grain yield and the cumulated BMD ( $r = 0.427^*$ ). The grain yield ( $t\ ha^{-1}$ ) was positively correlated with the RGR ( $r = 0.726^{**}$ ), NAR ( $r = 0.443^*$ ) and SLA ( $r = 0.536^{**}$ ) parameters during the generative growth phase and negatively with the LWR parameter ( $r = -0.440^*$ ).

Table 1. Effect of N fertilisation on the average values of RGR, NAR and LAR of maize plants during the vegetative and reproductive growth period (S.E. is in parentheses)

Maize hybrid	Vegetative growth period				Reproductive growth period			
	N <sub>0</sub>	N <sub>80</sub>	N <sub>160</sub>	N <sub>240</sub>	N <sub>0</sub>	N <sub>80</sub>	N <sub>160</sub>	N <sub>240</sub>
RGR ( $mg\ g^{-1}$ )								
<i>Mv 272</i>	74.8 (6.2)	76.2 (5.4)	77.7 (9.2)	71.6 (5.7)	36.3 (4.6)	39.4 (8.8)	40.0 (4.6)	39.9 (3.1)
<i>Mv 355</i>	81.0 (11.6)	90.9 (7.6)	81.6 (6.4)	79.1 (9.1)	34.4 (8.7)	45.5 (3.8)	44.7 (2.4)	43.1 (4.1)
<i>Maraton</i>	79.1 (8.5)	83.0 (8.2)	74.5 (8.0)	73.3 (3.6)	25.5 (7.3)	33.2 (6.9)	42.0 (3.4)	41.7 (1.8)
NAR ( $g\ m^{-2}day^{-1}$ )								
<i>Mv 272</i>	10.1 (1.7)	11.4 (2.3)	13.1 (2.4)	11.8 (1.8)	9.0 (2.4)	10.6 (4.3)	10.6 (3.7)	11.6 (2.7)
<i>Mv 355</i>	18.2 (5.8)	22.7 (3.3)	17.2 (4.5)	21.7 (4.8)	6.7 (5.1)	11.0 (3.0)	14.2 (3.3)	6.1 (2.9)
<i>Maraton</i>	10.5 (2.3)	13.0 (2.9)	12.1 (1.9)	12.4 (1.2)	5.0 (2.1)	7.0 (5.0)	10.6 (1.5)	11.8 (1.0)
LAR ( $cm^2\ g^{-1}$ )								
<i>Mv 272</i>	123.8 (18.9)	115.3 (20.6)	111.3 (30.2)	103.8 (17.1)	30.0 (5.4)	28.9 (8.9)	24.6 (4.4)	23.8 (4.1)
<i>Mv 355</i>	81.5 (23.7)	92.9 (35.2)	73.7 (13.0)	73.3 (19.1)	20.2 (6.0)	17.1 (2.1)	18.4 (2.9)	16.8 (2.9)
<i>Maraton</i>	115.8 (23.2)	114.5 (26.3)	100.6 (32.5)	87.4 (24.0)	37.3 (8.2)	31.0 (8.5)	28.8 (4.5)	29.6 (4.9)

## Conclusions

The effect of various levels of N fertilisation, differences between the N responses of maize hybrids and the effects of different years could be reliably described using the dynamics of dry matter accumulation in the whole maize plant and in the grain yield, the seasonal dynamics of leaf area, and the growth parameters of individual plants (RGR, NAR, LAR, LWR, SLA, BMD and LAD).

## Acknowledgements

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# Agriculture and Environment: Life Cycle Assessment of cow farming for High Quality milk production

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In recent years the concept of sustainability and the purpose to evaluate the effects of human activity or product manufacture on the Environment is increased. Milk production in farming involves several impacts because of the cow feed production and the sewage disposal. The objective of this study, carried out by Granarolo Spa (Bologna, Italy), a leader Italian dairy company, is to examine the total life cycle of production and processing of raw milk in farm, in order to quantify the potential environmental impact [1]. The study covers the production activities for “High Quality” raw milk, so labelled according to the requirements of Italian Law n. 169/1989. Granarolo has monitored the environmental effects of raw milk production by means of LCA (Life Cycle Assessment) methodology. These LCA data were used for EPD (Environmental Product Declaration) [2] of processed and packed milk for retail market.

## Methodology

LCA is an objective evaluation of products, processes or activity, carried out through the identification and quantification of energy, materials and wastes released into the environment. The evaluation includes the whole life cycle of the products, processes or activity, comprising extraction and the treatment of raw materials, manufacturing, transport, re-use, recycling and waste treatment (SETAC, 1990).

The LCA methodology includes four phases (ISO 14040 and 14044):

1. Goal Definition and Scope: this stage explains the reasons LCA has been done for. It describes the system analysed and the principal parameters: functional unit (defined as the quantified performance of a product system), system boundaries, allocation rules and quality data.
2. Life Cycle Inventory Analysis: the LCI consists of the collection of data and concerns: the resource use, energy consumption, emissions and wastes.
3. Life Cycle Impact Assessment: the purpose is to consider the LCI results to understand their environmental importance. LCIA classifies the inputs and outputs of the inventory into specific categories, and it models the inputs and outputs for each category into an aggregate indicator.
4. Life Cycle Interpretation and Improvement: is a procedure to identify, qualify, check and evaluate the information from the results of the LCI and/or LCIA of a product system.

The boundaries of the raw milk system analysed by LCA are illustrated in Fig. 1.

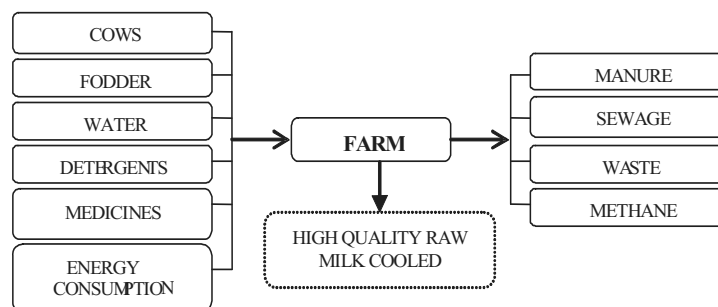


Fig. 1 – System Boundaries of raw milk production.

## Results

The Life Cycle Assessment of raw milk is carried out by IMPACT 2002+ methodology [3]. The IMPACT 2002+ methodology has several environmental indicators, but the main results that concerns

raw milk are Global Warming (measured in kg CO<sub>2eq</sub>), Eutrophication (measured in kg PO<sub>4</sub>) and Resources (measured in MJ<sub>primary</sub>). The functional unit is 1 liter of raw milk.

In particular the LCA of High Quality raw milk shows that:

- for the phenomenon of global warming significant impacts are associated to methane emissions produced by the digestive system of cows;
- for the phenomenon of eutrophication the use of chemical fertilizers involves the emission to soil of nitrogen and phosphorus;
- for the depletion of resources there is considerable energy consumption because of cows feeding and agricultural and zootechnical activities at the farm.

Granarolo applied LCA analysis in seven (7) selected farms. The selection criteria were applied according to the size of the herd, milk production, geographic location, with the aim to obtain a good representation of farms. These data are representative for 92.8% of the High Quality raw milk delivered to Granarolo S.p.A. Tab. 1 shows the results of LCA of 1 liter of raw milk from High Quality farms (mean values from data of 7 representative farms).

IMPACTS MEAN DATA FOR HIGH QUALITY RAW MILK	
INDICATOR	SUBSTANCES
0,32 kg CO <sub>2eq</sub>	<i>Principal emissions to air:</i> 0,29 kg CO <sub>2</sub> 0,016 kg CH <sub>4</sub>
0,0021 kg PO <sub>4</sub>	<i>Principal emissions to water:</i> 0,00089 kg phosphorus 0,0012 kg COD 0,02 kg nitrogen
5,72 MJ primary	<i>Principal resources with energy content</i> 0,063 kg crude oil 0,035 kg di natural gas

Tab. 1 – LCA results of High Quality raw milk.

Fig. 2 shows the global impact (0,45 ELU-Environmental Load Unit) on the basis of the aggregate indicators: Human Health, Ecosystem Quality, Abiotic Stock Resources and Biodiversity. The greater impacts are for Human Health (including GWP) and Abiotic Stock Resources (including energy consumption).

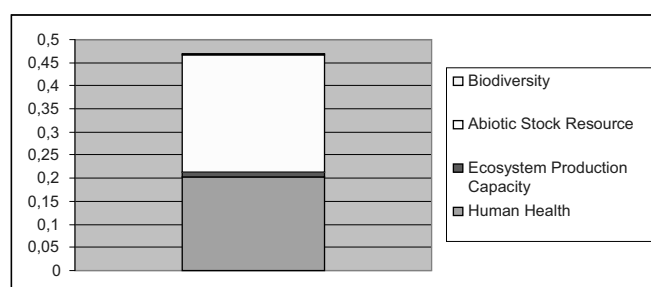


Fig. 2 – Environmental Impact of High Quality raw milk.

## References

- [1] Falconi F. et al., “Analisi del Ciclo di Vita di allevamenti Alta Qualità fornitori della Granarolo Spa”, DOC ENEA ACS P135 – 022 - pag. 1-229, Bologna 29/04/2006.
- [2] Swedish Environmental Management Council: “Requirements for Environmental Product Declaration (EPD)”; MSR 1999:2.
- [3] IMPACT 2002+ method. IMPACT 2002+ version 2.0 and 2.1; IMPACT 2002 (Pennington et al. 2005), Eco-indicator 99 (Goedkoop and Spriensma. 2000, 2nd version, Egalitarian Factors), CML (Guinée et al. 2002) and IPCC.

# Agriculture and Environment: Life Cycle Assessment of cow farming for organic milk production

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The objective of this study, carried out by Granarolo Spa (Bologna, Italy), a leader Italian dairy company, is to examine the potential environmental impact of the total Life Cycle of raw milk production in organic farms [1]. The aim is also to compare the environmental impacts of Organic milk with High Quality milk, both supplied by members of Granlatte Cooperative. The methodology used for this study is Life Cycle Assessment (LCA). These data were used for Environmental Product Declaration EPD of processed and packed milk for retail market.

## Methodology

LCA is an objective evaluation that includes the whole life cycle of the products, processes or activity, comprising extraction and the treatment of raw materials, manufacturing, transport, re-use, recycling and waste treatment (SETAC, 1990).

The LCA methodology includes four phases (ISO 14040 and 14044):

1. Goal Definition and Scope
2. Life Cycle Inventory Analysis
3. Life Cycle Impact Assessment
4. Life Cycle Interpretation and Improvement

The boundaries of the organic raw milk system analysed by LCA are illustrated in Fig. 1.

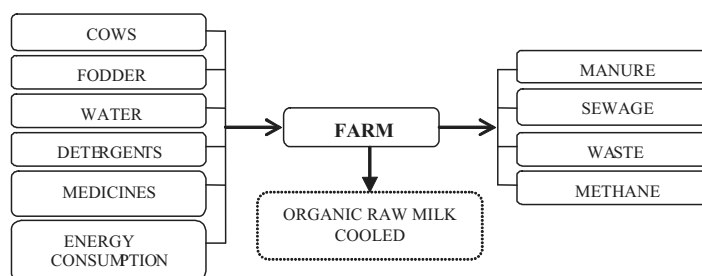


Fig. 1 – System Boundaries of raw milk production.

## Results

The Life Cycle Assessment of raw milk is carried out by IMPACT 2002+ methodology [2]. The functional unit is 1 litre of organic raw milk. The LCA of Organic milk shows some advantages: less emission of nutritious substances that involves lower eutrophication impacts because of use of natural fertilizers (manure and sewage), more CO<sub>2</sub> absorption by crops, and less energy consumption. Granarolo applied LCA analysis in five (5) selected organic farms. The selection criteria were applied according to the size of the herd, milk production, location (lowlands and hills) with the aim to obtain

a good representation of the different types of farms. These data are representative for 97.5% of the organic milk delivered to Granarolo S.p.A.

Tab. 1 shows the results of LCA of 1 litre of raw milk from Organic farm (mean values from data of 5 representative farms).

IMPACTS MEAN DATA FOR ORGANIC RAW MILK
ENVIRONMENTAL INDICATORS
0,12 kg CO <sub>2eq</sub>
0,00078 kg PO <sub>4</sub>
1,65 MJ primary

Tab. 1 – LCA results of Organic raw milk.

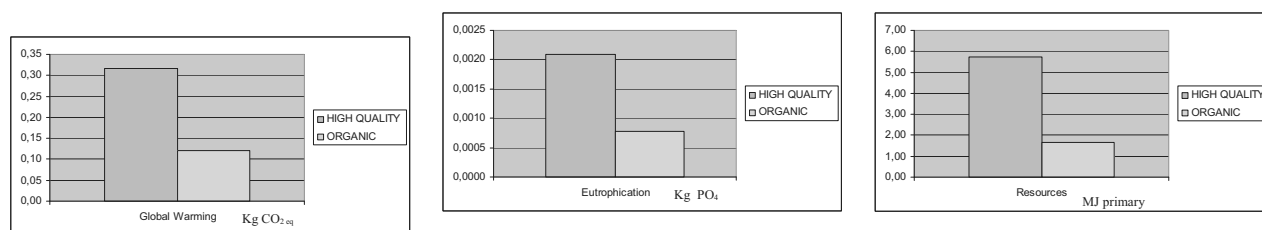


Fig. 2 – Comparison between Organic raw milk and High Quality raw milk ( 1 litre).

The Organic raw milk involves a lower impact for the three environmental indicators.

## References

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- [2] IMPACT 2002+ method. IMPACT 2002+ version 2.0 and 2.1; IMPACT 2002 (Pennington et al. 2005), Eco-indicator 99 (Goedkoop and Spriensma. 2000, 2nd version, Egalitarian Factors), CML (Guinée et al. 2002) and IPCC.
- [3] Falconi F. et al., “Analisi del Ciclo di Vita di allevamenti Alta Qualità fornitori della Granarolo Spa”, DOC ENEA ACS P135 – 022 - pag. 1-229, Bologna 29/04/2006.

# Nitrogen Fertilisation of First Year Maize after Alfalfa in the Ebro Valley

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Alfalfa–maize is a traditional crop rotation in several regions of the world and in particular in the Mediterranean irrigated areas of the Ebro Valley (N.E. Spain). It is well known that alfalfa is a good precedent for maize, for both the N left in the soil and for the ‘rotation effect’. However, a recent survey conducted in the Ebro Valley (Sisquella et al., 2004) shows that only 25% of maize producers reduce their N fertilisation rates after alfalfa. El Hout and Blackmer (1990) also observed similar behaviours in the EEUU.

Several studies conducted in North America (El-Hout and Blackmer; 1990; Morris et al., 1993) evaluated N fertilisation of maize after alfalfa. However, there are some differences in alfalfa and maize management between these areas and these of the Ebro Valley. Firstly, the number of alfalfa cuttings per year is higher in the Ebro Valley (5-7 cuttings vs 3-4 in the Midwest of USA). This higher biomass production in alfalfa could lead to higher N fixation (Gault et al., 1995). Secondly, irrigation leads to higher maize yields and N uptakes. So, we believe that the results reported in literature could differ in our conditions. The objective of this research was to study the effects of high-yielding alfalfa in the N fertilisation of the subsequent maize crop, under irrigated conditions in the Ebro Valley.

## Methodology

Two field experiments were conducted in Lleida (N.E. Spain). The trials were established after four-year-old alfalfa fields, under contrasting irrigation conditions and soils: i): a shallow soil (Petrocalcic Calcixerept, 60 cm depth) with flood irrigation in 2006 and ii): a deep soil (Typic Xerochrept, >1 m depth) with sprinkler irrigation in 2007. The experimental design consisted of randomized blocks with four replications. Six N rates were applied to the maize (0, 50, 100, 150, 200, and 300 kg N ha<sup>-1</sup>) in two sidedress applications. Maize (PR33P67) was seeded in early April at a density of 8 pl m<sup>-2</sup>. The plot size was 27 x 6 m. Maize grain yield (Table 1) was measured by harvesting 2 central rows of the plots. N uptake was estimated by multiplying plant biomass by plant N content. Initial (before sowing) and final (after harvesting) soil N content was measured (0-90 cm). We also determined the number of alfalfa roots before sowing (0-30 cm), their dry weight and N content. Statistical analyses were performed using general lineal procedures (SAS Institute, 1991).

## Results

Table 1. Maize yield, plant N uptake and residual soil N as a function of N rate, for the two trials.

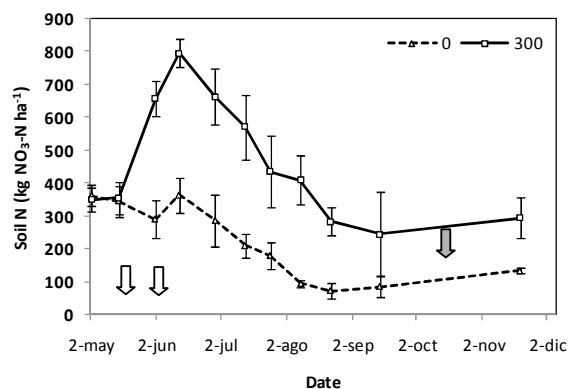
N rate kg ha <sup>-1</sup>	Gimenells 2006			Almacelles 2007		
	Maize yield Mg ha <sup>-1</sup>	Plant Uptake kg N ha <sup>-1</sup>	Residual soil N kg N ha <sup>-1</sup>	Maize yield Mg ha <sup>-1</sup>	Plant Uptake kg N ha <sup>-1</sup>	Residual soil N kg N ha <sup>-1</sup>
0	10.3	205 b	110	16.7	413	134 b
50	10.5	230 ab	110	16.6	429	128 b
100	10.7	209 b	127	16.3	445	184 b
150	11.4	231 ab	127	16.3	465	233 ab
200	11.5	258 a	143	16.3	469	221 ab
300	11.6	264 a	131	16.8	434	294 a
Mean	11.0	233	125	16.5	443	199
LSD (0.05)	ns	43	ns	ns	ns	92

In the first experiment (Gimenells 2006), the alfalfa stand at the end of the crop was 39 plants  $m^{-2}$ , containing in the upper part of the roots collected (0-30 cm) about 70-80 kg N  $ha^{-1}$ . The initial amount of soil inorganic N ( $NO_3-N$  plus  $NH_4-N$ ), before maize seeding was 114 kg  $ha^{-1}$ . On the other hand, in the second experiment (Almacelles 2007) before maize seeding there were 60 plants  $m^{-2}$  remaining in the field, containing about 200 kg N  $ha^{-1}$ . The initial soil N content of this trial was 220 kg N  $ha^{-1}$ . The N contained in alfalfa roots was relatively high and could become available for the crop as the root decomposition progresses.

Maize grain yields of both experiments are presented in Table 1. In Gimenells, the mean maize yield was 11 Mg  $ha^{-1}$ . In this case, alfalfa provided enough N to achieve more than 10 Mg  $ha^{-1}$  of maize without N fertilisation. There was little response of maize yield to N fertilisation, approx. 1.3 Mg  $ha^{-1}$  when comparing 0 and 300 kg N  $ha^{-1}$ . After harvest, the amount of residual N in the soil was of 125 kg N  $ha^{-1}$ , quite similar to the soil N content before seeding and without differences between treatments (Table 1). This fact suggests N losses when fertilising with high N rates.

In Almacelles, the average yield of the trial was 16.5 Mg  $ha^{-1}$ . There were no differences in maize grain yield between N rates. In this case, both 0 and 300 kg N  $ha^{-1}$  treatments yielded similarly (Table 1). This results are in agreement with other studies (El-Hout and Blackmer, 1990; Morris et al., 1993), which also found little or none response of maize yield to N fertilisation after alfalfa.

After maize harvest, residual soil N increased with increasing N fertilisation rates (Table 1). Soil  $NO_3-N$  evolution during the growing season is presented in Figure 1. The difference in residual N between unfertilised and 300 kg N  $ha^{-1}$  plots were about 160 kg N  $ha^{-1}$ . Since N uptake was similar between N rates (Table 1), this suggests possible N losses in high fertilised plots, increasing environmental risk and decreasing crop profitability.



**Figure 1.** Soil  $NO_3-N$  evolution in Almacelles (0-90 cm). Non-fertilised plots (spotted line) and 300 kg N  $ha^{-1}$  plots (solid line). White arrows indicate first and second sidedress. Grey arrow: maize harvest.

## Conclusions

These results suggest that N fertilisation of first year maize after alfalfa could be considerably reduced. In deep soils with a high amount of residual N there would be no need of N application, even for obtaining high grain yields ( $>16$  Mg  $ha^{-1}$ ). In shallow soils with flood irrigation, alfalfa could provide maize yields at least 10 Mg  $ha^{-1}$ , with a limited response to N application.

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# Long-term effects of catch crops, no-till and reduced fertilisation on nitrogen balance in 3 cropping systems in France

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Establishing catch crops, reducing N fertilization and tillage can decrease N losses in arable cropping systems. Catch crops are known to decrease nitrate leaching (Hansen *et al.*, 1997) while no-tillage increase storage in soil (Rossella *et al.*, 2007). Reduced fertilization could reduce leaching at long-term more than at annual scale (Gomez *et al.*, 2002). However the importance of the mitigation is not well documented on the long term. The aim of this study is to determine the long-term fate of nitrogen and quantify nitrogen fluxes to evaluate sustainability of these agricultural practices.

## Methodology

We studied the nitrogen fate in three long term experiments (16 years) in Northern (Boigneville, Thibie) and Western France (Kerlatic). Catch crops were tested in all sites (CC or NoCC), whereas tillage (conventional (CT) or no-tillage (NT)) was tested at Boigneville and reduced nitrogen rate at Thibie (conventional rate (N) or 65% rate (N-)). Crop uptake, nitrate concentration in porous cups and drainage in lysimeters were routinely measured. Soil organic nitrogen was measured after 16 years of experiment. The standard N balance was calculated as difference between inputs (fertilization, fixation and atmospheric deposition) and outputs (crop offtake). It corresponds to the sum of leaching, gaseous losses and storage in soil (Mary *et al.*, 2002). Then, the net balance was calculated by removing nitrogen leached from the standard balance to assess the sum of N immobilized in soil and N losses through gas emissions. The difference in net balance between treatments was compared to the difference in N storage after 16 years.

## Results

The mean balance was -11, +34, +24 and +53 kg N ha<sup>-1</sup> year<sup>-1</sup> at Kerlatic, Boigneville, Thibie (N- and N rates), respectively. The lower value was obtained in a rotation with high level of export (silage) and high drainage; the higher value was found in site with the highest return of crop residues.

The standard balance was similar in CC vs NoCC and NT vs CT whereas N leaching was much lower in CC vs NoCC and smaller in NT vs CT. Catch crops reduced quantity of leaching about 37% at Boigneville and Kerlatic where drainage was 150 and 600 mm yr<sup>-1</sup> respectively. At Thibie the reduction was higher with 60% of decrease with approximately the same drainage as Boigneville because uptake of catch crops was double. NT also reduced by 28% leaching.

Reducing nitrogen rate poorly decreased N leaching (8%). Implanting catch crops decreased nitrate concentration of drained water near or under 50 mg l<sup>-1</sup> while the two

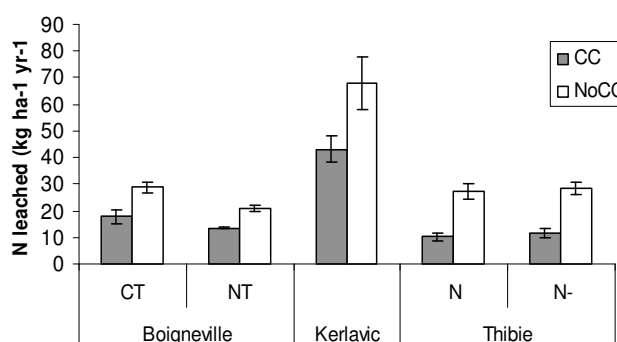


Figure 1 : Mean quantity of nitrogen leaching by year over 16 years

other practices were not enough efficient to decrease this concentration over this European threshold of nitrate concentration in drinking water.

The no-leached nitrogen can be found in crop, soil or can be lost in the atmosphere. Crop uptake was not significantly increased by CC. Then, to evaluate the fate of this nitrogen, we studied the difference in the net balance between CC and NoCC. It ranged from 129 to 274 kg ha<sup>-1</sup> after 16 years which corresponded approximately to the quantity of no-leached nitrogen in CC vs NoCC.

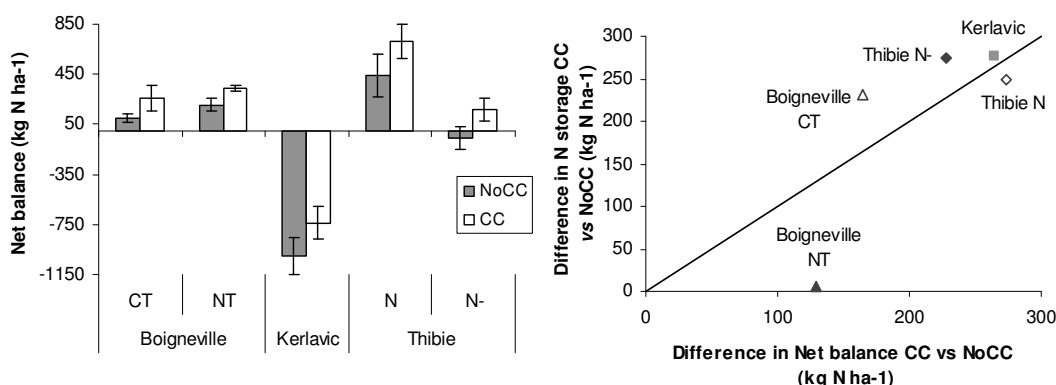


Figure 2 : (a) Net balance by treatments and sites over 16 years and (b) relation between net balance (CC -NoCC) and Nitrogen storage (CC-NoCC)

Implanting CC has a significant effect on N storage in soil. So, part of nitrogen retained by CC is probably store in soil. The difference in net balance between CC and NoCC was approximately equal to the mean difference of organic N stored in soil except in no-tilled plots where difference between CC vs NoCC was of 129kg N ha<sup>-1</sup> while difference in N storage was only 6 kg N ha<sup>-1</sup>. These results mean that decrease in leaching could lead to increase nitrogen storage in soil with an increase in carbon storage also (data not shown). In this study, it appears that majority of retained nitrogen is stored in soil and not lost in the atmosphere except in NT where retained nitrogen by CC could have been lost as gas. However, due to high variability in storage measuring, complementary experiments are necessary.

## Conclusions

Implanting CC was the most efficient way to decrease nitrogen leaching in long-term experiment. No-tillage less reduced nitrogen leaching. It appears that reducing nitrogen fertilization below conventional rate had low impact on leaching over 16 years of experiment. Decrease in leaching by implanting CC seems to lead to store more nitrogen in soil after 16 years of experiment on tilled plot. In NT plot, retained nitrogen by CC was not found in soil organic matter suggesting that this nitrogen can be lost as gaseous loss. Evaluation of gaseous losses with isotopic method at natural abundance and long-term modeling will allow evaluating more precisely the fate of nitrogen.

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# Nitrogen Release from Soil and from Incorporated Organic Residues as Affected by Biuret Presence in Urea Fertilizer

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Biuret is a known contaminant of urea fertilizers. It is mineralized by microorganisms at a much slower rate than urea, thus accumulating in the soil. Biuret is both stimulatory and inhibitory of plant growth. It can be either toxic to plants, when present in excessive amounts in soils, or it can stimulate plant growth, by enhancing soil nutrient availability, namely nitrogen (N), due to priming effect (Xue et al., 2004, 2005). Thus, the release of N from soil organic matter, as well as from organic residues applied to soils, may be influenced by biuret in soils. The evidence of a priming effect raises the hypothesis of a stimulatory effect on the mineralization of organic residues. The objective of the experiment was to evaluate the effect of biuret presence on the mineralization of organic residues applied to a sandy loam soil.

## Methodology

A laboratory aerobic incubation was performed using a Cambic Arenosol (FAO, 1998) mixed with dried and ground secondary pulp mill sludge (S), poultry manure (P) and composted pig manure (C) at rates equivalent to  $170 \text{ Kg N ha}^{-1}$ , based on its total Kjeldahl N (TKN) content, to evaluate the effect of biuret presence in a sandy loam soil, and in the N mineralization potential of organic residues, after years of urea fertilization, simulated under laboratory conditions ( $100 \text{ mg kg}^{-1}$ ). The three organic residues were mixed to the soil, with and without biuret present, and incubated aerobically, under controlled conditions, for 34 weeks. Mixtures were placed in plastic containers, wetted at 60% soil field capacity, kept in a thermostatically controlled cabinet at  $24 \pm 1^\circ\text{C}$ , and weekly aerated. Ten samplings, were performed at 0, 2, 4, 7, 11, 21, 46, 92, 170 and 238 days after setting, to determine mineral N ( $\text{N-NH}_4 + \text{N-NO}_3$ ), after extraction with 2 M KCl solution (Mulvaney, 1996). N content was determined by molecular absorption spectrophotometry using a Skalar segmented flow auto-analyzer. To estimate organic N mineralization, a one compartment first-order kinetic model (OCFO) ( $N_m = N_0 * (1 - \exp^{-k*t})$ ) was fitted to the incubation data.  $N_m$  represents the mineral N accumulated along time  $t$ ,  $N_0$  the potentially mineralizable N (PMN) and  $k$  the mineralization rate constant.

Table 1. Some chemical characteristics of the soil and of the three organic residues under study

	soil	Sewage sludge	Poultry manure	Composted pig manure
pH	6.12			
TKN ( $\text{g kg}^{-1}$ )	0.34	39.40	31.10	27.30
C/N		12.65	12.51	21.70

## Results

Figure 1 shows that, not only the OCFO model fitted well the mineralization of N from the organic residues tested, in the presence and absence of biuret in soil, but also that the presence of biuret led to a higher amount of N mineralized during the experiment. This effect was higher in treatments where secondary pulp mill sludge was applied. This residue has high amount of recalcitrant N (Cordovil et al. 2005). When the three residues were applied to the soil, an initial mineralization increment was

observed, probably as a result of mixtures wetting after application. It is well known that the addition of water is responsible for N mineralization flush, the every time it occurs. However, according to curve fitting shown in Figure 1, N mineralization in treatments where biuret was present in the soil, was more gradual over time, particularly in C treatments.

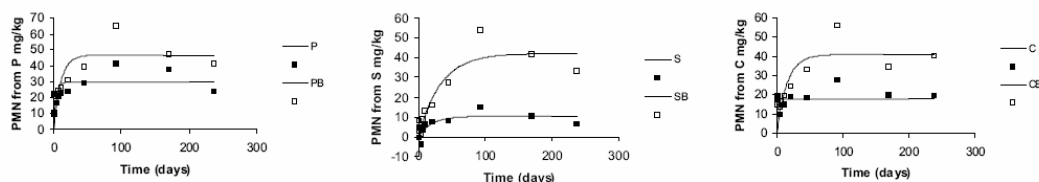


Figure 1: Potentially mineralizable N from poultry manure (P), pulp mill sludge (S) and composted pig manure (C) added to the soil. (■ without the presence of biuret, □ with biuret present in soil).

If we consider the amount of N mineralized from the residues alone, the presence of biuret in soil, seemed to have enhanced the net apparent mineralization of N from the organic residues under study, particularly in the first month of the experiment (Figure 2). Mineralization of N from poultry manure and composted pig manure, were enhanced by 10% on average and mineralization from pulp mill sludge increased 50% on average. Although this is not to be considered a priming effect, because the soil organic matter (SOM) was not under evaluation, the presence of biuret in soil enhanced N mineralization from each one of the organic residues tested. In fact a priming effect has been previously reported by Xue et al. (2005), regarding SOM.

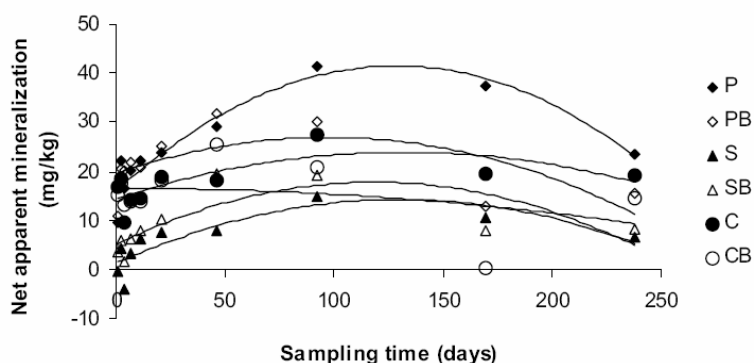


Figure 2: Net apparent nitrogen mineralization from the residues alone, in the presence (●, ◆, ▲) and absence (○, ◇, △) of biuret in soil (mg N kg<sup>-1</sup> residue).

## Conclusions

The presence of biuret in soil as a result of continuous use of urea as fertilizer in top dressings, has shown to enhance the potential N mineralization from organic residues.

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# Seed Yield and Plant Regeneration of Different Subclover Cultivars in Southern Italy

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Legumes are a fundamental resource for agro-ecosystem sustainability and because of their N-fixation capability they play an important biological role in the crop rotation and intercropping systems (Cazzato *et al.*, 2003). As for annual legumes, clovers and medics are the species most utilized for Mediterranean pasture improvement (subclover in particular) while medics are best adapted to alkaline soil. As cover crop of the orchards, legumes reduce the use of N fertilization and herbicides while, in the sloping ground, they mitigate the water erosion. In general, subterranean clover matures during May, but there are large number of varieties representing a wide range of maturation dates. Cultivars are grouped according to the length of time required to reach maturity or seed set, as early, early mid-season, mid-season, and late-(Murphy *et al.*, 1976). The earliest and latest strains may differ by as much as 60 days. Reseeding capability is a very important tool since it assures the species persistence throughout the years. Subclover seed yield is strongly influenced by the grazing management and the intensity of plant defoliation. Previous researches on seed production and self seeding pointed out that subclover can produce seed under close grazing, unlike most other annual clovers (Evers and Smith, 1988). In this paper are reported the results obtained in a 3-year period on the comparison of different cultivars of subclover belonging to 3 different species: *T. brachycalycinum* Katznelson and Morley, *T. subterraneum* L., *T. yanninicum* Katznelson and Morley, with the aim to determine cultivars earliness, seed yield and hardseededness along with other parameters involved in seed production.

## Methodology

The research has been performed for a 3-year period (1994, 1996, 1997) at the Gaudio di Lavello field, Basilicata Region. The experimental site had a sandy-clay textured soil, sub-alkaline, low in total nitrogen and high available phosphorus and exchangeable potassium. The climate is dry in the summer and has a typical Mediterranean distribution of precipitation from autumn to spring. A randomized complete block design with 3 replications and plot size of 1 m<sup>2</sup> was used comparing 12 cultivars of subclover 3 of which (Clare, Nuba and Rosedale) belonging to *T. brachycalycinum* (b-type), 7 (Denmark, Enfield, Goulburn, June, Karridale, Leura and Woogenellup) to *T. subterraneum* (s-type) and 2 (Gosse and Larissa) to *T. yanninicum* (y-type). Soil preparation was performed September 29 1993 ploughing the soil to a depth of 30 cm and fertilizing with 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as superphosphate. Sowing in row 20 cm apart, using a seed rate of 40 kg ha<sup>-1</sup>, was effectuated October 1<sup>st</sup> 1993. Emergence occurred October 8 1993, October 24 1994, August 30, 1995 and September 15, 1996. Every year at the end of emergence all the subclover cultivars covered uniformly the plot and weed contribution was not appreciable. The following parameters have been analyzed for a 3-year period: seed yield (SY), burr density (BD), seed/burr (S/B), 1000 seed weight (SW). Only in 1994 the cited parameters were measured at 2 different soil depths: 0-0.5 cm and >0.5 cm along with hard seed percentage (HSP).

## Results

### Earliness

Date of appearance of the first flower was comprised from April 3 (Woogenellup, s) to April 23 (Larissa, y) showing a range of about 3 weeks. Within *T. brachycalycinum*, Clare and Rosedale flowered within the 1<sup>st</sup> week of April while Nuba flowered 2 weeks later. Among s types cultivars Woogenellup was the earliest (April, 3) and Karridale the latest (April, 17); the 2 y types present in this trial, one (Gosse) was early (April, 10) and the other (Larissa) late (April, 23).

### Plant regeneration (PR)

PR, expressed as number of seedlings m<sup>-2</sup>, was determined at the end of emergence of each year. The mean values of a 4-year period ranged from 2808 seedlings m<sup>-2</sup> produced by Enfield (s) to 1555 seedlings m<sup>-2</sup> obtained with Nuba, (b). The cultivars belonging to *T. subterraneum* shown on the average a higher number of seedlings m<sup>-2</sup> (2416) than *T. brachycalycinum* (1618) and *T. yanninicum* (1804). However this character seems to be highly influenced by the environmental conditions of each year (cultivars x years interaction).

For more details of the above two parameters see Corleto and Cazzato (2008).

### Seed yield

SY as average of a 3-year period ranged from 0.86 t ha<sup>-1</sup> to 2.23 t ha<sup>-1</sup> (table 1). Rosedale (2.23 t ha<sup>-1</sup>) and Gosse (1.89 t ha<sup>-1</sup>) ranked 1<sup>st</sup> and 2<sup>nd</sup> place with no statistical difference among them. The above cultivars produced more burs per unit area (on the average 8,500 m<sup>-2</sup>) than cv. Larisa (4,167 m<sup>-2</sup>) that ranked the last place. The following cultivars (Rosedale, Gosse, Woogenellup, Clare, Nuba and Larisa) had higher 1000 seed weight comprised between 8.1 (Rosedale) and 7.1 g (Nuba). The cultivars Denmark (5.39), Junea (4.9), Leura (4.8) and Goulburn (3.6 g) showed the lowest values. Hard seeds (%) measured only in the first experimental year (1994) varied from 94.3% (Goulburn) to 44.1% (Clare). Seed burial influenced all the parameters studied. On the average 75% of SY and 79% of BD, were found on the superficial layer, while SW and HSP were higher in the deeper layer (7.4 and 72.9% respectively).

Table 1. Seed production and other parameters of different cultivars of sub-clover.

Cultivars	Seed yield <sup>(1)</sup> (t/ha)	Burs/m <sup>2</sup> <sup>(1)</sup> (n.)	1000 seeds Weight <sup>(1)</sup> (g)	Hard seeds <sup>(2)</sup> (%)
Rosedale (b)	2.23 A	8,419 A	8.1 A	87.6 AB
Gosse (y)	1.89 AB	8,605 A	7.2 B	76.4 BC
Woogenellup (s)	1.39 BC	5,525 AB	7.7 AB	59.3 CD
Clare (b)	1.33 BC	5,125 AB	7.8 AB	44.1 E
Nuba (b)	1.24 BC	5,547 AB	7.1 B	70.3 C
Junea (s)	1.20 BC	7,634 AB	4.9 D	90.6 AB
Denmark (s)	1.19 BC	6,665 AB	5.3 CD	67.2 C
Karridale (s)	1.12 BC	5,673 AB	5.7 C	73.3 C
Enfield (s)	1.08 BC	7,904 AB	5.7 C	52.4 DE
Goulburn (s)	0.98 BC	7,688 AB	3.6 E	94.3 A
Larisa (y)	0.90 C	4,167 B	7.2 B	69.7 C
Leura (s)	0.86 C	6,049 AB	4.8 D	68.6 C
Mean	1.28	6,583	6.3	71.2
<i>Soil depth<sup>(2)</sup></i>				
0-0.5 cm	1,1 a	6,052 a	6,3 b	69,4 b
> 0.5 cm	0,4 b	1,677 b	7,4 a	72,9 a

<sup>(1)</sup> Mean of 3-year period. <sup>(2)</sup> data collected in 1994

### Conclusions

All the cultivars tested produced satisfying amount of seeds even though they were subjected to plant defoliations effectuated up to the end of April. Rosedale and Gosse showed the highest SY along with high HSP. Clare, that is a popular cultivar in Southern Italy, produced a satisfying SY but with the lowest HSP that makes this cultivar more susceptible to false start. Seed burial reduced, on the average, rates of seed softening, according with the finding of other authors (Taylor and Ewing, 1996). Rosedale and Nuba, belonging to *T. brachycalycinum* as Clare, showed a high HSP and in a previous research (Corleto and Cazzato, 2008) produced high DMY along with Clare. It is suggested the use of Rosedale and Nuba for pasture improvement since they could reduce the risk of early seed germination due to erratic rains.

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# Influence of Cultivar, Nitrogen Input and Season on Biomass Yield of Fiber Sorghum

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Crops as energy sources have been boosted by recent developments in the energy global market, driven by rocketing demand from some fast growing economies, and by the widely perceived need for energy sources with low impact on the balance of carbon gases in the atmosphere. Among several crops suitable for Italian conditions, fiber sorghum can be competitive in terms of energy yield (Candolo and Meriggi, 2006). Dark tobacco types have fulfilled the role of cash crop for some hilly areas of South Italy, but reduced demand and an EU policy aimed at phasing out tobacco production have prompted to search for alternatives. With this aim we have assessed the biomass and energy yield of fiber sorghum in the conditions of the above areas, in the framework of an EU program for finding suitable replacements for tobacco.

## Methodology

Field trials were conducted in 2006 and 2007 on a clay loam previously cultivated with tobacco at Venticano (Avellino province), testing in a CRB design with three replicates and 30 m<sup>2</sup> plots two cultivars (H133 and H952) at two nitrogen inputs (100 and 200 kg ha<sup>-1</sup> N), one third broadcast before seeding and two thirds as topdressing, after thinning the density to 200,000 plants ha<sup>-1</sup>. Fertilization also included 60 kg ha<sup>-1</sup> phosphate (P<sub>2</sub>O<sub>4</sub>). The crop was seeded on May 5<sup>th</sup> in 2006 and on April 24<sup>th</sup> in 2007 and was twice irrigated in both seasons, supplying up to 75 mm in 2006, at an early stage of the crop, and up to 100 mm in 2007, at a later stage. A light cultivation after seeding was conducted for weed control. The biomass was harvested in the first decade of September in 2006 and in the last decade of August in 2007, two weeks after flowering, a stage reported to correspond to the peak of cumulated dry matter (Peyre, 1979). Biomass yield was determined by oven drying samples at 105 °C for 48 hours, and energy yield by the Mahler bomb calorimeter. The response was statistically analysed according to a general linear model, with season, cultivar and N input level as fixed effects, and model predictions are reported graphically. The R environment (R Core Team, 2007) and some functions of *climatol* (Guijarro, 2006) and *Hmisc* (Harrell, 2007) contributed packages were used for data analysis and graphics.

## Results

The two seasons were remarkably different: temperate and moist the 2006, with regular rains except for an initial dry spell; hot and dry the 2007 (yearly average temperature 3 °C higher and rainfall 243 mm lower), without rains for the two last months of the crop (figure 1).

Given this difference, the seasonal effect was predominant for crop growth and biomass dry yield, while canopy size was mostly determined by cultivar.

Dry biomass yield ranged between 21 and 35 t ha<sup>-1</sup>, increasing on average between the dry and the temperate season by 28% for the higher yielding H133 and by 21% for H952 (figure 2).

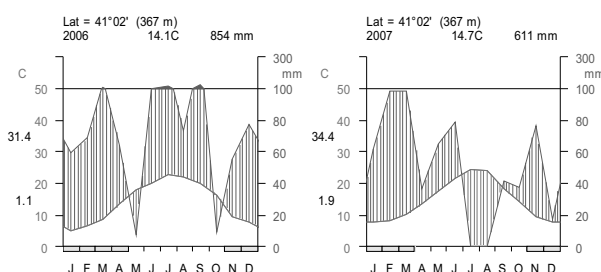


Figure 1. Walter and Lieth (1960) climatic diagrams for the trial area and years.

Crop response rate to N input for dry biomass was also higher in the temperate season (11.8 vs 10.8 kg kg<sup>-1</sup>). H133 outyielded H952 on average by 2 t ha<sup>-1</sup> (13%) in the adverse and by 6 t ha<sup>-1</sup> (19%) in the favorable season. Biomass dry yield increased in the temperate season even if the dry:fresh ratio decreased by about two percentage points and was further reduced by increasing N input (figure 3). Plant size increase was associated with reduced dry:fresh ratio of biomass in the wetter season, especially at the higher level of N input.

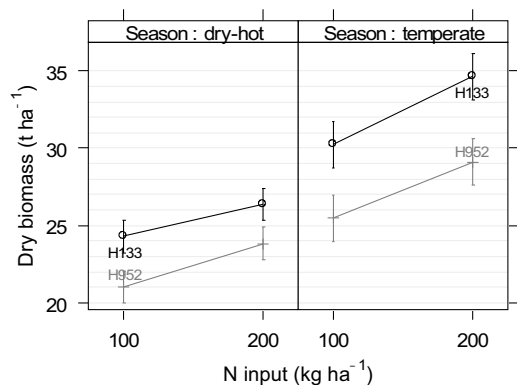


Figure 2. Effect of N input, cultivar and season on dry biomass yield of fiber sorghum. Means and 95% confidence intervals.

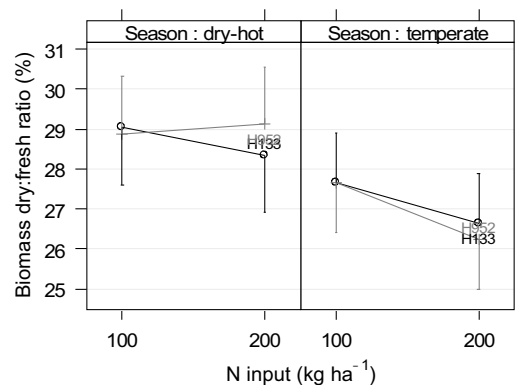


Figure 3. Effect of N input and season on the dry/fresh biomass ratio of fiber sorghum. Means and 95% confidence intervals.

Energy yield, determined only on the biomass from the dry-hot 2007 season, was influenced additively by cultivar and N input level (figure 4).

H133 performed better both for biomass and unit energy yield, giving 20% more energy per hectare. The lower unit energy yield imputable to increasing N input was more than compensated by the increase of biomass yield.

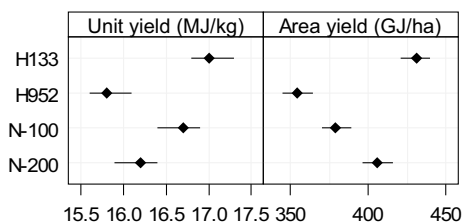


Figure 4. Energy yield by cultivar and N input level for the 2007 season. Means and 95% confidence intervals.

### Conclusions

Biomass and energy yields obtained from fiber sorghum in these trials are in the upper range of reported figures for Italian conditions and higher in comparison with some competing crops, allowing a positive economic balance for the crop (Candolo, 2006). The observed yield range could amount to crop values between 1,200 and 1,500 € ha<sup>-1</sup>, higher than sunflower outputs for similar environments. However, the marginal value of produce for N input was about a quarter of the fertilizer price, showing that N fertilization was of doubtful value in the conditions of the trials.

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# The Effect of Systemic Seed Treated Insecticides on Sunflower Yield

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## Introduction

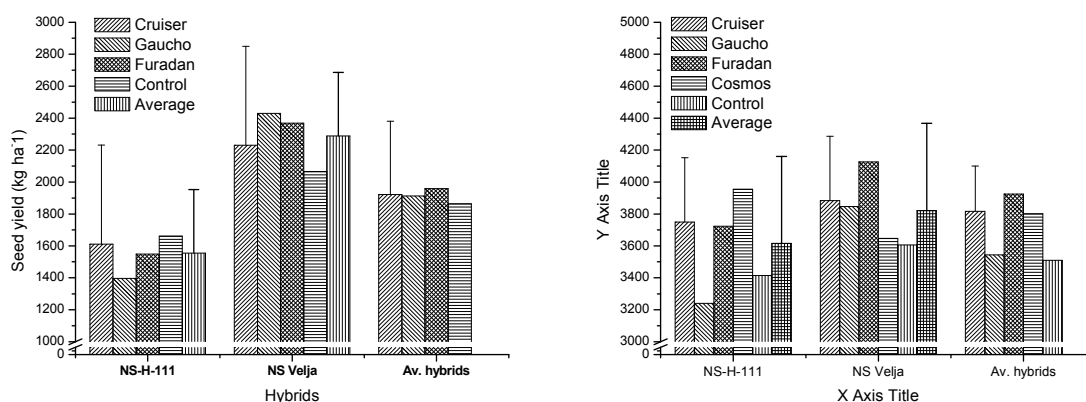
Number of plant is one of main yield component (Crnobarac et al. 2000), but it depends of damage seedling by insect. Chemical pest management is one of common cultural practice in sunflower production. By applying insecticides, also it should consider environmental and human labour protection as well as plant tolerance to toxicity of insecticides (Phillip, 2008). Advantage of systemic seed treated insecticides is its: lower applying doses, and seed toxicity, as well as longer effect for protect plant of all pest even to the middle of vegetation and reduce necessity of next insecticides treatments (Sekulic, et al. 2000)

## Methodology

Three years experiment (2005-2007) was conducted in field of Institute of filed and vegetable crops in Novi Sad, Serbia, on chernozem soil type, with “NS-seed” sunflower hybrids NS-H-111 and NS Velja. Seed of hybrids were treated with: contact insecticide Furadan, three systemic (Cruiser, Gaucho and Cosmos), and control without any insecticide. Four replication, with size of each elementary parcel of 28 m<sup>2</sup> was used. After harvesting seed and oil yield, oil content and percentage of harvested/sprout plants were processed like split plot design where hybrids were main and insecticides subplot.

## Results

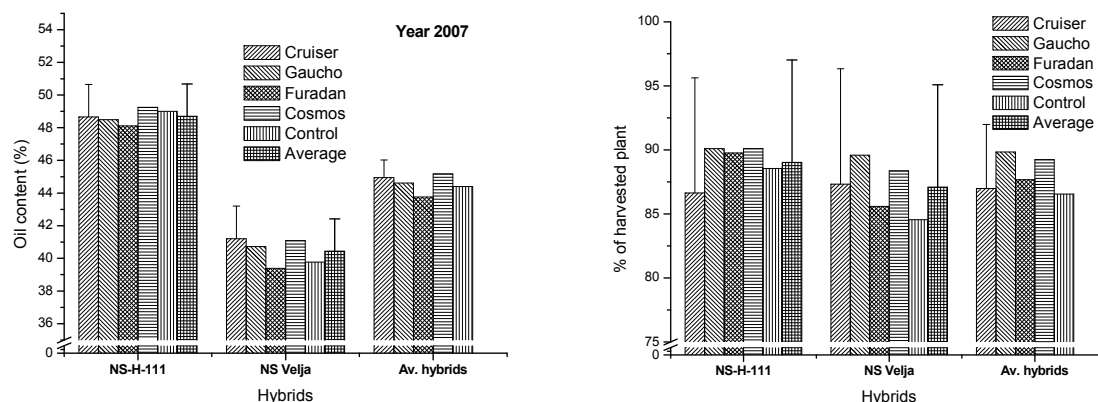
Hybrids had significant influence on seed yield only in 2005, on oil content and yield in two year and there were no significant effect on percentage of harvested/sprout plants. Variants of insecticides had no significant influence on seed yield in 2 year, while in 2006 control even had lower yield then variant with Furadan (Graph 1). Insecticides variants had no significant influence on oil content, oil yield and on percentage of harvested/sprout plants in all year (Graph 2 right).



Graph. 1. The effect of year, hybrid and insecticides on sunflower

seed yield (left 2005, right 2006 year)

In interaction hybrids\*insecticides on seed yield were significant differences only between hybrids in 2005, on oil yield in 2005 and 2007, and in oil content significant differences were between hybrids at each variant in two year and in 2006 in variant with Cosmos. Only in 2007 variant with Furadan had significant lower oil content than variants with Cruiser and Cosmos, but between Control, Gaucho and Furadan there were no significant differences (Graph 2 left).



Graph. 1. The effect of hybrid and insecticides on sunflower oil content (left) and % of harvested/sprout plant (right)

## Conclusions

Hybrids had significant influence on seed yield, oil content and yield and there were no significant effect on percentage of harvested/sprout plants.

Variants of insecticides had no negative influence on seed yield, oil content, oil yield and on percentage of harvested/sprout plants at all.

Considering environmental aspect and protection of human labor, better are systemic insecticides

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# Effects on Plant and Soil of Anaerobic Digestates

## Application: Results of a Two-Year Field Study on Fodder Crops

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Under anaerobic condition organic substances are broken down to simpler chemical components by a naturally occurring process. During decomposition methane is produced and digestate is obtained as by-product. Anaerobic digestates largely contain plant nutrients (N, P and K) and these residual materials can be used as organic fertilizers or as soil amendments and applied to cultivated soils. The agronomic management of digestate is also related to the presence of heavy metals and other inorganic contaminants. The utilisation of these materials could increase pollution risks, especially if homogeneous distribution and correct dosage are not respected. One of the possible solutions is to apply the organic amendments on fodder crops which are not directly used as food.

Objective of this study was to evaluate the agronomic performance of anaerobic digestates in comparison with mineral fertilisers on graminaceous and leguminous fodder crops.

### Methodology

A two-year field study (2005 and 2006) was carried out in the Experimental Farm "Agostinielli" in Rutigliano (Bari), Southern Italy. In permanent rain-fed meadows of alfalfa (*Medicago sativa* L.) and cocksfoot (*Dactylis glomerata* L.), three fertilizer treatments (two anaerobic digestates and one mineral) and the unfertilised control (CONTR) were compared in a randomized-block experimental design with three replications. In the first year (2005) two anaerobic digestate, based on pig slurry mixed with olive mill waste water (PSO) and on waste water from frozen food processing (WFF), were compared. In 2006, PSO was substituted with an anaerobic digestate amendment based on the waste water treatment of wine derived process (WWW). Based on the nutrient elements content of the anaerobic digestates applied in the two-year field experiments, the total amount of organic materials distributed is shown in Table 1.

**Table 1.** Anaerobic digestates applied in the two-year field experiment

	2005		2006	
	PSO	WFF	WWW	WFF
	t ha <sup>-1</sup>		t ha <sup>-1</sup>	
Alfalfa	29.1	14.5	7.1	11.1
Cocksfoot	20.9	7.7	5.4	6.6

In both the years, during the crops cycle, fresh weight, dry weight (oven-dried at 70 °C till constant weight), total N (Fison CHN elemental analyzer ) and P (Inductively Coupled Plasma-Optical Emission spectrometry -ICP-OES) contents of plants were determined, allowing the calculation of total N and P uptake (N and P content x biomass dry weight). Total content of heavy metals in plants (Cd, Cr, Fe, Zn, Cu, Ni and Pb) was also determined. Plant material was acid-extracted in a mixture of nitric acid and hydrogen peroxide, than placed in microwave oven. All extracts were analyzed by ICP-OES.

At the end of the experiment, three soil sub-samples (0-40 cm layer) were taken from each elementary plot, pooled in one sample for replication and treatment, air dried, ground to pass a 2-mm sieve and then analysed. The following soil characteristics were determined: total N (Kjeldhal digestion and distillation); available P (Olsen); total Zn, Ni, Cu and Pb (ICP-OES). Total organic carbon (TOC) was determined using the Springer and Klee method, while total extractable carbon (TEC) and humified organic carbon [C(HA+FA)] were measured.

The statistical analysis was carried out by using the General Linear Model procedures of the SAS package. Differences among the treatments were evaluated with the Duncan Multiple Range Test.

## Results

The total yield of alfalfa in 2005 was 9.47 t ha<sup>-1</sup> and 13.2 t ha<sup>-1</sup> in 2006. Furthermore, no significant difference was found among the fertiliser treatments in both years and during the whole cropping cycles. In cocksfoot, the cumulative dry matter at the end of cycles was 2.66 and 1.47 t ha<sup>-1</sup> for 2005 and 2006, respectively. The yield of CONTR treatment was lower than fertiliser treatments in both the years, confirming that the organic materials could be a consistent N source for fodder crop production, in accordance with the results found by Sullivan *et al.* (2003).

No significant difference was found among treatments in N and P content for both alfalfa and cocksfoot and in the two years field experiment. The values of heavy metals concentration were low in both alfalfa (Mc Crory *et al.*, 2005) and cocksfoot (Ortiz and Alcaniz, 2006). No statistical significant differences were found among treatments in the two years, with exception for Fe in 2005 and Zn in 2006 for alfalfa and Ni for cocksfoot in both the years.

No significant difference was found in soil characteristics and in total N and P content among the fertiliser treatments neither in alfalfa nor in cocksfoot. These findings were probably due to the chemical properties of the organic matter contained in both the anaerobic digestates utilised. In addition, the levels of organic C fractions did not substantially vary among fertiliser treatments, probably due to their high absolute values in the soil, which makes difficult to assess changes in the short-term period (two years) (Montemurro *et al.*, 2007).

## Conclusions

The results demonstrate that anaerobic digestates could be an effective sustainable option to provide nutritive elements for fodder crops and to decrease the supply of mineral fertilisers, because of their properties (high and low amounts of mineralisable N and lignin, respectively). Furthermore, the cumulative organic applications increased the yield throughout the two-year experimental period, due to a greater amount of nutrients supplied. The results pointed also out that there was not difference in the nutrient elements utilisation by the fodder crops between anaerobic digestates and mineral fertilisers. However, the application of organic materials slightly contribute to increase total organic C content of soils compared to the unfertilised control.

The results of our experiment indicate that anaerobic digestates applications in fodder crops did not accumulate heavy metals when plants were cultivated under short-term repeated application and that their recycling could represent a valid alternative to landfill disposal or to other waste management strategies.

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# Can Top Quality Tobacco Be Grown Saving Water and Fertilizers in Mediterranean Region?

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There is an increasing pressure on tobacco producers worldwide to grow their crops according to good agricultural practices, which are conservative for soil and water, and to reduce both the leaf content of nitrogen (N) related compounds (tobacco specific nitrosamines, TSNA) and environmental contamination. Dark fire-cured tobacco has been grown in Italy for centuries to produce the famous Toscano® cigar. We investigated different drip irrigation and N fertilization treatments on dark-fired tobacco grown to produce the wrapper of Toscano® cigars.

## Methodology

A field experiment was conducted on cv. Foiano in Central Italy during 2007. Two deficit irrigation treatments (50% ET<sub>c</sub>), imposed either using conventional deficit irrigation (DI<sub>50</sub>) or alternate row irrigation (ARI<sub>50</sub>, non conventional), were compared with a full irrigation treatment (100% ET<sub>c</sub>, control, FI). In the DI<sub>50</sub> treatment, plants received half amount of water, uniformly applied to both sides of the row, whereas in the ARI<sub>50</sub> treatment one-half of the root zone was wetted while the other half was maintained dry, thereby the half amount of irrigation water was applied; in particular, the wetted and dry zones were interchanged in two subsequent irrigations. One hundred and eighty kg N ha<sup>-1</sup> (dose commonly used by growers) and 120 kg N ha<sup>-1</sup> were used as fertilization rates. Seedlings were transplanted at 1.0 × 1.0 m distance on June 11. Plants were topped at the beginning of flowering (21 August), harvested on September 18 and fire cured in traditional burns. Total alkaloids, TSNA, reducing sugars, total nitrogen and nitrates were determined on cured product. Cured leaves were also evaluated by a blind panel for determination of some external quality traits like colour, body and elasticity by using a 1 to 3 scale (low to high scores for bad to good quality traits, respectively).

## Results

The yield of cured leaves of DI<sub>50</sub> and ARI<sub>50</sub> was 89 and 92% of that of the control, respectively. There was a significant interaction N fertilization × Irrigation for yield and the irrigation water use efficiency (IWUE, kg ha<sup>-1</sup> cured leaves mm<sup>-1</sup> water applied). In particular, yield of FI plants was not significantly

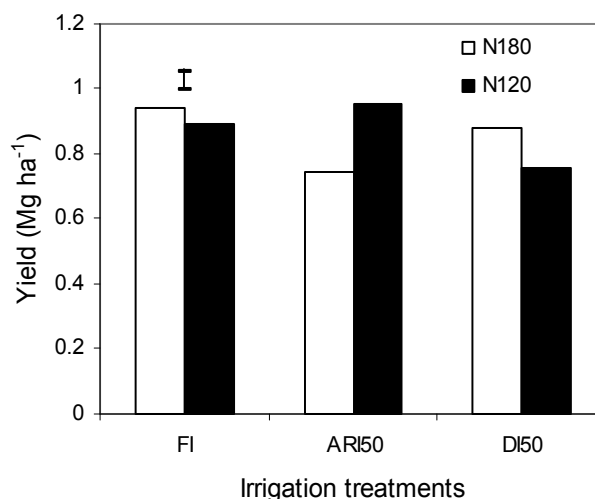


Figure 1. N fertilization × Irrigation interaction for yield of fire-cured wrapper leaves. Legend: FI, full irrigation (100% ET<sub>c</sub>); ARI<sub>50</sub>, alternate row irrigation (50% ET<sub>c</sub>); DI<sub>50</sub>, conventional deficit irrigation (50% ET<sub>c</sub>). N180, 180 kg N ha<sup>-1</sup>; N120, 120 kg N ha<sup>-1</sup>. The bar indicates least significant difference at P ≤ 0.05.

influenced by N treatments whereas, by contrast, plants grown under ARI<sub>50</sub> and DI<sub>50</sub> irrigation produced significantly more and less, respectively, with 120 than 180 kg N ha<sup>-1</sup> (Fig. 1). The mean weight of cured leaves was unaffected by N fertilization, although it appeared influenced by irrigation treatments (36.7, 34.4 and 30.2 g in FI, ARI<sub>50</sub> and DI<sub>50</sub>, respectively,  $P \leq 0.05$ , data not shown). Interestingly, there were no changes in size of wrapper fire-cured leaves since both leaf length or width did not vary significantly with N fertilization and irrigation treatments. As for the IWUE, both conventional and non conventional deficit irrigation increased it with respect to the FI treatment (Kang *et al.*, 2001) but, in particular, the IWUE of ARI<sub>50</sub> plants was specially favoured by the lowest N dose (Fig. 2). Analytical quality determinations showed no significant change in leaf composition due to treatments (irrigation and N fertilization) even though numerically lower nitrate and TSNA contents were measured with application of 120 than 180 kg N ha<sup>-1</sup> (3866.7 vs. 4667.3 mg kg<sup>-1</sup> of nitrates, respectively; 9.6 vs. 14.8 mg kg<sup>-1</sup> of TSNA, respectively). Finally, no difference was found in external quality traits between irrigation or N fertilization treatments. Therefore, despite 50% of water saved with respect to FI treatment, both conventional and non conventional deficit irrigation treatments determined only a negligible yield reduction with respect to FI treatment which was presumably due to their greater efficiency in irrigation water use than FI treatment (Tab. 1).

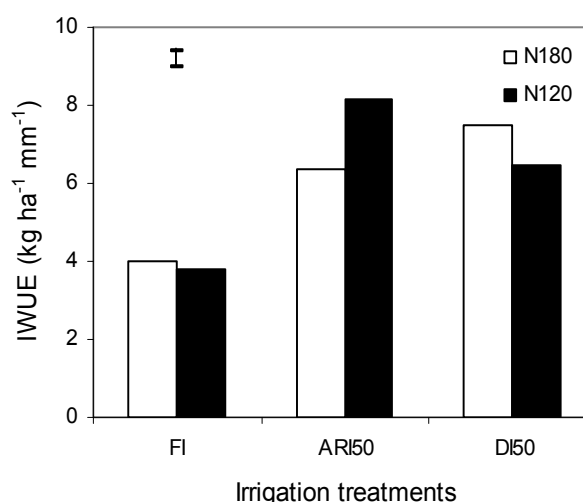


Figure 2. N fertilization x Irrigation interaction for irrigation water use efficiency (IWUE) calculated on yield of fire-cured wrapper leaves. Legend: FI, full irrigation (100% ET<sub>c</sub>); ARI<sub>50</sub>, alternate row irrigation (50% ET<sub>c</sub>); DI<sub>50</sub>, conventional deficit irrigation (50% ET<sub>c</sub>). N180, 180 kg N ha<sup>-1</sup>; N120, 120 kg N ha<sup>-1</sup>. The bar indicates least significant difference at  $P \leq 0.05$ .

Table 1. The amount of water saved and IWUE and yield changes relative to full irrigation treatment. Legend: FI, full irrigation (100% ET<sub>c</sub>); ARI<sub>50</sub>, alternate row irrigation (50% ET<sub>c</sub>); DI<sub>50</sub>, conventional deficit irrigation (50% ET<sub>c</sub>)

Irrigation treatments	Irrigation (mm)	Relative IWUE*		Relative yield		Water saved (%)
		N120	N180	N120	N180	
FI	233.6	1.0	1.0	1.0	1.0	0
ARI <sub>50</sub>	116.8	2.1	1.6	1.1	0.8	50
DI <sub>50</sub>	116.8	1.7	1.9	0.8	0.9	50

\* calculated on wrapper dark fire-cured leaves, only (wrapper dark fire-cured leaves are about one third of the total yield).

## Conclusions

In conclusion, the reduction of irrigation water by 50% and N fertilizer by one third did not determine relevant loss of yield or lack in quality characteristic of top quality wrapper dark fire-cured tobacco and could represent an effective way to save water and N in dark tobacco cultivation in Mediterranean region.

## References

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# Winter Cereal Response to Manure and Mineral N Fertilization

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Fertilization strategy of winter cereal crops in Catalonia depends very much on availability of manure by farmers for applications before sowing. The management of mineral N dressing application must be very different depending on the use or not of manure in the system. The aim of the work was to optimize N use in winter cereal fertilization under Mediterranean conditions.

## Methodology

A plurianual trial was established (La Tallada d'Empordà; NE Iberian peninsula) to determine the optimal fertilization strategy using organic (dairy manure) and mineral fertilizers. The experimental design was a split-plot with 3 replicates and a single plot surface of 60 m<sup>2</sup>. The experiment started in 2001 and data from years 2006 and 2007 are shown in this paper. Treatments consisted, since the initial, on dairy manure (0 and 30 Mg ha<sup>-1</sup> corresponding to 0 and 170-210 kg N ha<sup>-1</sup>) applications as a main factor. The secondary factor was the rate of mineral N applied at dressing (0, 40, 80 and 120 kg N ha<sup>-1</sup>). Every year yield, N crop uptake, crop nutritional status and mineral N content in soil (0-90 cm) at presowing, dressing and harvest were determined.

## Results

The highest yield (8,600 kg ha<sup>-1</sup>) in the trial was attained for the control treatment (without N mineral or organic application) on year 2007 (Table 1), probably due to crop lodging before the end of grain maturation in the treatments with N or manure application. When manure was applied, the best performing treatment was that without N dressing application (yield: 76 % from the unmanured one).

Organic N applied before sowing	Mineral N applied at dressing	Yield (kg ha <sup>-1</sup> )		Specific weight (kg hL <sup>-1</sup> )		N uptake (kg N ha <sup>-1</sup> )	
		2006 (wheat)	2007 (barley)	2006 (wheat)	2007 (barley)	2006 (wheat)	2007 (barley)
No manure	0	5060	8619	66,5	64,9	51,9	116,2
	40	5482	7994	66,6	64,1	62,2	115,5
	80	5758	7100	65,1	62,8	77,3	120,7
	120	5631	6152	62,2	62,1	85,5	106,5
30 t ha <sup>-1</sup> dairy manure	0	6946	6516	59,5	63,7	97,3	121,9
	40	6165	4627	62,4	62,3	103,7	96,0
	80	5530	5110	60,3	61,6	106,3	109,0
	120	5396	4544	61,2	62,7	105,4	99,4

Table 1.- Grain yield (kg ha<sup>-1</sup>), specific weight (kg hL<sup>-1</sup>) and N uptake (kg N ha<sup>-1</sup>) for the different manured and unmanured treatments on years 2006 and 2007. La Tallada d'Empordà.

The most productive unmanured treatment on year 2006 received 80 kg N ha<sup>-1</sup> at dressing (5,758 kg of wheat ha<sup>-1</sup>) although from an economic and quality (Table 1) point of view, the optimal unmanured treatment was that with 80 kg N ha<sup>-1</sup> applied at dressing (5,482 kg of wheat ha<sup>-1</sup>). Nevertheless, on year 2006, the most productive treatment (6,946 kg of wheat ha<sup>-1</sup>) of the trial was the manured with no

further mineral N application at dressing one, although its quality (specific weight) was under the normal standards. Its yield was 25 % higher than the optimal one in unmanured treatments. The yield achieved for low input treatments and N uptaken on unfertilized plots (Table 1) were possibly related with the high soil N mineralization on the soil (Domingo *et al.*, 2005). Nutrition status (Table 2) indicates a concordance between those measurements and the yield results achieved on year 2006 but not for year 2007, probably due to the effect of late plate lodging, after GPN measurements. Nitrate-N content in soil (Figure 1) showed a higher abundance on manured plots, mainly in the 0-60 cm soil depth.

Organic N applied before sowing	Mineral N applied at dressing	Relative plant nutrition index measured with GPN			
		14/03/2006	12/04/2006	16/02/2007	20/04/2007
No manure	0	95,56	93,79	94,35	98,35
	40	92,69	96,08	91,06	98,55
	80	92,86	98,49	92,98	99,08
	120	92,23	98,99	93,18	99,44
30 t ha <sup>-1</sup> dairy manure	0	96,92	97,98	96,92	99,48
	40	98,68	99,38	100,00	99,60
	80	100,00	100,00	98,25	100,00
	120	99,51	99,69	98,55	99,88

Table 2.- Relative (considering the highest value at each date as 100 %) index for plant nutrition status measured with reflectometer GPN ® at different dates on years 2006 and 2007. La Tallada d'Empordà.

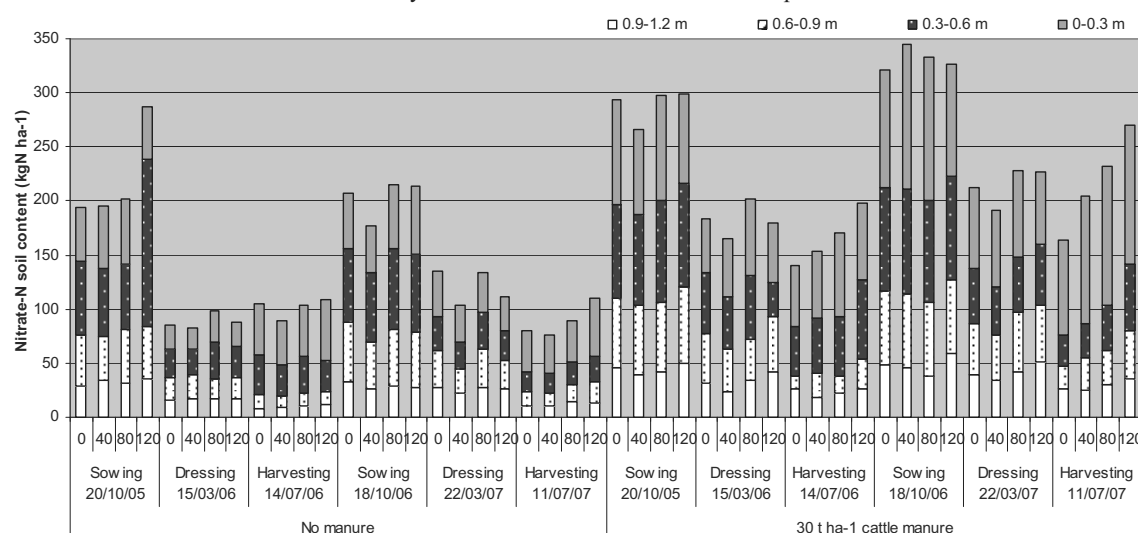


Figure 1.- Evolution of nitrate-N soil content for the different manured and unmanured treatments. Tallada d'Empordà.

## Conclusions

Optimal rate of N dressing fertilization for winter cereals (wheat and barley) was less than 50 kg N ha<sup>-1</sup> when only mineral N was applied and 0 kg N ha<sup>-1</sup>. When manure was used at an agronomical rate, no added N at dressing was necessary for an optimal yield under rainfed mediterranean conditions.

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# Long-term Effect of Different Crop Rotations and Soil Tillage Systems on Soil Organic Matter Content

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The soil organic carbon (SOC) pool is an important indicator of soil quality, and has numerous direct and indirect impacts on it. For example, increase in SOC pool in degraded soils improves soil structure and tilth, reduces soil erosion, increases plant available water capacity, stores plant nutrients, provides energy for soil fauna, purifies water, denatures pollutants, increases soil biodiversity, improves crop/biomass yields, and moderates climate. It makes soil a living ecosystem (Lal, 2007).

The concept of “soil quality” has recognized soil organic matter as an important attribute that has a great deal of control of many of the key soil functions. However, soil organic matter varies among environments and management systems, generally increasing with higher mean annual precipitation, with lower mean annual temperature, with higher clay content, with an intermediate grazing intensity, with higher crop residue inputs and cropping intensity, with native vegetation compared with cultivated management, and with conservation tillage compared with conventional tillage (Rasmussen, Collins, 1991; Franzluebbers, 2002; Hůla, Procházková *et al.*, 2008).

## Methodology

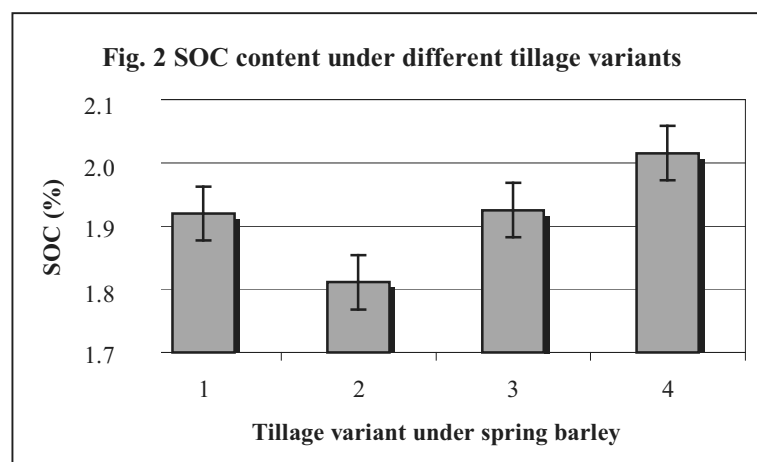
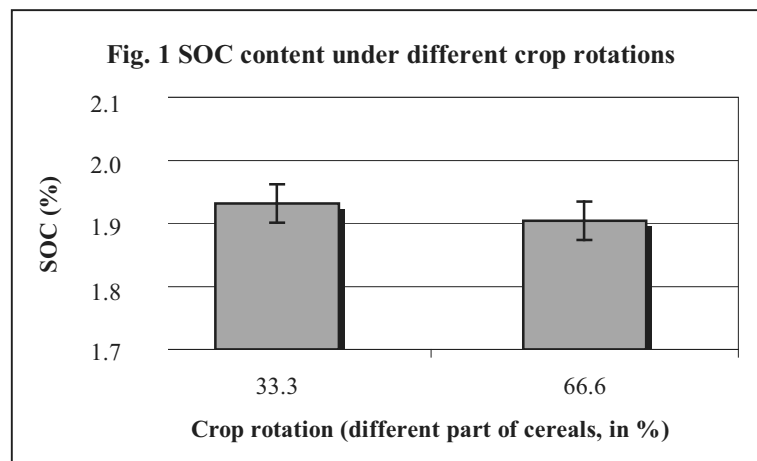
In the long-term field experiment were studied the effects of two crop rotations and variants of different intensity of soil tillage for spring barley with preceding crop sugar-beet on soil organic carbon content (SOC). Long-term experiment was established in year 1989 in sugar-beet growing region in the altitude of 225 m; the average annual temperature and sum of precipitation were 9.2 °C and 548 mm, respectively. Soil conditions: chernozem, loamy soil with neutral soil reaction and good reserves of phosphorus, potassium and magnesium.

Studied variants of crop rotations: 1/ with 33.3 % cereals part, 2/ with 66.6 % cereals part. Soil tillage variants for spring barley: 1/ ploughing to the depth 0.22 m, 2/ ploughing to the depth 0.15 m, 3/ direct sowing into no-tilled soil, 4/ shallow loosening to the depth of 0.10 m. Soil tillage variants for sugar-beet: 1/ fertilising of manure, ploughing to the depth 0.22 m, ploughing to the depth 0.28 m; 2/ fertilising of manure, ploughing to the depth 0.22 m, loosening to the depth of 0.40-0.45 m; 3/ fertilising of manure, ploughing to the depth 0.22 m, ploughing to the depth 0.28 m, sowing of freezing out catch crop; 4/ fertilising of manure, ploughing to the depth 0.22 m, sowing of freezing out catch crop. Sample depth: 1/ 0-0.1 m, 2/ 0.1-0.2 m, 3/ 0.2-0.3 m.

Results were statistically analyzed by means of variance analysis and Tukey's test ( $P \geq 95\%$ ).

## Results

SOC content value was always higher under crop rotation with 33.3 %. On the variant with ploughing to the depth 0.15 m under spring barley with sugar-beet preceding crop under ploughing to the depth to 0.22 m and soil loosening to the depth 0.40-0.45 m, was detected significantly the lowest value of SOC content in both crop rotations. Main results are presented in Figures 1-2. SOC content under sampling depth was similar in both crop rotations and each soil tillage variants - i.e. the highest value was reached in 0-0.1 m depth and the lowest value was reached under 0.2-0.3 m depth.



## Conclusions

Soil organic carbon is considered one of the keys to soil fertility. Soil organic carbon levels are affected by agricultural management practices and by complex processes where tillage systems have a strong influence on its stratification and evolution. Carbon sequestration in soil depends on turnover time too.

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## Acknowledgement

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# Effects of Different Techniques of Green Manure on N Balance of the Rotation Tomato-Melon

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## Introduction

Alternative strategies for satisfy N requirements of crops are crucial for organic farming. This aim could be successfully reached by green manure though it presents several uncertainties due to the need of coincidence of the biomass N mineralization and crop N uptake (Korsaeth, 2002). Besides, this microbially driven process is influenced by chemical composition of manure and pedoclimatic factors (Breland, 1994) that shows high variability among sites and years, thus increasing the uncertainties about N balance (Korsaeth, 2002).

Changing times of green manure burying may regulate their chemical composition (Kankanen *et al.*, 1998) and mineralization rate too (Yadvinder-Singh *et al.*, 1992). Legume biomasses are quickly mineralized in Mediterranean area if buried at early stages, while burying at late stages or burying mixed crops (legume crops+cereals) may reduce mineralization rates, because of higher C/N ratio and lignin content. In this trial, different techniques of green manuring were compared with the aim to evaluate the effects on yield and N balance of an extensive horticultural cropping system.

## Methodology

The trials were carried out during 2003-2004 and 2004-2005 in Southern Italy on a Clay-Loam Soil. The treatments were: C=non fertilized control, M=mineral fertilization with 130 kg ha<sup>-1</sup> of N for tomato and 150 kg ha<sup>-1</sup> for melon, F1=fababean early green manure, F2=fababean late green manure, FO1=fababean+oat early green manure e FO2=fababean+oat late green manure. All the data were subjected to ANOVA using a complete randomized block design with 4 replicates. Mean separation was made using LSD test. Early burying was made at the beginning of fababean flowering (April 8 and 6 in 2003 and 2004 respectively); late burying was made at the end of flowering (April 23 and May 3 in the 2 years). Cash crop transplanting (tomato in 2004 and melon in 2005) was made at the half of May. Plants were weighed at harvest (24/8 and 20/7 for tomato and melon respectively), and then oven-dried for chemical analysis. Soil samples were collected at three depths at sowing and harvest of cash crops. Mineral N fractions were extracted by HATCH method and measured by spectrophotometer while Kjeldhal and Walkely Black methods were used for total N and organic C.

N balance was made considering N buried, soil N mineralization, mineral N at transplanting and N uptake of crops. Soil N mineralization was estimated by N uptake of control + variation in soil mineral N between transplanting and harvest of control.

Rainfalls were very high during growth periods of the cover crops (609 mm in 2003-2004 and 655 mm in 2004-2005 from November to April), while they were low during growth periods of cash crops (135 mm in 2004 and 30 mm in 2005 from May to August).

## Results

On the average, F showed the highest N supply (94.5 kg ha<sup>-1</sup> on the average) and the lowest C/N (25.5 on the average) ratio as compared with FO (59.2 kg ha<sup>-1</sup> and 35.7 respectively). Late burying caused an increase both in N supply and in C/N ratio (101.1 kg ha<sup>-1</sup> and 34.0) as compared with early burying (52.7 kg ha<sup>-1</sup> and 27.3). F1 resulted the best green manure allowing to gain not different yield from M (37.1 vs. 28.5 t ha<sup>-1</sup> for tomato and 17.9 vs. 19.2 t ha<sup>-1</sup> for melon). FO caused lower yield because of lower N supply and higher C/N ratio, giving in some case lower yield than C (6.7 vs. 19.2 Mg ha<sup>-1</sup>)

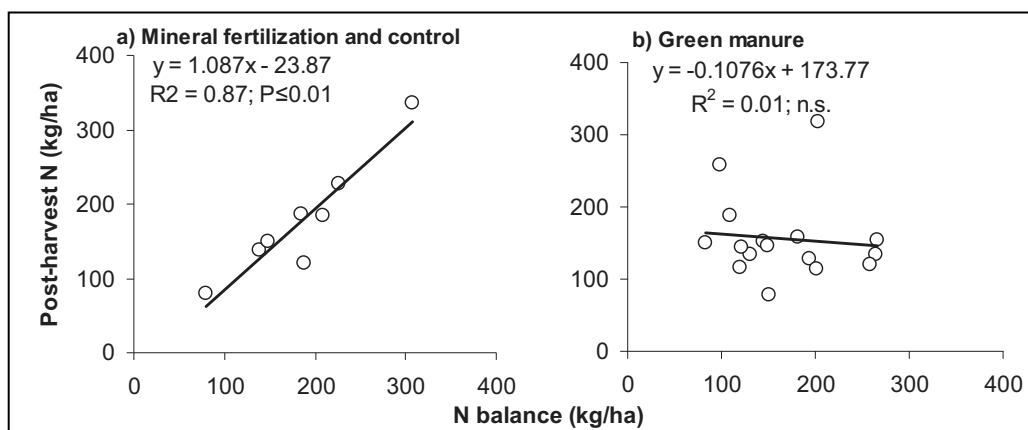
maybe because C supply may have reduced SOM mineralization too. N balance (Tab. 1) was positive, resulting in high soil content of mineral N in post-harvest period (Sept.-Oct.).

**Table 1. Uptakes and N surplus of tomato and melon**

Treatments	Tomato		Melon	
	Uptakes (kg ha <sup>-1</sup> )	N Surplus (kg ha <sup>-1</sup> )	Uptakes (kg ha <sup>-1</sup> )	N Surplus (kg ha <sup>-1</sup> )
<b>F1</b>	96.4 bc	146.4 b	28.0 b	155.9 bc
<b>F2</b>	101.1 b	224.5 a	27.4 b	195.1 b
<b>FO1</b>	78.3 bcd	158.2 b	20.1 c	112.4 c
<b>FO2</b>	66.0 d	168.6 b	17.7 c	171.3 b
<b>M</b>	128.3 a	222.3 a	37.4 a	281.2 a
<b>C</b>	72.2 cd	148.4 b	21.4 bc	110.6 c

Values with the same letters are not different at  $P < 0.05$

Because low decomposition of organic N buried with biomasses, no correlation for green manures was found between N surplus and soil mineral N at harvest of tomato (Fig. 1).



**Figure 1. Linear regressions between N balance and post-harvest soil mineral N.**

## Conclusions

Green manure with legume crops buried at early stages is able to gain yields not different from mineral fertilization. Late burying of manures leads to an increase in N supplies but reduces its availability for the following crop. This results in higher content of soil mineral N in post-harvest periods. In the environments with rainy autumn, not only mineral fertilization but also green manure can cause nitrate pollution, because large amounts of N from buried biomasses may be mineralized in the post-harvest period of the following crop, thus increasing the risk of nitrate leaching.

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# Wheat and Barley Floret Development in Response to Nitrogen and Water Availability

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## Introduction

Wheat and barley yields are quite variable under Mediterranean environments mainly due to the erratic rainfall regime. Part of the interannual variation could be managed with nitrogen (N)<sup>1</sup>. In addition, water use efficiency is largely affected by N management. That is why water and N are the two dominant factors controlling cereal yield in Mediterranean conditions. Most of the yield responses to water x N is related to differences in the number of grains m<sup>-2</sup>. Understanding the physiological bases of grain number responses to these two factors would allow a better understanding of yield determination in Mediterranean agrosystems. Analyses of crop developmental features<sup>3</sup> and responsiveness to radiation and photoperiod<sup>2</sup> affecting grains m<sup>-2</sup> showed that the outcome is a consequence of the process of floret primordia initiation followed by a strong mortality phase in which a small proportion of the maximum number of primordia initiated survive to produce fertile florets (which then become grains). To the best of our knowledge, no evidences have been provided on whether yield responsiveness to N and water is linked to the fate of floret primordia to produce fertile florets. This study aimed to clarify the main yield responsiveness to N under different water regimes is linked to the fate of floret primordia to produce fertile florets.

## Methodology

Cereals were grown in micro-crops in large containers (1 m height x 1 m<sup>2</sup> surface) filled with a sand:soil mixture at the University of Lleida (41°35'S, 58°29'W) during two growing seasons. Treatments consisted in the factorial combination of (i) two species (durum wheat, cv. Claudio, and barley, cv. Sunrise), (ii) two levels of N, an unfertilized control (N-) and a treatment fertilized (N+) [100<sub>(2006-07)</sub> or 250<sub>(2007-08)</sub> kgN ha<sup>-1</sup>] and (iii) two levels of water availability (irrigated and rainfed). Aboveground and soil samples were taken in jointing (DC 31); anthesis (DC 65) and maturity (DC 95). Floral development was measured in all florets of two basal, two central and two apical spikelets corresponding to a main-shoot spike throughout the stem elongation phase following a quantitative scale of spike development<sup>4</sup>. Data of floret development stage were regressed against thermal time and rates of floret development (Waddington units per degree day) were estimated for each treatment and experimental condition. For simplicity, in this presentation a linear relationship between developmental scores of floret primordial and thermal time was assumed (and thus in this presentation rates of floret development integrates environmental effects on both actual rates and effective duration of development progress). Due to higher-than-expected rainfall in the pre-anthesis phase of the first experimental year, for this experiment treatments were reduced to the combination of genotypes and N levels.

## Results

Nitrogen increased the number of fertile florets, although the pattern of spikelets in which the increased number of fertile florets took place varied with the experimental condition. While in the first experiment the improvement was evident in the extreme spikelets and not in the central ones, in the following year the number of fertile florets increased mainly in the central spikelets (see Fig. 1). Whether this is a consequence of the particular conditions of the years or a consequence of structural differences between the spikes of both years remains to be determined. The greater number of fertile florets in spikelets analyzed was also variable with the environment where the effect of the N was

evaluated: in 2006-07, higher N availability accelerated the initiation of floral primordia and reached a greater maximum number of primordia spikelet<sup>-1</sup> that determined a higher number of fertile florets *a posteriori*, whereas in 2007-08, irrigated and rainfed treatments respectively, the maximum number was independent of N that affected the survival of floret primordia to produce fertile florets (Fig. 1).

The rate of floral development in wheat was greater in all environments for central than for any of the extreme spikelets. The increased number of fertile florets at anthesis produced by N in all the three conditions (Fig. 1) was related to an effect of this factor on the rate of floret development, particularly for the floret positions in which the final number of fertile florets was defined in each of the spikelets considered (Table 1). The effect was not clear in the unique floret of barley spikelets.

### Conclusions

N availability in wheat increased the rate of development of the florets mainly at the distal positions, and thus increased the establishment of additional fertile florets in those spikelets. In Barley the dynamics of tillering might be more relevant than in wheat as determinant of the responses to N.

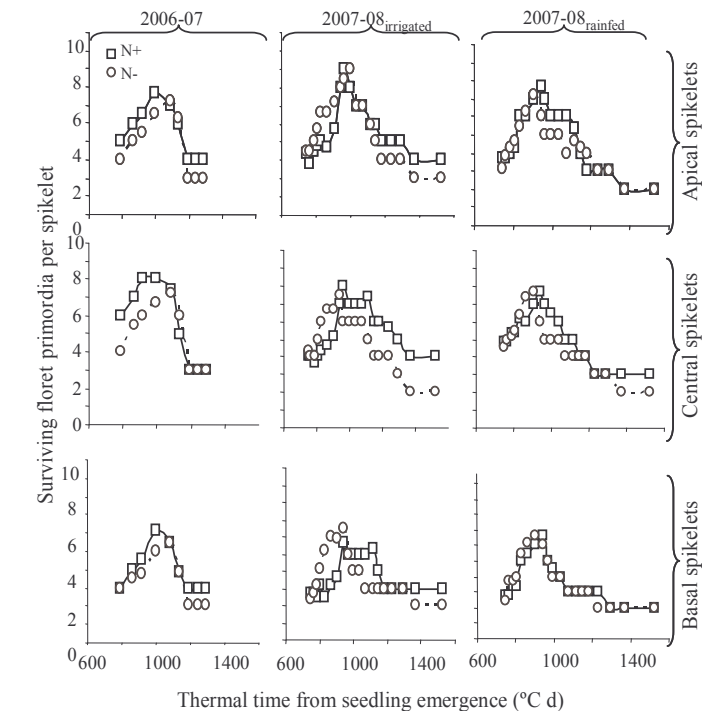
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**Fig. 1.** N effect on the developmental dynamics of floret primordia (those that continue developing from their initiation to anthesis) in each of the three experimental conditions and for each of the three spikelet categories considered

		Wheat				Barley
Basal		Floret 1	Floret 2	Floret 3	Floret 4	
2006-07	N-	1.46 ± 0.09	1.65 ± 0.09	1.58 ± 0.12		1.91 ± 0.03
	N+	1.45 ± 0.12	1.56 ± 0.10	1.76 ± 0.08		2.36 ± 0.13
2007-08 Irrigated	N-	1.56 ± 0.06	1.54 ± 0.06	0.99 ± 0.09		1.35 ± 0.10
	N+	1.33 ± 0.07	1.29 ± 0.09	1.29 ± 0.07		1.32 ± 0.07
2007-08 Rainfed	N-	1.74 ± 0.11	1.23 ± 0.12			1.31 ± 0.11
	N+	1.68 ± 0.08	1.72 ± 0.07			1.32 ± 0.09
Central						
2006-07	N-	1.41 ± 0.11	1.42 ± 0.07	1.52 ± 0.03		2.03 ± 0.09
	N+	1.45 ± 0.13	1.45 ± 0.09	1.50 ± 0.08		1.91 ± 0.20
2007-08 Irrigated	N-	1.41 ± 0.08	1.53 ± 0.07	1.24 ± 0.08	0.87 ± 0.07	1.33 ± 0.10
	N+	1.43 ± 0.08	1.59 ± 0.07	1.49 ± 0.06	1.22 ± 0.06	1.29 ± 0.07
2007-08 Rainfed	N-	1.55 ± 0.09	1.66 ± 0.09	1.12 ± 0.12		1.25 ± 0.11
	N+	1.57 ± 0.06	1.71 ± 0.07	1.61 ± 0.08		1.29 ± 0.10
Apical						
2006-07	N-	1.57 ± 0.12	1.62 ± 0.06	1.37 ± 0.09		2.10 ± 0.11
	N+	1.62 ± 0.10	1.68 ± 0.08	1.79 ± 0.13		2.06 ± 0.11
2007-08 Irrigated	N-	1.62 ± 0.06	1.40 ± 0.06	0.88 ± 0.08		1.52 ± 0.13
	N+	1.54 ± 0.06	1.45 ± 0.06	1.08 ± 0.07		1.46 ± 0.09
2007-08 Rainfed	N-	1.69 ± 0.11	1.25 ± 0.11			1.50 ± 0.12
	N+	1.61 ± 0.06	1.66 ± 0.09			1.49 ± 0.10

# Influence of Tillage on the Productivity of *Anethum graveolens* L. in a Semi-Arid Mediterranean Environment

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In dry farming areas, the water content in soil varies during the growth cycle. This variability is due to the annual climatic cycle and to tillage operations, which drastically alter both the total pore space and the relationship between macro and micropores (Godwin, 1990). Conservation tillage systems allow farmers to employ sustainable agricultural practices whilst at the same time save on supplies. Different tillage systems use specific tools at different intensities which affect the structural characteristics of the profile in different ways, at both a superficial and a deeper level (Lampurlanés, J. and Cantero-Martínez C., 2006).

This research was carried out in order to estimate the consequences of minimum tillage (at depths of 20 cm and 30 cm) on some biometric parameters in dill (*Anethum graveolens* L.) crops.

The result of this research indicate that minimum tillage can be a more productive dill farming practice than conventional tillage (at depths of 40 cm).

## Methodology

The experiment was carried out in 2006 in a field located near Segezia (FG) (latitude: 41° 23' 12": longitude 15° 29' 44"). The physical and chemical properties of the soil were: pH 7.5; Total N (g kg<sup>-1</sup>) 1.9; Total C (g kg<sup>-1</sup>) 20; Available P (mg kg<sup>-1</sup>) 31; Sand (%) 55; Silt (%) 36; Clay (%) 9.

The experiment considered the adoption of three cultivation techniques: (P1) minimum tillage to depths of 20 cm using a disk harrow; (P2) minimum tillage to depths of 30 cm using a disk harrow, (P3) conventional tillage by mouldboard ploughing to a depth of 40 cm and then a tillage to a depth of 20 cm using a cutter.

Tillage was carried out in January and a 100 units of ammonium nitrate were spread.

The dill was sown in alveolated polystyrene containers in greenhouses on 01/03/06. The seedlings were manually replanted in rows 40 cm apart on 19/4/06. A distance of 20 cm was left between each seedling. All the stolons were watered with a sprinkler immediately after replanting (about 400 m<sup>3</sup> of water/ha); another 4 interventions were carried out about every 15 days until the harvest (15 June). In total, 2000 m<sup>3</sup>/ha/year were irrigated. Two manual weeding operations were carried out. Before weeding floristic mapping was performed using the Bruan Blanquet method. At harvest, plants equal to one meter in length were taken from each test group and the following biometric parameters were determined from them: root length, stalk length, number of leaves, total fresh weight, fresh weight of the root, fresh weight of the stalk, fresh weight of the leaves, total dry weight. In order to calculate the dry weight, the product was kept in an oven at a temperature of 60 °C. All the data collected from the experiments were subjected to variance analysis using ANOVA statistical analysis.

## Results

The values of the parameters obtained during the three experiments show significant differences (Tab 1). A characteristic fact was the positive tendency in the influence of the tillage depth of P1 on the biometric parameters of dill in comparison with the remaining treatments (P2 and P3).

The average total fresh weight, fresh weight of the leaves, number of leaves and total dry weight for P1 were all significantly higher than for P2 and P3. Tillage did not have a significant tillage effect on root length. As regards the fresh weight of the stalk and stalk length, contrasting values were found between tests P1, P2 and P3, in test P3 there was no increase in fresh weight corresponding to the increase in the length. This means that the seedlings had longer stalks with smaller a diameter, the opposite was noted for test P1 where there were shorter stalks with a greater diameter were noted.

Table 1 – Variation of biometric parameters of dill in relation to tillage depth

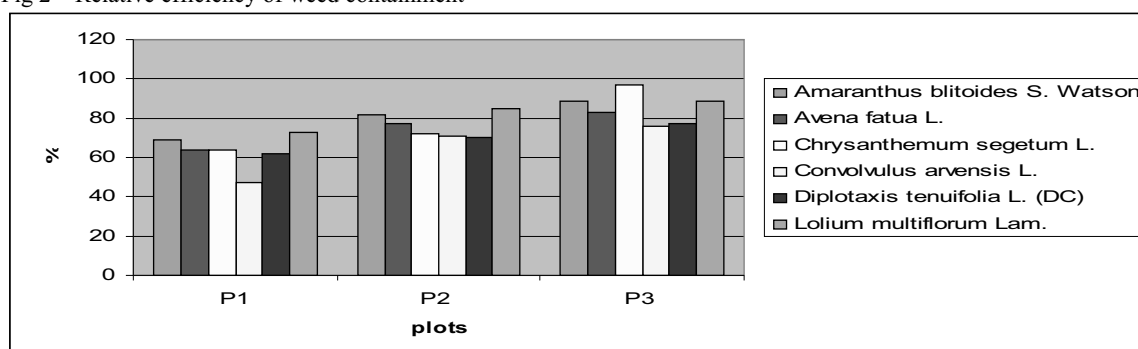
Tillage depth	Total fresh weight (g m <sup>-2</sup> )	Fresh weight of the root (g m <sup>-2</sup> )	Fresh weight of the leaves (g m <sup>-2</sup> )	Fresh weight of the stalk (g m <sup>-2</sup> )	Root length (cm)	Stalk length (cm)	Number of leaves (-)	Total dry weight (g m <sup>-2</sup> )
P1	752 A	121 a	367 A	511 A	22 n.s. <sup>a</sup>	72 ab	1799 A	127 a
P2	533 C	85 b	96 B	343 B	23 n.s.	68 b	770 B	77 c
P3	611 B	117 a	101 B	389 B	21 n.s.	78 a	1172 B	106 b

Values followed by the same letters in each column are not significantly different according to Tukey's ( $p < 0.05$  or  $p \leq 0.01$  capital letter) test. <sup>a</sup>Not significant.

The higher growth of dill in the P1 treatment compared with P2 and P3 might have been partly due to lower water loss. In general, infiltration is reported to be greater under no-tillage than in tilled soils (Radcliffe et al., 1988; Chan and Heenan, 1993; Azooz et al., 1996; McGarry et al., 2000) due to the larger number of macropores (Chan and Heenan, 1993; McGarry et al., 2000), increased fauna activity, which is responsible for many of these macropores (Logsdon and Kaspar, 1995; Suwardji and Eberbach, 1998) and accumulated organic matter forming a litter of residues (Radcliffe et al., 1988; Logsdon and Kaspar, 1995; Arshad et al., 1999).

The competition with weeds did not stop the growth of seedlings in P1 (fig 1).

Fig 2 – Relative efficiency of weed containment



## Conclusions

The trial using the minimum-deep ploughing appeared to be plausible, because it ensured the highest crop yields with better physical features. Kneading the soil by deep tillage had a negative influence on the growth of the dill. Results also indicate that minimum tillage can be a more productive dill farming practice than conventional plow tillage.

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# Effect of Winter Rye Catch-Crop on Growth and Yielding of Buckwheat

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Catch crops are introduced to crop rotations as effective methods of soil conditions improvement, particularly in simplified crop rotations. Studies conducted in different countries (Burgos *et al.* 2000, Chase *et al.* 1991) show that rye could play an important role in weed infestation control as well. There are a big number of weed species which sprouting and growth can be effectively limited by allelopathic compounds of winter rye. High affectivity of weeds control by rye biomass was confirmed in experiments conducted in Pulawy (Grabiński 2006), particularly to the *Chenopodium album*, *Stelaria media*, *Viola arvensis*, *Capsella bursa pastoris* and *Anthemis arvensis*. These results were the base to plan experiments to verify possibility of winter rye catch crop use in practice. Buckwheat was chosen as testing crop in the study because chemical methods of weeds infestation control was not worked out so far so eventual alternative methods of weed control could be useful not only in organic but in traditional (intensive) agriculture as well.

## Methodology

The field experiment was conducted in 2006 and 2007 on heavy loamy sand soil in Experimental Station Osiny of the Institute of Soil Science and Plant Cultivation – State Research Institute. The experiment was established using long belt method. Six experimental treatments (methods of seedbed preparation for buckwheat) were studied:

1. control object- without catch crop, preparation of field for sowing using plough;
2. above ground mass of catch-crop harvested and taken out from the field; preparation of field for sowing using plough;
3. winter rye catch crop in 2<sup>nd</sup> node incorporated into the soil using plough;
4. winter rye catch crop in 2<sup>nd</sup> node incorporated into the soil using rotary cultivator;
5. winter rye catch crop in the end of shooting stage incorporated into the soil using plough;
6. winter rye catch crop in the end of shooting stage incorporated into the soil using rotary cultivator.

The Słowiańskie winter rye cultivar was sown as catch-crop in the 3rd decade of September. Emergence time and appearing of particular growth stages and plant number per 1m<sup>2</sup> of buckwheat were observed. Weed infestation of studied objects were estimated in percentage in relation to control. The results were elaborated statistically.

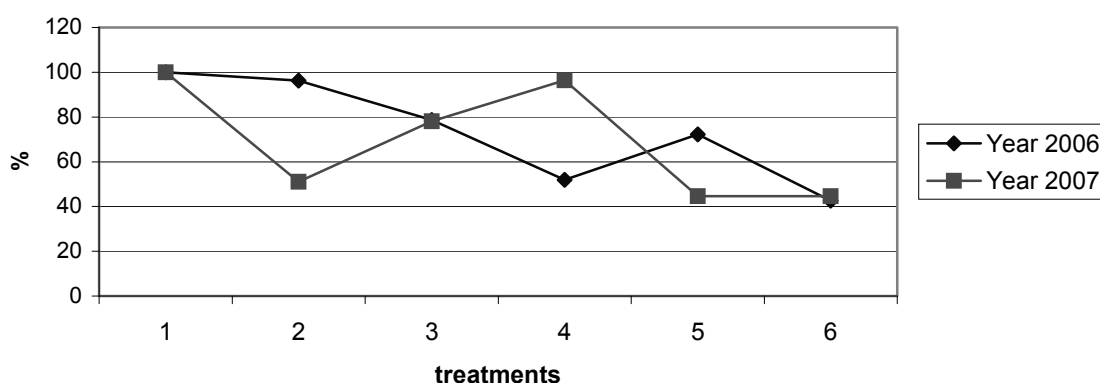
## Results

In spite of good soil moisture after sowing negative effect of winter rye catch-crop on emergency term of buckwheat was observed in the year 2006 only. This year 4-6 days delay of sprouting on object with winter rye biomass incorporated into the soil at stage of beginning of heading was observed. Probably, lack of such phenomenon in 2007 was related with very intensive rain (20 mm in short time) just after sowing of buckwheat which leached to the deeper layers of soil phenolic compounds created during rye biomass decomposition. But the two proper leaf of buckwheat and next stages occurred in almost the same time on all objects (differences not bigger than 1 day).

Biomass incorporation into the soil changed number of emerged plants, particularly strongly in the drier years (2006), in which number of plant on treatment with rye biomass were lower in the range 20-

30%. In the year 2007 with higher precipitation negative effect on plant number per unit area was weaker - differences in plant number between object with rye biomass and control not exceed 13%. Index of leaf area analysis shows that effect of rye biomass on crop plant was changing during vegetation. Just after beginning of buckwheat flowering (about 6 weeks after sowing) leaf area index on objects with the big mass of winter rye there be on heading stage incorporated into the soil was significantly lower than on objects with rye incorporated in 2<sup>nd</sup> node. Three weeks later (full blooming of buckwheat) the situation was quite different (higher leaf area index on object with the bigger mass of rye incorporated into the soil). The studies has been confirmed results of different authors that rye has had herbicide effect (fig.1). This effect was stronger on plots with rye incorporated into the soil at heading stage than on plot with rye incorporated at 2<sup>nd</sup> node. The most effectively weeds were defeated on object where biomass was mixed with the soil using rotary cultivator (object 4 and 6). It is interesting that in year with higher quantity of precipitation herbicide effect was observed on trial 2 - without above ground biomass of rye.

**Figure 1. Weed infestation in relation to control (as 100% weed infestation on control object was assumed) (treatment describe in *Methodology* chapter)**



In drier year (2006) yields of buckwheat were lower. In mentioned year the highest yield was obtained from control object and object with rye incorporated to the soil in 2<sup>nd</sup> node stage using rotary cultivator. But generally differences this year among particular trials were very small.

In the year 2007 with higher precipitation after sowing of buckwheat, yields were much higher than in the first year of investigations. The highest yields were obtained from trials where rye was incorporated at the beginning of heading stage of rye by using rotary cultivator.

### Conclusions

1. Winter rye catch crop influence on decrease of buckwheat emerged plant number.
2. The most intensively weed infestation of buckwheat is limited when winter rye catch-crop is incorporated into the soil at heading phase.
3. Winter rye catch crop can influence on buckwheat yielding negatively or positively depend on amount of precipitation.

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# Pasting behaviour, dough properties and bread quality of organic spelt-amaranth composite flours

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## Introduction

Due to excellent nutritional composition, pseudocereal grain amaranth (*Amaranthus* spp.) represents an interesting alternative as non-wheat material in composite flour in bread making (Breene, 1991; Bressani, 1994; Berghofer and Schoenlechner, 2002; Bavec and Bavec, 2006a). As amaranth also the spelt (*Triticum spelta* L.) can be classified as an alternative crop and is recognised as one of the most appropriate cereals for organic production (Bavec and Bavec, 2006b). Among studies on composite flour containing amaranth there are only some on dough characteristics and bread quality (Lorenz, 1981; Breene, 1991; Ayo, 2001), but they are all based on wheat flour, and virtually no research has been reported on composite spelt-amaranth flour. The aim of present study was to investigate the effects of amaranth substitution rates in white spelt based, organically produced, composite flours on rheological properties of dough and suitability for bread making.

## Methodology

White spelt flour (*Triticum spelta* L.) and wholemeal amaranth flour were produced and processed in accordance with the EU Council Regulation (2092/91) for Organic farming. Composite flours were prepared by replacing spelt flour with 10, 20 and 30% (w/w) of amaranth flour (AFS). Rheological properties of dough from blends were determined by farinograph and extensiograph using standard procedures ICC No. 115/1 and 114/1, respectively. Amylase activity was determined by amylograph according to the manufacturer instructions. The dough for bread was prepared with salt, yeast (2.5% per flour) and water according to farinograph water absorption values. Quality analyses of cooled bread samples (6 hours after baking) were carried out by measuring weight, loaf volume (determined by displacement of millet seeds in a constructed loaf volume meter) and calculating specific volume as a ratio between loaf volume and loaf weight. Hedonic sensory evaluation (scored from 1-unacceptable to 10-excellent) by 37 panellists was conducted the following day after baking.

The rheological analyses, amylograph and baking test were run in triplicates and sole white spelt flour was used for comparison. Statistical analyses were performed using the Statgraphic Centurion XV statistical program (Statgraphic, 2005), with the significance level set at  $P < 0.05$ . Duncan test was used to determine significance of differences among means.

## Results

Amaranth substitution affected pasting behaviour of slurry by increasing the peak gelatinisation temperature and time to peak viscosity, while initial gelatinisation temperature was affected and decreased only in 30% of substitution. Recorded maximum viscosity of composite flour suspensions were higher (from 682 to 702 AU) in comparison to control of 640 AU. Increasing levels of amaranth flour in blends increased water absorption from 60.0 to 62.5% and farinograph quality number mainly by increased development time and dough stability. According to extensogram values recorded after 45 minutes of dough resting the amaranth addition strengthened the dough by increasing resistance to extension and decreasing extensibility (Table 1).

Table 1 Amylogram, farinogram and extensogram characteristics of tested spelt and spelt-amaranth composite flours

Brabender amylogram values	ANOVA	Composite flour (white spelt:amaranth flour in %)			
		100:0	90:10	80:20	70:30
Maximum viscosity (AU)	**	640 <b>c</b>	692 <b>ab</b>	702 <b>a</b>	682 <b>b</b>
Begin of gelatinisation (°C)	**	61 <b>a</b>	61 <b>a</b>	61 <b>a</b>	58 <b>b</b>
Gelatinisation temperature (°C)	**	92 <b>d</b>	93 <b>c</b>	94 <b>b</b>	95 <b>a</b>
Time (min)	**	43 <b>d</b>	45 <b>c</b>	46 <b>b</b>	47 <b>a</b>
Farinogram values					
Water absorption (%)	**	60.0 <b>d</b>	61.2 <b>c</b>	61.8 <b>b</b>	62.5 <b>a</b>
Development (min)	**	2.4 <b>c</b>	3.7 <b>b</b>	4.0 <b>b</b>	4.8 <b>a</b>
Stability (min)	**	1.93 <b>c</b>	3.03 <b>b</b>	3.40 <b>a</b>	2.97 <b>b</b>
Farinograph quality number	**	36 <b>d</b>	54 <b>c</b>	62 <b>b</b>	67 <b>a</b>
Extensogram values (after 45' of dough resting)					
Energy (cm <sup>2</sup> )	*	34 <b>a</b>	36 <b>a</b>	31 <b>ab</b>	27 <b>b</b>
Resistance to extension (BU)	**	113 <b>c</b>	158 <b>b</b>	168 <b>ab</b>	181 <b>a</b>
Extensibility (mm)	**	178 <b>a</b>	144 <b>b</b>	122 <b>c</b>	103 <b>d</b>

\*\*, \*, ns - Significant at the 0.01, 0.05 probability level and non significant, respectively

Means within a row followed by different letter are significantly different (Duncan,  $\alpha = 0.05$ )

In comparison to control, amaranth substitution level of 10% resulted in bread of higher loaf volume and specific volume, while higher substitutions decreased the measures. As is revealed by scoring, amaranth addition had no detrimental effect on overall acceptability of bread up to 20%, and all other evaluated sensory attributes were not significantly influenced by amaranth flour substitution (Table 2).

Table 2 Baking performance and sensory evaluation of breads made from control and composite flours

Composite flour (spelt:amaranth in %)	Loaf volume (cm <sup>3</sup> )	Spec. volume (ml g <sup>-1</sup> )	Colour	Flavour	Aroma	Texture and appearance	Overall acceptability
ANOVA	**	**	ns	ns	ns	ns	*
100:0	1844 <b>b</b>	2.55 <b>b</b>	7.9	8.1	8.1	8.1	7.8 <b>a</b>
90:10	1937 <b>a</b>	2.72 <b>a</b>	8.4	8.2	8.8	8.8	8.2 <b>a</b>
80:20	1772 <b>c</b>	2.48 <b>c</b>	8.8	7.8	8.2	8.2	7.9 <b>a</b>
70:30	1633 <b>d</b>	2.28 <b>d</b>	8.3	7.2	7.5	7.8	6.9 <b>b</b>

Means within a column followed by different letter are significantly different (Duncan,  $\alpha = 0.05$ )

## Conclusions

Study indicates that amaranth flour addition altered examined pasting behaviour, rheological properties and bread quality of spelt based composite flours. According to obtained results substitution up to 20% of the white spelt flour in a formulation could be replaced by wholemeal amaranth flour.

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# Weed Harrowing and Inter-Row Hoeing in Organic Grown Quinoa (*Chenopodium quinoa* Willd.)

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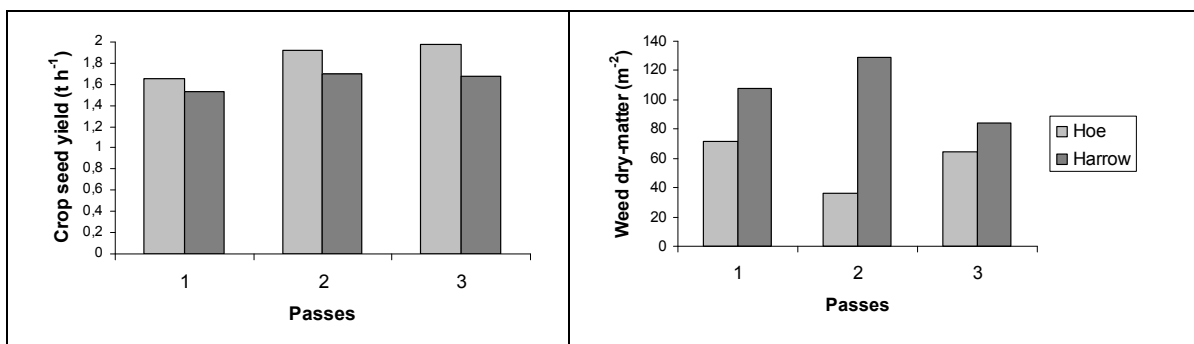
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## Introduction

Work on quinoa is on-going in several European countries (Jacobsen, 2003), and the general perception is that it should be considered as a crop in temperate climates holding a promising potential as a new protein crop for organic feed in Denmark. Trials in Denmark have demonstrated seed yields of 2-3 t ha<sup>-1</sup> with 12-16% protein content and 6-8% fat. Seed yields have varied considerably among years and locations, because the establishment of the crop, weed control, harvest, and post-harvest techniques have not yet been optimized.

## Results

Two experiments were carried out in 2004 and 2005, with three passes separated in time of either inter-row hoeing or weed harrowing. Hoeing and harrowing improved seed yield significantly in both years, however, hoeing gave higher yields (Fig.1). Weed dry-matter was reduced significantly by hoeing and harrowing, but hoeing was most efficient. The reduction of weed dry-matter was also unaffected by year. It is seen from Fig.2 that the protein content in seeds was lowest when weeds were not controlled, while an efficient control secures a significantly increased protein content in the seeds.



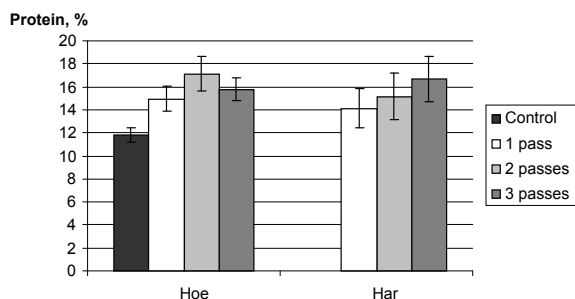
**Fig. 1** Effects of increasing number of passes with the hoe and the weed harrow on yield (left) and weed dry-matter (right) (means of two years).

## Discussion

Weed harrowing is an easy and quick method, where the crop needs to be ahead of the weeds if not being damaged by the treatment. By hoeing between the rows it is easier to go deeper into the soil profile without damaging the crop, and when driving fast some of the weeds in the rows will be covered by soil. It was demonstrated when comparing harrowing with hoeing on 50 cm spacing, that with both weeding strategies quinoa plants were lost. By weed harrowing the smallest quinoa plants were lost, because the treatment had to be fairly rough in order to combat the weeds. When hoeing plants were lost mainly from being covered by soil. For both treatments, in control plots with no weeding there were always more plants than in the treated plots. Weeds were

controlled better with hoeing than with weed harrowing (Fig. 1), but the results indicate that harrowing is a potential weed control method in quinoa because it reduced weed dry-matter about 40% without any obvious crop injuries. However, hoeing reduced weed dry-matter with about 70%. Future experiments may then serve to study whether harrowing should be used only as a supplement to inter-row hoeing, or as the main direct weed control method in narrow-rowed quinoa.

In these experiments with quinoa there was a significant negative effect from weeds on protein content. Protein content was low in the control plots (12%), due to competition for nitrogen from weeds, and increased when weeds were controlled with up to 17% protein. Seed yield after an efficient weed control was close to 2 t ha<sup>-1</sup>, which is on the same level as usually obtained in Denmark, even though earliness has been the main factor in the selection and breeding work during the last 15 years. Potential is higher than this. In Bolivia, for instance, average yield under naturally occurring extreme climatic conditions are 500 kg ha<sup>-1</sup>, but under experimental conditions 3 t ha<sup>-1</sup> is often obtained (Jacobsen et al, 2004).



**Fig. 2.** Protein content with different weeding strategy, 1-3 passes with hoe or harrow, respectively

In conclusion, this study shows that weed harrowing is not sufficiently effective to replace inter-row hoeing in quinoa, when the crop is established at 50 row distance. Weed harrowing, however, shows a potential to supplement inter-row hoeing in wide spaced quinoa or as a sole control method in narrow-rowed quinoa. False seed-bed and thereby delayed sowing showed poor results emphasizing the importance of early sowing. Also, it may be a combination of weed control with crop management systems that will provide acceptable levels of weed control in the future in quinoa.

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# Evaluation of Yield and Yield Components in Rapeseed (*Brassica Napus*) Cultivars in Eastern North of Iran

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## Introduction

More than 90 percent of Iranian domestic need for oil is imported, putting a heavy burden on country's economy. Rapeseed with having 40 percent oil and almost 39 percent protein in its meal and its adaptability to some regions in Iran has a high potential as an oil crop. Variety plays an important role in the yield obtained. Christomer(1996) showed that varieties have different yields in different climates. According to Torling(1991), fast initial growth, early flowering, short and thick stems, flowers without petals, resistant to seed abscission during harvest, more pods on the main stem, and fewer sub-branches are among the main traits which increase the yield. Jazmy(2004) found Hayola 42 to have the highest yield with 3560 Kg/ha in Iran. Farzin(2006) showed Cobra to be the most productive with 4130 kg/ha among 25 varieties.

## Methodology

An experiment on 10 cultivares was conducted using randomized complete block design with four replications in the eastern north of Iran in 2005-2006. These are Okp , Cobra , SLm , Orient , Colvert , Fornex , Alice , DP94 , Hayola42 and Syn1. Each plot had six rows of six meters with a spacing of 30 cm and 3 cm between plants. Plots were kept weed, pest, and disease free until harvest. A random sample of five plants from each plot was used for taking observation of different characteristics. The present study was carried on the eastern north of Iran.

## Results

### a. seed yield

The highest yield belonged to Cobra variety with 3917 kg/ha. The lowest yields belonged to Colvert and Alice. Farzin(2006), in a study carried out in Iran, obtained similar results with Cobra producing the highest yield.

### b. Plant height

There was a significant height difference among the varieties with SLm having 139.9 cm. As a result of defoliation, the remaining sources of photosynthesis are pod and stem. This makes having greater heights lead to producing more flowers and more pods, which increases the photosynthesis surface. Colvert, which has a good height and many branches, was among the most productive varieties in the experiment.

### c. Length of pod

SLm and Orient have the highest pod length and highest number of seeds per pod. There was no significant difference among the other varieties. The length of a pod is mainly determined by genetic factors rather than agricultural factors. Long pods tend to have more seeds, which means more yield (Rao ,1991). According to table 1, Colvert has the shortest pod length and the lowest number of seeds per pod.

### d. Sub-branch

Orient, Cobra, and Colvert had more sub-branches than the other varieties. Alice had the lowest number of sub-branches. The higher number of sub-branches has direct correlation( $r=0.43$ , table 2) to the increased amount of yield since sub-branches lead to inflorescence.

e. The number of seeds per pod

The highest number of seed per pod is produced by Orient and Okp, which have the highest yield.

Table 1: Traits mean comparison of rapeseed the varieties

The number of pod per plant	Plant height	Length of pod	Sub branch	The number of seeds per pod	Seed yield Kg/ha	Triats Variety
100.84 b	101.83 ab	8.967 ab	7.772 ab	26.47 a	2369 b	Okp
108.25 b	104.62 ab	9.7 a	8.107 a	24.26 ab	3917 a	Cobra
102.35 B	139.9 a	9.48 a	7.633 ab	21.82 ab	2701 b	SLm
131.4 ab	108.9 ab	9.707 a	8.7 a	25.9 a	3725 a	Orient
141.2 a	112.87 ab	8.97 ab	8.6 a	17.02 c	2231 c	Colvert
120 ab	114.67 ab	9.433 ab	6.99 b	21.82 ab	3045 ab	Fornex
79.1 d	76.92 c	8.715 b	5.8 c	16.72 c	2137 c	Alice
99.42 c	74.99 c	8.46 ab	7.3 ab	21.31 ab	2652 bc	DP94
78.3 d	99 bc	8.82 b	8.1 a	18.23 bc	2452 bc	Hayola 42
86.3 cd	84.3 c	8.3 b	7.82 ab	20.9 ab	2547 bc	Syn1

Table 2: Correlation among traits

Plant height	Length of pod	Sub branch	The number of seeds per pod	Seed yield	
			1	1	Seed yield
			1	0.19 n.s	The number of seeds per pod
		1	-0.225	0.43**	Sub branch
	1	0.26	0.49**	-0.011	Length of pod
1	-0.11 n.s	0.32**	0.16 n.s	0.21 n.s	Plant height
0.42**	0.2 n.s	0.725**	-0.22 n.s	0.43**	The number of pod per plant

There was a significant correlation among yield and the number of sub-branches and pod number per plants. The number of seed per pod and length of pod showed a high correlation( $r=0.49$ ). The highest correlation was obtained by the number of sub-branches and the number of pods per plant( $r=0.725$ ).

## Conclusions

Based on the results Cobra and Orient have the highest , among the studied varieties , yield. It is recommended that these varieties are used in the in the region and region whit similar climate.

These two varieties (Cobra and Orient) rank first in having traits which correlate highly with yield , that is sub branch and the number of pod per plant

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# Silage Yield and Protein Content of Common Bean Intercropped with Corn in Two Row-Replacements

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Common bean (*Phaseolus vulgaris* L.) is probably the most valuable source of plant protein for human consumption in many parts of Europe and contributes significantly to traditional cropping system (Santalla *et al.*, 2001). In addition, the use of corn (*Zea mays* L.) silage as feed for livestock has been a common practice in Europe and especially in the Mediterranean countries. However, because of the low crude protein content of corn it may be advantageous to grow corn and legume together (Gebeyehu *et al.*, 2006). In addition, nitrogen fixation from legumes can provide N for corn and create more sustainable cropping system (Maingi *et al.*, 2001; Yilmaz *et al.*, 2008). Intercropping of cereals with legumes improves soil conservation, enhances in some cases weed suppression, provides better lodging resistance, yield stability, hay curing, and forage preservation over pure legumes and may increase crude protein content, protein yield, and length of optimum harvest period over grasses (Anil *et al.*, 1998).

The objective of this study was to determine the effect of intercropping common bean with two different corn hybrids (an early maturing and a late maturing hybrid) on silage yield and protein content in four bean–corn mixtures in two row-replacements (i.e., 1:1 alternative rows and mixed bean:corn in the same row, respectively).

## Methodology

The experiment was carried out at the Farm of the Aristotle University of Thessaloniki in northern Greece during the 2007 growing season. The experiment was established in a calcareous loam soil (Typic Xerorthent). A traditional cultivar of common bean (cv. Plake Florinas; type IV bean with strong climbing ability), and two corn hybrids (i.e., Dunia 3655 with a FAO 410 maturity rating and PR34N43 with a FAO 500 maturity rating) in four bean–corn mixtures in two row-replacements (i.e., 1:1 alternative rows and mixed in the same row bean:corn, respectively) were used. Sole common bean and corn hybrids were planted at the rate of 80.000 plants per hectare, whereas the four mixtures were simultaneously planted at the rate of 40.000 bean plants and 40.000 corn plants per hectare. The row spacing was 75 cm for all treatments. The experimental design was completely randomized block with seven treatments and four replications. Pure stands and mixtures were harvested when corn was at approximately ½ kernel milk line stage. Silage yield (expressed as dry matter) was determined by harvesting one row of 4 m long from each plot. Samples of 1 kg biomass (of each species from each plot) were dried in the oven at 65° C to a constant weight to determine the relative water content, and prepared for chemical analysis. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25.

## Results and Discussion

The greatest dry matter yield was obtained by the sole crop of PR34N43 corn hybrid, whereas the lowest yield was obtained with bean sole crop (Table 1). The intercrops of bean with PR34N43 hybrid provided higher yield than bean intercrops with Dunia hybrid and also similar yield than Dunia sole crop. Moreover, similar yields were observed among mixtures with the same corn hybrid in both row

placements. In addition, the intercrops produced about 22–64% more yield than the bean sole crop, but 31.3 and 33.5% less than Dunia and PR34N43 corn sole crops, respectively. Similarly, other researchers reported that yield in intercrops of bean cultivars with corn was intermediate compared with yields of sole crops (Santalla *et al.*, 2001; Gebeyehu *et al.*, 2006).

Crude protein content was highest in monoculture bean (198.0 g kg<sup>-1</sup> DM) followed by bean-corn mixtures (107.0 to 121.3 g kg<sup>-1</sup> DM) which exhibited higher crude protein yield than the sole crop of corn hybrids (81 and 86 g kg<sup>-1</sup> DM) (Table 1). However, in most cases, a different trend was observed for the crude protein when it was expressed on a per area basis. Although crude protein content of bean was higher than corn and their intercrops, protein yield per hectare was similar for bean and corn (PR34N43) sole crops and their mixtures, because of the higher dry matter yield of corn compared with bean. On the contrary, Dawo *et al.* (2007) reported that bean-corn intercrops had higher crude protein yield per hectare than corn sole crop.

Table 1. Silage yield (expressed as dry matter), crude protein content and yield for sole stands and mixture of corn with common bean

Planting patterns	Dry Matter (Mg ha <sup>-1</sup> )			Crude Protein	
	Bean	Corn	Total	(g kg <sup>-1</sup> DM)	(kg ha <sup>-1</sup> )
Bean	11.74	-	11.74	198.0	2324
Corn I (Dunia)	-	20.87	20.87	86.0	1795
Corn II (PR34N43)	-	28.63	28.63	81.0	2319
Bean: Corn I (1:1)	4.91	10.02	14.93	118.9	1775
Bean: Corn I (mixed)	5.67	8.66	14.34	121.3	1739
Bean: Corn II (1:1)	4.01	15.20	19.21	107.0	2055
Bean: Corn II (mixed)	4.49	14.55	19.04	112.4	2140
LSD <sub>0.05</sub>	0.96	2.45	2.71	18.1	304

## Conclusions

The results of this study showed that mixtures of bean with corn (PR34N43 hybrid) in alternative rows (1:1) and in mixed rows had an advantage over the other two mixtures and the bean sole crop in terms of silage yield per hectare, whereas, these mixtures had a disadvantage over PR34N43 corn. In addition, these mixtures produced similar crude protein yield per hectare than bean and corn (PR34N43) sole crops.

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# Potassium Affects the Stem Architecture and Anatomy of Barley

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The ambition towards sustainable crop production and has brought new challenges. These include decreased use of fertilizers and thus increased nutrient use efficiency of crops, and decreased use of chemicals for plant protection. In contrast to the considerable research effort on nitrogen and phosphorus nutrition, potassium nutrition has been relatively neglected, even though it is one of the essential nutrients and the required amount of uptake by many crops is comparable to that of nitrogen. Uptake of potassium is mainly based on ion diffusion. This mechanism is sensitive to environmental factors, such as soil type, precipitation and temperature. The efficiency of the mechanism depends on the availability of potassium (Gaspar *et al.* 1994). However, it has also been shown that genotypic differences in potassium use efficiency exist (Woodend & Glass 1993). So potassium use efficiency could be incorporated in breeding programs leading to improved cost efficiency. An adequate level of potassium seems to prevent or at least reduce pathogen infections in cereals (Beringer & Koch 1980, Christensen *et al.* 1981). This increases the leaf area duration, and thus prolongs the period leaves supply assimilates to filling grains. Gaspar *et al.* (1994) reported a 4% increase in yield, and an 7% increase in 1000 grain weight of oat due to increased potassium fertilization. Similarly, in barley, the single grain weight increased with 1 mM potassium treatment due to increased availability of assimilates (Beringer & Haeder 1981). Further support was obtained by Andersen *et al.* (1992) who showed that potassium increased the root growth and vegetative mass of cereals resulting in increased photosynthetic capacity, especially during dry seasons. Often, the availability of assimilates determines the number of aborting grains in cereals (Peltonen-Sainio & Peltonen 1995). Lodging of cereal crops results from the combination of adverse weather conditions and inadequate standing ability of the plants. The latter may be due to weakness of lower internodes of the culm. The resistance of a culm internode to bending depends largely on its diameter and wall thickness and since potassium has a marked role in plant growth, it can be hypothesised that lodging could be reduced with optimal potassium fertilization. Our objective was to investigate the effect of potassium on plant and plant stand architecture, e.g. tillering, and also stem anatomy of barley.

## Methodology

Two experiments were conducted in the greenhouse. Barley (*Hordeum vulgare* L., cv. Barke and Saana) was grown in 7.5 L pots containing unfertilised peat. Potassium was applied as KCl from 25 mg K L<sup>-1</sup> soil up to 800 mg in 25 mg intervals. Other nutrients applied included N, Mg, Cu, B, S, Mn, Mo, Ca (Suomen salpietari and Täydennyslannos 1, Kemira Grow-How, Finland). The experiments were carried out as a completely randomised design with six replicates. The development of the plants was monitored from germination until full maturity. The number of tillers was recorded and plant parts, separated into main stem and tiller leaves, stems and heads, were weighed every second week until maturity. Grain number and weight were measured at maturity. Stem diameter and stem wall thickness were measured on the first, second and third internodes at heading, at anthesis and at yellow ripening. Stems were sectioned, stained with methyl blue and safranin O, viewed with a light microscope and photographed.

## Results

Even though, at the early stages of growth, the growth rate of plants under lowest potassium application rates was highest, after a few weeks the plants under highest potassium had the highest growth rate until maturity. The tiller head weight increased significantly with increased potassium fertilization (Table 1). Stem diameter and wall thickness (Figure 1) were lowest in the plants grown at the lowest potassium application rates. Moreover, the thickness of lignified cell walls of the sclerenchyma ring was also lowest in plants grown at the lowest potassium application rates. In mature plants, the potassium concentration in stems ranged from 25 to 55 mg g<sup>-1</sup> dw, in leaves from 55 to 77 mg g<sup>-1</sup> dw, and in grains from 0.26 to 0.31 mg g<sup>-1</sup> dw. In grains, the K concentration did not vary significantly between treatments.

Table 1. Dry weight of plants, single grain weight, number of head bearing tillers and grains, and tiller head weight of barley grown under different K application rates. Data are means  $\pm$  SE, n=6-40.

K applied mg L <sup>-1</sup> soil	Plant dry weight g	Grain weight mg	No. head bearing tillers per plant	No. grains per spike	Tiller head weight g
50	3.20 $\pm$ 0.39	28.9 $\pm$ 1.0	3.6 $\pm$ 0.5	13.3 $\pm$ 1.7	0.29 $\pm$ 0.06
100	4.67 $\pm$ 0.31	36.9 $\pm$ 0.8	4.5 $\pm$ 0.7	19.0 $\pm$ 1.3	0.38 $\pm$ 0.05
200	7.89 $\pm$ 0.75	56.9 $\pm$ 0.8	4.6 $\pm$ 0.8	21.8 $\pm$ 1.5	0.79 $\pm$ 0.13
800	9.63 $\pm$ 0.51	59.3 $\pm$ 0.8	3.4 $\pm$ 0.3	22.0 $\pm$ 0.8	1.31 $\pm$ 0.16

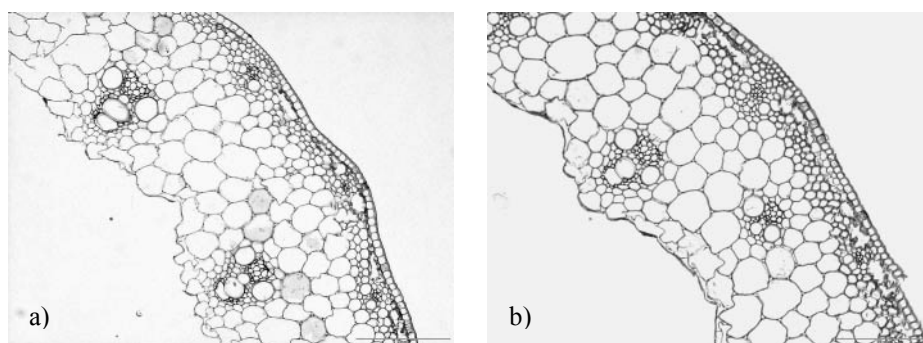


Figure 1. Sections of barley stems grown in a) 25 and b) 600 mg K kg<sup>-1</sup> soil, 25 x enlargement.

## Conclusions

Increased potassium application rates were associated with thicker stems and cell walls and resulted considerably larger grains. Potassium as a fertiliser possesses a marked role in the improvement of plant stand architecture and to decrease lodging.

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# Nitrogen Availability during Eggplant Crop Growth in Soil Amended With Defatted Seed Meals

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The amending of agricultural soils with defatted seed meals represents an interesting way to exploit the fertilizing potential of these byproducts of the biodiesel production (Lazzeri *et al.*, 2004; Shaine Tyson *et al.*, 2005). Knowledge of the dynamics of inorganic N in soil may help to establish the most suitable time for defatted meals incorporation, which leads to the improvement of crop N use-efficiency and possibly to a reduction in environmental impact of fertilizing practices. The aim of this study was to improve our general knowledge of N availability for horticultural crops amended with defatted seed meals by monitoring the inorganic N content in the cultivated layer of a soil cropped with eggplants (*Solanum melongena* L., cv. Lunga di Napoli), during the crop growth season.

## Materials and Methods

The field experiment was carried out in 2007 on a clay loam soil (8.8 g organic C kg<sup>-1</sup>, 1.0 g Kjeldahl N kg<sup>-1</sup>, pH 7.4, 34.6% sand, 36% silt, 29.4% clay, in the top 0-0.40-m soil layer) at the experimental station of the CRA-ORT, Battipaglia (SA), Italy. The experimental design was a randomized complete block with 2 replicates (plot area: 50 m<sup>2</sup>). Treatments were: Ethiopian mustard (*Brassica carinata* A. Braun) seed meal, sunflower (*Helianthus annuus* L.) seed meal, chemical fertilizer (as ammonium nitrate), unfertilized control. The seed meals were uniformly surface-spread on June 8 (at rate of 3 t ha<sup>-1</sup>, corresponding to an organic N supply of 155 and 142 kg ha<sup>-1</sup>, for *B. carinata* and *H. annuus*, respectively) and thoroughly incorporated into the top 0-15-cm soil layer the day after. The chemical fertilizer (150 kg N ha<sup>-1</sup>) was supplied in several fertigation events throughout the crop season. Control and meal-amended plots received the same amount of irrigation water as the chemically fertilized plots. Eggplants were transplanted on June 20 and removed on Oct. 24. The eggplant cultivation techniques were those typical of the Sele Valley (Campania region). Soil samples were collected at 0-0.20 and 0.20-0.40 m soil depth, on 6 dates, from just before seed meal incorporation to the day before the last harvest. Two samples (within-plot replicates) were collected in 2 plot positions, at each date. As we suspected that the irrigation water probably supplied a certain amount of N, data analysis was carried out on the difference between the inorganic N content in the treated plots and that of the control plot (*net inorganic N content*), for each block and for each sampling date, depth and plot position. We measured the inorganic N (nitrate + ammonium N) content colorimetrically. The statistical analysis was performed on log-transformed data (plus a constant value, to remove negative figures) using a mixed model for measurements repeated in time.

## Results

Significant differences in the soil net inorganic N content were observed between the plots amended with defatted seed meals and the plots supplied with the chemical fertilizer (Tab. 1). The net soil N content varied across the crop growth season, depending on the treatment (significant interaction Sampling date x Treatment). In particular, in the sunflower plots the net inorganic N content increased earlier in the growth season, and then decreased, whereas the opposite occurred in the *B. carinata* plots. Remarkable amounts of inorganic N were observed in the 0.20-0.40 m soil layer, despite the surface incorporation of the plant meals. In fact no significant differences were found in net inorganic N

content between the 2 soil layers. A great variability of inorganic N content was observed between- and within-plot sample replicates, in the meal-amended plots (data not shown). This variability, which was probably caused by spatial heterogeneity in distribution of the meals in the soil, may have reduced the statistical significance of the differences between the means.

Table 1. Net inorganic N content in the top 0.4-m soil layer, as the difference between inorganic N content in the treated and control plots at each sampling date, block, depth and plot position, during the eggplant growth season, summer 2007, at Battipaglia (Salerno, Italy).

Sampling date	Net inorganic N content (mg N kg <sup>-1</sup> dry soil) in soil with			Mean
	Chemical fertilizer	<i>B. carinata</i>	<i>H. annuus</i>	
7 June	0.3a	0.2a	-0.6a	-0.1C
14 June	0.1b	6.9a	17.7a	8.3B
25 June	-0.3b	10.2a	11.1a	7.0B
9 July	3.2a	12.2a	12.9a	9.4AB
9 August	20.4ab	25.6a	6.9b	17.7A
23 October	-0.3a	-0.7a	-0.4a	-0.5C
Mean	3.9B	9.1A	7.9A	

<sup>1</sup> With reference to the significant sources of variation (see text), upper-case letters were used for comparisons of the mean effects, lower-case letters for the comparison of first order interaction effects. The interaction effect was estimated at each date (comparison between treatments within each row). For each source of variation, means followed by the same letters are not significantly different for P<0.05, according to the Tukey-Kramer test for mean comparisons.

The observed differences between treatments may be explained partly by the different supply-timing of the N source, as the chemical fertilizer was distributed by drip irrigation in small amounts along the whole crop growth season, whereas the organic amendments were applied only as pre-planting treatments, at the beginning of the growth season. The reasons for the increase of net inorganic N in soil amended with sunflower meal, faster than that in soil amended with *B. carinata* meal, may be searched for either in a different tissue biochemical composition (Kumar and Goh, 2003) or in a temporary reduction of the activities of the soil microbial community due to some *B. carinata* biofumigant effect on nitrifiers (Bending and Lincoln, 2000). However, we did not observe any inhibiting effect of *B. carinata* meals on soil N mineralization potential in laboratory conditions (unpublished data).

## Conclusions

These preliminary field results support the hypothesis that defatted meals used as biofumigants not only may substitute synthetic chemicals in pest control but also supply the soil with organic N available for crop growth.

## Acknowledgements

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# Comparison of Dehydrogenase and Phosphatase Activities in Fallowed and Tilled Soils

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Results of our previous studies, based on a long-term field experiment, indicate that among various microbial and biochemical parameters measured activities of dehydrogenase and phosphatase as well as microbial biomass C are most often significantly correlated with chemical characteristics of soils and with crop yields (Gajda *et al.* 2000). In this study we measured activities of the above mentioned enzymes in two soils as influenced by fallowing and various soil tillage systems.

## Methodology

For the purpose of this study a long-term field experiment in Baborówko Agriculture Experimental Station (52°35' N; 16°38' E) was used. In this experiment 100m<sup>2</sup> plots were established within productive fields in which the following soil tillage systems are being used: - direct sowing (no-till), - reduced tillage (Ares harrowing 8cm deep), - conventional tillage (including deep plowing down to 30cm), and two fallowing systems (with or without green cut). Areas with two soil textures were selected for setting of the plots, and the soils were: light loamy sand and light loam. The soils were sampled in August of 2005, 2006 and 2007 at 0-5cm and 10-15cm soil depths. Composite samples from each soil and depth were thoroughly mixed, sieved through a 2 mm sieve and stored at 4° C for analyses within one week.

Dehydrogenase activity in the soils was determined using TTC as the substrate of this enzyme (Casida *et al.*, 1964) and acid phosphatase activity was measured by Tabatabai and Bremner method (1969) using p-nitrophenyl phosphate as the substrate. Sub-samples were used to measure soil pH(H<sub>2</sub>O) and soil water content after drying for 24 h at 105° C.

Two-way analyze of variance (ANOVA) and Tuckey's test was used to indicate significant difference between the treatments.

## Results

It has been found that the activity of both enzymes in the fallowed soils was higher than in the tilled or directly sown soils (Tab. 1 and 2), particularly in the case of fallowed soils, on which vegetation was cut twice a year and left on the soil surface, the activities were generally significantly higher than in the tilled soils. Each year at the sampling time the fallowed soil had higher moisture contents than the soils

Table 1. Dehydrogenase activities (μl H<sub>2</sub> g<sup>-1</sup> soil d. m.) in two soils as influenced by tillage and soil depth (mean for the years 2005-2007)

Tillage system	Light loamy sand		Light loam	
	0-5 cm depth	10-15 cm depth	0-5 cm depth	10-15 cm depth
Fallowing (green cut)	27.1	19,6	30.5	20,7
Fallowing (no cut)	19.2	15,6	26.8	16,1
No-tillage	11.8	5,3	16.7	11,0
Reduced tillage	11.3	4,4	19.4	12,5
Conventional (plough)	9.8	8,0	15.4	16,6
LSD (p = 0.05): for tillage (A)	11.3		11.8	
for depth (B)	5.0		5.2	
interaction AxB not significant				

under the other treatments, probably mainly due to the mulching effect of plant debris remaining on the surface of the fallowed soil and due to reduced plant growth (transpiration) on these soils. This was probably the main reason for the higher enzymatic activity of the fallowed soils.

Table 2. Acid phosphatase activities ( $\mu\text{g p-nitrophenol g}^{-1}$  soil d. m.) in two soils as influenced by tillage and soil depth (means for the years 2005-2007)

Tillage system	Light loamy sand		Light loam	
	0-5 cm depth	10-15 cm depth	0-5 cm depth	10-15 cm depth
Fallowing (green cut)	102	83	118	87
Fallowing (no cut)	81	74	113	83
No-tillage	82	60	83	62
Reduced tillage	76	57	79	59
Conventional (plough)	69	67	79	75
LSD ( $p = 0.05$ ): for tillage (A)	Not significant		34.6	
for depth (B)	“ “		15.3	
interaction AxB	“ “		Not significant	

Comparing effects the studied tillage systems it could be seen that both soils under the conventional tillage system (plowing) had lower activity of the enzymes in 0-5cm soil layer than the soils under no-till or reduced tillage system, while the opposite was true for 10-15cm soil layer, but the differences were not statistically significant.

The enzymatic activity of the soils at the depth 10-15 cm was, in general, significantly lower than that in the surface layer (0-5 cm), and it was dependent on soil tillage system. While the plowed soils had similar activities of the enzymes at both depths, clearly due to soil and plant debris mixing in the plow layer of this treatment, no-tilled and surface tilled (up to 8 cm deep) soils showed lower enzymatic activity at 10-15 cm as compared to that at 0-5 cm depth. These differences could also be explained by accumulation of OM and nutrients, higher microbial and other beneficial mulching effects of plant residues remaining on the soil surface in the case of no-till or reduced tillage treatments (Gajda *et al.* 2001, Martyniuk *et al.* 2006)

## Conclusions

Results of this study indicate that in fallowed soils activity of dehydrogenase and acid phosphatase is generally higher than in tilled soils. Comparing effects of the tillage systems it has been found that in the case of conventional (plough) system the activity of these enzymes is evenly distributed in the plough layer while in the case of no-till or reduced tillage it is higher in the upper (0-5 cm) soil layer than at lower depths.

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# Residual Effects of Legumes in Wheat-Based Cropping Systems in a Temperate Environment

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Sustainable agricultural systems preserve soil quality and biodiversity through an appropriate crop rotation. In Southern Europe's environments, non-irrigated agricultural systems depend on winter crops; with limited alternatives, farmers rely on short rotations of wheat and barley. One way to improve the sustainability of these systems is to introduce legume crops such as faba bean and field peas into the rotation. These species play beneficial effects including increased soil organic matter content, reduced incidence of root and leaf diseases in subsequent crops, reduced weed populations (Blackshaw *et al.*, 1994), increased P, K and S availability (Bullock, 1992), improved soil structure and stability (Karle *et al.*, 1994). Overall they may represent an alternative to commercial nitrogen fertilizers and provide a net input to soil N, particularly important in organic farming systems.

This work is aimed at investigating on the residual effect on wheat of two legume species (faba bean and field peas) at two different sowing time (autumn and winter) in a biannual rotation system wheat-pulse crops.

## Methodology

Field experiments were carried out over six years of research (2001-2007) at two locations of central Italy (Papiano: 42° 57' N, 165 m a.s.l.; Badiola: 43° 15' N, 257 m a.s.l.). The locations are geographically similar but Papiano is characteristic of production land of the valley bottoms, while Badiola is representative of more sloping production. Soil characteristics are: Papiano 8% sand, 56% silt, 36% clay, 1.5% o.m., pH 7.8, and at Badiola, 11% sand, 41% silt, 48% clay, 1.7% o.m., pH 8.0.

Several faba bean and field pea varieties were compared on a randomised block design with four replicates with plots of 1.5 x 8 m. The crops were compared during the autumn and winter sowing. Seeding rates were calculated to achieve a final target density of 45 and 70 plants m<sup>-2</sup> for faba bean and field peas, respectively. Plots consisted of 5 rows (1.5 m wide and 5 m long). In all years, locations and sowing times, soils were fertilized with 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. To estimate the residual effect of the two pulses, a soft wheat crop was introduced into the rotation following faba bean and peas during all years. To compare the improving effect of leguminous with respect to wheat grown in monocropping, over a period 2003-2007, two wheat-wheat rotation, one fertilized with 180 kg N ha<sup>-1</sup> (N<sub>180</sub>) and one unfertilized (N<sub>0</sub>), were compared on a randomised block design with four replicates.

Wheat yield data were subjected to ANOVA; an F test, in its simplest form, was used to assess the significance of the differences among thesis, after the sources of variance of the elementary thesis were broken-up and confronted (between sowing times, species, interaction sowing times × species).

## Results

At Papiano the yields of wheat grown after the two legumes in autumn sowing were significantly higher than yields of wheat grown after legumes in winter sowing. The effect of species was always significant, with faba bean favouring higher yields than field peas for the following soft wheat (table 1). Unfertilized monocropped wheat always produced less than wheat in rotation with the two leguminous

species, which in some cases produced similarly to wheat fertilized with 180 Kg N ha<sup>-1</sup> (figure 1). These results were also confirmed by the experimentation carried out at the second location.

Table 1. Yield of soft wheat obtained in rotation with faba bean and field pea in autumn and winter sowing at Papiano, over a period of 5 years.

Following crop		Wheat yield (t/ha)				
sowing time		2003	2004	2005	2006	2007
						<i>Average 2003-2006</i>
Faba bean						
autumn sowing		6.5	6.7	3.6	2.8	6.6
winter sowing		6.0	6.5	3.3	2.4	
<i>average</i>		6.3	6.6	3.5	2.6	4.7
Field peas						
autumn sowing		5.5	6.3	3.3	2.5	6.1
winter sowing		5.2	6.1	2.5	1.9	
<i>average</i>		5.4	6.2	2.9	2.2	4.2
Effects						
Sowing time		**	**	**	**	
Species		**	**	**	**	**
Sowing time x species		<i>n.s.</i>	<i>n.s.</i>	**	<i>n.s.</i>	
LSD (p<0.01)		0.26	0.20	0.19	0.19	0.22
LSD (p<0.05)		0.19	0.15	0.14	0.14	0.13

\*, \*\*, *n.s.*, significant for P≤0.05, P≤0.01 and not significant (Test F, ANOVA fix model).

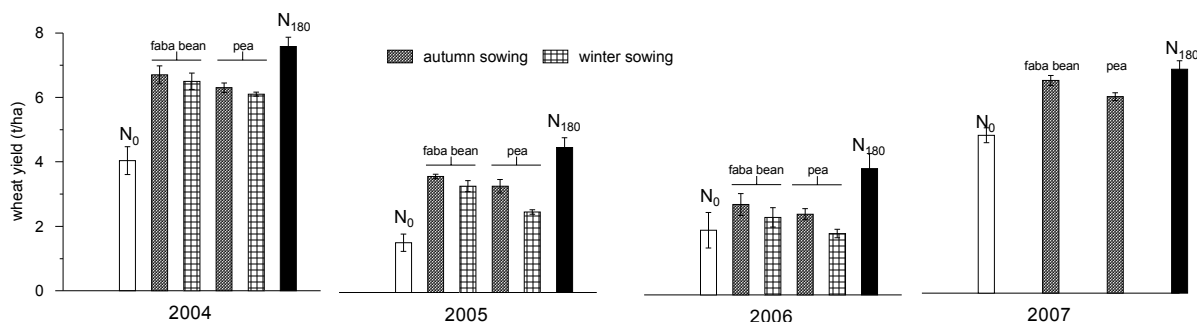


Figure 1. Yields of wheat grown after wheat (unfertilized and fertilized with 180 Kg N ha<sup>-1</sup>), faba bean and field pea at Papiano over a period of 4 years. Bars stand for fiducial intervals (P<0.05%).

## Conclusions

The amount of nitrogen released into the soil from the faba bean and field peas available for the succeeding wheat depends on climatic regimes and growing conditions. This study work highlights an higher capacity of the faba bean to release nitrogen into the soil than field peas. Besides higher yields of these pulse crops, autumn sowing allows both species a longer crop cycle and hence a higher amount of nitrogen released into the soil.

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# MSW compost and brackish water effects on tomato crop in mediterranean conditions

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The application of municipal solid waste (MSW) compost from organic fraction is an alternative to conventional landfill disposal, allowing organic material to be recycled for agricultural use at relatively low cost (Montemurro *et al.*, 2005). MSW compost is mainly applied to soil as an amendment, even if its mineral composition suggests potential as a mineral fertilizer, because of the soil distribution improves plant growth by supplying the sufficient nutrients to plants (Togun and Akanbi, 2003) and affecting the levels of nutritional elements in soil (Giusquiani *et al.*, 1988). Furthermore, Sikola and Yakovchenko (1996) indicate that the addition of MSW compost to soil could influence positively plant yield although this influence is not always directly related to the nutrients present in MSW compost but could depend on the general improvement of the soils physical properties. Tomato crop is classified as moderately sensitive to salinity, which means that it tolerates an electrical conductivity (EC) of the saturated soil extract up to about 2.5 dS m<sup>-1</sup> without any yield reduction (Maas, 1986). Conversely its cultivation under conditions of soil and water degradation is severely restricted (Cuartero and Fernandez-Munoz, 1999).

The aim of this research was to investigate the sustainability of MSW compost on tomato production and its possible application to reduce the negative effects of the saline water.

## Methodology

The field experiment was carried out at Metaponto (MT) in Southern Italy (40°24' lat. N; 16°48' long. E) during the summer 2007. The soil presented low content of both nitrogen (N) (1.03 g kg<sup>-1</sup>) and organic matter (12.1 g kg<sup>-1</sup>). The clay, silt and sand contents were 28.3, 40.4 and 31.3 %, respectively; water field capacity (-0.03 MPa) and permanent wilting point (-1.5 MPa) values were 25.6 and 12.3 % (percentage of soil dry weight), respectively.

Table 1. Some characteristics of the MSW compost

Total organic carbon	(g kg <sup>-1</sup> )	264.6
Total N	(g kg <sup>-1</sup> )	21.7
Total P	(mg kg <sup>-1</sup> )	11.2
C/N		12.2
C(HA+FA)	(g kg <sup>-1</sup> )	110.3
Degree of humification	(%)	78.4
Rate of humification	(%)	41.7
Index of humification		0.3
Moisture	(%)	18

Four-leaf tomato plants (7071 Tomito cultivar) were transplanted on May 7<sup>th</sup> on single row (plant densities were of 2 plants m<sup>-2</sup>). In a randomized block experimental design with three replications, the following four treatments were compared: mineral N fertilizer (180 kg N ha<sup>-1</sup>) and irrigation with fresh water (EC = 0.9 dS m<sup>-1</sup>) (FF); mineral N fertilizer (180 kg N ha<sup>-1</sup>) and irrigation with saline water (EC = 6.0 dS m<sup>-1</sup>) (SF); MSW compost and irrigation with fresh water (EC = 0.9 dS m<sup>-1</sup>) (FC); MSW compost and irrigation with saline water (EC = 6.0

dS m<sup>-1</sup>) (SC). The salinity was imposed by irrigating with water artificially salinized, using commercial salt (97% of NaCl). In all treatments the irrigation water, supplied by localized method, was applied when cumulated crop evapotranspiration value reached the 40% of available water capacity, re-establishment the 100%. The MSW compost was applied about one month before transplanting as amendment, its main characteristics are presented in Table 1. At harvest (106 days after transplanting), weight and number of fruits were measured, total and marketable yield, green, turn dark-coloured and overripe fruits, and dry matter of fruit were calculated.

## Results

The results of this research indicated that the total yield did not show significant differences in FF, FC and SC treatments (97.3, 103.2 and 102.5 t ha<sup>-1</sup>, respectively), while in SF the total yield was 16% lower than the mean of the other treatments. In particular the SC reached 17% and 23% of total and marketable yields

higher than SF indicating that the MSW compost application reduce the negative effects of saline water. Tomato yield was reduced by both decreased average fruit weight and low the number of fruits produced. The average weight of fruit was significantly higher in treatments with fresh water than in the saline ones (15.4 and 13 g, respectively), whereas, the number of fruits per m<sup>2</sup> was the highest in SC treatment and the lowest in FF and SF treatments (663, 522 and 525, respectively) (Tab. 2), in according with Cuartero and Fernandez-Munoz (1999). Fruits dry matter percentage was higher in the saline treatments than fresh water ones in according with several studies (Caliandro *et al.*, 2000). No significant difference was found in green, turn dark-coloured and overripe fruits, whereas in FC treatment the ripe fruits were the highest (Fig. 1).

Table 2. Total and marketable yields, fruits number, average fruit weight and dry matter

Treatments	Total yield (t ha <sup>-1</sup> )	Marketable yield (t ha <sup>-1</sup> )	Fruits (n m <sup>-2</sup> )	Average fruit weight (g)	Fruits dry matter (t ha <sup>-1</sup> )	Fruits dry matter (%)
FF	97.33 a	77.97 ab	522 b	15.23 a	5.51 b	7.06 b
SF	84.83 b	67.50 b	525 b	12.93 b	5.27 b	7.82 a
FC	103.17 a	91.06 a	589 ab	15.52 a	6.60 a	7.10 b
SC	102.50 a	87.80 a	663 a	13.26 b	7.01 a	8.17 a

The values followed by different letters are significantly different at P<0.05 (SNK).

## Conclusions

The results confirmed that MSW compost application could be a valid method both to distribute N and to sustain tomato productivity, in agreement with Bhattacharyya *et al.* (2003). The application of MSW compost as amendment to tomato crop also, in Mediterranean conditions, could provide as a N source and attenuate the deleterious effects of salt because the increase of organic matter improves the physical and chemical characteristics.

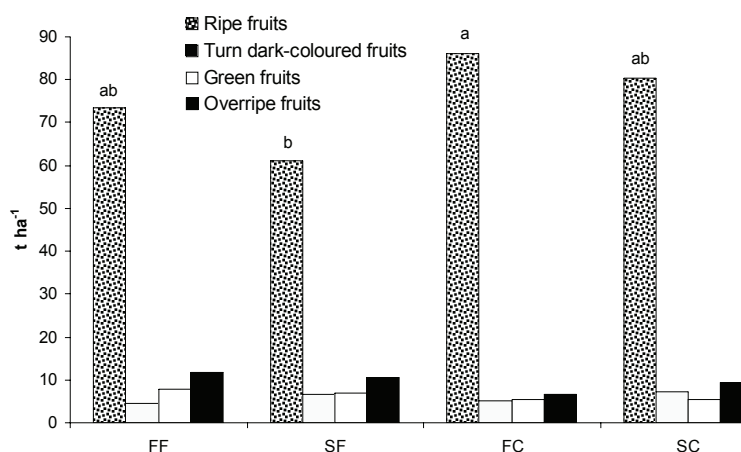


Figure 1. Ripe, turn dark-coloured, green and overripe yields of tomato

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## Acknowledgements

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# First Effects of Some Compost Fertilization Strategies on a Vegetable Crop Sequence, Soil Organic Carbon and Nitrate Release in Two Sites of Southern Italy

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The utilization of composted organic matter as fertilizer in vegetable crop systems has to face up to different needs: to define the quality of available composts; to set the adequate amounts of compost able to trigger a positive soil organic carbon balance and the related amelioration of its physical and biological properties; to increase the efficacy of nitrogen cycling in soil to satisfy either crop nutrients demand or to reduce the hazards of nitrate pollution of ground waters (Hargreaves *et al.*, 2008). The aims of our research were to validate some fertilization strategies with municipal solid waste compost through the evaluation of vegetable crops production in open fields, the soil organic carbon balance and the timing of nitrate release in soil. This research was funded by Campania Region.

## Methodology

Compost made by organic fraction source separated by municipal solid wastes was used as soil amendant in field trials located at Scafati (S) and Battipaglia (B), Campania Region, Southern Italy. Compost had chemical and physical characteristics in compliance with Italian law (D.L. n. 217/2006). Compost was rototilled in the first half of June. An annual vegetable crop sequence was scheduled with eggplant (*Solanum melongena* L.), cv Lunga Napoletana, in spring-summer 2007 cycle and endive (*Chycorium indivia* L. var. *latifolia*), cv Cuartana, in autumn-winter 2007/2008 cycle. Four fertilization strategies were compared: 1) 30 t ha<sup>-1</sup> of compost on a dry matter basis (C30), 2) 15 t ha<sup>-1</sup> of compost integrated with reduced doses (1/4 for eggplant and 1/2 for endive) of N fertilizers (C15+N), mineral fertilization (MIN), control not fertilized (CNF). The treatments were arranged in plots of 100 m<sup>2</sup> according to a randomized complete block with two replications. The initial level of soil organic C (SOC) and soil total N were 12.6 and 1.5 g kg<sup>-1</sup> respectively in site S, 9.4 and 1.2 g kg<sup>-1</sup> respectively in site B. These measures were measured again 11 months after compost amendment. The release of nitrates in the soil solution at 0-30 and 30-60 cm layers was measured in four times between August '07 and March '08. Samples of soil were collected in fixed points along the plant rows by gouge auger for stepwise sampling. Soil was extracted in 1:5 ratio. The nitrate content was measured using a colorimetric method by Auto-Analyzer. Mineral fertilization was restricted only to nitrogen, because in both site P and K concentration was high. Doses were defined according to fertilization guidelines of Campania Region. Eggplant received 150 kg ha<sup>-1</sup> in site (B), fractionated in 12 fertigations whereas it received 180 kg ha<sup>-1</sup> in site (S) in 3 topdressing distributions of ammonium nitrate. C15+N received ¼ of nitrogen supplied in MIN. In the same way, endive received 120 and 60 kg ha<sup>-1</sup> of N in MIN treatment in sites (B) and (S), respectively. C15+N received ½ of that amounts. A slow release product (Entec 26 solub) was used as fertilizer in pre-transplant of endive in both sites.

## Results

Figure 1 shows that the higher rate of compost (C30) maintained the initial SOC level at site S and gave an increase of 1.96 g kg<sup>-1</sup> C at site B. The half-dose of compost (C15+N) determined a negative balance (-0.6 g kg<sup>-1</sup>) at site S whereas favoured an increase of 1.40 g kg<sup>-1</sup> C at site B. SOC native mineralization at site S was higher than site B as pointed out by observation of negative SOC trends in MIN and CNF. Marketable yields of eggplant and endive were higher at site B than S (Tab. 1); MIN fertilization was the more effective in increasing yields, particularly at site B and during crop cycle of endive. Production in CNF was negatively influenced at site B more than site S due to the lower SOC content of the first one. C30 and C15+N gave similar yields at site B whereas at site S the integrated treatment appeared not adequate to sustain crop needs.

Finally, nitrate release in soil at 0-30 cm depth, showed in Figure 2, was, in both sites, the lowest in compost treated plots ( $C30 < C15+N$ ) but pointed out in MIN plots a peak over  $100 \text{ mg kg}^{-1}$  at site S in February.

Figure 1. Soil organic carbon content at the start of trials and after one year in Scafati and Battipaglia (Bars represent standard error of the means)

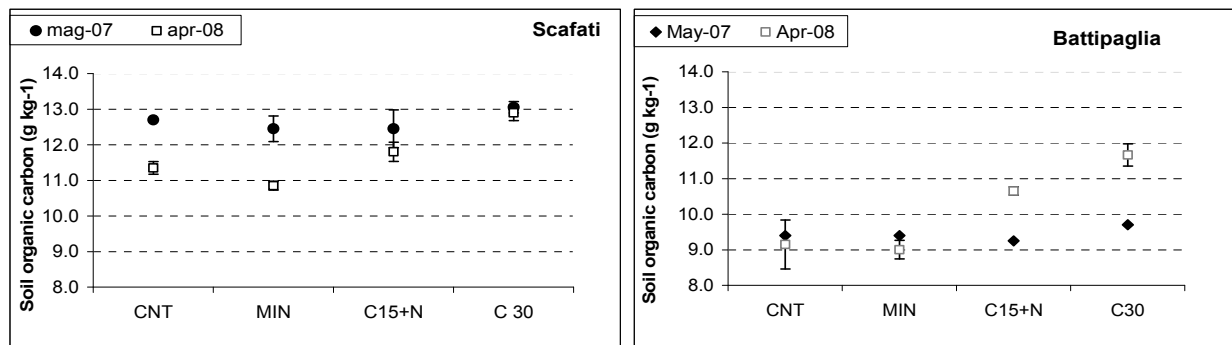
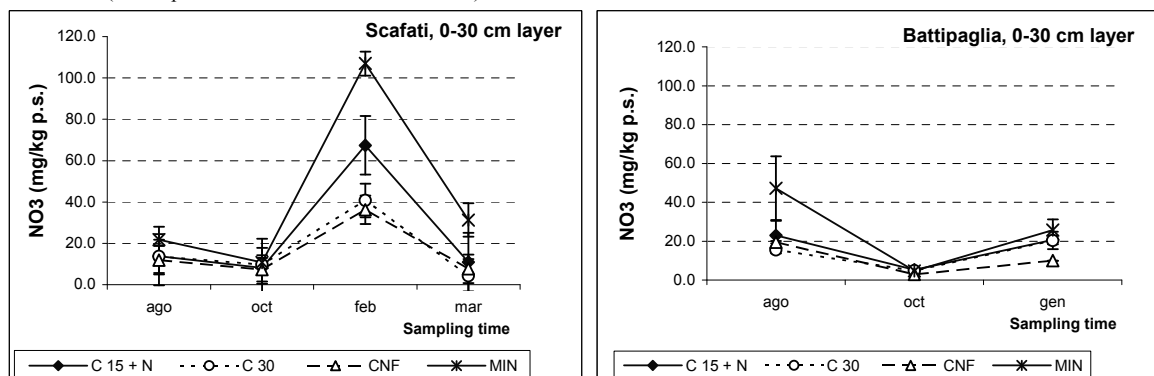


Table 1: Eggplant and endive yields as related to fertilization treatments and location in 2007/08.

Treatments	Marketable Yield (t ha <sup>-1</sup> )			
	Eggplant		Endive	
	Battipaglia	Scafati	Battipaglia	Scafati
MIN	39.6 a	29.0 a	38.6 a	17.7 n.s.
C 30	28.7 b	26.2 ab	26.1 b	14.2
C 15 + N	32.5 ab	22.6 b	28.1 b	9.1
CNF	23.9 b	27.8 a	16.2 c	12.7

Different letters indicate significant different values to Tukey HSD Test ( $P = 0,05$ ); n.s: not significant differences

Figure 2: Nitrates measured in the topsoil (0-30 cm) in different times of crop sequence as related to the fertilization treatments. (bars represent the standard error of means)



## Conclusions

Clay content (4,4 %) in soil of site S played a key role in explaining the high mineralization rate of compost organic carbon, in comparison to site B, where soil clay content is 22 %. Actually, it is known that the capacity of a soil to store organic matter is related to the association of SOC with clay and clay plus silt (2-20  $\mu\text{m}$  diam) particles (Carter, 2002). Soil nitrate release due to compost amendment was, often, lower than  $20 \text{ mg kg}^{-1}$  in both sites; relatively high soil nitrate fluxes were some time detected in mineral fertilized plots. In the first year, the combination of compost with low doses of mineral N did not increase crop yields more than C30 contrary to our preceding results.

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# The Effect of Crop Management Practices on Yields of Different Crops

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## Introduction

The farmer has to look for the most suitable agricultural system which respects climatic and economic changes. He has to decide if will choose ecological, conventional or integrated system. Each system can be with or without animal husbandry. Crop management practices include the choice of appropriate fore crop, crop stand establishment and soil tillage, fertilization and crop protection, harvest and post harvest processes.

## Methodology

The field trial is located in maize production area in the field experimental station of MUAF in Žabčice. This locality (179 m above sea level, 49°01' N, 16°37' E) is situated 25 km southwards from Brno (South Moravia region, Czech Republic). It is a warm and dry region with average annual temperature and precipitation of 9.2°C and 480 mm. According to the taxonomic system of soils of the Czech Republic, the soil in the field experimental station in Žabčice is classified as gleic fluvisol, which has developed on alluvial sediments of the Svratka River. These soils are without any marked diagnostic horizons and the parent substrate consisting of alluvial material is situated below a thin humus horizon. More marked symptoms of gley processes can be observed in the depth of below 0.6 m. In the course of the year, the groundwater level fluctuates between 0.8 – 2.5 m. As far as the soil texture is concerned, the soil is classified as heavy to very heavy.

This contribution is focused on results which are coming from model crop rotation acquired for system with animal husbandry in condition of dry area in the years 2004-2007. It includes 7-year crop rotation: lucerne – first year, lucerne – second year, winter wheat, silage maize, winter wheat, sugar beet and spring barley.

Three kind of soil tillage were compared in this field trial:

I. ploughing includes:

- a) stubble breaking – Kverneland stubble cultivator (working depth approx. 0.10 m)
- b) ploughing – middle deep (0,20 – 0,24 m) – mounted reversible plough Lemken
- c) sowing - cultivator mounted-pneumatic seed drill Accord (by maize and sugar beet pneumatic precision drill Kleine)

II. loosening

- a) stubble breaking – Kverneland stubble cultivator (working depth approx. 0.10 m)
- b) loosening – Kverneland stubble cultivator (working depth approx. 0.10 m)  
(manure is incorporated into the soil in sugar beet and maize)
- c) sowing - cultivator mounted-pneumatic seed drill Accord (by maize and sugar beet pneumatic precision drill Kleine)

III. direct sowing

- a) sowing – by cereals and lucerne cultivator mounted-pneumatic seed drill Accord (adjusted to depth of sowing, in sugar beet and maize the procedure is the same as variant II.)

Furthermore, the effect of fungicides was assessed in cereals. In the variant treated against leaf and ear diseases of winter wheat, fungicides were applied twice, at the beginning of stalk shooting (BBCH 32, TANGO SUPER - 84 g epoxiconazole + 250 g fenpropimorph) in the dose of 1.0 l/ha<sup>1</sup> and in the

growth stage of heading (BBCH 55, FALCON 460 EC – 250 g spiroxamine + 167 g tebuconazole + 43 g triadimenol) in the dose of 0.6 l/ha.

The grain yield in cereals, hay yield in lucerne, yield of dry biomass in silage maize and yield of sugar beet roots (by 16 % of sugar content) were evaluated. For statistical analyses the software UNISTAT for Excel version 5.0 was used.

## Results

The level of yield is corresponding with dry condition of this locality. During the observed period the average yield of winter wheat was 7.6 t/ha, by spring barley 6.2 t/ha, by silage 18.2 t/ha, by sugar beet 84.2 t/ha and by Lucerne 11.6 t/ha.

The differences in yields among variants of soil tillage were not statistically significant. The highest yields were on variant I – ploughing by all crops (exception spring barley). Lower yields were on variant II – with loosening and the lowest on variant III – direct sowing. These results showed Table 1. The effect of fungicide application was statistically significant. The yield of winter wheat treated with fungicides was 8.1 t/ha, non- treated 7.0 t/ha. In spring barley it was 6.4 t/ha respectively 6.1 t/ha.

By comparison of results from all occurred years we can conclude, that the highest yields were in 2004 and the lowest in 2007. For instance the yield by winter wheat was 8.76 t ha<sup>-1</sup> in 2004 and 6.40 t ha<sup>-1</sup> in 2007. The differences between years were statistically significant among all crops.

Table 1: Average crops yields in experiment 2004-2007 (t.ha<sup>-1</sup>; by cereals – yield of grain; by lucerne and silage maize – yield of dry matter, by sugar beet yield of roots by 16 % of sugar content)

		variants					
Crop	forecrop	soil tillage			application of fungicides		total
		I. plough ing	II. loosening	III. direct sowing	yes	no	
lucerne – first year	spring barley	9.72	8.70	8.01	-	-	8.81
lucerne – second year		14.59	14.83	14.10	-	-	14.51
winter wheat	lucerne	7.68	7.66	7.67	8.15 <sup>a</sup>	7.19 <sup>b</sup>	7.67
silage maize	winter wheat	19.16	17.79	17.75	-	-	18.23
winter wheat	silage maize	7.88	7.24	7.51	8.16 <sup>a</sup>	6.93 <sup>b</sup>	7.54
sugar beet	winter wheat	86.16	84.49	82.10	-	-	84.25
spring barley	sugar beet	6.38	6.38	6.04	6.41	6.11	6.27

Notice: different letters different letters (a, b) indicate significant differences at  $P \leq 0.05$

## Conclusions

The obtained results show, that the level of crop yields is more influenced by weather than by crop management practices. The differences among observed years were statistically significant.

## Acknowledgement

This work was supported by the National Agency for Agriculture Research as a project no. 1G46055 "Possibilities of limiting the drought impact by optimizing management practices in some field crops" and by the Ministry of Education, Youth and Sports of the Czech Republic as project no. 2B06124 "Reducing of impacts and risks on environment and information acquisition for qualified decision-making by methods of precision agriculture".

# Field Functional Diversity of Arbuscular Mycorrhizal Fungi in a Crop Rotation of *Trifolium alexandrinum* and *Zea mays*

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Soil microbes play a major role in the functioning of agroecosystems. Arbuscular mycorrhizal fungi (AMF) are beneficial microbes fundamental in soil fertility and plant nutrition, enhancing plant P and N uptake by means of their extraradical mycelium (ERM) spreading from mycorrhizal roots into the surrounding soil (Smith and Read, 2008). Many studies have been carried out with plants grown in sterile soil with or without AMF inoculation (Avio *et al.*, 2006), while little is known about mycorrhizal symbiosis in nonsterile soils, which can contain diverse microorganisms differently influencing plant growth. Furthermore, the impact of agricultural practices on mycorrhizal colonization and host plant response in the field is not yet clearly understood. Greenhouse and field studies were performed in order to assess inter- and intraspecific functional diversity of geographically different isolates of the AMF species *Glomus intraradices* and *Glomus mosseae*, and of indigenous isolates inoculated on *Trifolium alexandrinum* and *Zea mays*, in a 2-year crop rotation.

## Methodology

**Plant and fungal material.** The AMF used were: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolate IMA1 from UK and isolate AZ225C from USA, *Glomus intraradices* Schenck & Smith, isolate IMA5 from Italy and isolate IMA6 from France, inoculated singly (exp. 1, in greenhouse and exp. 2, in the field) or as a mixture (Mix) (exp. 2), and a population of indigenous AMF (Indy) (exp. 2). The indigenous population has been morphologically and molecularly characterized by Pellegrino *et al.* (2007). The plant species used were *Trifolium alexandrinum* L. cv. Tigri (exp.1 and exp.2) and *Zea mays* L. cv. Eleonora (exp. 2). **Greenhouse experiment.** Seeds of *T. alexandrinum* were sown into pots containing steam-sterilized soil and Terragreen. Pots were inoculated either with 90 ml of crude inoculum of one of the four isolates or with 90 ml of a sterilized mixture of them (control). All the pots received a filtrate to ensure a common microflora. The experiment was a completely randomized design with 5 inoculum treatments (fungal isolates and control), and 5 replicates. Three months after emergence, plant shoots were harvested, and *T. alexandrinum* dry weights were determined. Percentage of AMF colonization and total root length were assessed (Giovannetti and Mosse, 1980). N and P concentrations were assessed using Kjeldahl method and using the photometric method, respectively. **Field experiment.** In order to prepare the large quantity of inoculum to be used in the field experiment, AMF were reproduced in sterile soil and Terragreen, using *Z. mays* as host plant. The experimental field was prepared by digging and harrowing the soil, which was then inoculated with 0.7 Kg m<sup>-2</sup> of crude inoculum or with a sterilized inoculum mixture (control). The experimental design was a randomized block with seven inoculum treatments, three replicates, three harvests for *T. alexandrinum* and one harvest for *Z. mays* (one year after AM fungal inoculation). Experimental field soil and inocula were tested for mycorrhizal potential (MIP) (Pellegrino *et al.*, 2007) and spore density. **Short-term effect of AMF inoculum.** At each harvest, dry weight, percentage of AMF colonization, number of stems, seed weight, and N and P concentrations were assessed. **Long-term effect of AMF inoculum.** *Z. mays* dry shoot matter, percentage of AMF colonization, number of ears plant<sup>-1</sup>, grain dry weights, weight of 1000 seeds, grain N and P concentrations were assessed. Data of exp. 1 and exp. 2

were compared using one-way and two-way ANOVA, respectively. Data were transformed when needed and multiple comparisons were done with Tukey's B test.

## Results

**Greenhouse experiment.** The four *Glomus* isolates successfully established mycorrhizal symbioses with *T. alexandrinum*. Host benefits, calculated as dry weight increases, were 131, 149, 114, 121% for *G. mosseae* AZ225C and IMA1, *G. intraradices* IMA5 and IMA6, respectively. Plants colonized by *G. mosseae* showed higher shoot dry weights than those colonized by *G. intraradices*, while plants inoculated with *G. intraradices* showed a larger stem biomass. Root biomass and length were significantly affected by mycorrhizal symbiosis and differences in root length were observed at inter- and intraspecific level. All mycorrhizal *T. alexandrinum* plants showed significantly higher N and P shoot concentrations compared with nonmycorrhizal controls. Differences in shoot N and P concentrations were observed at the interspecific (*G. intraradices* > *G. mosseae*) and the intraspecific level (IMA5 > IMA6). N and P shoot contents of mycorrhizal plants were significantly higher than those of controls. Host benefits calculated as N content increases, were 129, 156, 143, 128%, and calculated as P content increases were 262, 292, 458, 249%, for AZ225C, IMA1, IMA5 and IMA6, respectively. Moreover, differences in P content between the two species (*G. intraradices* > *G. mosseae*) and within *G. intraradices* species (IMA5 > IMA6) were observed.

**Field experiment. Short term effect of AMF inoculation.** The different AMF isolates used as inocula showed a significantly higher infectivity compared to the natural experimental soil. After one month's growth, mycorrhizal colonization was significantly higher in inoculated plants (43.5%) than in controls (5.0%), but it did not affect ecophysiological parameters. *T. alexandrinum* shoot biomass were significantly affected by mycorrhizal symbiosis at the first and second harvests. Host benefits, calculated as mean dry weights of the two harvests, were 78, 47, 52, 15, 91 and 56% for AZ225C, IMA1, IMA5, IMA6, Mix and Indy, respectively. Shoot N and P concentrations and contents were significantly affected by mycorrhizal inoculation at each harvest. Moreover, an intraspecific variability in P content was observed in *G. intraradices*. Regrowth ability and seeds dry weight were significantly increased by mycorrhizal inoculation (by 68% and 84%, respectively). **Long term effect of AMF inoculation.** *Z. mays* inoculated plants showed a high root colonization even after two years. *Z. mays* shoot biomass, N and P concentrations were not affected by AMF inoculation, whereas grain dry weight, numbers of ears and 1000 seeds dry weight showed significantly higher values in mycorrhizal plots than in controls. Interestingly, differences between mixed and single inocula were also observed (Mix > Single). Grain N e P contents were affected by mycorrhizal inoculation: host benefits calculated as N content were 31, 50, 38, 38, 69, 80% and calculated as P content were 44, 44, 22, 44, 56, 67% for AZ225C, IMA1, IMA5, IMA6, Mix and Indy, respectively.

## Conclusions

The differential degree of host affinity found in the field may allow the selection of the most efficient plant – fungus combinations in terms of P and N uptake and growth parameters. The long term positive effects of mixed AMF inocula and of the indigenous population suggest the possibility of on-farm production of selected inoculum for low input and organic production systems.

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# Nitrogen Use Efficiency in a Potato Crop Fertilised with Compost

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Compost use in field horticultural crops is an interesting option for recycling organic wastes, with several environmental and economic advantages. It has been shown that compost can have positive effects on soil quality and can be considered as an important source of plant nutrients.

However, in order to introduce profitably compost in field horticultural crops, by managing correctly its application, it is necessary to know the time course of release of the main plant nutrients and their recovery efficiency. Few field studies have been done so far, on the release of nitrogen (N) after compost incorporation into the soil, and none has been carried out in Central Italy. Therefore, the objective of the present work was that of quantifying, at the field scale, mineral N release and plant uptake after the incorporation of compost into the soil in a potato crop (*Solanum tuberosum* L.) in Central Italy and to compare N use efficiency for different fertilisation strategies.

## Methodology

A 2-year field study (2005-2006) was conducted at the experimental farm of the University of Tuscia, on an Ando Eutric Cambisol with 32.6 % sand, 27.9 % silt, 39.5% clay, 1.5 % organic matter and 0.1% total N. The experiment included three different fertilisation strategies: only compost (PC100), only mineral fertiliser (PM100), combined fertilisation (50% compost and 50% mineral fertiliser, PC50), and an untreated control (P0), in a randomised blocks design with three replicates. Municipal waste compost was used at rates of 64 and 32 Mg ha<sup>-1</sup> for PC100 and PC50 respectively in the first year, and 77 and 38 Mg ha<sup>-1</sup> for PC100 and PC50 in the second year. These rates were determined by estimating that 10% of total N in the compost, i.e. 2.5 and 2.6% respectively in the two years, was available for the crop. Hence all fertilised treatments were assumed to receive the same N amount (150 kg N ha<sup>-1</sup> in 2005 and 180 kg N ha<sup>-1</sup> in 2006).

Compost was applied each year in spring and incorporated into the soil by disk-harrowing. Mineral N fertilisers (urea or ammonium nitrate), in the PC100 and PC50 plots, were split into two applications: at sowing and 45 days after sowing. Ground cover, leaf area index, above-ground and tuber biomass were measured during canopy development. N content of biomass components and soil were also measured, as well as the main soil physical variables influencing mineralisation: soil moisture (using TDR) and soil temperature. Three indices of N fertiliser efficiency were computed: calculated surplus (SUR), N use efficiency (Nuef) and apparent recovery (NRE) (Grignani *et al.*, 2000). The N mineralization rate (NMR) was calculated according to He *et al.* (2000), i.e. as soil organic N after compost incorporation minus soil organic N after crop harvest, divided by soil organic N after compost incorporation.

## Results

No significant differences were observed in the two years in biomass or tuber yield within the fertilised plots, with a mean value of 1.73 Mg ha<sup>-1</sup> dry weight (DW) for leaves and stems and a 32.8% lower value in the unfertilised control (Table 1).

Ground cover (GC) was similar among fertilised plots in the first year with a higher value

Table 1. Dry weight (DW) and fresh weight (FW) of potato plant organs (Mg ha<sup>-1</sup>). Means of 2 years  $\pm$  standard error.

Organs		P0	s.e	PC100	s.e	PC50	s.e	PM100	s.e
Leaves+stems	DW*	1.16 $\pm$ 0.07 b		1.68 $\pm$ 0.16 a		1.84 $\pm$ 0.06 a		1.67 $\pm$ 0.26 a	
Tubers	DW <sup>†</sup>	6.64 $\pm$ 0.38 b		9.22 $\pm$ 0.74 a		10.38 $\pm$ 0.24 a		10.72 $\pm$ 0.90 a	
Tubers	FW <sup>†</sup>	34.68 $\pm$ 2.00 b		50.89 $\pm$ 3.07 a		54.48 $\pm$ 0.90 a		56.06 $\pm$ 3.32 a	

\* = 69 days after emergence (DAE); <sup>†</sup> = 85 DAE; values within rows with different letters are significantly different (P < 0.05 Duncan's MRT)

for PM100 and PC50 (81% and 79 % GC respectively) as compared to PC100 (70% GC) at 46 days after emergence (DAE). The opposite was true at 35 DAE in the second year, with a higher value for PC100 and PC50 (61% and 55% GC respectively) than PM100 (49% GC). P0 treatment had lower GC values in both years. LAI values reflected GC results. Soil water content (0-30 cm) was substantially the same for PC100, PM100 and P0 with higher value for PC50 (+25%) in the first year. No significant differences were observed in the second year. Soil temperatures were higher in PC100 and PC50 than PM100 and sometimes P0.

Soil organic N, measured at different times, showed higher values in PC100 and PC50 as compared to P0 and PM100, generally with a decreasing seasonal trend, possibly due to mineralisation (Table 2).

NMR of compost was 37.2 and 34.5% for PC100 and PC50 respectively in 2005 and 15.3 and 7.0% in 2006. NMR of soil organic N were 17.8 and 23.8% for P0 and PM100 respectively in 2005, and -13.8 and -9.6% in 2006.

N uptake by the whole plant (NRt) was higher in PM100 and PC50 as compared to P0 and PC100 in both years (Table 3). N uptake by tubers (NRtub) was similar for fertilised plots. Actual N supplied through fertilisation (Nf) from compost was higher than originally estimated (10 %) in both years. SUR (= Nf-NRt) was higher for PC100 and PC50 in 2005 and for PC100 and PM100 in 2006. NUef [(DW in fertilised plots - DW in P0 plots)/Nf] and NRE [=100\*(NRt through fertilisation -NRt P0)/Nf] were significantly higher for PC50 and PM100 as compared to PC100 in both years. Besides, NUef and NRE values remained unchanged in time for PM100, while they increased significantly during the two years for both PC100 and PC50.

Table 2. Soil organic N (Mg ha<sup>-1</sup>) in the 0-30 cm soil layer during the 2 years

Date	P0	PC100	PC50	PM100
13/04/05	2.72 b	4.09 a	3.82 a	2.81 b
22/12/05*	2.24 a	2.57 a	2.51 a	2.14 a
13/04/06	3.04 b	6.15 a	4.69 a	3.00 b
02/08/06†	3.46 c	5.20 a	4.36 b	3.29 c

\*= 249 DAE; †= 109 DAE; values with different letters within rows are significantly different (P < 0.05 Duncan's MRT)

Table 3. Total N removal and N use indices for the different fertilisation strategies

Index*	Year	2005				2006			
		P0	PC100	PC50	PM100	P0	PC100	PC50	PM100
NRt	(kg ha <sup>-1</sup> )	113b	121b	145ab	180a	81c	132b	159a	162a
NRtub	(kg ha <sup>-1</sup> )	83a	86a	106a	138a	58b	97a	120a	125a
Nf	(kg ha <sup>-1</sup> )	0	208	197	150	0	259	148	180
SUR	(kg ha <sup>-1</sup> )	-113c	88a	52a	-30b	-81c	127a	-10c	18b
NUef	(kg SS kg N <sup>-1</sup> )	-	6b	13a	28a	-	19b	39a	28ab
NRE	(%)	-	4b	16ab	44a	-	20b	53a	45a

\* = see text for indices meaning and computation details; values with different letters within years in the same rows are significantly different (P < 0.05 Duncan's MRT)

## Conclusions

This study confirms the important potential role of compost as a source of N for crops. Mineralisation rates between 11 and 35% allowed for sufficient N supply to the potato crop. Compost N use efficiency increased considerably in the second year of compost application to the soil. The indices computed for assessing N fertiliser efficiency emphasized the advantage of repeated moderate compost application rates combined with mineral N fertilisation, in order to minimize nitrogen losses.

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# Effect of Different Intensity of Soil Tillage on Yields of Spring Barley

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Spring barley is a crop, which requires good physical conditions and soil structure, sufficient amounts of available nutrients and good aeration of soil. As far as the crops of spring barley are concerned, there is a wide scale of technological procedures of tillage and crop establishment available. Their choice must be done with regard to site conditions and inclusion of spring barley into the crop rotation (including the management of post-harvest residues of the forecrop and soil conditions after the forecrop harvest. It is possible to use not only conventional methods of soil tillage with ploughing but also minimum tillage with only shallow loosening of soil with disc or shovel cultivator; under very favourable conditions it is even possible to use direct sowing into non-tilled soil. In the Czech Republic, the conventional technology with ploughing is the most frequent method of crop establishment. For spring barley it is usually sufficient to perform a shallow ploughing to the depth of 0.15 – 0.18 m. Ploughing can be performed in practically all localities, on all sites and after all forecrops. It is suitable above all on those fields where it is necessary to plough-in greater amounts of post-harvest residues. The main disadvantage of this method consists in a higher consumption of energy and labour.

Recently, the minimum tillage technology is used also when growing spring barley. Results of many experiments as well as practical experience indicate that good results can be obtained above all in medium heavy soils with good structure and higher natural fertility in maize and sugar-beet growing regions. In heavy soils under more humid and colder climatic conditions the use of minimum tillage technologies is less suitable (Dryšlová *et al.*, 2006; Klem *et al.*, 2006; Zimolka *et al.*, 2006; Hůla, Procházková *et al.*, 2008).

## Methodology

The effect of different methods of tillage on yields of spring barley was studied in a long-term field experiment within the period of 1990 – 2007. Experimental results were obtained in the period of 1990 – 2005 and 2007. In 2006, the stands of spring barley were damaged by floods.

The experimental site is situated in the sugar-beet growing region in the altitude of 225 m; within the period of 1989 – 2007, the average annual temperature and sum of precipitation were 9.25 °C and 542 mm, respectively. Soil conditions: chernozem, loamy soil with neutral soil reaction and good reserves of phosphorus, potassium and magnesium. Spring barley was grown within the framework of three different crop rotations, always after sugar-beet, which was grown after silage maize, winter wheat and spring barley. Compared were always four variants of tillage: Variant 1 – ploughing to the depth of 0.22 m; Variant 2 – shallow ploughing to the depth of 0.15m; Variant 3 – direct sowing into non-tilled soil; and Variant 4 – disking of soil to the depth of 0.10 m. Application of fertilisers was the same in all experimental variants: 40 N, 30 P, 60 K kg.ha<sup>-1</sup>. Cultivars of barley: 1990 – 1996 Rubín; 1997 Akcent; 1998 – 2007 Kompakt. Plant protection was performed according to the valid current methodologies of the State Phytosanitary Administration. The experiment was established in four

replications using the method of sub-divided plots; the total area of one experimental plot was 300 m<sup>2</sup> and the size of sub-divided harvested plots was 22 m<sup>2</sup>.

## Results

Results of a long-term evaluation of effects of different methods of tillage on yields of spring barley are presented in the following table.

Effect of different methods of tillage on yields of spring barley (t.ha<sup>-1</sup>) grown in three crop rotations - mean values for the period of 1990-2005 and 2007

Variant of tillage	1. Maize sugar beet spring barley	2. Winter wheat sugar beet spring barley	3. Spring barley sugar beet spring barley	Mean
1. Ploughing 0.22 m	6.15	6.34	6.43	6.31
2. Ploughing 0.15 m	6.28	6.54	6.57	6.50
3. No tillage	6.25	6.44	6.52	6.40
4. Discing 0.10 m	6.28	6.40	6.40	6.36
Mean	6.27	6.43	6.48	6.39

1<sup>st</sup> Crop rotation - dt (tillage) p=0.05 0.180; p=0.01 0.218

2<sup>nd</sup> Crop rotation - dt (tillage) p=0.05 0.043; p=0.01 0.052

3<sup>rd</sup> Crop rotation - dt (tillage) p=0.05 0.121; p=0.01 0.147

The highest average yield of spring barley was obtained in the crop rotation spring barley – sugar beet – spring barley while the lowest one in the crop rotation involving crops demanding high amounts of water, i.e. silage maize – sugar beet – spring barley. The effect of different tillage technologies on yields of spring barley was statistically significant in all three crop rotations. When evaluating pooled results of all three crop rotations as recorded within the period of 1990 – 2005 and 2007, the highest average yield was obtained in Variant 2 (shallow ploughing to the depth of 0.15 m – 6.50 t.ha<sup>-1</sup>), followed by Variant 3 (direct sowing into no-tilled soil – 6.40 t.ha<sup>-1</sup>). The lowest average yield was obtained in the Variant 1 (ploughing to the depth of 0.22 m – 6.31 t.ha<sup>-1</sup>).

## Conclusions

Results of a long-term study on effects of various tillage technologies on yields of spring barley grown after sugar beet in chernozems soil in the sugar-beet growing region indicate that it is possible (and advantageous) to use also less intensive/minimum tillage technologies, which is characterised by a lower consumption of energy and labour.

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# Evaluation of the Effects of the Application of Dehydrated Pig Slurry on Wheat Production and on the Soil

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In Northeast Spain the expansion of the pig sector in the last thirty years has brought as a result the production of great amounts of pig slurry that exceeds field crops fertilization needs, therefore livestock effluents could significantly contribute to the polluting load and the progressive environmental deterioration of the region. Under such circumstances, it is necessary to treat pig slurry in order to reduce its polluting potential and/or facilitate the logistics of transporting the manure from the pig producer to the field. In the last years the implantation of six cogeneration treatment plants in our region that make full use of the energy, exceeding 90% of primary energy usage, and that treat 100,000 m<sup>3</sup> of pig manure per year each (46,000 pigs for plant) has facilitated a sound manure management. Although in our region some field experiments have been conducted to evaluate the fertilizer value and environmental impact of pig slurry as a fertilizer (Berenguer *et al.* 2008), little is known about the fertilizer value and eventual adverse environmental effects of dehydrated pig slurry produced in such plants. The aim of the present research was to evaluate the effects of a single autumn application of dehydrated pig slurry on wheat yield and soil nitrogen dynamics when applied to cover phosphorus and potassium crop fertilization needs.

## Methodology

Field experiments were conducted in the river Ebro valley at Almacelles on a calcixerept soil (at 0-30 cm, pH 8.1, soil texture: 171, 388 and 441 g kg<sup>-1</sup> of sand, silt and clay, respectively, and 21.4, 35 and 225 g kg<sup>-1</sup> of organic matter, P(Olsen) and K(NH<sub>4</sub>Ac), respectively) irrigated by a center-pivot. The crop received 250 mm of irrigation plus 207 mm of rainfall mostly in April when crop growth stage 43 (Zadoks *et al.* 1974). Experimental units were 10 m x 10 m plots. Dehydrated pig slurry (DPS) application rates of 2000 and 6000 kg ha<sup>-1</sup> (DPS2 and DPS6, respectively) were compared with a standard mineral treatment (M) and control plots (C) in a completely randomized experiment with three replicates. DPS analysis showed that it contained 64.7 % organic matter, 4% N, 5% P<sub>2</sub>O<sub>5</sub> and 7% K<sub>2</sub>O, and 225 and 1791 mg kg<sup>-1</sup> of Cu and Zn, respectively. Mineral treatment consisted of 67, 217 plus 233 kg ha<sup>-1</sup> of urea, DAP and KCl, respectively, also in a single application. Wheat (cultivar Dollar) was sown on November 29 2006 at 200 kg ha<sup>-1</sup> (inter-row spacing was 0.15 m) and harvested on July 12, 2007). Plants were harvested by hand, grain yield was estimated selecting ten random 2 m crop rows length, and yield components were estimated selecting two random 2 m rows length. Plant nitrogen uptake was calculated by multiplying grain and straw biomass by its Kjeldahl N relative content determined following standard methodology (Tan, 1996). Soil cores were collected each four weeks, 3 cores per plot at 0-30 and one at 30-60 cm depths from November 2006 to July 2007 using a soil-sampling tube (Veihmeyer, 1928). Soil nitrates were extracted using CaCl<sub>2</sub> 0.01 M (Kmecl, 2005). Soil NH<sub>4</sub><sup>+</sup> -N was not measured, since its content was negligible, as confirmed also by other authors (Villar-Mir *et al.* 2002). Total Cu and Zn determinations were performed following the standard method (AOAC, 2003). Analysis of variance and Student-Newman-Keuls multiple comparisons were performed using SAS package (SAS, 1991).

## Results

Analysis of yield showed that there were no significant differences in yield between treatments, being the average yield of 6170 kg ha<sup>-1</sup>. However the analysis of yield components showed that the number of ears m<sup>-2</sup> was lower in the control plots (623 ears m<sup>-2</sup> versus 777 ears m<sup>-2</sup>). There were no significant differences in the number of grains per ear (32.2 grains) and on 1000 grain weight (36 g). Plant analysis showed that there were not significant differences between treatments in the grain relative nitrogen content (24 10<sup>3</sup> mg N kg<sup>-1</sup>), however straw N relative content of DPS2 (11.1 10<sup>3</sup> mg N kg<sup>-1</sup>) was significantly higher than that of the other treatments (8.9 10<sup>3</sup> mg N kg<sup>-1</sup>). The results of soil core analysis showed that from December to March NO<sub>3</sub><sup>-</sup>-N content at 0- 30 cm depth was significantly higher in M treatment. There were no significant differences between C plots and DPS treatments. After harvest residual NO<sub>3</sub><sup>-</sup>-N content was negligible in all treatments. At the beginning of the experiment at 30 – 60 cm depth the NO<sub>3</sub><sup>-</sup>-N content was insignificant, it increases in January and then decreases so that after harvest its content is negligible in all treatments.

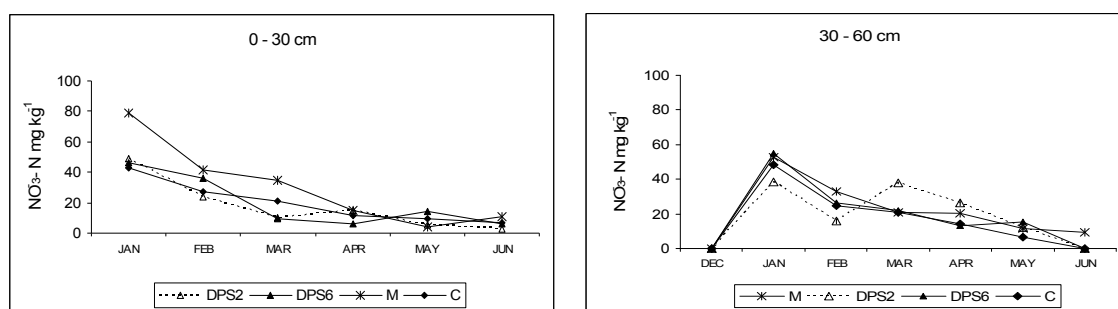


Figure 1. Dynamics of N-NO<sub>3</sub> from December to June at 0-30 cm and at 30-60 cm depth. M: mineral fertilizer treatment, DPS2 and DPS6: dehydrated pig slurry at 2000 and 6000 kg ha<sup>-1</sup>, respectively, C: control.

At 0-30 cm N-Kjeldahl content kept steady at about 3300 mg kg<sup>-1</sup>, there were no significant differences between treatments. However at 30 – 60 cm N-Kjeldahl content increases from 1800 a 3200 mg kg<sup>-1</sup>, since there are no differences between treatments that increase can not be attributed to DPS treatments. At 0 – 30 cm Cu and Zn total content were of 17.5 and 47 mg kg<sup>-1</sup>, respectively and at 30-60 cm depth 13.6 and 32.81 mg kg, respectively. No significant differences were appreciated.

## Conclusions

These preliminary results seem to show that under irrigation dehydrated pig slurry could be promising fertilizer in autumn applications for cereals. In north east Spain dehydrated pig Slurry could be an alternative to mineral phosphate and potassium application in autumn. At 2000 and 6000 kg ha<sup>-1</sup> rates crop performance and soil nitrogen dynamics are not significantly different from not fertilized plots.

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# Seed Yield And Oil Content In Flax (*Linum usitatissimum* L.), Cropped In Southern Environments

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## Introduction

The fat acid groups,  $\omega$ -3 (mainly  $\alpha$ -linoleic acid) and  $\omega$ -6 (mainly linolenic acid), have an important role in the human cardiovascular and tumoral illnesses prevention (Vecchini *et al.*, 2004; Thompson *et al.*, 2004). The  $\omega$ -6 reduces the amount of blood cholesterol, even if this reduction concerns the “good” cholesterol; besides, it has a low ability to reduce the plasmatic levels of triglycerides. The  $\omega$ -3 reduces the plasmatic levels of triglycerides, but it has a low ability in the bad cholesterol reduction. For this reasons, the optimal  $\omega$ -3/ $\omega$ -6 ratio must range between 2 and 4, to maximize the positive effects on human health.

The aim of this work is to evaluate the adaptability and therefore the productivity of flax (*Linum usitatissimum* L.), in two different Mediterranean environments, with the purpose to identify crop management and location that can ensure elevated oil yield and quality for animal food preparation.

## Methodology

In two Mediterranean locations, Foggia (Apulia region) and Cammarata (Sicily region), field experiments were carried out in two years, 2006 and 2007, with a spring sowing in the first year, autumnal and spring sowing times in the second year. Seed yield and oil seed composition were recorded. The soil of Foggia is a Vertic Calcixerept, the soil of Cammarata is a Chromic Haploxerert, both with xeric conditions. The climate is “accentuated thermomediterranean”, with temperatures below 0 °C in the winter and above 40 °C in the summer. Rainfall (500-600 mm) is mostly concentrated during the winter months.

In 2006 the sowing dates were April 6<sup>th</sup> in Foggia and 24<sup>th</sup> March in Cammarata, while the crop was harvested at the end of July in Foggia and 28<sup>th</sup> June in Cammarata. In Foggia, for the delay in sowing time and for the drought conditions, two irrigations in April and May were applied (total of 60 mm).

In the second year in Foggia the autumnal flax was sown on 17<sup>th</sup> November, the spring sowing on 19<sup>th</sup> March, and harvested on 21<sup>st</sup> June and 17<sup>th</sup> July, respectively. In Cammarata the autumnal sowing date was 8<sup>th</sup> December 2007, while for the spring sowing, the date was 20<sup>th</sup> January. The crop was harvested on 12<sup>th</sup> June and 2<sup>nd</sup> July for the autumnal and for the spring sowing crop, respectively.

## Results

In the first year no difference was observed between the two locations for seed yield, while Cammarata gave the best results in the second year for both sowing dates (Tab.1), with values slightly lower than those reported in Northern Italy (Casa *et al.*, 1999). The seed productivity was, in general, higher in the autumnal sowing times than spring one, for the longer crop cycle and for the temperature closer to the optimal values for reproductive phase. The amount of oil yield is function of seed yield and oil content, with these variables inversely correlated. The oil yield resulted higher in autumnal than spring sowing time and in Cammarata than in Foggia (Tab.1).

The oil of seed flax cropped in Foggia had a different fat acid composition, with an  $\omega$ -3 average content 5% greater than the seed oil obtained in Cammarata, but an  $\omega$ -6 average content lower of 8%. Consequently, the ratio  $\omega$ -3/ $\omega$ -6 resulted higher in Foggia, and this is a positive result from a qualitative point of view.

The pedoclimatic conditions of Cammarata were more favourable for flax cultivation, both for yield and quality, than Foggia, but the results need to be confirmed. The table 2 shows a strong correlation between the rain (and irrigation) felled down during crop cycles and the seed yield; in fact, the greater difference between the two locations is due to the cumulated rain felled down during the cropping cycles of autumnal and spring flax sowing times (on average, 644 mm in Cammarata and 490 mm in Foggia). This positive correlation has been compensated for the negative correlations with the average air temperature, probably because the higher is the rain, the lower is the air temperature. The percentage of  $\omega$  -3 and  $\omega$  -6 seem to not be influenced by climatic conditions.

**Table 1.** Seed yield, oil content and acidic composition of flax, cropped in Foggia and Cammarata in two years and sowing times. Different letters indicate significant difference at  $P>0.05$  (LSD test).

Seasons	Seed yield <i>t ha<sup>-1</sup></i>	Seed oil content %	Seed oil yield <i>kg ha<sup>-1</sup></i>	$\omega$ -6 content %	$\omega$ -3 content %	$\omega$ -3/ $\omega$ -6 -	$\omega$ -6 yield <i>kg ha<sup>-1</sup></i>	$\omega$ -3 yield <i>kg ha<sup>-1</sup></i>
<b>Spring 2006</b>								
Foggia	0.55	33.1 b	183.1	15 b	46.8 a	3.2 a	27.8	85.3
Cammarata	0.44	35.9 a	160.0	19.1 a	43.1 b	2.3 b	29.2	70.7
Mean	0.50	34.4	171.6	17.1	44.9	2.8	28.5	78.0
<b>Autumn 2007</b>								
Foggia	0.80 b	31.3 b	249.5 b	17.5	46.9 a	2.7	43.3 b	116.2 b
Cammarata	1.02 a	32.2 a	325.1 a	16.8	44.5 b	2.7	55.3 a	144.5 a
Mean	0.91	31.7	285.3	17.2	45.7	2.7	49.3	130.4
<b>Spring 2007</b>								
Foggia	0.38 b	31.9	120.8 b	16.4	45.1	2.8	19.8 b	54.6 b
Cammarata	0.77 a	32.1	246.7 a	16.8	44.4	2.7	41.5 a	109.7 a
Mean	0.58	32.0	186.7	16.6	44.8	2.8	30.7	82.2
<b>Foggia</b>	<i>0.58 b</i>	<i>32.1 b</i>	<i>184.4 b</i>	<i>16.3 b</i>	<i>46.3 b</i>	<i>2.9 a</i>	<i>30.3 b</i>	<i>85.4 b</i>
<b>Cammarata</b>	<i>0.74 a</i>	<i>33.4 a</i>	<i>243.9 a</i>	<i>17.6 a</i>	<i>44.0 a</i>	<i>2.6 b</i>	<i>42.0 a</i>	<i>108.3 a</i>

**Table 2.** Pearson correlation coefficients between rainfall and average temperature observed during crop cycle in both years, sowing dates and locations.

	Seed yield <i>t ha<sup>-1</sup></i>	Seed oil content %	Seed oil yield <i>kg ha<sup>-1</sup></i>	$\omega$ -6 content %	$\omega$ -3 content %	$\omega$ -3/ $\omega$ -6 -	$\omega$ -6 yield <i>kg ha<sup>-1</sup></i>	$\omega$ -3 yield <i>kg ha<sup>-1</sup></i>
Rainfall	0.91	-0.72	0.87	n.s.	n.s.	n.s.	0.85	0.88
AverageTemp	-0.75	0.56	-0.72	n.s.	n.s.	n.s.	-0.77	-0.71

## Conclusions

Despite the brevity of the experiment, encouraging results emerged on the flax oil yield and quality. The autumnal better than spring sowing time and Cammarata better than Foggia location, seem to be the first indications for the seed yield, oil content and  $\omega$ -3 and  $\omega$ -6 composition and yield.

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# Evaluation of Chlorophyll Meter Data for Nitrogen Fertilizer Management in Maize (*Zea mays* L.)

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In order to achieve high yield of maize (*Zea mays* L.) farmers in many parts of Iran tend to apply nitrogen fertilizer (N) in excess of the crop requirements. Excessive N input in the beginning of growth season is the major cause of the poor fertilizer efficiency. Only less than 20% of the total N uptake by maize occurs before V8. Environmental and economic considerations require that nitrogen fertilizer should be applied only in quantity and time which is strictly justified. Improving N management would eventually depend on the accuracy with which the N status of plant can be assessed. The usefulness of SPAD chlorophyll meter readings for plant N assessment is based on the direct proportionality between the leaf chlorophyll and the leaf N concentration. By using the critical value of SPAD it is possible to determine the best time for N application.

## Methodology

Field experiments were conducted in the 2005 and 2006 growing seasons on a loamy clay soil at experimental field of Lorestan University. Full season hybrid of maize (S. C. 704) was sown at a density of 72000 plants ha<sup>-1</sup>. Various levels of nitrogen fertilizer (0, 100, 200, 300, 400 and 500 Kg N ha<sup>-1</sup>) were applied in two experiments. Dynamics of chlorophyll content was measured indirectly using a chlorophyll meter (SPAD) on the youngest fully developed leave of maize. Weekly SPAD reading was taken in all plots starting from V6. Total nitrogen content (N %) in the maize leave and grain dried samples was determined using the Kjeldahl digestion technique. The aims of this study was to determine relationship between nitrogen fertilization and leaf chlorophyll content as measured using a SPAD chlorophyll meter and determining the critical value of SPAD for different growth stages of maize.

## Results

By increasing rates of N fertilizer, %N in grain and leaf increased. Increasing N fertilizer up to 400 kg ha<sup>-1</sup> significantly increased biomass of maize but higher N application did not increase this parameter further. The SPAD readings were significantly increased by N application up to 400 kg ha<sup>-1</sup>. Regression analyses indicated a significant quadratic plateau equation for SPAD values versus different level of N fertilizer. Regression analysis of the data showed that grain yield, biomass and nitrogen concentration in the leaf and grain of maize was linearly correlated with the SPAD readings (Tab. 1). The linear regression of the leaf N% and the SPAD values was highly significant at the VT, R1 and R2 growth stages and the same was when data for all stages were pooled.

Based on results using SPAD meter is not a good technique for early prediction of nitrogen status in maize, but it is very reliable at the growth stages after V11. Relationship between the marginal grain yield increase (in response to use higher amount of N fertilizer) and SPAD readings in every growth stage was negative (Fig. 1). Results showed that when SPAD reading at the VT growth stage was more than 53.3, any further application of nitrogen was not justified (Tab. 2). Similarly critical values of 44.1, 47.5 and 55.3 were calculated for V9, V11 and R1 growth stage respectively.

Table 1. Correlation between the SPAD readings at R1 growth stage and some major agronomic attributes of maize

Agronomic attributes	Regression equation	R <sup>2</sup>
Leaf Nitrogen (%)	$Y = 0.1079X^2 + 2.8742$	0.84
Grain Nitrogen (%)	$Y = 0.0044x^2 - 0.3591x + 8.536$	0.68
Grain yield (t ha <sup>-1</sup> )	$Y = -0.0153X^2 + 1.9552X + 49.191$	0.84
Maize biomass (t ha <sup>-1</sup> )	$Y = 0.0596X^2 + 6.3392X + 142.44$	0.72

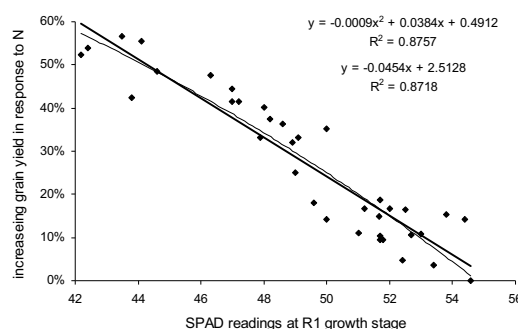


Figure 1. Relationship between grain yield increase in response to N application and SPAD readings at R1 growth stage

Table 2. Critical SPAD values for different growth stage of maize

Growth stage	Equations				Critical SPAD value
	Linear	R <sup>2</sup>	Quadratic	R <sup>2</sup>	
V9	$Y = -8.93X + 398.3$	0.19	$Y = 0.04X^2 - 12.5X + 473.6$	0.19	44.1
V11	$Y = -10.3X + 489.4$	0.52	$Y = -1.08X^2 + 85.3X - 1096$	0.53	47.5
VT	$Y = -4.58X + 244.3$	0.69	$Y = 0.14X^2 - 18.34X + 567$	0.70	53.3
R1	$Y = -4.54X + 251.2$	0.87	$Y = -0.08X^2 + 3.84X + 49.1$	0.87	55.3

## Conclusions

SPAD-based N fertilizer management can favor the application of the best agronomic technique for N fertilization. However, from a practical point of view, this method has some limitations since plant chlorophyll is affected by many factors and it is impossible to identify a universal meter reading that indicates sufficient N for all varieties of a specific crop. Therefore the reading must be calibrated for different varieties and environments. Moreover this method is not capable of detecting luxury N uptake since maize plants achieve maximum chlorophyll content irrespective of level of over fertilization. Based on results of this paper using SPAD meter is a good technique for prediction N status in middle and late growth stages of maize. At VT growth stage it is still possible to affect the grain yield of maize through the application of N fertilizer. Because Urea fertilizer is very soluble in water, farmers can use this fertilizer through fertigation at the middle of growing season, so also at this late stage it is still possible to use a critical value of SPAD to decide about time of N fertilizer application.

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# Soil Phosphorus Dynamic in an Olive Orchard Grown Under Different Weed-Control Systems

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Most of the olive orchards in Northeastern Portugal are on steep slopes. Tillage is the usual soil management system for these orchards. The combination of both these factors increases greatly the risk of soil erosion. To protect the soil, alternative weed-control systems, such as the use of post-emergence herbicides, must be implemented. As collateral effects, the weed-control systems may influence several aspects of soil fertility and crop nutrition, including P availability. The bioavailability of P in these soils is frequently low, mainly if they are acid, due to phenomena insolubility. Consequently, regular application of P fertilizers is needed to promote the growth of crops (Arrobas and Coutinho, 2002). The aim of this work is to study how the different soil surface management systems can affect the soil P dynamic in a rainfed olive orchard.

## Methodology

The field trial is located in Mirandela (NE Portugal). The climate is Mediterranean type, hot and dry during the summer growing season. The soil is Leptosol, sandy-loamy textured, with an organic matter content in the first 20 cm of  $6.1 \text{ g kg}^{-1}$  and  $\text{pH}_{(\text{H}_2\text{O})}$  (1:2.5) of 4.9. The field trial was established in 2001 with the following weed-control systems: i) glyphosate based herbicide (Gly), applied in April; ii) herbicide with a residual component (glyphosate + diuron + terbutylazine) (Res), applied in February; and iii) conventional tillage (CT). Annually, each individual tree received 0.5 kg of a compound fertilizer (10-10-10) that was spread beneath the tree canopy at the end of winter. Soil cores were taken in October 2007, beneath the trees and between rows, in the 0-5, 5-10 and 10-20 cm depth. Samples from the 20-30 cm layer were only collected beneath the trees. Soil P fractionation was performed according the Hedley et al (1982) procedure. Total P and Total Organic P were determined by the Saunders and Williams (1955) procedure. In the ANOVA, the weed-control systems were included as treatments and the sampling site, beneath the trees and between rows, as blocks. The means with significant differences ( $p < 0.05$ ) were separated by t-Student and Tukey HSD tests.

## Results

The results of soil P dynamic under the different weed-control systems and beneath the trees and between rows are presented in Table 1. The mean values of the more available P fraction (Resin-Pi +  $\text{NaHCO}_3\text{-Pi}$ ) were significantly higher beneath the canopy than between rows in the 0-5, 5-10 and 10-20 cm layers. The results identify a pool of labile inorganic P fraction beneath the trees. The presence of a pool of fertility under the trees was previously reported by Rodrigues et al (2005) as a result of fertiliser placement and nutrient recycling by leaves. Comparing the labile P fractions among the three weed-control systems, the values were lower in Gly treatment. The differences were statistically significant in the 5-10 and 10-20 cm layers. From the beginning of this experiment the olive yield and trunk perimeter were significantly higher in Gly treatment (Rodrigues *et al.*, 2006). The higher performance of trees on Gly treatment should imply a higher uptake of P and may justify the lower values found as inorganic P fraction. No significant differences were found among treatments or between sampling sites in  $\text{NaOH-Pi}$  fraction. However, the mean values tended to be lower in the Gly treatment. This soil is acid and this fraction represents P bounded to iron and aluminum. Quantitatively this is an important P fraction which could be relevant for the crop nutrition. This fraction is sensitive to changes in soil pH. In fact, the influence of the weed-control systems in soil pH was low at this time

(data not shown). Phosphorus extracted with HCl represents P bounded to calcium. The influence of sampling site and weed-control systems was not statistically significant for this P fraction. The upper soil layer (0-5 cm) presented the higher values, as a probable result of calcium application in the compound fertilisers. The organic P pool showed significant differences among the weed-control systems. In the 5-10 and 10-20 cm soil layers the organic P fraction was higher in Gly and lower in the CT treatment. Regarding total organic P, the values were higher in Gly and Res treatments. In the layer 5-10 cm, the values were significantly high in Gly treatment, and in the layer 10 to 20 cm both the herbicide treatments showed values significantly higher than that of CT treatment. Total P reflects the differences found in the other fractions, in particular the labile and organic ones.

Table 1- Soil P fractions in different sampling sites and under different weed-control systems

P fraction	depth (cm)	Sampling site		weed-control systems		
		Beneath trees	Between rows	Gly	Res	CT
Resin P + NaHCO <sub>3</sub> Pi (mg kg <sup>-1</sup> )	0 to 5	84.6 a	55.2 b	56.7 b	88.7 a	64.2 b
	5 to 10	46.9 a	36.1 b	25.8 b	52.3 a	46.4 a
	10 to 20	39.1 a	31.6 b	24.8 b	43.2 a	38.0 a
	20 to 30	20.5	-	16.4 a	24.1 a	21.0 a
NaOH Pi (mg kg <sup>-1</sup> )	0 to 5	153.9 a	137.8 a	110.5 a	171.9 a	155.1 a
	5 to 10	142.1 a	132.2 a	101.1 a	162.0 a	148.4 a
	10 to 20	129.2 a	121.9 a	88.5 a	157.3 a	130.8 a
	20 to 30	82.3	-	73.3 a	90.4 a	83.1 a
HCl P (mg kg <sup>-1</sup> )	0 to 5	192.8 a	106.3 a	128.4 a	213.7 a	106.6 a
	5 to 10	47.3 a	44.0 a	35.2 a	50.2 a	51.5 a
	10 to 20	43.9 a	38.3 a	37.8 a	42.0 a	43.5 a
	20 to 30	24.9	-	23.7 a	29.6 a	21.5 a
Organic total P (mg kg <sup>-1</sup> )	0 to 5	56.5 a	77.3 a	49.0 a	61.9 a	83.6 a
	5 to 10	126.1 a	105.0 a	182.7 a	103.6 b	60.3 c
	10 to 20	117.1 a	136.7 a	152.4 a	140.1 a	84.3 b
	20 to 30	87.3	-	47.9 a	114.3 a	99.8 a
Total P (mg kg <sup>-1</sup> )	0 to 5	507.9 a	441.1 b	409.4 b	582.3 a	431.9 b
	5 to 10	432.4 a	381.1 b	375.9 b	449.2 a	395.1 ab
	10 to 20	394.4 a	409.4 a	357.0 c	456.4 a	398.6 b
	20 to 30	305.4	-	167.8 b	481.4 a	267.2 b

For sampling site and weed-control systems separately, means followed by the same letter in row are not statistically different ( $p < 0.05$ ).

## Conclusions

The results showed a slight reduction in the more labile P fraction in Gly treatment, justified with the higher performance of trees and a probably higher P uptake on this plot. In Gly plot it seems not sustainable to maintain the higher olive yields if the P fertilization was not increased. A pool of P fertility was recorded beneath the trees as a result of fertiliser placement and P recycling in leaves.

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# Interaction of N, P, K as a Base of Sustainable Fertilizers Use

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## Introduction

In Poland in the last twenty years the consumption of nitrogen, phosphorus and potassium fertilizers and the relation between these three nutrients has changed considerably. At the threshold of economical and political transformation in 1989/90 the consumption of fertilizers dropped dramatically and the ratio P:N and K: N widened significantly. Since then the nitrogen consumption is increasing slowly.

Year	Consumption kg nutrient*ha <sup>-1</sup>			Relation, N = 1	
	N	P	K	P	K
1989	68,9	17,5	50	0,23	0,72
1992	33,9	5,3	13,3	0,16	0,39
1999	48,4	7,55	18,0	0,15	0,37
2007	65,3	11,1	25,8	0,17	0,39

The main objective of this paper was to simulate the long-term consequences of decreasing P,K rates on behalf of N rates on crops yield and soil fertility in permanent field experiments.

## Methods

Two long-term field experiments has been carried on since 1985 in two sites on medium-heavy soils with medium content of potassium and phosphorus. The first factor, established in 1985 has been six nitrogen rates and the second one P and K fertilization on null and recommended (optimal) levels. The experiments have been carried on in four course rotation (Table 1) in the years 2003 – 2006, with each crop in each year. These are the most important crops covering almost half of the area of better soils in Poland.

## Results

The average crop yields and nutrients uptake for the whole experimental period are presented in table 1 and 2. All crops responded positively on nitrogen fertilization and no one showed negative reaction on abandoning P and K fertilization. There was only a slight tendency for more negative reaction of winter wheat and winter rape for lack of potassium at higher rate of nitrogen fertilizers.

Table 1. Grain yields in kg ha<sup>-1</sup> depending on N, P, K fertilization

N levels	Winter wheat			Maize for grain			Spring barley			Winter rape		
	NPK	NK	NP	NPK	NK	NP	NPK	NK	NP	NPK	NK	NP
0	319.6	315.8	306.0	406.7	410.3	386.4	171.2	167.5	156.6	143.3	148.4	144.2
1	444.4	435.8	445.5	497.2	476.8	478.9	289.6	287.0	312.1	198.3	208.7	212.7
2	548.8	550.4	570.0	553.3	539.5	536.7	376.0	375.9	389.8	273.8	281.6	274.8
3	608.8	614.0	606.1	590.4	556.8	562.1	433.8	426.1	432.8	331.7	313.7	309.6
4	651.9	649.2	623.3	591.7	597.0	597.8	470.7	450.1	475.5	365.7	353.6	362.5
5	668.4	656.0	656.6	587.6	581.1	585.7	498.2	480.9	496.1	387.8	364.6	354.7

The uptake of P and K, like yield of grain increased significantly in line with increasing N rate and was practically independent of withdrawing these elements from fertilizer rates. Phosphorus and potassium balance was strongly negative in the treatments without these nutrients and with higher nitrogen rates (Table 3). In the treatments with P fertilization the balance of this element was positive but phosphorus surplus decreased significantly with increasing N rates. In the treatments with K fertilization potassium balance was positive to second level of nitrogen fertilization only.

Table 2. Nutrient uptake in kg ha<sup>-1</sup> depending on N, P, K fertilization

N levels	Winter wheat				Maize for grain				Spring barley				Winter rape			
	P uptake		K uptake		P uptake		K uptake		P uptake		K uptake		P uptake		K uptake	
	NP	NK	NP	NP	NP	NK	NP	NP	NP	NK	NP	NP	NP	NK	NP	NP
	K		K		K		K		K		K		K		K	
0	12.1	12.1	39.3	36.5	22.8	21.9	103	93.8	10.0	9.71	32.9	29.8	16.0	15.8	48.4	46.0
1	16.7	16.1	59.5	55.4	23.5	23.6	113	109	14.5	14.4	49.5	52.7	20.7	21.2	69.2	63.5
2	20.7	20.4	84.3	79.9	28.4	27.8	123	119	17.2	17.5	71.7	67.1	25.7	24.7	96.4	88.6
3	24.3	25.1	94.2	96.1	26.5	29.3	119	124	20.0	19.2	82.2	78.5	30.0	29.3	119	107
4	25.8	24.2	117	104	28.2	28.6	129	129	21.7	19.9	103	92.5	32.2	30.7	146	125
5	25.9	25.7	119	107	28.1	28.3	134	131	22.5	20.8	110	98.7	33.6	33.1	158	137

Table 3. Total uptake of nutrients in four-course rotation and the balance of these elements

N level	total P uptake kg ha <sup>-1</sup>		total K uptake kg ha <sup>-1</sup>		P balance kg/4 years		K balance kg/4 years	
	NPK	NK	NPK	NP	NPK	NK	NPK	NP
0	61.4	59.8	225	209	75.0	-59.8	144	-209
1	75.4	75.7	291	284	61.5	-75.7	80.3	-284
2	92.0	91.3	378	357	45.0	-91.3	-6.54	-357
3	101	101	419	412	36.2	-101	-43.2	-412
4	108	102	496	448	28.6	-102	-119	-448
5	109	108	524	474	27.1	-108	-150	-474

The contents of available phosphorus and potassium in soils decreased in the course of the experiment, though there was no straightforward relation between P and K balances and the differences between the content of nutrients in the soils at the beginning and at the end of experimental period (table 4).

Table 4. The content of available ( Egner – Riehm DL) P and K in the soils\*

N levels	P in 2003		P in 2007		K in 2003		K in 2007	
	mg kg <sup>-1</sup>	kg ha <sup>-1</sup>	mg kg <sup>-1</sup>	kg ha <sup>-1</sup>	mg kg <sup>-1</sup>	kg ha <sup>-1</sup>	mg kg <sup>-1</sup>	kg ha <sup>-1</sup>
0	66.0	297	58.1	292	125	565	110	497
1	67.3	302	51.4	231	128	576	97.9	440
2	54.8	247	41.3	198	104	469	84.0	378
3	55.1	248	41.3	186	105	471	78.7	354
4	52.3	235	43.9	197	99.5	448	83.5	376
5	53.2	239	42.7	192	101	455	81.3	366

\* left column in mg P, K kg<sup>-1</sup> soil, right column in kg P, K ha<sup>-1</sup> in the soil layer 0-30 cm

In four-course rotation crops optimally provided with nitrogen, in the period of 4 years have mined the soil from over 100 kg P and 450 kg K ha<sup>-1</sup>. It corresponds to the decreasing of about 45 kg available P and 100 kg available K ha<sup>-1</sup> in soil plough layer. It means that some amount of phosphorus and substantial amount of potassium had to be taken by crops from less available (fixed) forms of these nutrients. Nitrogen fertilization strongly promotes these processes.

## Conclusions

1. In spite of conducting both experiments on soils showing medium content of P and K there was no yield decrease after 4 years in the P null and K null treatments independently of nitrogen rates.
2. The uptake of P and K by crops increased significantly under influence of increasing nitrogen rates hence the nitrogen seems to be a driving force in plant supply with phosphorus and potassium.
3. With application of recommended rates of P and K the balance of phosphorus shows surpluses decreasing with increasing N rates and the balance of potassium at optimal N fertilization is negative.
4. In the treatments without P and K the balance of both elements shows serious deficiency, which in about 50% in case of P and 25% in case of K can be explained by decreasing content of available forms of these nutrients in the soil plough layer.

# Comparison of Soil Hydrological Properties in Conventional Tillage and No Tillage Systems

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Water saving is becoming progressively an important issue in agriculture and agricultural practices aimed at increasing the water efficiency of the system should be preferred. This work is aimed to evaluate the advantages of a No Tillage system against a Conventional Tillage system from the hydrological point of view to improve water management in Agriculture.

## Methodology

As a part of a larger medium term experiment carried out in Piedmont, Northern Italy, over a Typic Hapludalfs, coarse-loamy, mixed, nonacid, mesic soil, a No Tillage system was compared to a Conventional Tillage system. The following four treatments were part of the experiment:

1. No Tillage system after No Tillage system (NT NT)
2. No Tillage system after Conventional Tillage system (NT CT)
3. Conventional Tillage system after No Tillage system (CT NT)
4. Conventional Tillage system after Conventional Tillage system (CT CT).

The Conventional Tillage system was based on ploughing and two rotary tiller passages.

The hydrological measurements were performed about six months after the conversion from the old management to the new management at the end of the first season of cultivation of a maize crop.

The measurements performed were the following:

1. Soil bulk density
2. Macroporosity at different matric potentials near to saturation
3. Saturated hydraulic conductivity
4. Micromorfometric shape indexes of the aggregates having a diameters between 2 and 5 mm and between 5 and 10 mm.

The micromorfometric shape indexes here analysed were the Aspect, defined as the ratio between the longest and the shortest perpendicular axis of the aggregate, that refers how different the aggregate is from a circle, and the roundness, defined as the ratio  $4 \cdot \pi \cdot \text{area} / \text{perimeter}^2$  that refers how wrinkled the aggregate is.

All variables, except saturated hydraulic conductivity, were measured on 0-15 cm and 15-30 cm layers. Variables were compared in terms of confidential intervals and data were transformed when not normally distributed.

Figure 1: Bulk density and water retention curve of the different treatments on the two layers.

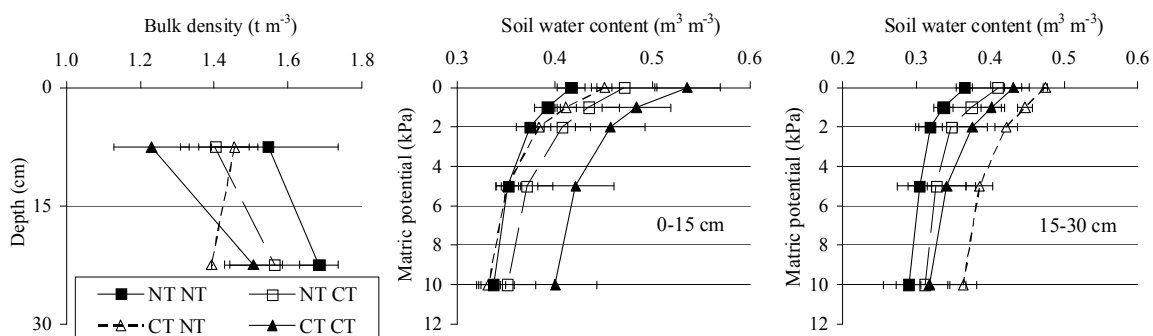
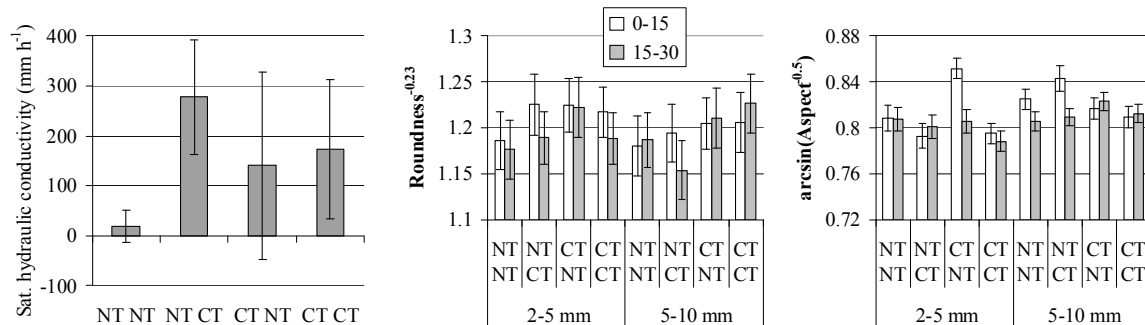


Figure 2: Saturated hydraulic conductivity in the 0-30 cm layer and micromorfometric shape indexes of the aggregates of two different dimensions in the 0-15 cm and 15-30 cm layers.



## Results

The increasing values of soil bulk density measured from CT CT system through NT CT system, to NT NT system, explained the progressive compaction of the soil not tilled since 6, 18 and 42 months. After 6 and 18 months without tillage the bulk density had not yet reached the value measured after 42 months. The CT NT system showed a different behaviour in respect to the other treatments probably because of the disruption of a reached equilibrium in a No Tillage system and the needs of more time to reach a new one.

The water retention curve measured near to the saturation confirmed the results showed by soil bulk density. The differences in porosity between the treatments was large in the very large pores and it decreased progressively considering pores of smaller equivalent diameters confirming previous results (Sacco *et al.*, 2000). NT CT system showed again an intermediate behaviour.

Saturated hydraulic conductivity showed values 9 times lower for NT NT in respect to CT CT treatment according to the greater bulk density. NT CT system showed values not statistically different from CT CT system while CT NT showed a very large variability probably because of the large porosity created in the second layer and showed by the values of soil bulk density.

Finally, micromorfometric shape indexes of the aggregates were significantly influenced by the treatments in the most of the analysed cases. In general soil aggregates in NT NT system were less spherical and more wrinkled than in CT CT system. Their shape indexes reflect the effect of the impact of the machinery on the CT CT system and the process of creation of new aggregates in the NT NT system.

## Conclusions

All the variables here presented are able to statistically differentiate the measured values in the two more differentiated situations that are represented by a No Tillage management against a Conventional Tillage management.

The higher compaction measured in the No Tillage management results in less large porosity and lower saturated hydraulic conductivity if compared to the Conventional Tillage management and then it should be able to maintain more water in the layers explored by the roots.

Finally, when a recent conversion from Conventional Tillage to No Tillage management is considered, a period of 18 months is not enough to reach the new equilibrium in terms of physical properties of the soil.

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# Pig Slurry Applications On Growing Alfalfa Under Mediterranean Conditions: Crop And Environmental Effects

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## Introduction and objectives

Pig slurry is a traditional organic fertilizer that is causing diffuse soil and water contamination problems in some particular areas of the Ebro Valley (Spain) due to the elevated spatial concentration of pig farms. Although the slurry is mainly applied to cereal crops, the application in alfalfa (*Medicago sativa* L.) could be a practical solution since it would broaden the areas and the time to be applied. Although there is some research about this agricultural practice (Ceotto and Spallaci, 2006; Lloveras *et al.*, 2004; Russelle *et al.*, 2001), the possible environmental effects have not been completely addressed and are of special interest in the vulnerable areas declared by the different the European Community countries. The objectives of this work were: (1) to evaluate the effect of two doses of pig slurry during the vegetative growth period on alfalfa yield and (2) to assess the effect of these applications on nitrate and dissolved phosphorous concentration in drainage water under irrigated conditions.

## Methodology

The experiment was carried out at the CITA experimental farm (Zaragoza, Spain) during 2007 in 12 drainage lysimeters of 5.2 m<sup>2</sup> x 1.2 m depth, filled up with a silt loam soil, that had been sown the previous year with alfalfa (cv. Aragón). The treatments established were a control (TC) without slurry application, a low-dose of slurry (LD=177 kg ha<sup>-1</sup> of N) and a high-dose of slurry (HD=354 kg ha<sup>-1</sup> of N). The experimental design was a randomized block design with four replicates. The TC received a winter application of 0-200-200 kg ha<sup>-1</sup> of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O mineral fertilizer. The slurry applications were split, half and half, following the first (April, 16<sup>th</sup>) and the third (June, 25<sup>th</sup>) cut. Irrigation was performed following the Penman-Monteith weekly evapotranspiration requirements and considering a leaching fraction of 20%. A total of 1,286 L m<sup>-2</sup> of irrigation was applied during the growing season. Drainage water was collected weekly and nitrate and dissolved phosphorous analyzed by colorimetry. The alfalfa was harvested 7 times during the growing period and the total aboveground dry matter measured after oven-dry at 65°C.

## Results

The effect of pig slurry applications on alfalfa yield was relatively unimportant since only in two out of the seven cuts (cuts number 2 and 5) the differences among the three treatments were significant (P<0.05). Over all the season, the cumulative yield of alfalfa in the LD treatment was slightly (around 6%), but significantly (P<0.05) higher than the control. Using contrast of significance, the pig slurry treatments yielded significantly more (23.4 Mg dry hay ha<sup>-1</sup> year<sup>-1</sup>) than the control (22.3 Mg dry hay ha<sup>-1</sup> year<sup>-1</sup>) similarly to the results obtained by Ceotto and Spallaci (2006) suggesting an apparent benefit of this practice that could be attributed to the lower energy cost of N reduction compared to symbiotic N fixation in the slurry-fertilized alfalfa.

Nitrate concentrations in the drainage water were very low (Figure 1a) during all the growing season in all treatments evaluated, generally below 2 mg L<sup>-1</sup> of NO<sub>3</sub>-N and very far from the 11.4 mg L<sup>-1</sup> limit of the E.C. policy for drinking water.

Using paired comparison between the two slurry treatments and the control (HD vs TC and LD vs TC) along the season, no significant differences were observed ( $P>0.05$ ) in the nitrate concentration of drainage water. When considering accumulated  $\text{NO}_3\text{-N}$  leached over the growing season no differences were found again among the treatments. Only 1.33, 2.95 and 1.05  $\text{kg NO}_3\text{-N ha}^{-1}$  were lixiviated for the TC, HD and LD treatments, respectively. These results strongly suggest a high ability of alfalfa to remove the nitrogen applied with the slurry.

Dissolved phosphorous concentrations in the drainage water (Figure 1b) were in general very low and within the European standards of drinkability (DOCE 75/440). No significant differences ( $P>0.05$ ) were observed among the treatments evaluated, and the risk of eutrophication of natural waters according the USEPA criteria would be low (lower than  $0.04 \text{ mg L}^{-1}$  of P). The averaged concentration of total P (data not shown) in the HD treatment was  $0.039 \text{ mg L}^{-1}$  which is also below the critical value above cited.

## Conclusions

The results, although preliminary, indicate the feasibility of pig slurry applications to alfalfa during the growing period under Mediterranean irrigated conditions, without detrimental effects on the quality of drainage water. A rational use of pig slurry in

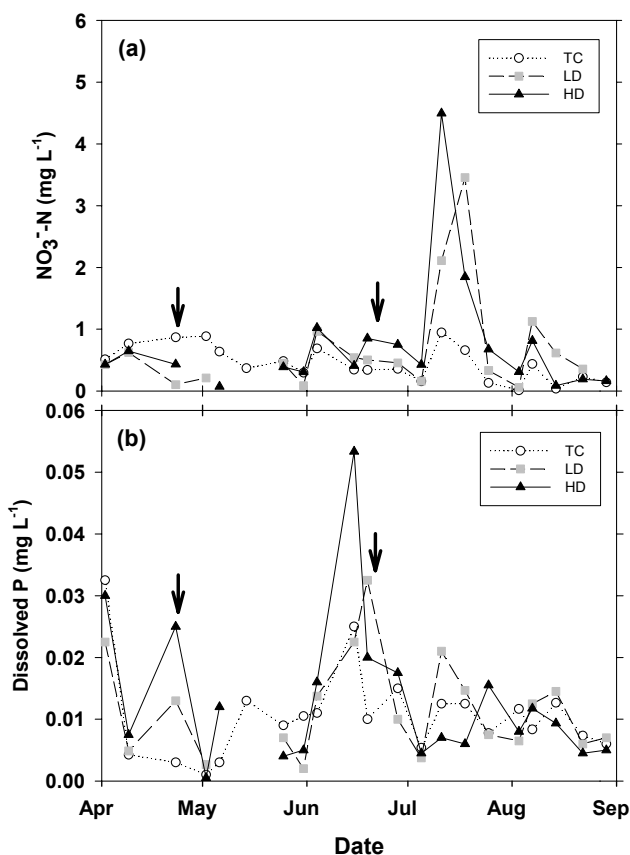
alfalfa, avoiding build-up of heavy metals and phosphorous in the soil, can slightly increase yield and contribute to a better management of pig slurry in agricultural areas avoiding excessive applications in cereal crops and broadens time and area for pig slurry applications.

## Acknowledgments

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**Figure 1.** Effect of the applied treatments to the  $\text{NO}_3\text{-N}$  and dissolved P ( $\text{mg L}^{-1}$ ) in drainage water during the growing season. The arrows indicate the date of slurry applications.

# Iron and proteins mobilization in *Vitis Vinifera* cultivars variously tolerant to iron chlorosis

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## Introduction

In dependence of their genotype tolerant rootstocks do not evidence iron deficiency symptoms of inter-vein chlorosis even in highly calcareous soils (Tagliavini et al., 1995). It can be presumed that such plants have an enzymatic redox equipment depending on the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio (Nenova and Stoyanov, 1995), such that makes the microelement in its active form available to the plant. Tolerant varieties can mobilize iron by determining a lower pH in the soil at root level, thanks to their ability to emit  $\text{H}^+$  and/or organic acids; in the latter case, iron is absorbed and transferred as a complex (Gessa et al., 1997). The present research was conducted with the aim of assessing the various rates of iron absorption by sensitive cultivars when grafted on a tolerant rootstock in an iron-deficient soil.

## Methodology

Two iron deficiency-sensitive table grape cultivars, "Italia" and "Vittoria" were grafted on a tolerant rootstock, "140 Ruggeri" (Berlandieri x Rupestris). Six plants per cultivar were labelled on healthy rootstocks (2.27 to 3.84 mg total chlorophyll  $\text{g}^{-1}$  fresh matter, fm: chlorosis absent) and two levels of chlorosis were identified at the grafting level, namely incipient (1.47 to 1.82 total chlorophyll  $\text{g}^{-1}$  fm) and evident (0.77 to 1.30 total chlorophyll  $\text{g}^{-1}$  fm). Plant development was followed from May to August, prior to fruit ripening time, with four monthly leaf samplings from apical shoots.

On the each leaf sample the extraction of the variously available iron forms: extra-cellular and soluble iron in the cytoplasm (A), mobile chloroplastic iron (B) and loosely linked chloroplastic iron (C) was done on fresh material, following Machold (1968). Strongly linked iron (D) was determined in the residue of the centrifugation. Total iron was analysed on plant dry matter (dm). All the iron forms were determined by atomic absorption spectroscopy analysis. Chlorophyll (Chl.) was determined according to Arnon procedure (1949); total nitrogen (N%) with the Kjeldhal method; ferritine (Ft.) with the method Seckbach (1968); protein content (Pro.) according to Bradford procedure (1976). Analytical data averaged from May to August, over 12 replications were statistically elaborated and one-way variance was found at 0.05 probability.

## Results

The values of N were linked to the level of chlorosis, with values growing according to chlorosis intensity. Proteins and proteic efficiency (%N proteic/total N), evidenced in healthy plants levels higher than those in chlorotic plants; with reductions proportional to chlorosis intensity (table 1). Ferritine values were lower in the leaves sprouting from the rootstocks, showing no chlorosis symptoms. In the two iron-inefficient cultivars ferritine values were progressively growing, showing the highest levels in the most chlorotic plants.

Ferritine percentage over the total protein content (% Ft/ total protein) resulted with higher values in the chlorotic plants respect to control (table 2).

Iron content, both total and active, resulted lower in chlorotic plants (table 2). The percentages of iron, both extra-cellular and cytoplasmic soluble and chloroplastic mobile, were higher in healthy plants; in chlorotic plants the highest iron percentages (referred to total iron content) were recorded in the strongly linked fraction (table 2).

The active iron percentage referred to the total iron content was increasing in healthy plants, while in mildly chlorotic plants and in those evidently chlorotic was progressively decreasing (table 2).

## Conclusions

In evidently chlorotic leaves a reduced proteic efficiency was detected along with an accumulation of nitrogen, depending on a more pronounced N translocation in its nitric form (Tang et al., 1991) and on a diminished nitrogenase and nitrato-reductase activity (Nenova and Stoyanov, 1995). Which demonstrates a reduced plant ability in the synthesis process, probably in dependence of a changed photosynthetic response; evidenced by the lower content in total chlorophyll in stressed plants as an indirect response to iron deficiency. Although total iron content resulted higher in non iron-deficient plants, the real impact of iron deficiency on plant dysfunction is given by the active form of this element, radically diminishing in those leaves showing inter-vein chlorosis, proportionally to deficiency intensity and plant sensitivity. The fact that in chlorotic plants iron is present but not available is confirmed by the high, progressively growing levels of ferritine, since in this case iron is strongly bound to the protein as an insoluble phosphate, related to the residual iron pool, indicating that the genome of the two cultivars is equipped to synthesize this protein (Grossman et al., 1992; Lobreaux et al., 1992). As a consequence, although the rootstock can be able to mobilize and absorb iron, thus meeting plant requirements, the scion is unable to use the element since it cannot reach a balance between its active and unavailable forms.

**Table 1** dm (g), N %, total iron and protein (mg g<sup>-1</sup> dm), proteic efficiency (PE), chlorophyll (mg g<sup>-1</sup> fm) as affected by chlorosis intensity and cultivars

chlorosis	dm		N %		protein		PE %		Chlorophyll		iron	
	I	V	I	V	I	V	I	V	I	V	I	V
<b>absent</b>	29.70 a	28.90 a	2.28 b	2.08 b	29.80 a	28.60 a	21.00 a	17.62 a	2.42 a	2.03 a	1.37 a	1.33 a
<b>incipient</b>	25.50 b	24.20 b	2.84 b	2.80 b	17.70 b	17.50 b	9.98 b	10.00 b	1.42 b	1.40 b	1.09 b	1.00 b
<b>evident</b>	21.00 c	20.90 c	4.76 a	4.58 a	14.20 c	13.00 c	4.90 c	4.60 c	0.92 c	0.83 c	1.12 b	1.26 a

**Table 2** Ferritine (mg g<sup>-1</sup> dm), % Ft/total protein and variation in various iron fractions (% on total) as affected by chlorosis intensity and cultivars

chlorosis	cultivar	Ferritine	% Ft/total proteins	iron				
				active	A	B	C	D
<b>Absent</b>	<i>rootstock</i>	0.90 d	2.98 e	89.35 a	40.00 a	40.43 a	8.13 d	11.48 e
<b>Incipient</b>	<b>I</b>	3.39 b	19.85 c	69.15 c	27.65 b	23.98 c	18.80 c	29.63 c
	<b>V</b>	2.22 c	14.13 d	76.97 b	26.60 b	32.63 b	19.07 c	21.73 d
<b>Evident</b>	<b>I</b>	4.66 a	36.28 a	58.03 d	15.95 c	12.63 d	31.13 a	40.38 b
	<b>V</b>	3.51 b	28.53 b	46.83 e	8.90 d	10.83 d	23.73 b	56.50 a

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# Determination of Critical Nitrogen Curve and Nitrogen Nutrition Index of Maize Grown in Southern Italy Under Different Irrigation Regimes

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Plant diagnostic methods of nitrogen (N) deficiency should be based on the definition of a critical N concentration ( $N_{ct}$ ), that is the minimum N concentration required to achieve maximum growth (Lemaire *et al.* 2008). The nitrogen nutrition index (NNI), that is measured using the ratio between the actual N concentration and  $N_{ct}$ , reportedly indicates if crop is above or below optimal nitrogen nutrition ( $NNI \geq 1$  or  $< 1$ , respectively)(Lemaire *et al.*, 1989). Water deficit can often induce a reduction of the nitrogen nutrition index. The main aim of the study was to determine  $N_{ct}$  of maize (*Zea mays* L.) grown under different irrigation regimes in Mediterranean region.

## Methodology

A field experiment was conducted at the experimental Farm of the University of Napoli in Southern Italy (40° 37' N; 14° 58' E) in 2005 to determine the  $N_{ct}$  of maize (*Zea mays* L.). Four N fertilization rates (0, 120, 180 and 240 kg ha<sup>-1</sup>) and three irrigation treatments were factorially combined. The irrigation treatments were: i) a full-irrigation treatment, with restitution of 100% ET<sub>c</sub> (FI); ii) a deficit irrigation treatment, with restitution of 50% ET<sub>c</sub>, in which the root zone was alternatively exposed to dry and wet cycles, that is alternate row irrigation (ARI<sub>50</sub>); iii) a not irrigation treatment (NI). The  $N_{ct}$  was determined by selecting data points for which the highest total above-ground biomass was obtained by the relationship between N concentration (N) and total above-ground biomass (W), both determined by sampling plants at 54 (rapid growth), 65 (beginning of male flowering) and 95 DAS (grain filling).

## Results

Grain yield of NI and ARI<sub>50</sub> treatments was 37 and 101% of that of FI plants, respectively, and, as for N fertilization, the greater yield was reached on the whole with application of 180 kg N ha<sup>-1</sup> (data not shown).  $N_{ct}$  varied from 0.9 to 2.8% when W ranged between 1.15 and 6.23 Mg ha<sup>-1</sup> (Fig. 1) and was unaffected by both irrigation and N fertilization treatments.

The NNI varied from 0.48 to 1.59. There was a significant interaction Irrigation x N fertilization for instantaneous NNI at 54 and 65 DAS (Fig. 2). In particular, at 54 DAS, the NNI did not vary significantly with N fertilization treatments under full irrigated conditions (NNI of 1.04, mean over N fertilization rates) whereas it increased significantly already with 120 (ARI<sub>50</sub>) or 240 kg N ha<sup>-1</sup> (NI)

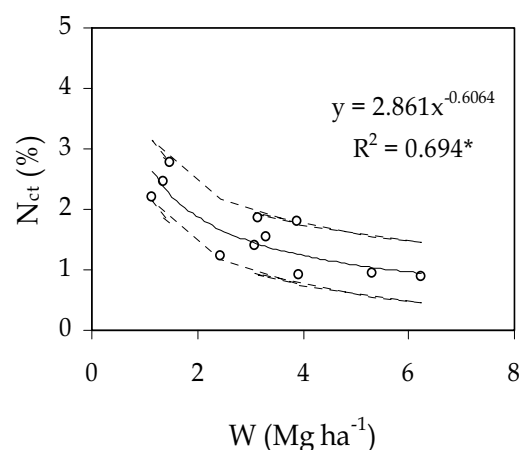


Figure 1. Critical N concentration ( $N_{ct}$ ) vs. total above-ground biomass (W), and critical N dilution curve. Calculation method of  $N_{ct}$  is reported in Methodology. \* significant at  $P < 0.05$ .

(Fig. 2 a). At 65 DAS the NNI increased significantly already with 120 kg N ha<sup>-1</sup> in FI and ARI<sub>50</sub> but not in NI plants and, surprisingly, it was significantly greater under NI than in FI and ARI<sub>50</sub> treatments

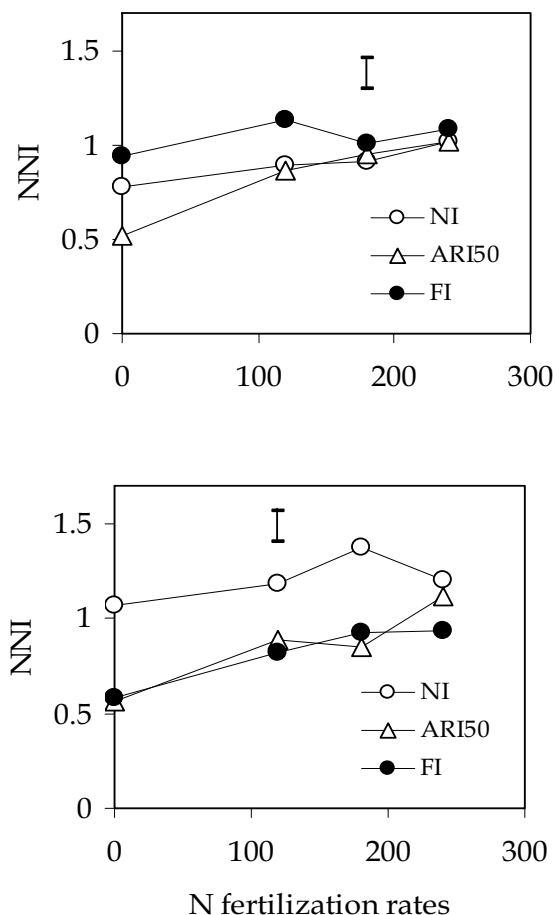


Figure 2. Irrigation x N fertilization interaction for nitrogen nutrition index (NNI) at 54 (a) and 65 (b) days after sowing (DAS). Bars indicate least significant difference at  $P \leq 0.05$ . Legend: NI, not irrigated; ARI<sub>50</sub>, alternate row irrigation (50% ET<sub>c</sub>); FI, full irrigation (100% ET<sub>c</sub>). N fertilization rates: 0, 120, 180 e 240 kg N ha<sup>-1</sup>.

Mediterranean region.

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## Aknowledgements

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with application of 0, 120 and 180 kg N ha<sup>-1</sup> (Fig. 2 b). Finally, at 95 DAS the NNI of ARI<sub>50</sub> was 1.07 vs. 0.86 and 0.99 of NI and FI, respectively ( $P \leq 0.01$ ) and, as for N fertilization, it increased significantly up to 180 kg N ha<sup>-1</sup> without any further increase with application of 240 kg N ha<sup>-1</sup> (data not shown).

Our results showed that FI plants were under not limiting N nutrition at the beginning of growth (54 DAS) but under sub-optimal condition (NNI < 1) in the mid season (65 DAS) with 0 or 120 kg N ha<sup>-1</sup> applied. As for ARI<sub>50</sub> plants, not limiting N nutrition conditions were reached later (at 95 DAS, during the grain filling period; NNI of 1.07, mean over N fertilization rates) which could explain why it did not produce less than FI.

## Conclusions

In the present study, each irrigation treatment showed a temporary N deficiency, since NNI was less than 1 at least in two out of three stages of growth under all irrigation treatments. As for N fertilization, the N status of the crop was close to optimal (NNI ≥ 1) generally with application of 180, 240 kg N ha<sup>-1</sup> or less depending on soil water availability.

Because of several variables involved and their interaction with the environment, we conclude that these results should be confirmed by additional work before considering the NNI as a reliable indicator of the level of N stress during the growing season of maize in

# Rule-based Handling of Hazardous Nitrogen

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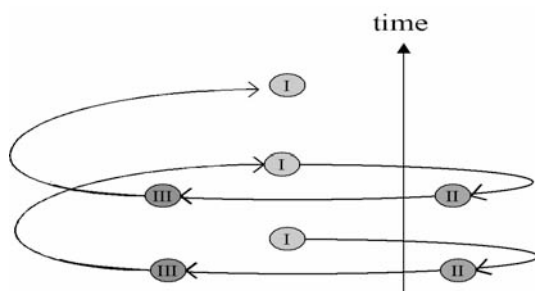


Fig. 1 - The recursive framework (details in the text).

grow over time through reiterations (Fig. 1), allowing for a continuous adaptation of agricultural productions systems as the business environment and society change. The same procedure was applied in this study to assess the behaviour of an array of CS run in the proximity of Lake Massaciuccoli - an area of Central Italy currently defined as “vulnerable area” under EU Directive 676/91 - as part of an action aimed at identifying possible responsibilities of farmers in  $\text{NO}_3$  contamination of waters (research developed in 2005-2006 under the Italian Ministry of Education, University and Research).

A rule-based, recursive framework is an ideal approach to support the design of cropping systems (CS). A framework of this type was proposed, arranged into three stages (Silvestri and Bellocchi, 2007): (phase I) prior evaluation (technical, problem-solving, farmer-driven stage), (phase II) posterior evaluation (institutional, environmental monitoring implemented when CS response deviates from expected behaviour), and (phase III) managing the change (participatory, dynamic rearrangement of CS). This sequence is meant to evolve and

## Methodology

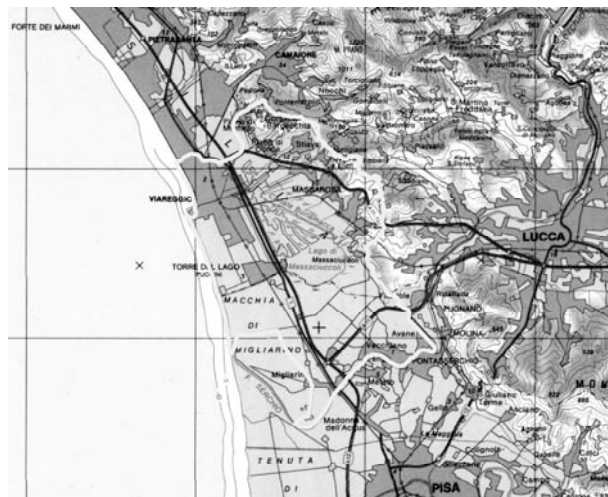


Fig. 2 - Map of the study area.

**Study area.** Lake Massaciuccoli is a shallow coastal lagoon, located within north-western Tuscany (Italy). It extends over  $\approx 690$  ha, with a storage capacity of  $\approx 9$  millions  $\text{m}^3$  and varying in depth from 1.5 to 2.0 m. Water input to the lake is limited to small rivers draining the hilly region to the east and neighbouring areas (from marshes restored long ago). The vulnerable area (Fig. 2) surrounding the lake covers over 14000 ha ( $\approx 50\%$  of which being cultivated).

**Phase I.** At each site, information was collected from the farmers by using common interview schedule and observational checklist. The aim was to characterize the technical choices of the farms, and to assess the structure and functions of CS. Farmers' behaviour was assessed on a 4-point scale: -2 (very negative), -1 (negative), 0

(standard), +1 (positive), +2 (very positive). Such assessment was based on: mechanization choices, management of irrigation, fertilisation options, composition and sequence of crops. A synthetic index (NSI: Nitrate Sustainability Index) was calculated at each farm to assess the potential hazard of nitrate contamination due to  $\text{NO}_3$  escaping from the fields.

**Phase II.** An environmental monitoring was carried out. Surface water bodies were monitored twice (May and November) for a range of 70 GPS-georeferenced sampling points within the basin. Nitrate level and an array of quality parameters were determined (Silvestri and Gorreri, 2006).

*Phase III.* Of the farms included in the project, a core sample (>50%) was selected where more detailed information was available. A focus group was created to debate potential changes to the CS management. Patterns and attitudes of sharing and acceptance of solutions were evaluated.

## Results

*Phase I.* In total, 45 farms were assessed covering over 1508 ha of suitable arable area (SAU), corresponding to more than 20% of the vulnerable area covered by farms. The farms extend over flat domains and 62% of businesses are family-runs. Average farm SAU is about 30 ha. The most commonly cultivated crops are cereals (56%), followed by industrial crops (14%), horticultural crops (6%) and woody crops (6%). Maize, with 37% of the cultivated area, is the most widely grown crop. Irrigation is performed over 40% of the SAU. NSI = -0.13 (slightly negative) was computed as weighed average across values from single farms. However, farms were registered with both NSI values largely lower (down to -0.9) and much higher (up to 1.25). NSI values >1 only affect less than 5% of the SAU. Favourable conditions ( $0 < \text{NSI} < 1$ ) were registered on about 20% of the SAU. For the most part (74% of the SAU), the fields are characterized by  $-1 < \text{NSI} < 0$  and only on 1% of the SAU was  $\text{NSI} < -1$ .

*Phase II.* Results from the environmental monitoring confirmed that contamination of surface waters by nitrates does exist. The phenomenon is less marked in the northern sub-area (less densely and intensively cultivated), where 3% of positive samples ( $\text{NO}_3$  concentration  $>1 \text{ mg L}^{-1}$ ) were detected (against 68% in the southern sub-area). Timing of sampling is also important:  $16 \text{ mg L}^{-1}$  on November versus  $5 \text{ mg L}^{-1}$  on May, as averages of all samples collected.

*Phase III.* For possible measures to be taken in order to limit agricultural contribution to pollution, farmers expressed their preferential choice (67%) for reduction of fertilisation rates. Splitting of fertiliser applications is also largely shared (54%). The idea of using slow release fertilisers and reducing irrigation usage are less appealing options. Only 1/3 of the farmers gave favourable opinion about a change in the crop patterns (e.g. reduction of the area allocated to maize, introduction of a catch-crop). This generally negative opinion keeps unchanged even in the expectation of compensating financial aids.

## Conclusions

Farming practices registered cover a range of diverse behaviours. The latter are in some cases potentially harmful for the water-environment of a vulnerable area. The environmental monitoring confirmed indeed the presence of non-negligible  $\text{NO}_3$  levels in surface water bodies, although not generalized over time and space. The management of fertilisation seems not to depart from rational decision-making; this indicates that alternative farming strategies should be based on a modification of crop patterns. Other actions (e.g. introduction of buffer strips) are even less accepted by the farmers.

Data (not shown here) from the agronomic stations placed in the basin, also provided evidence on the peaty nature of some soils and their importance in the release of nitrates from soil organic matter as consequence of natural mineralization. It would guess a critical stance towards the CS proposed and the start of a new evaluation cycle (Fig. 1) for a more advanced definition of sustainable and agreed agricultural strategies.

It is worth mentioning here that the Region Tuscany action program for nitrate vulnerable zones (Decree of the President of Regional Council n. 32/R of the 13/072006) entered into force on March 1, 2007. The plan set out obligations for the farmers to draw up fertilisation plans based on application of detailed N budgets, but it does not directly account for specific edaphic conditions related to the zone of interest. This research goes further to provide a comprehensive assessment about the condition of agricultural soils and farmer preferences in the Massaciuccoli area.

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# Changes in Soil Organic Matter Under Different Tillage Systems and Crop Rotations in Three Semiarid Areas of Castilla and Leon

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## Introduction

Soil organic matter (SOM) dynamics play a decisive role in soil quality, improving crop production and protecting environment (Bauer and Black, 1994), because it contributes to plant growth and development through its effect on the physical, chemical and biological properties of soils (Tabatabai, 1996). Conservation tillage improves soil quality. Minimum or no tillage enhances soil structure, increasing organic matter content, present in top layer of the soil (Kay, 1995), but tillage effects on soil organic matter dynamics are complex and often variable. The degree of these effects depends on soil type, crops, and tillage management systems. Crop rotations and the quality and quantity of crop residues on the soil surface can affect the increase of organic matter content (Wright and Hons, 2005). The objectives of this study are to evaluate the response of soil organic matter to different tillage systems and crop rotations in three semiarid areas of Castilla and Leon, Northern Spain.

## Methods

The experiments of study were carried out in a cereal producing areas of Viñalta, (Palencia) , from 1994 to 2001, Torrepadierne, (Burgos) from 1995 to 2001 and Zamadueñas (Valladolid) from 1997 to 2001. In Viñalta, the soil formation is classified as Typic Calcixerolls with 1,1% SOM and average annual precipitation is 400 mm. In Torrepadierne, the soil formation is classified as Typic Calcixerolls with 1,8% SOM and average annual rainfall is 450 mm. In Zamadueñas, the soil formation is classified as Typic Xerofluvents with 0,9% SOM and average annual rainfall is 400 mm,

The experimental design was a split plot with three tillage systems (conventional tillage, CT; minimum tillage, MT; no tillage, NT), as main factor and crop rotations (cereal/cereal, C/C; cereal/fallow, C/F; and cereal/legume, C/L) as sub-factor in two first locations. In Zamadueñas, the experimental design was split plot with four treatments of tillage systems (conventional tillage, CT; minimum tillage, MT; no tillage, NTd and NTTr, disk or tine furrow opener), as main factor and residue management packed and picked as sub-factor. The crop was winter barley.

The soil properties were determined at the beginning of the experiments. The soil samplings were taken from 0 to 15 and 15 to 30 cm depths in the three locations every two years to determine SOM content by Walkley and Black (1934) method. The data were analyzed as a split-plot, using the general lineal model (GLM) procedure.

## Results

The increases of SOM were very slow and variable, probably due to the short-time period of this study. Figure 1 shows soil organic matter content in different tillage systems and crop rotations after the sixth and fifth years from the beginning of the experiment in Palencia and Burgos respectively. In both locations, SOM contents were significantly different among tillage systems in the top fifteen cm soil layer. SOM content in minimum tillage was different and higher than this of the conventional and no tillage systems. Whereas in Palencia, SOM content in no tillage was similar than this of the conventional, in Burgos, this parameter was higher than this of the conventional tillage. Taking into account fifteen to thirty cm. soil layer, SOM contents were similar on the three tillage systems in both locations, however, in Burgos, the tendency of this parameter was the same than this in the first fifteen cm, where minimum tillage and no tillage showed higher SOM content.

Considering crop rotations (over all the tillage systems data), SOM content was significantly higher in cereal/legume followed by cereal/cereal and cereal/fallow respectively in the first fifteen cm of soil in both locations (Figure 1). The introduction of a legume or cereal crop in the rotation with winter cereal

had positive effects on SOM content especially for legume. Respect to fifteen to thirty cm. soil layer, we found the same tendency for SOM content where in cereal/legume this parameter was always higher.

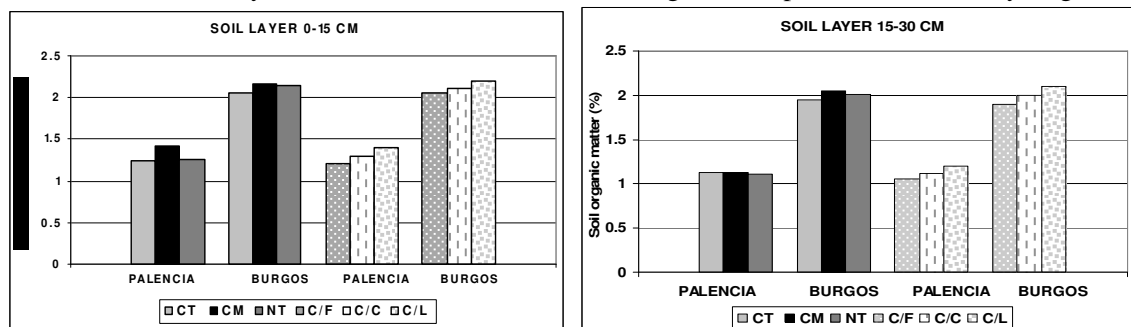


Figure 1.- Soil organic matter content in different tillage systems and crop rotations in two locations. CT: conventional tillage; MT: minimum tillage and NT: non tillage. C\_C: Cereal rotation; C\_F: Cereal/Fallow; C\_L: Cereal/Legume and L\_C: Legume/Cereal.

Figure 2 shows SOM contents in different tillage systems treatments and residues managements in the fourth years from the beginning of the experiment. SOM contents were significantly different between treatments of tillage systems. SOM contents in minimum tillage and in the non tillage treatments were different and higher than this of the conventional tillage in both depths. Management of residues, packed and picked had not effect on SOM content. Franzluebbbers and Arshad (1996) and Sombrero et al. (2006) reported that there may be little to increase in SOM content in the first years after changing conservation tillage, but a large increase in this parameter occurred in the next 5 to 10 years.

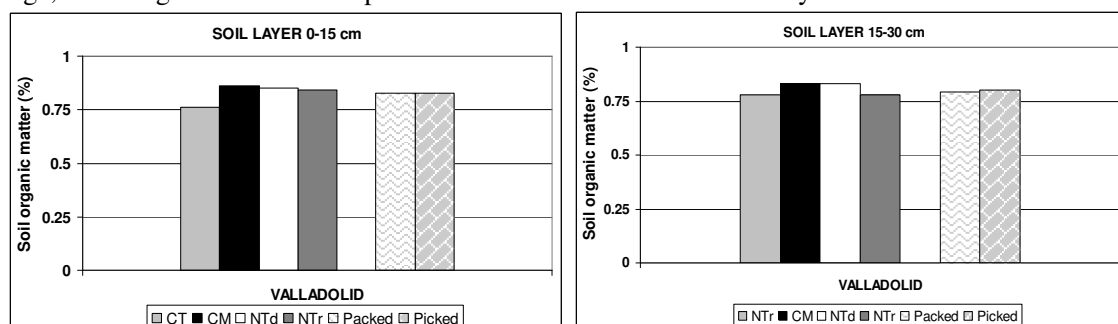


Figure 1. - Soil organic matter content in different tillage treatments and residue management. CT: conventional tillage; MT: minimum tillage and NTd and NTr: Non tillage disk or tine furrow opener. Residue packed and picked.

## Conclusions

Conservation tillage systems (minimum tillage and non tillage) and the introduction of legume or cereal in the rotation with winter cereal have had a positive influence on SOM content. Residues management had not effect on this parameter on this limited study. Thus, further research is needed to have better conclusions.

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# Economic Analysis of Crop Rotations in three Tillage systems in Semiarid Central-Northern Spain

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## Introduction

Rainfed crop production is low and instable in Central and Northern Spain because the precipitation is limited and irregularly distributed and the evaporation is high. The soils typically have low organic matter content and fragile structure. Traditionally, farmers use intensive tillage for seedbed preparation and weed control, but these practices have led to a significant decline in soil quality (Hernanz *et al.*, 2002). In the last two decades, they are adopting different tillage systems as minimum and no tillage, called conservation tillage, in order to minimise production costs and to improve soil quality. Economic benefit and the need of simplifying labour are the most important factors taken into account to adopt conservation tillage. In fact, these systems reduce the number of field operations and as consequence they save energy and farm equipment. Other advantageous environmental aspects of conservation tillage described in scientific publications are soil water conservation, soil protection, soil organic carbon and soil biological activity increase.

The objective of the present study was to analyse economically the effect of three tillage systems and crop rotation for cereal, fallow and legume production in semiarid central-Northern Spain. The studied variable was annual gross margin (total income less variable costs).

## Methods

The experiments were located in cereal producing areas Torrepadriene (Burgos), from 1994 to 2004, Alcalá de Henares (Madrid) and Olite (Navarra), from 1994 to 2006, and average annual rainfalls were 450 mm., 380 mm., and 433 mm respectively. In Torrepadriene, the soil is classified as Typic Calcixerolls. In Alcalá de Henares and in Olite the soils are classified as Calcic Haploxerepts.

The experimental design was a split plot with tillage systems (conventional tillage, CT; minimum tillage, MT; and no-tillage, NT) and crop rotations (cereal/cereal, C/C; cereal/fallow, C/F; and cereal/legume, C/L). For seedbed preparation: CT consisted of fall mouldboard ploughing to a depth of 30 cm, followed by two passes of a spring tine cultivator (10–15 cm depth), MT consisted of chisel ploughing to a depth of 15 cm and NT system, weeds were sprayed with 1l/ha glyphosate. Management crops (sowing, fertilisers and treatments) were applied at rates used in every area where experiments were located. Cereal and pea grain yields were determined from two 25m long rows mechanically harvested at maturation. Vetch crop was harvested when it was flowering. Forage was estimated by hand delimiting areas in each plot whose samples were 4 m<sup>2</sup> and weighing the above-ground material. The results for the three tillage systems and three rotations were subjected to an economic analysis on an annual basis over the 1995–2004 periods. Nine and seven years have been considered to calculate yield means of tillage systems and of crop rotations respectively in all locations. The data were analyzed as a split-plot, using the general lineal model (GLM) procedure (SAS Institute, 1990).

## Results

Crop cereal yield means showed statistical differences between locations (Figure 1). In Madrid and Burgos, crop production was mainly affected by growing season precipitation. When precipitation was below or above usual one and irregularly distributed, crop yields were very low or considerably higher than normal yields respectively (Data not showed). However, crop production was not so dependent on precipitation in Navarra where production was higher and more regular than in the other locations.

In general, in all locations, crop cereal yield was not significantly different between tillage systems nevertheless, only in Madrid, crop yield average was different and lower in monoculture for minimum tillage. The introduction of a fallow or legume crop in the rotation with winter cereal had positive effects

on cereal yield for all the tillage systems but especially better for conservation systems. This effect depended on locations: weeds infestation in Madrid and Burgos limited yield in cereal monoculture in conservation tillage mainly for minimum tillage (Sombbrero *et al.*, 2004). Meanwhile in Navarra, fallow did not present significant difference in no tillage because as precipitation was abundant during the trials, water was not a limiting factor. The response of crop legume yield varied with location. Legume yield was similar in all tillage systems in both locations Madrid and Navarra, whereas in Burgos yield showed statistical differences between tillage systems. Conventional tillage had higher yields than non tillage and minimum tillage respectively.

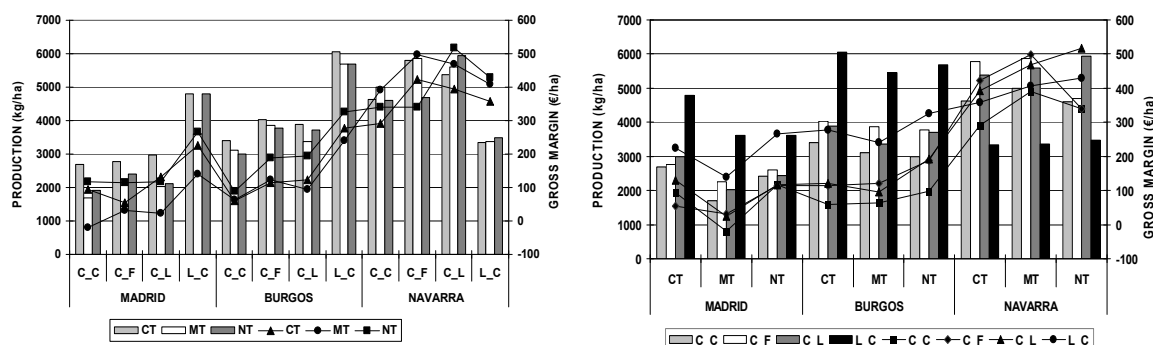


Figure 1.- Cereal and legume production and gross margin means in different tillage systems and crop rotations in three locations. CT: conventional tillage; MT: minimum tillage and NT: non tillage. C\_C: Cereal rotation; C\_F: Cereal/Fallow; C\_L: Cereal/Legume and L\_C: Legume/Cereal. Columns and lines show production and gross margin respectively.

Figure 1 shows the nine year mean gross margins of each crop and tillage system in three locations. Gross margins behaviour was different for cereal and legume crops among tillage systems, crop rotations and locations. In Madrid, NT provided higher gross margin than CT and MT respectively in monoculture cereal and in cereal/fallow rotation. Gross margin was 24 and 60 euros higher in NT than in CT for the two rotations respectively. However, gross margin was 14 euros lower in NT than CT in cereal/legume rotation. Legume gross margin was 40 euros higher in NT than CT, but 86 euros lower in MT. In Burgos, NT had the highest gross margin when compared with CT and MT in the three rotations. Gross margin was 39 and 35 euros in cereal monoculture, 74 and 65 euros in cereal/fallow and 71 and 97 euros in cereal/legume in NT than in CT and MT respectively. Gross margin was similar in CT and MT in monoculture and cereal/fallow, however, gross margin was lower in MT than CT in cereal/legume. Legume gross margin was 48 euros higher in NT than CT, but 38 euros lower in MT. In Navarra, MT had highest gross margin in the three crop rotations. Gross margin was 99, 76 and 77 euros higher in MT than CT in monoculture cereal, cereal/fallow and cereal/legume respectively. Gross margin in NT was 48 and 125 euros higher than CT in cereal monoculture and cereal/legume; however, it was 83 euros lower in NT compared to MT. Legume gross margin was 72 and 22 euros higher in NT than CT and MT respectively; it was 50 euros higher in MT than CT.

## Conclusions

Conservation tillage had a positive effect on gross margins across rotations depending on locations. Gross margins tended to be higher for NT than CT and MT for monoculture cereal and cereal/fallow in Madrid and Burgos. However, gross margins were higher for MT than NT and CT in the three crop rotations studied in Navarra. In this location, the cereal/fallow rotation was less profitable in NT than in the other tillage systems.

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# Yielding of *Festulolium Braunii*–*Trifolium Pratense* Mixtures Depending on the Nitrogen Fertilization

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Forage grasses in mixtures with red clover are one of the most important fodder source in cattle breeding. The mixtures are characterized higher yields and better quality of dry matter than grasses and leguminous plants in pure stand (Jolliffe, Wanjan 1999, Zannone et al. 1986). Intensification of agricultural production is related to the development of new species and varieties. *Festulolium* hybrid is combining such characters of ryegrass as productivity, growth potential and feeding quality, and from fescue stress resistance in wintering and resistance to drought during the growth period (Kaltofen et al. 1990, Thomas, Humphreys 1990).

The objective of this research was to evaluation the yielding of *Festulolium braunii* cultivated in mixtures with red clover depending on the nitrogen fertilization.

## Methodology

Field experiment was carried out in the years 2005–2007 in Poland at the Institute of Soil Science and Plant Cultivation–Agricultural Experimental Station Grabow (Mazowieckie Voivodeship). Stands on grey-brown podsolc soil, on very good rye complex (pH 7.04,  $P_2O_5$ –148,  $K_2O$ –114,  $Mg$ –40  $mg\cdot kg^{-1}$ ) were established. The percentage of red clover (v. Nike) sown in mixture with *Festulolium* (v. Sulino) was 80%. Trials were sown in the 14<sup>th</sup> of April 2005. The plots were fertilized as follows: P-22, K-66  $kg\cdot ha^{-1}$ . The factor was level of nitrogen fertilization 0, 60<sub>(20+20+20)</sub>, 120<sub>(40+40+40)</sub> and 180<sub>(60+60+60)</sub>  $kg\cdot ha^{-1}$ . Share of cuts of green mass was depended on the weather course during plant vegetation mainly. Swards were cut two times in sowing year, three times in the first year of utilization and four times in the second year of utilization. Dry matter and nitrogen content in tissues (modified Kjeldahl) were measured.

## Results

The factor taken into consideration in the research was not the only one that effected on growth, development and the yields of mixtures. Weather conditions during growing season were additional important factor. The biggest yields of dry matter were obtained in 2007 year which was characterized by the most favourable distribution of precipitation. The sum of precipitation was higher by 30% than many-year average. The yields of dry matter lower by about 38% were obtained in 2006 year in which in the second half of June and whole July drought and high temperatures appeared. As a consequence this conditions the yield of second cut was very small and lots of plant *Festulolium* were died out (tab. 1). It is possible to say that *Festulolium* is on drought sensitive species. The similar opinion was got by Wilman et al. (1998).

The study found that yields of dry matter were significantly dependent on the doses of nitrogen fertilization. The highest yield gave mixture fertilized of nitrogen dose 60  $kg\cdot ha^{-1}$ . Yield of dry matter was 4,7 t per ha higher than that of mixture without fertilization. Increasing of the nitrogen dose didn't caused increasing yields of dry matter. The least advisable was mixture without fertilization. The yield of total protein didn't significantly dependent on the nitrogen fertilization however the mixtures fertilized of doses N-60 and N-120  $kg\cdot ha^{-1}$  gave 6-7% higher yields of total protein than other mixtures.

Table 1. Botanical composition of the mixtures (%)

Level of fertilization with nitrogen (kg N ha <sup>-1</sup> )	Species	The first year of utilization (2006)									The second year of utilization (2007)			
		Sowing year (2005)*		cuts										
		I	II	I	II	III	I	II	III	IV				
0	FL	43.3	89.7	54.7	12.3	30.0	25.9	10.9	10.0	47.3				
	RC	56.7	10.0	45.0	86.7	67.0	73.8	88.8	90.0	52.4				
60	FL	52.8	82.8	60.9	7.2	26.8	50.5	20.6	12.5	57.7				
	RC	47.2	16.4	39.1	92.5	68.4	46.3	79.1	87.5	42.0				
120	FL	47.2	79.7	68.1	11.2	45.9	52.9	36.5	22.8	62.2				
	RC	52.8	19.5	31.9	88.8	52.0	45.7	61.5	77.2	37.8				
180	FL	54.4	80.8	74.4	14.6	46.5	71.3	34.2	15.2	70.8				
	RC	45.6	18.8	25.6	84.4	52.5	28.0	64.8	84.8	29.2				

FL – *Festulolium*, RC – red clover, different to 100% - weeds.

\* In sowing year doses of nitrogen fertilization weren't differentiated

Table 2. Yields of dry matter and total protein of *Festulolium*/red clover mixtures

Level of fertilization with nitrogen (kg N ha <sup>-1</sup> )	Years of utilization			Total yields (2005-07)
	2005	2006	2007	
dry matter yield (t·ha <sup>-1</sup> )				
0	3.98	9.52	16.10	29.60
60	4.74	11.16	18.40	34.30
120	4.54	11.26	17.79	33.58
180	4.17	11.20	18.38	33.74
LSD (α=0,05)	0.645	1.613	i.d.*	4.808
total protein yield (kg ha <sup>-1</sup> )				
0	682.6	1,427.6	2,798.8	4,909.0
60	880.3	1,665.1	2,686.5	5,231.9
120	712.7	1,707.2	2,865.2	5,285.1
180	709.8	1,506.4	2,714.8	4,931.0
LSD (α=0,05)	i.d.			

\* i.d.-insignificant difference

## Conclusions

The yields of *Festulolium*/red clover mixtures were significantly depend on the doses of nitrogen fertilization. The most advisable was mixture fertilized of nitrogen dose 60 kg·ha<sup>-1</sup>. This mixture gave the highest yield of dry matter and total protein. Increasing of the nitrogen dose didn't caused increasing yield of dry matter. The last advisable was mixture without fertilization.

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# Productivity and Forage Quality of *Festulolium Braunii*–*Trifolium Pratense* Mixtures Depending on the Share of Components

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The significance of short-term grasslands in modern agriculture is far-reaching and multifaceted. The legume-grass mixtures, apart from preparing exceptionally good sites for successive crop plants, also meet the requirements from “ecological forecrops”. Yielding potential of mixtures depends on productive possibilities its components, that is determined by habitat conditions and competition of plants (Jelinowska, Staniak 2007, Zanone et al. 1986). From the point of view of the level and quality of crop, the optimal proportion of leguminous plants in the mixture sward should be between 30% and 50% (Charles, Lehmann 1989, Kessler, Lehmann 1998).

The objective of this research was to evaluation the yielding and quality of dry matter of *Festulolium* cultivation in mixtures with red clover depending on the share of components.

## Methodology

Field experiment was carried out in the years 2005–2007 in Poland at the Institute of Soil Science and Plant Cultivation – Agricultural Experimental Station Grabow (Mazowieckie Voivodeship). Stands on grey-brown podsolic soil, on very good rye complex (pH 7.0,  $P_2O_5$ –187,  $K_2O$ –124,  $Mg$ –30  $mg\cdot kg^{-1}$ ) were established. Trials were sown in the 14<sup>th</sup> of April. The factor was percentage of red clover in sowing mixtures (40, 60, 80%). The mixtures were fertilized as follows  $N-60_{(20+20+20)}$ ,  $P-22$ ,  $K-66_{(33+33)}$   $kg\cdot ha^{-1}$ . As a control there were plots with *Festulolium* v. Sulino ( $N-180$   $kg\cdot ha^{-1}$ ) and red clover v. Nike ( $N-0$   $kg\cdot ha^{-1}$ ) in pure sowing. Swards were cut two times in sowing year, three times in the first year of utilization and four times in the second year of utilization. Chemical composition of plants: dry matter, total protein, crude fibre, crude fat, crude ash and macro elements were determined.

## Results

The study found that the yields of dry matter and nutritive value of mixture were significantly dependent on the share components. The highest yield of dry matter, independently from the year of utilization was obtained from mixtures containing 80% of red clover. This mixture gave 38% higher total yields of dry matter than festulolium and 8% higher than red clover in pure stands. The last advisable was mixture containing 40% of red clover however the smallest yield of dry matter gave festulolium in pure stand (fig. 1).

The mixtures gathered from 6 to 26% more total protein than festulolium and from 6 to 21% fewer than red clover in pure stand. The opposite relation was obtained refer to crude fibre. The mixtures accumulated from 3 to 8% fewer crude fibre than festulolium and from 3 to 8% more than red clover in pure stand. The mixture containing 80% of red clover characterised by the highest nutritive value. The content of total protein was the highest as well as the content of crude fibre was the lowest when compared with other mixtures (tab. 1). The last advisable was mixture containing 40% of red clover. The content of macroelements was at the similar level in all mixtures (tab. 2).

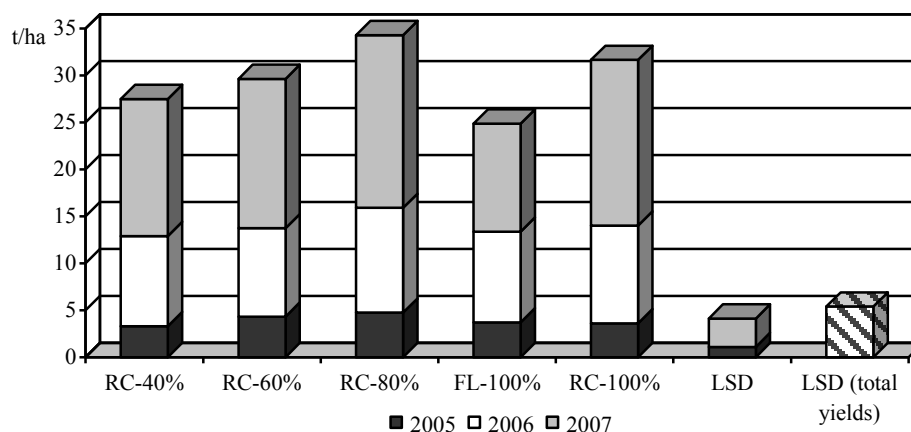


Fig. 1 Yields of dry matter of *Festulolium* (FL), red clover (RC) and their mixtures

Table 1. Percentage of nutrient elements in dry matter (average from years 2005-2007)

Share of red clover in sowing (%)	Dry matter	Total protein	Crude fibre	Crude ash	Crude fat
40	25.95	13.87	23.25	8.08	4.09
60	24.69	15.41	23.48	7.78	3.26
80	24.55	16.42	22.41	7.85	3.22
Festulolium 100%	27.38	13.04	24.21	8.33	4.22
Red clover 100%	23.98	17.47	21.83	7.84	3.50
LSD ( $\alpha=0,05$ )	3.367	3.315	2.304	i.d.	i.d.

\* i.d.-insignificant difference

Table 2. Percentage of macroelements in dry matter (average from years 2006-2007)

Share of red clover in sowing (%)	P	K	Ca	Mg
40	0.29	3.00	0.82	0.22
60	0.30	3.05	1.17	0.25
80	0.32	2.87	1.22	0.24
Festulolium 100%	0.34	3.34	0.72	0.18
Red clover 100%	0.28	2.74	1.68	0.32
LSD ( $\alpha=0,05$ )	i.d.	i.d.	0.539	i.d.

\*i.d.-insignificant difference

## Conclusions

The yields of dry matter and nutritive value of mixtures were significantly dependent on the share of components. The mixture containing 80% of red clover gave the highest total yield of dry matter. This mixture also characterised by the highest nutritive value. The last advisable was mixture containing 40% of red clover.

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# Effect of N Fertilisation on the Growth Dynamics of Winter Wheat Varieties

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Although the fertiliser response and the maximum yield are both genetically determined characters (Balogh et al. 2007), they may be influenced to a considerable effect by environmental and agronomic conditions. The yield of winter wheat depends both directly and indirectly on numerous growth parameters, which in turn are fundamentally determined by the environment and the genetic background of the varieties. One of the most important environmental factors for winter wheat production is the nutrient supply. Pepó (2005) demonstrated that dry matter production and LAI were chiefly responsible for the yield of the genotypes examined, and found a strong correlation between crop fertilisation and LAI in winter wheat. A close correlation between the yield and the leaf area were also demonstrated in winter wheat by Lönhard et al. (1988). The size of LAI was also influenced by the biological traits of the varieties (Petr et al. 1985).

## Methodology

Experiments involving growth analysis and growth dynamics studies were carried out on a winter wheat stand grown on chernozem soil with brown forest residues in Martonvásár during the unusually mild winter of 2006/2007. In the two-factor long-term experiment the effect of fertilisation and genotype on the dynamics of dry matter accumulation and leaf area and on the spike number and grain mass per square metre was studied in a crop rotation where winter wheat was grown after pea. In the split-plot design the N treatments (0, 80, 160, 240 kg ha<sup>-1</sup>) were in the main plot and the variety (Mv Toborzó – extra early, Mv Palotás – early, Mv Verbunkos – medium early) in the subplot. Samples were taken at weekly intervals. Leaf area was measured with LI-3100 and AM300.

## Results

Spring development began extremely early in the extremely mild winter of 2006/2007. Differences were observed in the time course of growth and phenological phases for varieties with different growing periods.

The significant effect of mineral fertilisation on the dry matter production and leaf area index (LAI) was perceptible throughout the experiment for all three varieties, though the level of significance varied in the course of the growing season ( $P=0.1$ –5%) (Fig.1).

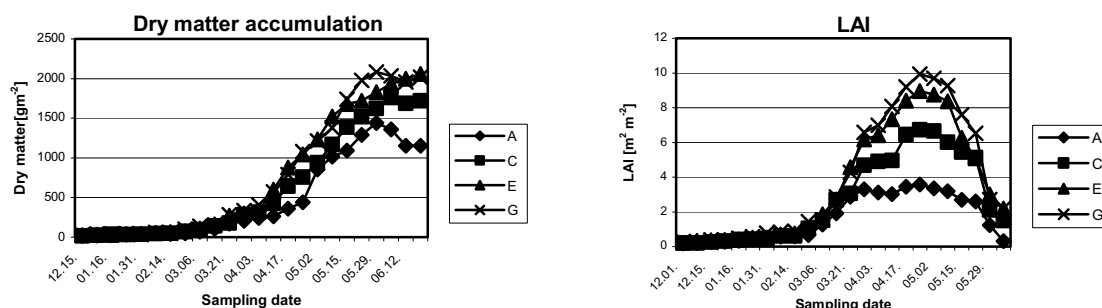


Fig. 1. Dynamics of dry matter accumulation, dry spike mass and leaf area index over time for Mv Palotás at different N levels (2007). Treatments: A: 0, C: 80, E: 160, G: 240 kg ha<sup>-1</sup> N

The most favourable treatment proved to be the 160 kg ha<sup>-1</sup> N level, where the greatest dry matter production was recorded at harvest. In the case of the medium early variety Mv Verbunkos the date of maximum dry matter production differed with the N level. This variety continued to respond to additional N supplies up to the 240 kg ha<sup>-1</sup> level, but the 160 kg ha<sup>-1</sup> rate was the most favourable for the grain yield. Here, too, the untreated plots exhibited losses by harvest.

There were no substantial differences between the three genotypes in the maximum value of dry matter production per square metre, but the ratio of productive to reproductive dry matter varied for the three varieties. Mv Palotás had the greatest grain mass per m<sup>2</sup> (680 g), followed by Mv Verbunkos (670 g) and Mv Toborzó (640 g). All three varieties produced the maximum values at the 160 kg ha<sup>-1</sup> N level. The grain mass per m<sup>2</sup> differed significantly at all four N levels. Due to the exceptional weather conditions the LAI values reached their maximum values 2–3 weeks before flowering, as the withering of the lower leaves began very early.

Differences between the values of LAI<sub>max</sub> were observed for both the genotypes and the N levels (Table 1). There was no significant difference between the 160 and 240 kg ha<sup>-1</sup> levels, but both were far higher than the values recorded in the control and 80 kg ha<sup>-1</sup> plots. Mv Verbunkos exhibited lower values than Mv Toborzó and Mv Palotás.

The growth parameters biomass duration (BMD) and leaf area duration (LAD) were determined for the sampled plants. The cumulative values of these parameters increased with a rise in the N rate and also differed between the varieties (Table 1).

Table 1. Values of LAI<sub>max</sub>, cumulative BMD and cumulative LAD for the three genotypes at different N levels.  
1: Mv Toborzó, 2: Mv Palotás, 3: Mv Verbunkos

N-level [kg ha <sup>-1</sup> ]	LAI <sub>max</sub> [m <sup>2</sup> m <sup>-2</sup> ]			ΣBMD [g day <sup>-1</sup> ]			ΣLAD <sub>LA</sub> [m <sup>2</sup> day <sup>-1</sup> ]		
	1	2	3	1	2	3	1	2	3
0	3.1	3.6	4.8	213.5	186.6	206.8	0.54	0.6	0.64
80	7.4	6.7	7.1	264.3	230.3	240.7	1.02	0.93	0.94
160	10.6	9.0	7.7	287.3	261.3	259.2	1.25	1.14	1.02
240	11.5	9.9	8.1	303.9	272.6	273.9	1.23	1.24	1.1

## Conclusions

The dynamics of plant dry matter accumulation was determined mainly by the nutrient supply level. All three varieties exhibited similar dynamics and the maximum values did not differ significantly at the same nutrient levels. Considerable differences were observed between the varieties in the case of LAI and reproductive parameters (dry spike mass). The growth parameters having the greatest influence on yield (dry matter production, LAI) were most favourable at the 160 kg ha<sup>-1</sup> N fertiliser level, while only a much smaller increase was caused by the 240 kg ha<sup>-1</sup> rate. This excessive rate led to losses in dry matter (Mv Palotás), spike mass (Mv Palotás and Mv Verbunkos) and grain mass (all three varieties) compared with the 160 kg ha<sup>-1</sup> rate.

Reproductive growth (dry spike mass) was fundamentally determined by the genotype. From this point of view Mv Palotás gave the best fertiliser response to the 160 kg ha<sup>-1</sup> N supply level, which was optimum for all three varieties.

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# Optimisation of Water and Nitrogen in Irrigated Permanent Meadows in the Province of Reggio Emilia

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## Introduction

Permanent meadows located in the provinces of Parma and Reggio Emilia (Northern Italy) are extremely important for the production of Parmigiano – Reggiano cheese. Traditionally, this crop is irrigated by means of flood irrigation. In recent years, the reduced availability of irrigation water has meant that it has become necessary to optimise water use for crops with high irrigation requirements like permanent meadows.

The purpose of this research was to evaluate the possibility of using the sprinkling method to optimise irrigation. The influence of nitrogen fertilisation has also been studied over a three year period, to verify the environmental effects of the different irrigation methods.

## Methodology

A split-plot experimental design with four replicates was set up on a permanent meadow. The soil was a loamy-skeletal, mixed, mesic, Typic Ustochrepts according to Soil Taxonomy. The main factor was the irrigation system (sprinkling vs. flood irrigation) while the secondary factor was the fertilisation rate (0, 100 and 150 kg N ha<sup>-1</sup>) for a total of 24 plots. Each of the plots were 5 m wide and 25 m long.

Water from the Enza river was used for flood irrigation while water from the farm well was used for the sprinkling method. The quantity of water used for each irrigation was calculated by the software “IRRIPRATO V2T” developed by Canale Emiliano Romagnolo (CER,2005). The seasonal volumes employed for the irrigation are shown in table 1.

Table 1: Seasonal volumes used for irrigation

Irrigation method	Seasonal irrigation volume (m <sup>3</sup> ha <sup>-1</sup> )		
	2005	2006	2007
Sprinkling method	2400	3362	4734
Flood method	21060	17885	18157

The crops were harvested five times over the first two years but only 4 harvests were possible in 2007 because of adverse weather conditions. At each harvest measurements were made of dry-matter yield and forage nutritional composition and soil samples were taken at two depths (0-25 cm and 25-50 cm) to measure nitrates content by ionic chromatography.

## Results

Forage dry matter yield (table 2) was influenced by the fertilisation rate in each year. In contrast there were no significant differences deriving from the irrigation method used except in the first year partially due to initial difficulties in the set up of the sprinkling method. In the last two years the sprinkling method produced a slightly higher dry matter yield but this was not statistically significant. N fertilisation significantly increased forage yield in all three years, with average increments of 18%, 17% and 45% respectively for 2005, 2006 and 2007. N2 rate was slightly more productive than N1, but the extra 50 kg ha<sup>-1</sup> did not have a statistically significant effect.

The irrigation method did not seem to influence the nutritional composition in any of the three years as can be seen in table 3. On the other hand, the nitrogen fertilisation significantly modified the forage composition: crude protein content was higher and NDF lower when no nitrogen was applied: in fact on the fertilised plots nitrogen selectively promoted the development of graminaceous species to the detriment of legumes, lowering the protein content of the forage.

Table 2: Forage dry matter yield (Mg ha<sup>-1</sup>)

<b>Irrigation method</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>
Sprinkling method	11.69 b	12.49	9.29
Flood method	12.60 a	11.39	9.05
<b>Significance</b>	<b>**</b>	<b>n.s.</b>	<b>n.s.</b>
<b>Fertilisation rate</b>			
N0: 0 kg N.ha <sup>-1</sup>	10.84 b	10.71 b	7.07 b
N1: 100 kg N.ha <sup>-1</sup>	12.77 a	12.21 a	9.97 a
N2: 150 kg N.ha <sup>-1</sup>	12.83 a	12.90 a	10.48 a
<b>Significance</b>	<b>***</b>	<b>**</b>	<b>***</b>
<b>Interaction Irrigation x Fertilisation</b>			
Significance	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>
Field mean	<b>12.15</b>	<b>11.94</b>	<b>9.17</b>

Table 3: Forage crude protein (% DM) and NDF (%DM) content

<b>Irrigation method</b>	<b>2005</b>		<b>2006</b>		<b>2007</b>	
	Crude Protein	NDF	Crude Protein	NDF	Protein	NDF
Sprinkling method	13.19	51.48	11.96	55.30	10.15	57.84
Flood method	12.80	52.10	12.22	54.70	9.85	57.64
<b>Significance</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>
<b>Fertilisation rate</b>						
N0: 0 kg N.ha <sup>-1</sup>	13.40	49.42a	12.82a	52.47a	10.24	56.21a
N1: 100 kg N.ha <sup>-1</sup>	12.68	52.55b	11.52b	56.09b	9.56	58.40b
N2: 150 kg N.ha <sup>-1</sup>	12.90	53.40b	11.94b	54.44b	10.20	58.61b
<b>Significance</b>	<b>n.s</b>	<b>**</b>	<b>***</b>	<b>***</b>	<b>n.s</b>	<b>*</b>
<b>Interaction Irrigation x Fertilisation</b>						
<b>Significance</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>

Throughout the three years of the study the level of nitrates in soils always remained below 20 mg kg<sup>-1</sup> for the upper soil layer (0-25cm) and below 12 mg kg<sup>-1</sup> for the 25-50 cm layer. No significant differences were found either for the irrigation method or the fertilisation rate.

## Conclusions

There is no evidence from the above results that the use of the sprinkling irrigation method has negative effects on permanent meadows if irrigation is carried out at the proper time, supplying the right water quantity. The economic and technical aspects of the use of this irrigation method on permanent meadows requires further study as well as continuing observations on the botanical composition of the sward to confirm there are no observable effects over a longer period (Paris et al.,1992).

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# Ammonia Volatilization in Winter Rain Fed Cereal Fertilized with Pig Slurry

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When studying the nitrogen cycle for fertilization purposes the “*unaccounted for*” nitrogen (denitrified, volatilized and lixiviated) is often up to 40% of the total. With the goal of improving nitrogen use efficiency, the objectives of this study where: to estimate the influence of the fertilizer type and dose on volatilization, as well as the effect of environmental conditions on emissions.

## Methodology

Volatilization was sampled after the application of either pig slurry or ammonium nitrate to the crop on the following occasions:

- 2003-04 at dressing,
- 2004-05 at dressing,
- 2005-06 at pre-seeding and at dressing,
- 2006-07 at pre-seeding and at dressing.

Sampling has been done on some of the plots (see table) of the fertilization experiment of the project (RTA04-114-C3) according to their suitability for the comparison of ammonia volatilization from slurry with that occurring from ammonium nitrate applied at an equivalent dose.

Plot (m <sup>3</sup> /ha)	Treatment	Pre-seeding (kg N/ha)			Dressing (kg N/ha)			Total (kg N/ha)		
		total	organic	NH <sub>4</sub> <sup>+</sup> -N	total	organic	NH <sub>4</sub> <sup>+</sup> -N	total	organic	NH <sub>4</sub> <sup>+</sup> -N
N0	Control									
rN3	AN	30		15				90		45
P22 (20)	Fattening slurry	128	28	100				128	28	100
PN8 (30)	Sow slurry	165	37	129				165	37	129
P42 (80)	Fattening slurry	626	143	483				626	143	483
N13	AN				90		45	90		45
N3 (20)	Fattening slurry				158	41	117	158	41	117
N8 (90)	Sow slurry				244	83	161	244	83	161
N5 (60)	Fattening slurry				425	110	315	425	110	315

Ammonia has been sampled by means of static open chambers (Schjoerring and Mattsson, 2001), threefold, from immediately after the application till the emission was similar to the background.

## Results

The larger the applied dose of slurry, the lower the losses of  $\text{NH}_3\text{-N}$  (in %) since once the soil surface is covered, a larger dose does not imply a larger volatilization.

In general, the losses are larger (in %) from the mineral fertilizer than from slurry and after the dressing application (spring, about 35% of the applied  $\text{NH}_3\text{-N}$ ) than at pre-seeding (winter, about 15% of the applied  $\text{NH}_3\text{-N}$ ).

Volatilization takes place mainly during the first days after application (3 days after dressing and 7 days at pre-seeding). About 70% of the losses (at pre-seeding), and about 50% (after dressing) of the total  $\text{NH}_3\text{-N}$  volatilized during the whole sampling period.

The losses from fattening pig slurry are larger than those from sow slurry, since with sow slurry, the total N application was between 20% and 30% larger, but volatilization was only a 5% larger. This can be explained by the difference in  $\text{NH}_4^+\text{-N}$  and dry matter concentration, leading to a larger infiltration of sow slurry.

## Conclusions

Regarding the effect of the type of N source on volatilization:

- volatilization from mineral fertilizer is larger, in percentage, than from slurries,
- volatilization from fattening slurry is larger than from sow slurry,
- volatilization is maximum during the first 3 days after pre-seeding and during the 7 days following dressing fertilization.

Regarding the effect of the applied fertilizer dose on volatilization:

- volatilization (in mass) increases with the dose, but decreases as percentage of the applied N.

Regarding the influence of environmental conditions on volatilization:

- volatilization increases with temperature and either increases or decreases with rain depending on the effect of rain, either increasing infiltration or wetting the dry surface and therefore, re-starting volatilization.

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## Acknowledgements

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# Optimum Density in Maize Varies Across Environments and Density Affects the Influence of Stand Uniformity on Productivity

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Optimum plant population density for maximum crop yield (i.e., yield per unit area) may differ from hybrid to hybrid in maize (*Zea mays* L.) due to strong genotype x density interactions (Farnham, 2001), and in turn hybrids exhibit strong density-dependence (Fasoula et al., 2005). The impact is blunted when hybrids are characterized by improved yield potential per plant as expressed under very low density that excludes plant to plant interferences for resources and thus approaches absence of competition (Tokatlidis et al., 2005). On the other hand, absence of uniformity in the stand adversely affects grain yield productivity (Fasoula et al., 2005). Moreover, as density increases stand uniformity deteriorates (Tokatlidis et al., 2005). Objective of this work was to investigate: (i) if optimum density of a given hybrid differs across growing seasons, (ii) the role of yield potential per plant in this differentiation, and (iii) how density affects the degree of relationship among stand uniformity and productivity.

## Methodology

Experimentation was conducted during 2006 and 2007 with seven short-season hybrids (FAO ≈400) in Romania under rainfed conditions, and seven long-season (FAO ≈700) hybrids in Greece with irrigation. The experimental design was a split-plot with three densities (low, medium and high) as main plots, randomized in complete blocks, and the hybrids as subplots. Each block was replicated three times. Densities were 2.5, 4.2 and 8.4 plants/m<sup>2</sup> in Romania, and 3.13, 6.13 and 8.33 plants/m<sup>2</sup> in Greece. Experimentation also included an adjacent honeycomb experiment (Fasoulas et al., 1995) where hybrids were evaluated under very low density assumed to (1.12 and 0.74 plants/m<sup>2</sup> in Romania and Greece, respectively) to assess their yield potential per plant. The optimum plant density for maximum crop yield of each hybrid was calculated on the basis of the four densities through the slope b of the linear regression analysis of natural logarithm of grain yield per plant over plant density {ln(Y<sub>p</sub>)=α-bD}, where Y<sub>p</sub> is the yield per plant in grams and D is the plant density in plants/m<sup>2</sup>, with the optimum plant density being equal to 1/b (Tollenaar 1992). Stand uniformity was also estimated through coefficient of variation (CV) of individual plants for grain yield, main ear length and kernel row number in both split-plot experiments of Romania and in that of 2007 in Greece.

Table 1. Yield potential per plant (YP<sub>p</sub>) averaged across two years in g/plant, and optimum density (Dopt) and its differentiation of each hybrid across two seasons in plants/m<sup>2</sup>

Romania					Greece				
Hybrid	YP <sub>p</sub>	Dopt			Hybrid	YP <sub>p</sub>	Dopt		
		2006	2007	2006-07			2006	2007	2006-07
Rom1	419	3.5	5.7	2.2	Gr1	455	5.8	5.9	0.1
Rom2	449	3.8	6.9	3.1	Gr2	846	5.3	5.0	0.3
Rom3	265	4.5	7.7	3.2	Gr3	704	6.2	5.1	1.1
Rom4	462	3.8	6.5	2.7	Gr4	776	5.5	5.3	0.2
Rom5	422	3.7	6.2	2.5	Gr5	869	5.2	5.8	0.6
Rom6	338	4.1	6.1	2.0	Gr6	576	5.5	6.7	1.2
Rom7	464	3.5	6.0	2.5	Gr7	401	10.2	8.7	1.5

## Results

In both countries average yield of the split-plot experiments was significantly higher in 2007 compared with 2006, particularly in Greece (6006 vs 5357 and 12320 vs 9247 kg/ha in Romania and Greece,

respectively). Optimum density differed either among hybrids at the same season or among the same hybrid across the two seasons (Table 1). Although optimum density estimation by this method is, in part, an artefact as it is strongly influenced by the range of densities included in analysis, differences among hybrids did not vary (Tollenaar, 1992). Differentiation of optimum densities raises queries for risk of biased judgment when comparative evaluation of different hybrids for their crop yield potential is sought in a single density. On the other hand, more favourable conditions in 2007 than 2006 in Romania led to higher yields accompanied by considerably higher optimum densities. Different optimum density for the same hybrid across environments also causes confusion about what density should be applied by farmers. As far as yield potential per plant ( $YP_p$ ) is concerned, generally higher values were accompanied by lower optimum density differentiation, except for hybrids Rom6 and Gr3. This impact is indicative of less density dependence. Excluding these hybrids simple correlation coefficients were negative ( $r=-0.50$ , and  $-0.89$ ) with the second one being significant. Improved yield potential per plant broadens the optimum density rendering the hybrids less density-dependent (Tokatlidis et al., 2005).

Table 2. Simple correlation coefficients between yield per ha and CV values within each density across 7 hybrids and three replication per hybrid (n=21) \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$

Density	Romania						Greece		
	g/plant		Ear length		Kernel row		g/plant	Ear length	Kernel row
	2006	2007	2006	2007	2006	2007			
Low	-0.03	-0.20	-0.43	0.08	-0.35	-0.29	-0.36	0.22	-0.39
Medium	-0.32	-0.29	-0.34	-0.25	-0.63**	-0.22	-0.62**	-0.27	-0.64**
High	-0.50*	-0.48*	-0.59**	-0.26	0.07	-0.61**	-0.68***	-0.52*	-0.68***

Density affected stand uniformity, as well. Increased density was followed by increased CV values for all the traits tested. For example, in case of grain yield per plant of Romanian hybrids average CV values were 14.7, 20.6 and 36.5% in 2006, and 22.7, 27.3 and 31.0% in 2007 at the low, medium and high density, respectively. Regarding Greek hybrids average CV values of the main ear length were 5.0, 7.6 and 9.7% at the low, medium and high density, respectively. There was found a tendency of negative relationship among yield per unit area and CV values (Table 2). However, simple correlation coefficients increased as density increased, being significant at the high density (except for two out of the nine  $r$  values). At the middle density three out of the nine  $r$  values were significant, whereas at the low density none of the  $r$  values was significant. Consequently, the necessity of modern hybrids to be planted in high densities to obtain maximum crop yield because they lack improved yield potential per plant (Tokatlidis et al., 2005), render them vulnerable to acquired (environmental) variability, and hence deteriorates their stability. In turn, the development of density-independent hybrids though improving yield potential per plant appears a realistic and beneficial goal.

### Conclusions

Improved yield potential per plant renders hybrids less density-dependent. The results highlight the necessity for density-independent hybrids with broadened optimum density for the following reasons: (i) to avoid the risk of biased judgment when different hybrids are comparatively estimated for their crop yield potential, (ii) to blunt the confusion of farmers about what density to apply (iii) density-independent hybrids could be cultivated under lower densities so that stand uniformity is improved, and the negative association among yield per unit area and the degree of non-uniformity is also blunted.

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# The N Nutritional Status of Processing Tomato Grown after Green Manures

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Green manure is a fundamental practice in organic agriculture as it represents an opportunity to optimize N fertilisation without turning to external inputs. Several field trials have shown that green manures are an appropriate fertilisation technique for organic maize in Central Italy (Benincasa et al., 2007), but the use of green manures on N fertilisation of vegetables crops has not been yet fully evaluated. This field research aimed to evaluate the N nutritional status of transplanted processing tomato grown after different green manures in comparison to organic or mineral fertilisation.

## Methodology

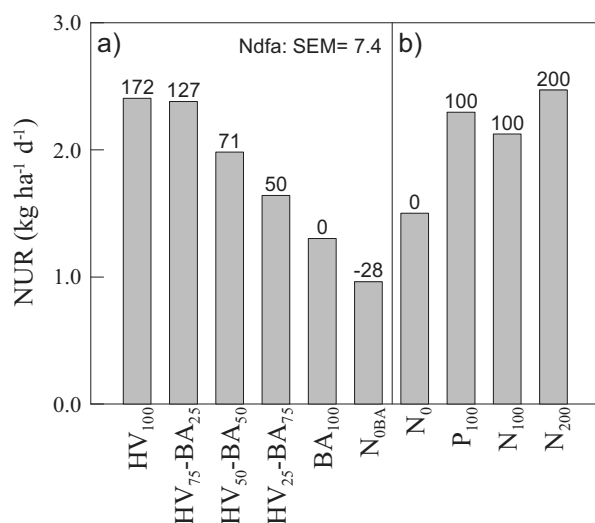
A field experiment was carried out in 2005/2006 at the Experimental Station of the Department of Agricultural and Environmental Sciences of the University of Perugia (43° N, 165 m a.s.l.).

In a randomized block design with 4 replicates, processing tomato (*Lycopersicon esculentum*, Mill. cv PS1296) was grown after different green manures and in soil left bare until transplanting (controls). The green manures were represented by hairy vetch (*Vicia villosa* Roth., HV) and barley (*Hordeum vulgare* L., BA) cultivated as monocultures at full sowing density (HV<sub>100</sub>, 200 seeds m<sup>-2</sup>, and BA<sub>100</sub>, 400 seeds m<sup>-2</sup>) and as mixtures obtained by using a fraction of the full sowing density, namely 75% of vetch + 25% of barley (HV<sub>75</sub>-BA<sub>25</sub>), 50%+50% (HV<sub>50</sub>-BA<sub>50</sub>) and 25%+75% (HV<sub>25</sub>-BA<sub>75</sub>). In fertilised control plots, 100 and 200 kg ha<sup>-1</sup> of N as urea (N<sub>100</sub> and N<sub>200</sub>) and 100 kg ha<sup>-1</sup> of N as poultry manure (P<sub>100</sub>) were applied. Unfertilised control plots (N<sub>0</sub>) and additional BA<sub>100</sub> plots where the barley above ground biomass was removed (N<sub>0BA</sub>) were used to estimate the soil base N fertility. The dry matter and the N accumulated in green manures and in tomato were determined by destructive samplings and Kjeldhal analysis. The N actually supplied by green manures, corresponding to the N derived from the atmosphere (N<sub>dfa</sub>, kg ha<sup>-1</sup>), was estimated as difference between the total N accumulated in the green manures and that accumulated in BA<sub>100</sub> according to Müller and Thorup-Kristensen (2001). The Nitrogen Uptake Rate (NUR, kg N ha<sup>-1</sup>d<sup>-1</sup>) of tomato was estimated as the derivative of the Gompertz functions applied to the actual N accumulated during the crop cycle.

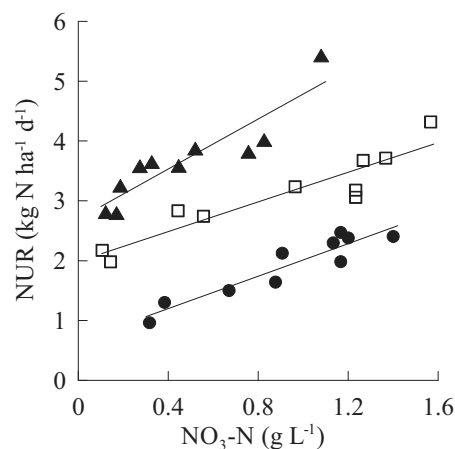
The NO<sub>3</sub>-N concentration in the petiole sap was determined by a ion-specific electrode meter (Cardy-meter, Spectrum Tech. Inc., USA). The N status (i.e. limiting/optimal/luxury N consumption) of tomato plants was evaluated over the whole crop cycle by comparing the actual N percentages with the critical N dilution curve reported by Tei et al. (2002).

## Results

The N<sub>dfa</sub> supplied by green manures increased with the seed rate of vetch (SR<sub>HV</sub>) at sowing (N<sub>dfa</sub>=0.844 SR<sub>HV</sub>-0.5, R<sup>2</sup>=0.973). After green manures the NUR of tomato as recorded during the exponential growth phase (i.e. 32 Days After Transplanting) was strictly dependent on N<sub>dfa</sub>, while in fertilised controls NUR was not affected by N rate (Fig. 1). During early growth (i.e. until 57 DAT), NUR was highly correlated to the NO<sub>3</sub>-N concentration in the petiole sap (Fig. 2), but the NUR corresponding to a given NO<sub>3</sub>-N concentration clearly increased with time. This suggests that the NO<sub>3</sub>-N concentration in the plant tissues tended to be steady despite the root expansion enhanced N uptake and NUR.



**Fig.1** – Nitrogen Uptake Rate (NUR, kg N ha<sup>-1</sup> d<sup>-1</sup>) of tomato at 32 days after transplanting after green manures (a) and in control treatments (b). Values above bars are the N derived from atmosphere (Ndfa, kg ha<sup>-1</sup>) after green manures, the N removed in N<sub>0BA</sub> and the N supplied in the controls.

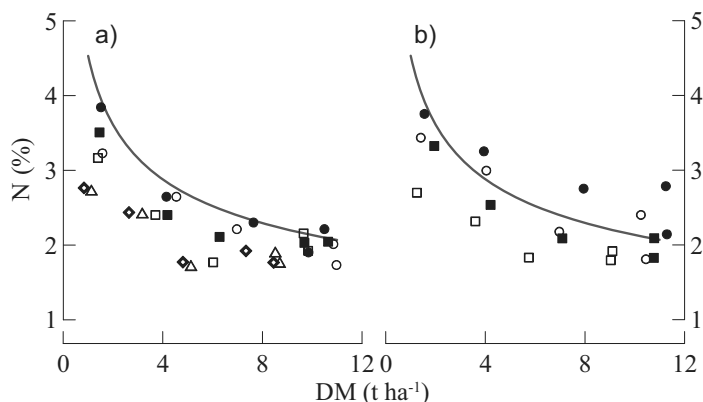


**Fig.2** – Correlation between NO<sub>3</sub>-N in the tomato petiole sap and the Nitrogen Uptake Rate (NUR) as recorded at 32 DAT (●,  $r=0.939$ ,  $p=5.8 \times 10^{-5}$ ), 42 DAT (■,  $r=0.929$ ,  $p=1.0 \times 10^{-4}$ ) and 57 DAT (▲,  $r=0.906$ ,  $p=3.1 \times 10^{-4}$ ).

The N consumption of tomato plants has to be considered optimal after green manures with a proportion of vetch  $\geq 50\%$  and in P100 and sub-optimal both after green manures with high proportion of barley and in unfertilised controls (Fig. 3a). On the contrary, urea at both N rates promoted a luxury N consumption (Fig. 3b).

### Conclusions

The use of mixtures with high proportion of vetch ( $>50\%$ ) allows an optimal N nutritional status of processing tomato without promoting luxury N consumption. The Nitrogen Uptake Rate was highly correlated with the nitrate concentration in the petiole sap, confirming that in tomato the sap test is a reliable method to detect the mineral N availability also after green manures (Taber, 2001).



**Fig.3** – Nitrogen concentration (N%) vs Dry Matter (DM, t ha<sup>-1</sup>) of tomato after green manures (a): HV<sub>100</sub> (●), HV<sub>75</sub>-BA<sub>25</sub> (○), HV<sub>50</sub>-BA<sub>50</sub> (■), HV<sub>25</sub>-BA<sub>75</sub> (□), BA<sub>100</sub> (△), N<sub>0BA</sub> (◇); and in the controls (b): N<sub>200</sub> (●), N<sub>100</sub> (○), P<sub>100</sub> (■), N<sub>0</sub> (□). The curves represent the critical N concentration for tomato as found by Tei et al. (2002).

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# Alfalfa Effects on Soil Fertility

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The importance of leguminous crops in enhancing soil fertility in a rotation with cereals is well known. What is still uncertain is the mechanism of this improvement and how long it lasts. Basically, it can depend on the soil enrichment in organic matter, on the amount of N fixed by rhizobia or on both (Levin et al., 1987). In many parts of the World a pluriennial stand of leguminous forage crop is considered fundamental for the sustainability of agricultural systems (Russelle et al., 2007). The scarce literature on the topic, however, reports contrasting results, and in some instances the benefits of meadows revealed not so long (Toderi et al., 1985).

## Methodology

A long-term experiment, started in 1966 and still conducted in the eastern Po Valley (Cadriano, BO) compares 2-year rotation (corn-wheat CW) with 9-year rotation (alfalfa, alfalfa, alfalfa, C-W, C-W, C-W), combined with two rates of cattle manure supply (none and 20 t ha<sup>-1</sup>year<sup>-1</sup> of fresh manure) and 3 mineral fertilization rates (N0P0, N1P1, N2P2) in a split-plot replicated twice (56 m<sup>2</sup> sub-plots) on a medium textured soil, with low organic matter content. The effects of alfalfa on the subsequent successions of corn and wheat are shown and compared to the corn and wheat crops inserted in 2-year rotation (without alfalfa). Discussed data are the averages of four cycles of the 9-rotation.

## Results

The influence of alfalfa on corn grown after its interruption was marked, and was still clear on wheat cropped after corn. These results, however, varied with fertilization regimes (table 1). In unfertilized control, alfalfa precession gave 1.8 t ha<sup>-1</sup> more grain in next corn (+35%) and plus 1 t ha<sup>-1</sup> in subsequent wheat (42%). Manure supply without mineral fertilization increased yield in a similar way (table 1). In both crops the effects of manure and alfalfa precession were not additive. Indeed, the yield difference between amended plots after the meadow and the control was lower than what expected by the sum of the single influences. The intensification of mineral fertilization drastically reduced alfalfa benefits to succeeding cereals. Thus the meadow effects on cereal grain production are likely of nutritional nature.

		(2-years rot.) No manure No alfalfa	(9-years rot.) No manure Yes alfalfa	(2-years rot.) Yes manure No alfalfa	(9-years rot.) Yes manure Yes alfalfa
Corn	N0 P0	5.2	7.0	7.2	8.2
	N1 P1	8.2	8.5	9.1	9.2
	N2 P2	8.9	9.2	9.4	9.1
Wheat	N0 P0	2.4	3.4	3.4	4.2
	N1 P1	5.1	5.7	5.7	6.1
	N2 P2	6.0	6.3	6.1	6.1

Table 1. Effects of the interaction: alfalfa precession (yes or no) x manure supply (yes or no) x mineral fertilizer rates (NP) on the grain yields of corn (t ha<sup>-1</sup> at 15.5% moisture) and wheat (t ha<sup>-1</sup> at 13% moisture) crops. Averages of 4 rotational cycles, with compared crops grown in the same years.

Alfalfa effects gradually decreased during successive years from the meadow interruption. Table 2 reports the effects of the interaction alfalfa precession and mineral fertilization as a function of the time passed after meadow interruption, in absence of manure application in order to exclude any interaction between the effects of manure and meadow. Without any fertilizer supply corn planted soon after alfalfa (i.e. corn inserted in 9-year rotation) produced 62% more grain than corn after wheat (in the 2-year succession); but this difference more than halved in corn grown 3 years (+26%) and 5 years

(+23%) after alfalfa interruption. For subsequent wheat crops the benefits of alfalfa lasted a little longer. They gave 45%, 43%, and 33% yield increases in the 2<sup>nd</sup>, 4<sup>th</sup>, and 6<sup>th</sup> years after the end of the meadow. All the residual effects of alfalfa almost disappeared when mineral fertilizers were applied, even at the lower rate. Therefore, again, they can be ascribed to a greater nutrient availability in the soil, probably due to the N-rich alfalfa roots and plant residues that remain in the soil.

	Years after alfalfa	Mineral Fertilizer rates					
		N0 P0		N1 P1		N2 P2	
		(2-years rot.) no alfalfa	(9-years rot.) yes alfalfa	(2-years rot.) no alfalfa	(9-years rot.) yes alfalfa	(2-years rot.) no alfalfa	(9-years rot.) yes alfalfa
Corn	1	5.06	8.21	8.44	8.86	9.12	9.52
	3	5.08	6.39	7.53	7.89	8.13	8.65
	5	5.70	7.01	9.39	9.50	9.84	10.36
Wheat	2	2.80	4.06	5.14	6.27	5.99	6.52
	4	2.24	3.21	5.49	5.88	6.40	6.72
	6	2.53	3.37	5.48	5.83	6.37	6.30

Table 2. Effects of the interaction: alfalfa precession (yes or no) x mineral fertilizer rates (NP) on the grain yields of corn and wheat crops (t ha<sup>-1</sup> at 15.5 and 13% moisture, respectively) as a function of the time passed after alfalfa interruption. Averages of 4 rotational cycles, with compared crops grown in the same years and in absence of manure application.

## Conclusions

From a productive point of view the effects of a monophyte meadow of the leguminous alfalfa on the subsequent cereal crops appeared similar to a normal rate of manure supply. Similarly to manure, moreover, the benefits of the pluriannual forage crop seem linked to a greater and prolonged availability of nutrients for the succeeding crops. That availability, probably due to the organic matter that remains in the soil incorporated in roots and plant debris, was significant on the N-greedy corn planted soon after the meadow interruption, but resulted more prolonged on wheat crop. Anyway, all these effects resulted easily surrogated by adding mineral fertilizers to the crops following alfalfa, at application rates not even high.

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# Assessment of the Behavior of the Nitrification Inhibitor DMPP: a 3-yr Irrigated Ryegrass Field Experiment

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The results of our previous field work in the last 10 years show that the behavior of the nitrification inhibitor (NI) DMPP, is not always consistent in field conditions, mainly due to the experimental error associated with spatial variability and soil sampling for N determinations. Nevertheless, crop N response is common when compared to unfertilized plots. Previous pot experiments provided more consistent results, with significant differences related to quality issues (expressed as protein or nitrate content), total N uptake, N efficiency indexes, the reduction of nitrate leaching risk, and some nutrients uptake such as phosphorus. From the farmer's point of view, forage production and economical aspects are the most important. Today, DMPP fertilizers are offered at a similar price compared with the same formulation without NI, and therefore, any advantage that comes from the use of NI needs to be considered as an improvement on plant nutrition. The addition of NI to different types of slurry has been the subject of numerous recent studies and was recommended by Amberger (1990) to prevent groundwater pollution, especially if slurry is applied in late autumn or winter. It is known that N concentration (Crude protein level) in ryegrass forage increase as applied N increase (Lippke et al, 2006). The objective of this study was to determine the effects of DMPP applied with inorganic fertilizers and with pig slurry on the yield, nitrogen recovery, and mineral composition of ryegrass grown on a calcareous irrigated soil.

## Methodology

A highly intensive monitored field experiment was conducted from 2005 to 2008 at the Torreneral farm (41°44'N, 0°57'E; Lleida, Catalonia (NE Spain), the soil was very deep, coarse loamy, mixed, calcareous, mesic, Typic Calcixerept). Westerwold ryegrass (*Lolium multiflorum* Lam., Cv. Trinova) was sown each year in September. Ryegrass was harvested three times each year. At the beginning of the 3<sup>rd</sup> year no fertilizers were applied in order to assess the residual N effects. The results have been analysed until the second cut of the third year. Treatments were: (1) Control, without N application, (2) ANS-26 (125 kg N/ha after each cut), (3) ENTEC-26 (ANS +DMPP) (125 kg N/ha, after each cut) (4) pig slurry (PS) (5) pig slurry with DMPP. The apparent recovery of N by the ryegrass (NREC) was calculated as described by Greenwood and Draycott (1989). Ammonia losses and nitrogen leaching were also determined (data not shown). In summer 2005, the upper 30 cm of the soil profile contained 24 g of organic matter kg<sup>-1</sup>, which is a relatively high value for irrigated semiarid land; there was also 13.9 mg of NO<sub>3</sub>-N kg<sup>-1</sup>, which constitutes a moderate nitrate content. The soil pH was 8.1 and the CO<sub>3</sub>Ca equivalent was 110 g kg<sup>-1</sup>. The Soil Test P (Olsen method) value was high (29±7.9 mg P kg<sup>-1</sup>), and the soil test K value was also interpreted as high (220 ± 74.4 mg K kg<sup>-1</sup>). Data were analyzed using the GLM procedure in the SAS System for Windows 9.0.

## Results

Unfertilized ryegrass (control) produced 23.2 t of accumulated dry matter (DM)/ha in these 8 cuts, and the macronutrient uptake was 451 kg N/ha, 66.2 kg P/ha and 661 kg K/ha. There were non significant differences between treatments in most of the parameters analyzed. The ENTEC treatment produced, on average, the highest accumulated DM production 41.8 t/ha, and nutrient export was 1223 kg N/ha, 124.5 kg P/ha and 1549 kg K/ha. Fertilizers (mineral and pig slurry) generated an increase between

58.5 and 80.2 % of accumulated dry matter in the 8 cuts analyzed. The aboveground removal ratio for N:P:K was 8:1:11.3 (fertilized treatments). Average apparent N recoveries for the 8 cuts were 88.3% and 64.8 % for Entec and ANS-26, respectively. Accumulated NPK uptake was significantly lower for the unfertilized treatment. Accumulated phosphorus uptake was greater in the nitrification inhibitor treatments. No significant effect was observed on accumulated potassium uptake due to NI. Average concentration of P was 0.41% for pig slurry plus DMPP treatment, significantly different from the two mineral fertilizer treatments. The greater P uptake due to DMPP, an indirect advantage of its use, might be explained by the fact that DMPP improves the mobilisation and uptake of P from the rhizosphere, probably due to the presence of ammonium ions in the soil solution, which might reduce soil pH (Pasda *et al.*, 2001). Total N applied (see table) for pig slurry treatments refers only to ammoniacal nitrogen content. NREC was higher for inorganic fertilizers compared with pig slurry. Average nutrient concentrations were 27.7 g N kg<sup>-1</sup>, 3.3 g P kg<sup>-1</sup>, and 37.1 g K kg<sup>-1</sup> on a dry matter basis. Average forage K content was similar between treatments. Only the control was significantly lower. The last cut (May, 5<sup>th</sup>, 2008) showed a significant difference on forage K content between pig slurry and mineral fertilizers due to K content of the pig slurry (data not shown) and probably to a lower K soil content.

Treatment	Accum. DM (Mg ha <sup>-1</sup> )	Preplant test (NO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> ) (kg N ha <sup>-1</sup> )	N Total applied (kg N ha <sup>-1</sup> )	N Accum. uptake (g N ha <sup>-1</sup> )	NREC (%)	Accum. P uptake (kg P ha <sup>-1</sup> )	Accum. K uptake (kg K ha <sup>-1</sup> )
Control	23.2 b	220	0	451 b	-	66.2 c	661 b
ANS-26	36.7 a	197	875	1,018 a	64.8 ab	113.0 b	1,411 a
Entec-26	41.8 a	181	875	1,223 a	88.3 a	124.5 ab	1,549 a
Pig slurry (PS)	36.8 a	234	1100	973 a	47.0 b	141.3 ab	1,533 a
PS + DMPP	38.9 a	261	1113	1,127 a	61.5 ab	159.2 a	1,607 a
Differ.	**	NS		**	#	**	**

\*\* significantly different at the 0.01 level; NS Not significant;# significantly different at the 0.1 level

## Conclusions

These results indicated that nitrogen fertilization significantly increased forage production. The NI DMPP improves the recovery fraction of Nitrogen and the aboveground P absorption. The highest nutrient exported by ryegrass was potassium. After three years of cropping ryegrass in a soil with a high K content, the last cut presented a clear response to K applied with the pig slurry. Soil K test at the end of the study will help us to confirm this hypothesis.

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# Potential Herbicidal Effect of Oregano, Rosemary and Rue on some Mediterranean Weed Species

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## Introduction

Vegetal world contains a high number of molecules that are supposed to have insecticidal, fungicidal or herbicidal effect that deserve to be properly used as phytosanitary products (Regnault-Roger *et al.*, 2002). Some vegetable oils and formulations of fatty acids are already known to possess herbicidal properties and have been used commercially (Singh *et al.* 2005). Tworkoski (2002) pointed out that essential oils can be used as viable weed control technology under organic farming systems. Among natural plant products, volatile essential oils and their constituents have attracted much attention because of their phytotoxicity and allelopathic property (Batish *et al.*, 2004). Campiglia *et al.* (2007) showed that essential oils of cinnamon, lavender and peppermint inhibited seed emergence in the case of ryegrass, wild mustard and redroot pigweed. Previous work (De Mastro *et al.*, 2006) reported preliminary observations about the inhibitory effects on germination and growth of some weeds caused by oregano

This study aims at assessing the potential inhibition capacity of three aromatic plants (oregano, rosemary and rue) on seed emergence and plant growth of five different weed species.

## Material and methods

This experiment was conducted under a shadow net green house at the experimental field of Agriculture Faculty - Bari University (Italy). *Origanum vulgare* L. (OR) (*O. vulgare* L. spp. *viridulum* (Martrin-Donos) Nyman x *O. vulgare* L. spp. *hirtum* (Link) Iestwaart), *Rosmarinus officinalis* (RO) and *Ruta graveolens* (RU) dry biomasses were mixed to the soil. Seven groups of treatments with three different doses and an untreated control were used (Table 1). Soil was previously sterilized in oven at 85°C for 24 hours then put in a 4 liters plastic containers with a diameter of 25 cm in which a tomato plant (cv LOGAN) was transplanted and were irrigated equally during the study.

Table 1: Tested treatments

Weight of dry biomasses of tested species incorporated in 1 kg of soil (g)																					
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21
OR	60	40	20	0	0	0	0	0	0	30	20	10	30	20	10	0	0	0	20	13.33	6.67
RU	0	0	0	60	40	20	0	0	0	30	20	10	0	0	0	30	20	10	20	13.33	6.67
RO	0	0	0	0	0	0	60	40	20	0	0	0	30	20	10	30	20	10	20	13.33	6.67

100 seeds of each weed species (*Amaranthus retroflexus* L., *Chenopodium album* L., *Convolvulus arvensis* L., *Portulaca oleracea* L. and *Lolium perenne* L.) were sown at a depth of about 5 mm. Each treatment was replicated three times. 30 days after transplanting, plant number and dry weight were assessed for each weed species. The collected data were subjected to analysis of variance according to a completely randomized design using the Statistical Analysis System Version 9 (SAS V9); the significance of differences between treatments was determined by Duncan's multiple range test.

## Results and discussion

The use of aromatic plant biomasses buried in the soil determined a significant reduction of weed seed emergence and plant growth of *A. retroflexus* and *L. perenne*. On *A. retroflexus* all treatments showed an inhibition effect on both of seed germination and plant growth. Oregano alone was the most efficient with a reduction of 74% and 84% respectively in respect to the control. On *L. perenne* treatments with OR and OR + RU showed a significant difference compared to the control, OR was the most efficient with about 30% reduction for plant number and 68% on plant weight. No significant difference was obtained on *P. oleracea*. No effect was observed on *C. arvensis* and *C. album*.

Table 2: Effects of treatments groups on weed seed emergence and growth

Treatments group	Inhibition of emergence and plant growth (%) (1)					
	<i>A. retroflexus</i> number	<i>A. retroflexus</i> weight	<i>L. perenne</i> number	<i>L. perenne</i> weight	<i>P. oleracea</i> number	<i>P. oleracea</i> weight
OR	74.17 a	84.34 a	29.70 a	68.2 a	57.41 a	33.33 a
RU	59.38 abc	75.19 ab	9.52 b	-1.38 c	43.52 ab	22.22 ab
RO	32.00 d	56.25 bc	8.02 b	21.93 bc	-30.56 bc	11.11 ab
OR + RU	63.06 ab	81.68 a	25.48 a	48.06 ab	40.74 abc	48.15 a
OR + RO	52.63 bc	48.23 c	-3.52 b	23.87 bc	-44.44 c	-40.74 b
RO + RU	41.16 cd	67.69 abc	-2.95 b	-3.20 c	24.07 abc	44.44 a
OR + RO + RU	62.41 ab	75.32 ab	-2.83 b	16.06 bc	18.52 abc	14.81 ab
Control	0 e	0 d	0 b	0 c	0 abc	0 ab

(1) Values with different letters are statistically significant at 0.05 (Duncan's test).

## Conclusions

This study shows that the use of aromatic plant biomass mixed to soil could have a high potential to control weed emergence and plant growth. The Mediterranean area represents an arsenal of aromatic plants that can be screened with the objective of finding new natural bioherbicides. In this study oregano had the strongest inhibition activity on seed emergence of the tested weed species; thus, it would be recommended to test its effect on other weed species that cause major economic damage to the Mediterranean agriculture. The inhibition of weed seed emergence may have a practical meaning because these species are economically important in the Mediterranean area. So far, the obtained results make feasible further *in vivo* and *in vitro* researches as well as in open field to assess weed seed emergence and plant growth in order to develop an appropriate technology of essential oil and aromatic plant application for weed control.

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# Use of Defatted Seed Meals of *Helianthus annuus* L. and *Brassica carinata* A. Braun as Amendments on Escarole Endive

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The increase of biodiesel production in the next future will increase the production of defatted seed meals. These meals are rich in organic matter, nitrogen and phosphorus and, therefore, are potentially suitable for amendment and nutrition in agriculture. Moreover, meals obtained from crucifer plants can be very useful for soil biofumigation because *Brassica* spp. meals contain glucosinolates with biocidal properties (Lazzeri et al., 2004).

In this research are described results about the use of *Helianthus annuus* and *Brassica carinata* defatted meals, this last with biocidal activity, for amendment and fertilization of escarole endive (*Cichorium endivia* L.).

## Methodology

Trials were performed from Winter 2007 to Spring 2008 at Battipaglia (Salerno District, Italy) at the experimental station of C.R.A.-Research Center for Horticultural Crops, in a plane area of Sele Valley, on a clay loam soil. The experimental design was a randomized complete block with 2 replicates (plot area 50 m<sup>2</sup>). The following treatments were compared: sunflower seed meal (*Helianthus annuus* L.), Ethiopian mustard seed meal (*Brassica carinata* A. Braun), chemical fertilizer (MIN) and unfertilized control (CNT). Seed meals were uniformly surface-spread on November 12 and thoroughly incorporated in the first 0.20 m soil layer. Meals were used at a rate of about 2.5 (sunflower meals) and 2.3 (*B. carinata* meals) t ha<sup>-1</sup>, equivalent to the number of nitrogen units added in the mineral fertilized plots (120 kg N ha<sup>-1</sup>). Chemical fertilizer was supplied as “Entec 26”, a soluble ammonium sulphate plus 3,4 DMPP, an inhibitor of nitrification. Escarole endive variety Cuartana was trans-planted on November 13, with a density of 60.000 plants per ha<sup>-1</sup>, adopting bins with distance of 0.40 m between the rows, 0.33 m between the plants on each row and 1 m among the bins.

On January 23, soil samples were collected from plots of all the treatments at 0.20 m soil depth, to measure the impact of meals on some soil biological activities such as total hydrolase, dehydrogenase, phosphatase, sulphatase and  $\beta$ -glucosidase activities. Total hydrolase activity was assayed by fluorescein diacetate (FDA) hydrolysis and measuring the absorbance at 490 nm of released fluorescein (Schnürer and Rosswall, 1985). Dehydrogenase activity was assayed by measuring absorbance at 464 nm of iodonitrotetrazoliumformazan (INTF) obtained by reduction of 2(p-iodofenil)-3-(p-nitrofenil)-5-fenil tetrazolium chloride, after incubation for 48 h at 37°C (Von Merck and Schinner, 1991). Phosphatase, sulphatase and  $\beta$ -glucosidase activities were assayed, respectively, by 4-nitrophenyl phosphate, 4-nitrophenyl sulphate and 4-nitrophenyl  $\beta$ -D glucopyranoside hydrolysis and measuring absorbance at 398 nm of released para-nitrophenol (Tabatai and Bremner, 1969).

Harvesting was performed from assay areas (two for each replicates) of 7 m<sup>2</sup> each; total and marketable yield and unit weight of the tufts were measured.

All data were statistically analysed by ANOVA and means separated by Duncan's test. Statistical analyses were performed using the software MSTAT-C (Michigan State University, USA, 1988).

## Results

At harvest, performed on April 10, total and marketable yields obtained with *H. annuus* meal were 39.0 and 33.2 t ha<sup>-1</sup> whereas total and marketable yields obtained with *B. carinata* meal were 39.0 and 31.7 t ha<sup>-1</sup>, respectively (Table 1). Yields obtained with meals were statistically not different respect to total and marketable yields obtained with mineral fertilizer (45.2 and 38.6 t ha<sup>-1</sup>) and were significantly higher than yields obtained from control plots (19.5 and 16.2 t ha<sup>-1</sup>).

Total hydrolase and dehydrogenase activities were highest in the plots treated with the meals. In particular, hydrolase activity was significantly highest in the plots treated with *H. annuus* meal respect to mineral fertilized and control plots (Fig. 1a). No differences among treatments were observed for phosphatase activity whereas sulphatase activity was significantly highest, respect to fertilized and control plots, for the treatment with the meals (Fig. 1b). Respect to control plots,  $\beta$ -glucosidase activity was significantly highest for the treatment with meals or mineral fertilizer.

Table 1. Total and marketable yield of enviva escarola and weight of the tufts obtained in this trial.

Treatments	Total yield (t ha <sup>-1</sup> )		Marketable yield (t ha <sup>-1</sup> )		Unit weight of the tufts (g) (from tot. yield)		Unit weight of the tufts (g) (from mark. yied)	
Mineral fertilizer	45.2	A	38.6	A	753.2	A	643.2	A
<i>H. annuus</i> meal	39.0	A	33.2	A	656.8	A	558.8	A
<i>B. carinata</i> meal	39.0	A	31.7	A	649.9	A	527.7	A
No fertilized	19.5	B	16.2	B	324.2	B	269.3	B
<i>General mean</i>	<i>356.6</i>		<i>299.0</i>		<i>596.0</i>		<i>499.7</i>	

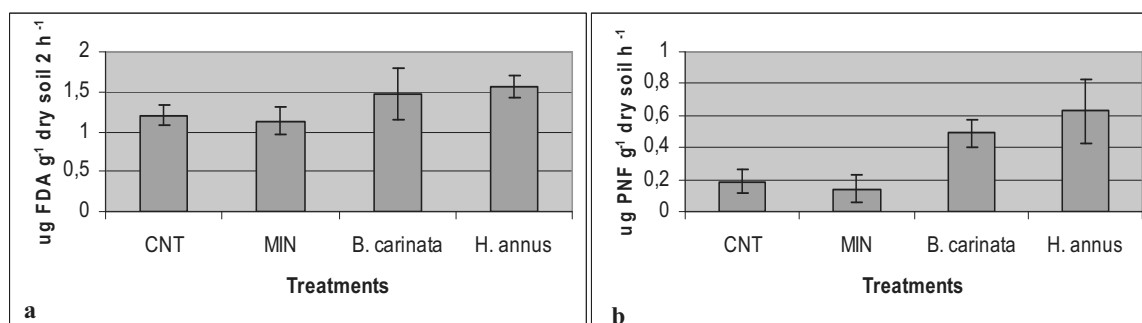


Fig. 1. Total hydrolase activity (a) and sulphatase activity (b) measured on January in this trial.

## Conclusions

Results indicate that *H. annuus* and *B. carinata* defatted meals are suitable for fertilization of escarole cultivated in open field in Winter-Spring cycle, probably because nitrogen is released slowly, as observed for nitrogen mineral fertilizer Entec 26 (containing nitrification inhibitors) used in this study. Results indicate, potentially, an increment of the major part of the biological activity indexes in the soil treated with the meals. However, the impact of the meals on soil biological activities during cold season was lowest respect to biological activities registered when meals are distributed during Summer-Autumn cycle (Zaccardelli et al., 2007).

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# Productivity of Cabbage by Intercropping System in Organic Vegetable Production

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## Introduction

Sustaining yield and income are more important objectives for farmers with limited resources than maximizing either yield or income. The improvement of agricultural sustainability favours the maintenance of the intercropping systems (Guvenc, 2006). Intercropping is an efficient soil conservation practice due to the increased ground cover that it provides, as well as the exploitation of different soil layers due to the different depth of the root systems of the two species (Zimmermann, 1996; Jarenyama et al., 2000). Intercropping, through more effective use of water, nutrients and solar energy, can significantly enhance crop productivity compared to the growth of sole crops (Midmore, 1993). Many studies have indicated that intercropping with different vegetables was more productive and profitable than sole cropping because of the complementary effects of intercrops (Brown et al., 1985; Olasantan, 1991).

Investigation was carried out to determine the effect of different intercropping systems on growth, morphological characteristics, yield of white cabbage in organic farming and to compare leaf photosynthetic rate of cabbage in sole stand and in intercropping systems.

## Methodology

Field experiment was conducted at the University Agriculture Centre Pohorski dvor, Maribor, Slovenia (46° 39'N, 15° 41'E) on loamy sand soil. A randomized complete block was used as the experimental design with four replications. Plot size was 4.5 x 3.6 m. In the study, white cabbage (*Brassica oleracea* L. var. *capitata* f. *alba* DC.) spacing was 60 x 75 cm in both sole-cropping and intercropping. White cabbage (cv. 'Kranjsko okroglo') was used as the main crop, and lettuce (*Lactuca sativa* L. var. *capitata* DC), tomatoes (*Lycopersicum esculentum* Mill.), celery (*Apium graveolens* L.), bush bean (*Phaseolus vulgaris* L. var. *communis*), leek (*Allium porrum* L.) and red beet (*Beta vulgaris* L. ssp. *rubra* L.) were used as intercrops. For all crops with the exception of red beet and bean the seedlings were used. Light-saturated photosynthetic rate, i.e. rate of CO<sub>2</sub> exchange in the leaf chamber ( $A_{max}$ ) of single cabbage leaf in sole crop and intercropping systems was measured 4 times during growth period of cabbage with Lcpro+ portable photosynthesis system. The crops were harvested when they reached marketable size and quality. Non wrapped leaf number and weight per plant, head diameter, head height, head weight and yield for cabbage were measured after harvest. LER (Land Equivalent Ratio) of different cropping systems based on cabbage, as an index of intercropping efficiency, was also evaluated. Furthermore, some growth parameters and yield for intercropped vegetables were determined. All measurements were effected from centre rows, after border rows were discarded to avoid edge effects.

## Results

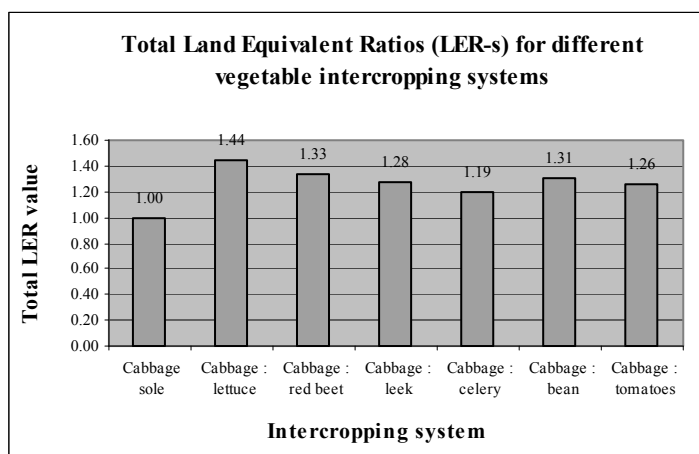


Table 1: Mean measured values for light-saturated single leaf photosynthetic rate ( $A_{max}$ ) in cabbage.

Treatment	$A_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
Cabbage sole	9.23 <sup>b</sup>
Cabbage : tomatoes	11.46 <sup>a</sup>
Cabbage : red beet	11.87 <sup>a</sup>
Cabbage : leek	11.92 <sup>a</sup>
Cabbage : bean	12.84 <sup>a</sup>
Cabbage : celery	13.71 <sup>a</sup>

Means followed by the same letter are not significantly different at the 95 % confidence level (Duncan test,  $p \leq 0,05$ )

Figure 1: Total LER values for different vegetable intercropping systems.

Different intercropping systems compared with sole cabbage cropping have significant effect on average plant weight, biomass production, total and market yield (data not shown). There were no significant effects of intercropping system on the main morphological characteristics in cabbage. The measurements of cabbage leaf photosynthetic rate showed significantly higher mean photosynthesis rate of cabbage grown alone compared to cabbage grown in association with other vegetable species (see Table 1). The highest market yield of cabbage was obtained in plots with cabbage and red beet intercropping and the highest average head weight in plots of cabbage intercropped with lettuce (data not shown). LER values were over 1.0 in all intercropping treatments and reached the highest value 1.44 in case of cabbage and lettuce intercropping (see Figure 1).

## Conclusions

The preliminary results have indicated that cabbage based intercrop treatment might increase productivity in organic field vegetable production. This efficiency might be attributed to component crops difference in growth duration so that their maximum requirements for growth resources occur at different times. In this study, the differences of growth rhythm, time of maturity, morphological characteristics or resource use of cabbage as sole crop and intercrops might have reduced competition between component crops because of complementary effects of intercrops. We can also assume that higher leaf photosynthetic rate in intercropped cabbage could be to some extent realized in the improved canopy photosynthesis.

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**SUB SESSION 1.2**  
**TECHNOLOGIES FOR BIODIVERSITY CONSERVATION**

1.2a - ECOLOGICAL INFRASTRUCTURE – THE INTERACTION BETWEEN NATURAL  
RESOURCES AND AGRICULTURE

Chairmen: Hartmut Stützel and Olaf Christen



# Relationships between natural conditions and crop production. Case study for Poland.

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## 1. General information concerning Poland (level NUTS 1)

Poland counts about 38.1 mln inhabitants and covers the area of 31.1 mln ha, including 19.1 mln ha of rural land, which gives about 0.42 ha per person. This area looks quite sufficient to provide food self-sufficiency of the country, but the natural conditions for agricultural production are much worse than in Western Europe. It results from dominance of light, sandy soils and unsuitable climatic conditions. Soils in Poland are of glacial origin, what means that they are very heterogonous, deeply leached, acid and poor in plant nutrients.

Scandinavian glacier entered Polish area 3 times and it formed the oldest Polish soils on the South (Mindel glaciations) and the youngest soils in North areas (Wurm glaciations). The soils considered as very light (0-10% of <0.02 mm fraction) and light (11-20% of <0.02 mm fraction) cover 59.5% of arable land and soils considered as very acid (pH<4.5) and acid (pH 4.6-5.5) cover 56.6% of arable land. Almost 40 % of soils show very low and low content of available phosphorus and half of them very low and low potassium content. The climate in Poland is transient between maritime and continental which means its instability. The mean temperature and rainfall are lower and vegetation period shorter than in Western Europe. The average length of vegetation period is about 210 days and is comparable with Scandinavian countries. Practically some intensive crops as winter barley or corn for grain are not recommended for cultivation in the North-East from Vistula river . The average annual temperature oscillates between 6 °C and 8.8 °C. The precipitation is the main source of water resources. Its average annual volume is 500-600 mm in the lowlands and 1200-1500 mm in the uplands and mountains.

The environmental potential for agricultural production is described by Land Quality Index LQI generated at the Institute of Soil Science and Plant Cultivation at Pulawy, Poland. The LQI is an aggregated indicator based on the score for soil quality, agro-climate, soil moisture index and land relief according to their relative magnitude in the impact on crop productivity. In the valorisation of agricultural land soil quality is the main denominator of land quality as defined by texture of soil profile and location within the terrain. Across the Poland LQI ranges between 31 and 111 scores. Comparison of agricultural land quality in Poland and in some European countries shows that in Poland there is only 0.25 ha per 1 inhabitant of the same quality agricultural land as in Belgium, the Netherlands and Germany.

The structure of rural land (59% of Poland total area) is predominated by agricultural land (15.8 mln ha). The crop structure is dominated by cereals. Wheat is the most popular cereal in Poland.

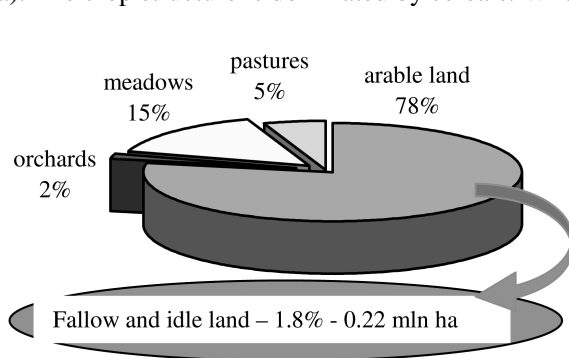


Figure 1. Structure of agricultural land in Poland

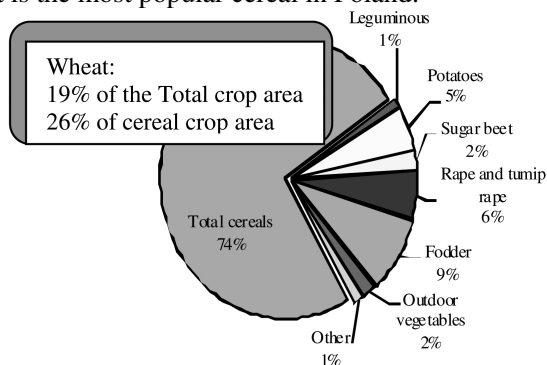
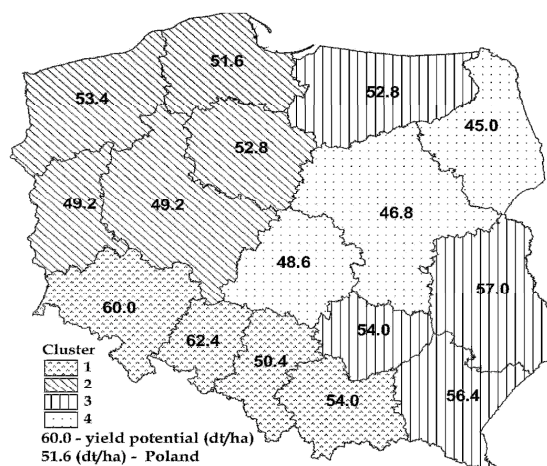


Figure 2. Crop structure in Poland

The cereal yields are close to the world average (about 30 dt per ha) but it is only half of the yield in Western Europe. Current level of mineral fertilization (123.3 kg of NPK per ha of arable land and 55 kg CaO per 1 ha) and the utilization of chemical plant protection substances (1.32 kg of the active ingredient) in Poland comparing to European standards should be assessed as moderate. It will not have a detrimental impact on the quality of agricultural land and agricultural products. From this point of view, production is environmentally safe.

## 2. Analysis of the factors influencing cereal productivity in regions (level NUTS2)

It was decided to conduct this analysis on the regions levels due to the availability of the data. The analysis for 16 regions of Poland was performed by cluster method according to the furthest neighbor (complete linkage). The analysis allowed to divide the area of Poland into four clusters of different cereal productivity dependent on agriculture land potential. Yield potential of cereals was defined as the productivity of arable soils attained in differentiated soil and climate conditions and the best management practices. The calculations considered the cereals share in crop structure of regions and share of soil suitability complexes in structure of arable land. The highest cereal yield potential was found in the regions of South Poland (cluster 1) and in the regions of South-Eastern Poland (cluster 3). However the best utilization of the potential is in regions of Western and North-Western Poland (cluster 2) and South Poland (cluster 1).



The highest yields are obtained in South of Poland. The four regions included into cluster 1 are characterized by the highest values of the utilization of agricultural land, LQI, rainfalls and use of mineral fertilizers. The highest index of agricultural land potential utilization is noted in Western and North-Western Poland with the smallest share of poor soils and the highest use of mineral fertilizers. The smallest cereal productivity is in both Eastern and Central regions. However regions of cluster 3 are characterized by high LQI value and small use of fertilizers. Regions of cluster 4 are distinguished by small LQI values due to high share of poor soils, relatively small amount of rainfall.

Figure 3. Cereal yield potential in regions of Poland

Table 2. Characteristics of regional diversification of agriculture potential in Poland

Cluster	Actual yield of cereals	Utilization of yield potential	Land Quality Index	Actual rainfall sum	Use of NPK fertilizers	Negative bonitation score of soil fertility
-	dt ha <sup>-1</sup>	%	-	mm	kg ha <sup>-1</sup>	-
1	37.6	72.1	76.4	780	110.4	42.4
2	31.4	73.6	66.4	586	114.4	37.8
3	27.7	60.3	70.0	638	68.3	48.7
4	24.8	63.5	59.1	563	76.7	58.1

## Conclusions

The regions of Poland are differentiated in agricultural land potential and its realization. Cereal yield potential ranges from 45 to 62.4 dt ha<sup>-1</sup> and its utilization ranges from 60.3 to 78.4%. The differences should be explained by both natural (soil and climate) conditions and production intensity.

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# Physiological Determinants of Grain Nitrogen in Wheats Released Since 1940 in Mediterranean Spain

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## Introduction

In wheat and other cereal crops, yield and grain N concentration (GNC) have been negatively correlated (e.g. 1, 2) due to a dilution effect that a higher number of grains per m<sup>2</sup> (higher yielding cultivars) produce to the concentration of N in the grains. Understanding the bases behind grain N accumulation in the grains could help breeders to break the barrier that the negative relationship between both traits have been producing, clarifying future avenues to improve yield and GNC simultaneously or at least improve one of them without negative effect on the other.

GNC depends mainly on the remobilization of the stored N accumulated in vegetative organs before anthesis (4). The ability of the crop to export N from vegetative tissue may come from either improved capacity of the grain to accumulate N or through greater N supply to the grains (2). In general, GNC decreased with the year of release of the cultivars due to a dilution effect (e.g. 1, 3) that revealed an important degree of source-limitation for grain N accumulation. However, breeding has modified the source-sink balance, a fact that could imply changes in the degree of source-limitation that wheat in general is reported to have for grain N accumulation.

This study aimed to clarifying the main physiological determinants of grain N accumulation in wheat under Mediterranean conditions. For this, the effect of breeding and source-sink ratios treatments (post-anthesis trimming or pre-anthesis shading) were analysed under different experimental conditions.

## Methodology

Seven field experiments were carried out at north-eastern Spain during the 2005/06 and 2006/07 growing seasons. Three of the experiments were conducted at Giménells (41°37'N, 0°22'E, 248m) under high-inputs and well irrigated conditions (Exp. 1, 2 and 3), other two experiments at Giménells were under moderate-inputs and rainfed conditions (Exp. 4 and 5), while the other two experiments were at Foradada (41°51'N, 1°0'E, 407m) under low-inputs and rainfed conditions (Exp. 6 and 7).

Three bread wheats (Aragon 03, released in 1940; Estrella, 1960; Anza, 1978) and an advanced line (ID-2151) developed by IRTA were compared. They were selected from a previous study (5) as representative of their times under cultivation in Mediterranean Spain but also they fit well the overall trends of breeding effects from 1940 to 2005.

The experiments were grouped as i) descriptive experiments (Exp. 1, 3 and 6) which explore breeding trends in the four cultivars, ii) post-anthesis trimming experiments (Exp. 2 and 7) that consisted of the factorial combination of the four cultivars and two source-sink ratios during grain filling (control and trimmed spikes: all the spikelets from the upper half of the spikes in a 1.2-m<sup>2</sup> quadrant were hand trimmed seven to ten days after anthesis) and iii) pre-anthesis shading experiments (Exp. 4 and 5) that consisted of the factorial combination of two contrasting wheats (Aragon 03 and ID-2151) and two pre-anthesis shading treatments (un-shaded control and shading from jointing to anthesis with a black shade cloth that decreased light intensity by  $75 \pm 2.3\%$ ).

At anthesis and maturity all plots and subplots, depending on the experiment, were sampled. Samples consisted of all plants in 50 cm of central rows at anthesis or 100 cm at maturity. From these materials, leaf blades, stems (including leaf sheaths), spikes and grains (the last one only at maturity) were separately, oven-dried, and milled. Nitrogen was determined by a micro-Kjeldahl method.

## Results

N content per grain (mgN grain<sup>-1</sup>) was significantly different between cultivars in Exp. 1, 3 and 6, showing that the newer the cultivar, the lower the N content per grain. These differences were not

evident for the grain N yield (GNY) and total N accumulated by the crop ( $\text{kgN ha}^{-1}$ ) at maturity.

All cultivars increased significantly their N content per grain in both experiments in response to the trimmed treatment, showing an increased of 33 and 40% (mean of all cultivars) of the N content per grain in Exp 2 and 7, respectively. The degree of source-limitation for N accumulation in the grains increased with the year of release of the cultivars (and so with the grain number per  $\text{m}^2$ ) in both experiments from 23% in the oldest cultivars to 48% (mean of both experiments) in the most modern line. However, the increase in N content per grain did not compensate the GNY loss due to the trimmed treatment and so control plots had higher GNY in both experiments for all cultivars.

Pre-anthesis shading increased significantly the N content per grain in both cultivars and experiments (Exp.4 and 5), being the N content per grain 21% in Aragon 03 and 53% (mean of both experiments) in ID-2151. Again, when the number of grains per  $\text{m}^2$  increased (modern line), the degree of source-limitation for N accumulation in the grains was increased. However, GNY was 43% (mean of both cultivars and experiments) higher in the un-shaded control than in shaded plots. Thus, the increased in N content per grain did not compensated the yield loss due to shading.

Summarizing the results from all experiments it can be seen that final N content per grain depends strongly on the source-sink ratio established at around anthesis between the number of grains set and the amount of N absorbed at this stage by the crop (Figure 1). Thus, breeding improving yield through increases in grain number per  $\text{m}^2$  reduced the N content per grain provoking the above mentioned dilution effect. This dilution effect is further confirmed by the reverse effect produced by grain number reductions due to shading or directly trimming the spikes after anthesis.

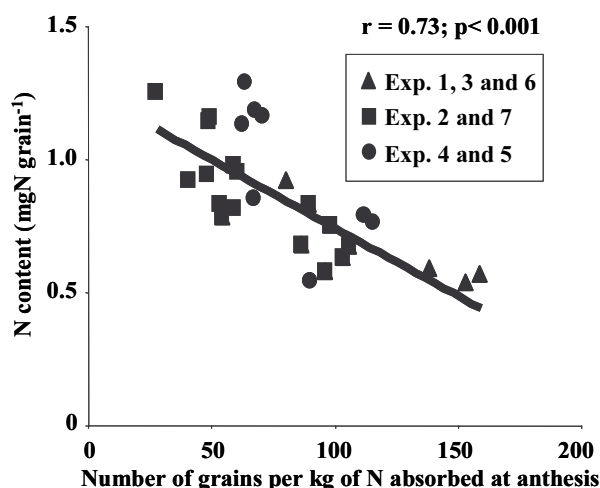


Figure 1. Relationship between the N content per grain and the ratio between the number of grains per  $\text{m}^2$  and the amount of N absorbed at anthesis for the descriptive experiments (triangles; Exp. 1, 3 and 6), post-anthesis trimming experiments (squares; Exp. 2 and 7) and pre-anthesis shading experiments (circles; Exp. 4 and 5).

## Conclusions

Grain N accumulation in wheat under Mediterranean conditions is mainly determined by the N source-strength at anthesis. Then increases in grain number per  $\text{m}^2$  produces a dilution effect on the N content per grain.

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# The Economic, Agronomic and Energetic Efficiency of Conservation Tillage in Organic Systems of Western France

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## Introduction and objective

Several authors have studied conservation tillage, from agronomical, economical and societal viewpoints, as an alternative approach for sustainable management of arable crops, but references from organic systems are less numerous. The aim of this study was to evaluate the influence of conservation tillage (shallow ploughing, direct drilling, reduced tillage) under organic farming conditions on yield, weeds, economical aspects, and energy balance using different indicators.

## Methodology

Four different types of tillage seedbed management were compared on a network of farm fields over 2 years: mouldboard ploughing – MP, reduced tillage – RT and minimum tillage – MT. The four situations studied were the following:

- A: RT (11cm) vs. MP (23 to 27 cm) with a wheat crop followed by a lupine
- B: RT (18cm) vs. MT with wheat and triticale
- C: MP (25cm) vs. RT (10cm) with maize and faba bean
- D: MP (20cm) vs. RT (15cm) with wheat and maize

Soils were loam. Weeds were collected on six plots of 0,25m on each field and the dry matter weighed, at the end of winter and at harvest. Yields were measured at maturity. Management practices were recorded by the farmer including time spent and machinery used. The following costs were calculated: mechanisation, labour, input and crop production costs as well as net profit, and competitiveness ratio. For energy evaluation, “Planete” software was used.

## Results

Reduced tillage did not lead to significantly increased weed development. High weed levels observed at harvest in second year B and first year D were instead caused by the absence of mechanical control. Sowing method was more important than tillage technique in determining weed levels. For example, higher levels of weeds were observed in MP sown linearly than in broadcast sown RT.

Yields depended on weed levels but were not correlated with soil management. No significant yield differences were recorded between treatments (Fig1). The only case of MT showed considerable weed problems.

The variability between situations as regarded profit margins and production costs per unit of produced grain was better explained by yield variability than by varying production costs. Production costs depended on two main factors: traction time, and use of co-ownership, which together diminish mechanisation costs and therefore production costs.

Reductions in energy consumption and production costs as a result of conservation tillage techniques were variable depending on farms and crops. The time spent for traction is key: mechanisation costs represented 60% of total costs (Fig2), and fuel consumption 57% of total energy consumption. Economic and energetic (Fig3) gains were not systematic with RT and MT, when classic machinery was used.

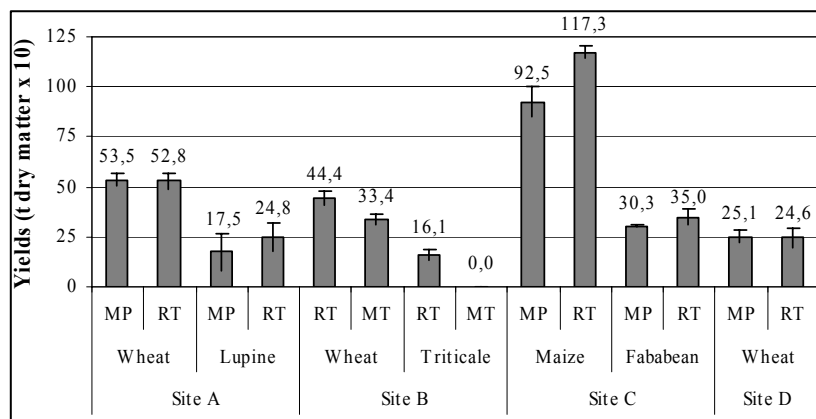


Fig1 – Average yields according to site, treatment and crop

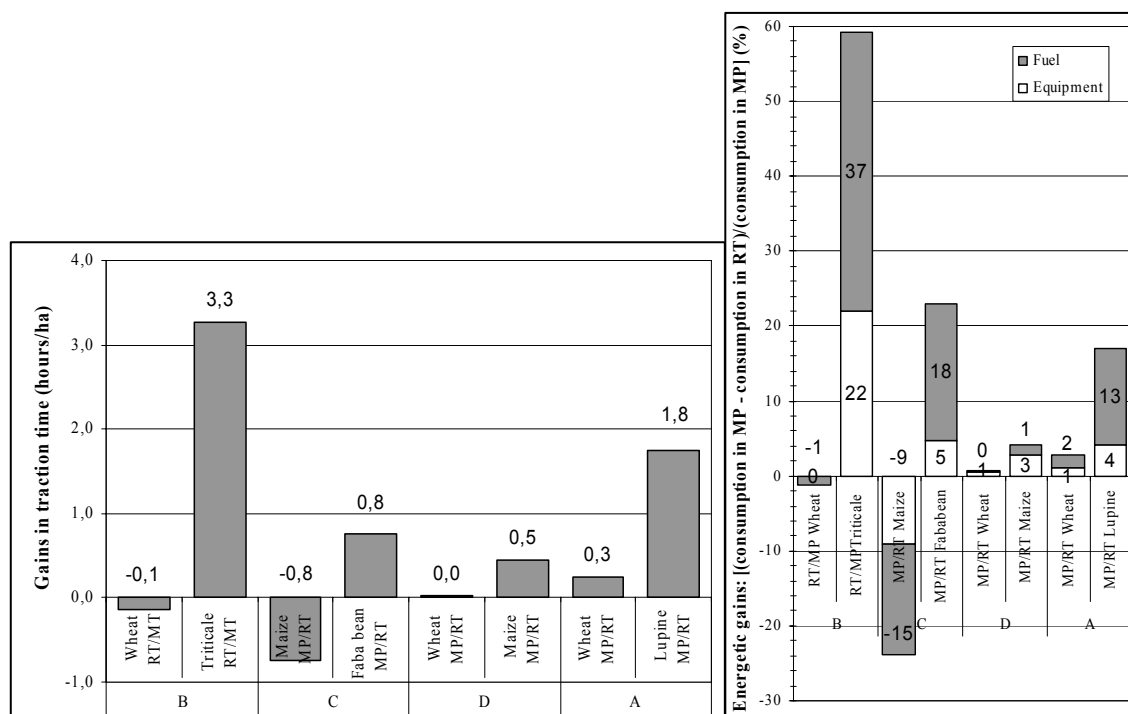


Fig2 : Gains in traction time (traction time MP – traction time RT or MT)

Fig3 : Gains in energy consumption with RT or MT compared to MP.

## Conclusions

This study would need to be extended over a longer period, and results also compared at the rotation scale. Finally, the different criteria recorded should be weighted according to the farmer's objectives.

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# Particle-Size Distribution and Associated Organic Matter under Different Cropping Systems and Tillage Practices in a Semi Arid Environment

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The aim of this study was to evaluate the long-term impact of different management practice and tillage tools on :1) changes in soil carbon stock with different cropping systems and soil tillage managements; 2) aggregate size distribution and organic carbon concentration of each fraction; 3) organic matter composition and stability

## Methodology

The research was carried out at the Pietranera farm, located in southern part of central Sicily (Italy) (37°32'74" N / 13°31'53" E; elevation 236 m; mean annual precipitation 481 mm; mean air temperature 19 °C) on a soil of a long term experiment. The soil is classified as a fine-clayey, calcareous, mixed, xeric Chromic Pelloxerert with a slope of 4%. The soil were sampled before the start of the experiment and had 471 g kg<sup>-1</sup> clay, 225 g kg<sup>-1</sup> silt, 304 g kg<sup>-1</sup> sand, 18,1 g kg<sup>-1</sup> SOC (soil organic carbon), 1,29 g kg<sup>-1</sup> N, and a pH of 8,1. The twelve plots (twenty years long term trial) used in this test area follow two crops rotations, wheat-wheat (W) and wheat-bean (WB), with three different soil managements: 1) traditional (CT = conventional tillage) consisting of a ploughing at 30-35 cm depth; 2) dual layers tillage (DL) with a chisel at 40 cm and 15 cm depth; 3) no tillage (NT). Soil sample were taken at 0-20 cm depth, air dried and sieved at 2 mm. Wet aggregate-size fractions, with no chemical dispersion were isolated by mechanic shaking of 50 g air-dry 2 mm sieved sample of soil on a column of 2000, 1000, 500, 250, 75 and 25 µm sieves. After the physical fractionation, we distinguished three main fractions: 75-250 µm (large microggregates), 25-75 µm (small microaggregates), < 25 µm (silt and clay fraction). The relative distribution of nitrogen and organic carbon content as well as the <sup>13</sup>C natural abundance were measured for these fractions. Cumulative carbon input of the cropping systems were calculated according to Kong et al. (2005).

## Results

The carbon content in the different experimental plots does not vary greatly, ranging from 18,3 g kg<sup>-1</sup> up to 21,2 g kg<sup>-1</sup>. The highest carbon content values were found under the wheat monocropping system with no-tillage (WNT) (21,2 g kg<sup>-1</sup>) and conventional tillage management (WCT) (20,8 g kg<sup>-1</sup>), respectively. Comparing the three different soil management techniques, NT shows a higher influence on organic carbon accumulation (20,4 g kg<sup>-1</sup>), than the CT and dual layer (DL) (19,7 g kg<sup>-1</sup> and 18,3 g kg<sup>-1</sup>). The estimated cumulative C input, ranged from 6,5 Mg C ha<sup>-1</sup> in WBDL to 7,0 Mg C ha<sup>-1</sup> in WCT. Annual SOC sequestered is higher for W than WB (0,16 Mg C ha<sup>-1</sup> yr<sup>-1</sup> vs. 0,05 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, p<0,05). Among the different soil managements techniques the DL seems to be less effective in soil organic carbon protection. Regarding the different soil aggregates, the <25 µm

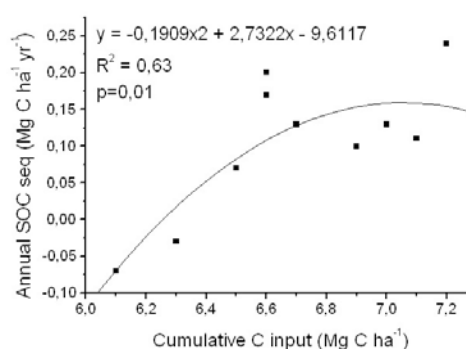


Fig. 1. Relationship between annual sequestered soil organic carbon and cumulative carbon input across the 2 different cropping systems and 3 different soil tillage management practices.

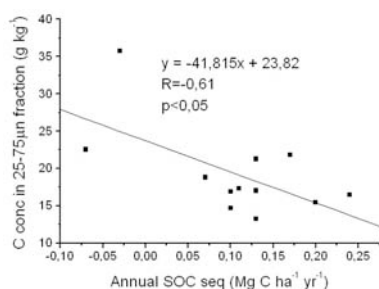


Fig. 2. Relationship between annual sequestered soil organic carbon and SOC concentration in 25-75 µm fraction of all

the 75-250 µm aggregate size show a C enrichment (23,9 g kg<sup>-1</sup>), while the corresponding values of the 25-75 µm and 0-25 µm fractions do not differ greatly from the bulk soil concentration (19,3 g kg<sup>-1</sup> and 19,8 g kg<sup>-1</sup> respectively). According to Gerzabek et. al. (2001), we compared C and mass distributions between size fractions and found that large microaggregates (75-250 µm) were enriched in organic C, whereas small microaggregates and silt and clay fraction showed no enrichment or depletion (0,98 and 0,99). The amount of annual sequestered SOC, is a consequence of the cumulative carbon input (fig.1), and, when compared to C the concentration in different soil size fractions, a trend of decreasing annual SOC sequestration with increasing soil size aggregates can be detected (fig.2). The importance of the < 25 µm fraction in characterising the bulk soil features is pointed out by the amount and quality of organic matter stored in small microaggregates. The strong correlation between the bulk SOC concentration and SOC concentration in the <25 µm fraction is only due to wheat residues, since no correlation between the bulk SOC and the SOC concentration in < 25 µm fraction was found under WB rotation (fig 3).

## Conclusions

Fifteen years of conservative tillage and crop rotation did not result in significant differences in C accumulation or aggregate stability (the non-existing differences are not mentioned before in the text). Among the different tillage techniques, NT seems to improve the organic carbon content, mostly due to the contribution of the C stored in small microaggregates and the silt-clay fraction. 75-250 µm fraction show an enrichment of C (C/mass ratio = 1,20). The strong relationship between δ<sup>13</sup>C values of the bulk soil and <25 µm fraction under wheat cropping systems demonstrates the predominant contribution of these crop residues to the composition of organic matter. The higher nitrogen concentration in soils samples under wheat, both in bulk and in < 25 µm fraction, suppose a greater stability of the associated organic matter (by N-bonding mechanisms to mineral surfaces). The comparison of our data with those of other long-term agricultural experiments sites suggests that cropping systems in Sicily have lower efficiency in sequestering C from added C inputs.

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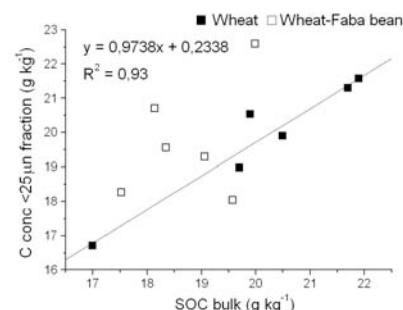


Figure 3. Relationship between SOC concentration of bulk soil and SOC concentration in <25 µm fraction distinguished for wheat and wheat-faba bean

# Assessment of Crop Management Using Agro-Ecological and Economic Indicators

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The sustainability of agricultural systems is frequently evaluated with indicators. Each indicator deals with one aspect of sustainability. The results are frequently presented separately, while an integrated evaluation could benefit from the calculation of a single sustainability index.

The objectives of our work are: i) to set up and test a framework for the economic and environmental sustainability assessment based on indicators, ii) to apply it on commercial farms in the Sud Milano Agricultural Park (Italy), and iii) to integrate these indicator values into a global index.

## Methodology

During the period 2005 – 2006, seven farms were monitored through face-to-face interviews with farmers. A set of indicators was selected from literature (Castoldi and Bechini, 2006; Castoldi et al., 2007) and applied to 131 fields, grouped by crop succession type: continuous maize (Mc), maize with other crops (Mo), continuous rice (Rc), rice with other crops (Ro), permanent meadows (PM), and other cereals (Ce). The indicators were grouped in five classes, describing the management of:

- i) economic resources (variable costs [VC], gross income [GI], and gross margin [GM]);
- ii) nutrients (N [NS] and P soil surface balances [PS]);
- iii) energy (energy inputs [EnIN] for gasoline, lubricants, pesticides, fertilisers, seeds and machinery; energy output [EnOUT], and energy gain [EnG]);
- iv) pesticides (Load Index, calculated for several non target organisms: algae [LIa], crustaceans [LIc], fish [LIf], and rats [LIr]);
- v) soil (crop sequence indicator [CS], that evaluates the goodness of each previous-successive crop combination; soil cover index [SC], that evaluates the percentage of soil cover by crops in one year; and soil organic carbon indicator [OCI], that evaluates if the management on a specific soil tends to accumulate or deplete soil organic carbon).

In order to provide a global evaluation of the systems, the indicator values were aggregated into an index to facilitate the use of complex information by non-experts. To do this, optimum and unsustainable ranges for each indicator were derived from literature or from expert knowledge, and a specific sustainability function (Fig. 1) was then used to convert each indicator value into a sustainability index, which equals 1 if the indicator value is in the optimum range (between  $S_{opt1}$  and  $S_{opt2}$ ; Fig. 1), and 0 if it is in the unsustainable range (below  $S_{min}$  and above  $S_{max}$ ; Fig. 1). In order to avoid a sharp boundary, the sustainability index assumes intermediate values when the indicator lies between the sustainable and unsustainable, described by a user-defined linear or non-linear function, defined as

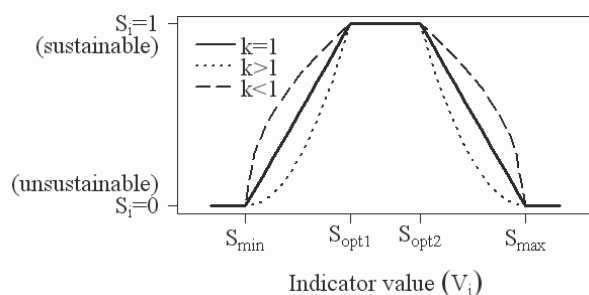


Fig. 1. A general type of function used to convert indicator values ( $V_i$ ) into sustainability scores ( $S_i$ ).

$S_i = ((V_i - S_{min}) / (S_{opt1} - S_{min}))^k$  for the left side of the curve, and  $S_i = ((V_i - S_{max}) / (S_{opt2} - S_{max}))^k$  for the right side. The exponent  $k$  produces a stretch of the curve: when  $k > 1$ , the sustainability

changes rapidly close to optimum range, while when  $k < 1$  the sustainability changes rapidly close to the unsustainable range;  $k > 1$  is used when indicator values outside the optimal range produce an immediate reduction of sustainability: this situation is rare in biological systems with buffer systems and vice versa. A value of  $k = 1$  is used when it is not possible to discriminate between the above-mentioned cases. The  $S_i$  were averaged by indicator class and by field in order to obtain a global sustainability index ( $S_g$ ) ranging from 0 to 1.

## Results

The results of the indicator (Table 1) show that rice- and maize-based successions have good economic performances, due in the first case to the high price of the products, and in the second to the high yields.

Rice-based successions have high potential impact on environment due to intensive use of pesticides and to low energy production, while the maize-based have generally high nutrient surpluses due to the large use of fertilisers and manure. Organic carbon is rather good in Mc due to the high amounts of residues and manures applied. Permanent meadows have very low environmental impact (low energy consumption, low nutrient surpluses, no pesticide applied), but poor economic performance. Permanent meadows obtained the highest  $S_g$  (Fig. 2), with moderate values for economic and energy indicators. Continuous rice obtained the lowest  $S_g$ , while maize was intermediate, with good economic and energetic performance.

## Conclusions

The indicators chosen for the analysis describe a large range of economic and environmental issues and make it possible to clearly separate and characterise different cropping systems. The procedure for their calculation is transparent and sound, and can be applied for *ex-ante*, *ex-post* and monitoring procedures of sustainability assessments. It can be easily expanded by adding other indicators, and can be tailored by changing the thresholds used to calculate  $S_i$ .

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Castoldi N. et al. 2007. Agro-ecological indicators of field-farming systems sustainability. II. Nutrients and pesticides. *Ital. J. Agrometeorol.*, 12:6-23.

Table 1

Average of indicators calculated for 131 fields monitored over a two-year period.

Crop succession <sup>1</sup>		Mc	Mo	Rc	Ro	PM	Ce
# fields monitored		50	21	24	3	31	2
<u>Economic indicators</u>							
VC	€ ha <sup>-1</sup>	583	445	692	466	145	188
GI	€ ha <sup>-1</sup>	1616	1284	2052	1736	876	951
GM	€ ha <sup>-1</sup>	1033	840	1360	1270	731	763
<u>Nutrient management indicators</u>							
NS	kg N ha <sup>-1</sup>	182	72	75	55	64	-18
PS	kg P ha <sup>-1</sup>	38	0	-5	-15	0	-12
<u>Energy management indicators</u>							
EnIN	GJ ha <sup>-1</sup>	27.8	22.0	22.6	18.8	13.5	10.7
EnOUT	GJ ha <sup>-1</sup>	364	257	193	205	156	127
EnG	GJ ha <sup>-1</sup>	337	235	170	186	143	117
<u>Pesticide toxicity indicators</u>							
LIa	TOX ha <sup>-1</sup>	108	106	259	144	0	0
LIc	TOX ha <sup>-1</sup>	1.4	15.5	7.6	4.1	0.0	0.0
LIf	TOX ha <sup>-1</sup>	2.2	2.4	8.5	7.6	0.0	0.0
Llr	TOX ha <sup>-1</sup>	1.5	0.8	8.5	3.6	0.0	0.5
<u>Soil management indicators</u>							
CS	0-10	2.0	4.6	1.0	4.1	10.0	3.5
SC	0-1	0.35	0.48	0.33	0.41	1.00	0.45
OCI	0-10	6.3	4.6	4.3	2.1	10.0	1.4

<sup>1</sup> For the abbreviations of crop successions, see text.

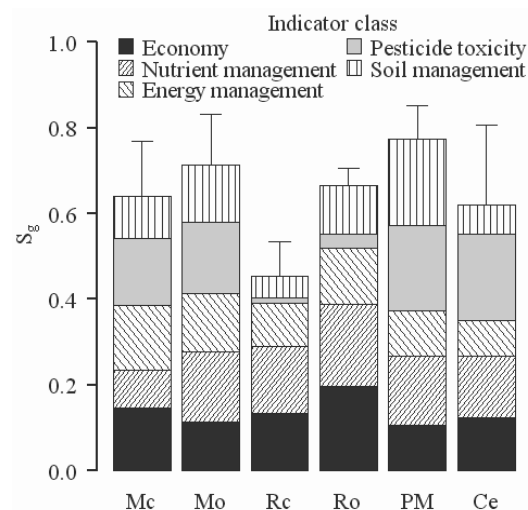


Fig. 2. Contribution of the five indicator classes to the global sustainability index ( $S_g$ ). For the abbreviations of crop successions, see text.



# Agricultural Land Use and Optimization of the Ecological Network Pattern: the Apulian “Monti Dauni Meridionali” Case Study

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The concept of “ecological network” is an important issue in the frame of the European nature conservation policy; a progressive expansion of its target is quite evident today: from a strategy to protect biodiversity, wildlife and natural habitats this new paradigm is becoming an overall approach to land planning in accordance to nature. This concept is supposed to be an effective response to the fragmentation of natural systems, a degradation process mostly caused by human activities, and a means to restore the ecological connectivity. Connectivity relates to the organism possibility to pass through the diverse landscape patches, driven by the inherent suitability of each patch as well as by the pattern of the network itself, intersperse into a particular milieu. Connectivity, therefore, is an emerging landscape property resulting from the specific interaction between organism and environment. Avoid fragmentation and promote connectivity is the key to correctly design an ecological network; the present work is an attempt to identify a methodological procedure in this direction.

## Methodology

The reference geographical area comprises “Monti Dauni Meridionali” and the subtended plain (193,119 ha wide), in the Apulia region, South-East Italy; it is characterized by a mosaic of agricultural patches also including important natural zones (“Community Importance Site” according to the European “Habitat” Directive 1992/43). The cartographic base of the study is a land cover/land use map at a scale of 1:10.000 (Ufficio Piano, Foggia Province), structured according to the Corine, level 4, classification. A geographic information system (Arcview 9.2) was employed, together with the software Fragstat 3.3 (McGarigal and Marks, 1994) in order to estimate landscape metrics. A map of biotopes was produced, properly merging land cover classes; fourteen biotope typologies were obtained as a whole. A “naturalness degree” was defined for each biotope according to a cardinal ordering methodology which assigns values ranging from 0 (minimum naturalness) to 1 (maximum naturalness). Six representative animal species, belonging to different biotope typologies, were selected: *Picus viridis* is distinctive both of forest and agricultural areas with significant natural vegetation; *Miliaria calandra* is typical of agricultural land; *Emberiza cirrus* is dominant in mixed agricultural and natural lands; *Potamon fluviatile*, *Motacilla cinerea* and *Cettia cetti* are mostly linked to riparian vegetation. Each biotope was then related to every single animal species according to specific ecological properties (niche functions), assigning a suitability score of 0, 1, 2 or 3 (Boitani, 2002). Multiplying the species suitability score by the naturalness degree of each biotope, a preliminary potential single species suitability map was produced. The six maps so obtained were then overlaid each other thus yielding a general potential suitability map. Diagnostic connectivity indicators, derived from the landscape ecology approach, were calculated in order to compute the actual landscape connectivity.

## Results

The general potential suitability map was the starting point of the following landscape analysis and interpretation of the resulting “ecological network” structure and function. The riparian vegetation areas are the most suitable, in a broad sense, to animal species, while the irrigated crop fields the less attractive (apart from built up areas). The fourteen biotope typologies were clustered and ranked into 5

classes, according to their suitability score, from 1 (minimum suitability) to 5 (maximum suitability); the resulting biotope aggregation is reported in *Table 1*. “Landscape metrics” were computed in order to assess connectivity according to the following landscape properties: composition, fragmentation, aggregation and interspersions with respect to each suitability cluster (*Table 2*). The composition index *Pland* is related to the surface percentage share of the landscape; the fragmentation indexes are expressed by the proportional number of patches (*NP*), the proportional patch density (*PD*) and the edge density (*ED*); the patch aggregation properties are stated by the landscape shape index (*LSI*) while a measure of patch adjacencies is linked to the interspersions and juxtaposition index (*IJI*). Cluster 2, characterized by the most intensive agricultural crop, resulted the dominant class (highest *Pland*) and the most intersperse (highest *IJI*), so it could represent the *matrix* of the “ecological network”. Cluster 3 and 5 showed the minimum landscape share (lowest *Pland*) but also the minimum fragmentation (lowest *NP*, *PD* and *ED* values); since cluster 3 turned out to be the most aggregated (lowest *LSI*) as well as greatly intersperse (high *IJI*), its patches could effectively play the role of *stepping stones* or *secondary nodes* in the frame of the “ecological network”. Cluster 5, consisting of patches with the highest suitability, characterized

**Table 1 Clustering and ranking of biotopes according to their suitability score**

Rank	Clusters of biotopes
1	Bare rock, Fruit trees, Vineyards, Irrigated land
2	Olive grows, Bush and garrigue, Non-irrigated arable land
3	Coniferous reafforestation, Pasture and natural grassland, Complex cultivation pattern, Lakes and basin
4	Land principally occupied by agriculture with significant areas of natural vegetation, Prevalent broad-leaved forest
5	Riparian vegetation areas

**Table 2 Landscape Ecology Indicators (acronyms explained in the text)**

Clusters of biotopes	<i>Pland</i> (%) composition	<i>NP</i> (%)	<i>PD</i> (n/100 ha) fragmentation	<i>ED</i> (m/ha)	<i>LSI</i> (-) aggregation	<i>IJI</i> (%) interspersions
1	10.2	20.3	0.43	12.70	44.21	24.41
2	77.8	23.7	0.50	31.05	40.67	89.14
3	3.0	9.6	0.20	4.53	28.93	69.73
4	7.3	32.5	0.69	13.61	55.58	35.65
5	1.7	13.9	0.29	5.28	45.26	39.56
Landscape	100.0	100.0	2.11	33.60	39.03	66.34

emphasized several functional limitations and structural constraints. Similarly, cluster 4, which contains biotopes of relevant suitability, but is greatly fragmented (highest *NP* and *PD*), the most disaggregated (highest *LSI*) and among the less intersperse; as a consequence, its potential role of *primary node* in the network is very poor and should be greatly reinforced. Finally, cluster 1 contains the less natural biotopes and could be associated to cluster 2 in its *matrix* role.

## Conclusions

The methodology applied was able to clearly highlight the features of strength and weakness related to the planning of the ecological network. Different structural and functional role of the network components have been identified together with the limitations and constraints that must be considered in order to improve landscape connectivity.

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- This work has been carried out in the frame of the research project “CyberPark 2000”, POR Puglia 2000-2006, Measure 6.2, Action C.*

# Cropping Sequences and Soil biodiversity. Case Study of Intensive Cropping Systems in the East Po Valley

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Agricultural land practices as well as the semiarid conditions of the Mediterranean cropping areas are negative factors that can increase microbial degradation in these areas (Bastida et al. 2006). The loss of biodiversity and the consequent loss of functioning attributes of soil microbial communities is the second cause of land degradation in Mediterranean agricultural soils. Soil microorganisms are effective indicators of soil quality, since microbial diversity is broadly related to land use (Ibekwe et al., 2002). Soil microbial diversity is the main factor in soil suppression of soil borne pathogens, for this reason, it is considered an important soil health indicator. Although fungi represent the greater part of soil microbial biomass (Lin and Brookes, 1999) they play a basic role in influencing macro-aggregate formation and their C assimilation efficiency is markedly higher than that of bacteria (Bailey et al., 2002), they have not yet been used as widely as soil biological indicators as bacteria. This is mainly due to the difficulties in applying molecular analysis tools due to the limited availability of reference sequences for fungal strains in DNA databases and the taxonomic accuracy of reference strains in these databases as well as the difficulties in isolating, enumerating and identifying groups of fungi that differ as regards their functioning in soil and their biological characteristics (Frankland et al., 1990).

A study of the impact of cropping sequences on microbial communities has been carried out using soil fungi as bio-indicators to investigate the possibility of their use in monitoring loss of biodiversity and the impact of cropping sequences on fungal communities.

## Methodology

The study was carried out in the east Po valley in the agricultural district known as the “Pianura Bolognese e alto Ferrarese” (latitude 44° 12’ - 44° 80’ N), an intensive potato growing area. Ten fields were chosen on the basis of the previous crop rotation and the following criteria:

- five samples, named ‘potato sites’, were in fields on farms with a long history of specialized potato production,
- five samples, named ‘rotation sites’, had been following a 4 year rotation, or fallow-rotation system without a potato crop, for the last twenty years.

The soil texture of sampling sites varied from clay loam to silty-clay loam soil. The pH was 8.1-8.2 and the SOM for the sites varied from 0.8 to 1.6%. The soil samples were subjected to analysis of fungal communities using the soil dilution plate method. After a three day-incubation period, colonies were counted by visual observation on transparent agar disks, including the germinated fungal propagules within the soil suspension. Each colony forming unit (CFU) observed was identified and the relative frequency of each species was expressed as a percentage of the total CFU for each community.

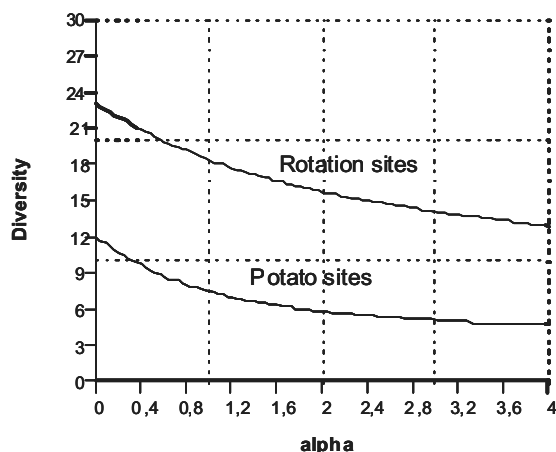
*Diversity.* Pooled data on fungal communities from potato and rotation sites were compared using two different tests: the diversity t test, which compares the Shannon index, based on richness (n of species), and a diversity profile, a test based on abundance data (number of individuals in taxon, species in this case, relative to the total number of individuals).

*Similarity* of fungal communities was compared with multivariate methods using the ANalysis Of SIMilarity test (ANOSIM), with the Bray Curtis distance and computed on 10000 permutations, and

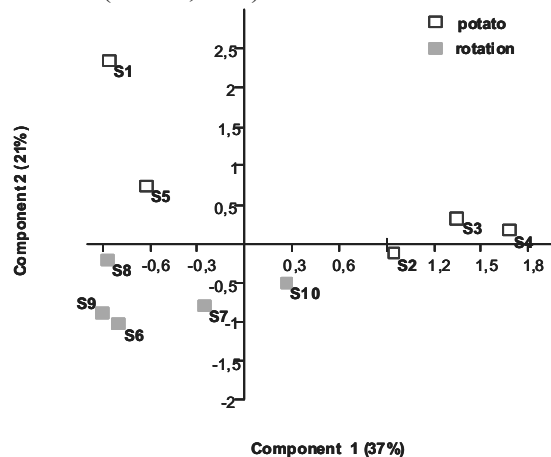
the principal component analysis (PCA) with 1000 bootstrap replicates. The PAST program software (Hammer et al., 2001) was used for all the diversity analyses.

## Results

**Diversity:** The diversity of fungal communities from rotation and potato sites differed significantly ( $P > 0.001$ ) according to both the diversity tests applied. Diversity t test based on the Shannon index (H) was higher in the rotation ( $H = 2.89$ ,  $n = 23$ ) than in the potato sites ( $H = 1.99$ ,  $n = 12$ ).



**Fig. 1** Diversity profile performed on pooled data of communities soil fungi in rotation and potato sites.



**Fig. 2** Principal component analysis of ten sites according to their community composition.

The diversity profile, which compared abundance data, confirmed the difference in diversity between the two communities, in agreement with the previous test (Fig. 1). On the basis of diversity analysis, potato sites showed the lowest richness and the highest abundance data. The Berger Parker dominance (the number of individuals in the dominant species relative to total individuals in the community) for potato sites was significantly higher than for rotation sites. Berger Parker dominance is considered a good indicator of the 'disturbance' of an environment (Shaw et al., 1983). Thus potato communities showed a lower diversity and a higher degree of disturbance than rotation sites. In addition, the most abundant species in potato sites were *Fusarium solani* and *oxysporum*, two well known agents of potato rotting in storage.

**Similarity:** According to the ANOSIM test the composition of fungal communities in rotation sites differed significantly from those of potato sites. This was confirmed by PCA which delivered rotation and potato sites in two opposite quadrants of the Y axis (Fig. 2).

## Conclusion

Cropping sequences deeply affected fungal communities of the two systems compared and specialized potato cropping systems showed a significantly reduced diversity as compared to rotation. The findings of this study suggest that the ecological approach can be used to evaluate changes in fungal diversity associated with agricultural management and to monitor the decline in biodiversity in agricultural areas on the verge of soil degradation as those in the east Po valley.

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# A method to Appraise Multiple Agro-Environmental Functions in Farming Regions

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In the latest decades there has been a growing interest by local managers in implementing and monitoring agro-environmental policies on their farming areas. This generates a need to develop approaches on landscape agricultural functions taking into account, at broader level than the farm, the effects of agricultural practices on the environment (Deffontaines, 1998, Benoît *et al.*, 2007). In this context, we propose a method for appraising the spatial variability of the potential fulfilment of a combination of agro-environmental functions (hereafter EAF) in farming regions.

## Material and method

The method is multiple functions oriented in the perspective of multifunctional policies. It is focused on spatial variability of both environmental functions and agricultural responses, because regions sustain different farming systems inducing different environmental effects (Benoît *et al.*, 2007). We propose a three step method: (a) to select a range of land-cover and bio-physical indicators describing the potential fulfilment of AEF either from political and scientific documents (e.g. Bonari, 1993) either from statistical analysis (correlation, PCA), (b) to characterize the spatial distribution of these indicators in a farming region, creating a geographical database by means of common databases (e.g. Corine Land Cover, DEM, hydrological maps) and (c) to differentiate homogeneous areas for potential functions fulfilment through a multivariate geographical clustering analysis (Hargrove and Hoffman, 2005). The method has been tested in two farming regions of central France (Combrailles, 1763 km<sup>2</sup>) and Italy (Collina interna di Grosseto, 1144 km<sup>2</sup>). Because of the large size of farming areas, we refer to a basic pixel of 250 ha as a common spatial unit for the characterization; this maximizes internal conditions variability and minimizes errors from common databases; it also makes possible further observations of farming practices. In spite of different farming systems (beef cattle versus ovine cattle/cereals) and biophysical conditions (continental climate/mountainous plateau versus Mediterranean/hilly inland), some regional policies target common AEF as the conservation of landscape heterogeneity and the preservation of surface water quality from nitrate pollution; meanwhile other ones are site-specific as the conservation of landscape openness in Combrailles and the preservation of soil fertility from erosion in Collina interna di Grosseto.

## Results

The proposed method shows two interesting results in the two farming regions concerning spatial variability of AEF combinations.

Firstly, a limited number of indicators are strongly related to AEF potential fulfilment. Starting from 10 and 15 indicators for the two farming regions, we succeeded to reduce then to 5 and 6 through PCA (table 1); they explain respectively 68% and 64% of the variance of the French and Italian farming areas (470 and 334 pixels) through the two first PCA components. Some indicators are site-specific, e.g. concerning the geomorphology (mean altitude in French, mean slope in Italy); while others are commons, e.g. water drainage density and land cover diversity. Moreover, the selected indicators inform on the potential fulfilment for a single AEF (table 1); while in others they are relevant for several AEF. These indicators of multiple EAF can have a positive (or negative) link to their potential fulfilment (table 1).

Secondly, in the two regions there is an internal diversity of farming areas presenting common conditions for AEF potential fulfilment. Four clusters for each region resulted from the performed

HCA. They are characterised by (a) different combinations of bio-physical and land cover indicators (table 1), signifying contrasted conditions for the potential fulfilment of AEF; and (b) a different spatial distribution of the indicators, showing some localized and homogeneous areas presenting the same conditions for AEF fulfilment as well as some isolated ones at their edges.

**Table 1: indicators used to perform the Hierarchical Cluster Analysis (HCA) in the French (F) and Italian (I) farming regions and their relationships with the potential fulfilment of the two common agro-environmental functions.**

	HCA indicator	Description	Farming region	Relationship with the potential contribution to AEF	Kind of the relationship
Bio-physical indicators	Range altitude (m)	Difference between max. and min. pixel elevation	F	+ Landscape heterogeneity	Different land cover suitability
	Mean altitude (m)	Average pixel altitude	F	- Landscape heterogeneity	Different land cover suitability
	Mean slope (%)	Average pixel slope	I	- Nitrates in surface water - Landscape heterogeneity	(a) water and nitrates runoff, (b) different land cover suitability
	Water drainage density (m/ha)	Ratio between mean river length and pixel area	I, F	- Nitrates in surface water + Landscape heterogeneity	(a) proximity between fields and rivers, (b) land cover diversity and fragmentation
	Soil permeability (%)	Ratio between pixel area of good soil permeability and pixel area	I	- Nitrates in surface water	Water and nitrates runoff
Land cover indicators	Grassland (%)	Ratio between grassland and pixel area	F	± Nitrates in surface water ± Landscape heterogeneity	(a) number of buffer zones, (b) land cover diversity
	Arable land (%)	Ratio between pixel arable land and pixel area	I	± Nitrates in surface water ± Landscape heterogeneity	(a) field fertilization, field surfaces (b) different land cover suitability, field fragmentation
	Woodland (%)	Ratio between pixel woodland and pixel area	I	+ Nitrates in surface water ± Landscape heterogeneity	(a) number of buffer and unfertilized zones, (b) different land cover suitability
	Land cover diversity (value)	Number of different and non-adjacent land use patches within a pixel	I, F	+ Nitrates in surface water + Landscape heterogeneity	(a) number of buffer zones, (b) different land cover suitability

+ means a positive relationship, - a negative relationship and ± a positive or negative owing the frame conditions; a and b refers to the two AEF considered (a = nitrates in surface water, b= landscape heterogeneity)

## Conclusions

Our results show the interests of a spatial analysis to compare regional and local farming contributions to agro-environmental functions. For agronomical research, it is helpful to point out comparable and relevant indicators of agro-environmental functions fulfilment; for local managers, it opens possibility to compare farming regions and areas in terms of their potential contribution to these functions.

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# Response of Microbial Activities to Additional Metal Amendment to Long-Term Heavy Metal Contaminated Soils

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## Introduction

Smelters and mines are of the greatest and often the oldest industrial sources of soil heavy metal contamination. Negative effects of heavy metals on microbial communities and activities have been many times reported (i.e. Brookes, 1995; Giller et al., 1998; Paton et al., 2006). On the other hand, microbial functions in soils are typically quite resilient towards toxic effects of metals and are often found to recover after an initial inhibition by high metal inputs (Holtan-Hartwig et al., 2002). Development of tolerance towards heavy metals by microbial communities is a commonly known fact (Gadd, 1992) which may have various explanations, such as long term genetic modifications, spread of resistance genes among microorganisms or replacement of metal sensitive strains by tolerant strains. The aim of this research was to evaluate the ability of the soil microbial communities in a long-term contaminated soil to tolerate the additional Cd and Pb treatment.

## Methodology

Long-term heavy metal contaminated and non-contaminated soils sampled near the lead smelter of Příbram (Czech Republic) were used for the experiment. The smelter has been in operation for over 200 years, in 1982 a stack with efficient filters was built. The contaminated (4.06 mg Cd kg<sup>-1</sup> soil, 1138 mg Pb kg<sup>-1</sup> soil), and non-contaminated soils (0.65 mg Cd kg<sup>-1</sup> soil, 74 mg Pb kg<sup>-1</sup> soil), were treated with Cd (40 mg kg<sup>-1</sup> soil) or Pb (500 mg kg<sup>-1</sup> soil) as Cd(NO<sub>3</sub>)<sub>2</sub> or Pb(NO<sub>3</sub>)<sub>2</sub>. The control soil was amended with Ca(NO<sub>3</sub>)<sub>2</sub> in concentration corresponding to NO<sub>3</sub><sup>-</sup> applied to metal-treated variants. The soils were incubated for 66 days in closed containers under stable temperature and humidity conditions. The microbial biomass C, respiratory activity and qCO<sub>2</sub> were periodically determined during incubation. The measurements of the soil microbial biomass C (B<sub>c</sub>) were performed using the fumigation-extraction method according to Vance et al. (1987) procedure. The respiratory activity was determined as the amount of organic C released as CO<sub>2</sub> after absorption in NaOH and precipitation with BaCl<sub>2</sub> and was analysed by titration with HCl. The metabolic quotient (qCO<sub>2</sub>) was calculated according to the Anderson and Domsch (1990) equation:  $qCO_2 = \mu g\ CO_2-C \cdot \mu g\ C_{Bc}^{-1} \cdot h^{-1}$ .

## Results and discussion

At the beginning of the experiment, the microbial biomass C was larger in the non-contaminated soil in comparison with the contaminated one. At day 66 of the incubation, a decrease from 9% to 25% of microbial biomass C was found for all variants in the contaminated soil, whereas in the non-contaminated soil a decline between 32% and 42% could be observed (Fig 1). The Pb-treated variants showed the greatest decrease of the microbial biomass in both soils. Therefore, the microbial biomass in the contaminated soil tolerated better the additional metal loading and also the 66 days running incubation under our experimental conditions than in the non-contaminated soil. The results indicate that during a long-term exposure to toxic elements the microbial communities showed a good resilience to heavy metals (Holtan-Hartwig et al., 2002) and to other environmental effects. The metal sensitive strains were possibly substituted by tolerant strains (Gadd, 1992).

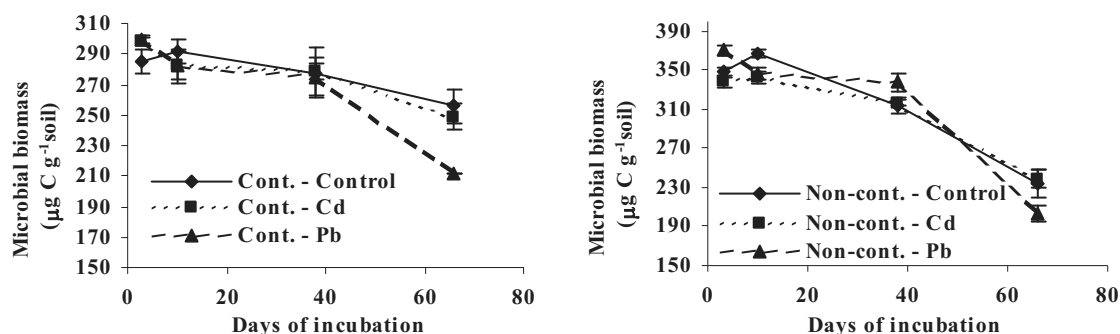
In comparison with the non-contaminated soil (Tab 1), the respiratory activities and qCO<sub>2</sub> were lower in the contaminated soil in all studied treatments, which suggests a lower respiratory efficiency under long-term metal contamination. The highest respiratory activities and qCO<sub>2</sub> were found at the beginning of incubation, thereafter they decreased in all studied variants. The Cd-treated variants did

not show significant differences compared to their control soils at the beginning of the incubation. However, higher respiratory activity of Cd-treated soil in comparison with the control soil was found at the end of experiment in the non-contaminated soil. Significantly higher respiratory activity and  $qCO_2$  compared to the control variant was also observed in the Pb-treated variant in the contaminated soil at the end of the incubation. The obtained results suggest that a long-term contamination with heavy metals decreased the respiratory activities and  $qCO_2$ , the additional fresh metal input, in our case particularly Pb, increased significantly the respiratory activities and  $qCO_2$  indicating the major energy requirements of soil microbial communities under additional soil pollution as it was suggested by Brookes (1995) and Giller et al. (1998).

**Tab 1:** The respiratory activity and  $qCO_2$  in non-contaminated and contaminated soil at day 3 and 66 of the incubation with Cd or Pb. The letters indicate a significant difference according to Duncan ANOVA test.

	Day of incubation		Day of incubation	
	3	66	3	66
	Respiratory activity ( $\mu\text{g C g soil}^{-1} \text{ h}^{-1}$ )		$\text{qCO}_2$ ( $\mu\text{g C } \mu\text{g Bc}^{-1} \text{ h}^{-1}$ )	
Non-contaminated soil				
Control	1.543 <sup>b</sup>	0.334 <sup>a</sup>	0.0044 <sup>b</sup>	0.0015 <sup>a</sup>
Cd-treated	1.516 <sup>b</sup>	0.375 <sup>b</sup>	0.0045 <sup>b</sup>	0.0016 <sup>a</sup>
Pb-treated	1.328 <sup>a</sup>	0.364 <sup>b</sup>	0.0036 <sup>a</sup>	0.0018 <sup>ab</sup>
Contaminated soil				
Control	1.153 <sup>a</sup>	0.252 <sup>a</sup>	0.0041 <sup>a</sup>	0.0010 <sup>a</sup>
Cd-treated	1.128 <sup>a</sup>	0.262 <sup>a</sup>	0.0038 <sup>a</sup>	0.0011 <sup>a</sup>
Pb-treated	1.242 <sup>b</sup>	0.334 <sup>b</sup>	0.0041 <sup>a</sup>	0.0018 <sup>b</sup>

**Fig. 1:** The microbial biomass C in non-contaminated (Non-cont.) and contaminated (Cont.) soil during the incubation with added Cd and Pb. The vertical bars represent the deviation standard.



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# Soil Mineral N Content and Dynamic After Three Years of Conventional, Low Input and Organic Farming in a Mediterranean Environment

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## Introduction

Evaluation of the environmental and economic sustainability of farming systems in different pedoclimatic and crop conditions is crucial in order to define future and effective soil management strategies and crop sequence choice. Soil mineral nitrogen content and dynamic monitoring represents a powerful means being able in highlighting short and medium term changes and indicating not only variations in soil residual fertility, and therefore in crop productivity, but also potential pollution risks (groundwater contamination, human health risks). Moreover it is particularly important when comparing nutrient availability from different sources (i.e. mineral vs organic). The aim of the present study was to assess the effect of different input farming systems (conventional, low input and organic farming) and crop types on soil N content and dynamic in a Mediterranean environment.

## Methodology

A field trial was started in 2001 in Southern Italy at the experimental farm “E. Pantanelli” of Bari University (Policoro-MT: 40°13' North, 16°41' East) on a soil characterised by a quite high fertility with an average content, in the top 0.4 m, of organic C, total N, available P and CaCO<sub>3</sub> of 18.3 g kg<sup>-1</sup>, 1.96 g kg<sup>-1</sup>, 17.35 mg kg<sup>-1</sup> and 8.84 g 100g<sup>-1</sup>, respectively. A three year crop rotation (oilseed rape – chickpea – wheat) was managed under conventional (synthetic fertilizer and pesticide use based on conventionally recommended rates), low input (synthetic fertilizers reduced by one half of the recommended rate and pesticide use reduced by some mechanical weeding) and organic farming (no synthetic fertilizer and no pesticide application; N applied as humified farmyard manure with rates close to the low input system). Crop rotation was performed in contemporary phases. Treatments were arranged in a strip plot design with 3 replicates, with cropping systems as main plot factor and crops as subplot factor; plot surface was of 168 m<sup>2</sup>.

At the end of the first rotation cycle, soil samples were collected two times in the plots previously cropped to wheat and oilseed rape, at three soil layers (0-0.20 m; 0.20-0.40 m; 0.40-0.60 m). Moreover in the second sampling time, soil samples were collected also from the 0-0.40 m soil layer in the plots previously cultivated with chickpea. The first sampling was carried out before wheat and oilseed rape sowing (Autumn 2004) and the second at the end of the winter period before chickpea was sown (April 2005) in order to evaluate the residual fertility and the movement of NO<sub>3</sub>-N along the soil profile as consequence of rainfall and crop presence. On field moist soil samples, NO<sub>3</sub>-N and NH<sub>4</sub>-N contents were spectrophotometrically quantified, after extraction with a 0.025M CaCl<sub>2</sub> solution. Afterwards on average air dried and 2 mm sieved soil samples, total N, organic C, available P and exchangeable K were determined. Data were statistically analysed through three way ANOVA (farming system x crop x depth) and afterwards through two and one way ANOVA in order to investigate the significant interaction effects; mean comparison was carried out through SNK test, p=0.05.

## Results

At the first sampling time, soil NO<sub>3</sub>-N concentrations resulted significantly influenced by farming system (16.67 mg kg<sup>-1</sup> for the conventional system against 10.09 and 9.9 mg kg<sup>-1</sup> for the low input and

organic systems, respectively) and depth (average content of  $14.71 \text{ mg kg}^{-1}$  for the 0-0.40 m layer against  $7.24 \text{ mg kg}^{-1}$  for the lower layer) but showed a different behaviour along the soil profile (significant interaction farming system x soil depth). In the organic system the highest concentrations were observed in the top layer (0-0.20 m), whilst in the conventional system the highest values were found in the 0.20-0.40 m layer. No difference was found as consequence of the previous crop (wheat or oilseed rape) in the measured parameters except for a higher soil moisture after wheat in the organic system ( $20.17$  against  $18.29 \text{ g } 100\text{g}^{-1}$  on wet weight).

In April, the positive action of the crop in “catching” the  $\text{NO}_3\text{-N}$  in the soil was evident and highly significant: average contents of  $2.69 \text{ mg kg}^{-1}$  were observed where oilseed rape was growing against values of  $11.94 \text{ mg kg}^{-1}$  in the plots where chickpea was not yet sown. Similar results were obtained by considering the three crops in relation to the 0-0.40 m layer ( $12.27 \text{ mg kg}^{-1}$  where the soil was bare against  $2.87$  and  $2.27 \text{ mg kg}^{-1}$  where oilseed rape and wheat were respectively growing). The  $\text{NO}_3\text{-N}$  concentration was also significantly influenced by the farming system, with a different behaviour in relation to the crop presence. In particular, in the bare soil the highest values were found in the soil conventionally managed ( $17.9 \text{ mg kg}^{-1}$  against an average value of  $8.95 \text{ mg kg}^{-1}$  for organic and low input treatments) while in the cropped soil the lowest values were found in the organic system ( $1.73 \text{ mg kg}^{-1}$  against an average value of  $3.17 \text{ mg kg}^{-1}$  for conventional and low input treatments).

A trend along the soil profile was observed in the bare soil with nitric N values higher on average in the 0.20-0.40 m layer, and for the conventional system higher in the 0.20-0.60 m layer, letting hypothesize a  $\text{NO}_3\text{-N}$  movement due to the winter rainfall. Total rainfall recorded in the experimental field during the interval between the two samplings was of  $138.9 \text{ mm}$ . Finally a significantly higher  $\text{NH}_4\text{-N}$  content was observed in the organic treatment in the bare soil at the second sampling time.

Crop yield recorded in 2005 were not significantly influenced by the cropping systems compared, although higher values were observed in the conventional management for oilseed rape and chickpea.

### Discussion and conclusions

The differences in soil  $\text{NO}_3\text{-N}$  content at the end of the three year rotation cycle observed in this experiment were mainly due to the total amount of inputs applied. Indeed, the higher inputs supplied in the conventional system determined a higher nitric N content in the soil ( $16.67 \text{ mg kg}^{-1}$ , averaged across the 0-0.60 m layer) while lower contents were found in the soil managed under organic and low input systems ( $9.9 \text{ mg kg}^{-1}$ ) that received lower and similar amounts of N. Moreover, while similar concentrations were found in the first layer for the three cropping systems compared ( $11.72\text{-}14.43 \text{ mg kg}^{-1}$ ) the significantly higher concentrations observed in the 0.20-0.40 m layer ( $25.2 \text{ mg kg}^{-1}$ ) in the conventional system can be attributed to the amount of N exceeding the crop uptake capacity and moved to the deeper layer by the rainfall.

Crop presence showed a crucial action in catching mineral N in the soil and therefore attenuating the previous differences and subtracting  $\text{NO}_3\text{-N}$  to potential leaching risk. In fact, in the soil still bare contents similar to the first sampling were observed on average but with the higher concentrations in the 0.20-0.40 m layer for the low input and organic systems, in the 0.20-0.60 m layer for the conventional.

The not significant effect of the different input supply on crop yield can be attributed to the high average and quite unusual, for Southern Italy environments, fertility of the soil where the experiment was carried out. In a less fertile environment different results could have been obtained.

### Acknowledgment

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# Greenhouse Structure Techno-Economic Material Selection and Simulation; SUGREMEMA Low Cost Greenhouse Case Study

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The poor technological development in the developing markets' agricultural sector impacts in a determinant way our environment, our economy and our alimentary safety. Mexico as developing market lives a similar situation e.g. for the year 2004 to 2005 the corn production felt 6% [SAGARPA, 2007] and just the 10% of the annual sow area could be grow due the climatic injuries [SAGARPA, 2006]. The Mexican agricultural sector plays a crucial role in the country social security and it is the only way to obtain a sustainable alimentary independence.

Until recent years for the agricultural industry, the use of greenhouse technology had been considered to satisfy necessities and expectations of developed markets, even though they represent only 14% of the world market. Developing markets are experiencing accelerated growth in populations and income, and they are becoming more important to define the future of the world agricultural business. In Mexico the greenhouse industry has been growing rapidly, from 50 hectares in 1991, to 350 in 1997 and 1000 in 2001 [SAGARPA, 2006]. Rapid growth is prognosticated to persist as open field producers face new challenges and demand from North America for greenhouse grown products. Although still with severe problems in different areas as know how; the economics of the industry are allowing for better built and better managed projects, resulting in higher quality products.

## Methodology

In the Tecnológico de Monterrey, Molina et al [2007] developed a reference framework to integrate the life cycle of mechanical products, in this paper this methodology was instantiated in order to achieve cost reductions during the life cycle of a low cost greenhouse where the key factor for a successful design is the correct sustainable material selection e.g. the steel commonly used in greenhouse structure suffer an cost increment in January of this year of about 30% in Mexico, representing a huge barrier for the introduction of low cost greenhouses. The opportunity area for this market is to develop a customizable greenhouse that covers the low-income-farmer's specific necessities.

The Methodology for Rapid Needs Coverage Identification, this is the phase of the instantiated reference framework that aims to identify the greenhouse needs of the selected market and the possible needs coverage by means of structural techniques (See Fig. 1).

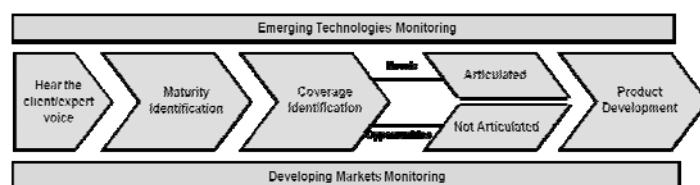


Fig. 1 Rapid Necessities Coverage Identification Methodology

Molina et al. [2007] proposed a reference model that enables the creation of a partial and particular model to set up an Integrated Product, Process and Manufacturing System development processes (IPPM) that is the second phase of this reference framework. This Reference Model was structured in three dimensions: Processes that are a description of the entities that will be developed, it can be a product, process and/or manufacturing system; Stages that are the set of activities, based on the product lifecycle phases that were condensed in conceptualization, basic development, advanced development

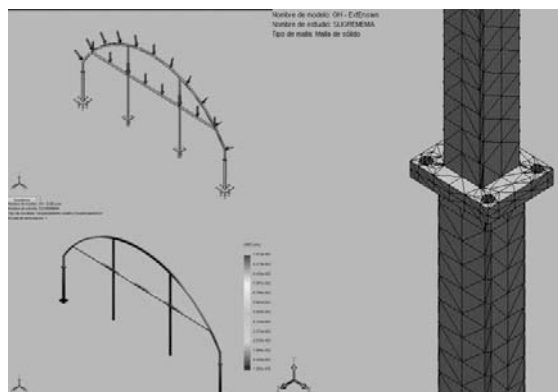


Fig. 2 Finite Element Analysis

material (bamboo sticks); a techno-economic analysis was developed. Three phases were proposed in order to configure a particular integrated model: i)PhaseI–Definition of the project: identification of the project's scope according to the reference map; ii)PhaseII–Partial model definition, from a set of proposed activities, the evaluation and selection of the suitable activities is made; iii)PhaseIII–Particular model definition, consist on the translation of each one of the activities into the particular case under study.(See Fig. 3).

and launching. For each stage, three kinds of activities can be identified: analysis, synthesis and evaluation; for this study we select the correct design and material selection for the structure of our low cost greenhouse named SUGREMEMA-Pro for the northeast Mexican market; a finite element computer aided test (COSMOS<sup>TM</sup>-SolidWorks<sup>TM</sup>) of 5 materials was carried out using the production data and environmental characteristics of six tomato producers of the community of Palmitos Nuevo Leon Mexico (See Fig. 2). The materials tested were steel SAE 1025, steel SAE 1035, steel SAE 1045, a nano-composite material and an organic

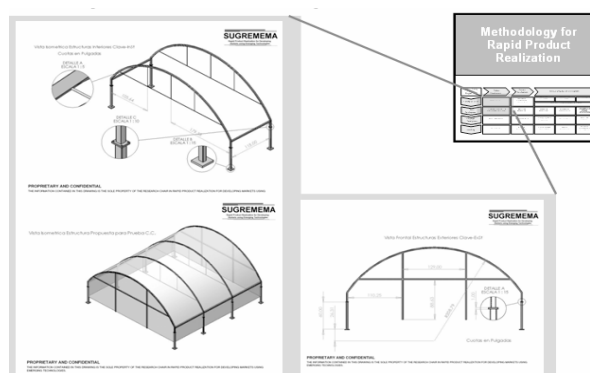


Fig. 3 Methodology for Rapid Product Realization

## Results

SUGREMEMA-Pro Tunnel					
Material	Von Mises Test Max. [N/m2]	Resultant Max. Displacement [mm]	Min Security Factor	Mechanizing Prize	Price Volatility
Steel SAE-1025	2.336E+10	5.805	4.4	Low	High
Steel SAE-1035	2.353E+10	6.004	4.3	Low	Medium
Steel SAE-1045	2.502E+10	6.014	3.9	Medium	High
Nanomaterial	1.211E+10	7.748	8.5	High	Low
Bamboo	3.900E+09	12.326	1.9	Low	Low

## Conclusions

The best material for the selected design (SUGREMEMA-Pro Tunnel) was the Steel SAE-1035. It was interesting to find that the bamboo sticks are a good option, but its security factor is very low. The methodology presented here results very useful for the design of greenhouses and could be used with other design scenario. This research is a contribution for the Rapid Product realization for Developing Markets Using Emerging Technologies Research Chair, Registration No. CAT077. The authors wish to acknowledge the support of the Tecnológico de Monterrey, Monterrey Campus.

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# Integrated Assessment of Agricultural Systems – a Modular System for Agricultural and Environmental Modelling (SEAMLESS-IF)

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## Introduction

Agricultural technologies and agricultural, environmental and rural development policies are increasingly designed to contribute to the sustainability of cropping and farming systems and to enhance their contributions to sustainable development at large. The effectiveness and efficiency of such policies and technological developments in realizing desired impacts could be greatly enhanced if the quality of their ex-ante assessments were improved. *Four key challenges* and requirements to make research tools more useful for integrated assessment in the European Union have been defined (Van Ittersum et al., 2008): **(a)** overcome the gap between micro-macro level analysis, **(b)** decrease the bias in integrated assessments towards either economic or environmental issues, **(c)** ensure reusability of models and their use for indicator assessment and **(d)** overcome hindrances in technical linkage of models. Tools for integrated assessment must have multi-scale capabilities and preferably allow application to a broad variety of policy questions. At the same time, to be useful for scientists, the framework must facilitate state-of-the-art science both on aspects of the agricultural systems and on integration. This paper presents the design of a framework for agricultural systems (SEAMLESS Integrated Framework) and discusses the implications for cropping and farming systems modelling.

## Methodology

SEAMLESS-IF has been developed as a component-based system. This supports the process of composing different model chains depending on the application purpose and to facilitate synthesis of scientific knowledge in the domain of agriculture and its environment in relevant model components. The components in SEAMLESS-IF consist of a pan-European data base with data on soils, weather, farming systems, agro-management, prices and trade flows, an indicator framework and a number of quantitative models to assess the indicators. The quantitative models simulate various aspects of the system at different levels of organization and scale (Van Ittersum et al., 2008):

- APES (Agricultural Production and Externalities Simulator) is a modular simulation platform calculating agricultural production and externalities at field level.
- FSSIM (Farm System Simulator) is a bio-economic farm model quantifying the integrated agricultural, environmental and socio-economic aspects of farming systems, using APES outputs.
- EXPAMOD (Extrapolation Model) is used for up-scaling the outcomes from FSSIM to the European scale, in the form of price-supply relationships.
- CAPRI (Common Agricultural Policy Regional Impact Analysis), an existing model but adapted to SEAMLESS-IF, is a comparative static equilibrium model providing information on price-supply relationships, solved by iterating supply (from EXPAMOD) and market modules.

The model components, database and indicators are linked into model chains in SEAMLESS-IF. Through the use of ontologies the conceptual consistency of inputs and outputs of the various components is ensured (Wien et al., 2007), while a technical linkage is enabled through the use of OpenMI (Verweij et al., 2007).

### Example application

The framework allows application to specific regions to assess location specific policies or agro-technical innovations, such as those of the water or nitrate framework directive (see e.g. Belhoucette et al., these proceedings) and to the entire European Union to assess EU wide implications of policy reforms, such as those negotiated under the World Trade Organisation. For the latter we provide an example in this paper, i.e. the integrated assessment of a trade liberalisation proposal by the so called G20 group of developing countries at the current Doha Round of the World Trade Organisation. We take the year 2013 as time horizon of the assessment, for which a baseline and policy scenario is defined. The baseline scenario for 2013 is interpreted as a projection in time covering the most probable future development of the European agricultural policy, based on the Luxemburg Agreements on the Common Agricultural Policy Reform, and including all future changes already foreseen in the current domestic, EU and international legislation (e.g. sugar market reform). The baseline is used as a reference point for counterfactual analysis. The policy scenario implements the G20 proposal on tariff reduction for agricultural products and the additional abolition of subsidized exports by the EU.

The application of the *market* model CAPRI within SEAMLESS-IF under the policy scenario results in changes of agricultural market indicators such as prices and corresponding production and consumptions quantities. Prices of products where the original degree of protection is relatively small (cereals, oilseeds or pork meat) do not decrease much, whereas highly protected products like beef and dairy show larger price reductions. The decrease is differentiated by region due to variations in the development of profitability of products competing for limited resources such as land. In a next step, the *farm* model FSSIM within SEAMLESS-IF simulates consequences of the price changes due to the liberalisation proposal, in terms of the supply of commodities at farm level, as well as the associated production plans, input use and a range of externalities including nitrogen surplus and emissions, pesticide use and irrigation water use. The farm model uses information on the various, current and alternative, agricultural activities which can be practiced on the major farming systems across the EU, i.e. annual and perennial cropping activities and livestock activities. Externalities of activities can be assessed using the *cropping system* modelling platform (APES).

### Conclusions

SEAMLESS-IF is an innovative modelling system designed to address the introduced *four key challenges* as to quantitative scientific tools for integrated assessment. As such it presents an operational method of how systems analytical tools developed in the agronomic community can be integrated in more holistic and multi-disciplinary frameworks for integrated assessment of agricultural systems. Initial applications demonstrate the merits of this approach; the presentation will also highlight the significant investments needed from research institutions and individuals to allow for integration.

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# Biodiversity as a Measure of Differences between Organic and Conventional Farming: a Case Study

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Natural ecosystems are a structural and functional association of biotic-abiotic components within a physic-chemical environment. Such a dynamical aspect gives to natural surfaces the ability to maintain high qualitative properties of constitutive resources (water, air, soil), of land (landscape) and of habitat (for animal and vegetal species). These properties are fundamental to the concept of sustainability (Lewandowski et al., 1999) and biodiversity is a way to estimate them in a farm context (Bockstaller and Girardin, 1997) and for a comparative use (Caporali et al., 2003).

Biodiversity indicators are largely used for detecting and evaluate the amount and effectiveness of natural surfaces within a farm (Hole et al., 2005). In the following study indicators at plot scale and farm scale were used to compare the effects of organic and conventional management on occurrence of spontaneous vegetation.

## Methodology

Biodiversity is a property of a vegetated surface and is commonly evaluated analysing homogeneous (and broadly delimited) surfaces. These surfaces may range from few square meters to hectares and may be cultivated or not. In the cropped case biodiversity is given by weeds, which spatial frequency distribution is observed in the early summer. Spontaneous vegetal formations in farms commonly involve infrastructures as field margins and edges, so that biodiversity evaluation requires a former identification and classification of different structures, requiring a farm-wide survey to identify homogeneous surfaces and successively to recognize vegetal species of each of them. The composition of such formations includes herbaceous, shrubs and trees and can be described in terms of layers. In this case the Braun-Blanquet (1932) method was used.

For the plot-scale biodiversity, indicators selected are herbaceous species richness **Ich**, namely the number of species for unit area, and diversity **IDh** obtained by means of the Shannon-Weaver transformation ( $ID = - \sum [Ps \log Ps]$ , Ps being the species coverage). Similar indices were obtained for the spontaneous vegetal formations, namely species richness (**ICv**, same as before) and diversity (**Idv**), where bushes and trees are included. For the purpose, two couples of farms were identified, to compare a plain to a hilly scenario. In fact, in the hillside, Italian agriculture is more fragmented than in plain and more inclined to move towards organic management. Each couple of farms, an organic and a conventional one, were closed to one another so as to have similar soil, climate and crop production schemes. The farms were all from north-eastern Italy (Province of Bologna) with full and cultivated areas reported in table 1.

In the farms, plots with the same crop (winter wheat) were selected within ordinary crop rotations of the area, including legumes in the case of organic farming. Sampling was performed by means of the moving square method (a 20 cm side frame have been used) to randomly identify sampling areas.

A preliminary survey over the farm surface also allowed to identify 8 vegetal formations in the plain scenario and 17 in the hillside.

Table 1 – Size of farm included in the analysis (SAT: Total Farm Surface, SAU: Cropped Surface, in ha)

Farm	Plain - Conv.	Plain - Organic	Hilly - Conv.	Hilly - Organic
SAT	40.1	36.2	73.0	17.9
SAU	38.7	33.0	33.4	13.7

## Results

Over the plots any diffusion of weeds were observed both in the conventional and in the organic scenarios. The majority of species found, 24 in total, are dicots and the number of individuals, as expected, are sensitively greater in the organic case than in the conventional (Hole et al., 2005). As it can be observed from table 2 (first row), the difference between plain and hilly scenario is also greater, a difference mitigated by means of the diversity index (table 2 - row 2).

In the natural formations 75 and 97 species were found, respectively in the plain and hillside. The number of individual for unit surface (IC) was greater in the plain than in the hilly scenario, but the difference between the farm management regime was also relevant.

These results support already known results of literature (Caporali et al., 2003), even if it is not common to find a marked causality of biodiversity in organic farming (Hole et al., 2005).

Table 2 – Values of indicators computed in the farms

Farm	Plain - Conv.	Plain - Organic	Hilly - Conv.	Hilly - Organic
ICH	5.0	18.0	40.0	51.0
IDh	1.61	1.84	1.65	2.36
ICv	24.7	75.9	5.49	25.3
IDv	0.70	1.91	1.12	3.58

## Conclusions

Floristic analysis developed in this investigation proved to be easy both in terms of survey and index computability, even if it requires to work at a certain period of the year (late spring in middle latitudes) and a former background in species recognition skills is required. Finally, all the computed indices showed sensitivity to both location (plain vs. hilly) and farm management (conventional vs. organic).

## Acknowledgments

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# Is Soil Analysis Useful to Farmers for Managing P Fertilisation? An Interview-based Analysis with Annual Crops Growers in South-West France

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Phosphorous (P) is a limited fertiliser resource for which world reserve are quickly depleting. There is therefore an important need for redesigning P fertilisation management and, first of all, to understand farmers' fertilisation management practices. Such an analysis of farmers' practices was implemented, that aimed at (i) describing farmers' P fertilisation practices, (ii) evaluating these practices in term of input-output plot P balance and (iii) understanding the reasons of their practices. This paper presents the first results of this farmers' practices analysis.

## Methodology

Interviews with farmers were realised in spring 2008 with 38 farmers in South-West France (Bordeaux region) about their P fertilisation practices. The study area included three main types of soil: poor sandy soils, sandy-loam soils and clay soils. The main crops were maize, cereal, sunflower and canning vegetables. One third of farmers grew animals. Farmers were asked their crop management practices, including their crop yields and their P management, and were asked to detail their P fertilisation decision making. Soil analysis, when available, were collected. To evaluate farmers' practices, input/output P balances were performed at the plot scale for each crop of each farmer. Balances were computed as fertiliser and manure supplies multiplied by their composition minus crop yield multiplied by crop P composition (Comifer, 2007).

## Results

### *Farmers' practices evaluation*

Figure 1 shows the distribution of farmers' P fertiliser amounts. Fertiliser amounts were rather homogeneous among crops, except for spring beans that were significantly more fertilised. Figure 2

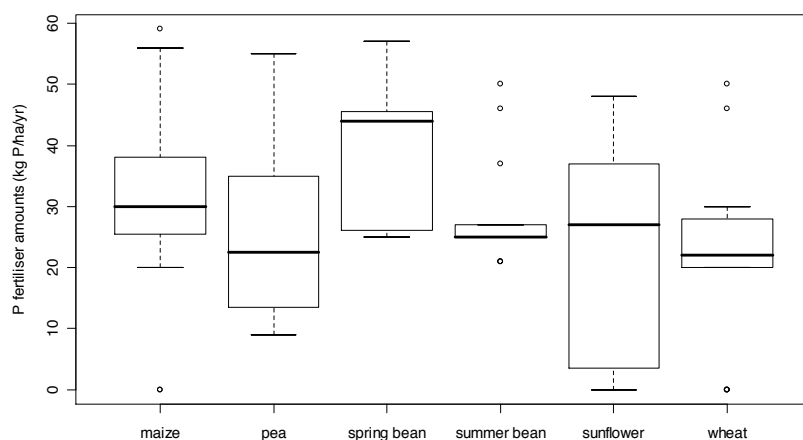


Fig1: Box plot of farmers' P fertilisation (kg P/ha/yr). Statistical units are farmers

shows the input/output balances for each considered crop. Balances were positive for canning vegetables, and were null for maize not receiving manure, wheat and sunflower. But maize balances were strongly positive for cases where manure was applied to the crop. Such balances may have strong environmental negative impact. Therefore, farmers' practices were carefully analysed to

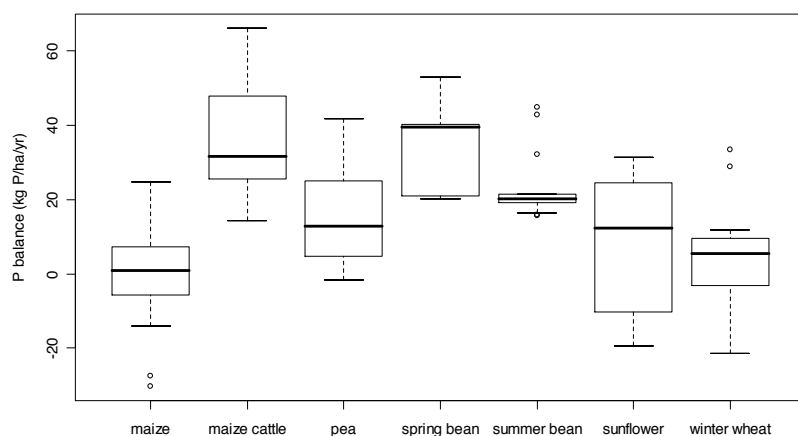


Fig2: Input/output P balances (kg P/ha/yr). Statistical units are farmers. Maize cattle: maize plots receiving cattle manure.

understand farmers' decision making.

### Detailed analysis of practices

A detailed analysis of farmers' practices showed that no farmer use soil analysis as a tool for P fertilisation decision even if all interviewed farmers regularly performed soil analysis on their different plots. On the contrary, P fertilisation was managed

on a yearly and species basis: for a given species, a given P fertiliser amount was applied to all the plots of the farm cropped with that species, unrelated to soil P composition. Moreover, farmers who grew cattle did not consider P contained in manure when deciding their P fertilisation. This led, for

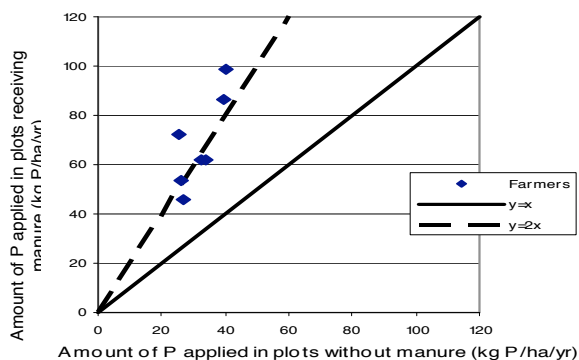


Fig3: P amount supplied to the different maize crops of the same farms receiving or not cattle manure (kg P/ha/yr).

example for maize crop, to supplying about twice the amount of P that was currently applied on the other plots of the same farm (Figure 3).

Farmers' practices analysis also showed that different types of P fertiliser supply were performed, as represented in Table 1. Most farmers localised their P fertilisation *i.e.*, applied P fertiliser on the row for large row spring crops such as maize or beans. Phosphorous fertiliser localisation was supposed, according to 60 % of farmers, to help early crop growth and to give a resistance to poor weather conditions and pest attacks.

Crop	Fertiliser supplying mode (% of farmers)			
	Localised supply	Localised + broadcast supply	Broadcast supply	No supply
Maize	42	28	25	6
Green pea	0	0	100	0
Spring bean	43	57	0	0
Summer bean	77	15	8	0
Winter wheat	0	0	76	24
Sunflower	9	9	55	27

Table 1: P fertiliser supplying modes. Statistical units are farmers

This study also underlined the poor role of the soil analysis as a decision tool for farmers. This should question agronomist research teams who focus on the soil analysis as a unique decision tool for P management.

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### Conclusions

This study suggested the risk of the negative environmental impact that farmers' P management practices may have on water quality, particularly in cases of cattle breeding. It suggested that more detailed analysis should focus on cattle breeders' P management: what are the different driving forces explaining the input/output balances of this type of farms?

# Conservative Agronomical Practices: a Way to Support the Crops Production and the Environment

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## Introduction

Conventional agriculture is mainly characterized by deep and repeated soil tillage, mineral fertilizer and chemical herbicides applications, crop residue burning and continuous cropping. Such practices can cause soil degradation through loss of organic matter as result of progressive deterioration of soil fertility (Convertini *et al.*, 1997), soil erosion and compaction, leaching and runoff of fertilizers and pesticides that pollute surface and ground water. Rational tillage systems and greater application of organic fertilizers are indispensable for improving resources sustainability, yet their long-term effects must be assessed, especially in semi-arid and Mediterranean conditions, characterized by a hot, dry climate and rainfall usually scanty and unevenly distributed during the year.

Therefore, it should be useful to apply conservative and eco-compatible agricultural practices, able to increase soil organic matter, improve soil chemical and physical properties (Bazzoffi *et al.*, 1998) and also reduce ecological problems, due to the use of high level of chemical fertilizers.

On this basis, and bearing in mind the more advanced principles of sustainable development and eco-compatible agriculture, our three-year field research in Southern Italy was aimed to determine the effects of reduced agronomical practices (shallow soil tillage and organic-mineral fertilizer application) on growth parameters, production and quality of durum wheat.

## Materials and methods

The three-year field study, started in the autumn of 2004 and ended in 2007, took place at Foggia (Southern Italy, 41° 27' lat. N, 3° 04' long. E, 90 m above sea level) in a typical area of Mediterranean environment, on a cereal-livestock farm. The soil of the experimental field is a silty-clay Vertisol of alluvial origin, classified as Typic Chromoxerert, Fine, Mesic, by Soil Taxonomy USDA.

The climate is “accentuated thermomediterranean” (UNESCO-FAO classification), with rains concentrated mainly in the winter months.

On crops of durum wheat (*Triticum durum* Desf.), cropped in rain-fed conditions, the following two management strategies were studied: i) Conservative (CONS), with shallow tillage at 15-20 depth, combined with organic-mineral nitrogen (N) fertilizer, and ii) Conventional (CONV), with traditional soil tillage at 40-45 cm and mineral N fertilizer. The N fertilizer (70 kg N ha<sup>-1</sup>) was broadcasted, as mineral or organic-mineral, always as a top dressing, in February-March.

The experimental design was a randomized complete block with three replicates and elementary plots of 350 m<sup>2</sup>. At harvesting, the most important quanti-qualitative parameters (grain and straw yields, plant height, harvest index, 1000 seeds weight, hectolitre weight and protein content) were determined. Statistical analysis of variance was carried out using the SAS procedures (SAS Institute, 1998). Differences among the means were analyzed at the P≤0.05 probability level, applying the Least Significant Difference (LSD) and the Duncan Multiple Range Test (DMRT), for two and more than two means comparison, respectively.

## Results and discussion

The weather during the trial period was characterized by great variability. With reference to the interannual cycle “September-July”, the rainfall were 484, 599 and 520 mm, respectively for 2004/05, 2005/06 and 2006/07 trial years, vs. the 553 mm of the long-term averages 1952-2004. The average monthly temperatures were 16.0, 16.5 and 16.2 °C, for the same three years, vs. the 15.5 °C of the long-term period.

Table 1 shows that the best grain and straw yields, plant height, 1000 seeds weight, hectolitre weight and protein content were obtained in the 2005, followed by the 2007, trial years in which the rains were lower than 2006, but better distributed during the wheat cropping cycles.

With reference to the management strategies (Table 1), it is particularly interesting to note that grain yield and protein content of the CONS treatment, even if lower than those of the CONV one, did not show any significant difference, with values of 2.31 t ha<sup>-1</sup> vs. 2.74 t and 9.61% and 10.63% for both wheat parameters and agronomical practices, respectively. Similar results, but with significant differences, were found for grain yield by Lòpez-Bellido et al. (2001) and for grain protein content by Lòpez-Bellido et al. (1996) in Mediterranean environments, while Campbell et al. (1998) report that the tillage system has no effect on grain protein content.

On the contrary, significant differences were recorded for straw yield (3.37 t ha<sup>-1</sup> and 2.60 t, for CONV and CONS, respectively) and plant height (63.12 and 58.22 cm, for the same treatments, respectively).

### Conclusions

Despite the effect of the treatments were greatly affected by seasonal weather, the experiment allowed some interesting results coming from the application of conservative practices. In fact, the responses obtained during the trial period have pointed out the possibility to apply a lower level of agro-techniques, almost always without compromise the qualitative and quantitative wheat performance, confirming the findings of Montemurro and De Giorgio (2005) and Maiorana et al. (2007) in other crops.

Furthermore, considering that the slightly lower grain yields could be balanced by the lowest production costs and pollution risks, these practices appear very satisfactory from both the economical and the environmental points of view.

**Table 1. Effect of experimental treatments on quanti-qualitative parameters of durum wheat**

	Grain yield (t ha <sup>-1</sup> )	Straw yield (t ha <sup>-1</sup> )	Plant height (cm)	HI	1000 seeds weight (g)	Hectolitre weight (kg)	Protein content (%)
<i>Years</i>							
2005	3.26 a	4.84 a	64.73 a	0.40 b	47.31 a	83.97 a	11.69 a
2006	1.41 b	2.62 b	49.83 b	0.34 b	39.68 b	79.97 b	9.84 b
2007	2.90 a	1.49 c	67.45 a	0.66 a	40.65 b	79.47 b	8.82 b
<i>Treatments</i>							
CONS	2.31	2.60 b	58.22 b	0.48	42.96	81.13	9.61
CONV	2.74	3.37 a	63.12 a	0.46	42.13	81.14	10.63

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# Evaluation of Bread Wheat Cultivars on Organic Fields in Northern Italy

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From the data of the Ministry of Agriculture, there has been an incredible growth in organically managed land in Italy over the past years. By December 2006, there were 1.150.000 hectares of agricultural land organically managed, of which 20% grown with cereal, particularly durum wheat, barley and oat, followed by bread wheat, rice and rye. Bread wheat farmers, who decided to convert to organic farming can thus, find a profitable option in growing this cereal, lately supported by the extraordinary price increase for wheat grain (+ 73% between 2006 and 2007).

In this contest, the CRA-Research Unit for Cereal Selection and Valorisation of Plant Variety (CRA-SCV) has carried out, in the last three years, field experiments in organic fields of northern Italy, comparing different varieties of bread wheat with the scope to identify and recommend to farmers those which perform best (in term of yield, yield stability and quality attribute) under organic conditions.

## Methodology

In the growing seasons 2004-05, 2005-06 and 2006-07, thirty-six varieties of bread wheat were compared for agronomic parameters (grain yield, heading time, plant height, resistance to main diseases, 1000 seed weigh and test weight) in eleven northern Italy organic fields. In addition to agronomic performance, grain samples for the 2004 and 2005 sites were evaluated for protein content, test weight and SDS sedimentation volume (SDSS). Each year, field experiments were conducted for 20 varieties in eight organically managed fields using a lattice design with three replicates. Plots of 10m<sup>2</sup> were seeded at rate of 450 viable seed m<sup>-2</sup>. Agronomics practices varied by locations and years and, when used, fertilisation and weed control were performed in accordance with the Organic Production Standards. Varieties analysed are the mainly grown in the country and represent the effort of the south-European wheat breeding in the last 20 years. For each year, in addition to four testers representative of the quality classes (*Hard Wheat-Frumenti di Forza* FF, *Superior Bread Wheat-Frumenti Panificabili Superiori* FPS, *Bread Wheat-Frumenti panificabil* FP, *Biscuit Wheat-Frumenti da Biscotto* FB), varieties to be compared were chosen between those which performed best in the annual conventional varieties testing (in terms of yield, protein content and resistance to main diseases). Because cultivars are added and dropped from testing each year, different genotypes have been evaluated over years and only 10 and 14 are in common for the seasons 2004-06, 2005-07 respectively. For each agronomic parameters recorded in each locations analysis of variance has been performed.

## Results

Among premium quality wheat cultivars (*Hard Wheat*-FF and *Superior Bread Wheat*-FPS) Blasco was the best performing across years and locations in terms of yield and quality characteristics (test weight and 1000 seed weight), followed by Nomade, Palladio and Serpico although the experimentation revealed their low yield stability in the locations considered. Among the *Bread Wheat* FP quality class, for stable and high productivity, cultivars to be mentioned are Aubusson, Africa, Guarni, Isengrain and PR22R58, while for good and stable quality characteristics, Agadir and Palesio.

Among locations, the best performing in term of yield over years are Fiume Veneto (PN), Lonigo (VI), S. Pancrazio (PR) and Santa Giuletta (PV); the former two have recorded the higher protein content as well.

In northern Italy environments, the main factors influencing overall breadmaking quality (test weight, protein content, and SDSS) are generally lower than those observed in conventional management (Corbellini et al. 2006). Moreover, in our trials, premium quality wheat cultivars achieved protein content higher than those observed for FP and FB, although none of the FF and FPS varieties reach the value expected for the class, while values for FP and FB are within the expected ranges of the classes. The low protein value of premium quality varieties could be related to the fact that they need higher level of N to express their quality potential when compared to lower quality bread wheat cultivars, and this could limit their recommendation for organic production.

To confirm the hypothesis that old cultivars could be better adapted to organic environment as they have been bred during period of low-input agriculture, two cultivars (Abbondanza and Autonomia A) released before the seventies were evaluated in the organic fields in 2005. The results obtained, especially in terms of yield, do not support this hypothesis, as the two varieties were the least productive throughout the experimentation. These results are consistent with those obtained by other authors (Poutala et al. 1993, Kitchen et al. 2003) that show how old cultivars, at least in terms of yield are not better than newer for organic farming. In qualitative terms, however, it has to be noticed that these two lines recorded the highest protein content of the whole experimentation associated with a good gluten, which could be associated to a more efficient utilization and relocation of N. Although the higher quality do not compensate for the low yield, thus excluding old cultivars to be ready recommend to farmers for organic production, further investigation are needed to fully understand and exploit the potential of this material for organic agriculture.

## Conclusions

Among the cultivars evaluated, it is possible to recommend, within all the quality classes, varieties which perform best in term of yield in organic managed field although not all meet the quality characteristics of the class. This is particular true for the premium quality wheat cultivars (*Fruenti di Forza* and *Fruenti Panificabili Superiori*) which show lower quality values (protein content and SDS sedimentation volume) when compared to those obtained in conventional farming, especially during the less favourable years. Nevertheless, with particular attention to the agronomics practices which improve the availability of N during the critical stage of development, farmers could choose between a diverse panel of varieties suitable for organic production, preferably between the modern rather than those released not in recent years

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# Agro-Environmental Risk Analysis at Landscape Scale: Limits for a Sustainable Land Management

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## Introduction and objective of the paper

Research activities on optimal relations between agricultural development and environmental protection is one of the main agronomic challenges from the 80s ahead. Thenceforth production goals and claims for appropriate incomes for the farmers have been sided by the awareness that they cannot be split from environmental degradation risks. These risks result mainly from widespread pollution sources, intensification of agriculture, over-exploitation of resources, as well as from abandon in marginal areas. In this context we present a study aimed to integrate an agricultural system assessment with an agro-environmental risk analysis. Our focus has been to assess the agro-environmental risk at a landscape scale in order to identify the development limits in the study area. This has been done to support transformation processes of the current productive system toward a more “sustainable agriculture” (Bonari, 1995). The research has been carried out in the Grosseto Province (central Italy) where the environmental complexity have substantially affected the local socio-economical activities and, above all, the agricultural development (Pacciani, 2003).

## Methodology

The methodology has been based on a qualitative assessment of the agro-environmental risk on the arable land of the Grosseto Province. Erosion and loss of organic matter have been assumed as principal parameters to characterize the risk. They have been chosen after a bibliographical analysis crossed whit the results from surveys to the stakeholders (cooperatives and producer associations). This preliminary phase of the research has pointed out soil conservation as the foremost environmental limit for the development of the agricultural system in the study area (Galli et al., 2007). The analysis has not estimated quantitatively these risks. We have studied, instead, their effect on agro-environmental opportunities and limits for farming activities. We have employed a rule-based model analysis also called “cognitive model” since rules are defined by expert knowledge of phenomena. These kind of models are used usually for regional studies, so on

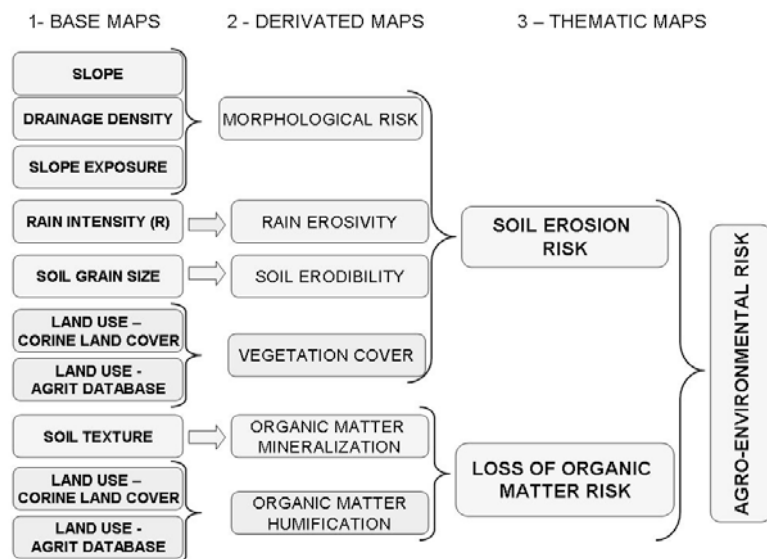


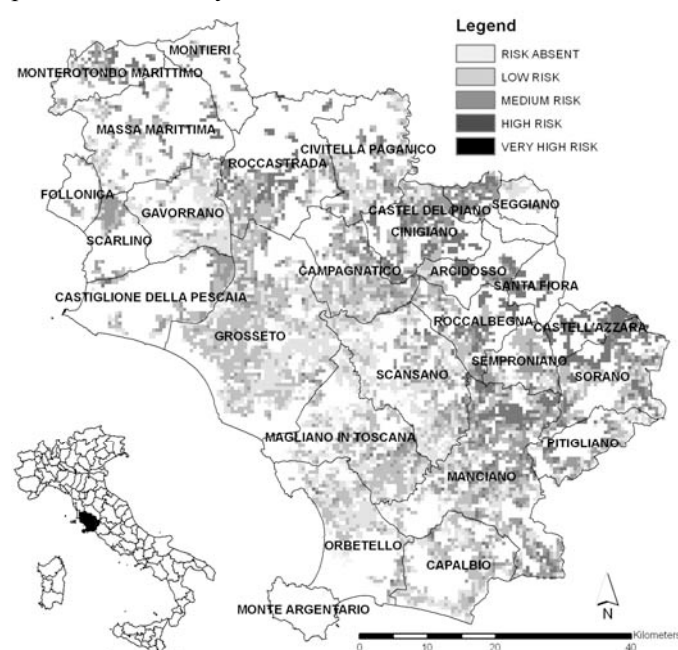
Fig. 1: Scheme of the methodology.

extensive spatial scale, where input data for quantitative modelling are not available (Kheir et al., 2006). In our study we have employed a hierarchical procedure (Fig. 1). Firstly, a set of “base” maps (1) of the parameters needed to analyse each risk has been classed and ranked as function of risk levels (2). Secondly, “derived” maps have been re-classed on five levels of increasing hazard (3) through the natural breaks method (Jenks, 1967); these levels have been defined in order to stress differences for environmental vulnerability within the territory. Finally, the outputs have been interpreted to locate “action areas” wherein proceed to design new development models.

## Results

As shown in Fig. 2, the distribution of risk classes is not homogeneous on the territory, although there is a gradient moving from coast to internal hills area. Areas with no agro-environmental risk are as expected lower than the others; they cover only 3% of overall arable lands in the Province. The largest part of the territory can be considered at “low” or “medium” risk, whereas “high” and “very high” risk

areas represent about 30% of the territory. Areas in fourth and fifth class are concentrated on mean and high hills zone, mainly on the south-east areas, where more than 35% of the arable lands has a very high vulnerability level. On these outputs we can distinguish four sub-areas with different risk classes distribution: the coastal zone, with a low/medium risk level; two hills areas (northern and eastern) with a medium risk level; the “Amiatine” hills with a high risk level.



**Fig. 2: Thematic map of agro-environmental risk in Grosseto Province.**

Sub-areas	Null	Low	Medium	High	Very high
Coastal zone	3.2	38.9	44.0	13.1	0.8
Northern hills	3.8	28.9	35.0	22.1	10.2
Eastern hills	3.1	30.2	34.4	22.4	9.9
Amiatine hills	0.5	13.6	25.5	24.5	36.0

**Tab. 1: Percentage distribution of risk classes in the sub-areas**

## Conclusions

The identification of four sub-areas through the assessment of the agro-environmental risk has provided a support to specify enhanced development models. In the sub-areas with lower risk intensive development models can be promoted achieving high quality productions (hard wheat, horticulture). On the contrary, areas with higher risk should be addressed toward conservative development models, providing alternations and recovering traditional productions (i.e. forage-livestock chain) thus improving the organic matter conservation.

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# Response of Sweet Maize to Different Cultivation Systems and Nitrogen Mineralization in the Soil

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## Introduction

Cultivation systems and mulch have an important influence on growth and yield of vegetables and field crops. In many cases the use of mechanical weeding equipment was investigated. The rotary hoe is an effective tool in controlling weeds in an integrated weed management approach or for organic sweet maize production (Leblanc et al, 2006). On the other hand according to Kwabiah (2004) plastic mulches are used at planting as a simple, cost effective, soil warming and season extending technique that ultimately improves stand establishment and hastens maturity. The plastic mulch acts like a glass house by capturing and retaining daytime solar radiation and reducing heat loss at night, producing a mini-greenhouse effect. Neeteson et al. (2001) expose the facts on large amounts of nitrogen remains in the soil after harvesting the crops. Application of the recommended rates of fertilizers to other field vegetables, however, may leave large amounts of residual soil mineral nitrogen (Nmin), especially after crops that are harvested before maturing as it is the case of sweet maize.

The aim of this research was to investigate the effects of mulch and cultivation systems with different hoeing intensity during the growing period on growth, yield and nitrogen mineralization in the soil.

## Methodology

Field experiments were conducted at the University Research Centre (Faculty of Agriculture) in Maribor, Slovenia (46° 39'N, 15° 41'E) on a loamy sand soil in years 2005 and 2007. Factorial arrangement of a randomised block design with four replications was used. Treatments were: control plot (without hoeing - Co), hand cutting (mulching) of weeds (Cu), hoeing 1 × (H1), hoeing 2 × (H2), hoeing 3 × (H3), hoeing 4 × (H4), hoeing 5 × (H5), hoeing 6 × (H6) times during the season, plots with black polyethylene foil (F) and plots with straw (S). The basic plot had an area of 19, 6 m<sup>2</sup>. The preceding crop was clover. Plant density was 8 plants/ m<sup>2</sup> with the distance between the rows 0, 7 m and in the row 0, 15 m. Before sowing Nmin soil analysis have been done (to a depth of 0, 6 m). With regard to results of Nmin analysis plots were fertilized with organic nitrogen fertiliser Biosol (target value of N – Nmin = 200 kg N ha<sup>-1</sup>). Crops were observed and attended through the seasons. Samples for measuring nitrogen levels in the soil were taken two times during the season (in the stage of teaselling and after harvesting – milk stage). Statistical analyses were done using the ANOVA procedure (statistical program Statgraphics® Centurion XV).

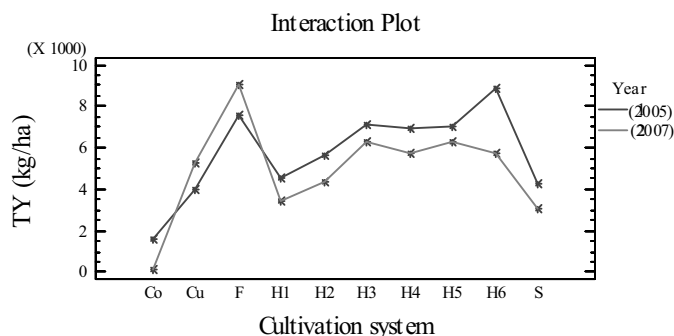
## Results

The cultivation system showed significant influence on mean total (TY) and marketable yield (MY) (P=0,001) and also year affected MY (P=0,001) and TY (P=0,05). TY and MY were significantly influenced by cultivation systems × year interaction (P=0,05). In both years TY and MY by foil use were in the group with the highest yields. The TY was significantly higher in plots with foil use and H6 compared to the control. MY was also significantly higher in plots with foil use, H6 and H4 compared to the control.

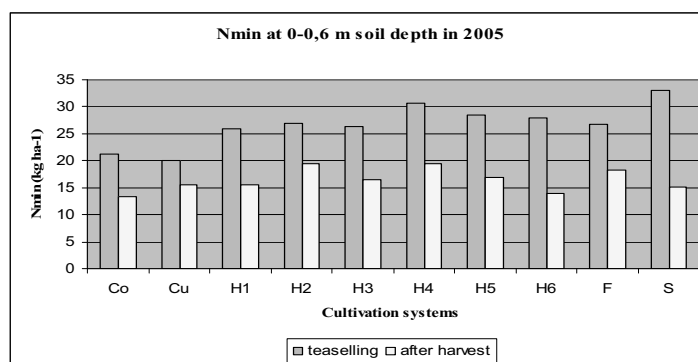
**Table 1: Effect of years and different cultivation systems on total (TY) and marketable cob yield (MY) of sweet maize (kg ha<sup>-1</sup>)**

	TY	MY
<b>Year (Y)</b>	*	**
<b>Treatment (T)</b>	**	**
<b>Interaction (YxT)</b>	*	*
<b>Year</b>		
2005	5736,6 a	4942,3 a
2007	4935,6 b	3699,2 b
<b>Treatment</b>		
Co	854,2e	660,4d
Cu	4611,6d	3872,4c
H1	4009,6d	3344,4c
H2	4993,0dc	4160,7cb
H3	6702,2cb	5725,8ba
H4	6332,9b	5306,8a
H5	6646,1b	5316,3ba
H6	7276,8ba	5601,4a
F	8275,5a	6299,7a
S	3659,4d	2919,6c

Legend: TY – total yield (cobs with husk)  
MY – marketable yield (cobs without husk)  
\* significant indicated at P=0,05, \*\* at P=0,01  
a,b,c means within column followed by the same letter are not significantly different at the 95% confidence level (Duncan's test)



**Figure 1: TY as affected interaction of treatments (year 2005 and 2007)**



**Figure 2: Average Nmin values (NH<sub>4</sub>-N and +NO<sub>3</sub>-N) to 0,6 m depth during the vegetation period (teaselling and after harvest) in the year 2005**

Preliminary results showed that the Nmin to depth 0,6 m (in development stage of teaselling in June) were higher in treatments with H4 and treatment with straw use. The highest Nmin content by straw use was probably due to humidity under straw, which results in intensive mineralization in the summer time. Low yields in plots by straw use can be linked with intensive process of decomposition of straw. Average Nmin rests after harvesting in all treatments were low (average 16,4 kg ha<sup>-1</sup>).

## Conclusions

Two year results have confirmed that mulch and hoeing intensity have influence on the plant growth and nitrogen levels in the soil. In organic farming, sweet maize could be cultivated with frequent hoeing (3 – 6x) in the season or by using black foil to attain higher yields. Regarding the Nmin results in 2005 the conclusion about the effect of hoeing and mulch system on N mineralization in the soil should be considered with caution and some further investigations will be needed in the future.

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# The Weed Flora Infestation and Yielding of Winter and Spring Wheat in Organic System

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Weed infestation in crops cultivated in organic system is one of the most common agrotechnical problems for many farmers. On the other hand some authors suggest that it is possible to maintain weed density on the level that doesn't influence the yield (Janczak-Tabaszewska and Tyburski 1999, Feledyn-Szewczyk and Duer 2007). The weed control strategy in organic farming is based on indirect methods, containing crop rotation, choice of species and cultivars with bigger competitiveness ability due to weeds, using of undersown crops and direct methods: mechanical, biological, physical (Eisele 1998, Davies and Welsh 2001, Hauggaard - Nielsen H. et al. 2006). Connection of these measures should result in increasing the dominance of crop canopy on weeds. The aim of the research was the analysis of weed flora infestation and yielding of winter and spring wheat cultivated in organic crop production system.

## Methodology

The study was conducted in the years 2005-2007 at the Experimental Station of Institute of Soil Science and Plant Cultivation - State Research Institute in Osiny (Lublin province, Poland), where these plants were grown in 5-field crop rotation. According to organic agriculture rules any mineral fertilisers and chemicals were not use on these fields. In winter wheat weeds were controlled in mechanical way using a weeder whereas in spring wheat the undersown crop (the grass with white clover and lucerne) was an element of weed regulation. The analysis included number of weeds and their dry matter in dough stage as well as the grain yield of winter and spring wheat.

## Results

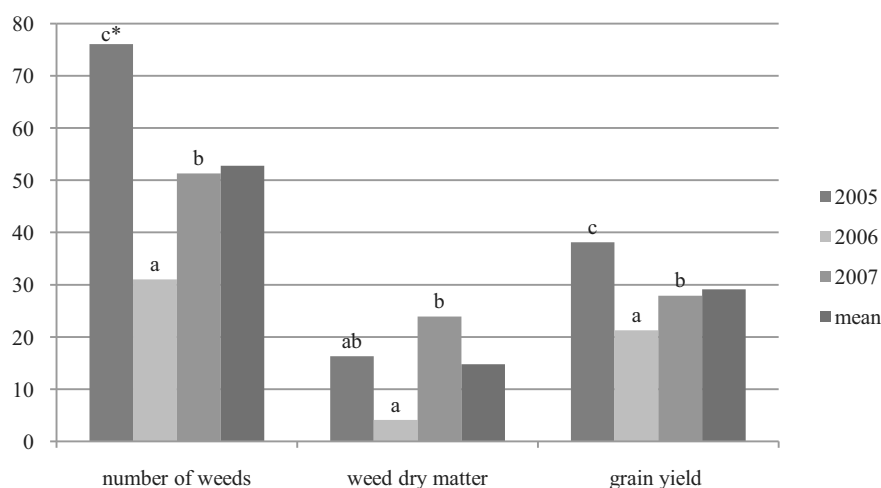


Fig. 1. Number of weeds (plants  $\cdot$  m<sup>-2</sup>), weed dry matter (g  $\cdot$  m<sup>-2</sup>) and grain yield (dt  $\cdot$  ha<sup>-1</sup>) of spring wheat with undersown crop cultivated in organic system

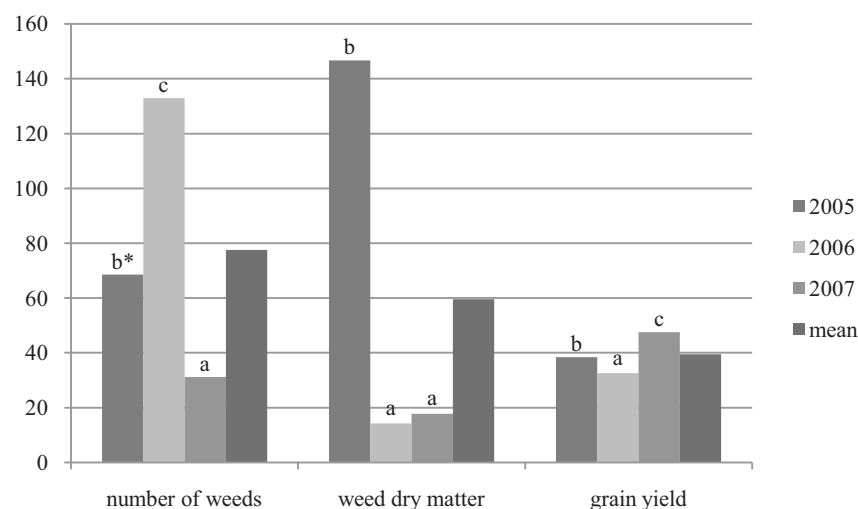


Fig. 2. Number of weeds ( $\text{plants} \cdot \text{m}^{-2}$ ), weed dry matter ( $\text{g} \cdot \text{m}^{-2}$ ) and grain yield ( $\text{dt} \cdot \text{ha}^{-1}$ ) of winter wheat cultivated in organic system

\* values marked by the same letter are not significantly different at  $\alpha=0,05$  (LSD Tukey)

The level of weed infestation was higher in winter wheat than in spring wheat (fig. 1-2). Low weed density ( $31\text{-}76 \text{ plants} \cdot \text{m}^{-2}$ ) and dry matter ( $4\text{-}24 \text{ g} \cdot \text{m}^{-2}$ ) in spring wheat suggest good competitive ability of spring wheat canopy with undersown crop (fig. 1). The effectiveness of weed control in spring wheat was enough to maintain weed infestation on the level that doesn't reduce the grain yield.

In winter wheat canopy only in 2005 year the weed infestation was so big that it could decrease the yield ( $147 \text{ g} \cdot \text{m}^{-2}$ ), which was caused by domination of *Papaver rhoeas*, but in the next years dry matter of weeds was smaller than  $20 \text{ g} \cdot \text{m}^{-2}$  (fig. 2). The grain yield in winter and spring wheat in 2006 was affected by drought during vegetation period.

## Conclusions

The weed infestation of wheat cultivated in organic system depended on effectiveness of direct and indirect methods of weed regulation, especially harrowing in winter wheat and undersown crop in spring wheat. The results showed that it is possible to maintain the density of weeds and their dry matter on the level that doesn't decrease the grain yield of wheat in organic farming.

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# Green Manure as a Tool for Improving C and N Balance of Mediterranean Cropping Systems

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## Introduction

It is well known that high input cropping systems may cause the decay of soil fertility, thus limiting crop yield and soil resilience. Furthermore the typical environmental conditions of Mediterranean area (high temperature coupled with high moisture in autumn and spring periods) usually enhance this trend because of an increased SOM degradation. In this context, Green Manures, especially with legume crops, may represent a useful tool for improving soil fertility also reducing external energy inputs because they allow to increase soil N and P availability for the following crop and at the same time, contribute to the conservation of SOM and soil biological, physical and chemical properties (Mc Vay et al., 1989). On the other side, winter non leguminous crops, thanks to their high C up N ratio could improve C storage in soil, thus resulting also effective in capturing post-harvest soil N, so reducing autumn N leaching (Uppendra, 2007). A mixture of legume and non legume cover crops could be more suitable to both increase organic matter and reduce the potential N leaching. Nevertheless, adequate quantities of C and N are not easy to predict, because biotical (weeds) and a-biotical factors (rainfall and temperature) could limit growth and N-fixing of the crops used for green manuring. This paper shows the results of a two year trial on the effects of different green manure treatments (leguminous, non leguminous and mixtures) on C and N balance and yield of maize.

## Methodology

The experiments were carried out on maize in Torre Lama Farm of Naples University (Southern Italy 43°31'N, 14°58'E) in 2004-2005 and 2005-06 on a Clay-Loam Soil. Rainfalls were high during growth periods of cover crops (442 mm in 2004-2005 and 635 mm in 2005-2006), and low during growth periods of maize (65 mm in 2005 and 250 mm in 2006). The treatments were M= Mineral fertilization; C= Not fertilized Control; F and FO= respectively Fababean and Fababean+Oat; V and VO= Hairy Vetch and Hairy Vetch+Oat. All the data were subjected to ANOVA (complete randomized block with 4 replicates) and means separation was made using LSD test. F, V and O were sown with 160, 50 and 200 kg ha<sup>-1</sup> of seeds respectively, while the doses were halved for mixtures. Green manure crops were sown on 28/11/04-25/11/05, cut and buried at early flowering on 28/4/05 and 17/5/06. Yield of above-ground biomass of cover crops was affected by cumulative rainfall during the growing period and by the competitive effect of weeds and of consociated species. 2005 was characterized by the lowest biomass yield and N inputs because of water surplus from November 2004 to April 2005. The differences of N inputs were not significant between F and V (100 and 179 kg ha<sup>-1</sup> of N in 2005 and 2006, on the average). Maize was sown on 29/4/05 and 22/5/06 at 0.80 m x 0.20 m and harvested at full ripening on 8/9/05-11/10/06. Biomasses were weighted, splitted in their components and oven-dried. Soil was sampled at three depths at seeding and harvest of maize. Mineral N was measured by HACH® method, total N and organic C by Kjeldhal and Walkely black methods. N mineralization from soil was estimated by N uptake of control+soil mineral N variation between sowing and harvest.

## Results

In both the years, the highest maize yield was gained with M (10.1 t ha<sup>-1</sup> on the average), followed by F and V (7.5 and 7.9 t ha<sup>-1</sup> respectively). The lowest values were recorded for O (4.0 t ha<sup>-1</sup>), C (5.2 t ha<sup>-1</sup>)

and mixed manures (5.1 and 6.0 t ha<sup>-1</sup> for FO and VO). In the first year, M gave the highest N uptake (175 kg ha<sup>-1</sup>) followed by V (129 kg ha<sup>-1</sup>), while no differences were recorded among the other treatments that ranged from 65 to 95 kg ha<sup>-1</sup> of N. N recovery (Tab. 1) for V was at the same level of M (65% and 88% respectively), thanks to the low C/N ratio of its biomass, while it was the lowest for mixtures, because of the low decomposability of their biomasses. M gave the highest uptake values in 2006 too (163 kg ha<sup>-1</sup> of N), while all the other treatments showed lower N uptake; there was no difference among all the treatments in N recovery in 2006. N surplus (supplies–uptakes) showed negative values for C and O in both years, because of the low N inputs. Values for the other treatments were not different from zero in 2005 and positive in 2006. Of course, N balance showed higher values for all treatments, if N from mineralization is added (99 kg ha<sup>-1</sup> in 2005 and 55 kg ha<sup>-1</sup> in 2006).

**Table 1. N balance (Su = supplies; Up = uptakes) and N recovery of maize cycle**

Treatments	2005		2006	
	Su - Up (kg ha <sup>-1</sup> )	N recovery (%)	Su - Up (kg ha <sup>-1</sup> )	N recovery (%)
<b>O</b>	-31.7 b	31.9 b	-48.2 de	7.8
<b>OM</b>	54.9 a	23.2 b	14.0 b	34.2
<b>F</b>	7.5 ab	20.2 b	84.7 a	18.3
<b>FO</b>	8.0 ab	-8.7 b	78.2 abc	2.2
<b>V</b>	-12.6 b	88.9 a	50.8 abc	36.0
<b>VO</b>	-14.1 b	-12.5 b	48.0 abc	21.1
<b>M</b>	-27.6 b	65.5 b	-3.5 d	57.3
<b>C</b>	-105.1 c	---	-71.8 e	----

Values with the same letters are not different at  $P < 0.05$

### Conclusions

This two year experiment showed that legume crop green manure can give a maize yield not different from mineral fertilization. The application of high quality organic input such as Vetch (with low lignin/N and C/N ratio) could provide a more efficient use of nutrients by releasing N quickly for food crops. Green manuring made with mixed crops and pure oat cause a significant reduction in yield of the following crops, because of lack in available N due to high C/N ratio of biomass. Nevertheless application of lower quality plant residues (high in lignin and low in N), such as oat, may maintain or increase SOC, and build-up soil organic N in the slowly turning pool (Sanchez, 1989), but only additional N fertilization can make this tool sustainable for farm economics. High N surplus at harvest was assessed for all treatments including C, showing that nitrate leaching and ground water pollution could involve organic farming too. Growing winter N catch crops between the summer crops may be one way to reduce this problem. Those crops can uptake the residual inorganic N present in soils at the end of the summer growing season as well as that, which will be mineralized during the following winter period, till the beginning of the next summer season (Rodrigues, 2001). Furthermore extreme variability in biomass and N supplies with green manure suggest to integrate this tool with other techniques of soil fertility management.

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# Can the Reduction of Nitrate Leaching Decrease the Consumption of Fossil Energy?

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Two of the most important issues in modern agricultural systems are: i) to improve nitrogen (N) management and ii) to reduce the consumption of non-renewable fossil energy resources and the emissions of greenhouse gases. Within a project aimed at improving N management in intensive farms in northern Italy, we conducted a simulation study to evaluate if reduced nitrate leaching is accompanied by decreased fossil energy consumption.

## Methodology

We conducted a survey on six farms (dairy and arable) located in Lombardy region (northern Italy) and representative of different soil and climate types, different crop and animal production systems, and different management types. During the survey we collected data about farm management, using a structured questionnaire (Fumagalli et al., 2008). To simplify data management, farm fields were aggregated into homogeneous areas for soil type and crops management. The indicators that we have calculated for each homogeneous area were the nitrate leaching (using a cropping systems simulation model) and fossil energy use. Energy use efficiency was calculated as the ratio energy output / energy input. Energy input is the sum of direct and indirect energy consumption. Direct energy is that contained in fuel and lubricants consumed for the agronomic operations (including the transport of machinery and products from farm to field and vice versa). Indirect energy is the energy content of: pesticides applied, chemical and organic fertilisers and seeds. Fuel and lubricants consumption were estimated by using a mechanisation model that takes into account the soil type and the tractor-implement combinations (size and expected work rate) detected and recorded for each individual crop operations. For each production factor, updated energy equivalents were derived from the literature (Castoldi, 2008). The energy content of the manure was calculated by multiplying the amount of manure-N applied by the mineral fertiliser equivalent (= apparent nitrogen recovery of manure-N / apparent nitrogen recovery of mineral-N), and by the energy content of mineral N. Energy output was obtained by multiplying the energy content in above ground crop dry matter by a calorific coefficient. As in many cases the soil surface balance was positive (indicating excess), we defined alternative N management strategies to reduce this surplus. To this purpose, we have used a cropping systems simulation model to define adequate doses of sidedress inorganic N application for maize silage and to evaluate if inorganic N application for winter forage crops could be eliminated. We also introduced a winter catch crop following maize. We present the results for one non-irrigated dairy farm with silt – clay soil types and elevated annual precipitation (1150 mm on average).

## Results

Model outputs for actual and improved N management scenarios for two homogenous area with two different cropping systems (continuous silage maize and double-cropping system with maize and Italian ryegrass) are compared. In both cropping systems simulated crops yields remain almost unchanged in the improved compared to the actual scenario. Introducing a catch crop following

continuous silage maize and reducing sidedress N inorganic application for maize resulted in a substantial reduction in nitrate leaching (Table 1 and Figure 1). The energy use efficiency did not change because the limited use of mineral fertilisers is compensated by tillage operation for winter cover crop (Figure 2). Also in the double cropping system nitrate leaching decreased significantly after the reduction of sidedress N inorganic application for maize and the elimination of inorganic N application for Italian ryegrass. The increase of energy use efficiency is due to the decrease of energy content of mineral fertilisers.

Table 1. Comparison between actual and improved N management for two cropping systems: average of simulated crop yield and nitrate leaching (and standard deviation) and energy indicators.

Cropping System	Scenario	Fertilisation (kg N/ha)	Crop yield (t DM /ha)	Nitrate leaching (kg N / ha)	Energy inputs (GJ/ha)	Energy outputs (GJ/ha)	Energy outputs / inputs (GJ/GJ)	E out – E in (GJ/ha)
Continuous silage maize	Actual	110 (lm) 211(mn)	15.1 (4.2)	156 (69)	29	268.5	9.2	239
	Improved (with catch crop)	110 (lm) 110 (mn)	14.8 (3.9)	17 (17)	29	263.2	9.0	234
Double-cropping systems: Italian Ryegrass + silage maize	Actual	Silage maize: 110 (lm), 156 (mn)	10.8 (3.6)	178 (89)	43	324.6	7.4	281
		Italian ryegrass: 71 (lm), 42 (lm)	5.1 (0.7)					
	Improved	Silage maize: 110 (lm), 52 (mn)	10.8 (3.6)	38 (35)	35	324.6	9.2	289
		Italian ryegrass: 71 (lm)	5.1 (0.7)					

lm = liquid manure; mn = mineral fertilisers

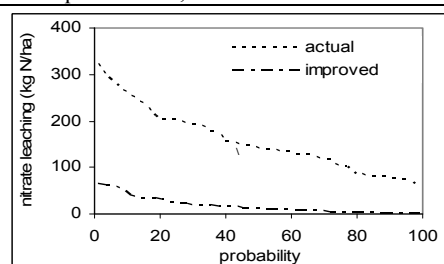


Figure 1. Breakthrough probability exceeding N leaching level for two scenarios of continuous silage maize.

## Conclusions

Results of first analysed farm show a possibility of leaching reduction using improved management. Catch crops do not allow for improvement of energy efficiency while the reduction of mineral fertilisers use does it. Results for six more farms are being analysed using the same methodology.

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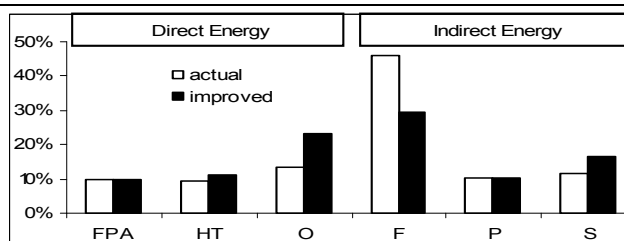


Figure 2. Percentage partitioning of simulated direct (FPA = fertiliser and pesticide applications; HT = harvest and transport; O = other crop operations) and indirect (F = fertilisers; P = pesticides; S = seeds) energy inputs in continuous silage maize.



# Evaluation of Winter Cereals for Their Suitability to Organic Farming Systems

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The adaptation of plant species to organic farming systems is affected by many factors including competitiveness to weeds and resistance to natural occurring foliar diseases. Cereals that confer a high degree of crop competitive ability, especially against aggressive weeds, are highly beneficial in organic agriculture and other farming systems that aim to reduce the reliance on herbicides by integration of chemical and cultural methods (Mason and Spaner, 2006). Additionally, competitive cultivars will be beneficial in situations where herbicide resistance has been developed. There has been great variation among the winter cereals regarding the rate of growth and development, resistance to diseases as well as the agronomic traits. The aim of this study was to perform a relative evaluation of five winter cereals for their competitiveness to broad-leaf and grass weeds, susceptibility to powdery mildew and various agro-physiological traits under field conditions.

## Methodology

The experiment was carried out at the farm of the Cereal Institute of Thessaloniki, Greece during the 2004-2005 growing season. The winter cereals examined were bread wheat (*Triticum aestivum* L. subsp. *aestivum*), durum wheat (*Triticum turgidum* L. subsp. *durum*), triticale (*X Triticosecale* Wittmack), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.). Three cultivars from each species were used. Plants were arranged in the field using a split plot experimental design with four replications. Cereal species were used as main plots and cultivars as subplots. Subplots were consisted of 7 rows, 4 m long each with a distance between rows of 0.25 m. The sowing was done in 23 of November using the recommended seed rate for the area. The weeds that were developed in the field were mainly the annual grasses *Anena sterilis*, *Lolium spp.* and *Phalaris spp.* and the annual broad-leaf *Veronica hederifolia*, *Sinapis arvensis* and *Papaver rhoeas*. Competitiveness to weeds was taken using a scale of 0 (competitive plants) to 9 (non-competitive plants). Susceptibility of plants to powdery mildew was assessed using a scale from 1 to 9, where 1 indicated no symptoms and 9 highly susceptible plants. Plant height at the booting stage, anthesis and maturity, resistance to lodging, and time required for the development of the various plant stages were determined. All data were subjected to analysis of variance (ANOVA) and the means were separated using the least significant difference test.

## Results

Significant differences among plant species were observed for early growth and plant height. Early growth was on average higher for oat and accompanied by higher plant height early in the spring (63 cm). On the other hand, bread wheat showed the lowest mean early growth and plant height in the spring (50 cm). Oat and barley was found to have the highest competitiveness to weeds (8.25) and durum wheat the lowest (6.4). Competitiveness to weeds was associated to plant height, as indicated by the significant positive correlation found between the two parameters ( $r=0.674$ ,  $P<0.01$ ). These results are in agreement with previously reported researches showing that taller cereal plants reduced weed

biomass more than did shorter plants and thus were more competitive (Blackshaw et al., 1981, Balyan et al., 1991). Significant variation among cereal species was also detected for the resistance to powdery mildew. Bread wheat cultivars showed on average the highest resistance (3.9), whereas barley and oat the lowest (5.9 and 5.7, respectively). Averaged across cultivars, barley and oat plants required lower number of days to maturity (153d), which is mostly due to the shorter duration of grain filling rather than to earlier heading (Table 1).

Table 1. Early spring plant height, competitiveness to weeds, powdery mildew susceptibility, and vegetative and filling period duration of various cultivars of five winter cereals.

Plant species	Cultivar	Early spring plant height (cm)	Competitiveness to weeds (1-9)	Powdery mildew susceptibility (1-9)	Vegetative period duration (days)	Seed filling period duration (days)
Barley	Konstantinos	40	7.5	7.3	130	26
	Persephoni	61	8.8	5.3	121	30
	Kos	63	8.5	5.3	124	28
	Mean	55	8.3	5.9	125	28
Bread wheat	Orpheas	66	7.3	3.8	123	33
	Elisabet	51	7.3	3.8	126	32
	Oropos	49	6.3	4.3	130	27
	Mean	55	6.9	3.9	126	31
Durum wheat	Athos	58	7.5	4.5	126	30
	Mexicalli	46	6.0	5.3	121	35
	Papadakis	46	5.8	6.0	121	36
	Mean	50	6.4	5.3	123	34
Oat	Pallini	51	7.5	3.8	134	20
	Phlega	71	8.5	5.5	121	32
	G-018839	66	8.8	7.8	122	31
	Mean	63	8.3	5.7	125	28
Triticale	Thisbi	51	7.5	4.3	120	38
	Vronti	53	6.3	4.5	119	38
	Niovi	59	7.0	4.8	118	39
	Mean	54	6.9	4.5	119	38
LSD <sub>0.05</sub> <sup>1</sup>		6.3	1.5	0.87	2.2	3.1
LSD <sub>0.05</sub> <sup>2</sup>		5.0	1.1	0.44	1.8	1.6

<sup>1</sup> For comparisons between cultivars for a given plant species; <sup>2</sup> For comparisons between means of plant species.

## Conclusions

Significant variation among and within winter cereals examined was found for all traits studied. Farmers can choose the most appropriate plant species and cultivars using economic parameters and suitability for adaptation to organic farming systems based on criteria such as the weed competitiveness and the resistance to foliar diseases. By this point of view, oat and barley was found to have an advantage compared to other cereal species examined in this study.

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# The Efficiency of Stubble Catch Crops - Comparison of Two Seasons with Different Meteorological Conditions

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The stubble catch crops, sown in late summer and early autumn after the harvest of main crop, are introduced to cropping systems with prospect of several benefits, especially soil protection from erosion and retention of nitrogen. However, the expected positive effects directly depend on the success of catch crops, that is the ability to produce substantial amount of above-ground and below-ground biomass before the onset of winter. The establishment and growth of stubble catch crops is limited chiefly by the length of growing period determined by (sum of) temperatures, radiation, the occurrence of early frost and by water availability in some years. Poor growth diminishes expected positive effects. To use catch crops effectively and to broaden the diversity of suitable species better understanding of the impact of variable weather conditions on their growth and efficiency is needed.

The aim of the study was to compare the performance of several species of common or prospective stubble catch crops in respect to their effect on soil nitrogen content.

## Methodology

In the field experiment 10 plant species, including both overwintering and frost susceptible ones, were evaluated: white mustard (*Sinapis alba* L.), phacelia (*Phacelia tanacetifolia* Benth.), radish (*Raphanus sativus* L. var. *oleiformis*), buckwheat (*Fagopyrum esculentum* L. Moench.), millet (*Panicum miliaceum* L.), sea kale (*Crambe abyssinica* Hochst. Ex. R.E.FR.), semipermanent rye (*Secale cereale* L. var. *Multicaule* Metzg.), common canary grass (*Phalaris canariensis* L.), safflower (*Carthamus tinctorius* L.), mallow (*Malva verticillata* L.) and Westerwold ryegrass (*Lolium multiflorum* var. *Westerwoldicum*). Bare soil was included in the scheme as a control treatment. The experiments were established at two sites with different soil and climate conditions (heavy and medium soils, lower and higher average temperatures and precipitation) after winter wheat, on two terms of sowing, viz. summer one – in the middle of August and delayed one – in the middle of September. The crops were left to overwinter. The above-ground biomass of the crops was determined on two terms: 45 days after emergence and at the end of growth period (end of November). Nitrogen concentration of aerial dry mass was determined and nitrogen yield was calculated. Soil was sampled for the content of mineral N in 0-90 cm before and at the end of winter, nitrate concentration of soil solution sampled with suction cups in bare soil and under phacelia or mustard was determined. Further, the rate and uniformity of emergence, development rate, the height of plants, occurrence of frost damage and weed infestation were determined in the experiment. The temperature at selected crops was measured at 30 minutes interval. The data of two seasons 2006/07 and 2007/08 are available, the experiment will continue further two seasons.

## Results

In the poster the results from both sites are presented, in the contribution only data from Praha-Ruzyně are shown (Figure 1). The efficiency of catch crop species is evaluated using data on crop establishment, biomass yield and nitrogen uptake before winter, mineral N content in soil and the concentration of nitrate in soil solution. The results showed a great variability of biomass and N yield among species (more than 100 %), in their reaction to delayed sowing term and weather conditions. As expected, biomass yield was strongly related to amount of nitrogen fixed in crops ( $R^2=0.95$  and  $0.96$  in years 2006 and 2007, resp.) and high yields resulted in a low soil mineral N content before winter.

White mustard, radish, phacelia, crambe and buckwheat had the highest biomass and the N yield in both years and on both terms of sowing (except for buckwheat) in comparison with other species. The crops also showed good emergence under dry conditions in 2006. The species sensitive to low temperatures, buckwheat and millet, were killed as soon as in November by occurrence of only one or two-day night ground frost in both years. This trait decreases their protective functions during winter. On the other side, soil cultivation in spring is easier due to low amount of crop residues. The similar results were obtained from the other site - Lukavec at Pacov (potato growing region).

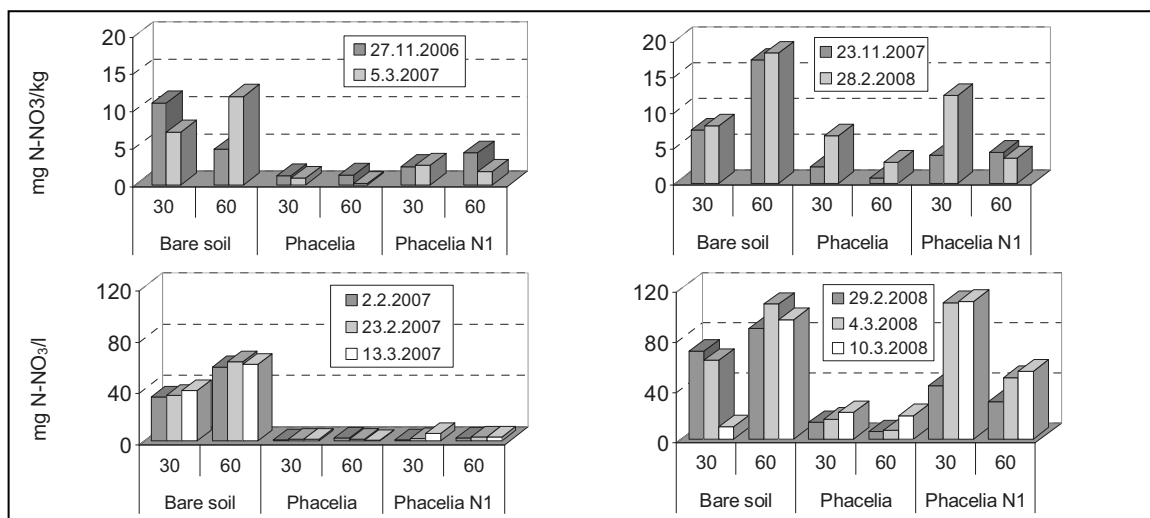


Figure 1. The content of nitrate N (in mg/kg dry soil) in soil in autumn and at the end of winter, and the concentration of N nitrate (mg/l) in soil solution on two or three terms in layers 0-30 cm (30) and 30-60 cm (60) under bare soil and phacelia crop. "Phacelia N1" indicates treatment with additional 50 kg N/ha applied at the start of growth.

Phacelia and other catch crops depleted most of supply of soil mineral nitrogen (mostly nitrate) in soil to depth of 60-90 cm before winter in both years (Fig. 1). The autumn and winter 2006/07 were exceptionally warm and almost without frost so that most of the examined species survived winter and were able to deplete soil nitrate. Further, the soil moisture was low that reduced winter leaching. The conditions resulted in a high soil and soil solution nitrate contents under bare soil and low ones under phacelia crop. In the season 2007/08 the favourite meteorological conditions enhanced growth of the crops but lower temperature and ground frost in early November killed or heavily injured (mustard, phacelia, crambe) plants of most species, except for radish, regrass and younger plants of mustard, phacelia, crambe from delayed autumn sowing term. We suggest that a warm and wet weather during the most of winter months 2007/08 enhanced the mineralization of soil organic matter and dead plant material of catch crops, especially that of leaves. It resulted in higher content of nitrate N in early spring 2008 in comparison with year 2007 and less tight correlation between soil nitrate N content and nitrate concentration in soil solution in 2008 ( $R^2 = 0.65$ ) than in spring 2007 ( $R^2 = 0.98$ ).

## Conclusions

The results showed great variability in biomass and N yield among species of stubble catch crops, and in the reaction to weather conditions and delayed sowing term. Weather during autumn and winter affected survival of the catch crops and the nitrate concentration at the end of winter suggesting variable effect of non-overwintering species on expected reduction of risk of nitrate leaching.

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# Forage Yield and Analysis of Competition in Cereal-Legume Intercrops

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Intercropping legumes with cereals for forage production is a sustainable technique showing several environmental benefits. In the Mediterranean area, most annual legumes, such as common vetch are sown with grain cereal to improve growing conditions and forage harvesting. Small grain cereals provided support for the climbing vetches, improved light interception and facilitating mechanical harvesting. A balanced botanical composition is needed, however, because legumes are of great importance to the nutritive value of the forage mixture (Thomson et al., 1990). Interspecific competition can often reduce yields and the growth rate of intercropping compared with cereal sole cropping. The objectives of this study were (i) to compare the yields from mixed stands of vetch and four cereals and (ii) to examine the relationships of competition in oat-common vetch intercrops at three seeding ratios for forage yield at two stages of maturity.

## Methodology

Field experiment was conducted during 2007-08 growing season at the Centre for Cereal Research in Foggia (Italy) (41°32'N, 15°43'E) using one annual legume: common vetch (*Vicia sativa* L. cv. Morava) and four cereal species: oat (*Avena sativa* L. cv. Genziana and cv. Rogar 8), barley (*Hordeum vulgare* L. cv. Diomede), triticale (*xTriticosecale* Wittmack cv. Catria) and dicoccum (*Triticum dicoccum* S. cv. Davide). The forage species were grown in mixtures containing one cereal and one legume at one seeding ratio. Only for the vetch-oat mixture three seeding ratios were examined. Plots size were 10.2 m<sup>2</sup> arranged in randomized complete block design with three replications. The row spacing was 17 cm and in the intercrop the seeds were mixed and sown together. The monocrops and the mixtures were harvested at the early flowering stage (EF=plants with 10% of flowers) and at the early pod-setting (EP=pod of 3 cm) of vetch. Plant height (cm), dry matter yield (DM) (g m<sup>-2</sup>) and botanical composition (%) were evaluated. Aboveground biomass was determined by harvesting plants at ground level from a 1 m<sup>2</sup> net plot. To determine botanical composition, plants in 1 linear meter per plot were harvested, hand-separated, counted and dried 72 h at 60°C for dry matter determination. Relative yields of both species were calculated as the ratio of yields in mixture to yields in monocrop. Relative yield total (RYT; the sum of both relative yields) was used as the criterion for mixed stand advantage as both legume and cereal were desired species. The value of unity is the critical value, with the intercrop favoured above and the monocrops favoured below that value (Vandermeer, 1990).

## Results

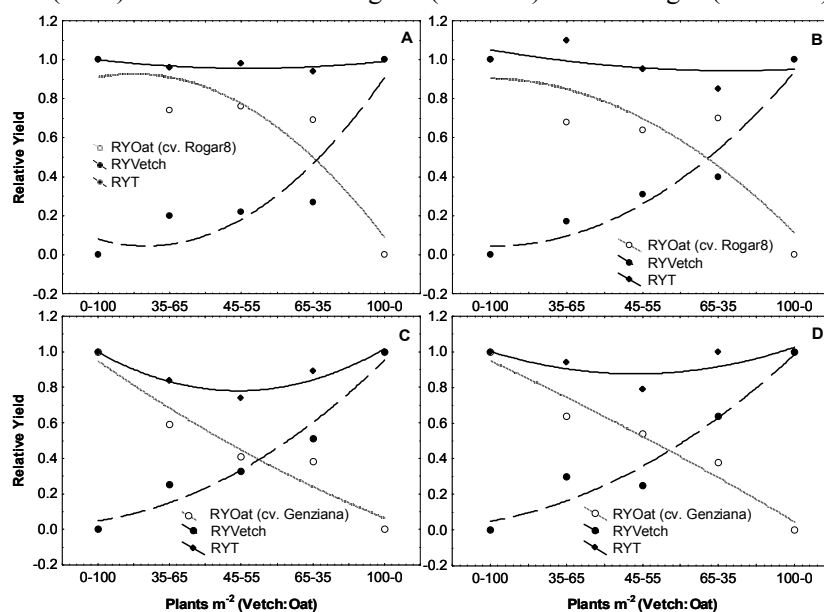
The net effect of cereals in the intercrops was to produce about 0 to 40% and about 47 to 113% more DM than the sole crop of common vetch under EF and EP stages, respectively. On the contrary, the intercrops produced about 12-65% more dry matter than the common vetch sole crop, but 4-21% less than cereal sole crops. Dry matter yields of mixtures of common vetch with oat were not affected by seeding ratio of vetch but proportions of common vetch decreased as the percentage of cereal seed increased in the mixtures (Table 1). Barley monoculture provided greatest forage yield, whereas the greatest RYT (1.10) was calculated in the common vetch-oat (cv. Rogar 8) intercrop at the 35:65 seeding ratio under EP treatment (Fig. 1). In this case, the total relative yield value exceeded unity, which indicates an advantage of intercropping over sole cropping in terms of using the environmental resources for plant growth.

Table 1. Plant height (PH), dry matter (DM) and vetch contribution (%) of sole crops and intercrops of common vetch with four cereals at different seeding ratios during 2007-2008 at Foggia, Italy.

Crop	Seed Ratio	PH (cm)		DM g m <sup>-1</sup>		Vetch (%)	
		Vetch	Cereal	EF	EP	EF	EP
Oat (cv. Genziana)	100		78	100	95.0	167.8	
Vetch:Oat	35:65	77	97	70	87	76.8	143.2
Vetch:Oat	55:45	72	108	64	97	71.7	144.5
Vetch:Oat	65:35	83	118	70	88	80.5	125.8
Oat (cv. Rogar8)	100		90	113	112.8	177.2	
Vetch:Oat	35:65	75	100	88	107	101.2	135.7
Vetch:Oat	55:45	81	108	87	107	106.2	142.8
Vetch:Oat	65:35	90	112	92	108	103.0	163.2
Barley	100		105	112	140.5	180.5	
Vetch:Barley	65:35	92	103	95	112	109.7	194.5
Triticale	100		83	113	113.0	177.2	
Vetch:Triticale	65:35	93	105	82	105	97.2	133.7
Dicoccum	100		102	115	135.0	174.2	
Vetch:Dicoccum	65:35	92	105	90	112	131.3	166.5
Vetch	100	86	115			93.5	91.2
LSD <sub>0.05</sub>		9	8			36.3	14

<sup>†</sup>EF, early flowering of vetch; <sup>§</sup>EP, early pod-setting of vetch.

Figure 1. Replacement series diagrams with relative yield (RY) for oat and vetch and relative yield total (RYT) of the mixtures during EF (A and C) and EP stages (B and D).



## Conclusions

The preliminary data of the present study seem to highlight that intercropping of common vetch with oat, barley, triticale and dicoccum affects dry matter yield of the individual species.

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# Effects of Partial Root Drying on Nitrogen Distribution in Potato

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To overcome the drought problems efficiently, an innovative sustainable irrigation technique called Partial Root Drying (PRD) was proposed. The PRD results for different plant species demonstrated the benefit of this method in terms of improved water-use and to maintain yield (Loveys et al. 2000). The aim of the research was to investigate the effects of PRD irrigation on nitrogen distribution and yield of potato grown in the field conditions and to compare these effects with conventional irrigation practice (full irrigation).

## Methodology

The potato (*Solanum tuberosum* var. Liseta) trial was conducted during 2007 year in the opened field situated in the vegetable farm (Salad Centre) near Serbian capitol Belgrade. Channel water was used for irrigation and two irrigation treatments were tested: FULL subsurface drip irrigation (FI), control treatment where plants were irrigated daily and the soil water content was kept close to field capacity and PRD subsurface drip irrigation, where one side of the row was irrigated with 70% of FI, while the other was kept dry. The PRD treatment was imposed during the period of tuber filling and tuber maturation. The time for PRD shifting was between 3 and 5 days depending on soil water content (measured by TDR). Soil N status (N<sub>min</sub>) was measured by Kjeldahl method (Page et al. 1982) during season within the layer of 20-40 cm soil depth. Nitrogen content in the shoot and tuber (bulk values) was measured by Kjeldahl method (Jones, 1990), although yield was calculated as final tuber fresh mass. Water use efficiency (WUE) was calculated as the ratio of tuber fresh mass and amount of applied water (irrigation water plus precipitation during measured period). The measured traits have been analyzed for statistically significant differences by Student's unpaired *t*-tests (Sigma Plot 6.0 for Windows - SPW 6.0, Jandel Scientific, Erckhart, Germany).

## Results

N content in plant material and soil is presented in Table 1. Measurements of N content in the leaves and stems showed that the N concentration was progressively reduced during a growing season until final harvest when the lowest values were measured in both irrigation treatments. These results indicated that translocation of N (from leaves to tubers) occurred during growing season of potato plants. During the tuber filling stages the N content in the leaf and stem was very similar, although in the last period of growing season the N concentrations in the tubers tended to increase. Comparison between irrigation strategies showed that N content in tubers at final harvest was *ca.* 12% higher in PRD than in FI irrigated plants. These results are in accordance with results of Shahnazari et al. (2008) and they indicated that PRD treatment could be beneficial from the point of increasing the N content in tubers. Measurements of N content within the layer of 20-40 cm soil depth showed that plants were optimally supplied with N<sub>min</sub>. However, comparison of different applied irrigation strategies showed significant differences between full and PRD treatments. These differences were the biggest at the end of sampling period when the N content in the root zone was 50% lower in PRD than in FI. Shahnazari et al. (2008) founded lower residual N in PRD than in FI treatments confirming improved soil nitrogen availability during the late phases of growing season.

Table 1. The effects of applied irrigation treatments on N content in different plant organs and in the soil

Treatment	Days after emergence the plants	Leaf (%)	Stem (%)	Tuber (%)	Nmin (mg/kg)
Full	45	4.98	2.84	1.63	33.5
	60	3.54	2.44	1.36	22.8
	75	3.45	1.75	1.60	16.1
	90	3.46	2.26	1.55	55.6
	105	2.72	1.90	1.60	67
	Tuber final harvest			2.18	48.2
PRD	45	4.60	2.48	1.67	32.1
	60	3.97	2.25	1.41	19.5
	75	2.91	2.18	1.69	18.8
	90	2.32	1.72	1.77	25.4
	105	2.89	1.92	1.71	44.2
	Tuber final harvest			2.45	20.7

The effects of different irrigation treatments on yield at final harvest and water use efficiency (WUE) are presented in Table 2. At final harvest yield was classified as total and marketable (fresh weight of tuber with diameter 4-6 cm). Results showed that the total and marketable yield was decreased in PRD plants (*ca.* 8%) comparing to FI, although WUE was increased (*ca.* 10%). PRD treatment did not only improve WUE efficiency but also saved water for irrigation by 30%. Similar results were obtained by Shahnazari et al. (2007; 2008).

Table 2. The effects of applied irrigation treatments on yield and WUE

Treatment	Yield (t/ha)	Marketable yield (t/ha)	WUE (kg/m <sup>3</sup> )
Full	45.31 ± 3.14	43.10 ± 2.36	11.38 ± 0.79
PRD	41.78 ± 2.02	40.00 ± 2.38	12.47 ± 0.60

## Conclusions

Results for N content in different plant organs indicated that PRD treatment could improve allocation of N from shoot to tuber at final harvest, although soil N data pointed out that PRD treatment may also being beneficial from the point of increasing the N-use efficiency. Further investigations of the use of PRD as fertilization strategy would be very worthwhile.

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# Effect of Pea/Spring Cereals Intercrops on Yield and Quality Parameters of Crops in Organic Farming

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In recent years much attention has been paid in the world to the research and development of organic farming system, which is considered to be one of the safest in terms of environment and consumer. Intercropping of cereals and grain legumes is a neglected theme in agricultural science and practice in both conventional and organic (Dahlmann, Fragstein, 2006). The purpose of intercropping is to generate beneficial biological interactions between the crops. Intercropping can increase grain yields and stability, more efficiently use available resources, reduce weed and plant health (Hauggard-Nielsen et al., 2001, Jensen et al., 2006). Legumes may also provide cost savings by reducing the requirement for nitrogen application (Anil et al., 1998). In Lithuania, organic farming is a relatively new farming form which is on a rapid increase. The experiment was aimed to ascertain the influence of pea/spring cereals intercrops on spring crops yield in various crops combinations and yield quality.

## Methodology

The experiments were carried out in 2007 at the Lithuanian Institute of Agriculture in different experimental sites, soil and cultivation conditions: in Dotnuva on a loamy *Endocalcari-Epihypogleyic Cambisol*. Soil pH 7.5, humus content was 2.3 per cent, available P 75 mg kg<sup>-1</sup> and K 125 mg kg<sup>-1</sup>, in Joniskelis on clay loam *Endocalcari-Epihypogleyic Cambisol*. Soil pH 6.4, humus content was 2.2 per cent, available P 52 mg kg<sup>-1</sup> and K 200-220 mg kg<sup>-1</sup> and in Perloja on light-textured *Hapli-Albic Luvisol*. Soil pH 4.7, available P 67 mg kg<sup>-1</sup> and K 115 mg kg<sup>-1</sup>.

In the first year of experiment peas (*Pisum sativum* L.) and cereal: wheat (*Triticum aestivum* L.), spring barely (*Hordeum vulgare* L.), oat (*Avena sativa* L.), triticale (x *Triticosecale* Wittm.) were sown in the mixtures 50:50 or mono crop and grown for grain in organic condition. The layout was a randomised block design with three replicates. Peas and cereals were harvested for grain at complete ripeness stage. Nitrogen was determined by the Kjeldhal method. The data of experiments were statistically processed using analysis of variance.

## Results

The results obtained in the first experimental year showed that in Dotnuva on loamy the productivity of pea-spring barley and pea-spring wheat mixtures was significantly higher than that of other mixtures and than that of cereal monocultures, except for spring wheat (Table 1). In Joniskelis on clay loam and in Perloja on sandy loam the highest yielding were pea and spring barley and pea and oats mixtures. Weed incidence in the crops depended on a crop species and cereal growth stage. Legumes exhibited the weakest weed suppressive power. Cereal cultivation in a monoculture or in a mixture with legumes did not have any exceptionally significant effect on the disease incidence in the crops. Since the observed trends in the reduction in the severity of individual cereal diseases were not always consistent. Analysis of grain quality

showed that the intercropped cereals N concentration was increased compared to mono cropped cereals in all experimental locations.

**Table 1.** Yield data and nitrogen content in peas and cereals dry mater in the sowing year, 2007

Treatments	Species of cereals	Dotnuva			Joniskelis			Perloja		
		Grain yield t ha <sup>-1</sup>	Nitrogen in the grain yield		Grain yield t ha <sup>-1</sup>	Nitrogen in the grain yield		Grain yield t ha <sup>-1</sup>	Nitrogen in the grain yield	
			N % DM	N kg ha <sup>-1</sup>		N % DM	N kg ha <sup>-1</sup>		N %	N kg ha <sup>-1</sup>
Pea	pea	4.23	3.63	154	3.84	3.66	127.9	0.75	3.25	22.05
Peas + Spring wheat	pea	0.72	3.67	26.5	1.04	3.55	31.5	0.28	3.35	8.19
	cereal	3.77	2.44	92.2	3.44	2.20	64.1	1.07	2.52	24.08
	total	4.49		118.7			95.6	1.35		32.37
Pea + Spring barley	pea	0.65	3.69	24.0	0.39	3.61	12.0	0.45	3.36	12.92
	cereal	3.30	2.24	73.9	3.38	2.20	62.4	0.57	2.32	11.05
	total	3.95		97.9			74.4	1.02		23.98
Pea + Oat	pea	0.63	3.74	23.6	0.22	3.60	6.7	0.06	3.38	2.01
	cereal	2.38	1.84	44.1	4.15	1.84	65.4	1.37	1.83	22.41
	total	3.01		67.7			72.1	1.43		24.42
Pea + Triticale	pea	0.84	3.69	30.8	0.97	3.62	30.0	0.16	3.41	4.72
	cereal	2.73	2.29	62.4	2.78	2.50	59.2	1.26	2.22	27.45
	total	3.57		93.2			89.2	1.42		32.18
Spring wheat	cereal	4.65	1.98	92	3.77	2.05	65.1	1.37	2.17	25.32
Spring barley	cereal	3.30	2.05	68	4.23	1.90	68.4	0.72	2.09	12.68
Oat	cereal	3.24	1.78	65	4.69	1.70	66.9	1.62	1.86	25.55
Triticale	cereal	3.77	2.04	77	4.51	2.47	94.5	1.27	2.11	19.01
R <sub>05</sub> (total yield)	total	0.373		10.4	4.30		18.23	0.179		4.419

## Conclusions

The productivity of spring cereal monoculture or pea-cereal mixtures depended on the species of cereals and varied between different experimental sites, soil and cultivation conditions. The data generated over one year are not sufficient either to corroborate or contradict the trends, therefore the experiments should be continued.

## Acknowledgements

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# Genotypic Variation for Nitrogen Accumulation and Translocation in Spring Sown Chickpea under Mediterranean Conditions

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Chickpea (*Cicer arietinum* L.) is an important food legume of rainfed farming systems of the Mediterranean basin providing high quality protein for human diets. It is also used as feed for livestock and has a significant role in agricultural systems contributing to the sustainability of production and reducing the need for N fertilization through fixing atmospheric nitrogen (Singh, 1997). Several studies have shown the superiority of winter sowing compared to spring sowing as for the seed yield. However, in cooler Mediterranean areas, such as those of Northern Greece, winter sowing usually resulted in crop failure due to freezing temperatures during the winter period. Frost risk can be avoided by shifting the sowing to the spring.

Considerable amounts of nitrogenous compounds accumulate in the vegetative organs of chickpea before the seed filling period. By this time, the N assimilatory processes progressively decrease and fail to meet the seed's N requirement. Therefore, N must be redistributed from the vegetative tissues to the seeds to meet the deficits. The objective of this study was to assess the genotypic variation in N accumulation and translocation in spring sown chickpea under Mediterranean conditions.

## Methodology

A field experiment was carried out at the farm of Democritus University of Thrace in Orestiada (41°33'N latitude, 26°31'E longitude, 33 m altitude), Greece, during the 2003 growing season. Five chickpea cultivars, Amorgos, Serifos, Andros, Kassos and Thiva were sown on 12 March using a RCB design with six replications. Plots were 4 m long and consisted of 10 rows, 25 cm apart.

The experimental area was uniformly fertilized with 60 kg P<sub>2</sub>O<sub>5</sub>/ha in the form of superphosphate just before planting and incorporated into the soil. Plant samples, composed of an inner row 1 m long, were taken at the beginning of seed growth (R5) and maturity. The plants were cut at the ground level and were separated into vegetative parts (leaves, stems and pod walls) and seeds. All plant samples were dried at 70 °C and weighed. Nitrogen content was determined in all plant samples by Kjeldahl procedure. Nitrogen translocation (NT), nitrogen translocation efficiency (NTE) and contribution of nitrogen translocation to seed nitrogen (CNTS) were calculated according to Koutroubas et al. (1998).

## Results

Differences among cultivars in total aboveground dry matter were observed in both sampling dates (data not shown). Both vegetative and seed dry matter contributed to these differences. Total aboveground nitrogen content increased between the beginning of seed growth and maturity in all cultivars, indicating soil N uptake by the plants during this period (Table 1). Averaged across cultivars, the mean N accumulation after the beginning of seed growth was 72.1 kg/ha. Total N content of vegetative parts decreased dramatically after the beginning of seed growth, suggesting active nitrogen translocation to the seeds during the filling period. Cultivars differed significantly with respect to N translocation parameters. The net amount of N translocated from vegetative tissues to the seed ranged

from 69.8 to 145.3 kg N ha<sup>-1</sup> and the N translocation efficiency from 68 to 87%. Nitrogen translocation was significantly correlated with total dry matter and nitrogen content at the beginning of seed

Table 1. Nitrogen accumulation and N translocation related parameters for five chickpea cultivars.

Cultivar	Total N content (kg/ha)				NT (kg/ha)	NTE (%)	CNTS (%)
	Beginning of seed growth	Maturity stem	Pod wall	Seed			
Amorgos	123.4	15.5	13.9	232	94.0	76	40
Serifos	167.0	11.5	10.2	170	145.3	87	86
Andros	130.6	9.8	7.2	145	13.5	87	78
Kassos	184.2	13.7	11.8	201	158.6	86	79
Thiva	100.8	16.4	14.5	194	69.8	68	37
LSD <sub>0.05</sub>	29.4	2.6	3.8	27	29.3	6	15

growth ( $r=0.979$ ,  $P<0.01$ , and  $r=0.990$ ,  $P<0.01$ , respectively, Fig. 1), indicating that higher amount of nitrogen at R5 stage resulted in higher N translocation. The percentage of seed nitrogen content at maturity that came from translocation by the vegetative tissues during the filling period ranged from 37 to 86% and it was tended to be lower for the cultivars with greater seeds. Kurdali (1996) showed that N remobilization accounted for 81% of N in pods of rainfed chickpea, while Davis et al. (2000) using pot experiments reported that 62-85% of grain N is derived from vegetative tissues.

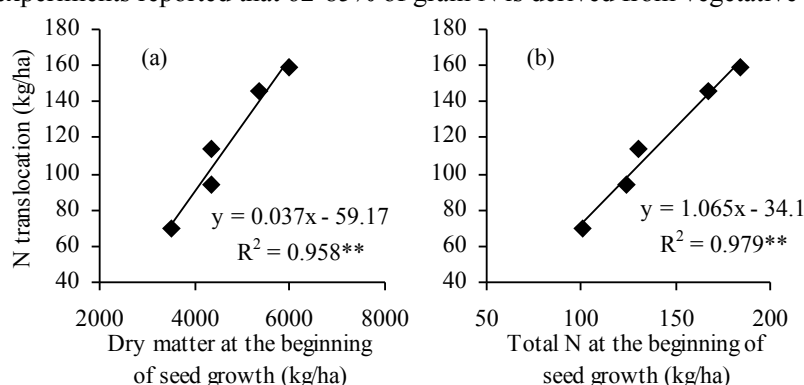


Fig. 1. Relationships between nitrogen translocation and (a) dry matter and (b) total N at the beginning of seed growth in chickpea.

## Conclusions

Results indicated the importance of nitrogen translocation from the vegetative plant tissues to the seed during the filling period in spring sown rainfed chickpea under Mediterranean conditions. Nitrogen translocation was found to be positively correlated with the vegetative nitrogen accumulation up to the beginning of seed growth. Therefore, cultural practices facilitating growth and nitrogen uptake by plants at early stages seems to improve the N nutrition of seeds.

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# Effects of Agro-Climatic Factors on Grain Yield and Quality of Winter Wheat

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Recently, due to climate change and prolonged vegetation period in autumn in Latvia and elsewhere (Harrison P. A., Butterfield R. E., 1996, Olsen J. et al., 2000), planting of winter wheat in conventional sowing terms sometimes result in vigorous tillering and strong outgrowing of plants and occurrence of snow mould in spring, causing death of most plants. At the same time winter-hardiness which is dependent on winter wheat plant status prior to wintering as well as on the variety adaptation ability to variable meteorological situation between years and in the period of wintering should be considered. Moreover, new varieties characterized with different growth and development indices are constantly introduced into production. The growth, development and productivity of field crops and particularly of winter crops (winter wheat) are greatly influenced by climate change (Knapowski T., Ralcewicz M., 2004., Lupu C., 2001). Therefore the goal of this research was to come to new conclusions regarding response to different sowing time and stand density shown by winter wheat varieties of different origin.

## Methodology

The 3-year field experiments (2005 – 2007) were established at the Study and Research Farm „Peterlauki” on silt loam brown lessive soil (sod calcareous). Three varieties of winter wheat (*Cubus*, *Tarso*, *Zentos*) in 4 sowing times with a 10-day interval from the end of August till the end of September with three sowing rates – 300, 400 and 500 germinating seeds per 1 m<sup>2</sup> with four replications were included in the experiment. Fertilizer treatments – preplant application of P<sub>2</sub>O<sub>5</sub> - 60 kg ha<sup>-1</sup>, K<sub>2</sub>O - 90 kg ha<sup>-1</sup>, split nitrogen topdressing N 150 kg ha<sup>-1</sup> – in spring after renewal of vegetation N 90 and N 60 kg ha<sup>-1</sup> at the beginning of stem elongation stage. Yield data were expressed at 100% purity and 14% moisture. Three-factor analysis of variance was used to determine yield significance level. Coefficients of variation were calculated between years for each factor. Quality tests were performed on the harvested grain average sample from each treatment. The following grain quality indices were measured: crude protein content by Kjeldahl procedure, gluten content and quality according to Perten, sedimentation value according to Zeleny, Hagberg falling number according to Hagberg–Perten as measure of the degree of alpha-amylase activity and 1000 kernel weight according to ISTA method.

## Results

The 3-year research results indicate that all winter wheat varieties included in the experiment ( $B \gamma_{0.05} = 0.366$ ) showed higher grain yields (8.5 – 9.4 t ha<sup>-1</sup>) and were more stable between years in treatments the sowing time of which was the second half of September till the end of September ( $A \gamma_{0.05} = 0.417$ ), i.e. with sowing time deviation by 10-14 days compare to conventional sowing time. Sowing performed in late August resulted in significantly lower grain yields of winter wheat. Winter wheat *Cubus* turned out to be most sensitive to early sowing. For this variety, the 3-year average grain yield obtained in the 1st sowing time was by 17% lower compare to the 2nd sowing time.

During comparatively long after-sowing period till the end of autumn vegetation plants of winter wheat *Cubus* vigorously tillered and strongly outgrew. This winter wheat variety also turned out to be most unstable between years regarding sowing time. Other winter wheat varieties also showed significant yield increase in the subsequent sowing times, yet it was considerably lower – 10-12% compare to the 1st sowing time. Winter wheat *Tarso* was less sensitive to sowing time, except early sowing. Grain yield was greatest with the variety *Zentos* obtained in the 3rd and 4th sowing time, i.e. in

the third decade of September (beginning and end). On the whole, all the winter wheat varieties included in the experiment produced higher ( $8.5 - 9.5 \text{ t ha}^{-1}$ ) and more stable yields of grain between years in treatments the sowing time of which was the second half of September – the 3rd and 4th sowing time (Ruza A., Kreita D., 2008).

The protein content (Fig.), gluten content as well as Zeleny index in all the investigated winter wheat varieties were higher in treatments sown in earlier terms. Yet grain yield obtained in later sowing terms showed higher quality indices in treatments with lower sowing rates. On the whole, sowing time deviation to a later period has a tendency to lower most significant quality (protein and gluten content, sedimentation value) indices in food grain.

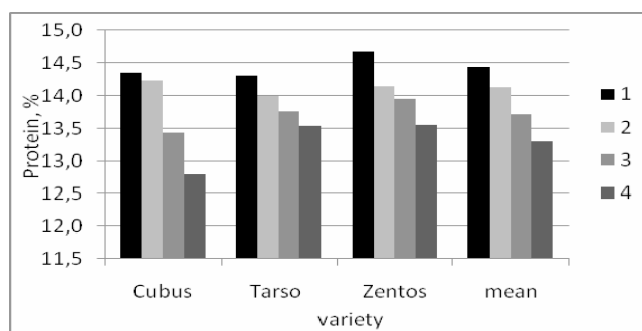


Fig. Effect of sowing time on protein content in grain

As the yield of grain was harvested timely and in weather conditions good enough, falling number in all treatments was medium high to high – it could be also considerably lower, yet it does not cause a particular problem in food industry.

### Conclusions

The 3-year yield averages practically smooth over annual deviations caused by variable meteorological conditions. Under different weather conditions, expression of some yield affecting factors is also different as indicated by yield variation coefficients. Among sowing terms between years, smallest fluctuations in variation coefficient below 8% were observed for the 3rd sowing time (around 20 September). For the rest of sowing time, fluctuations in variation coefficients between years were already considerably greater, particularly in the 1st sowing time.

Yield quality indices (grain size, protein and gluten content, sedimentation value, etc) are greatly dependent on annual meteorological situation and genetic character of the variety. At the same time, a tendency towards decreased protein and gluten content in the winter wheat grain with the increase of grain yield is observed in treatments with almost all sowing terms and for all investigated varieties particularly referring to winter wheat *Tarso* with grain yield level of about  $10 \text{ t ha}^{-1}$ .

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# The Usability of Alfalfa (*Medicago ssp* L.) Saponins for Bean Aphid Control in Faba Bean

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Occurrence of agrophages, especially bean aphid, is the main factor limiting the yields of faba bean. The control of this pathogen in organic farming is difficult because there are no registered insecticides against this insect. As a consequence almost 50% of total grain and green mass yield can be lost if no control will be applied (Barlov et. al. 1977). Because in organic farming it is prohibited to apply conventional insecticides it is necessary to seek new solutions of biological control of bean aphid. The attempt to use natural biologically active substances (bio-pesticides) can be treated as one of the solutions of this problem. Alfalfa saponins which can inhibit growth and development of different fungus, as well as control some insects, can be an example of such bio-pesticides (Adel et. al. 2000, Puszkar et. al. 1994, Szczepanik et. al. 2001).

Evaluation of possibilities to use alfalfa saponins to control bean aphid in faba bean cultivated in organic farming is the aim of the research. It is assumed that this biological preparation (alfalfa saponins) should have similar efficiency as conventional insecticides.

## Methodology

The study was conducted in years 2005 and 2007 in the Agricultural Experimental Station Kępa near Pulawy. Saponins extracted from the aboveground parts and roots of three alfalfa species (*M. sativa*, *M. lupulina*, *M. arabica*) was included in the research. Two different concentrations (a- 1%, b- 1,5%) of saponins was taken into account, and two control objects - without insecticide treatment, and control – Pirimor 500 WG. Bean aphid were counted on every plot on fifty plants. Observation was conducted every seven days begin appearance first insects to loss bean aphid colony. The evaluation of efficacy aphid control to Abbott's formula (Abbott 1925)

## Results

The results of experiment found that used agents against bean aphid had important influence on the yielding of faba bean (tab. 1). The highest yield of faba bean seeds was obtained on the object where used the Pirimor WG. The yield of seeds was higher at about 13% than control without protect. On the object where applied the sum of saponins in 1,5% concentration, the yield of seeds was higher than on the control object without applying insecticides (significant differences) but it was smaller than on the object with applying the Pirimor 500 WG.

The yield of seeds was a little higher after application of saponins sum extracted from roots than after applying sum of saponins extracted from the aboveground parts of alfalfa. Applying of Pirimor 500 WG insecticide allowed to limited occurrence of aphid bean the most effectively. There weren't found any insects on the faba bean plants after application of Pirimor 500 WG. The sum of saponins extracted from the aboveground parts and roots of three alfalfa species applied in 1% and 1,5% concentration allowed to limited occurrence of aphid bean insignificant (by about 16%). According Puszkar et al. (1994) the most effectively control of hop aphid on hop was obtained after applying sum of saponins in 0,2% concentration. A little higher effectiveness was found by application the sum of saponins

extracted from the aboveground parts and roots of *Medicago lupulina* than *Medicago sativa* and *Medicago arabica* (tab.2).

Table 1. Yield of seeds faba bean in dependence on cultivation system protection

Alfalfa saponins concentration (%)	Aboveground parts			Roots	
	M. sat.	M. lup.	M. ara.	M. sat.	M. lup.
1.0	2.29 a	2.30 a	2.38 c	2.32 a	2.34 a
1.5	2.36 c	2.39 c	2.52 d	2.47 c	2.41 c
means	2.32	2.35	2.45	2.40	2.38
Control - without insecticide treatment	2.26 a				
Control – Pirimor 500 WG	2.90 b				

Table 2. The evaluation of efficiency aphid control to Abbott's formula (%)

Specification	I term	II term	III term
aboveground parts			
M. sativa 1%	12.8	5.6	68.2
1.5 %	26.4	11.1	72.7
M. lupulina 1%	14.6	8.3	77.3
1.5%	20.0	13.9	77.3
M. arabica 1%	7.3	2.8	72.7
1.5%	12.8	16.7	72.7
roots			
M. sativa 1%	16.4	16.7	77.3
1.5 %	20.0	16.7	77.3
M. lupulina 1%	18.2	13.9	81.8
1.5%	20.0	16.7	81.8
Control- without insecticide treatment	0	0	0
Control – Pirimor 500 WG	100	100	100

## Conclusions

Application of the sum of saponins extracted from the aboveground parts and roots of *M. sativa*, *M. lupulina* *M. arabica* in concentration of 1 and 1.5% only slightly decreased the occurrence of aphids on the plants of horse bean. Average efficiency of aphid control 7 days after application of saponins amounted to 16%, whereas in the third term was significantly higher and amounted to 75%. Application of Pirimor 500 WG was the most efficient method of sugar beet aphid control. Saponins extracted from roots were more efficient than from the aboveground parts. Higher efficiency of aphid control was observed for saponins extracted from the aboveground parts and roots of *M. lupulina* than from *M. sativa* and *M. Arabica*.

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# Evaluation of Nitrogen Fixation in Horsebean (*Vicia faba minor* Beck) as Affected by Sulphur Fertilization in a Hilly Area of Basilicata Region (Italy)

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Sulphur is one of the essential nutrients for plants and it is a fundamental element in the nitrogenase enzymatic complex, being part as a basic constituent of the metallic cofactors affecting electron transfer in the nitrogen fixation process (Brady and Weil, 1996). The need of sulphur to optimise nitrogen use and improve yield and yield quality is increasing and it will be an important factor in the next future (McGrath *et al.*, 2002). Soil sulphur content impoverishment was observed due mainly to environmental policies aimed to reduce gas pollutants in the atmosphere and will become a phenomenon of great importance (Scherer, 2001). In spite of this and of its biological importance sulphur has not received enough consideration. The aim of this research was to study the effect of sulphur on plant growth and nitrogen fixation with the objective to re-introduce legumes in hilly areas of southern Italy where they can play an important role in soil fertility management.

## Methodology

A two years field trial (2002/2003-2003/2004) was conducted in a hilly area of Southern Italy at the experimental farm of the Crop Systems, Forestry and Environmental Science Department of the University of Basilicata (Guardia Perticara – 700 m a.s.l.) on a deep loamy-clay soil with available sulphur content lower than 10 mg kg<sup>-1</sup>. The experiments were performed on horsebean (*Vicia Faba minor* L.) “Scuro di Torrelama”. In both years the sowing was made in the first ten days of November and the cultural practices were defined on the results of previous researches in the same environment. The randomized blocks experimental design was adopted with four replication and elementary plot of 15 m<sup>2</sup>.

The soil was fertilized with 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> at ploughing while sulphur was applied at the end of winter in both years. In the first year was applied elemental sulphur (35 kg ha<sup>-1</sup>) while in the second year calcium sulphate and it was added at a higher rate with respect to the previous year (30 and 60 kg ha<sup>-1</sup>). The amounts of nitrogen fixed by horsebean was determined at flowering stage on the total shoot biomass using <sup>15</sup>N dilution technique and adopting durum wheat (cv “Appio”) as reference plant (Chalk and Ladha, 1999). In the second year the relieves were made also on the shoot biomass. At harvesting grain yield and yield components were determined.

## Results

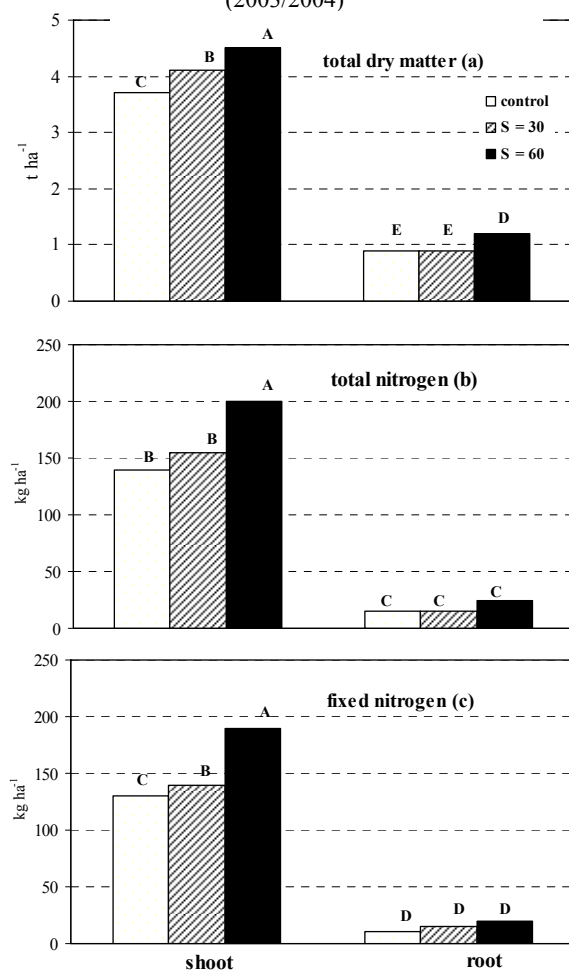
The rainfall was higher in 2002/2003 (803 mm) with respect to 2003/2004 (644 mm). In both years the temperatures were always superior to 0°C. The second year was characterized from the better rainfall and temperatures time-courses, especially during the Spring.

Table 1. Relieves during the first year of the trial (2002/2003)

	Relieves at flowering			Relieves at harvest		
	shoot dry matter (t ha <sup>-1</sup> )	total nitrogen (kg ha <sup>-1</sup> )	Fixed Nitrogen (kg ha <sup>-1</sup> )	fertile pods per plant (n)	Seeds per pod (n)	grain yield (t ha <sup>-1</sup> )
Control	4.1 a	133.0 a	117.8 a	6.1 a	2.0 a	1.3 a
S (35 kg ha <sup>-1</sup> )	4.6 a	151.8 a	129.0 a	7.5 a	2.7 a	1.4 a

Values marked by the same letter aren't significantly different according to the Duncan Test

Figure 1. Relieves at flowering  
(2003/2004)



The results of the first year are reported in Table 1. Biomass production and total fixed nitrogen at flowering resulted slightly higher in the thesis with sulphur even no statistically significant differences were observed. The same trend was recorded for the data at harvesting. Yield results were not significantly different in the first year, probably due to the type of sulphur fertilizer (elemental sulphur) and its delay in distribution, both factors limiting availability for the plants.

In fact in the second year when sulphur was applied as calcium sulphate, readily available for the plant, significant effects of fertilisation were registered on growth and N<sub>2</sub> fixation of horsebean at flowering (Figure 1). The differences were higher for the shoot biomass, especially when sulphur was applied at the maximum dose compared to the control.

Productive results confirm the trend of relieves carried out during the cultural cycle. In fact the treatment with the highest sulphur dose showed higher values for the grain yield and yield components compared to the control (Table 2).

### Conclusions

Results pointed out a positive effect of sulphur fertilization in a soil poor in its content on nitrogen fixation and plant growth. The productive results confirm the trend of relieves carried out during the cultural cycle.

Anyway it is not clear if sulphur has direct effect on the nitrogen fixation process or has indirect effect, through a better vegetative growth. More work is needed to discriminate between direct and indirect effects on this important biological process.

Table 2. Relieves at harvest in the second year of the trial (2003/2004)

	plant height (cm)	Pods per plant (n)	fertile pods Per plant (n)	seeds per pod (n)	grain yield (t ha <sup>-1</sup> )
Control	83b	6.9B	6.5B	2.8b	1.6B
S (30 kg ha <sup>-1</sup> )	85b	7.1B	6.6B	3.0b	1.8AB
S (60 kg ha <sup>-1</sup> )	92a	10.6A	9.8A	3.6a	2.0A

Values marked by the same letter aren't significantly different according to the Duncan Test

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# Long Term Replant Effect on Soil Microbial Diversity in Cropping Systems of Southern Italy<sup>\*</sup>

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Intensive cultivations (orchards, vineyards, vegetables, cereals crops) dominate agriculture in the Mediterranean area. Soil microbial diversity is broadly related to land use and it represents one of the main components of soil quality in agricultural soils. Indeed, it is the main factor involved in soil suppressiveness of soil-borne pathogens, agents of decline in vegetable crop yields (Nitta, 1981) in intensive systems as well as replant problems (May and Abawy, 1978) in orchards (Mazzola, 2002). A study of the microbial diversity of two intensive cropping systems, peach tree orchards and vegetable crops, was carried out in the same area to evaluate how much soil microbial communities are affected by crop and soil management in fields with common soil characteristics and the same environment. The results of this study were analyzed using the ecologic approach.

## Methodology

The study was performed in an agricultural area (Eboli) of the Salerno province in Campania (Lat 40° 36' N, Long 15° 4' E), on plots chosen for homogeneity of pedo-climatic characteristics, with soil organic matter content varying from 1.4 to 2.5 %.

The fungal community of the cultivated layer was adopted as a bio-indicator. Vitroplants of rootstock GF677, the most commonly used peach tree rootstock in Italy, were grown on soil samples in a green house for 70 days to assess root endophytic soil fungal communities and the level of replant disease.

Soil samples were collected in orchards on replanted soil and

five peach orchards (2-3 replants in the last 30-years; soil management: sod system with permanent vegetative cover)

five fields intensively cultivated with vegetables. (2-3 cycles per year in the last 35 years and periodic intervals with a cereal crop).

The soil samples were analysed for soil fungal communities using the soil dilution plate method. Colonies were counted by visual observation on transparent agar disks, including the germinated fungal propagules within the soil suspension. Each colony forming unit (CFU) observed was counted and identified and the relative frequency of each species was expressed as a percentage of the total CFU for each community.

*Diversity.* Pooled data of fungal communities from peach orchards and vegetable plots were compared using: the diversity t test, which compared the Shannon index, strongly based on richness, and the diversity profile, a test based on abundance data.

*Similarity* of fungal communities was compared using the ANAlisis Of SIMilarity test (ANOSIM) with the Bray Curtis distance and computed on 10000 permutations.

The PAST program software (Hammer et al., 2001) was used to perform all the analyses.

## Results

Diversity in the two cropping systems differed significantly according to the t test and the diversity profile supported the robustness of this result. Diversity in the peach orchards was significantly higher than vegetable plots with a much higher species richness than vegetable crops (Shannon index and number of taxa in Table 1). On the contrary, the diversity indices based on evenness (Evenness  $e^H/S$  ,

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Table 1. Diversity indices pooled data of fungal communities from vegetable and peach tree sites. Approximate 95 percent confidence intervals computed with a bootstrap procedure on 1000 random samples

Diversity Indices	Peach	Vegetable	Boot p(eq)
Taxa S	34	17	0
Dominance	0.05139	0.08078	0.004
Shannon H	3.226	2.657	0
Evenness $e^H/S$	0.7403	0.8381	0.093
Berger-Parker	0.1094	0.1474	0.235

both cases (0.05 and 0.08) suggesting that the species were quite equally distributed in the communities analysed. In addition, the Parker Berger index, which shows the largest species in proportion with all the species in a community, is considered an useful and sensitive indicator of disturbance in a biological environment (Shaw et al. 1983). Therefore, on the basis of these results, it was possible to conclude that peach tree and vegetable crops showed different fungal biodiversity but a common balance status.

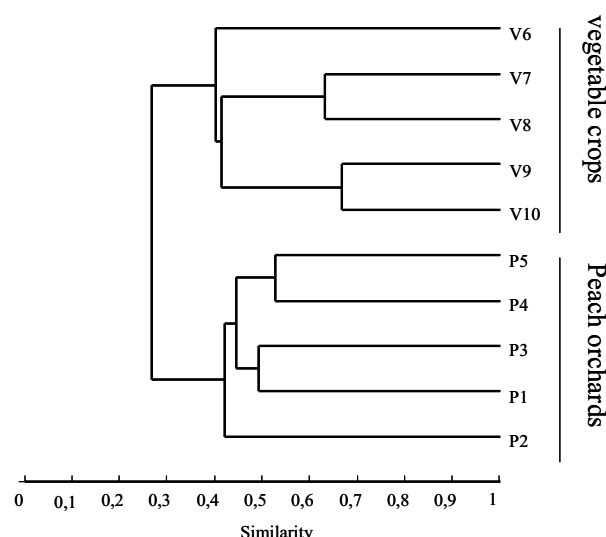


Figure 1. Bray Curtis cluster analysis of ten sites under study according to composition of fungal communities. Bootstrap analysis carried out on 1000 repetition counts.

According to the ANOSIM test the composition of fungal communities in the peach orchard differed significantly from that of vegetable plots while it did not differ significantly within the two groups. Cluster analysis delivered this difference dividing the ten sites in two homogeneous groups corresponding to the two cropping systems. (Fig. 1). Rootstock GF677, grown in peach orchard and vegetable crop soils, did not show any growth reduction and no symptoms were observed on the roots after two months. Indeed, no root discoloration or any other symptom was observed on the rootstock and no species known to be an agent of replant problems were isolated from the roots.

## Conclusions

Peach orchards showed a higher soil microbial diversity than vegetable plots, but

the balance of fungal communities in the two cropping system was very similar. This could be due to the presence of a permanent vegetative cover in the orchards and the variable crop sequence which guaranteed diversity of the fungal communities, contrasting the increase in dominance (prevalence of one or a few species). The deep difference between the community compositions in the two cropping systems and the high similarity observed within the five sites with the same crop, showed that intensive management strongly affect fungal community composition. However, the soil management of peach orchards may have guaranteed microbial diversity, avoiding the increase in fungal agents of root rot specific for peach which were not recorded in the test on soil health of peach orchards.

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# Effect of Soil Texture on Microbial Biomass under the Same Environment

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The amount of microbial carbon in soil is directly related to the total organic carbon (TOC), their ratio varies according to the environmental conditions (temperature and rainfall in particular) and is strongly affected by soil tillage. For this reason, soil microbial activity, measured by indirect methods based on carbon dioxide evolution after fumigation (e.i. substrate-induced respiration method), represents a widely used indicator of soil quality. However, those methods do not provide complete information of the behaviour of the single population (fungi and bacteria) under the environmental conditions where the trials are conducted. The microbial biomass is strongly related to soil texture, pH, organic C and N content and positively related to soil moisture. Soil fungi represent the greatest part of microbial biomass (Lin and Brookes 1999), and their C assimilation efficiency is markedly higher than in bacteria, playing a primary role in C sequestration. In addition, fungi are involved in macro-aggregate formation (Bossuyt et al., 2001) and it is well known that their presence in soils is an indicator of soil disturbance (van der Wal et al., 2006). The aim of the present work was to evaluate the effect of soil texture on fungal biomass, as determined by direct microscopic measurement, and composition without the biasing influence of different temperature and rainfall patterns.

## Methodology

The soil used in the present study was sampled from a set of lysimeters (1x1x1 m) containing 4 types of soils (sand, sandy loam, sandy clay and clay) originally collected from several areas in the region of Emilia Romagna, Italy. The lysimeters were established in 1976 and were left unmanaged and hence covered by natural weeds for the last eight years. Soil samples of the upper layer (0-0.25 m) were subjected to the following analysis i) Soil Organic Matter content (SOM) with Walkley-Black method; ii) quantitative analysis of free fungal propagules (expressed as Colony Forming Units, CFU; g<sup>-1</sup> soil) was recorded by soil dilution plate method in water agar. Fungal propagules were identified upon their transplantation after their transplant and growth on PDA (Potato Dextrose Agar); iii) Fungal biomass size was measured by fluorescence microscopy, using a microscope Leica with fluorescence at 200 x magnification. Photomicrograph samples preparation and image processing were carried out according Liu et al., 2001 using the software CMEIAS ver. 1.27. Fungal biomass was expressed as fungal area g<sup>-1</sup> soil as it is directly related to the volume of fungal biomass (Jenkinson et al., 1975).

One way analysis of variance was performed on quantitative data (CFU g<sup>-1</sup> soil and fungal area), while qualitative data were handled by Biodiversity Pro, a software for ecological statistics available in web.

## Results

Fungal biomass differed significantly among the four soil textures ( $P \leq 0.01$ ). Based on recorded fungal area, and considering a thickness of fungal bodies varying from 4 to 10  $\mu\text{m}$ , the volume of fungal biomass was in the order of 1 mm<sup>3</sup> g<sup>-1</sup> soil. Clay soil showed the highest fungal biomass size, sandy loam and sandy clay soil showed a significantly lower biomass, and, sandy soil has the lowest fungal biomass size (Tab. 1). SOM content was relatively high across soil types, ranging from 1.2% in sandy to 4.5% in clay soil, probably as a result of the long term fallow the lysimeters underwent. Moreover the SOM content was highly and significantly related to fungal biomass ( $R = 0.85$ ). Most of fungal

bodies observed by fluorescent microscope were propagules, in some cases aggregated among them, while hyphae were not frequently observed (Fig. 1). This was confirmed by quantitative data of free propagules which were highly and significantly correlated with biomass data ( $R=0.94$ ). The composition of fungal communities of four soil types did not differ. *Fusarium* spp. and *Humicola* sp. were the most represented and dominance of four communities was quite low ( $< 0.3$ ), suggesting that fungal communities were balanced.

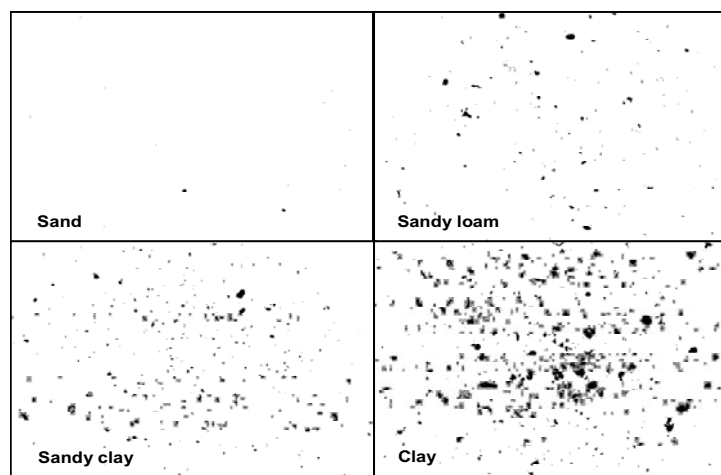


Figure 1. Images of fungal area of for 4 types of soil acquired by fluorescence microscope and transformed to black and white micrographs.

Soil texture	Area g soil <sup>-1</sup> (mm <sup>2</sup> )	LDS test (95%)
Sand	110	c
Sandy loam	207	b
Sandy clay	179	b
Clay	280	a

Table 1. Area of soil fungi obtained processing photomicrographs by CMEIAS

### Conclusions

Our findings indicate that soil texture strongly affect microbial biomass, and therefore, are in good agreement with observations by Kiem et al. (1997). The high correlation between quantitative data of soilborne fungi using two different methods suggested that both methods are good at estimating fungal biomass in comparative studies. The low differences observed among the composition of soil fungal communities of in the 4 soils under study suggested that environment and soil organic matter buffered the influence of soil granulometry on fungal diversity

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# Soil Carbon Contents and CO<sub>2</sub> Fluxes Measurements: How to Assess Carbon Storage Under No-Tillage Systems in Temperate Conditions

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The management and enhancement of soil organic carbon (SOC) is a key issue for agriculture as it is related to soil fertility, as well as for environment as it concerns soil carbon (C) sequestration. Reduced and no tillage practices are potential ways to increase SOC. Retention of soil C is also beneficial for soil physical, chemical and biological properties, and is essential if soils are to be used as a repository of C to mitigate atmospheric CO<sub>2</sub> increases (Lal, 1997).

Although tillage is assumed to have a major influence on soil C retention, the extent to which tillage enhances the transfer of soil C to the atmosphere is uncertain. This study gives elements to compare SOC storage under different tillage systems.

## Methodology

This study is mainly based on a review of literature ((Paustian *et al.*, 2000 ; Smith *et al.*, 2005, Arrouays *et al.*, 2002). Around 80 experimental data were plotted to assess soil carbon pool influenced by agricultural activities. The most common methods used in the field are : (i) measuring CO<sub>2</sub> fluxes from soil during the cropping cycle especially after tillage or fertilizing operations and (ii) measuring SOC over a several-year-cropping situation. Depending on the duration of the experiment, either synchronic or diachronic analyses are possible to estimate the carbon storage.

## Results

According to literature, in temperate conditions, no-tillage or reduced tillage systems are efficient to store carbon into the soil. This analysis also gives a range of SOC potential for no-tillage and reduced tillage. The mean value (Figure 1) is close to 300 kg C ha<sup>-1</sup> yr<sup>-1</sup> as a mean value or 150 kg C ha<sup>-1</sup> yr<sup>-1</sup> according to linear regression.

It is also important to notice that no-tillage and reduced tillage generally correspond to large amounts of organic restitutions (plant residues, cover crops) that enhance humification.

Tillage operations are also known to lead to a rapid release of carbon dioxide (CO<sub>2</sub>). The review of literature shows a great variability of soil CO<sub>2</sub> fluxes values (from 1 to 10-fold roughly). However, most published studies have been related to the effects of soil preparation disturbance, such as mechanical-powered fall or spring plowing, chiseling, or disking on CO<sub>2</sub> emissions. CO<sub>2</sub> flux from soil determines the extent to which carbon (C) deposited as plant litter is retained in the soil. From a methodological point of view, interannual SOC data seem to be easier to obtain and more efficient to understand carbon storage under different tillage systems.

However, due to the potential area to be converted to reduced tillage or no-tillage, this practice may be interesting to promote soil C storage. Lastly, the variability of measurement and the lack of long-duration plots are important limits to assess C storage potential (only one 30-year-old experiment in France, for example)

## Conclusions

According to this study, and besides the well-known agronomic standpoint, no-tillage and reduced-tillage are also beneficial regarding its C storage balance. However, C sequestration (Bernoux *et al.*, 2006) for a specific agro-ecosystem in comparison with a reference one, should be considered as the result (for a given period of time and portion of space) of the net balance of all greenhouse gases, expressed in C-CO<sub>2</sub>, computing all emission sources at the soil-plant-atmosphere interface. As a consequence, comparing this reduced and no-tillage system with traditional ploughing systems means not only taking into account the carbon storage but also the resulting greenhouse effect gas fluxes such as CH<sub>4</sub> and N<sub>2</sub>O fluxes (Six *et al.*, 2002). Lastly, some authors (Bellamy *et al.*, 2005) reported the possible effect of global warming on enhancing soil organic matter mineralization with an increased release from high carbon content soils and independently from carbon protection in soils. Further research on storage duration (soil carbon protection in stable aggregate, effect of global warming, other greenhouse effect gases, carbon release kinetics) should be led.

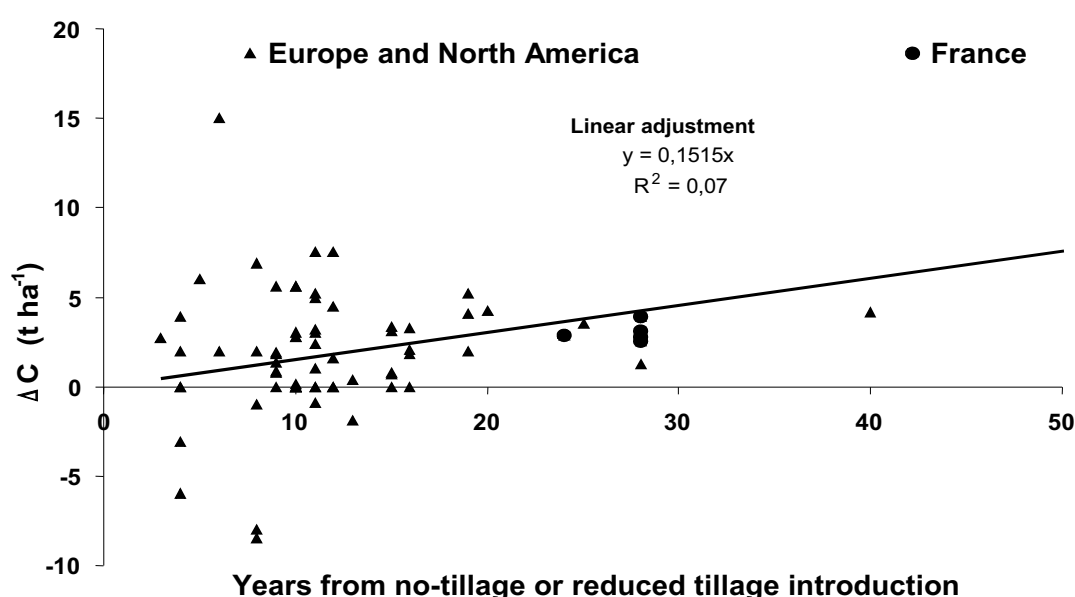


Figure 1 : Soil carbon pool storage influenced by tillage system: reduced or no-tillage versus ploughing systems (compilation of 80 values in temperate conditions France, Europe and North America), adapted from Arrouays *et al.* (2002) and Smith *et al.* (2005).

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# Relationship Between Soil Physical and Chemical Properties and Physical Quality Index

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The depth of soil tillage effects physical and chemical status of soil environment. Simplifications of tillage, including no tillage system, lead to increase of soil density, compaction and humidity. It reduces also the rate of soil organic matter mineralization and emission of CO<sub>2</sub> to atmosphere and protects soil against erosion. The big number of soil parameters influencing the physical state of soil complicates quantification of soil physical quality. Recently developed in the Institute of Soil Science and Plant Cultivation in Pulawy, Poland by Dexter (Dexter 2004 a,b,c) index of soil physical quality S gives possibility of such quantification. Soil physical index S is already defined and it equals to the slope of the soil water retention curve at its inflection point. The purpose of this study was to investigate the effect of tillage to different soil depths on the index of soil physical quality S in relation to soil physical and chemical properties in comparison to both successive and cut fallow.

## Methodology

The investigations were performed in the Experimental Station Baborówko in Poland in the years 2004 - 2006. The experiment has started in 2002, when three fields for rape/lupin – winter wheat – spring barley crop rotation were separated. Additionally two fallow fields with no mechanical interference into soil: successive fallow and green cut fallow were set up. Soil samples were collected to 100 cm<sup>3</sup> metal cylinders from the cultivated fields of wheat at three different soil depths: 30 cm – traditional plough system, 8 cm – reduced system and no tillage – direct sowings. Directly in the field the following measurements of physical soil properties were taken: soil penetration resistance, soil CO<sub>2</sub> flux. The other physical and chemical soil properties were estimated in soil samples taken from 0-5, 10-15 and 20-25 soil profile layers: soil stability - on the base of readily dispersible clay, bulk density, actual water content, soil reaction, organic carbon content, soil available potassium and phosphorus content, water retention characteristics of the soil and mineral nitrogen in 0-30, 30-60 and 60-90 soil layers. The water retention curves were fitted to the van Genuchten (1980) equation. Values of index of soil physical quality S was obtained according to Dexter (2004) equation. Results of measurements and analyses were statistically analyzed by ANOVA. Means were compared by Tukey test and considered significant at P=0.05.

## Results

Table 1. Organic carbon and mineral nitrogen content and soil reaction. Mean of 2004-2006

Treatments	Organic carbon			Nmin			pH <sub>KCl</sub>		
	(%)			(kg·ha <sup>-1</sup> )					
	soil layer (cm)								
	0-5	10-15	20-25	0-30	30-60	60-90	0-5	10-15	20-25
Successive fallow	1.46	1.25	1.23	30.6	14.9	7.1	6.3	6.4	6.4
Cut fallow	1.48	1.31	1.36	25.3	8.7	5.5	6.0	6.4	6.6
Direct sowing	1.24	1.07	1.07	40.6	20.8	20.0	5.7	6.2	6.3
Reduced tillage	1.39	1.22	1.03	52.8	27.3	16.2	5.9	6.3	6.4
Conventional tillage	1.34	1.19	1.09	49.8	29.3	13.9	6.1	6.6	6.6
LSD <sub>(0.05)</sub>	n.s.*	n.s.	n.s.	14.28	15.06	10.24	0.45	0.27	n.s.

\*n.s. not significant difference

Table 2. Physical soil properties. Mean of 2004-2006

Treatments	Bulk density Mg·m <sup>-3</sup>			Readily dispersible clay g·100 g <sup>-1</sup>			Penetration resistance MPa			S index
	soil layer (cm)			0-5	10-15	20-25	0-5	10-15	20-25	
Successive fallow	1.63	1.77	1.71	0.36	0.49	0.34	1.34	2.92	3.65	0.031
Cut fallow	1.59	1.72	1.73	0.25	0.36	0.34	1.84	2.94	3.57	0.041
Direct sowing	1.57	1.64	1.63	0.47	0.70	0.57	1.37	2.86	3.58	0.048
Reduced tillage	1.50	1.66	1.65	0.28	0.37	0.37	1.18	2.99	2.57	0.054
Conventional tillage	1.57	1.56	1.64	0.44	0.56	0.55	1.06	2.57	3.57	0.059
LSD <sub>(0.05)</sub>	0.098	0.139	n.s.	n.s.	0.266	n.s.	0.212	0.213	n.s.*	-

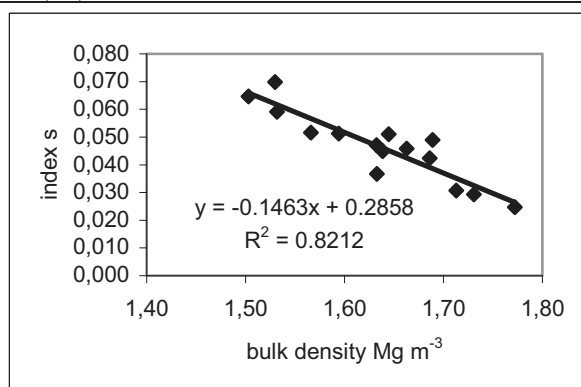


Figure 1. Effect of bulk density on soil quality index S

### Conclusions

Tillage measures into different soil depth influenced the variability of soil properties. Soils left as both successive and cut fallows showed significantly smaller mineral nitrogen content in 0-30 and 30-60 cm layers than soils of traditional and reduced tillage systems. Soil of cut fallow was characterized also by the highest compaction in the 1-10 cm layer and significantly higher CO<sub>2</sub> flux between soil and ambient air than soil of reduced tillage system. In comparison to reduced tillage (8 cm depth) and direct sowing (2-3 cm depth) soil of traditional tillage distinguished by higher CO<sub>2</sub> flux and the tendency to higher available phosphorus and potassium content. Our research showed that S index can be used as an index of soil physical quality that enables the differentiation of soils and the effects of different management treatments and conditions to be compared directly. This would be helpful in protection of rural ecosystems and proper management of the production capacity. Studies on different soils and management systems are needed to better assessment and validation the S parameter as an indication of the soil physical quality. Its a easy determination that makes it especially useful for assessing and monitoring the effects of the use and management systems in terms of degradation and recovery of the structure as well as the physical quality of the soils.

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# The influence of different biopreparations upon yield and other characteristics of winter wheat and spring barley

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The effects of biopreparations are favourable for the depression of phytopathogenic cereal fungi. Therefore, for the treatment of winter wheat and spring barley, we used contemporary biopreparations of different proveniences (Polyversum, Supresivit, and Trianum P). Winter wheat was cultivated in a different stand establishment, spring barley only with a conventional establishment. We evaluated the yields, as well as the infestations with pathogenic fungi, after the biopreparation treatments.

## Methodology

For the winter wheat stand establishment, we used three different soil tillage techniques: conventional tillage, no tillage, and minimum soil tillage. For the spring barley, only the classic conventional tillage was used. We used two biopreparations produced in the Czech Republic: Supresivit (based on the effect of *Trichoderma harzianum*), and Polyversum (based on the effect of *Pythium oligandrum*). One other biopreparation of Dutch provenance, Trianum P, is based on another strain of the same fungus as is used in Supresivit. Results from this latter biopreparation are only from 2007. The biofungicides were applied both as a seed treatment and as a mixture with the mineral fertilizers, during vegetation. The fertilizers which we used in the mixtures were ammonium nitrate with limestone (ANL) for winter wheat, and ammonium sulphate (AS) for the spring barley. The concentrations used were: 1g per 1kg of seeds or fertilizer for Supresivit and Trianum P; 3 g Polyversum per 1 kg seeds or fertilizer. Plant and soil samples were taken at one month intervals. After smears from the plant surface taken, we evaluated the number of microfungi or their numbers on the plant surface.

Table 1 The grain yields of winter wheat and spring barley from evaluated variants (average 2003 - 2007)

Variant	Methods of stand establishment							
	Winter wheat				Spring barley			
	a		b		c		a	
	(t.ha <sup>-1</sup> )	(%)	(t.ha <sup>-1</sup> )	(%)	(t.ha <sup>-1</sup> )	(%)	(t.ha <sup>-1</sup> )	(%)
1. control	7.64	100.0	7.50	100.0	7.44	100.0	6.39	100.0
2. fertilizer + Polyversum	7.91	103.5	7.73	103.0	7.67	103.1	6.68	104.5
3. fertilizer + Supresivit	8.05	105.4	7.94	105.9	7.86	105.6	6.72	105.2
4. fertilizer + Trianum P <sup>x)</sup>	8.24	107.8	8.03	107.1	7.95	106.8	6.93	108.4
5. seed + Polyversum	7.99	104.6	7.75	103.3	7.73	103.9	6.65	104.1
6. seed + Supresivit	8.00	104.7	7.86	104.8	7.83	105.2	6.75	105.6
7. seed + Trianum P <sup>x)</sup>	8.20	107.3	8.02	107.0	7.98	107.2	6.88	107.7
Mean grain yield:	<b>8.00</b>		<b>7.83</b>		<b>7.78</b>		<b>6.71</b>	
The effect of biopreparates:	<b>105.6</b>		<b>105.2</b>		<b>105.3</b>		<b>105.0</b>	
The effect of technology:	<b>100%</b>		<b>97.9 %</b>		<b>97.3 %</b>		<b>-</b>	

## Methods of stand establishment for winter wheat:

a - conventional tillage

b - no tillage - direct sowing into untilled soil, covered with mulch

c - minimum tillage - sowing into shallow tilled soil, with chopped (of pre-crop) straw incorporated

x) Results are only from the year 2007

## Results

On the basis of our 5 years of experiments (Table 1), one can observe that the use of biopreparations had an influence on the yield of all experimental variants. Use of Supresivit in co-application with mineral fertilizers caused an increase of grain yield in winter wheat by about 5.4 - 5.9%, compared with the non-treated control; depending on the soil tillage technology employed. The effect of the seed-treatment increased yield approximately 5%. The Polyversum biopreparation had a constant, although not the greatest, effect on the yields, with 3.0 - 4.6% (on the level of statistical significance). Winter wheat treated with Triatum P along with fertilizer ANL had the highest grain yield in the variant of conventional tillage (+ 7.8 %) in comparison to fertilized but with biopreparations non-treated control. The different tillage technologies showed their influences upon the yields. If the conventional variant (classic tillage) is assigned 100%, then direct sowing into non-tillage soil was 97.9 %, and the variant with superficial tillage with chopped straw of the pre-crop incorporated into the soil about 97.3 %.

For spring barley grain in the five-year field experiments, the best yield results were with the following variants: fertilizer (AS) in a mixture with the biopreparation Supresivit (+5.2%), and in the seed-treatment of +5.6%, compared with the control non-treated variant (no biological treatment). The Polyversum biopreparation did not have the greatest effect, but did have a positive influence on yield. In 2007, the variant with Triatum P caused an increase of about 8.4%, which was statistically significant.

We also studied the influence of phytopathogenic fungi (such as the genus *Fusarium*, *Septoria*, *Drechslera*, *Pseudocercospora*, *Gaeumannomyces*, *Rhizoctonia*, etc.), and their influence on the state of plant health. On both cereals, we found these fungi either in the spots on the leaves or ears. Differences were found between winter wheat and spring barley because the parcels with spring barley were only prepared with conventional tillage. The greatest effect on reduction of the evaluated phytopathogenic fungi was on those variants with winter wheat, using minimum tillage (sowing into shallow tilled soil) and incorporating chopped pre-crop straw. The quantity of semi-pathogenic fungi in the soil was depressed to about 10 - 12% in the treated variants of winter wheat. The effects on the variants with spring barley, after conventional tillage, were very similar.

## Conclusions

The winter wheat yield was highest with Triatum P. Supresivit, after conventional tillage of the soil, had 5.4 - 7.8% more yield than the control. We also found that the yield of spring barley after seed-treatment with Supresivit increased by about 5.2 - 5.6%. In 2007, we used the biopreparation Triatum P, with an effect on the spring barley of about 8.4%. From these results, it is evident that the effects of the biopreparations also created budgetary savings, and decreases in amounts of chemical fungicides used (which had impacts on maintaining a healthy environment). The most effective biopreparation was Triatum P (with only the one year experiment) and Supresivit (over the 5 years), in both cereals. The conventional technology demonstrated to be the most effective from all of the methods used, which is useful for agronomical praxis. The number of fungi was highest after minimum tillage, but was not statistically significant. From soil extracts, we assessed the number of soil micromycetes per 1 gram of soil (by the method of soil extraction and then cultivation on artificial medium). The number of soil semi-pathogenic micromycetes (such as the genus *Fusarium*) was found to be significantly lower, when compared with the non-treated variants.

## Acknowledgement

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# Selected Indicators of Sustainability on Organic Farms in the Czech Republic

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The aim of presented work was to figure out values of selected indicators on organic farms in the CR and find out how these values are influenced by farming system.

There are some limitations valid for use of fertilisers in organic farming and furthermore some farmers in the Czech Republic themselves apply lower amounts of farmyard fertilisers because of situation on the farm and in the region. Therefore nutrients management is a factor of great importance for maintenance of soil fertility and long term stability and productivity of agrosystem.

Other up to date question is energy. It can be supposed that organic farming, as more extensive system, has lower energy inputs but also energy outputs. Important is efficiency of utilisation of this energy for production.

## Methodology

The research involved 3 organic farms in the period of 3 years. Farms represent different structures: farm 1 without any use of fertilisers (mineral or farmyard) only with legumes and intercrops in crop rotation, farm 2 with intensive use of farmyard manure and farm 3 with medium use of farmyard manure but very diversified production structure.

In the article are presented results of N, P, K balance and energy balance. To count out these indicators was used model Repro (Hülsbergen, Diepenbrock 1997).

## Results

N balance on level of the whole farm was only very slightly higher than the limit set as  $\pm 25 \text{ kg} \cdot \text{ha}^{-1}$ . There was only one extreme value occurred on farm 2. (See Table 1.) The most homogenous results and closest to optimum values were reached on farm 2. Here all nitrogen was provided by legumes in crop rotation. On farm 2 was found great variability of N balance on the level of single fields occurring each year. In the case of nitrogen we should take into account its great mobility in the soil and therefore high N saldo means possible environmental threat. On the other hand, whole amount of N was applied in organic fertilisers (green manure, farmyard manure) that release the nutrient gradually in several years. High N saldo could be dangerous especially when it occurs for several years on the same field as it happened on 2 fields on farm 2 and on 1 field on farm 3.

In the most cases P balance values didn't fit into the optimum limit set as  $\pm 7 \text{ kg} \cdot \text{ha}^{-1}$ . Because P is nutrient not very much mobile in the soil, it is necessary to assess its balances in long-term view. In the whole period of assessment there were all plots on farm 1, 6 (from 45) plots on farm 2 and no plots on farm 3 with permanent negative balance.

K balance on level of the whole farm was under the optimum limit set as  $\pm 20 \text{ kg} \cdot \text{ha}^{-1}$  at least in 2 years of 3 on all farms. Similarly to P balance, it could be possible threat in case of long-term trend. Permanent negative balance occurred on 12 plots (from 45) on farm 2 and on 2 plots (from 36) on farm 3.

**Table 1:** Nutrient balances and their extreme values (kg.ha<sup>-1</sup>)

	OF1			OF2			OF3		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
<b>N Balance</b>	<b>2.73</b>	<b>6.57</b>	<b>8.43</b>	<b>30.33</b>	<b>85.33</b>	<b>1.86</b>	<b>18.56</b>	<b>21.23</b>	<b>5.58</b>
min	-2.32	-11.55	-14.48	-135.33	-87.57	-494.31	-47.24	-53.18	-37.11
max	19.01	79.28	86.21	234.00	285.57	245.31	118.17	153.78	87.18
<b>P Balance</b>	<b>-9.62</b>	<b>-9.72</b>	<b>-31.11</b>	<b>-0.23</b>	<b>14.46</b>	<b>12.76</b>	<b>1.42</b>	<b>-0.05</b>	<b>-6.29</b>
min	-13.68	-18.02	-15.50	-25.60	-23.79	-54.81	-19.65	-31.00	-13.45
max	-3.47	0.00	-5.49	90.94	57.30	99.52	34.98	76.99	1.42
<b>K Balance</b>	<b>-10.98</b>	<b>-46.38</b>	<b>-26.06</b>	<b>-42.16</b>	<b>63.37</b>	<b>-26.19</b>	<b>14.89</b>	<b>-23.28</b>	<b>-29.24</b>
min	-19.80	-133.72	-115.00	-196.00	-191.85	-772.66	-120.55	-230.00	-74.12
max	-4.77	0.00	-5.94	272.43	268.41	308.74	161.91	74.14	9.87

Energy balance put as Output/Input rate fluctuated from 6.6 to 16.2 on farm 2. Variable efficiency of energy use was found also on farm 3 (from 6.8 to 12.72) and the most stable and also the best results had farm 1 (from 14.54 to 16.05). Important in organic farming from the point of view of energy is more frequent occurrence of reestablishment of crops stands especially after winter that happened on farm 2. The structure of energy inputs on organic farms shows Figure 1. There are no inputs in mineral fertilisers and pesticides, which are significant in conventional farming (Valtyniova, Kren 2008), but the highest energy demand is for mechanized measures.

### Conclusions

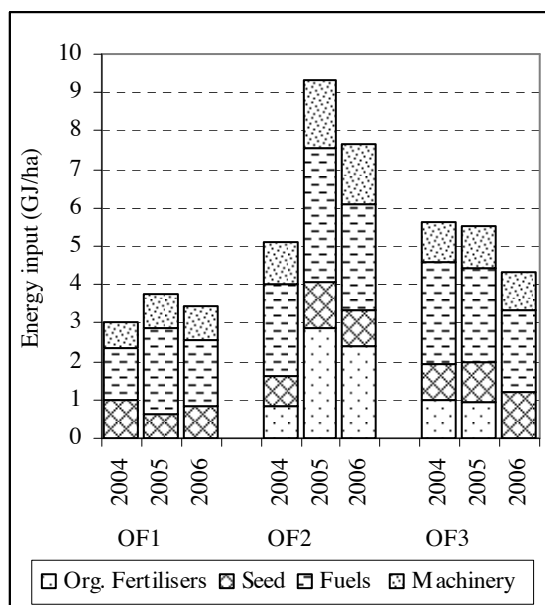
The most homogenous results in nutrients balances reached farm 3 with medium level of organic fertilisers and high diversity of production. The most problematic seems to be K balance, because it is negative in most years on many plots. It is important to monitor nutrient balances in longer time period and check their influence on the soil nutrient pool.

Energy efficiency was highest on farm 1 because of minimum inputs used. Instable energy balance is caused by fluctuating yields on the farms and relatively often occurring need for reestablishment of crop stand after winter especially on farm 2.

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**Figure 1:** Structure of energy inputs (GJ.ha<sup>-1</sup>)

# Assessment of Eco-Agro-Forestry System Benefits Through Economic and Socio-Environmental Indicators

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## Introduction

Rural landscape is rapidly changing in Italy during the last years. The abandonment of arable land, urban expansion and high resources withdrawal put increasing pressure on the agro-forestry ecosystems. Land use change have led to disturbance especially referring to the control of hydrological cycle along with the increasing risk of flooding, erosion, drought vulnerability, and land degradation. Soil and water resources are becoming finite and vulnerable to the increased pressure placed on them by an ever raising urban population and land cover change. Moreover, as on the whole world, the agriculture crisis is now turning in a food crisis. The European model of agriculture, considered a cornerstone of the future UE rural policy, is closely linked with the concept of multifunctional character of sustainable agriculture and could enhance the rural development in a post productive society. Briefly stated, the concept of multi-functionality recognises many potential benefits to the agro-forestry ecosystems among which the more relevant are: the carbon sequestration, food and biomass production, flooding and erosion protection, runoff reduction through the soil infiltration of rainfall, delivery of bio-capacity for resident people, facilitation of the waste cleansing, utilization of the no cost, endless radiation energy for food and biomass production. The linkage between environment and agriculture can be expressed through the functions connected with the land socio-economic and environmental conditions. These functions, contributing to environmental balance and to non marketable services supply, are representing benefits for farmers, for the rural community and for the whole society.

## Logical frameworks

Solving conflicts of the environment versus socio-economic development requires a new comprehensive approach which reconciles the different interests and integrates them into a sustainable development. An integrated *analysis* of the spatio-temporal socio-economic and ecological consequence of the land use and climate change is a fundamental prerequisite to set up the process of identify and solve the clashes existing between food production, natural resources and rural well-being. Yet the social and environmental *objectives* are the keystones of decision making process. Once the goals settlement is completed an *action plan* comprehensive of a wide range of activities to be carried out to counteract the agriculture *constraints* and *negative externalities* needs to be defined. The most serious agriculture constraints in our Country include low income, cultivated land abandonment, farmer ageing, land cover/climate change and urbanisation that causes rural occupation decreasing. Policy intervention is needed to support the rural social and cultural values in view of the development process and some guide lines have to be provided to decision makers to allow them to select among many options in order to achieve the scheduled goals. A decision support system has to be developed through the adoption of a set of indicators assessing the socio-economic and ecological conditions.

## Results

The study area includes the NUTS2 region of Lazio (17,065 Km<sup>2</sup>) characterized by complex topography with different climatic zones along the elevation gradient. Some indicators depicting

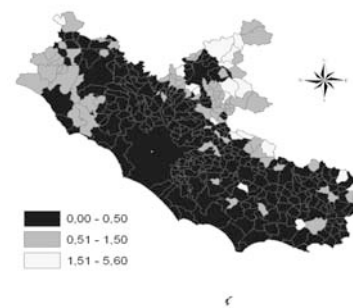
agriculture constraints, benefits, and negative externalities are here proposed (Tab. 1) as tools of the decision making process and to plan the needed activities to be implemented with the aim to overcome the ecological unbalance. An application of the *agro-eco-balance* indicator applied to Latium region municipalities (Tab. 2) and its spatial variation map are here reported. (Fig. 1)

**Tab. 1 Agro-eco-balance indicators of constraints, benefits, and negative externalities of agriculture.**

Dimension	Indicators	Measurement unit	Source
Constraints	Population density	people km <sup>-2</sup>	National Census of Population
	Time change in Aridity index	%	Meteorological statistics
	Loss in cultivated land	%	National Census of Agriculture
	Farmer ageing	% farmer > 55 y.-old	National Census of Agriculture
	Farm marginalisation	%	National Census of Agriculture
Benefits	Protection from erosion	sensitivity classes	Corine Land Cover
	Bio-capacity estimate	%	National Censuses
	Productivity of the agriculture	euro ha <sup>-1</sup>	ISTAT - National Accounting
	Cultivated land under protection	%	National Census of Agriculture
	Land under agri-environ. measures	%	MIPAAF
Negative externalities	Over-exploitation of ground-water	%	National Census of Agriculture
	Over-grazing	%	National Census of Agriculture
	Soil compacting	sensitivity classes	National Census of Agriculture
	Fire risk	%	Ministry of Agriculture
	Estimated soil erosion rate (ERO)	t ha <sup>-1</sup> y <sup>-1</sup>	Ministry of the Environment

**Table 2 – Agro-eco balance indicator by province and elevation in Latium.**

NUTS-3 Province	Mountain zones	Hilly areas	Lowlands	Province
Viterbo	-	0.42	0.60	0.43
Rieti	1.04	0.41	-	0.49
Rome	0.54	0.13	0.03	0.26
Latina	0.21	0.26	0.09	0.21
Frosinone	0.53	0.16	-	0.30
Latium	0.72	0.25	0.13	0.39



**Fig. 1 Distribution of agro-eco-balance indicator**

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# The Efficacy of Reduced Doses of Herbicides CALLISTO 480 SC + ATPLUS 463 and BASAGRAN SUPER on *Fallopia convolvulus*

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## Introduction

Black bindweed (*Fallopia convolvulus*) is an annual spring weed, which is common in all crops and production regions of the Czech Republic. Due to its high abundance and good adaptability this weed is classified as an important weed species not only in the Czech Republic but also abroad. The use of chemicals (herbicides) is one of possibilities how to control it. However, herbicides belong to substances showing significant negative effects on the environment. Regarding efforts how to reduce this environmental load it seems to be suitable to use those methods, which enable to define an optimum dose of herbicide and their efficacy on concrete weed species in individual stages of their development. Such methods have been used for many years in the Dutch institute Plant Research International Wageningen. The so-called Minimum Lethal Herbicide Dose Method (MLHD) enables to calculate low doses of herbicides on the base of measuring of photosynthetic efficiency. The MLHD method enables to calculate the minimum doses of herbicides belonging to the group of photosynthesis inhibitors with regard to the required control of individual weed species in their developmental stages on a given plot (Haage et. al., 2002).

## Material and Methods

In years 2005–2007, a small-plot trial was established in the Field Research Station Žabčice, Mendel University of Agriculture and Forestry Brno, Czech Republic, situated 25 km southwards from Brno with Lat/Long coordinates (49°01' N; 16°16' E). The objective of this experiment was to determine efficacy of graduated doses of herbicides CALLISTO 480 SC + ATPLUS 463 (mesotrione 480 g l<sup>-1</sup>) and BASAGRANU SUPER (bentazone 480 g l<sup>-1</sup> + 150 g l<sup>-1</sup> of activator) on the weed species black bindweed (*Fallopia convolvulus*) occurring in a grain maize crop. The herbicide CALLISTO 480 SC + ATPLUS 463 was applied in doses of 0.25 – 0.1875 and 0.125 l ha<sup>-1</sup> and the herbicide BASAGRAN SUPER in doses of 2.0 – 1.5 and 1.0 l ha<sup>-1</sup>. The field experiment itself consisted of seven variants with four replications (the size of one experimental plot was 21 m<sup>2</sup> (i.e. 3x7 m).

CALLISTO 480 SC is a systemic herbicide containing as effective agent mesotrione (480 g l<sup>-1</sup>), which belongs to the group of triketons. It is used for pre- and postemergent killing of annual dicotyledonous and grass weeds in maize crops. The effect is visible within 3 to 7 days after its application. Leaf etiolation and necroses are typical symptoms of its weed-killing action (bleaching effect).

BASAGRAN SUPER is a contact postemergent herbicide containing as effective agent bentazone 480 g l<sup>-1</sup> + 150 g l<sup>-1</sup> activator, which belongs to the group of photosynthesis inhibitors. Its effect is manifested through etiolation of leaves, inhibition of photosynthesis, and disturbances of transport of electrons and of specific CO<sub>2</sub> reactions. Its effect is visible within three days after the application. In maize crops, it is used for control of annual dicotyledonous weeds.

Prior to maize sowing, the plots were treated with urea in the dose of 120 kg N ha<sup>-1</sup>. Maize hybrid Ribera (FAO 410) was sown on 10 May 2005, 28 April 2006, and 24 April 2007 with a four-row precise sowing machine Kleine Multicorn adjusted to the depth of 0.06 m and row spacing of 0.75 m. Winter wheat was cultivated as a forecrop of maize. Herbicides were applied by the Solo 432 motor sprayer on 6 June 2005, 6 June 2006 and 4 June 2007. The application pressure was 0.3 MPa and the dose of water 300 l ha<sup>-1</sup>. Maize was in the developmental stage of the four true leaf (black bindweed – BBCH 17 – had seven true leaves). The efficacy of graduated doses of herbicides CALLISTO 480 SC + ATPLUS 463 and BASAGRAN SUPER was estimated by means of the apparatus PS1 Meter, which can measure percentage of damage of the photosynthetic apparatus within the range of 0 – 100%. Low

values characterize healthy, undamaged plants while high ones indicate that the plants were damaged (Tab. 1). The extent of the damage of the photosynthetic apparatus was measured on the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day after the application of herbicides. Experimental plants were harvested on 19 October 2005, 23 October 2006 and 2 October 2007. Harvested samples were weighed and the obtained results were converted to the yield at 15% of moisture content.

Table 1: Categorization of the damage of photosynthetic apparatus (MLHD PS1 2004)

Values measured with the apparatus PS1 meter	Expected weed-control efficacy
0 – 15	None
15 – 30	Low (reduction of photosynthesis by 20 %)
30 – 50	Medium (reduction of photosynthesis by 40 %)
>50	High (reduction of photosynthesis by more than 40 %)

## Results

Using the analysis of variance it was found out that within the period of three experimental years the measured values were highly significantly influenced by year, dose of herbicide and date of measuring. The performed Tukey test ( $P = 0.95$ ) revealed statistically significant differences in PS1 values between the year 2005 on the one hand and years 2006 and 2007 on the other as well as between the control and other experimental variants treated by graduated doses of herbicides CALLISTO 480 SC + ATPLUS 463 and BASAGRAN SUPER. In experiments with BASAGRAN SUPER there was a statistically significant difference in measured PS1 values between the registered dose (2.0 l ha<sup>-1</sup>) and the half dose (1.0 l ha<sup>-1</sup>). In case of the herbicide CALLISTO 480 SC + ATPLUS 463 there was no significant difference in PS1 values measured after the application of individual doses. In experiments with both herbicides the PS1 values increased gradually on individual dates of measuring. The highest killing effect of herbicide CALLISTO 480 SC + ATPLUS 463 on black binder was observed on the 5<sup>th</sup> day after the application while in case of herbicide BASAGRAN SUPER the maximum effect was observed already on the third day after the application. On these dates the LD 50 values were exceeded after all doses of tested herbicides so that it can be concluded that their weed-killing efficacy is very good. All tested doses of both herbicides were very efficient and reliably killed all black binder plants in this developmental stage. When evaluating the yield of grain (converted to 15 % water content) by means of variance analysis, a highly significant effect of the year was observed. However, on the other hand there were no significant differences in the yield of grain among individual variants of graduated doses of both herbicides. The highest average yield of all experimental variants (14.95 t ha<sup>-1</sup>) was recorded in 2006. In 2005 the average yield was 13.52 t ha<sup>-1</sup> and the lowest one (9.95 t ha<sup>-1</sup>) was recorded in 2007. All measured values were converted to 15% humidity of grain.

## Conclusions

Results of three-year experiments demonstrated that as far as the efficacy of herbicides CALLISTO 480 SC + ATPLUS 463 and BASAGRAN SUPER on black bindweed (*Fallopia convolvulus*) in the stage of seven true leaves (BBCH 17) was concerned it was quite sufficient to apply only half doses (i.e. 0.125 and 1.0 l ha<sup>-1</sup>) instead of registered ones (i.e. 0.25 and 2.0 l ha<sup>-1</sup>) without any negative effects on grain yield of maize. It was found out that the most suitable date for estimation of graded doses of the herbicide CALLISTO 480 SC + ATPLUS 463 was the fifth day after its application. As far as the herbicide BASAGRAN SUPER was concerned, such estimation can be performed already on the third day after the application.

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**SUB SESSION 1.2**  
**TECHNOLOGIES FOR BIODIVERSITY CONSERVATION**

**1.2b- VEGETATION AND SEED BANK DYNAMIC**

Chairman: Witold Grzebisz



# Critical Period of Weed Control in Soybean (*Glycine max* L. (merr))

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## Introduction

Weeds are one the most important limiting factors of soybean yield through competition for resources including light, nutrient and water. Critical period for weed control (CPWC) is a key component of an integrated weed management program (Knezevic et al. 2002). This is the period during the life cycle of a crop when it must be kept weed free in order to prevent a specific level of yield loss (Van Acker et al. 1993). Weed presence before and after CPWC should not significantly reduce yields (Martin et al. 2001). The CPWC is useful for making decisions on the need for and timing of weed control (Knezevic et al. 2002; Van Acker et al. 1993). The critical period of weed control influenced by the many factors that affect weed interference intensity, including the diversity of weed species, weed density, distribution and emergence periodicity, the nutrient status of the soil, weather and cultural practices (Hall et al. 1992; Swanton and Weise 1991). The objective of the present study was to determine the critical period of naturally occurring weed in soybean.

## Methodology

Experiment was conducted at a loam soil with a pH of 7.7 in Karaj (35° 49' N and 51° 50' E, 1321masl), Iran in 2002. The experiment was established in a RCBD with four replications. Treatments included different early-season and late-season interference durations of naturally occurring weed. Increasing durations of weed interference were established by delaying weed removal time to V2, V4, V6, R1, R3, and R5. Late-season weed interference durations were achieved by increasing length of weed-free period in appropriate plots up to each of the above phenological stages and then keeping the plots weed-infested till the end of the growing season. In addition, season-long weed-free and season-long weed-infested controls were included to give a total of 14 durations of crop-weed interference. Plots consisted of four 8-m rows, spaced 0.60m apart. Soybean population was adjusted to 200,000 plants ha<sup>-1</sup> by over seeding at the time of planting and thinning to the target density at V2. Destructive soybean plant harvests were made every two-week starting at 15 DAE. Leaves of harvested plants were separated from the stem and leaf area of leaves was measured. The whole plants were then dried at 80°C for 72 h. At crop maturity, grain yield was determined.

All data were analyzed statistically using GLM procedure in SAS (SAS Institute, 2000). Treatment comparisons for all traits except weed biomass were made using nonlinear regression analysis. The increasing duration of weed interference on relative yield was described by the logistic equation, while the Gompertz equation was used to describe the influence of increasing length of the weed-free period on relative yield (Van Acker et al., 1993).

## Results

Results indicated that weed removal time had significant effect ( $P<0.01$ ) on weed density and biomass. By increasing length of the weed-free period, total weed density significantly reduced compared to the full-

season weed-infested control. By increasing length of the weed competition period, weed biomass also increased significantly while increasing duration of the weed-free period had reverse effect. These findings were in agreement with that of Burncide (1997).

A reverse relation found between weeds and soybean biomass; i.e. by increasing crop-weed interference duration, weed biomass increased while crop biomass decreased. Tollenaar et al. (1994) also came to the same conclusion. It was revealed that by increasing length of crop - weed interference period, soybean LAI decreased so that the greatest LAI was achieved in the full - season weed-free control (Figure 1). Figure 1, however, implied that progress in soybean growth stages and increment in its LAI had reduced radiation penetration into the canopy and thus inhibited normal growth and dry matter accumulation of weeds. Considering 5% as the acceptable yield loss, the beginning and end of the CPWC for soybean were determined 24 DAE(V4) and 65 DAE (R4), respectively (Figure 2). Longer duration of the CPWC in the present study compared to other studies could be attributed to lower competitive ability of soybean and higher competitive ability of weeds. Early-season growth rate in soybean is low which provides an advantage to weeds in terms of resource acquisition. Weed composition in the present study consisted of different grass and broadleaved species that caused high competition pressure on soybean.

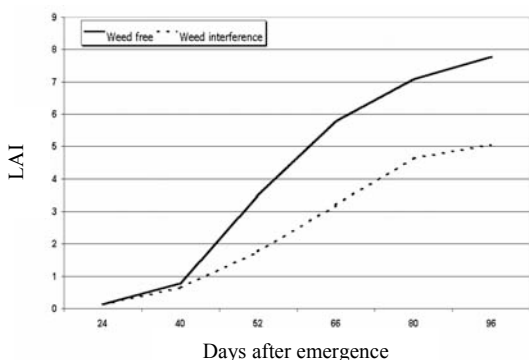


Figure 1. Soybean LAI in weed-free and weed-infested treatments.

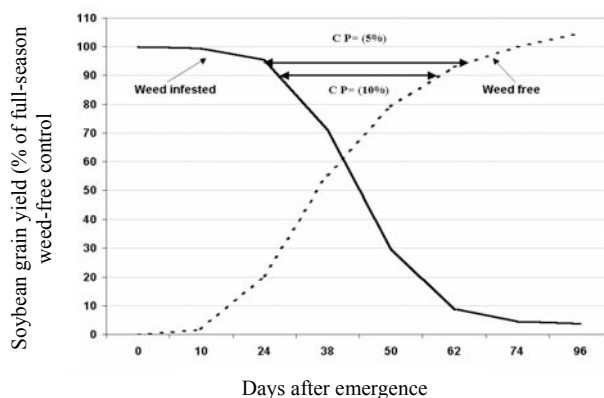


Figure 2. The CPWC in soybean.

## Conclusions

Weed interference significantly reduced (82.5%) soybean yield compared to the full - season weed - free control. A CPWC of 41 days, from 24DAE to 65 DAE, was determined for soybean in the present study.

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# Evaluation of Germination of Wild Plants in Order to Return this Species to the Nature

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The differences between wild and agricultural crops are in different level of seed endogenous and exogenous dormancy. Wild plants have large variability at every seed measured traits, especially germination can be strongly modified by environmental conditions. The basic aim after wild plants' seed collecting is the evaluation of their germination in order to return this species to the nature, i. e. for species-rich meadows' restoration in Bílé Karpaty Mountains, which were destroyed by bad farming system. For wild plants' sowing and seed mixtures preparation the (approximate) information about germination percentage of different species is needed. Standard testing methods used by agricultural laboratories are not suitable on account to very long time of gradual sprouting and contamination by fungi, which disallow to obtain the accurate results. For this purpose an effort for developing of special quick orientation test was made. In our experiments the one of the goals was to find a fast method for wild plants' germinability testing, which would give - after one week testing - similar or better results as testing by standard methods after several weeks.

## Methodology

The following species were analysed: *Anthoxanthum odoratum*, *Brachypodium pinnatum*, *Betonica officinalis*, *Daucus carota*, *Galium verum*, *Plantago media*, *Prunella vulgaris*, *Ranunculus acris*, *Salvia pratensis*, *Sanquisorba officinalis*, *Securigera varia*, *Serratula tinctoria*, *Thymus pulegioides*, *Valeriana collina* and *Vicia tenuifolia*). The twelve repetitions in every type of test were applied, sowing one hundred seeds of every species in every repetition. Germinability was analysed in Petri dishes with filter paper or in cultivation pots 40 x 60 x 10 cm with the soil or with the quartz sand. Petri dishes were placed in climatic chamber and pots for cultivation were placed in the greenhouse with 25°C, 13 h of day and 12 h of night. Pots were covered by plexiglass sheets to stop humidity lost. Humidity under plexiglass sheets was kept by spraying of deionized water. In the presented experiments following types of treatment before sowing were applied:

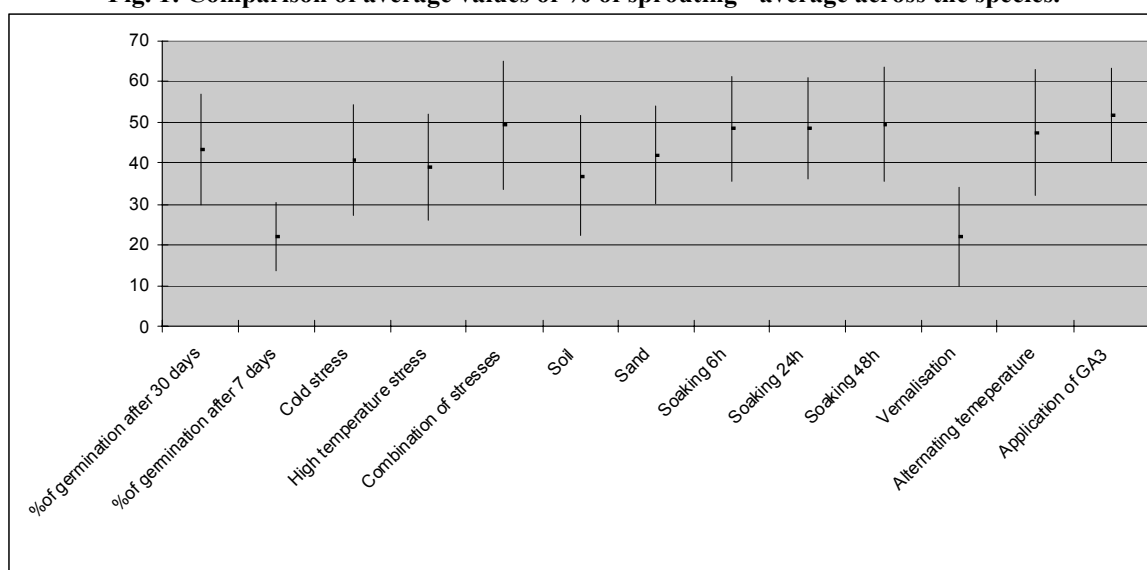
**Cold stress** = -1°C for 48 hours, **High temperature stress** = 32 °C for 48 hours, **Combination of stresses** = 40 °C for 24 hours, 0°C for 24 hours, **Soil** = sowing to the soil, surface was slightly covered by fine sand, **Sand** = sowing to the sand, surface was slightly covered by fine sand, **Soaking** = soaking before sowing – three variants: 6h (Soaking 1), 24 h (Soaking 2) a 48 hod (Soaking 3). After soaking seed was sown to the sand, **Vernalization**= 0 °C for one month, humidity 90-100%, dark; **Alternating temperature** = soaking for two days (distilled water, filter paper), after that alternating of 30°C for 12 hours and 5°C for 12 hours and finally sowing to the soil. As **control variant** standard laboratory method for germination testing was applied (Petri dishes and filter paper) for one month, 25°C, light regime 12 h day and 12 night.

## Results

As follows from obtained results (fig 1), the best methods are (1) two days soaking in distilled water or (2)

combination of stresses (alternating 12 hours of 30°C and 12 hours of 5°C) followed by sowing to the damp sand with regular humidification of sand surface by distilled water (Fig. 1). This two methods are very fast - there is the possibility to obtain very good results after one week testing and those two methods can give objective information for sowing on the basis of germination power. But in case of analysis of absolute percent of germination there is necessity to use combination of stresses or soaking for one month to reach the total possible germination level. The difference between results after one month testing and after one week testing are statistically non significant. It follows from the statistical analysis that six repetitions of this two best methods can give information with statistically non significant variability of obtained average values.

**Fig. 1: Comparison of average values of % of sprouting - average across the species.**



## Conclusions

It is well known, that there exists the best method of germination testing according to the biological reason for every species. But the goal of presented work has been satisfied-to develop fast method for wild plants' germinability testing, which would give after one week of testing very similar or better results as long-time testing by standard methods used by agricultural laboratorie.

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# The Seed Bank Dynamics as a Tool for Evaluating the Pasture Improvement Efficiency in a Mediterranean Environment

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## Introduction

The ecology of seed bank is very informative of the vegetation development and persistence (Leck *et al.*, 1989) and of the conservation of plant communities and species, mainly depending on the incidence of the persistent fraction of the seed bank (Diemont, 1990). The composition of the herbaceous community, and its soil seed bank, in the Mediterranean rangelands is highly influenced by pasture management, climatic and topographic conditions, productivity levels (Osem *et al.*, 2002). In this paper, with the aim of evaluating the efficiency of a legume-based over-sown pasture in Central Sardinia, the seed bank dynamic was observed during three years.

## Methodology

The trial was carried out during 1997-2000 at Usellus (Western Sardinia, Italy). The soil is clay-sandy sub-acid, with low N and P<sub>2</sub>O<sub>5</sub> content, and the climate is Mediterranean semi-arid with average annual rainfall of 610 mm. In October 1997, 4.8 ha of low-quality pasture was oversown with an annual legumes mixture (subclover 'Trikkala' and 'Clare' and burr medic 'Anglona') and fertilised with 80 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 20 kg ha<sup>-1</sup> of N. The germinable seed bank (GSB) was assessed in 100 intact soil cores (8 cm Ø x 4 cm depth) and separated in the following fractions (Perez *et al.*, 1998): a) RG (germination in watered field conditions); b) HT (21 days of test duration, 8/16 h of darkness/light and 20/30°C of T° daily cycle); c) LT (21 days of test duration, 3 - 5°C constantly); d) GA (application of 600 mg/kg of gibberellic acid, 35 days of test duration, 8/16 h of darkness/light and 15/35°C of T° daily cycle). GSB was obtained by summing RG+HT+LT+GA. The transient seed bank (TSB) was calculated by summing RG, HT and LT fractions. GA was considered as a contribution of the GSB to the persistent seed bank. The composition of the overlaying vegetation was estimated using an HFRO sward stick, by recording 4 leaf contacts at the vertices of 1200 random quadrats (20 x 20 cm).

## Results

In the improved pasture, GSB increased respectively of 46%, 174% and 36% in each of the three years (table 1). This increase was mainly due to the high contribution of spontaneous grasses to the TSB, particularly in the 2<sup>nd</sup> year. A good establishment of the introduced legumes was observed in the 1<sup>st</sup> year, when the 22% of GSB was represented by subclover and burr medic seeds, vs. less than 10% in the unimproved test. A reduction of the introduced legumes occurred in the last two years, as spontaneous grasses seeds increased. Despite of the decline of its presence in GSB after the first self-reseeding, burr medic showed a high and quite constant contribution to GA, with 29, 25 and 21% , respectively in the three years. In the improved pasture, only burr medic maintained a constant pattern of germination throughout the years. Summer occasional rains in the 2<sup>nd</sup> and 3<sup>rd</sup> year caused an early germination of subclover (*false break*), causing its reduction within the TSB. The spontaneous grasses dominated the TSB, mainly in the last two years, as a response both to the higher soil N content and to the better rainfall regimes. The TSB corresponded quite well to the overlaying vegetation composition (table 2) observed two months later in the field (November), indicating that this fraction is a good estimate of the actual vegetation.

Table 1 – Number of germinated seeds (on 100 samples) and percentage of pasture species belonging to different seed bank fractions in the 3-years trial. Values in italics are referred to the unimproved pasture.

Year	Treatment	Germinated seeds		Grasses		Subclover		Burr medic		Other legumes		Other species	
1997-98	RG	4206	<i>2203</i>	49	<i>48</i>	17	<i>10</i>	6	<i>1</i>	5	<i>3</i>	23	<i>38</i>
	HT	478	<i>397</i>	23	<i>25</i>	2	<i>2</i>	-	-	18	<i>9</i>	56	<i>64</i>
	LT	69	<i>43</i>	23	-	11	-	8	-	25	<i>54</i>	33	<i>46</i>
	GA	322	<i>107</i>	5	<i>13</i>	12	<i>6</i>	29	<i>16</i>	17	<i>47</i>	37	<i>19</i>
GSB composition (%)				43	<i>43</i>	15	<i>8</i>	7	<i>1</i>	8	<i>7</i>	27	<i>41</i>
1998-99	RG	9083	<i>3150</i>	79	<i>66</i>	3	<i>1</i>	2	-	7	<i>14</i>	9	<i>18</i>
	HT	437	<i>245</i>	43	<i>41</i>	1	-	-	-	11	-	44	<i>57</i>
	LT	368	<i>170</i>	49	<i>35</i>	3	-	4	<i>3</i>	4	<i>6</i>	41	<i>56</i>
	GA	583	<i>250</i>	6	<i>4</i>	10	<i>8</i>	25	<i>12</i>	10	<i>20</i>	48	<i>54</i>
GSB composition (%)				73	<i>59</i>	3	<i>1</i>	3	<i>1</i>	7	<i>13</i>	14	<i>25</i>
1999-00	RG	6634	<i>3391</i>	73	<i>74</i>	4	-	4	<i>3</i>	7	<i>4</i>	11	<i>19</i>
	HT	714	<i>1263</i>	17	<i>24</i>	2	-	1	-	1	-	82	<i>72</i>
	LT	182	<i>181</i>	28	<i>38</i>	3	-	5	<i>3</i>	13	-	51	<i>59</i>
	GA	411	<i>188</i>	8	<i>13</i>	4	-	21	-	10	<i>10</i>	58	<i>77</i>
GSB composition (%)				64	<i>60</i>	4	<i>0</i>	5	<i>2</i>	7	<i>3</i>	21	<i>34</i>
Source of variation													
Year				***	*	***	*	n.s.	n.s.	***	***	***	n.s.
Treatment				***	***	***	*	***	**	***	**	***	***
Year*treatment				***	*	**	**	n.s.	*	***	***	***	***

n.s. = not significant; \* = significant for  $P \leq 0.05$ ; \*\* = significant for  $P \leq 0.01$ ; \*\*\* = significant for  $P \leq 0.001$ .

Table 2 – Floristic composition of the transient seed bank (TSB), gibberellic acid fraction (GA) and overlaying vegetation in autumn.

Component	TSB (%)	GA (%)	Vegetation (%)
Grasses	42	5	48
Subclover	15	12	22
Burr medic	5	29	5
Other legumes	7	17	3
Other species	25	40	22

## Conclusions

The analysis of the transient and persistent seed bank short-term dynamics confirmed to be worth to forecast the efficiency of the pasture improvement (Cocks, 1992). This methodology shows reliability in estimating TSB for predicting the composition of the potential overlaying vegetation.

In terms of contribution to the persistent seed bank, the results confirm the highly conservative role of the hardseeded legumes and dormant seeds, in buffering against the quick changes in the species composition (Fenner, 1985, Pake and Venables, 1996).

An early determination of the GSB composition could be useful for an efficient pasture management aimed at exploiting the capability of some legumes to contribute to the persistent seed bank and improve the pasture quality and soil fertility.

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# Modelling the Effects of Cropping Systems on Weed Emergence: How to Develop a Multi-specific Model?

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In the past, weed control in fields mainly relied on herbicide applications. Because of the resulting environmental problems, it is now necessary to take into account all cropping system aspects to optimise weed control strategies. Modelling is essential to design and evaluate integrated cropping systems for weed management because of the large variation in agricultural practices and their complex interactions. Such a model, ALOMYSYS (Colbach *et al.*, 2006), was developed for *Alopecurus myosuroides* and we are now working on a multi-species version. This model is based on a succession of life-stages linked by functions depending on cropping system effects, in interaction with climate and soil environment. Extrapolating the mono-specific model to diverse weeds by studying every species is unfeasible. Our objective therefore was to search for relationships between species biology (*i.e.* model parameters) and traits. A trait is considered as any morphological, physiological or phenological feature measurable at the individual level (Violle *et al.*, 2007). In the present paper, pre-emergent shoot growth in the soil and pre-emergent seedling mortality due to soil clods were studied, being the main emergence processes depending on tillage *via* seed depth and soil structure. For each processes, generic relationships were searched for between the model parameters and traits by studying a few contrasted weeds. Seed mass has already been identified as a key trait for early growth in wild species and could be related to the shoot growth. Pre-emergent mortality due to soil obstacles could be indirectly linked to the shoot diameter and morphology (Sinha & Ghildyal, 1979), which differ between mono- and dicotyledons. Consequently, the nine species studied differed for their seed weight, shoot diameter and taxa. The experimental results were completed with literature data obtained with similar protocols for three other species.

## Methodology

Shoot elongation was measured on freshly germinated seeds planted in pots filled with fine sand and nutrient solution. The pots were placed in growth chambers. Regularly, 5 pots were sampled and their shoot length was measured. A Weibull equation was fitted to shoot growth with time and maximum shoot length and growth rates were estimated.

In the mortality study, germinated seeds were planted in trays and then covered with 2 cm of soil; clods with longest axis varying from 20 to 50 mm were placed exactly above each germinated seed. In a first experiment, clods were placed on the soil surface while in a second one, fine soil was added to cover them. Each treatment was replicated six times (24 seeds per replicate) for three different clod sizes and for each of the nine species. When the maximum shoot length was reached, the trays were opened, the percentage (P) of blocked seedlings was counted, and the following equation was fitted for each species and clod position (*i.e.* buried or on soil surface) as a function of clod size CS (mm):

$$P = 100 \left[ 1 - e^{-a(CS - CS_0)} \right] \text{ if } CS \geq CS_0 ; P = 0 \text{ if } CS < CS_0$$

where  $CS_0$  is the clod size (mm) below which no seedling was blocked and  $a$  the slope of the curve.

## Results

Fig. 1 shows that shoot growth followed a sigmoid curve and the Weibull equation could be fitted satisfactorily for all species. Maximal shoot length was related to seed dry mass (tab.1). The species also differed in their maximal shoot growth rates. The rates were slower than  $0.5 \text{ mm} \cdot ^\circ\text{C}^{-1} \cdot \text{day}^{-1}$  for *Matricaria perforata* and reached  $2.2 \text{ mm} \cdot ^\circ\text{C}^{-1} \cdot \text{day}^{-1}$  for *Avena fatua*. Positive correlations existed

between the maximal shoot growth rate and either the seed mass or the maximal shoot length (tab.1). The percentage of blocked seedlings increased with clod size; it was larger for buried than for surface clods and differed between species (fig. 2). The  $CS_0$  and  $a$  parameters estimated from these experimental results were correlated with the shoot diameter measured during the shoot elongation experiment (tab.1). Relations differed for mono- and dicotyledons. Consequently, for species with a high shoot diameter, the mortality due to clods was relatively small, in contrast to species with a smaller shoot diameter.

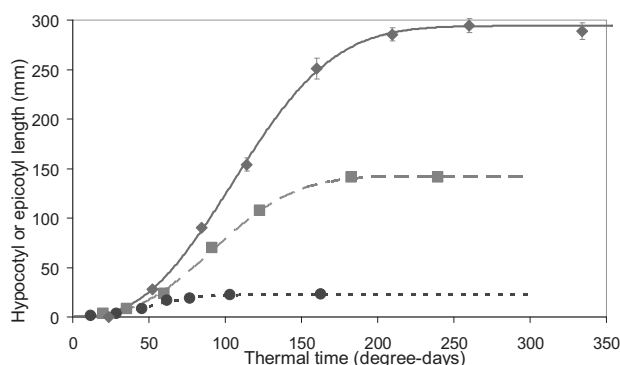


Fig. 1. Example of shoot growth in the dark for three species (*Matricaria perforata* (---●---), *Geranium dissectum* (---■---) and *Avena fatua* (—◆—)) measured in a pot experiment (symbols) and fitted with a Weibull equation (lines).

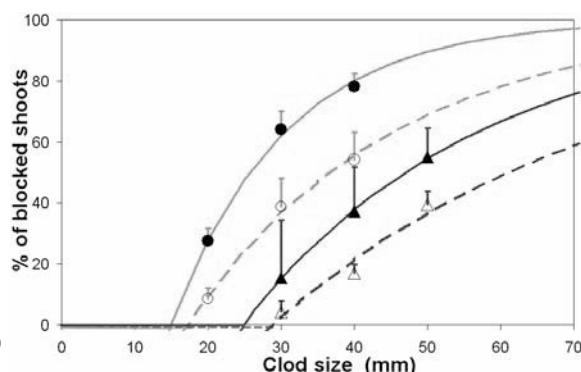


Fig. 2. Observed and fitted percentages of *Amaranthus hybridus* (circles) and *Geranium dissectum* shoots (triangles) blocked under surface (open symbols) or buried soil clods (closed symbols) as a function of clod size.

Table 1. List of the fitted equations between traits and pre-emergent growth and mortality parameters.

Lengths and diameters are in mm, masses in mg.

\* The factor "mono-/dicotyledon" was significant at  $p$ -value=0.09.

ns: not significantly different from zero at  $\alpha$ =0.05.

#### Shoot and root growth

Maximal shoot length = $68.8 \cdot \text{seed dry mass}^{0.51}$	$R^2$
shoot $v_{\max} = 0.710 + 0.067 \cdot \text{seed dry mass}$	0.95
shoot $v_{\max} = 0.48 + 0.0045 \cdot \text{maximal shoot length}$	0.49
	0.55

#### Seedling mortality under soil obstacles

<b>Surface clods:</b>		
• $CS_0 = 22.56 \cdot \text{shoot diameter} + 7.80$		0.83
• $\text{Log}(a) = -2.38 + a_{\text{taxa}} \cdot \text{diameter}$		
with $a_{\text{taxa}} = -0.89$ for monocotyledons and $-2.23$ for dicotyledons		0.54
<b>Buried clods:</b>		
• $CS_0 = -2.5 + \text{taxa effect} + 29.0 \cdot \text{shoot diameter}$		
with $\text{taxa}^* \text{ effect} = -5.8$ for monocotyledons and $+5.8$ for dicotyledons		0.87
• $\text{Log}(a) = -1.87 + a_{\text{taxa}} \cdot \text{diameter effect} + \text{taxa effect}$		
with $a_{\text{taxa}} = 0.32$ ns for monocotyledons and $-2.33$ for dicotyledons		0.99
with $\text{taxa effect} = -1.30$ for monocotyledons and $0$ for dicotyledons		

## Conclusions

The measured maximal shoot lengths were contrasted for the studied weeds and represent the species maximal emergence depth in soil. The stability of the relationship with seed mass enables us to use this trait as a predictor of the seed response to seed depth when analysing new species. Pre-emergent shoot elongation rate was positively related to the maximal shoot length and, to a lesser degree, to seed mass. Deeply buried seeds can then emerge but that their emergence will take time. The study of the shoot ability to bypass soil clods indicated that the studied species differed in their sensitivity to clod size but the general tendencies were the same. Species with a narrow shoot diameter and monocotyledons were more frequently blocked by clods than dicotyledons and species with larger diameters.

These results will be included into a plurispecific model in order to synthesise and to quantify the effects of tillage on emergence. The presented study confirmed that this approach can be used to predict the response of any weed species to soil aggregates and seed depth, with a few easily-measured traits, *i.e.* taxa, seed mass and shoot diameter.

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# Weed Population Dynamics In Maize Crop Characterized By High And Low Weed Density

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## Introduction

In the last years the growing interest in productive systems “sustainable or characterized by a limited (integrated or low-input) or null (organic) use of systematic herbicides” have led both to the reduction of mineral manuring and the use of environmental sustainable sources of nutritive elements like organic manuring, legume green manure, and on the other hand the reduction of chemical control and/or the employment of mechanical and physical weed control. Crops with a spring-summer cycle are those more subject to such problems, since they have high productive potential, for which it is necessary to employ greater energy resources (fertilization, irrigation, tillage practices, chemical products). Furthermore agricultural managements can have a strong impact on the weed flora composition and dynamics (Bàrberi *et al.*, 2001). It is, in fact, probable that the use of organic manuring or green manure could favour the emergence and the development of some weed species on other species (Liebman *et al.*, 2000). In this regard the aim of this work was to assess in maize crop, managed with high and low levels of agricultural input, the variation of weed population dynamics.

## Methodology

The experimental trials were carried out from 2005 to 2007 in central Italy (42°57'N-12°22'E, 165 m a.s.l.) on a clay-loam soil. In each year a randomized block design with 3 replicates was used to assess the variation of weed population dynamics in maize crop characterized by: 1) low weed density (LWD) due to conventional practices with mineral manuring (240 N kg/ha) and pre-emergence chemical weed control; 2) high weed density (HWD) due to organic manuring (80 N kg/ha) without weed control. Weed seedbank was estimated at the establishment of the trial in 2005 before maize sowing and at the crop harvest, while in 2007 only at the crop harvest. Three soil samples were taken with a manual soil corer of 0.04 m diameter at 3 different depths: 0-0.10, 0.10-0.20 and 0.20-0.30 m in the central area of plots of about 75 m<sup>2</sup> (6m x 12.5m). The soil samples were dried at 15°C to avoid seed germination. Seeds from each soil sample were extracted from the soil matrix with “Seed Filter Separator” (Pannacci *et al.*, 2007) and subsequently counted and analysed by binocular 20X. Weed density was assessed 4 weeks after chemical treatment and at the crop harvest in 4 rectangles (0.50 m x 0.75 m) per plot. The assessment of weed seed rain was carried out placing 6 traps along a diagonal line in the 4 central inter-rows of each plot. Each trap was made up of 2 aluminium trays (l: 0.15 m, L: 0.20 m x h: 0.04 m) which were left on the field from the end of July to the crop harvest and emptied every 21 days in 2005 and every 12 days in 2007. Post-dispersal weed seed predation by vertebrates and invertebrates of *Amaranthus retroflexus* L., *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Beauv. species was estimated from mid August to late September with maize at growth stage from 73 to 93 BBCH, for a total of 5 sampling periods. Fifty seeds of each weed species were lightly glued to sandpaper cards (45 x 90 mm) dusted successively with a fine layer of sieved soil. 9 seed cards of each weed species per each sampling period were placed in each plot of about 75 m<sup>2</sup>, in accordance with a completely randomized experimental design with 3 replicates: 4 seed-cards were placed without any cages (to estimate total seed predation = vertebrates + invertebrates) and 2 seed-cards were inside a cage with aluminium net with mesh of 3 mm (cage excluding any predators).

## Results

The initial weed seedbank population assessed in both treatments was not significantly different, with average values of 52738 seeds  $m^{-2}$  in 0-0.30 m depth and 17151 seeds  $m^{-2}$  in 0-0.10 m. The main weed species in 0-0.30 m depth were *A. retroflexus* (10190 seeds  $m^{-2}$ ), *C. album* (10134 seeds  $m^{-2}$ ) and *Portulaca oleracea* (31762 seeds  $m^{-2}$ ). Considering total weed density, in 2005, HWD was characterized by a greater number of broadleaved weeds (22 plants  $m^{-2}$  in 2005 and 77 plants  $m^{-2}$  in 2007) that contributed significantly to total weed seed production with 46661 seeds  $m^{-2}$  reported as an average over the two year period, since the values were not significantly different. On the other hand in LWD weed density was of 0.5 plants  $m^{-2}$  due to chemical control and seed production was absent in both years. The predation rate, as an average of *Amaranthus retroflexus*, *Chenopodium album* and *Echinochloa crus-galli*, in both years, was significantly different between the treatments with average values of 68% in HWD and 42% in LWD, while significant differences were not assessed between the years inside each treatment.

Table 1. Assessment of population dynamics in maize with high and low weed density (HWD and LWD) in 2005 and 2007 (standard error is reported in brackets)

Parameter Values			HWD		LWD	
Maize crop cycle			1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle	1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle
Before sowing	Initial seedbank n. x 10 <sup>3</sup> m <sup>-2</sup>	(0-0.30 m)	56.4 (3.1)		49.1 (3.6)	
		(0-0.10 m)	1.9 (1.0)		15.4 (1.9)	
During crop growth	Weed plants n. x m <sup>-2</sup>		22 (4.8)	77.0 (2.5)	0.5 (0.2)	0.5 (0.1)
	Seed production n. x 10 <sup>3</sup> m <sup>-2</sup>		47 (3.0)	46.7 (1.5)	0	0
	Seed predation rate		0.7 (0.3)	0.7 (0.4)	0.4 (0.1)	0.4 (0.2)
After crop harvest	Seedbank n. x 10 <sup>3</sup> m <sup>-2</sup>	(0-0.10 m)	12.7 (2.4)	28.6 (4.9)	7.8 (2.5)	7.1 (1.1)
		(0-0.30 m)		81.5 (11.6)		20.5 (2.3)

In 2007, HWD final weed seedbank was significantly greater (81529 seeds  $m^{-2}$ ) than LWD seedbank (20495 seeds  $m^{-2}$ ). Both seedbank were characterized mainly by *A. retroflexus* (16306 seeds  $m^{-2}$  in HWD and 3963 in LWD), *C. album* (12739 seeds  $m^{-2}$  in HWD and 3114 in LWD), *P. oleracea* (49200 seeds  $m^{-2}$  in HWD and 12909 in LWD), while *P. lapathifolium* and *F. convovulus* were recorded in both with a lower seed number. In HWD *E. crus-galli*, *D. sanguinalis* and *S. viridis* were present among grass species, while in LWD only *D. sanguinalis* was recorded. Concerning weed number species recorded in HWD and LWD seedbank, no significant difference was determined.

## Conclusions

These results suggest that chemical control is the best way to reduce seedbank while crop systems, managed without herbicides, are subject to a weed population dynamics that could render the control of the weeds ever more difficult over a long time because of the strong increase of weed seedbank, weed flora and seed production. Moreover it is important to note that weed seed predation has been considered until now a underestimated parameter for the seed losses in weed seedbank dynamics. In fact a predation rate of 68% is an important factor to contain the weed seedbank in presence of high a level of seed rain.

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# Predation by Ground Beetles on Weed Seeds Exhumed From Soil Bank

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## Introduction

Ground beetles (Carabidae: Coleoptera) are predators of the seed of herbaceous plants scattered on the ground, but prefer that of certain species. Seed eating carabids aggregate in weedy agricultural fields and their role in decreasing the seed output of weeds on farmland is generally recognized. The choice differs between carabid tribes and at the species level is determined by the relative size of the seed to that of the carabid and other still incompletely understood qualities of the seed. However, foraging beetles encounter both freshly dispersed seed and seed exhumed from the soil bank. In contrast to preferences for particular species of seed the preferences for freshly dispersed vs. soil bank stored seed were never considered nor experimentally investigated. Here we address the effect of burial on seed acceptability in two generalist granivorous carabid species abundant in Central Europe, *Harpalus affinis* and *Pseudoophonus rufipes*.

## Methodology

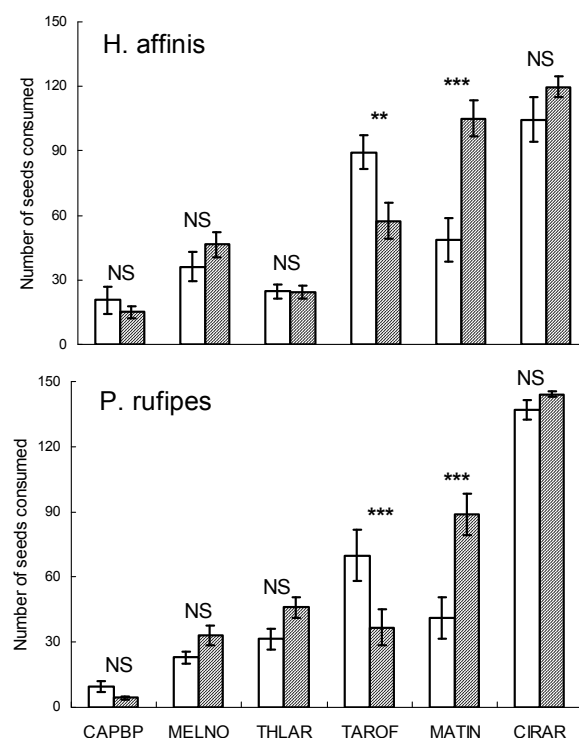
The seed of six species of weed, all common in fields of the Czech Republic, was collected in 2002. One half of the seed of each species was frozen at -20°C, and the other half buried in a field for 6 months (mixed with sieved soil and wrapped in pieces of nylon fabric). Both kinds of seed of six species were offered to carabids in a cafeteria experiment done in Petri dishes (250 mm in diameter, 50 mm height), each containing a 2 cm layer of soil. Each Petri dish contained twelve trays (area 6.2 cm<sup>2</sup>) in which particular species and kinds of seed were offered. The trays were filled with white plasticine and thirty seeds pressed into the plasticine to a depth of half their transverse diameter. Consumption of seed was measured over 6 days.

## Results

The consumption (Fig. 1) significantly varied with seed species but was similar in both carabid species which had similar preferences for particular species of seed. The differences in consumption of "fresh" and "buried" seed were not significant for seed of *Capsella bursa-pastoris* (CAPBP), *Melandrium album* (MELNO), *Thlaspi arvense* (THLAR) and *Cirsium arvense* (CIRAR). In *Taraxacum officinale* (TAROF) the consumption of buried seed was significantly lower and in *Tripleurospermum inodorum* (MATIN) smaller than in fresh seed. More data in Martinkova et al. (2006). There were large differences in consumption of particular species of seed but little difference between the two species of carabid whose preferences are generally determined by the relative size of the carabid and the seed. There was no difference in the consumption of "fresh" and "buried" seed of *C. bursa-pastoris*, *M. album*, *T. arvense* and *C. arvense*. That is, burial for half a year did not change the quality of this seed for the two carabids. There was a significant difference in the consumption of fresh and buried seed of *T. officinale* and *T. inodorum*. The consumption of *T. officinale* seed decreased because it deteriorated during burial, i.e. some of the seeds germinated or rotted. This was expected because *T. officinale* has a transient seed bank, which does not persist in the soil for more than a year. In contrast, the consumption of *T. inodorum* seed increased following burial. The change was possibly caused by the removal of a

repellent chemical from the surface of the testa, which may protect the freshly dispersed seed from predation by carabid beetles. We conclude that ground beetles may readily accept seed from soil bank but in the spring and autumn when most seeds become liberated by ploughing their positive effect is limited.

Fig.1 Consumption of fresh (open bars) and exhumed (cross-hatched) seeds of six weed species. The differences in consumption of "fresh" and "buried" seed of weed species is indicated \*\*\* (significant at  $p < 0.001$ ), \*\* (significant at  $p < 0.005$ ) and NS (not significant).



## Conclusions

The predation on seed from the soil bank and the effect of burial on seed acceptability have been studied in two generalist granivorous carabid species, *Harpalus affinis* and *Pseudoophonus rufipes*. In a laboratory cafeteria experiment adults were offered with fresh (stored frozen after dispersal) and buried (for 6 months in the soil under field conditions) seed of six common weed species. Significantly more of the buried than fresh seed was consumed in *Tripleurospermum inodorum*, probably because the repellent seed surface was abraded during the stay in the soil. In four weed species the consumption of fresh and buried seed did not differ. Ground beetles may consume seed from soil bank in the spring and autumn when it becomes liberated by ploughing.

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# Changes in Weed Community as Affected by Tillage Systems in a Semi-Arid Environment

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Development of improved weed management systems requires more knowledge on how weed species respond to changing agronomic practices. Changes in tillage practices can cause shifts in weed species and densities. Furthermore, information on the association of weed species with tillage systems has practical significance for future research in weed management. Stimulation of weed germination by tillage can depend in part on environmental conditions, such as rainfall and temperature. Where conditions are not suitable, tillage may be ineffective in inducing germination. In this sense, one of chief limiting factor of crop yield in agro-systems with a semi-arid environment is the scarce irregular rainfall distribution in addition to tillage system. In this study, three tillage systems were evaluated in a semi-arid agro-system of central Spain over a two year with different rainfall distribution.

## Methodology

The study was conducted at the INIA experimental farm “La Canaleja” located in Alcala de Henares (Madrid). The experimental site is characterized by a semiarid continental climate (minimum and maximum average annual temperatures are 6 °C in the winter and 23 °C in the summer), with an average total annual rainfall of 470 mm, and were conducted in two successive growing seasons, 2006 and 2007, to assess the effects of three tillage systems on weed community: 1) conventional tillage (CT), 2) minimum tillage (MT) and 3) no-tillage system (NT). The experiment consists in 60 trials divided in four randomized complete blocks, with three different tillage systems, and five replications. Weeds were counted by species in twenty samples randomly chosen with quadrant of 0,25m<sup>2</sup>. Analysis of variance was carried out on relative densities of the main species and total weed density for each tillage system, and weed density data were used to compare total community densities by tillage system using the Newman-Keuls multiple range test for the comparison of treatment means. All statistical analyses were performed using the software package STATGRAPHICS ® (Statgraphics Plus for Windows, 1998).

## Results

The weed community presents in this experiment was comprised of 23 species consisting of summer annuals, winter annuals, perennials, and volunteer crops. Mean total weed density data over two years is presented in Table 1 to give an overview of weed densities in this study and the overall effect of various tillage treatments, and also one analysis of variance on relative density of weeds confirmed that eight main weed species differed significantly among tillage system., these primary species were followed over the duration of study and have shown in Figure1.

In 2006, there were not differences in total weed density between tillage systems used, and high rainfall in October and November favoured weeds emerging later and perennial species. However, the growing season 2007 was characterized by the scarcity of precipitation in autumn. Under these conditions, NT and MT plots produced a higher total weed density than the conventional tillage. High rainfall occurred in spring favoured early-emergence weeds, such as *Papaver roheas* (L.) and weeds with extended patterns of emergence such as *Hypocoum procumbens* (L.). The decreased water evaporation from the soil due to the residual cover under NT and MT systems could have increased the soil water content in

comparison with CT, and that this could be the reason for the increased weed density in NT and MT plots.

### Conclusions

The results show that season distribution of rainfall restricted the effectiveness of the management practices. Furthermore, tillage systems have influence in the specialization of weeds in different environmental conditions and the application of this knowledge will allow farmers to better manage their existing weeds and more efficient use of available tillage tools in combination with other weed control practices.

Year	Tillage system p-value	Conventional Tillage	Minimum Tillage	No Tillage
2006	0.8955 (n.s.)	34.09 A	35.13 A	35.90 A
2007	0.0018 ***	24.00 a	34.81 b	37.46 b

Table 1. Effect of tillage system on total density of weeds (plants.m<sup>-2</sup>). Different letters show significant differences according to Newman-Keuls multiple range test (p<0.05).

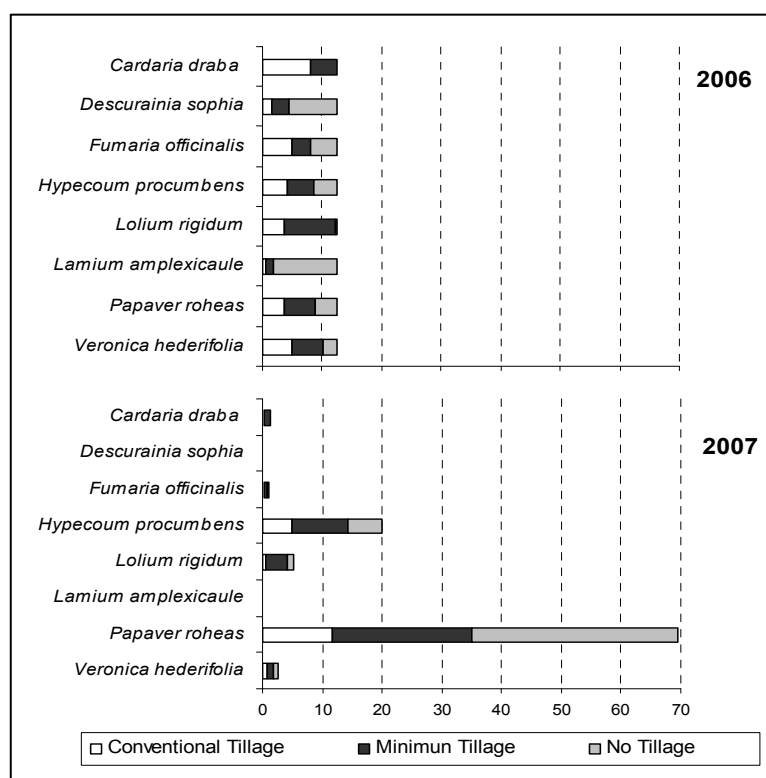


Fig1. Specific relative contribution to weed density total comparing three tillage system.

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# Ecological Features of *Ambrosia* spp Seeds from Genetically Modified Soybean Fields

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Sole reliance on a single herbicide over a long period of time to control weeds results in weed population that are resistant to that herbicide. Glyphosate is a non-selective post-emergence herbicide that controls many annual and perennial narrow-leaved and broad-leaved weeds. *Ambrosia artemisiifolia* (annual ragweed) and *Ambrosia trifida* (giant ragweed) are important weeds which cause great damage in field crops, in particular in the USA, where glyphosate resistant biotypes have been observed in genetically modified (Roundup Ready) soybean fields. Glyphosate resistant weeds are of major economic importance all over the world because of large scale use of glyphosate. The present study was conducted to investigate seed ecology (seed morphological characteristics, dormancy, germination and emergence) of suspected resistant (SR) and susceptible (S) biotypes of *A. artemisiifolia* and *A. trifida* collected in Roundup Ready (RR) soybean fields from USA.

## Methodology

Seeds of suspected resistant (SR) *A. artemisiifolia* and *A. trifida* were collected from US RR-soybean fields repeatedly treated with glyphosate. Farmers claimed an incomplete control of both biotypes and soybean yield reduction due to heavy infestation (approximately 5-20 plant m<sup>-2</sup>). Seeds of susceptible (S) populations were collected from areas (never treated with glyphosate) adjacent to RR soybean fields. For each biotype the weight of 100 seeds was determined. The main morphological seed features, such as the length of major and minor axes (cm), perimeter (cm), surface area (cm<sup>2</sup>), elongation, roundness and compactness factors, were determined by image analysis (Kodak Digital Science 2-D software). For dormancy trials, seeds were stratified in wet sterilized sand for 1, 2, 3 and 4 weeks at 4°C and germinated at 25°C (12 h light / 12 h darkness). For germination trial, seeds (stratified in wet sterilized sand for 4 weeks at 4°C) were germinated on moist filter paper in Petri dishes at 10, 15, 20 and 25°C (12 h light/ 12 h darkness). Germinated seeds (defined as cotyledon appearance) were counted daily. Germination counts were stopped when final germination percentages were reached (2-4 weeks as a function of temperature). Minimum temperatures for seed germination were calculated using the “x intercept” method previously utilized on other species (Wiese and Binning, 1987). Germinated seeds were counted and removed daily from Petri dishes. For emergence trials, pre-germinated seedlings of *A. artemisiifolia* and *A. trifida* were planted in boxes filled with sand at the depth of 1, 2, 4 and 6 cm and 1, 3, 6 and 9 cm, respectively. The boxes were placed at a constant temperature of 25 °C (12 h light / 12 h darkness). A seedling was considered as emerged when the shoot appeared from the sand surface. All the experiments were conducted according to a randomized complete design with 4 replications (each replicate of 50 seeds) and were repeated twice.

## Results

The seed weight of *A. artemisiifolia* ( $0.43 \pm 0.01$  g) and *A. trifida* ( $5.36 \pm 0.44$  g) S biotypes was statistically different ( $P < 0.05$ ) as compared to that observed for SR biotypes of annual ( $0.28 \pm 0.02$  g) and giant ragweed ( $4.39 \pm 0.24$  g). The dimensional parameters (seed major and minor axes, perimeter and surface area) confirmed for both species that seeds were bigger in S biotypes than in SR ones (data not shown). The shape of S biotype seeds differed from that of SR biotypes, being the later more elongated and less spherical.

Seeds of SR and S biotypes of *A. artemisiifolia* exhibited a similar dormancy, while differences in seed dormancy between *A. trifida* S and SR biotypes were observed (Table 1). In particular, when exposed for 7 and 14 days at low temperature (4 °C) in a wet environment, the S biotype of *A. trifida* showed significantly more dormant seeds than SR biotype. In contrast, SR and S biotypes of *A. trifida* did not differ in seed dormancy when exposed at low temperature for time intervals of 21 and 28 days.

As regards temperature requirement for germination after seed stratification at low temperature for 4 weeks, the optimum temperature was 25°C for both S and SR biotypes of annual ragweed. In addition, in the whole range of tested temperatures, no germination differences between S and SR biotypes were observed (Table 1). Also for both S and SR biotypes of giant ragweed the optimum germination temperature was 25°C. No differences in germination between S and SR biotypes of giant ragweed were found at 20 and 25 °C, while at 10 and 15°C the germination of SR biotype was 2.65-4 times lower than S biotype (Table 1). Germination data at different

temperatures were employed to calculate the minimum (or threshold) temperature for germination: S and SR biotypes of *A. artemisiifolia* showed similar values (5.7 and 4.4°C, respectively), whereas the threshold temperature of *A. trifida* S biotype (5.4°C) was half as compared to that of SR biotype (9.4°C)

Finally, no significant differences in emergence as a function of seed depth were observed between S and SR biotype of *A. artemisiifolia*: the great proportion of emergence was observed at 1-cm seed depth, while for deeper sowing a strong reduction of emergence was found. At seed depth greater than 2 cm, no emergence occurred (Table 1). At 1- and 3-cm depth similar emergences were observed for both S and SR biotypes of *A. trifida*, while at 6-cm depth the emergence of S biotype (16.7%) was 2.5 times higher than SR biotype (6.7%). At seed depth greater than 6-cm no emergence was observed for both S and SR biotypes (Table 1).

## Conclusions

The present study evidenced that seed morphological features and weight of giant and annual ragweed SR biotypes (smaller seed with elongated shape) differed from those observed for the respective S biotypes (bigger seeds of spherical shape), sampled in non-agricultural areas. Considering that SR biotypes were sampled in RR soybean fields with sod seeding management, it is plausible that small seeds have been selected for their best adaptation to this particular agro-technique (emergence from soil surface and avoidance of seed predators).

No substantial differences were observed in the seed ecology of S and SR biotypes of *A. artemisiifolia*. This evidence suggests that the failed glyphosate control under field conditions could be due to a physiological resistance phenomenon: investigations are in progress in order to verify this hypothesis. In contrast the seed ecology of *A. trifida* SR biotype was different as compared to S biotype. In particular, the seeds of SR biotype were more dormant and exhibited macro-thermal characteristics with respect to S biotype. Consequently, SR biotype of giant ragweed may avoid glyphosate control for their ecological features due to the delayed dynamic of emergence. On the other hand investigations are in progress in order to establish the respective role of physiological resistance and ecological features in determining the resistant response of this biotype towards glyphosate. On the whole, obtained results are in agreement with Di Tommaso (2004) who demonstrated that ragweed from agricultural areas evolved biotypes characterized by different seed ecology with respect to biotypes from urban areas where anthropic disturbs were less frequent.

Table 1: Seed dormancy, germination at different temperatures, threshold temperature and emergence (see “Methodology” section) of *A. artemisiifolia* (AA) and *A. trifida* (AT) S and SR biotypes. Values are means  $\pm$  SD.

	AA (S)	AA (SR)	AT (S)	AT (SR)
Non-dormant seeds (%) after 7 d	14.0 $\pm$ 4.0	13.3 $\pm$ 1.2	26.0 $\pm$ 4.0	20.0 $\pm$ 2.0
Non-dormant seeds (%) after 14 d	21.3 $\pm$ 3.1	18.0 $\pm$ 2.0	27.0 $\pm$ 3.1	19.3 $\pm$ 1.2
Non-dormant seeds (%) after 21 d	30.0 $\pm$ 9.2	33.3 $\pm$ 4.2	30.0 $\pm$ 9.2	33.3 $\pm$ 4.2
Non-dormant seeds (%) after 28 d	38.7 $\pm$ 6.1	40.0 $\pm$ 5.3	44.7 $\pm$ 3.1	44.7 $\pm$ 5.0
Germination (%) at 10°C	5.3 $\pm$ 3.1	2.7 $\pm$ 1.2	10.7 $\pm$ 3.1	2.7 $\pm$ 1.2
Germination (%) at 15°C	18.0 $\pm$ 2.0	15.3 $\pm$ 2.3	24.7 $\pm$ 3.1	9.3 $\pm$ 4.2
Germination (%) at 20°C	32.0 $\pm$ 3.5	28.0 $\pm$ 4.0	37.3 $\pm$ 4.2	34.0 $\pm$ 2.0
Germination (%) at 25°C	38.7 $\pm$ 6.1	40.0 $\pm$ 5.3	44.7 $\pm$ 3.1	44.7 $\pm$ 5.0
Threshold temperature (°C)	5.7	4.4	5.4	9.4
Emergence (%) at 1-cm depth	53.3 $\pm$ 5.8	56.7 $\pm$ 5.8	86.7 $\pm$ 5.8	86.7 $\pm$ 5.8
Emergence (%) at 2-cm depth	6.7 $\pm$ 3.3	3.3 $\pm$ 5.8	nd	nd
Emergence (%) at 3-cm depth	nd	nd	50.0 $\pm$ 10.0	53.3 $\pm$ 5.8
Emergence (%) at 4-cm depth	0.0	0.0	nd	nd
Emergence (%) at 6-cm depth	0.0	0.0	16.7 $\pm$ 5.8	6.7 $\pm$ 5.8
Emergence (%) at 9-cm depth	nd	nd	0.0	0.0

nd= not detected

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# Influence of Fodder Crop Rotations on the Potential Weed Flora in Maize Crops in Irrigated Lowlands of Lombardy, Italy

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The adoption of rational fodder crop rotations, with the rediscovery of the beneficial effect of the meadow, is viewed as a possible means to reduce the impact of farming systems characterised by highly intensive cropping and animal husbandry. A diversified crop management, as it is the case with composite crop rotations, is expected to exert a positive effect in controlling the weed flora, thereby increasing the sustainability of farming. The current study examined by the 'seedling emergence' technique the potential weed flora in maize crops cultivated in different rotations, at the end of 12 years since establishment of a long-term crop rotation trial in Northern Italy.

## Methodology

The experiment was carried out in Lodi, in the alluvial Po valley of Northern Italy (45°19' N, 9°30' E, 81 m asl), on a sandy-loam soil of the mollic Hapludalf family, with sub acid pH (6.2), low in nitrogen, organic matter, and exchangeable potassium, and with good provision of assimilable phosphorus. The climate is typical of the lowlands of North-western Italy, with average annual rainfall of about 800 mm and average annual daily temperature of 12.5 °C. The trial compared two fodder crops, namely grain maize (*Zea mays* L.) (Gr) and double crop of autumn-sown Italian ryegrass (*Lolium multiflorum* Lam.) + spring sown silage maize (IsM), cultivated in four rotations at different degree of intensification: i) an annually-repeated double crop (R1); ii) a three-year rotation (R3): autumn-sown silage barley (*Hordeum vulgare* L.), Italian ryegrass + silage maize, and grain maize; iii) a six-year rotation (R6): 3 years of Italian ryegrass + silage maize and 3 years of meadow (Ladino white clover, *Trifolium repens* L., + tall fescue, *Festuca arundinacea* Schreb.; iv) a continuous grain maize cropping (CM); a permanent meadow (PM) was used as a control treatment (Tomasoni et al. 2003).

The management of each rotation was mostly used in lowlands of Northern Italy, in terms of nutrient levels, herbicide doses and soil tillage methods. Each year four border irrigations, of ca. 1,000 m<sup>3</sup> ha<sup>-1</sup> volume each, were provided. The experimental design was a strip-plot with three replications in as many blocks, the main plots being represented by the rotation and the sub-plots by the compared crops. The size of an elementary plot was 10 x 6 = 60 m<sup>2</sup>. At the end of the second cycle of rotations, after 12 years, three soil cores were randomly collected from each plot at 0-20 cm depth, placed into round plastic trays where they remained 15 months to assess the magnitude of the seed bank by the 'seedling emergence' technique (Roberts, 1981). The number of emerged seedlings (expressed per square meter) was taken as an estimate of viable seed content in the soil (Cardina and Sparrow, 1996). Prior to the analysis of variance (ANOVA), the seedling counts were submitted to square-root transformation [ $\sqrt{(n+1)}$ ] of the values; all the statistical analysis were performed by the SAS software.

## Results

The results of the number of emerged weed seedling (Table 1) showed that the largest occurrence of the potential weed flora

Table 1. number of emerged weed seedlings m<sup>-2</sup> in different crop systems

Crop-rotations	Weed seedlings
Gr - CM	36040
Gr - R3	22440
IsM - R1	11220
IsM - R3	8840
IsM - R6	7650
PM (control)	4703
LSD <sub>(0.05)</sub>	5516

was in grain maize, particularly when it was cultivated in continuous cropping (Gr-CM) with 36,040 weed seedling  $m^{-2}$ . The number of weed seedling decreased to 22,440 when grain maize was cultivated in the three-year rotation (Gr-R3). The Italian ryegrass + silage maize showed the highest values of weed seedling in the annually repeated double crop (IsM-R1) with 11,220 plants  $m^{-2}$ . When in longer crop rotations (R3 and R6), its potential weed flora was not different from that of the permanent meadow. The autumn-sown Italian ryegrass in the double crop seemed able to reduce weed stock. The PM displayed the lowest value of weeds with 4,703 plants  $m^{-2}$ .

Figure 1 shows the trend of the weeds in the R6 rotation, particularly the outstanding ‘cleansing’ effect of the rotated meadow ( $R^2=0.97$ ) and the opposite effect of increase of the weeds caused by the Italian ryegrass + silage maize double crop in the subsequent three years ( $R^2=0.99$ ).

Identification of the weed species, according to the descriptions in Viggiani (1990) and Viggiani and Angelini (1993), yielded 28 species in total, but the number of abundant species was limited to six species (*Digitaria sanguinalis* Scop., *Poa annua* L., *Arabidopsis thaliana* Heynh., *Panicum* spp., *Portulaca oleracea* L. and *Stellaria media* Vill.), accounting for almost 90% of the emerged seedlings. Table 2 shows that the remarkable weed emergence in grain maize was largely determined by the presence of *D. sanguinalis*, *Panicum* spp. and *P. annua*, whereas in the Italian ryegrass + silage maize double crop, mostly in R1, the weed emergence was characterized by the presence of *A. thaliana*, *P. annua*, *Panicum* spp and *P. oleracea* (Table 2).

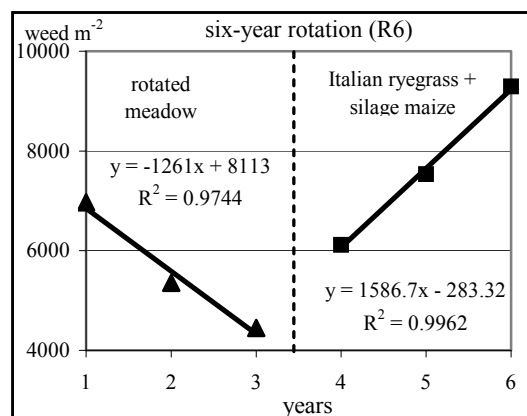


Fig. 1 Number of emerged weed seedlings  $m^{-2}$  in the rotated meadow and Italian ryegrass + silage maize components in the R6 rotation across years

## Conclusion

The crop rotations tested in this study exerted a marked influence on the potential weed flora, both quantitatively and qualitatively.

Long and short rotations had opposite trends with respect to the weed presence and the meadow showed an outstanding ‘cleansing’ effect, opposite to the Italian ryegrass + silage maize annual double cropping, that produced a very fast increment of the weed seed bank.

Table 2. Weed species emergence (% of presence)

Weed species	Italian ryegrass + silage maize			Grain Maize		
	R1	R3	R6	CM	R3	PM
<i>D. sanguinalis</i>	3	14	11	51	66	30
<i>A. thaliana</i>	35	25	23	8	6	5
<i>P. annua</i>	22	19	30	17	8	7
<i>Panicum</i> spp	1	19	2	6	14	11
<i>S. media</i>	6	6	6	4	1	13
<i>P. oleracea</i>	14	6	8	6	1	11

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# The Changes in Weed Infestation of Spring Barley Caused by Soil Tillage

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Change of soil tillage influences agrophytocoenosis. According to SKUTERUD ET AL. (1996) weeds react on reduced soil tillage technologies by increasing of number of winter weeds and perennial weeds. Increasing of late spring weeds is not so significant. Number of monocotyledonous weeds was in case of reduced soil tillage increasing more than in case of dicotyledonous weeds.

## Methodology

The actual weed infestation was assessed in two field trials where three kind of soil tillage were used (conventional tillage – CT, minimum tillage – MT and no tillage – NT). Conventional tillage was used in both cases before establishment of field trials. First trial was established in 2001 and the observation was done in 2002 – 2004. The 5-year crop rotation was applied in the first trial (winter wheat, winter oilseed rape, winter wheat, grain maize, spring barley). The second field trial was established in different location in 2003 with 7-year crop rotation (lucerne – first year, lucerne – second year, winter wheat, silage maize, winter wheat, sugar beet, spring barley). The weeds were counted on the area of 1 m<sup>2</sup> in spring barley stands before herbicide application during the three year period.

## Results

The number of weeds was decreased on NT (from 7.9 pc m<sup>-2</sup> to 5.3 pc m<sup>-2</sup> in second year and to 4.2 pc m<sup>-2</sup> in third year. The changes in weed infestation on MT and CT were similar (MT: 7.7 pc m<sup>-2</sup> in first year, 9.1 pc m<sup>-2</sup> in second year and 6.4 pc m<sup>-2</sup> in third year; CT: 7.8 pc m<sup>-2</sup> in first year, 9.6 pc m<sup>-2</sup> in second year and 4.0 pc m<sup>-2</sup> in third year). The observed differences were not statistically significant. Different soil tillage influenced also the occurrence of weed species in spring barley stands. The higher occurrence on NT was connected with *Echinochloa crus-galli*, *Conyza canadensis*, *Lamium amplexicaule*, *Cirsium arvense*, *Viola arvensis*, *Tripleurospermum inodorum*, *Veronica polita*, *Stellaria media*. Minimum tillage was more suitable technology for *Descurainia sophia*, *Amaranthus sp. a* *Veronica hederifolia*. The weed species as *Chenopodium album*, *Ch. hybridum*, *Consolida regalis* a *Fallopia convolvulus* were the most common on conventional tillage.

The changes in weed infestation were different in second trial. The variant with NT had 2.9 pc m<sup>-2</sup> in first year, 1.8 pc m<sup>-2</sup> in second year and 19.9 pc m<sup>-2</sup> in third year. Similar results were found on MT (2.5 pc m<sup>-2</sup> in first year, 4.0 pc m<sup>-2</sup> in second year and 19.2 pc m<sup>-2</sup> in third year) and CT (5.0 pc m<sup>-2</sup> in first year, 3.4 pc m<sup>-2</sup> in second year and 15.9 pc m<sup>-2</sup> in third year). Statistically significant differences were not found. The differences were observed in weed specimens: the most often weeds on NT were: *Convolvulus arvensis*, *Lactuca serriola* *Polygonum aviculare*, *Tripleurospermum inodorum*; on MT *Capsella bursa-pastoris*, *Datura stramonium*, *Galium aparine*, *Malva neglecta*, *Plantago major* and on CT *Echinochloa crus-galli*, *Fallopia convolvulus*, *Chenopodium album*, *Chenopodium hybridum*, *Lamium amplexicaule*, *Persicaria lapathifolia*, *Silene noctiflora*, *Stellaria media*, *Thlaspi arvense*, *Veronica polita*.

Tab. 1 Average number of weed species from the first field experiment

Weed species	Variants of tillage and year								
	NT			MT			CT		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
<i>Amaranthus sp.</i>		0.04		1.44	0.80	0.44	0.68	0.20	
<i>Capsella bursa-pastoris</i>	2.24	0.04	0.92	0.76	0.12	2.52	0.48	0.08	1.44
<i>Chenopodium album</i>	0.04	0.60	0.24	0.48	4.68	0.40	3.96	4.44	0.28
<i>Chenopodium hybridum</i>					0.40			0.64	
<i>Cirsium arvense</i>	0.80	0.12	0.04	0.36			0.12		0.12
<i>Conyza canadensis</i>	0.64	0.16			0.36				
<i>Echinochloa crus-galli</i>		0.40	1.60		0.32	1.28	0.08		
<i>Fallopia convolvulus</i>			0.04	0.12	0.44	0.16	1.36	3.48	2.12
<i>Lamium amplexicaule</i>	0.04	0.36	0.20		0.24	0.08		0.04	
<i>Tripleurospermum inodorum</i>	1.40	0.04	0.28					0.08	
<i>Veronica hederifolia</i>	1.96	1.88	0.48	4.24	1.08	0.60	0.32	0.32	
Other species	0.76	1.68	0.40	0.36	0.68	0.92	0.76	0.32	0.04

Tab. 2 Average number of weed species from the first field experiment

Weed species	Variants of tillage and year								
	NT			MT			CT		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
<i>Amaranthus sp.</i>	1,92		11,46	0,33		9,13	1,08		3,58
<i>Capsella bursa-pastoris</i>						0,21			
<i>Cirsium arvense</i>	0,50	0,75	0,75	0,75	0,71	1,50	0,67	0,04	1,04
<i>Convolvulus arvensis</i>			0,67	0,04		0,33	0,04		0,04
<i>Echinochloa crus-galli</i>				0,83			1,71		0,54
<i>Fallopia convolvulus</i>	0,04		0,17		0,25	0,13	0,04	0,38	0,29
<i>Galinsoga parviflora</i>			3,58			4,83			5,08
<i>Galium aparine</i>		0,08	1,13	0,04	1,21	1,08	0,21	0,13	0,08
<i>Chenopodium album</i>	0,17			0,08		0,04			0,42
<i>Chenopodium hybridum</i>						0,08			1,13
<i>Lactuca serriola</i>	0,13		0,21						
<i>Persicaria lapathifolia</i>					0,04	0,54		0,04	1,08
<i>Silene noctiflora</i>		0,29	0,04	0,21	1,13		0,96	0,50	0,08
<i>Sinapis arvensis</i>	0,04	0,42	0,42	0,00	0,38	0,71	0,04	0,83	1,00
<i>Sonchus oleraceus</i>			0,79	0,17					
<i>Thlaspi arvense</i>					0,04				0,13
<i>Tripleurospermum inodorum</i>	0,13	0,21							
<i>Veronica polita</i>		0,08	0,71		0,25	0,54	0,13	1,54	1,42
Other species			0,04			0,13	0,13		

## Conclusions

The obtained results showed that the change of soil tillage influence more weed specimens than the number of weeds.

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# Changes of Seed Germination During the Year

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Each plant species (cultivars) has typical germination season to optimise successful reproduction and survival. The reasons of this biological phenomenon are known, but during the year there is something like biological clock, because of the existence of the influence on the level of seed germination exists. This physiological phenomenon has large influence on rapidity of germination and on the germinability.

## Methodology

The eight species of crops were tested in laboratory experiments in order to analysis of changes rapidity of germination and germinability during the year. The four repetitions in every type of test were provided. Sowing of one hundred of seeds of every species in every repetition was provided. Germinability was analysed in Petri dishes with filter paper. Petri dishes were located in climatic chamber with the standard temperature 21°C. Regularly humidification of filter paper with spirituous water was provided.

Laboratory experiences were realized since October 2006 to September 2007. For experiments were used 8 selected crops: spring Wheat (*Triticum aestivum* L.), winter Wheat (*Triticum aestivum* L.), Fodder mallow (*Malva verticillata* L.), White sweet clover (*Melilotus albus* Medic.), Canary grass (*Phalaris canariensis* L.), Rye (*Secale cereale* L. var. *multicaule*) and Purple tansy (*Phacelia tanacetifolia* Benth.) The laboratory experiences with selected crops were provided simultaneously in research institutes in Praha-Ruzyně and in Research Institute for Fodder Crops Troubsko. The experiments were provided every three weeks (from 16. 10. 2006 to 17. 9. 2007).

## Results

It follows from the obtained results that in both research institutes the very similar results were obtained. It is possible to conclude on the basis of one week germinability for average values for all measured crops, ( fig 1) that minimum rapidity of germination in autumn exists (November) and maximum is in before spring (January to February).

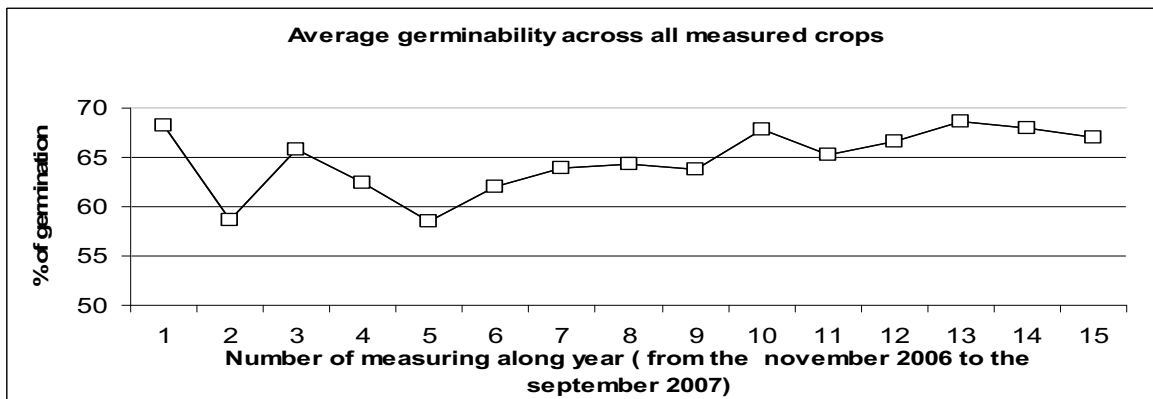
Basic information about the total germinability of all analysed crops during the year is in fig.2. The germination of winter wheat, spring wheat, Canary grass and Rye oscillated minimally, but the germination of Fodder mallow, White sweet and clover has fluctuating curve during individual measuring. Fig.1 and Fig 2 are average result of two localities.

## Conclusions

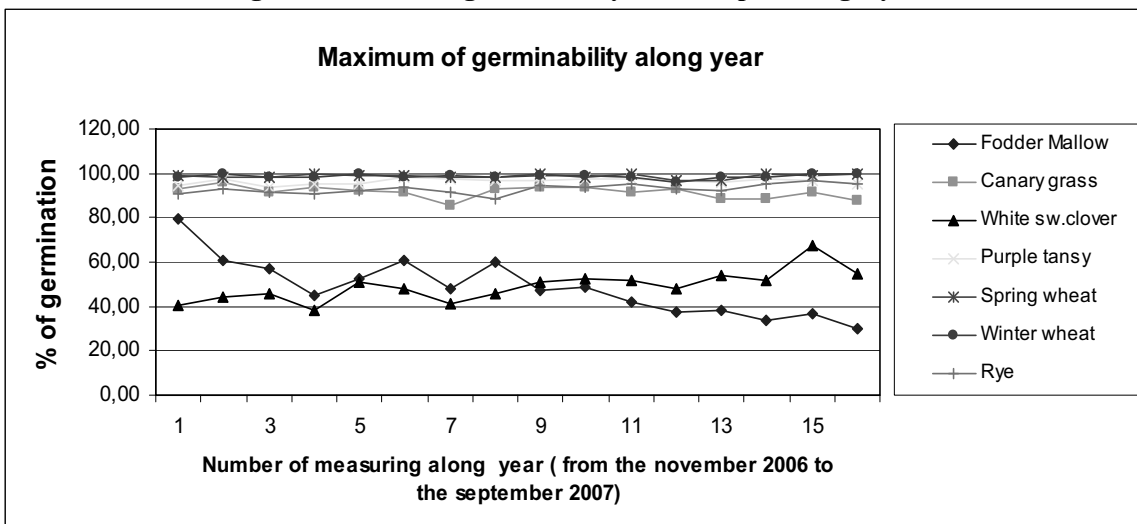
It is possible to conclude that typical common curve of rapidity of seed germination within a year exists. The cultivars of some crops and wild plants have large differences within a year in a level of germination rapidity, i.e. there is problem of determination of acceptable time of seed testing for commercial purposes

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**Fig.1: One week germinability of all species during year(Average across all measured crops)**



**Fig.2: Maximum of germinability of all crops during a year**



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# Influence of Abiotic Stresses on the Winter Wheat Sprouting Plants

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Availability of water and efficiency of water utilisation for germination is one of the basic factors which influence field emergence rate. Water uptake is essential for enzyme activation-for using of reserve seed storage material. The basic aim of the presented work is to present the effect of standard conditions and stress conditions, on the germination and water utilisation at this phase of development. The possibilities of lessening the influence of abiotic stresses on measured traits by application of 24-epibrassinolide were tested as well.

## Methodology

Pot experiments (mixture soil: sand; 1:1). In all experiments the same seed stock was used and the chosen varieties had different pedigrees. Experimental conditions are given in the following survey:

The influence of the spray application of the  $10^{-9}$  M solution of 24-epibrassinolide at the beginning of plant flowering on different traits of plants and especially seeds was also analysed in both conditions (standard conditions: temperature 20°C per day, 15°C per night and soil water content 70%. Stress conditions, temperature 33°C per day, 20°C per night soil water content 10-20%). The next step was an analysis of development of the root system in the filial generation after influence of abiotic stresses. The following stepwise analysis was applied for this purpose. Seed surface was sterilised in 1%  $\text{Ca}(\text{ClO})_2$  for 5 minutes. Grains for experiments were rinsed with deionised water before use. Plants were cultivated under standard conditions in standard growth chamber. After 5 days of germination at low temperature (5°C) with a day/night period 18/6 hrs average standard sprouting plants were selected (light intensity was  $490 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  (400W lamp). Five replications of experiment were performed.

At harvest - two weeks after the beginning of growth in growth chamber- plant shoots were separated from the roots and various parameters of shoots and roots were measured by standard methods. The images of the root system were analysed by image analyser. Chemical composition - total starch and damaged starch granules - were analysed by enzyme methods by Megazyme Kits (Megazyme 2004a, 2004b), protein content by Kjeldahl method and content of lipids by Soxhlet method. Nutrient content was analysed by AAS-4 flow analyser. The content of net energy was measured with automatic dry combustion calorimeter MS 10 A of Laget - ( $\text{kJ.g}^{-1}$  DM). Analysis of water utilisation by seeds of different provenance was provided by the rapidity of water uptake into the immersed seeds to the first third of volume of seeds to the deionized water of every cultivar and of every provenance. At the end of every hour of “watering”, the weight of the seeds was measured step by step to the maximum of possible acceptable amount of water, and percentage of germination of the seeds and maximum of possible growth of juvenile plants from water contained in seeds was analysed. After the maximum possible absorption of water, the seeds were located to the climatic chamber with temperature of 30°C and with drought conditions, and analysis of water loss was performed every hour.

Phytohormones were extracted and purified according to Dobrev and Kamínek (2002). Indolyl acetic acid and ABA were determined using two-dimensional HPLC according to Dobrev *et al.* (2005).

## Results

As follows from the obtained results, large differences among cultivars in water uptake in the stress environment to the seeds in wet conditions and after this procedure water loss from the seeds in drought conditions with high temperature exist (i.e. simulation of soil environmental conditions). Application of 24-epibrassinolide counteract cultivar differences at the effects of water uptake and decreases rapidity of loss of water at drought conditions. It is also possible to conclude, that in the seeds from standard conditions differences among cultivars in water uptake and water loss in drought conditions exist. Every cultivar has different necessary level of water content for sprouting and growth of sprouting plants-i.e large differences among cultivars at water utilisation exists.

It is very important for velocity of field emergency and decrease of difference between seed laboratory sprouting and field emergency. Rapidity of growth of sprouting plants decreases the level of influence of stressful environmental conditions.

The application of 24-epibrassinolide counteracted cultivar differences in the water uptake, but did not affect the velocity of loss of water without substantial effect in drought conditons. Application of 24-epibrassinolide during seed growth or during flowering has substantial positive effect on the percentage of germinability in standard and similarly in stress environment.

Net energy content decreases in all experiments with stress. 24-epibrassinolide regenerate the net energy in all cases. It is not possible on the basis of the first preliminary data of hormone contents in seeds to draw any conclusion on the correlation between the hormone content and capacity to germinate and take up water, but in case of analysis of necessary seed water content for germinaton large cultivars differences were obtained.

Chemical analysis of total and damaged starches show the same trend in standard conditios:- decrease after treatment with 24-epibrassinolide. Opposite situation was obtained at stress conditions in changes of proteins content - decrease to 95.3 % in standard conditions and increase up to 113.7 % in stress conditions after treatment with 24-epibrassinolide

## Conclusions

The availability of water and efficiency of water utilisation for germination is one of the basic factors that influence field emergence rate and following plant growth. The large variability in water use efficiency of seeds of different species and cultivars exists. Similar results between spring and winter wheat were also obtained ( $r = 0.78^*$ ). Provenance and the application of 24-epiBR have a substantial effect on contamination of the seeds by spores of fungi. In the case of phytohormone content it is not possible, to draw any conclusion on the correlation between the hormone content and the capacity to germinate and take up water.

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# The Influence of Morphological Features of Different Winter Wheat Varieties on Competitiveness Due to Weeds

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The strategy of weed regulation in organic system is based on using non-direct methods such as crop rotation and increasing the competitiveness of crops due to weeds (Eisele and Köpke 1997). The results of other authors show that the competitive ability of cereals depends on morphological features, such as: leaf area, leaf angle, tillering, early growth rate and height which influence the light penetration through the canopy and the emergence of weed seedlings (Christensen 1995, Seavers and Wright 1999). There is an opinion that modern wheat varieties are less competitive than older types (Lemerle et al. 1996). The use of herbicides to control weeds has allowed breeders to select high yielding, short varieties with high harvest index, but which in the presence of weeds are lower yielding (Didon 2002). The aim of the research was the evaluation of the influence of morphological features of selected winter wheat varieties on the competitiveness due to weeds.

## Methodology

The study was conducted in the years 2005-2007 at the Experimental Station of Institute of Soil Science and Plant Cultivation - State Research Institute in Osiny (Lublin province, Poland). On the fields used in organic way for 11 years some winter wheat varieties have grown in pure sowing (actually cultivated: Kobra, Mewa, left out of variety register: spelt wheat - variety Schwabenkorn and R-12) and the mixture (Kobra+Kris+Tonacja and Kobra+Roma+Mewa). Weeds were controlled in mechanical way using a weeder. The analysis included plant height, tillering, total leaf area, leaf area index (LAI), mean foliage tip angle (MTA), number of plants and ears and wheat dry matter. Moreover the assessment of weed infestation was carried out in dough stage.

## Results

The most favourable parameters increasing the competitiveness of wheat canopy due to weeds, such as the biggest leaf area, tillering, LAI index, height and dry matter of wheat characterised spelt Schwabenkorn (tab. 1). From the modern varieties Mewa was the tallest variety with large tillering. The mixture of Mewa with Kobra and Roma had the bigger number of plants and ears on unit area. Mewa and spelt Schwabenkorn had horizontally disposed leaves which shade the soil and results in decreasing of weed seedlings emergence. The number of weeds and their dry matter were the lowest in these two varieties and the mixture of Mewa with Roma and Kobra (fig.1). Kobra, mixture of varieties Kobra + Kris + Tonacja and spelt R-12 showed less favourable parameters of growth and development and the highest level of weed infestation (tab.1, fig.1).

## Conclusions

The results showed different competitiveness of winter wheat varieties due to weeds as a result of differences in morphological features and architecture of canopy. Mewa and spelt Schwabenkorn characterised by the most favourable morphological parameters influenced the competitiveness due to weeds and the lowest level of weed infestation which suggests that these varieties can be the most useful for organic farming. Kobra, mixture of varieties Kobra + Kris + Tonacja and spelt R-12 showed less favourable parameters of growth and development and the highest level of weed infestation. Bigger competitive ability due to weeds had mixture of higher varieties Kobra, Roma, Mewa than Kobra, Kris, Tonacja.

Tab. 1. Growth and development parameters of some winter wheat varieties cultivated in organic system (mean from 2005-2007)

Parameters	Growth stage of wheat	Varieties					
		Kobra	Mewa	Mixture (Kobra, Kris, Tonacja)	Mixture (Kobra, Roma, Mewa)	Spelt Schwaben-korn	Spelt R-12
Total leaf area (cm <sup>2</sup> )	tillering	51.9	50.8	33.8	58.1	71.2	35.6
Total tillering	tillering	4.1	4.8	3.6	4.8	6.7	4.3
	shouting	2.9	3.1	3.0	2.8	4.8	2.8
Height (cm)	shouting	56.2	61.0	53.4	56.1	71.8	51.6
	dough	78.1	85.3	79.1	87.3	129.2	75.0
Leaf area index (LAI)	shouting	2.5	3.7	2.7	3.9	4.1	1.9
	earring	3.1	4.2	2.9	4.1	4.1	2.5
Mean foliage tip angle (MTA)	shouting	62	57	63	60	62	61
	earring	54	52	60	57	52	58
Number of plants per 1 m <sup>2</sup>	tillering	388	418	327	467	343	329
	shouting	218	243	149	265	172	255
Number of ears per 1 m <sup>2</sup>	dough	446	482	298	538	517	428
	shouting	468	485	383	463	570	387
Wheat dry matter (g · m <sup>-2</sup> )	shouting	468	485	383	463	570	387
	dough	1250	1272	999	1214	1393	1152

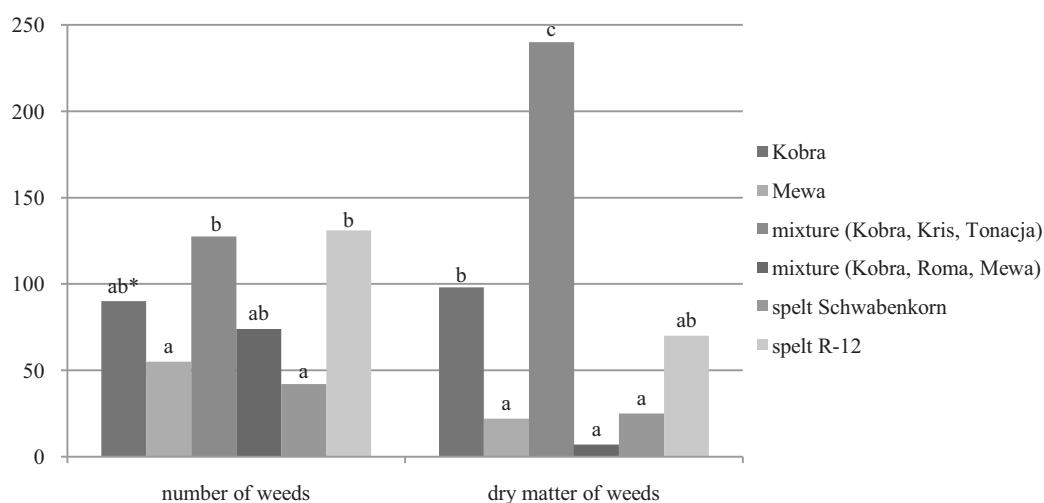


Fig. 1. Number of weeds (plants · m<sup>-2</sup>) and dry matter (g · m<sup>-2</sup>) in different varieties of winter wheat cultivated in organic system (mean from 2005-2007)

\* values marked by the same letter are not significantly different at  $\alpha=0,05$  (LSD Tukey)

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# Ground Beetle Predation Selectively Decreases Numbers of Weed Seeds Before Entering Soil Bank

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## Introduction

In central Europe, ground beetles (Carabidae) are important component of arthropod communities of many habitats. In the Czech Republic there exist c. 550 species of which only few live on arable land, those capable of withstanding agricultural practices. The field species thus share many common features including food preferences. Most species accept different animal prey but a large proportion of species is also phytophagous, both in adult and larval stage, and accept fragments of green plant organs, fruits, pollen, fungi. Feeding on seeds (granivory) was discovered more than 100 years ago and confirmed by many authors, however, the preferences for particular species of seed were studied only accidentally. We addressed seed consumption and preferences of carabid species eating seed from the ground of arable fields because predation is important, may remove 1,000–4,000 seeds m<sup>-2</sup> d<sup>-1</sup> and selectively influence the quantity of seeds of particular species entering the soil seed banks (Honek et al. 2003).

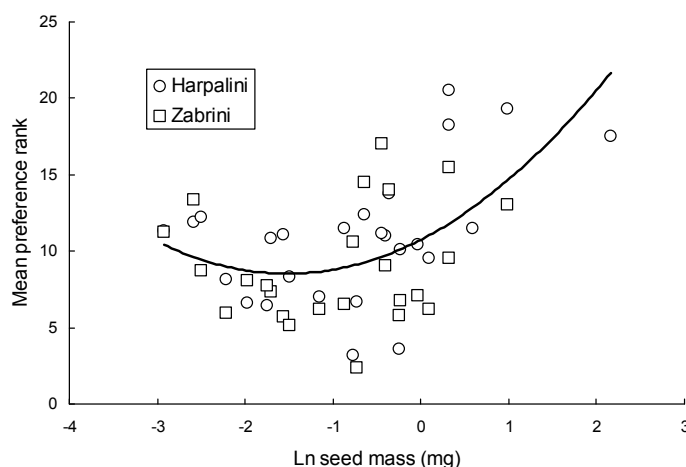
## Methodology

Seed preferences were investigated in adults of granivorous carabid species of the tribus Zabritini (17 species) and Harpalini (10 spp.). All species were locally and temporarily abundant on agricultural land and their adult body size ranged between 4 mm (dry mass 1 mg) and 15 mm (36 mg). The selection of species of seed was made deliberately to cover evenly the range of mass between 0.01 to 10.0 mg. The seed was exposed to carabid predation in small trays (surface area 6.2 cm<sup>2</sup>) filled with white plasticine where seeds were pressed into the plasticine half of their transversal diameter deep. Preference experiments were made in Petri dishes (250 mm in diameter), each containing a 2 cm layer of sieved soil and trays with 28 species of seed. Seed consumption was recorded for 5 days. Standardized data on consumption of seed species by particular carabid species were subjected to Detrended Correspondence Analysis (DCA) using CANOCO.

## Results

Ordination of carabid species using DCA revealed that preferences of both tribus were well separated. The Zabritini preferred seed of *Taraxacum officinale*, *Tripleurospermum inodorum*, *Crepis biennis*, *Capsella bursa-pastoris*, *Descurainia sophia* and *Lepidium ruderae*, the Harpalini preferred *Cirsium arvense*, *Viola arvensis* and *Cichorium intybus*. There was a strong positive relationship between the average mass of the preferred seed species and the body mass of carabids, however, there was no difference between Harpalini and Zabritini. The average preferences of Harpalini and Zabritini for particular species of seed varied with the size of the seed (Fig. 1). More data in Honek et al. (2007)

Fig. 1 Preference (mean rank of consumption of a particular species of seed, the most consumed species of seed =1) for 28 species of seed and two tribes of carabids



## Discussion

Although size was important factor of preference, there were consistent differences between Harpalini and Zabrinini species. Harpalini species accepted a wider range of seed species than Zabrinini species of similar size because Harpalini are generalist species, ubiquitous and tolerant to a range of environment conditions while Zabrinini species are specialists. Each tribe was also specific by preference for particular seed species. The preferred seed of most Harpalini species were *Cirsium arvense* and *Viola arvensis*, which both were rejected by Zabrinini, while in Zabrinini the universally preferred species was *T. officinale* (Honek et al. 2005). The importance of body size of carabids as well as size of seed for determining preference follows from the way how seed is consumed. Adult carabids grasped the seed into mandibles, crushed and discarded the testa, and ate edible germ and cotyledons. The range of the grasp is determined by the span of the mandibles which is correlated with body size. The beetles probably eat as large seed as they can grasp and ignore the small one. The size of the carabid thus limit both the upper and lower sizes of accepted seed.

## Conclusions

Most of the weed seeds scattered on the ground or buried in the soil die before germination. Mortality has several causes among which a very important one is predation by invertebrates, particularly polyphagous ground beetle species (Coleoptera, Carabidae). The preferences were determined in a cafeteria experiment using 28 species of weed seeds and adults of 27 species of central European field carabids mainly belonging to the tribes Zabrinini and Harpalini. Mass of the preferred species of seed eaten was positively related to carabid body mass. In the average the most preferred were species of seed weighing 0.1 mg and the preferences also differed between both carabid tribes.

This work was supported by project MZe CR QG 50081.

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# Alternative Materials to Black Polyethylene as Mulching in Processing Tomato: Behaviour and Weed Control

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Plastic mulches have been used commercially on vegetables since the early 1960s. Black polyethylene is the most widely used for controlling weeds due to its excellent properties and low cost. However, an important problem associated with the use of this material is removal from the field after crop. Plastic mulches do not break down and should never be disked or incorporated into the soil for implying a serious risk for the environment. However, the process of recovering and recycling them later is difficult due to the fact that approximately 80% of the weight is non-plastic material (González et al., 2003). For this reason, the use of different degradable and non-contaminating materials as alternative to black polyethylene is increasing in the last few years. These materials are basically composed of polysaccharides such as cellulose and starch, paper or straw (Chandra and Rustgi, 1998). The aim of this work was to analyze the behaviour of seven mulches of different composition (biodegradable and polyethylene) and the effect on weed control in an open-air tomato crop.

## Methodology

A field experiment was conducted in a processing tomato crop (*Solanum lycopersicum* L. cv. Perfectpeel) in Ciudad Real, Central Spain (4°2' W, 38°59' N, altitude 640 m). A randomised complete block design was adopted with four replications and nine mulch treatments: black polyethylene mulch (PE, 15µm), two starch-based biodegradable mulches (Mater-Bi®, Novamont, 15µ; Biofilm®, Barbier, 17µm), one oxo-biodegradable mulch (Enviroplast®, Genplast, 15µm), two paper mulches (Mimcord®, Mimgreen, 85 g/m<sup>2</sup>; Saikraft®, Saica, 125 g/m<sup>2</sup>), one barley straw mulch and two different check treatments, consisting of manual and no weed control, respectively. Each basic plot consisted in a single 15 m long row spaced 1.5 m apart, with beds 0.80 m wide. Plants were separated by 0.20 m within a row, which supposes a total population of 75 plants per basic plot. Number of weeds was controlled in each treatment at 21, 42 and 63 days after transplanting (DAT). At the last sample date, controls of weed cover, expressed as percentage of soil, and dry weight of aboveground parts of weeds were measured too. Efficacy in weed control was calculated following Abbott's formula: Efficacy (%) =  $(1 - Ta/Ca) \times 100$ , where *Ta* is the infestation in each treatment and *Ca* is the infestation in the check treatment (no weed control). The deterioration of the exposed and buried mulching films was evaluated during the crop cycle by means of a visual scale, ranging from 1 to 9: 1 corresponded to the mulch material completely deteriorated, and 9 to the film practically intact. The results of weed control efficacy presented as percentage were transformed according to Bliss formula. Data were subjected to analysis of variance and a Duncan's multiple range test ( $P < 0.05$ ) was applied to the significant results.

## Results

Weed infestation, basically caused by *Matricaria camomilla*, *Convolvulus arvensis*, *Sorghum halepensis* and *Amaranthus albus*, was low and very homogeneous in all the plots with the exception of the no weed control treatment (Table 1, Fig. 1). Although straw counted the highest number of weeds among the weed control treatments, the weed cover was low because the weeds which reached the

surface were weakened. Efficacies in relation to weed cover and dry biomass at 63 DAT were similar in all the treatments except on straw and significantly different with respect to no weed control. Biodegradable materials deteriorated gradually throughout the growing season (Table 2), although performed successfully their functions. In relation to the buried portions, PE remained practically intact, followed by Enviroplast®. Biodegradable materials suffered a strong deterioration at the beginning of the crop cycle, being practically disappeared at 69 DAT. Mater-Bi® presented an intermediate behaviour.

Table 1. Effects of mulch type on weed number, cover and dry biomass in a processing tomato crop at 63 days after transplanting (DAT).

Treatment	Weeds/m <sup>2</sup>	Cover (%)	Dry biomass (g/m <sup>2</sup> )	Efficacy		
				Weeds	Cover	Dry biomass
Polyethylene	0.0 a	0.0 a	0.0 a	100 a	100 a	100 a
Enviroplast®	0.0 a	0.0 a	0.0 a	100 a	100 a	100 a
Biofilm®	2.2 a	0.6 ab	1.1 ab	95 a	99 a	100 a
Mater-Bi®	0.6 a	0.1 a	0.2 a	98 a	100 a	100 a
Saikraft®	1.6 a	0.9 ab	0.5 a	96 a	99 a	100 a
Mimcord®	1.3 a	0.4 a	0.3 a	96 a	100 a	100 a
Straw	10.0 b	2.1 b	2.5 b	78 b	98 b	88 b
Manual weed control	0.7 a	0.2 ab	0.3 a	98 a	100 a	100 a
No weed control	43.8 c	73.3 c	451.0 b	0 c	0 c	0 c

For each parameter, treatments followed by different letters differ at  $P < 0.05$ .

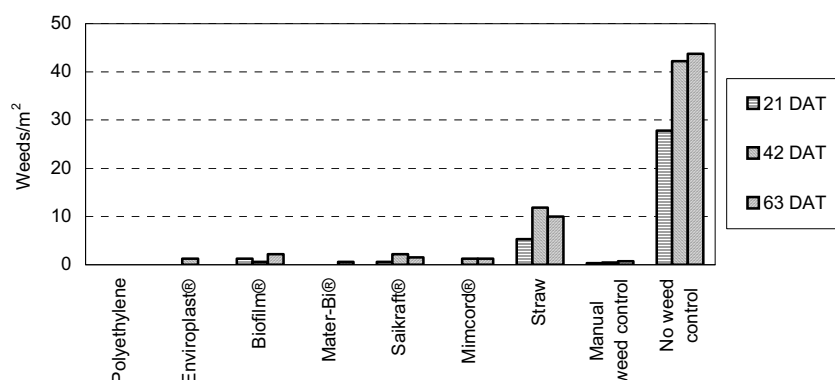


Figure 1. Evolution of weed population in the mulch treatments in a processing tomato crop.

Table 2. Deterioration evolution of the exposed and buried mulches throughout the crop cycle.

Treatment	Exposed mulch			Buried mulch		
	34 DAT	48 DAT	80 DAT	12 DAT	41 DAT	69 DAT
Polyethylene	8.0 a	7.3 ab	6.5 a	9.0 a	8.9 a	8.8 a
Enviroplast®	8.0 a	7.8 a	5.8 a	8.9 a	8.1 a	7.6 a
Biofilm®	7.8 a	3.5 c	1.0 b	8.3 b	2.5 c	1.6 c
Mater-Bi®	8.0 a	5.0 c	1.5 b	8.3 b	6.6 b	4.0 b
Saikraft®	7.8 a	5.3 bc	1.3 b	6.6 c	1.3 d	1.0 c
Mimcord®	7.8 a	5.3 c	1.0 b	6.4 d	1.6 d	1.0 c

Visual scale: 1 (film material completely degraded) to 9 (film practically intact).

For each parameter, treatments followed by different letters differ at  $P < 0.05$ .

## Conclusions

Biodegradable films control weeds similarly to polyethylene and deteriorate in a short time, specially when they are buried, instead of being left on soils or burnt without any control, with the risk of the environment it supposes. For this reason, the use of these materials for mulching is recommended in vegetable crops.

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# Effect of nitrogen fertilization, cultivar and species on attractiveness and nuisibility of two major pests of winter oilseed rape (*Brassica napus* L.): pollen beetle (*Meligethes aeneus* F.) and stem weevil (*Ceutorhynchus napi* Gyl.).

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The pollen beetle (*Meligethes aeneus* Fabricus) (Coleoptera: Nitidulidae) and the stem weevil (*Ceutorhynchus napi* Gyl.) are two of the major pests of winter oilseed rape (WOSR) (*Brassica napus* L.) (Brassicaceae) in Europe (Alford et al., 2003). The pollen beetle adults feed on pollen from flowers and buds, and females lay their eggs on buds suitable for oviposition (2-3 mm long) (Nilsson, 1994). Rape stem weevil females lay their eggs in the stem, deforming the stem considerably, and thereby damaging the crop. Serious yield losses, over 70 %, can result from pollen beetle attacks due to bud abortion (Nilsson, 1987; Ekbom & Borg, 1996). The main actual way to control these pests is the intensive use of pesticides treatments, but the emergence of metabolic resistance to pyrethroids have made more severe. New strategies for insect pest management appear to be a key issue for WOSR crop. The objectives of this study is to understand the effects of crop management (i.e. nitrogen fertilization, cultivar and species) on crop attractiveness and pest nuisibility in order to develop new control strategies.

## Methodology

In literature host quality is considered as an important factor of host's selection mechanism. So N fertilization and different cultivar and/or species may play a significant role in attractiveness of the crop through "push-pull" actions. Furthermore nitrogen application and cultivar and/or species may have an important effect on compensation mechanisms and on pest's nuisibility. In 2007, a split plot testing three fertilizations levels (differing by time and rates of application; N0: 80 kg/ha in one early application, N1: 160 kg/ha in two early applications and N2: 160 kg/ha in two late applications), two cultivars (*cv.* Campala and grizzly) and two pesticides treatments (total protection and no protection) as main factors, was conducted at Grignon, France. In 2006, a experimental trial with a 6 m *Brassica rapa* border was conducted in Versailles.

Crop status was described by different relevant variables such as nitrogen accumulation, phenological stage, height, weight, stem diameter and isothiocyanates (ITC) concentrations. Glucosinolates analyses were only realised at one stage, and were done using HPLC methods. Pollen beetle populations were counted weekly on 30 plants for each modality. Stem weevil populations were estimated through the number of oviposition stings on the main raceme. The nuisibility of pollen beetle attacks was evaluated through yield components and differences with the control (total pollen beetle protection).

## Results

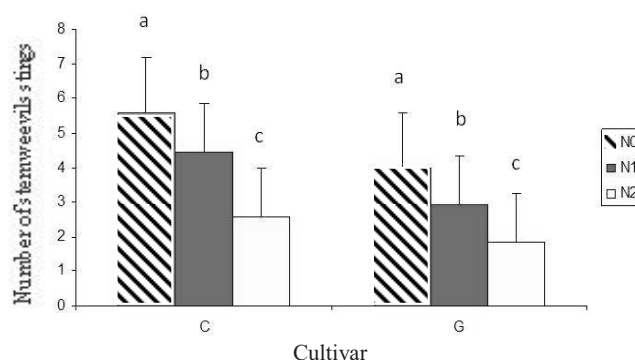
First of all, no significant differences on the total number of adults beetle were observed between the two cultivars (Figure 1a). But we observed significant differences between Campala and Grizzly on the number of pollen beetle per plant at each stage. Campala being the more attractive until growth stage

(GS) 3.5, and Grizzly being more attractive to GS 3.5 until GS 4.5 (data not shown). Secondly we found a relationship between nitrogen fertilization and crop attractiveness through the nitrogen status of the plant. In fact pollen beetle were found numerously on plants of the nitrogen modalities N0 and N1 (Figure 1a). An important effect of phenological stage on host plant selection was also recorded. The more advanced stage being the more colonised by adults. Species seems to have a great importance because more pollen beetle was found on turnip rape (*Brassica rapa*) than on WOSR (*Brassica napus*). Phenological stage effect on the number of pollen beetle was found between the two different species and show same tendencies as described previously. We also found a cultivar effect and a nitrogen fertilization effect on stem weevils' attacks (figure 1b). In deed Campala was more attractive, and plants of the modalities N0 and N1 showed more stem weevils' stings. Glucosinolates contents of edible parts at GS 4.5 differed between the two crop cultivars and are related to pollen beetle's infestations levels. Nevertheless glucosinolates contents were not related to nitrogen fertilization level at this stage.

No effect of N fertilization nor cultivar was reported on the number of damaged buds. But nitrogen fertilization interacts with pollen beetle nuisibility through compensation mechanisms principally occurring on seed weight.

	Mean number of pollen beetle
<b>Cultivar</b>	*
(F value)	2.76
<b>Campala</b>	4.48a
<b>Grizzly</b>	5.06a
<b>Nitrogen</b>	***
(F value)	12:69
<b>N0</b>	4.9a
<b>N1</b>	5.7a
<b>N2</b>	3.6b
<b>Blac</b>	NS
<b>N*Cultivar</b>	NS
<b>Dates</b>	***

**Figure 1a:** Effects of the different factors on mean number of pollen beetle for all dates (5 different dates), their F value and their means.\*\*\*: P<0.001; \*\*: P<0.05; \* P<0.1.



**Figure 1b:** Mean number of stem weevil's stings on the main raceme for the two cultivars and the 3 N modalities ( $\pm$  SE).

## Conclusions

The effect of nitrogen fertilization (time and rate) has never been reported neither in the attractiveness nor in the capability of plants to cope with a serious attack of pollen beetle and stem weevils. We demonstrate an important interaction between attack's dynamics of pollen beetle and stem weevils, and nitrogen fertilization through compensation mechanism and host quality. Despite, cultivar has often been investigated, the dynamic effect of this attractiveness of a cultivar has never been reported. Moreover ITC effects have never been reported in field's experiments yet. Those results have to be confirmed but can help to built WOSR cropping systems in order to reduce pesticides treatments.

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## **SESSION 2**

### **NEW FRONTIERS IN CROP PRODUCTION**



## **SUB SESSION 2.1**

### **FUNCTIONAL FOODS AND NUTRACEUTICALS**

Chairman: Giovanni Dinelli





# New Analytical Perspectives for the Agronomic Research in the Field of Functional Food and Nutraceuticals

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At present, one of the principal research lines in Food Science and Technology is the development of new functional foods. A food can be considered “functional” if, besides its nutritious effects, it can improve the state of health or well-being or reduce some disease risk. In our laboratory, we have studied for years different natural sources of functional ingredients including plants, spices, algae, etc. Nowadays, the use of genetically modified organisms (GMOs) has seen a great increase in agriculture and food industry. Thus, genetic engineering is mainly used to improve resistance of crops to plagues or pesticides. However, in the new generation of GMOs significant changes in other constituents will be deliberately introduced to generate new functional foods (e.g., increasing fatty acids, amino acids content, polyphenols, vitamins, or reducing undesirable constituents, etc), requiring the development of more powerful and informative analytical procedures. In this work, an original analytical strategy is proposed able to provide information on the composition of **GMOs** based on **metabolomics studies** carried out by **capillary electrophoresis-mass spectrometry** (CE-MS). The goal of this work is, therefore, to carry out a comparative profiling of metabolites found in transgenic varieties vs. their corresponding isogenic wild lines grown under identical conditions [1,2]. To do this, a complete analytical strategy is developed that combines metabolites extraction from samples, separation by capillary electrophoresis and chemical characterization by on-line electrospray-time of flight-mass spectrometry (CE-TOF-MS).

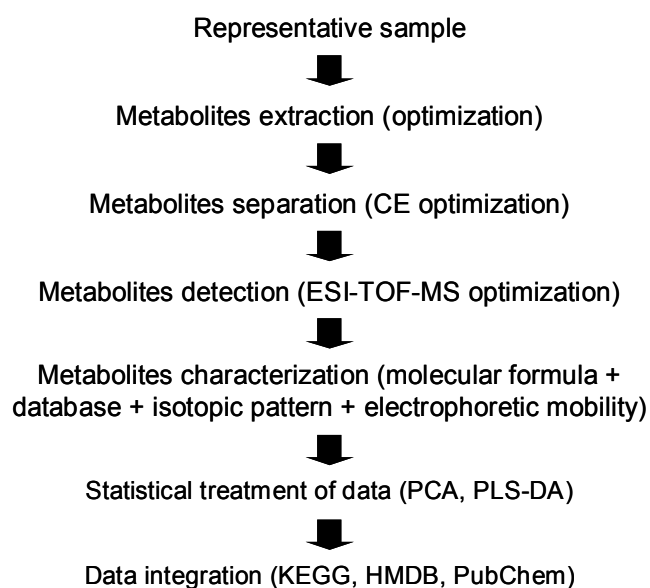
## Methodology

About 2.0 g of wild and GM maize flour were weighed out and extracted with 10 ml of methanol/water mixture (50:50) in ultrasonic bath for 10 min. After sonication, samples were centrifuged for 5 min (3000 rpm) and liquid phases were filtered through 0.45  $\mu$ m filter. Liquid phases were taken to dryness in a rotary evaporator, redissolved in 0.5 ml of methanol/water (50:50) and injected into CE-TOF-MS. Analyses were carried out with a P/ACE 5010 CE apparatus from Beckman Instruments coupled with an orthogonal electrospray interface (ESI, model G1607A from Agilent Technologies, Palo Alto, CA, USA) to a time of flight microTOF MS detector from Bruker Daltonik (Bremen, Germany). An uncoated fused-silica capillary with 50  $\mu$ m i.d., 375  $\mu$ m o.d. and 80 cm of total (and detection) length was used. The CE-TOF-MS conditions were: a solution composed of 5% formic acid in water at pH=1.9 was used as separation buffer in CE; isopropanol:water (50:50, v/v) as sheath liquid flowing at 0.24 ml/h together with 0.4 bar of N<sub>2</sub> as nebulizing gas and dry gas at 180°C and 4 l/min. Other TOF settings were: 50  $\mu$ s for the transfer time and 5  $\mu$ s as prepulse storage. The MS scan range was 50-450 m/z.

## Results

Samples from three genetically modified lines of maize and their respective wild (conventional) varieties of maize grown under the same conditions were studied in this work. Genetic modification on transgenic maize was based on the inclusion of the Cry1Ab gene, which provides resistance to some worm plagues through the synthesis of a Bt protein. An example of the results obtained can be seen in

Table 1. Thus, 27 metabolites were easily found and tentatively identified. Tentative identification was carried out based on the highly accurate mass determination provided by CE-TOF-MS, which allowed generating a more probable molecular formula for each metabolite. This molecular formula was then introduced in e.g., Kegg, Chempider or PubChem databases to obtain the metabolite identification. These assignments were corroborated using isotopic pattern simulations. Moreover, the expected electrophoretic mobility of each compound at the separation pH was also used to further corroborate the assignments. Figure 1 shows an scheme of the work flow followed in this work.



**Figure 1.** CE-ESI-TOF-MS work-flow used in this work for the comparative metabolomic study of wild vs. transgenic maize.

## Conclusions

In the present work, a complete analytical methodology (including metabolites extraction, CE-TOF-MS analysis and data evaluation) has been developed to comparatively study the metabolic profile of conventional and GM maize. This method allows the tentative identification of a large number of compounds, including isoflavones, aminoacids, carboxylic acids and other analytes. The results show that some of the detected metabolites do not change, while other show significant quantitative differences in their intensities in the conventional and GM corn, what seems to indicate that they can be used as biomarkers for GM monitoring. L-carnitine and stachydrine were identified as overexpressed metabolites in all the studied genetically modified maize varieties.

**Table 1.** Metabolites found by CE-TOF-MS in wild and GM corn samples, relative molecular masss (Mr) and their peak area ratio ( $A_{GM}/A_{wild}$ ).

Metabolite	Mr <sub>wild</sub>	Mr <sub>GM</sub>	Theoretical Mr	Tentative compound	$A_{GM}/A_{wild}$
1	319.230	319.219	319.225	C <sub>18</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub>	1.071
2	146.043	146.041	146.105	3,5-Diaminocaproate	1.611
3	264.104	264.095	264.101	Subaphyllin	1.047
4	103.102	103.098	103.101	Choline	0.772
5	174.113	174.107	174.113	Arginine	1.589
6	155.071	155.066	155.071	Histidine	0.972
7	135.056	135.051	135.054	Adenine	0.786
8	103.063	103.060	103.065	GABA	0.456
9	161.107	161.100	161.106	L-Carnitine	4.631
10	89.046	89.043	89.049	Alanine	0.652
11	105.040	105.039	105.042	Serine	0.872
12	117.081	117.079	117.080	Valine	0.797
13	129.081	129.076	129.080	Homoproline	0.659
14	131.096	131.090	131.094	Leucine	0.993
15	137.083	137.048	137.049	Trigonelline	0.911
16	245.237	245.226	245.148	beta-Alanyn-L-arginine	0.563
17	119.060	119.055	119.059	Threonine	0.908
18	115.072	115.064	115.065	Proline	0.683
19	147.054	147.050	147.054	Glutamic acid	1.116
20	187.064	187.057	187.120	7-Keto-8-aminopelargonic acid	0.569
21	143.097	143.093	143.094	Stachydrine	2.509
22	181.074	181.065	181.073	Tyrosine	0.807
23	133.039	133.037	133.039	Aspartic acid	1.236
24	437.239	437.221	437.231	Lunarine	1.225
25	342.105	342.094	342.095	1-Caffeoyl-beta-D-glucose	1.005
26	279.085	279.076	279.089	Graveoline	0.518
27	214.011	214.000	214.095	Pyrimidine nucleoside	1.130

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# Fluctuation of Isoflavone Concentration in Soybean Varieties Under Conventional and Organic Farming

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Polyphenols have a wide range of secondary metabolic compounds, which enter several fundamental processes in plant physiology. Some polyphenols, such as lignins, flavonoids, and others, have good effects on human health. Soybean is unique among the legumes, in that it is a concentrated source of isoflavones. Soybean contains three major isoflavone aglycones (i.e., daidzein, genistein, glycitein) and their corresponding glucosides, malonyl-esters and acetyls (Jackson et al., 2002). Ingestion of isoflavones is associated with many health benefits (Sarkar and Li, 2004). Soybean seed is made up of a large embryo containing two primary organ systems – germ and cotyledons – surrounded by a seed coat of maternal origin, and their isoflavone accumulation varies in both quantity and type. During maturation, the embryonic cells synthesize proteins and secondary metabolites, among which are isoflavones. The latter are considered natural active compounds in plant defence, produced against unfavourable conditions, and their amount is expected to vary with agronomic management. Until now, as regards isoflavone production, the effects of organic farming and low input have not been clearly recognised in soybean. In this framework, the aim of this research was to evaluate the influence of the environment, agricultural management (conventional vs. organic) and choice of variety on soybean isoflavone production in the seed sites.

## Methods

The experiment was conducted at Legnaro (Padova, NE Italy) in 2005 and 2006, during which 35 (first year) and 28 (second year) commercial soybean varieties were cultivated. The two types of management were organic and conventional. The soils in question contained 2.5% and 1.5% organic matter, respectively. Inoculated seeds were cultivated in four-row plots each 10 m long, at a seed density of 40 seeds/m<sup>2</sup>. The experimental design was a randomised complete block with three replicates. For isoflavone analysis, samples (60 seeds) were lyophilised and fractionated; germ and cotyledons were isolated, immediately milled, and then frozen to –20 °C. Samples of 0.1 g were treated with 7 mL of aqueous methanol solution (80% v/v, 2 h, room temperature). The residue was removed after centrifugation, yielding a clear supernatant. The end-solution was filtered (0.2 µm) and analysed by high performance liquid chromatography (HPLC) with a UV sensor (diode array) (Spectra Physics Analytical, Inc., Fremont, CA). The analytical column was a RP-C18, 40 mm long, with an internal diameter of 4.6 mm (Cluzeau, Sainte Foy La Grande, France) and was kept at 30 °C. The mobile phases were a water solution of 0.05% trifluoroacetic acid (v/v) (solvent A) and pure acetonitrile (solvent B). Gradient elution was performed according to the procedure of Murphy et al. (1999), with minor modifications as previously reported by Hubert et al. (2005): solvent B increased from 0 to 15% in 2 min., to 18% in 4 min., to 24.5% in 26 min., to 40% in 7 min, to 50% in 1 min, and lastly to 100% in 6 min. Analysis of variance for total isoflavones of cotyledons and germ was only performed on 23 varieties, which were cultivated in both years.

## Results

Cotyledons had lower isoflavone concentrations than germ (main effect: 11.9 vs. 1.89 mg g<sup>-1</sup>), but contained the major fraction of seed isoflavones (85%), due to their greater weight (80.4% vs. 2.2% of germ and 17.4% of seed coat). For cotyledons, all factors studied had significant effects, as well as

interactions, on single (data not shown) and total isoflavones (Table 1). Similar effects were detected in the germ, although the type of management was not significant; for germ isoflavones, the interaction among factors was also less significant. Major attention now focuses on cotyledons as the main structures for nutraceutical purposes. In conventional farming, cotyledons have more elevated values than in organic farming (average of varieties: 1.96 vs. 1.82 mg g<sup>-1</sup>), a fact that has also been associated with greater yield of grains (two-year mean: 3.92 vs. 3.72 t ha<sup>-1</sup>) and therefore of isoflavones. The lower soil fertility (organic matter) of conventional fields may have promoted the synthesis of these secondary metabolites. Cotyledons showed differences among varieties. In general, the highest values were found in Zen, Aires, Hilario and Fiume, whereas M10 and Sakai had the lowest values in both

Table 1. Statistical analysis (ANOVA) for factor effects and their interaction on total isoflavones in seed components.

\*\*\* P≤0.001; \*\* P≤0.01; \* P≤0.05; n.s. = not significant

Factors	Germ	Cotyledons
Year	***	***
Management	n.s.	***
Variety	***	***
Year×Management	n.s.	***
Year×Variety	n.s.	*
Management×Variety	*	*

agricultural types (Fig. 1). Soybean varieties showed small differences in their performance under conventional and organic managements, although the C.V. in organic cultivation was reduced (18.8% vs. 22.4%). The different climatic conditions of years markedly influenced concentration of isoflavones, much more than the method of cultivation (22% variation vs. 8%). The main isoflavone in cotyledons was daizein, and it was significantly correlated with total isoflavones (R<sup>2</sup>=0.84).

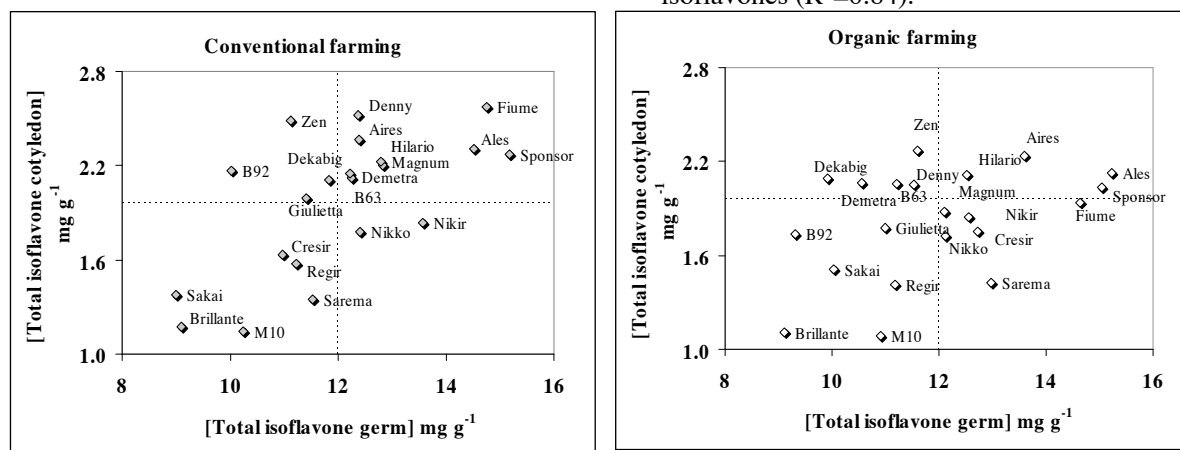


Fig. 1. Concentration of total isoflavones in germ and cotyledons (mean of 2005 and 2006 growing seasons) of soybean varieties in two agricultural systems. Dotted line: means calculated in conventional farming.

## Conclusions

Isoflavone is influenced by many factors, of which the ones mainly responsible, in order of magnitude, were variety, environmental conditions, and agronomic practices. Although germ is a by-product of small weight, its high isoflavone concentration suggests its use in soybean manufactures (soyfood). Further research is needed to understand the mechanisms of isoflavone accumulation in soybean in relation to gene control in various growing stages, for better application of agronomic practices.

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# Iodine Fortification for the Development of Functional Foods

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Iodine is an essential microelement for human health, and the Iodine Deficiency Disorders (IDD) are believed to be one of the commonest human health problems (Welch and Graham, 1999). The synthesis of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by the thyroid gland is the physiological role played by iodine in the human body. Therefore serious iodine deficiency also leads to functional and developmental abnormalities such as hypothyroidism. Hypothyroidism causes physical and mental retardation in infants and children. In neonates, iodine deficiency causes perinatal mortality, infant mortality and low birth weight. Severe iodine deficiency in the fetal and neonatal periods can lead to cretinism, which is characterized by stunted growth, mental and other neurological retardation, and delay in the development of secondary sex characteristics. In adults, it causes a reduction in the mental function and lethargy (Delange 1994)

Major staple crops contain insufficient concentrations of several essential elements like Iodine, to meet the Recommended Dietary Allowance (RDA) (DellaPenna 1999). A wide variety of foods, especially vegetables, contain low levels of iodine, while the best sources are fishes and other sea animals; therefore the natural iodine fortification of vegetables, by means of irrigation water, can be a part of strategy of functional food constitution.

Unlike mammals, iodine is a non-essential element for higher plants, and they can be adversely affected by low concentrations, although many studies have shown that plants can accumulate iodine (Mackowiak and Grossl 1999; Zhu et al. 2003; Dai et al. 2004; Dai et al 2006).

A previous study with the aim to establish the threshold of toxicity and to push to the maximum the amount of iodine uptaken by pot-grown plants demonstrated that crop plants and vegetables can accumulate high levels of iodine (Caffagni et al., submitted). The present study was aimed to determine the accumulation of iodine in tomato fruits, as well as in potato tubers in field trials after different treatments and levels. The final aim is to establish a field protocol for incorporating iodine into the above mentioned food crops, avoiding any detrimental effects on yields.

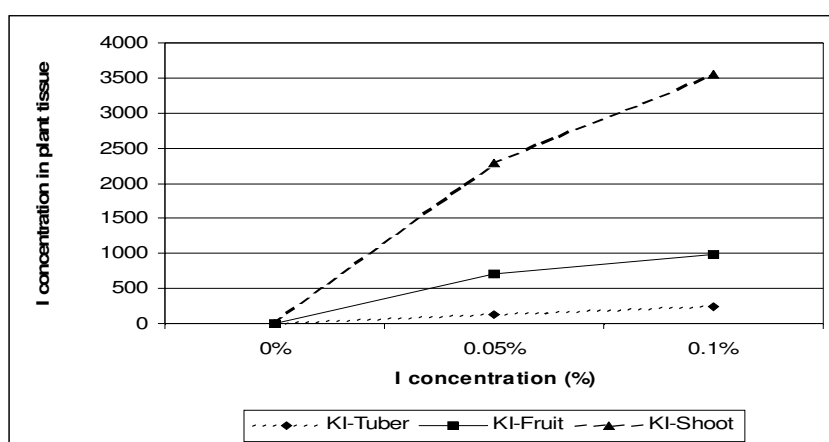
## Methodology

Particularly two horticultural crops, such as tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) were used. Plants, in field conditions, were treated with potassium iodide in different concentrations, in 2007, and in 2008 in Italy. Iodine concentrations in fruits and tubers was measured using the USEPA Method 3052 (HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub>, microwave digestion) and an inductively coupled plasma mass spectrometry (ICP-MS) analysis procedure. Iodine concentrations were analyzed after treatments, after harvest at commercial maturity, and after a storage period.

## Results

Previous results on pot-grown potato and tomato showed that, even in presence of phytotoxicity, high amounts of Iodine could be accumulated into different plant tissues (Fig. 1). In the first year of field trials none of the treatments provided sufficient iodine in the tubers and in the fruits to reach acceptable levels of iodine in a normal diet, although the plant species surveyed accumulated iodine. In 2008 the concentrations in treatments have been increased for all species, so as to provide iodine in a normal diet to meet 20-25% of human dietary requirements. In 2007 no detrimental effect of iodide on plant growth and yield was observed. The analyses of iodine content in different part of the plants are in progress.

Fig 1: Iodine concentration in tomato fruits and shoots, and in potato tubers on dry weight basis ( $\mu\text{g/Kg}$ ) after treatment with potassium iodide (0.05 and 0.1% in water) to pot potato and tomato.



## Conclusions

This study demonstrates that iodine can be effectively accumulated in the edible parts of tomato and potato, in order to supplement dietary iodine. A standardized protocol of iodine fortification for the fruit and vegetable species surveyed remains to be set up.

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# Application of Neural Networks for the Prediction of Lavender's Essential Oil Quality from Environmental Growing Conditions and Analytical Data

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The production of lavender has an important economic value in the cosmetic and pharmaceutical industries (Corbetta F. *et.al.*, 2001). Moreover, this crop has an ornamental importance for landscape. In order to improve production, we investigated the relationship among lavender's essential oil quality, environmental conditions of the growing locations and analytical components of the essential oil.

The aim of the study was the evaluation and quantification of the effects of environmental conditions (temperature, rainfall, insolation, soil traits) on oil quality and its prediction through neural networks (Richard J. *et al.*1991), to be applied in the identification of the best areas for the cultivation of lavender. Another goal was the interpretation of the chemical compound patterns of the oil samples for the prediction of the quality perceived by the panel test. By regression analysis the most important compounds for the perceived quality from sniffing analysis has been also identified.

## Methodology

We collected the complete floral and stems from 10 plants during two productive years (2006-2007) in 13 different zones (Giovanardi *et al.*, 2007). These locations have been classified into four homogeneous areas: mountain, hill, high plain and low plain. Productivity and quality of essential oils and flowers has been evaluated. We also collected soil traits (pH, cationic exchange capacity, organic carbon, soil organic matter, granulometry), and climate data (temperature, wind velocity, humidity, rainfall, daily insolation).

Essential oil samples were extracted by steam distillation. Essential oils were analyzed by gas chromatography (Shellie R. *et.al.*, 2002) and the quality evaluated by sniffing panel tests (1=worst, 4=best).

Using regression analysis some relationships between environmental conditions and quality judgement and compounds important for the quality judgment have been found.

A neural network and related software have been developed to predict oil quality from analytical data (figure 1).

Analysis (%)		
a-thujene	s1016	
(Z)-Ocimene	s1240	
3-octanone	s1244	
(E)-Ocimene	s1257	
p-Cymene	s1268	
Hexyl acetate	s1278	
(Z)-Linalool oxide	s1422	
(E)-Linalool oxide	s1444	
NI Terpene (204)	s1525	
NI Terpene (204)	s1544	
a-Cadinol	s2186	

Fig. 1: Software application for the quality estimation by the trained net.

## Results

By processing panel test results and environmental data we found a correlation between quality and daily insolation, temperature and rainfall (figure 2 and 3)

We also analyzed the impacts of different compounds in the quality judgment and found eight chemicals with a positive impact ( $\alpha$ -thujene, Z-ocimene, E-ocimene, 3-octanone, hexyl-acetate, Z-

linalool oxide, E linalool oxide, cadinol) and one with a negative impact (P-cymene). We also successfully trained a neural network using panel test results and analytical gas chromatography data.

## Conclusions

The results underline the relationships between environment and lavender's essential oil quality. The quality is higher when mean daily insolation increases until a specific threshold and then decreases (insolation is lower in the mountain than the others areas) (figure 2). Considering other environmental parameters, analysis shows an inverse relation in oil quality and the amount of rainfall, but a direct relation with oil quality and temperature (figure 3).

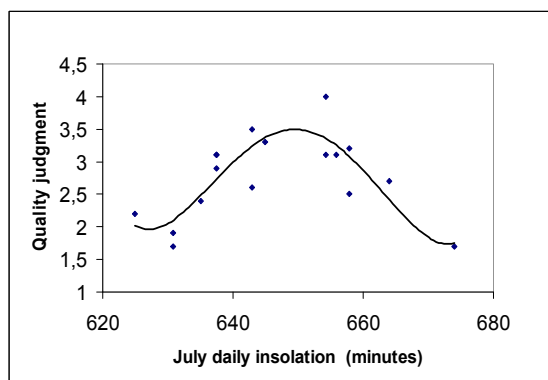


Fig.2: Relationship among quality judgment and daily insolation.

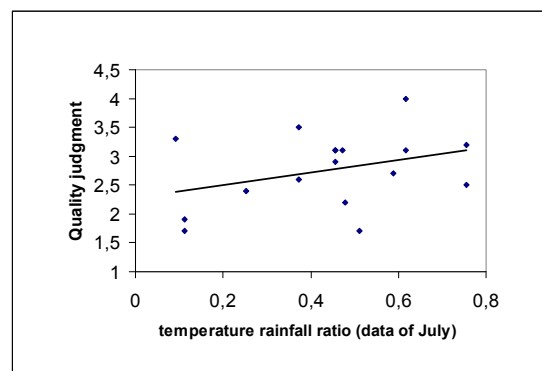


Fig.3: Relationship among quality judgment and temperature/rainfall ratio of July.

Tab. 1: Results of panel test.

These results are significant in constructing a map of the best areas to grow lavender. Through regression analysis we also found eight compounds that have a positive impact in the panel test judgment, and one which has a negative effect.

We developed a neural network for the prediction of lavender's essential oil quality from environmental growing conditions and analytical data; the results, in accordance with the panel test (table 1), highlighted the hill area as the best one for oil quality.

Areas	Quality judgment (From 1 to 4)		
	Medium	Minimum	Maximum
Low flat	2,98	2,5	3,2
High flat	2,96	2,4	3,3
Hill	3,05	2,6	3,5
Mountain	1,95	1,7	2,2

## Acknowledgements

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# Production of Vanillin from Wheat Bran Hydrolyzates Via Microbial Bioconversion

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Aromatic flavors production via biotechnological routes is of great interest as the products can be labeled as natural according to the EU legislation. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most important and widely used flavour in the food industry. Natural vanillin is obtained from *Vanilla planifolia*, which, however, can supply only less than 1% of the annual market demand (Walton et al. 2003). The majority of vanillin currently on the market is chemically synthesized from guaiacol or lignin, but the product is of lower quality and cannot be defined as natural (Walton et al. 2000). Biotechnological production of vanillin via microbial bioconversion of substrates such as eugenol or ferulic acid is a feasible alternative way of obtaining vanillin (Priefert et al. 2001).

In addition, the possibility of employing agro-industrial by-products such as wheat bran as ferulic acid sources (Mathew et al. 2007) may represent an interesting opportunity of valorising them. Previous studies have shown that resting cells of an *Escherichia coli* recombinant strain carrying the genes involved in the bioconversion of ferulic acid to vanillin of *Pseudomonas* origin (*E. coli* JM109/pBB1) can bioconvert the ferulic acid present in crude wheat bran hydrolyzates into vanillin (Di Gioia et al. 2007). Vanillin produced, however, is quickly converted into vanillyl alcohol (Di Gioia et al. 2007), thus reducing the vanillin yield of the process. Aim of this work is to try to increase vanillin molar yield from ferulic acid obtained from wheat bran.

## Methodology

Ferulic acid was obtained from wheat bran water suspension by enzymatic hydrolysis with a mixture of two commercial enzymes, i.e. Fungamyl Super AX<sup>®</sup> plus Celluclast BG<sup>®</sup> (Di Gioia et al. 2007). The biocatalyst employed for the bioconversion of ferulic acid into vanillin was a recombinant *Escherichia coli* strain containing the genes for the transformation of ferulic acid into vanillin (*E. coli* JM109/pBB1) (Barghini et al. 2007). Bioconversion of ferulic acid by resting cells of *E. coli* JM109/pBB1, previously grown on LB medium, was performed as already described (Di Gioia et al. 2007).

Ferulic acid was recovered from wheat bran hydrolyzates by adding the ion exchange resin Amberlite IRA<sup>®</sup> 95 (6%w/v, 50rpm, 4h) to the crude hydrolyzate, after hydrolyzate removal, the resin was washed with ethanol additioned with 4% HCl for 1h at room temperature. The alcoholic ferulic acid rich extract thus obtained was neutralized by using NaOH 2N and concentrated through evaporation in rotavapor and the solution was diluted in saline buffer in order to obtain the desired ferulic acid concentration.

## Results

In order to investigate whether high ferulic acid concentration could be the cause of the reduced vanillin yield obtained in bioconversion experiments with crude hydrolyzates as described in the previous work (Di Gioia et al. 2007), experiments were performed by increasing the hydrolyzate ferulic acid concentration with the addition of 100 mg/L of ferulic acid both to the undiluted wheat bran hydrolyzate (thus containing 300mg/L of ferulic acid and 30g/L of reducing sugars) and to the hydrolyzate diluted at 50% in saline buffer (thus containing 200mg/L of ferulic acid and 15g/L of

reducing sugars). Also in these experiments, the yields were not high (Fig. 1) and reached a maximum of 50% in the diluted hydrolyzate, suggesting that the low bioconversion yield obtained with the use of crude wheat bran hydrolyzate cannot be ascribed to the high ferulic acid concentration.

Therefore, a possible inhibitory role of carbohydrates present in crude hydrolyzates was investigated: ferulic acid was recovered from wheat bran hydrolyzates by using an ion exchange resin (Amberlite IRA<sup>®</sup> 95) and then used as bioconversion substrate. This method allowed a recovery of ferulic acid of 80% and a removal of reducing carbohydrates of 90%. The recovered ferulic acid was diluted in order to obtain 50, 100 and 200mg/L of ferulic acid, corresponding to 0,25 0,5 and 1,0 g/L of reducing carbohydrates respectively. The bioconversion made on this matrix with resting cells of *E.coli* JM109/pBB1 showed a rapid conversion of ferulic acid into vanillin thus obtaining a molar yield of about 70% (Fig.2). This suggests that the elimination of carbohydrates had a positive effect on the bioconversion process.

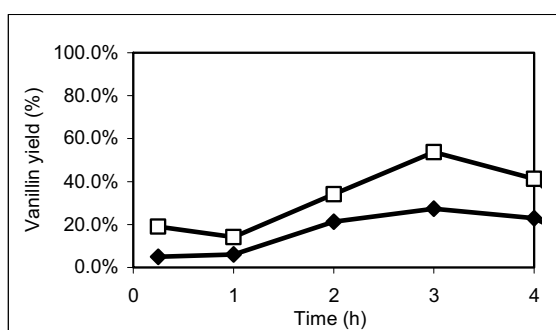


Figure 1: Vanillin molar yields obtained after the addition of 100 mg/L of ferulic acid to wheat bran hydrolyzates undiluted (◆) and diluted at 50% in saline buffer (□).

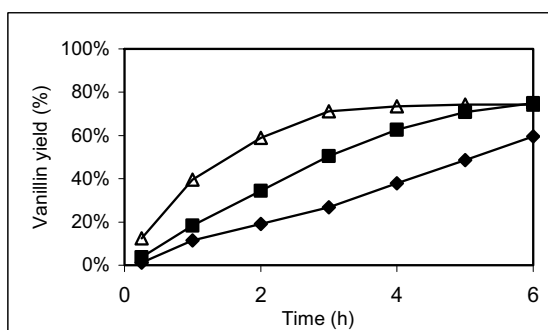


Figure 2: Vanillin molar yields obtained from 0.25 mM (Δ), 0.50 mM (■), and 1.00 mM (◆) of ferulic acid recovered employing ion exchange resins Amberlite IRA<sup>®</sup> 95.

## Conclusions

Bioconversion yields of ferulic acid obtained from wheat bran hydrolysis can be greatly increased by recovering ferulic acid from the hydrolyzates with the use of ion exchange resins. This procedure allowed carbohydrate elimination from the bioconversion matrix thus allowing to conclude that carbohydrates may play an inhibitory effect in the bioconversion process.

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# Functional Properties of Wheat: Phytochemical Profiles of Old and New Varieties

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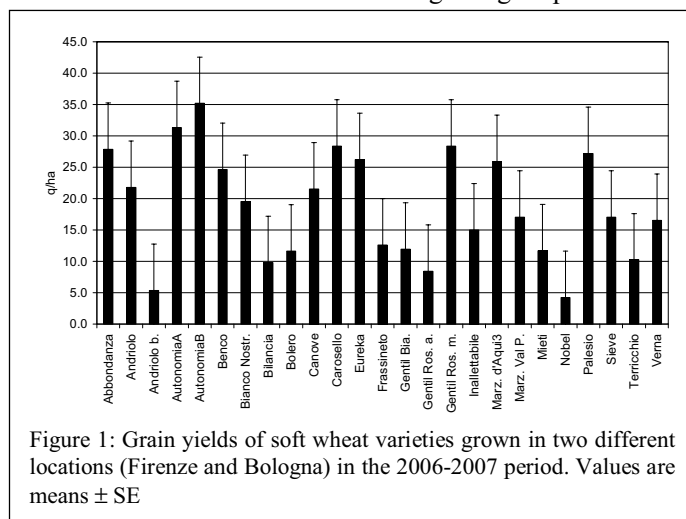
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Numerous lines of research have categorized the cereals, within the context of a balanced diet, as having a protective function in human health. The relationship between the consumption of whole grain cereals and the reduction of chronic illness, such as cardiovascular disease and cancer, strongly indicates the importance of functional properties contained in these foods. Such beneficial effects are partly attributed to the unique phytochemical content of whole grains (Thompson, 1994). More recent studies on the health benefits of functional products from wheat have become increasingly more focussed on the importance of introducing phytochemicals through the use of different varieties (Abdel-Aal and Wood, 2004). As a consequence, there is a renewed interest in the ancient varieties, particularly with regard to potential nutraceutical qualities.

The aim of this research was to analyze the variability of the phytochemical profiles in a collection of soft and durum wheat, comprised of old and modern varieties and to find out possible relationships among genotype, growing location (Bologna and Firenze, Italy) and quali-quantitative content of bioactive compounds in whole grains.

## Methodology

The trials were run in two different growing locations (Bologna and Firenze, Italy) in 2006-2007 on 35 Italian wheat varieties. The investigated germplasm consisted of 19 old and 6 modern soft wheat



(*Triticum aestivum* L.) varieties and of 7 old and 3 modern durum wheat (*Triticum durum* L.) varieties. The wheat genotypes were cultivated under low-input growing systems in a randomized block design. For each genotype and growing site, the following investigations were carried out: grain yield (q/ha), hectolitic weight (kg/hl), 1000-kernel weight (g), proteins (%), gluten (%), lipids (%), resistant starch (%), carotenoids ( $\mu\text{g}/100\text{g}$ ), polyphenols ( $\text{mg}/100\text{g}$ ), flavonoids ( $\mu\text{mol}/\text{g}$ ), lignans ( $\mu\text{g}/\text{g}$ ).

## Results

In field trials carried out in both locations (Bologna and Firenze) old soft wheat varieties highlighted higher grain yields than the modern ones,  $19.9 \pm 2.5$  and  $15.1 \pm 4.5$  q/ha, respectively (Figure 1). Among the new varieties, Eureka and Palesio had the highest grain yield values (more than 25 q/ha). The old varieties showed a great variability and five of them (ie Abbondanza, Autonomia A, Autonomia B, Carosello, Gentil rosso mutico) had yield values ranging between 28.5 and 35.4 q/ha. An opposite trend was observed for grain yields of durum wheat cultivars: the mean value for modern varieties was  $39.0 \pm 7.6$  q/ha whereas for old genotypes the mean yield was  $31.8 \pm 7.3$  q/ha. As regards the hectolitic weight and

the 1000-kernel weight of soft and durum wheat varieties, values were higher in the old genotypes as compared to the modern ones. This could be attributed to the fact that old populations are probably less affected by the applied low-input growing conditions.

**Proteins.** No significant differences were observed among old and modern genotypes of soft wheat as well as of durum wheat.

**Gluten.** The highest gluten values were observed in wheat cultivars grown in Firenze:  $71.1 \pm 0.1\%$  for *T. aestivum* varieties versus  $58.8 \pm 0.2\%$  for the same cultivars grown in Bologna and  $61.8 \pm 0.1\%$  for *T. durum* genotypes versus  $57.7 \pm 0.2\%$  the same cultivars grown in Bologna. Moreover, the mean gluten content of old varieties (soft and durum wheats) was significantly higher than the gluten content of modern cultivars in both growing locations.

**Lipids.** In soft wheat varieties, the mean values were not affected by the growing location and the old varieties showed lipid contents higher than the modern ones ( $3.0 \pm 0.21\%$  and  $2.3 \pm 0.4\%$ , respectively). As concerns the durum wheat cultivars no differences in lipids were observed as a function of the growing site, whereas the old genotypes had lipid percentages lower than those detected in the modern cultivars ( $2.0 \pm 0.1\%$  and  $2.3 \pm 0.1\%$ , respectively).

**Resistant starch.** In both soft and durum wheat cultivars the values of resistant starch were significantly higher in old populations ( $1.5 \pm 0.7\%$  for *T. aestivum* and  $4.1 \pm 0.8\%$  for *T. durum*) than in modern ones ( $0.5 \pm 0.3\%$  for *T. aestivum* and  $2.8 \pm 0.4\%$  for *T. durum*).

**Carotenoids.** The total carotenoid content was higher in old soft wheat populations as compared to modern ones ( $202.9 \pm 0.3$  and  $149.99 \pm 0.4 \mu\text{g}/100 \text{ g}$  seed, respectively). Conversely, the modern cultivars of durum wheat had carotenoids amounts higher than the old varieties. In both soft and durum wheat the most abundant carotenoid was lutein, followed by neoxanthin, violaxanthin and zeaxanthin.

**Polyphenols.** As concerns *T. aestivum* cultivars the polyphenol content was affected by both growing location (Bologna and Firenze) and genotype (old and modern varieties). Old and modern genotypes cultivated in Bologna had a total polyphenol two times higher than the same cultivars grown in Firenze. On the whole the old genotypes ( $100 \text{ mg}/100 \text{ g}$  in Firenze and  $250 \text{ mg}/100 \text{ g}$  in Bologna) showed values higher than the modern ones ( $90 \pm 9.5 \text{ mg}/100 \text{ g}$  in Firenze and  $200 \pm 11.5 \text{ mg}/100 \text{ g}$  in Bologna). As concerns *T. durum* cultivars, in both locations, the total polyphenol content was higher in the modern varieties ( $150.4 \pm 8.8 \text{ mg}/100 \text{ g}$ ) as compared to the old ones ( $90.5 \pm 9.8 \text{ mg}/100 \text{ g}$ ).

**Flavonoids.** The total flavonoid content was affected by both growing location and genotype. In particular the old cultivars and the Bologna growing conditions yielded high total flavonoid values in soft wheat genotypes (mean value  $1.65 \pm 0.2 \mu\text{mol}$  catechin eq./g). Conversely, in durum wheat cultivars, the highest amounts of flavonoid were detected in the old genotypes cultivated in Firenze (mean value  $1.50 \pm 0.5 \mu\text{mol}$  catechin eq./g).

**Lignans.** Qualitative analyses (performed only in soft wheat genotypes) highlighted remarkable differences between modern and old cultivars for the type of aglycon lignans and for the number of glucosidic forms. In particular, three lignan aglycon forms, namely arctigenin, isolariciresinol and syringaresinol, were exclusively identified in old wheat varieties. Moreover, the total lignan content in old varieties was approximately two times higher than the lignan content of modern ones ( $5.0 \pm 1.3$  and  $2.6 \pm 0.2 \mu\text{g/g}$ ) (Dinelli et al., 2007).

## Conclusions

On the whole, results highlighted that investigated old wheat varieties may represent a rich source of genetic diversity, especially with regard to functional properties. The unique composition in biologically active compounds of the old cultivars may suggest to expand their uses into a wide range of regular and specialty products, distinguished by their added value, based on health properties.

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# Application of Calcium and Magnesium Improves Yield and Essential Oil Yield of Oregano (*Origanum vulgare ssp. hirtum*)

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Oregano is the most valued spice and is the common name for a general aroma and flavor primarily derived from more than 60 plants species used all over the world as a spice. One of the most important species that is grown commercially is *Origanum vulgare ssp. hirtum* (syn: *O. heracleoticum* L.) which is endemic to the Mediterranean area. Oregano is a crop species which is well adapted to dry land conditions and to calcareous soils. Although, oregano is well adapted to a wide range of growing conditions and soils, nutritional disorders caused by Mg and Ca deficiency can be quite common especially on acid soils where Mg and Ca availability is significantly reduced (Marschner, 1995). While severe symptoms of Mg and Ca deficiency may not be found frequently it is known that Mg and Ca deficiency without the appearance of any visible foliage symptoms can significantly limit the yield of many crops (Marschner, 1995). The objectives of this study were to determine the effects of foliar Ca and Mg applications on growth yield and essential oil content of oregano.

## Methodology

The experiments were carried out at two different farms in the county of Kilikis one located at Cherso (Latitude 41,093, Longitude 22,727) and the other one located at Eptalofos (Latitude 41,000, Longitude 23,033) in Greece during the 2004-2005 growing seasons. The soil type at Cherso was a sandy clay with a pH=6.5 and low concentration of exchangeable Ca and Mg. In Eptalofos the soil type was sandy loam with pH=5.8 and low concentration of exchangeable Ca and Mg. The experimental design was completely randomized block with five treatments (0, 0.5% Ca, 1% Ca, 1% Mg, 2 % Mg) and five replications. Ca and Mg were applied three times during the growing period the first application was conducted at the initiation of stem elongation and the other two at two weeks interval. The following parameters were determined: plant height at harvest, chlorophyll content using a SPAD chlorophyll meter (Minolta, Japan), final yield, concentration of essential oils, and essential oil yield.

## Results

Calcium and Mg application affected plant height as the plants were shorter at the control treatment and increased with Ca and Mg application by an average of 10% at the two locations compared with the control. There was no statistical significant difference among the two rates of Ca and Mg and the only difference that was observed was between the control and the Ca and Mg treatments (Table 1). Plant height a characteristic that is affected by Ca and Mg deficiency (Marschner, 1995). Ca deficiency can result in shorter branches and banded petioles, deformed leaves, and abnormal fibers on cultured ovules. The terminal bud often dies and many lateral branches which have short internodes and enlarged nodes develop (Marschner, 1995).

Chlorophyll concentration was affected by Ca and Mg application as it was increased by an average of 11 % compared with the control with Ca and Mg applications at both locations (Table 1). During Mg deficiency there is a decline in chlorophyll content which is because chlorophyll degradation (Marschner 1995). Also Mg deficiency causes interveinal chlorosis of the older leaves and the export of carbohydrates from source to sink sites is impaired there is a decrease in the starch content of storage tissues (Marschner, 1995; Mengel et al., 2001).

Final yield increased in both locations with Ca and Mg applications. There was an average increase of 22% with Ca and Mg compared with the yield of the control treatment. Similar effect of Mg on yield was found by others in other plant species such as soybean, sugar beet, creeping bentgrass (Vrataric et al., 2006; John et al., 2003). The amount of information is minimal for the effect of Ca on

Oregano is a herb and is graded by the buyer according to its aroma and appearance. The essential oil content was not affected by the Ca and Mg application (Table 1). However, the essential oil yield was increased with the application of Ca and Mg indicating that it is more profitable for the farmers and the industry to apply Ca and Mg.

Table 1. Effect of Ca and Mg application oregano (*Origanum vulgare ssp. hirtum*) on plant height (cm), chlorophyll content, yield essential oil content and essential oil yield in two locations (Eptalofos and Herso) and in two growing seasons (2005, 2006).

Treatments	Plant height (cm)	Chlorophyll	Yield (Mg ha <sup>-1</sup> )	Net yield (leaves+flowers) (Mg ha <sup>-1</sup> )	Essential oil content (% DW)	Essential oil yield (lt/ha)
<b>Herso</b>						
control	57.75a <sup>†</sup>	42.66a	3.05a	1.63a	5.4a	88.02a
0.5 % Ca	63.25b	47.52b	3.51b	1.95b	5.7a	111.15b
1 % Ca	61.72b	47.36b	3.51b	1.96b	5.6a	109.76b
1 % Mg	58.25a	47.62b	4.11c	2.21b	5.2a	114.92b
2 % Mg	61.83b	46.7b	3.48b	1.99b	5.3a	105.47b
<b>Eptalofos</b>						
control	59.17a	42.58a	2.91a	1.46a	5.5a	80.3a
0.5 % Ca	63.4b	46.54b	3.69b	1.79b	5.6a	100.24b
1 % Ca	64.17b	47.68b	3.48b	1.86b	5.3a	98.58b
1 % Mg	63.87b	47.1b	3.56b	1.9b	6.1a	115.9b
2 % Mg	64.5b	47.62b	3.76b	1.9b	5.8a	110.2b

<sup>†</sup>Numbers followed by the same letter in a column are not significantly different (Tuckey's HSD test,  $P=0.05$ ).

## Conclusions

Calcium and Mg application affected plant height as the plants were shorter in the control treatment and increased with Ca and Mg application by an average of 10% compared with the control. Chlorophyll concentration was affected by Ca and Mg application as it was increased by an average of 11 % compared with the control with Ca and Mg applications at both locations. There was an increase 22% with Ca and Mg in both locations compared with the control treatment. In addition, the Ca and Mg application affected essential oil yield but they did not affect essential oil content. These results show that Ca and Mg application can affect the growth, yield and essential oil yield of oregano especially when it is grown in acid soils.

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# Antioxidant Activity in Wheat Grains With Different Ploidy Levels

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The people's interest in natural and healthy food products has led to a renewed interest in hulled wheat of *Triticum* species, commonly known as “farro”. This interest is due both to the rediscovery of traditional foods and to the adhesion to a healthy and equilibrated diet (De Vita et al., 2006). “Farro” was recognized as a very healthy cereal and is recommended in the diet of people suffering from allergies, colitis and high blood cholesterol (Stehlow et al., 1994).

Our work was aimed at investigating a relevant nutritional and health-related aspect, namely the Antioxidant Activity (AA), of three different “farro” species differing in the level of ploidy

- *T. monococcum* spp. *monococcum* [2n], *T. turgidum* spp. *dicoccum* [4n] and *T. aestivum* spp. *spelta* [6n],

as well as, for comparison, the AA of three corresponding free-threshing (or naked) species

- *T. monococcum* spp. *sinskajae* [2n], *T. turgidum* spp. *durum* [4n] and *T. aestivum* spp. *vulgare* [6n].

In particular, AA has been evaluated in hydrophilic and lipophilic extracts obtained from grain wholemeal flour. The former contained freely soluble hydrophilic antioxidants like freely soluble phenols and flavonoids. The latter contained carotenoids, tocotrienols, and tocopherols.

AA in these extracts was evaluated in some detail by using the new LOX/RNO method, based on the reaction between the soybean lipoxygenase isoenzyme-1 (LOX-1) and p-nitrosodimethylaniline (RNO) (Pastore et al., 2000), recently designed by our lab. This method is able to give an integrated evaluation of AA by simultaneously assessing reducing power, scavenging of radicals (LOO<sup>•</sup>, LO<sup>•</sup>, <sup>•</sup>OH) and antiperoxidative activity of a mix of antioxidants (Pastore et al., 2003, 2004). In this report, we also compare results from LOX/RNO method with that obtained by an initial evaluation carried out by using the well-known ABTS and DMPD methods (Pellegrini et al., 1999; Fogliano et al., 1999), primarily able to assess reducing power of the sample, as well as the ORAC method that provides a measure of the antioxidant capacity against peroxyl radicals (Ou et al., 2001).

## Methodology

Plants of the different species were grown in a glasshouse under natural light during the 2006-07 growing season at Foggia and the dehulled grain samples were milled using a laboratory mill.

Hydrophilic and lipophilic extracts were obtained from wholemeal flours as reported in Pastore et al. (2003) and Panfili et al. (2003), respectively. LOX/RNO methods: the reaction between soybean LOX-1 and RNO was monitored spectrophotometrically at 25°C following the absorbance decrease at 440 nm due to RNO bleaching as reported by Pastore et al. (2000); AA determination is based on the property of antioxidants to inhibit the rate of RNO bleaching (Pastore et al., 2004). AA was obtained by calibrating the method on increasing concentrations of Trolox, a hydrophilic vitamin E analogue used as reference antioxidant. AA of hydrophilic, lipophilic extracts as well as the sum of the two components (Total Antioxidant Activity, TAA) were expressed as µequivalents of Trolox/g of whole flour (µeqT/g). Mean values ± standard deviation (n=3) are shown in Table 1. A first study was also

carried out by using ABTS, DMPD and ORAC methods as reported by Pellegrini et al. (1999), Fogliano et al. (1999) and Ou et al. (2001), respectively.

## Results

LOX/RNO method showed no ploidy dependent response; differences were instead observed between hulled and naked species.

*T. dicoccum* showed the highest TAA value, basically due to the contribution of freely hydrosoluble antioxidants, whereas the contribution of lipophilic antioxidants obtained by hexane-ethylacetate extracts is lower. *T. spelta* and *T. monococcum* spp. *sinskajae* followed in the ranking list, showing similar TAA values, but this derives from an opposite contribution of AA of hydrophilic and lipophilic antioxidants.

The cultivated wheats (durum and bread wheat), along with the *T. monococcum* spp. *monococcum*, showed the lowest TAA values (Table 1).

**Table 1.** TAA in the different species evaluated with the LOX/RNO method

Species	AA <sub>LOX/RNO</sub> (μeqT/g)		TAA
	AA of hydrophilic antioxidants	AA of lipophilic antioxidants	
<i>T. dicoccum</i> (4n)	360 ± 65	141 ± 11	<b>501 ± 76</b>
<i>T. spelta</i> (6n)	256 ± 11	104 ± 12	<b>360 ± 23</b>
<i>T. monococcum</i> spp. <i>sinskajae</i> (2n)	115 ± 12	210 ± 8	<b>325 ± 20</b>
<i>T. aestivum</i> (6n)	128 ± 10	146 ± 14	<b>274 ± 24</b>
<i>T. monococcum</i> spp. <i>monococcum</i> (2n)	72 ± 4	148 ± 11	<b>220 ± 15</b>
<i>T. durum</i> (4n)	106 ± 8	73 ± 12	<b>179 ± 20</b>

Preliminary results showed that ORAC method, based on the scavenging of free radicals, also highlights “*Triticum dicoccum*” as the best species in terms of TAA, while the DMPD and ABTS methods, that evaluate the reducing power, cannot evidence the relevance of “*Triticum dicoccum*” (data not show).

## Conclusions

The results point out the advantage of *T. dicoccum* in terms of TAA, deriving from hydrophilic antioxidants having high radical scavenging activity. Interestingly, modern wheats showed lower TAA values than other species and the ploidy level does not seem to correlate with TAA, as evaluated by LOX/RNO method. Further studies are in progress *i)* to take into account the bound phenolic component, which is relevant in conveying antioxidant activity to cereal grains (Adom and Liu, 2002) *ii)* to better understand the environmental and genetic impact in determining grains TAA and *iii)* to evaluate intraspecific variability.

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# Production of vaccines in plants: Safety first!

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## Introduction and objective

Production of pharmaceuticals in plants is up to 50 times more cost effective than in fermenters through micro organisms. Plant derived drugs are an interesting alternative for developing countries where people cannot afford sufficient medical treatment. Therefore it makes sense to use this approach for synthesis of vaccines. Vaccines can be delivered as antigens either for peripheral or oral immunization. Immunoprotective antigens of bacterial pathogens, carcinogenic viruses but also protozoan parasites are available, and allow for increased mucosal immunization.

We are determining the viability to produce antigen subunit-vaccines against tuberculosis and mycobacterial pathogens (TB), the carcinogenic virus (HPV) and toxic E.coli stems (ETEC) in inexpensive production facilities – by plant transformation. Transformation of plants with genes carrying selected antigens of the respective pathogen allows producing immunogenic proteins on the field with high yields. However, transformation of the nucleus leads to transgenic pollen spreading these antigenic pharmaceuticals to the environment. Our solution to this ecological problem consists in the alternative to transform the chloroplasts. In contrast to the nucleus tobacco plastids are rarely contained in the pollen, and thus cannot be dispersed in the vicinity. An additional advantage of this extremely precise and predictable technique is that these cell organelles also allow an inducible regulation of protein expression through usage of a specially designed transcriptional system.

## Methodology

Transformation using plant leaves: Based on our experience with various insertion sites within the tobacco chloroplast genome, a vector was developed, which has shown to produce high chloroplast transformation frequencies. The antigen containing vectors are delivered on gold particles into fresh, fully expanded leaves using a DuPont PDS1000He biolistic gun. Homoplasmic transgenic tobacco plants were recovered after a minimum of two cycles of callus regeneration on spectinomycin containing media (500mg/L).

Integration of the antigenic *mmpI* reading frames (TB), L1-antigen (HPV) and LT-B gene (ETEC) into the plastid genome occurs through homologous recombination.

For construction of plastid expression vectors we designed antigenic polyprotein cassettes consisting of multiple antigenic epitopes as reviewed by Sette et al. (2001). These authors also suggested the use of GPGPG spacer sequences to increase the recovery of functional fusion peptides. However, to avoid concomitant direct repeats that can induce unwanted recombination we use different synonymous nucleotide triplets coding for Gly and Pro.

## Results

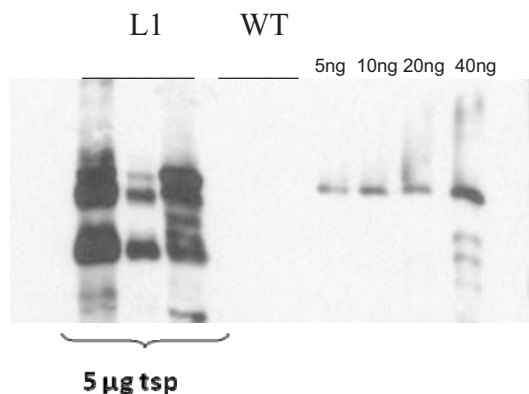
In order to produce the antigenic epitopes chloroplast transformation vectors were constructed, which contain synthetic expression cassettes for increased transcription and translation efficiency (Ye et al. 2001). On transcriptional level these constructs are combined with a spectinomycin selection cassette. All regenerates were selected on a modified MS medium containing spectinomycin. Positive transformants containing the *aadA* cassette and the gene of interest were identified by PCR analysis.

Fig.1 shows two transformants containing the L1 antigen with different phenotypes. Some transformed regenerates show a chlorotic phenotype with growth inhibition and sterility, which can pose a severe constraint to seed production.

Further Western blot analysis proved successful expression of the recombinant proteins. Transformants were then tested on correct folding of the fusion protein. Transplastomic plants contained ca. 1 % L1 protein of total soluble protein (TSP).



**Fig.1** A and B: Two transformants containing the L1 antigen of HPV. Transformant A shows a severe chlorosis and growth reduction. Transformant B similar to wildtype.



**Fig.2** Western blot analysis using immune-reactive antibodies showed signals with L1 transformants (3 lanes left) indicating correct conformation of the antigenic protein.

Lane 4 and 5 contain WT. For comparison of protein amounts lanes 6, 7, 8, 9 were loaded with 5ng, 10ng, 20ng and 40ng L1 protein isolated from viral particles of HPV.

## Conclusions

Our work has shown that it is possible to produce immunogenic antigens in tobacco plants to an interesting level. Genotypes containing the L1 antigen varied vastly in their phenotypes: Sterility, growth reduction and chlorotic phenotype in transplastomic plants can be overcome: Instead of constitutive expression we propose a time-specific regulation of Ag expression. Thus, our further work is aimed to set up an inducible antigen expression system (Lössl et al. 2005), which is regulated through chemical induction. Taken together these novel approaches allow for a controlled expression of transgenes with pharmaceutical impact, and thus production of vaccines in plants will soon become a reasonable alternative to bacterial fermenters.

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# Fresh Tomato Packinghouse Waste as Lycopene Biosource

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The waste management system of fresh fruit packinghouses, one of the first links in the food-supply chain abundant in Europe, has not been adequately studied. The negative environmental and economic aspects related to a not adequate bio-wastes utilisation are most serious for the food categories that generate large amounts of wastes and byproducts containing highly valuable, biologically active compounds. The potentially recoverable value of these wastes, can be obtained by the extraction of high added-value compounds that can be used as natural food additives. Within the Regional Research Program (POR SICILIA 2000-2006) we monthly collected waste samples from several packinghouses in order to achieve information on the variations in waste composition and on the content of high added-value compounds.

## Methodology

Packinghouses (waste sources) were selected in south eastern Sicily (Italy), since almost one-third of Italy's national production of greenhouse tomatoes (510,000 Mg year<sup>-1</sup>) is harvested in this area. We selected four tomato packinghouses (named  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) and we monthly sampled the wastes produced from December 2006 until July 2007. The waste samples, 8 time replicated, were analysed for the determination of lycopene content using the extraction method of Sharma and Le Maguer (1996) and a HPLC determination modified from Gregory et al. (1987) and Subagio et al. (1996). To evaluate the influence of both waste source (farms) and time (months), we used two-way analysis of variance (ANOVA), with a completely randomized design assuming the packinghouse and the month as additive factors. Because a potential waste management system would more easily receive unsorted waste produced by packinghouses, we also used one-way ANOVA, assuming that only the month was the studied factor and imposing a randomized block design with the four packinghouses as blocks, and then testing again using a completely randomized design. Nevertheless, because the selection of packinghouses to be involved in a system that recovers value from the wastes could affect the economical and technical feasibility of a more efficient waste management system, we applied the previously described one-way ANOVA approach that we used for the month, with packinghouse as the factor; again, we first used a randomized block design, with the eight sampling dates as blocks, and then we used a completely randomized design.

## Results and Discussion

Significant effects of both studied factors (farms and months) were reported on lycopene content in waste material (tab. 1), even in the severest analysis of variance conditions (with mean square errors growing from 46.0, calculated in the TwoWay ANOVA, up to 154.2 or 201.3, respectively for ANOVA with Time or Farm as the unique experimental factor). In the average of all the sampling dates, statistically significant differences were obtained in relation to packinghouses and a +38% was reported in  $\alpha$  and  $\delta$  (49.7 g Mg<sup>-1</sup>) compared to  $\beta$  and  $\gamma$  (36.0 g Mg<sup>-1</sup>). The lycopene content picked on the summer sampling dates of July and June, not statistically differentiated (57.5 g Mg<sup>-1</sup>), whereas on March, May and February lower amounts were measured (32.5 g Mg<sup>-1</sup> for the three levels not differentiated). According to the linear increase of lycopene content in tomato fruit during ripening (Riggi et al., 2008), the relevant lycopene amount, ascertained on June and July, was primarily expected in relation to the high amount of red ripened fruit fraction observed in waste material (data not

Table 1 - Lycopene content (g Mg<sup>-1</sup>) in the wastes as a function of farms and months, main ANOVA results, and related least-significant differences for the treatments and their interaction.

	Farm				Average
	$\alpha$	$\beta$	$\gamma$	$\delta$	
December	49.7	39.6	39.9	44.0	43.3
January	51.2	41.3	39.1	53.7	46.3
February	49.4	20.5	25.7	38.6	33.6
March	35.9	23.6	23.4	45.4	32.1
April	60.8	22.9	24.2	54.8	40.7
May	39.2	23.5	22.2	42.5	31.9
June	75.7	55.6	43.3	49.6	56.1
July	44.1	66.3	64.5	60.5	58.9
Average	50.7	36.7	35.3	48.6	
ANOVA	2-way		1-way		
Experimental design	(1)	(2) <sup>a</sup>	(1)	(1)	
			Time	Farm	
Month	$F^b$	76.7	33.3	22.9	---
	$P^c$	***	***	***	---
Farm	$F^b$	88.7	38.5	---	20.3
	$P^c$	***	***	---	***
MSE		46.0	106.1	154.2	201.3
Mean comparison test (LSD <sub>0.05</sub> )					
Time		3.3	5.1	6.1	---
Farm		2.4	3.6	---	4.9
Farm×Time		6.7	---	---	---

<sup>a</sup> Within the randomized block design, Month and Farm were alternately considered as factors or blocks.

<sup>b</sup> Variance ratio Fisher coefficient

(1) Completely randomized

(2) Randomized block

knowledge and the stimulation of new cultural and management approaches will be required. In this view, assuming the potential importance of tomato packinghouse wastes as a lycopene biosource available quite year round, our results represent a first step towards quantifying the potential economic benefits from the extraction of this compound.

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reported); whereas less easily explaining was the low value measured on February, when the carotenoid rich fraction assumed intermediate value between the other months (data not reported). The few data reported in literature on seasonal fluctuations of carotenoid levels in greenhouse tomatoes, refer to fresh red marketable products, collected directly in greenhouse or purchased from retail food stores (Raffo et. al. 2006; Toor et al., 2006). Even if they did not evidence clear seasonal trends of carotenoid content, some of them underline the low lycopene content measured in summer time under environmental conditions similar to our experiment (Raffo, et al., 2006; Toor et al., 2006). The nature of the analysed material within our research and in particular the seasonal fluctuation of the different waste fraction, may explain the observed high summer lycopene amount in tomato waste. Moreover, the results of multiple regression conducted by Toor et al. (2006), showed that, even if the increase in temperature over the summer months had a negative effect on the lycopene content, in the meanwhile the solar radiation had a slightly positive effect. Therefore, they hypothesized that some additional cooling of the greenhouse during the peak hours of solar radiation could increase the lycopene content of tomatoes.

## Conclusions

In order to improve the management of bio-wastes, the increasing of scientific

# The Antioxidant Potential of Wheat Milling Fractions

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Regarding the fact that antioxidants are considered one of the main contributors to food functionality, and having in mind that bread and other wheat products are frequently consumed worldwide, we decided to investigate antioxidative potential of some wheat milling fractions produced in our country, namely wheat flour type 400, 500 and 850, whole wheat flour and wheat bran, with the aim to predict preferences in functional food production, especially between different types of flour.

## Methodology

*Material.* Commercially available wheat milling fractions from the local market were used.

*Extraction.* Samples were mixed three times subsequently with 3x100 ml of 96% ethanol, by shaking at room temperature for 1 h. Combined extracts were dried by vacuum-evaporator to weight the yield and resolved in 96% ethanol to obtain 10 ml volume.

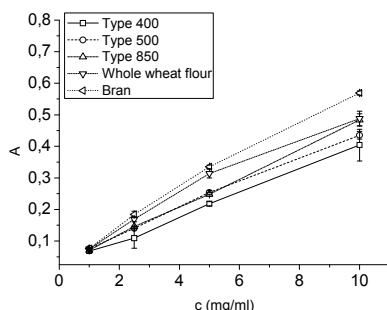
*Methods.* Total phenolic content, expressed as gallic acid equivalents (GAE) was performed according to the method of Singleton et al. (1999). Antioxidant activity (AOA) was estimated by monitoring of the oxidative loss of  $\beta$ -carotene in a  $\beta$ -carotene/linoleic acid emulsion (Moure et al. 2000). DPPH· scavenging activity was estimated according to the modified method of Hatano et al. (1988). IC<sub>50</sub> value (mg/ml) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial DPPH· under the experimental conditions given. It was obtained by interpolation from linear regression analysis. Chelating activity on Fe<sup>2+</sup> was measured according to the method of Decker and Welch (1990). Reducing power was assessed according to the method of Oyaizu (1986). All tests were performed in triplicate and given as mean  $\pm$  SD.

## Results

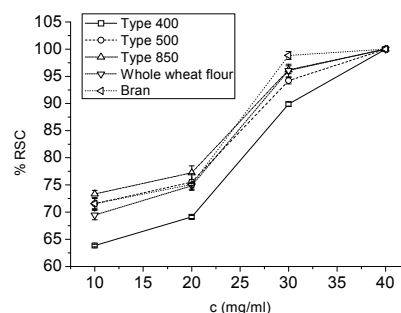
The highest content of total phenolics (Table 1) was obtained for wheat bran, while the lowest for flour type 400. Our results are in accordance with the previously published data, in which is stated that the most of the antioxidative potent compounds are located in the outer layers, i.e. in wheat bran (Liyana-Pathirana & Shahidi, 2007). Significantly different IC<sub>50</sub> values were obtained for different types of flour in DPPH scavenging test (Table 1). Also, significant differences between investigated types of flour in AOA test were obtained at lower concentrations of the extracts (Figure 1). At all investigated concentrations flour type 400 had the lowest AOA activity. Investigated extracts of milling fractions exhibited almost identical chelating activities (Figure 2). In the reducing activity test significant differences were found only for higher concentrations (Figure 3) where flour type 400 had the lowest, and bran possessed the highest activity.

Extracts	Total phenolics ( $\mu\text{g GAE/g}$ extract)	DPPH $IC_{50}$ (mg/ml)
Type 400	$19.45 \pm 0.35^a$	$39.36 \pm 0.31^c$
Type 500	$37.08 \pm 0.15^b$	$34.24 \pm 0.26^b$
Type 850	$50.76 \pm 0.48^c$	$31.59 \pm 0.20^a$
Whole flour	$137.19 \pm 1.69^d$	$31.26 \pm 0.06^a$
Bran	$410.63 \pm 6.98^e$	$31.64 \pm 0.33^a$

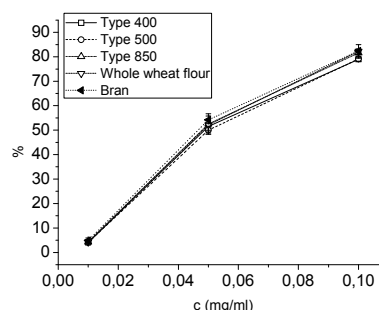
**Table 1.** Total phenolic content and scavenging activity on DPPH $\cdot$  of ethanolic extracts of different milling fractions



**Figure 2.** Reducing activity of ethanolic extracts of different milling fractions



**Figure 1.** Antioxidant activity of ethanolic extracts of different milling fractions



**Figure 3.** Chelating activity on  $\text{Fe}^{2+}$  of ethanolic extracts of different milling fractions

## Conclusions

According to our results, wheat in general is not an abundant source of plant antioxidants, but significant differences between the extracts of wheat milling fractions were found. Finally, regarding all mentioned above, wheat flour type 850 could be considered as better source of antioxidants than flour types 400 and 500 for functional food production.

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# Vegetative Production And Indigo Yield Of Woad (*Isatis tinctoria* L.) And Dyer's Knotweed (*Polygonum tinctorium* Ait.) Under Irrigation In Central Italy

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## Introduction

Woad (*Isatis tinctoria* L., *Cruciferae* family) and dyer's knotweed (*Polygonum tinctorium* Ait., *Polygonaceae* family) are potential alternative crops for indigo production adaptable to irrigated agriculture of several areas of Italy. However, little information is available on the water requirements for growing these crops under irrigated conditions, particularly with regard to increasing their vegetative growth and the production in their leaves of indigo dyes and their natural precursors. Aim of the present study was to analyze the crop coefficient (Kc), the crop water requirement (CWR) for these specific crops and location and the effects of irrigation on leaf yield and indigo production.

## Methodology

A 2-year field study was undertaken to evaluate the response to irrigation of woad and dyer's knotweed cultivated as spring crops. The research was carried out at the Experimental Centre of Dep. Agronomy, University of Pisa (Pisa countryside, Central Italy, N43°41';E10°23') on a typical Xerofluvent, silt-loam soil, representative of the low Arno river plain. *I. tinctoria* and *P. tinctorium* plants at 4<sup>th</sup>-6<sup>th</sup> true leaf stage were transplanted in the field in the spring (8<sup>th</sup> May 2002 and 18<sup>th</sup> April 2003) with a crop density of 330,000 plant ha<sup>-1</sup> (0.3x0.1m) and 120,000 plant ha<sup>-1</sup> (0.3x0.3m) for woad and dyer's knotweed respectively. Six irrigation levels (T<sub>100</sub>, T<sub>80</sub>, T<sub>60</sub>, T<sub>40</sub>, T<sub>20</sub> that received a seasonal water amount equivalent to 100, 80, 60, 40, 20% of ET<sub>c</sub> (actual evapotranspiration) and a rain-fed control T<sub>0</sub>) were compared in a randomised block design experiment with four replications. Two watertable microlysimeters installed in the central plots of each species were employed to estimate the crop water consumption (ET<sub>c</sub>) during the growing season while monitoring the climatic parameters and the phenological crop development. The reference evapotranspiration (ET<sub>0</sub>) was estimated by class A evaporimeter according to FAO method. For the irrigation supply, the water was delivered daily by an automated drip irrigation system. Daily meteorological data and crop water requirement (CWR) were automatically collected and recorded. The seasonal crop water consumption ET<sub>c</sub> (m<sup>3</sup> ha<sup>-1</sup>), and K<sub>c</sub> (K<sub>c</sub>=ET<sub>c</sub>/ET<sub>0</sub>) were evaluated. Mineral fertiliser was applied before planting at rates of 100/100/100 kg ha<sup>-1</sup> of N/P/K. Further 50 kg ha<sup>-1</sup> of N were supplied after the first (dyer's knotweed) or the first and second harvest (woad). Plant and leaf fresh and dry yield (t ha<sup>-1</sup>) were carried out on total plot area, excluding the outer rows. Measurements made on multiple harvests were summed to estimate crop seasonal yield. Indigo from *I. tinctoria* leaf samples has been determined spectrophotometrically in ethyl acetate at 600 nm according to Stoker et al., 1998. Indigo precursor indican from *P. tinctorium* leaves has been determined by HPLC-ELSD method according to Angelini et al., 2003. The theoretical indigo amount obtainable from the complete reaction of indoxyl was quantified by stoichiometric calculations.

## Results

The two growing seasons were characterised by contrasting rainfall distributions during spring and summer in comparison with a long-term set of data. The total rainfall recorded from May to August was 221mm in 2002 and 22mm in 2003 against 181mm as long-term data. The ET<sub>0</sub> values from April/May to October/November recorded in 2003 were always higher than 2002. The ET<sub>c</sub> values were different in the two years according with the different trend of the ET<sub>0</sub>. For woad K<sub>c</sub> maximum values of 0.46-0.47 have been reached at the end of August when the vegetative development of the plant

rosette was over 30cm diameter. The seasonal woad CWR recorded from April to November differed significantly between the two seasons being significantly higher (+37%) in 2003 than 2002 (3327 vs 2080 m<sup>3</sup> ha<sup>-1</sup>). For woad leaf dry matter (DM) production was not significantly affected by irrigation level both in the 2002 wet and in the 2003 dry growing seasons. In 2003 leaf fresh yield increased significantly as irrigation was increased incrementally from 0 to 40%ETc; water application over 40%ETc had no increased benefit. Even if leaf DM production and indigo yield were unaffected by the level of irrigation, going from T<sub>0</sub> to T<sub>40</sub> leaf and indigo yield increased 16% in 2003 (Table 1).

**Table 1.** Effect of different irrigation levels on mean fresh (tFWha<sup>-1</sup>) dry leaf yield (t DW ha<sup>-1</sup>) and indigo (kg ha<sup>-1</sup>) productions on *Isatis tinctoria* in 2002 and 2003 growing season.

Irrigation Treatments	2002 <sup>(1)</sup>			2003 <sup>(2)</sup>		
	Leaf t FW ha <sup>-1</sup>	Leaf t DW ha <sup>-1</sup>	Indigo <sup>†</sup> kg ha <sup>-1</sup>	Plant t FW ha <sup>-1</sup>	Leaf t DW ha <sup>-1</sup>	Indigo kg ha <sup>-1</sup>
T <sub>0</sub>	94.92	8.90	162.73	52.66 b	8.14	59.62
T <sub>20</sub>	107.94	10.48	174.12	61.52 ab	8.08	64.90
T <sub>40</sub>	101.29	8.99	149.76	71.34 a	9.46	69.10
T <sub>60</sub>	99.70	9.40	139.66	67.04 a	8.64	76.40
T <sub>80</sub>	101.75	9.77	168.77	65.12 a	8.52	75.70
T <sub>100</sub>	99.61	9.03	143.24	63.23 a	8.27	82.30
Mean	100.87	9.43	156.38	63.48	8.52	71.34
Significance (LSD)	NS	NS	NS	*(10.02)	NS	NS

<sup>(1)</sup> Harvest dates: 17 June; 29 August; 09 Sept.; 11 Nov.; <sup>(2)</sup> Harvest dates: 04 June; 04 August; 11 Sept.; 19 October.

Dyer's knotweed Kc values differed significantly with the crop growth stage reaching the maximum value of 0.7/0.8 at full vegetative development. Seasonal CWR recorded from April/May to October was 2413 and 4767 m<sup>3</sup> ha<sup>-1</sup> for 2002 and 2003 respectively. Irrigation significantly influenced seasonal plant and leaf dry and fresh yield as well as indigo production in both years. A significant decrement of yield was observed with irrigation level below 20%ETc in comparison with T<sub>40</sub>, T<sub>60</sub>, T<sub>80</sub> and T<sub>100</sub>. Irrigation rates higher than 40%ETc did not affect significantly plant/leaf and indigo yield (Table 2).

**Table 2.** Effect of different irrigation levels on mean dry whole plant, leaf (t DW ha<sup>-1</sup>) and indigo (kg ha<sup>-1</sup>) yield of *Polygonum tinctorium*.

Irrigation Treatments	2002 <sup>(1)</sup>			2003 <sup>(2)</sup>		
	Plant t ha <sup>-1</sup>	Leaf t ha <sup>-1</sup>	Indigo <sup>†</sup> kg ha <sup>-1</sup>	Plant t ha <sup>-1</sup>	Leaf t ha <sup>-1</sup>	Indigo kg ha <sup>-1</sup>
T <sub>0</sub>	9.76 c	4.66 d	214.6 b	7.89 b	2.85 b	110.18 b
T <sub>20</sub>	13.22 b	5.81 c	293.2 a	10.32 b	3.72 b	158.81 b
T <sub>40</sub>	16.39 a	6.87 a	324.4 a	15.58 a	6.71 a	268.16 a
T <sub>60</sub>	15.60 ab	6.84 ab	327.1 a	18.19 a	7.23 a	317.96 a
T <sub>80</sub>	14.92 ab	5.97 bc	325.2 a	15.86 a	5.99 a	281.69 a
T <sub>100</sub>	16.51 a	6.61 abc	313.9 a	16.72 a	6.16 a	306.97 a
Mean	14.40	6.13	298.7	14.09	5.44	240.6
LSD (P= 0.05)	2.86	0.89	39.9	1.78	0.88	94.3

<sup>(1)</sup> Harvest dates: 04 July; 03 October; <sup>(2)</sup> Harvest dates: 07 July; 3 September; 28 October

## Conclusions

*I. tinctoria* gave multiple harvests with high leaf yields, both under rain fed and well watered conditions. Despite its good capacity to grow and to re-grow after harvesting, *I. tinctoria* showed lower indigo potential than *P. tinctorium*, both as amount of indigo per leaf weight and as indigo production per unit area. However, *I. tinctoria* appeared to be drought tolerant, while *P. tinctorium* was very sensitive to water stress with a 64% reduction in indigo production observed in T<sub>0</sub> respect to T<sub>100</sub> in the driest 2003 growing season. Therefore, *P. tinctorium* appears to be more productive in non limiting water conditions, thus making appropriate irrigation plans (*i.e.* 40%ETc corresponding to 1907 m<sup>3</sup> ha<sup>-1</sup> in the driest season) necessary to achieve sustainable high yields.

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# Evaluation of Native Populations of *Gentiana* (*Gentiana lutea* ssp. *Symphyandra*) of the Friulan Pre-Alps

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*Gentiana* is an important medicinal herb employed both in the liquor industries and also for some pharmaceutical preparations. Its success in the sector of the alcoholic beverages is due to the fact that the root of *Gentiana lutea* contains some of the most bitter substances present in nature. It grows spontaneously on mountain pasture lands and it is present in different sites of pre-Alps, Alps and Appenines. In Friuli Venezia Giulia region (northeast Italy) there are two different sub-species: *Gentiana lutea* ssp. *Symphyandra* e *Gentiana lutea* ssp. *Vardjanii* (Bezzi, Aiello, 1998).

With the aim to develop a productive chain for the propagation and cultivation of the *Gentiana lutea* a preliminary evaluation of the native populations is required involving the development of an efficient propagation technique for the species, the molecular characterization of the native ecotypes and the localization of the most suitable sites for a gentiana crop. Several spontaneous populations of *Gentiana lutea* L. were identified especially in the pre-Alps where the seed was collected in order to propagate the species in greenhouses. The seeds of *Gentiana lutea* are subject to dormancy, therefore three different techniques are employed to remove seed dormancy in order to evaluate the most effective one (Aiello, Bezzi, Tartarotti, 1987). The plants obtained applying this technique are used to prepare several testing grounds located in different areas and altitudes of the mountains in the region, where different cultivated ecotypes and some native ecotypes were compared.

## Methodology

After detecting different sites of spontaneous growth in Friuli Venezia Giulia, on the basis of various excursions, biometric measures were carried out on spontaneous plants of *Gentiana lutea* in various phenological stages and the seed was then harvested when the capsule of the flowering stem had become dehiscent. Three different methods have been applied to overcome seed dormancy in order to propagate the plants and introduce into experimental cultivation in various areas of Friuli Venezia Giulia: growth regulators, such as gibberellic acid (GA<sub>3</sub>), *in vivo* chilling and *in vitro* chilling.

After the treatments, seeds were sown in greenhouses under controlled conditions of temperature and humidity, then the plants were transplanted and brought to testing grounds during the months of May/June.

Testing grounds were prepared using seeds collected in 7 of 18 regional spontaneous stations identified, and also seeds from other parts of the Alps belonging to ecotypes already brought into cultivation or spontaneous of certain extra-regional areas.

## Results

The most remarkable results to break down the yellow gentian seed dormancy were obtained through the gibberellic acid method. Seeds were soaked for different times (trial A-6h and trial B-24h) in different GA<sub>3</sub> solutions (50 ppm, 100 ppm, 150 ppm). Processed seeds were then washed under water. The statistical analysis obtained, showed a significant effect of GA<sub>3</sub> concentration, hours immersed under water and of the ecotypes on germination mean. In particular the trial B-24 h; 150 ppm GA<sub>3</sub> has been the best: germination mean increased gradually both from 6h to 24h of treatment and from 50 ppm to 150 ppm GA<sub>3</sub> concentration (Fig. n.1). The ecotype Cuarnan obtained the best results, up to 70% of germination mean, which is significantly different from the data obtained by Flajel, Mezzomonte and Valinis ecotypes (Fig. n.2).

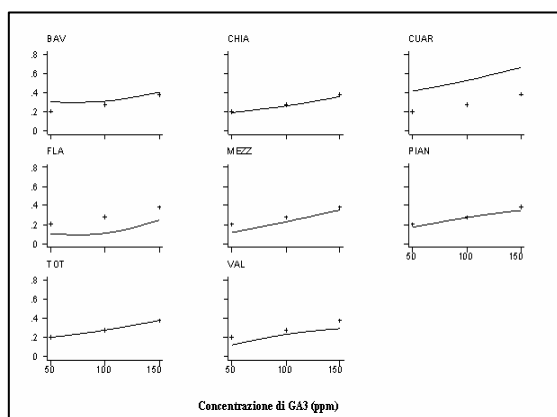


Fig. n.1- Trend of germination mean percentage for different ecotypes depending on GA<sub>3</sub> concentration

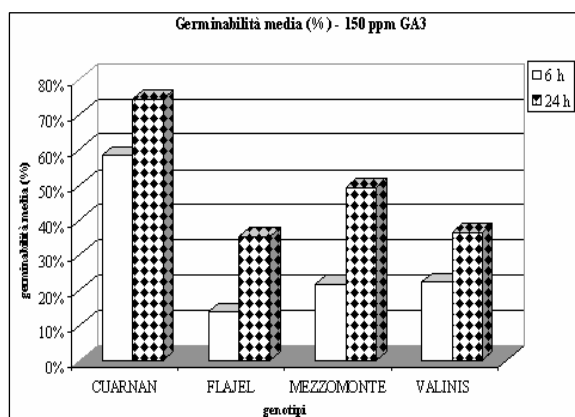


Fig. n.2 - Germination mean percentage for ecotypes: Cuarnan, Flajel, Mezzomonte, Valinis, with 150 ppm GA<sub>3</sub> concentration

## Conclusions

Trials with a growth regulator, gibberellic acid (GA<sub>3</sub>), show how this method, besides being fast, is also the most effective way to break down the seed dormancy of spontaneous yellow gentians harvested in different mountain areas of the region. The soaking in a solution concentration of 150 ppm GA<sub>3</sub> for an immersion of 24 hours has been the optimal combination for all ecotypes, and Cuarnan has obtained the highest germination values. Statistical results confirmed the influence of GA<sub>3</sub> concentration, the time of treatment and genotype on germination media. Whether in relation to immersion time (6h and 24h) both in relation to the GA<sub>3</sub> concentration (especially 50 ppm and 150 ppm) the trials carried out were significantly different.

## Acknowledgements

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# Isoflavone and Protein Contents in Soybean Under Irrigation and Nitrogen Fertilisation Management

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There is awareness that proper agronomic practices are the main key to production for several field crops. In soybean, both grain yield and accumulation of secondary metabolites (e.g., isoflavones) depend on several factors, including environment, variety, and agronomic practices (Lozovaya et al., 2005). Yield and its main components (e.g., carbohydrates, proteins, fatty acids) are not as sensitive to changes in agronomic practices as secondary metabolic compounds (Berger et al., 2002). Soybean isoflavones are polyphenol compounds derived from polypropanoid metabolism, which accumulate in various forms in the seeds. Isoflavones have recently attracted the attention of the pharmaceutical industry as a good source of phytoestrogens. Soybean proteins and isoflavones have positive effects on human health if they are correctly integrated in human diet. Consumption of soy-foods is associated with many health benefits, such as lowered risk of breast, prostate and colon cancer (Lacombe et al., 2000). In poor countries, soybean protein may become a substitute for animal milk in the form of soy-milk.

This study examines the effects of cultivar choice, supplementary irrigation and nitrogen on isoflavone and protein production in soybean, and their relation with nodule activity (N fixation, ureide production).

## Methods

A two-year field trial was carried out in 2006 and 2007 at the experimental farm of the University of Padova at Legnaro (NE Italy). Three factors, i.e., cultivar, irrigation and nitrogen fertilisation, were examined, following a split-split-plot experimental design with 4 replicates. Two cultivars were grown: Ales, with generally high isoflavone concentration and low protein content; and Nikir, commonly having high protein content and low isoflavone concentration. Irrigation regimes were: optimal water supply (100% of ET<sub>m</sub>, maximum evapotranspiration) and rainfed (controls). Soil moisture was monitored over the growing season by means of TDR (Time-Domain Reflectometer) equipment. The two fertilisation levels were: 100 kg N ha<sup>-1</sup> and unfertilised (controls), given in the form of urea in the R3 phase. The reason for the nitrogen supply was to improve N nutrition when nitrate reductase activity is presumed to fall and nitrogenase has not reached its peak. Foliar ureides was measured in the R5 phase in both varieties by the Herridge method (Herridge et al., 1982). The root system of 10 plants was sampled by trench dipping (0.3 m depth). Roots were washed and nodules separated with tweezers. Nodules were spread on a white background and photographed with a Canon 350 EOSD digital camera, and images analyzed with ImageJ open-source software, revealing their main geometric features (i.e., major and minor axes, perimeter, section area). The concentrations of 12 isoflavone molecules were measured in germ and cotyledons separately by HPLC (Hubert et al., 2005). Isoflavone concentration in seeds was measured on main stalk and lateral branches separately. Protein was measured according to N Kjeldahl×6.25.

## Results

Irrigation generally increased the concentration of total isoflavones in cotyledons, especially in lateral branches (year 2006: in laterals 2.05 vs. 1.82 mg g<sup>-1</sup>, i.e., +12.6%; in main stalk 1.60 vs. 1.50 mg g<sup>-1</sup>, i.e. +6.6%) but not in the germ (Fig. 1). Nitrogen supply had the effect of reducing total isoflavone

concentration by about 5% in cotyledons, irrespective of seed site, but had no influence on the germ. Isoflavone composition was variously affected by both irrigation and fertilisation. For instance, daidzin increased in irrigated plots and decreased in N-fertilised ones; genistin increased under irrigation but was less affected by N supply. As regards aglycone forms, daidzein was only sensitive to fertilisation and genistein was more sensitive to irrigation.

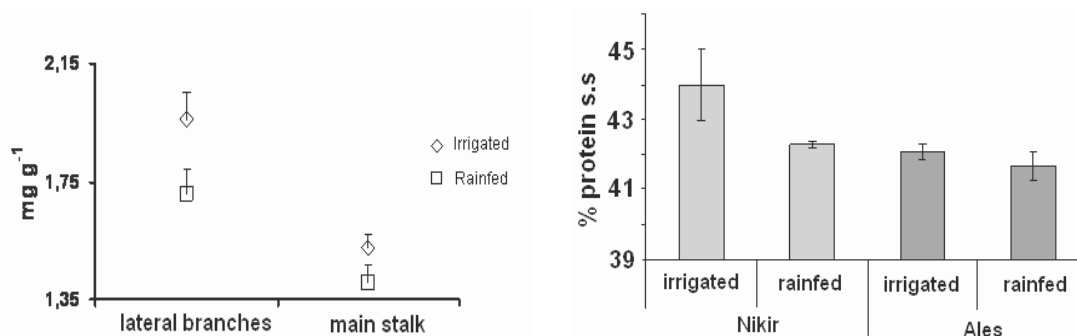


Figure 1. Effects of irrigation on total isoflavones (main effect) in two seed sites (left) and seed proteins (right) in two commercial soybean varieties. Vertical bars: standard error.

There was a negative correlation ( $R^2=0.51$ ) between foliar ureide concentration and isoflavone concentration of cotyledons in unfertilised treatments (a common condition in soybean). A significant negative correlation was also found between the size of nodules (i.e., area of the median section) and foliar ureide ( $R^2=0.53$ ), whereas the correlation ( $R^2=0.62$ ) between nodule size and total isoflavone concentration was positive. These effects match the results of Espinosa Victoria et al. (2000), who showed that phenylalanine-ammonia-lyase and chalcone-synthase (major enzymes responsible for activation of isoflavone biosynthesis) are less active at elevated N-fixation efficiency.

Seed protein was positively influenced by irrigation, but no effect was observed for nitrogen fertilisation. The *Ales* variety was only marginally improved by irrigation, whereas *Nikir* increased about by 5% in relative terms (Fig. 1).

## Conclusions

Irrigation, a practice only occasionally applied in soybean production in NE Italy, can profitably increase cotyledon isoflavone concentration for nutraceutical uses. These data also show that isoflavones can be increased by stimulating stalk branching through lower seed density at sowing. This has the effect of exposing laterals – which mature later – to lower temperature regimes, a fact known in the literature (e.g., Berger et al., 2002) for increasing isoflavone synthesis. Low nodule efficiency, in the form of low ureide production, and poor soil fertility (nitrogen) are favourable conditions for increasing isoflavone accumulation in soybean seeds.

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# Olive Mill Wastewaters as a Renewable Resource for Production of Biodegradable Polymers Through a Biological Anaerobic-Aerobic Process

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## Introduction

Polyhydroxyalkanoates (PHAs), and particularly the copolymer poly( $\beta$ -hydroxybutyrate/ $\beta$ -hydroxyvalerate) [P(HB/HV)], are bioplastic produced by bacteria or plants whose properties are similar to these of polypropylene (Dionisi *et al.*, 2004). Despite that, high PHA production costs, mainly due the employment of pure cultures and synthetic specific substrates, actually limit their diffusion (Reddy *et al.*, 2003). In this research, an innovative anaerobic-aerobic integrated process for PHA production, employing a mixed microbial consortium such as an activated sludge and fed with a non-cost renewable resource such as olive mill wastewaters (OMWs), was studied. This process consists of three stages. In the first anaerobic stage OMWs are fermented to obtain an effluent rich in volatile fatty acids (VFAs), which represent the substrate for PHA production. In the aerobic second stage, the VFA-rich anaerobic effluent is fed to a sequencing batch reactor (SBR) inoculated with an activated sludge, by alternating excess and lack of substrate ("feast and famine conditions") so to enrich the mixed culture of the PHA-producing microorganisms, able to store the polymer during the feast phase and to use it for growth during the famine phase. This stage operates at high organic loads so to maintain strong selective pressure on the sludge. Finally, in the third aerobic batch stage the excess sludge selected within the second stage is fed with the effluent of the OMW acidogenic fermentation at a considerably higher organic load in order to saturate its PHA storage capacity.

## Methodology

The OMW (COD: 37 g/L) acidogenic fermentation was performed in an up-flow anaerobic packed-bed biofilm reactor (PBBR) consisted of 2.5 L-hermetically closed and thermostated (25°C) glass column equipped with a recycle line. High-porous ceramic cubes (Vukopor S10) were employed as the packing material. The reactor, originally inoculated with the anaerobic OMW-digesting microbial consortium employed in a previous research (Bertin *et al.*, 2004), was fed at HRT and OLR of about 1,4 day and 26 g/L/day, respectively (considering the actual reaction volume). COD, total phenols concentration, amount and composition of the produced biogas were periodically determined (Bertin *et al.*, 2004).

The second stage was studied in a lab-scale aerobic mechanically-stirred SBR (working volume 1 L, temperature 25°C). The length of the SBR cycle was 2 hours, consisting of a feed phase (10 min), a reaction phase (109 min) and a withdrawal phase of the mixed liquor (1 min). No settling phase was performed and all excess biomass was withdrawn with the mixed liquor. In this way, biomass retention time was equal to the hydraulic retention time (1 day). The effluent from the OMW fermentation stage was diluted by mean of a mineral medium (Dionisi *et al.*, 2004) in order to feed the reactor with an OLR of 8.5 gCOD/L/day. The pH of the SBR was controlled at 7.5 by CO<sub>2</sub> additions from a compressed gas cylinder. Biomass concentration, volatile suspended solids (VSS) and PHAs (as reported in Braunegger *et al.*, 1978) were periodically measured.

The third stage of the process consisted in batch assays carried out within the SBR reactor, fed with different concentrations of the same anaerobic effluent, corresponding to about 1, 3, and 9 times the usual load in a single SBR cycle. During the tests, the reactor was sampled at regular intervals and no new feed was given for at least 2, 4, or 6 hours.

## Results

The COD concentration of the anaerobic effluent was about 34 g/L, indicating that only a little fraction (about 8%) of the influent substrate was lost as methane and produced biomass. The anaerobic fermentation stage increased the VFA percentage in the OMW from about the 18% to about the 32% of its COD, reaching the value of about 11 g<sub>COD</sub>/L.

The VSS concentrations in the SBR at 30 minutes from the beginning of the cycle and at the end of the cycle were quite similar, with a corresponding biomass productivity of about 5.3 g<sub>COD</sub>/L/day. Even if VFAs were only a fraction of the overall COD of the fermented OMWs, a selective pressure on the enrichment of mixed cultures able to store PHAs was maintained in the reactor. The PHA content in the biomass, at 30 minutes from the beginning of the cycle, was around 4% (mg<sub>COD</sub>/mg<sub>COD</sub>). Importantly, the stored polymer rate and yield (about 0.16 g<sub>COD</sub> of PHA/ g<sub>COD</sub> non-polymer biomass/h and 0.36 COD/COD, respectively) obtained with the fermented OMWs were similar to those obtained with synthetic mixtures of VFAs applied in preliminary experiments (in the range 0.18-0.39 g<sub>COD</sub> of PHA/ g<sub>COD</sub> non-polymer biomass/h and 0.34-0.53 COD/COD, respectively).

Concerning the third-step batch test carried out at the highest load, the polymer was rapidly and almost linearly stored and reached its maximum concentration (about 1.6 g<sub>COD</sub>/L) within 2.5 hours. An initial faster COD removal rate was observed, probably due to the rapid conversion into PHAs of the readily COD biodegradable fraction (such as VFAs and alcohols) contained in the fermented OMW. Indeed, a mass balance indicated that the maximum amount of PHAs theoretically produced from the fed VFAs was about 0.9 g<sub>COD</sub>/L. Thus, substrates different from VFAs were also stored into PHAs. Polymer content linearly increased at increasing feeding concentrations, so demonstrating the non-inhibitory nature of fermented OMWs on PHAs production. Particularly, only slight decrease of the PHA storage yield in the biomass was observed, so that PHA content in the biomass at the highest load was higher than 35% (mg<sub>COD</sub>/ mg<sub>COD</sub>).

## Conclusions

Within the first stage of a biological three-stage PHA producing process, carried out in an anaerobic PBBR, OMW fermentation allowed a large production of VFAs (representing the substrate for PHA synthesis) whose concentration increased to about 11 g<sub>COD</sub>/L, representing the 32% of the overall effluent COD. Such a percentage was enough so that a selective pressure on the enrichment of mixed cultures able to store PHAs was obtained in the second stage, carried out in a SBR. The final process stage was carried out at increasing anaerobic effluent loads (from 1 to about 9 times the load of a single SBR cycle). The non-inhibitory nature of the fermented OMWs was demonstrated, so suggesting the possibility of operating the process at higher OLR. Moreover, almost half of the produced PHAs derived from substrates other than VFAs, indicating that fermented OMWs contained other soluble COD able to support the polymer production.

In conclusion, the proposed process is promising for the simultaneous treatment of OMWs and their valorization as a renewable resource for PHA production.

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# Utilisation of Native Substances for Crop and Seed Production

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Stress abiotic factors, especially drought and high temperature affect basic metabolic plant pathways of the plants, chemical composition of seeds, yield, seed traits and traits of sprouting plants. These changes of the seeds have influence on vigour of sprouting plants and efficiency of water utilisation.

For this purpose utilisation of native substances for crop and seed production is alternative for seed production under stress conditions.

Unicum is really new homeopathic plant growth and immunity stimulator. Unicum is a highly effective complex of biologically active organic substances extracted and processed from the greenery of mountain coniferous trees and intended for maximum realization of plants yielding potential by improving their growth, development and non-specific overall resistance to a wide scale of stresses ( biotic and abiotic).

AVA is new fertilizer with very slow release and uniform diffusion of minerals. AVA is a highly concentrated fertilizer of wide scale of mineral and trace elements in a form of water-soluble phosphate glass with an ability to operate a self solution process in the soil in dependence with the time of the year (temperature) and without the risk of their overfertilizing.

The Bion 50 WG or Bioalgeen-S90 or are products of brown seaweeds. The Bion 50 WG or Bioalgeen-S90 have positive influence on the root development especially in drought conditions.

## Methodology

The aim of this study was to evaluate main effect of The Bion 50 WG, Bioalgeen-S90, fertilizer AVA or Unicum as a growth biostimulators and possibilities of this products to decrease influence of biotic and abiotic stresses. During three years (2004, 2005 and 2006) the pot experiment were conducted in a randomized complete block design in four replicates with a three factorial arrangement of treatments. For control field experiments were conducted.

The second basic aim of the presented work is comparison of the effect of above named substances in the standard conditions and in the stress conditions, i.e. under influence of drought and high temperature during vegetation period on the traits of seeds, on the differences between laboratory determined germinative capacity and field germination (= field emergence rate), on the water use efficiency, water content of plants and on the contamination by fungi. In all experiments the cultivars of wheat, haricot, buckwheat, jointing sleeve and rose mallow were used.

## Results

According to the large volume of results, only short commentary is presented. Stress abiotic factors, especially drought and high temperature affect basic metabolic plant pathways of the plants, chemical composition of seeds, yield, seed traits and traits of sprouting plants. These changes of the seeds have influence on vigour of sprouting plants and efficiency of water utilisation, but application of tested

native substances has positive effect-decrease of influence of abiotic stresses.

The application of Unicum or Bio-Algeen S-90 (or combination of this natural components) during vegetation period has substantial positive influence on plants which growth under stress conditions. Application of designated natural chemical components improve in the filial generation effect of water utilisation during seed germination, i.e., the rapidity of water uptake, decreases rapidity of water loss at drought conditions and capability of the plants to growth at minimum possible water content. After application of this substances large decrease of contamination by fungi exists.

The Bion 50 WG and Bio-Algeen S-90 are product of brown seaweed and has very positive influence on root development and water efficiency utilisation. Application of combination of „Bioalgeen“ products and Unicum has also positive effect on the plant stress tolerance.

There is possibility to conclude that above named natural active substances can be utilised in seed production at selected crops, especially under stress conditions. Similar results were obtained after application of fertilizer AVA. These changes of the seeds are connected with change of vigour of sprouting plants and efficiency of water utilisation.

## **Conclusion**

Utilisation of native substance give promising results in the stress conditions and it is also possible to use this method for ecological production of the seeds .In standard conditions influence isn't significant

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# What is „Fate“ of 24–Epibrassinolide After its Application on Plants Before Flowering?

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Seeds of the analysed wheat cultivars have a natural content of 24-epiBL. The application of this phytohormone on the plants' surface at the beginning of flowering leads to a substantial increase of this phytohormone content in the seeds. This transport of 24-epiBL to the seeds has previously shown a positive effect on the vigour, and other traits, of the seeds.

## Introduction

Seeds are used especially for protein production, pharmacy, production of carbohydrates, oil baked products, livestock and plant production. The possibilities of understanding the relationship between seed development, environment and quality at the molecular, cellular, physiological and agronomical level, are the basic aim of seed science. The interactions between abscisic acid (ABA), gibberellins (GA) and brassinosteroids (BR) are regulating the key processes that determine dormancy and germination. Absciscic acid inhibits germination and gibberellins, but on the other hand, brassinosteroids promote germination. The basic aim of the presented work is to answer the question of whether the transport of 24-epibrassinolide, after its application on the wheat plants at the beginning of flowering, into the developed seeds exists, i.e. if better seed quality, especially seed vigour, is only an effect of better metabolic plant pathways after application of this hormone or if this hormone is transported to the seeds.

## Methodology

### Greenhouse experiments:

The greenhouse pot experiments with winter wheat cultivars Estica, Samanta and Ebi were performed. One portion of seeds (C) was used as a control experiment, without any treatment. The second portion of seeds (F) was treated with a spray of  $10^{-9}$  M solution of 24-epibrassinolide at the beginning of plant flowering. The third portion of seeds (S) was soaked in  $10^{-9}$  M solution of 24-

epiBL for 30 minutes. After soaking, the seeds were washed and dry seeds were used as a control for the analysis of 24epiBL content. In all experiments the same seed stock was used and the chosen varieties had different pedigrees in order to avoid the influence of common parents. In the harvested seeds, the extraction and purification of brassinosteroids and UPLC-MS/MS analysis of Brassinosteroids was provided (Swaczynova et al. 2007).

## Results

As it is seen from the results, transport of 24epi-BL from the shoot to the seed exists after the application of  $10^{-9}$  M solution of 24-epiBL at the beginning of flowering. However, as follows from the obtained results, all seeds naturally contain some amount of 24-epiBL and 24-epicastasterone (24-epiCS). After foliar application, the content of 24-epiBL is significantly higher, about 5 times. However, after soaking in the same solution for only 30 minutes, the content of 24-epiBL and also 24-epiCS is much higher, between 25 and 250 times, in comparison with non-treated seeds.

Conditions	Cultivar	24-epibrassinolide	24-epicastasterone
C	Ebi	8.21±1.14	2.67±0.48
C	Samanta	6.01±0.89	3.75±0.53
C	Estica	9.48±1.12	3.17±0.20
F	Ebi	47.21±5.21	3.41±0.23
F	Samanta	53.70±4.82	2.92±0.27
F	Estica	49.97±5.01	2.01±0.37
S	Ebi	615.15±87.34	72.85±8.04
S	Samanta	1590.37±18.55	167.75±4.67
S	Estica	1273.32±99.56	86.04±17.01

## Acknowledgments

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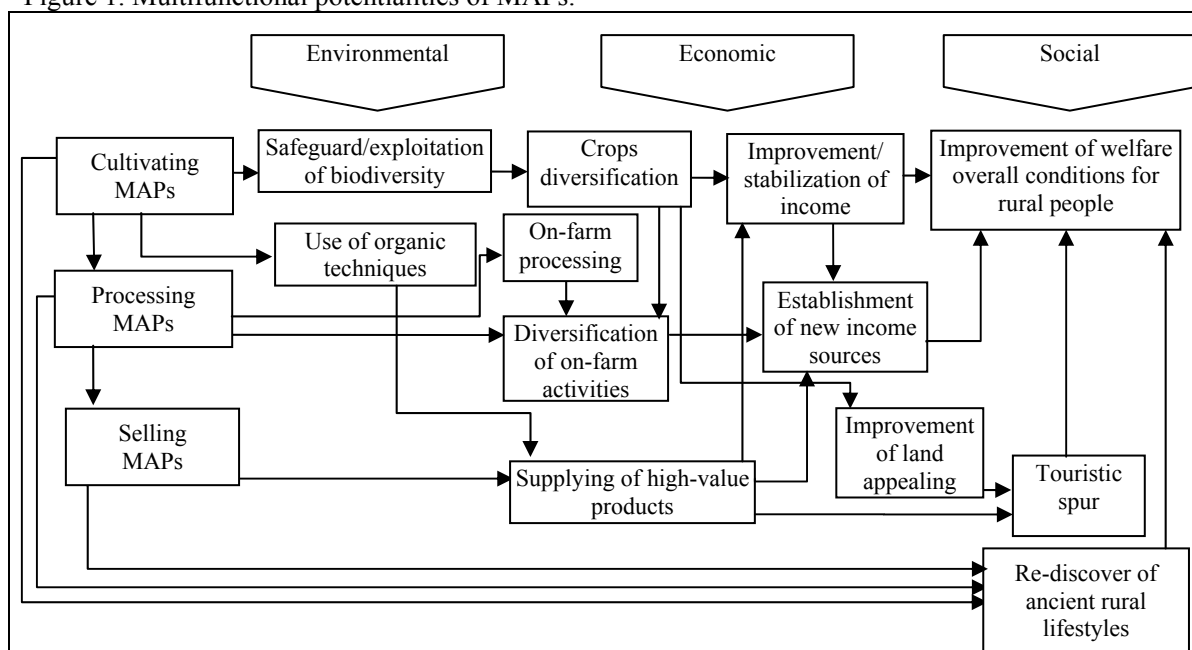
# Multifunctional Role of Medicinal and Aromatic Plants: Perspectives and Constraints

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Multifunctionality is one of the major concerns in agri-environmental issues. All over-national organizations, such as EU, FAO and UN, unanimously claim that the opportunity to supply goods and services other than the “classical” ones is one of the basic steps in order to promote a sustainable and effective development of rural areas. In many Mediterranean environments, often called to severe marginality conditions, such a goal is especially important – and somehow especially difficult to achieve. Medicinal and Aromatic Plants (MAPs) may play a decisive role in this, and their potentialities involve the environmental, economic and social benefits that are, *sensu* FAO (1999), identified as crucial for a multifunctional development of agricultural activity. Fig. 1 illustrates a broad relational scheme of the major issues that are linked to the cultivation – and related economic activities – of MAPs inside rural territories.

Figure 1. Multifunctional potentialities of MAPs.



First, it should be pointed out that there is a tight relationship between MAPs and biodiversity. Besides the obvious consideration that they represent a crucial component in wild flora, their cultivation allows to supply the market without depleting the natural stands. As in many places on Earth, also in the Mediterranean areas MAPs have been representing for centuries the basic sources for food and medicines for local populations. In the oldest utilization form, they were collected from the wild - a practice that did not endangered species until it was not too massive. Today, the increasing interest of industry towards some wild plants has in some cases led to a depletion of natural populations, and

many species all around the world are presently at risk of extinction. In many tropical and subtropical areas, the alarming levels of deforestation and ecosystem degradation have strongly contributed to a decline in MAPs populations. It is obvious that the cultivation on a medium-large scale of the plants that bear a major interest for industrial purposes could be an important step in order to safeguard their natural populations. This concern has a great importance for many species native to the rainy forests of Amazonia, but it is also important for many Mediterranean plants, since a depletion in natural stands has been claimed already for some wild population of Rosemary, Spanish Arnica, Gentian and so on.

The major economic benefit of growing MAPs is linked to the fact that their production may fit in agro-industrial pathways that involve various industrial sectors. Based on the *a priori* choices that farmers may perform, on market needs, on cultural and local tendencies, the introduction of MAPs inside usual cropping systems may represent a land use option to provide new income streams: e.g. Rosemary herb is a commercial item *per se*, but a cultivation of Rosemary may also sustain honey production and beekeeping, or supply the raw matter for further industrial processing (manufacturing of plant extracts, liqueurs, perfumes, antioxidants, and so on). Such a movement of rural economy may lead to decisive economic benefits for farmers.

Finally, MAPs fit very well in organic farming systems, due both to their generally low request of outer inputs (Demarco et al., 1999), and to the enhancement of their “naturalness” content, for which buyers are often willing to pay a considerably higher price.

### **Multifunctionality in Mediterranean farming systems.**

In traditional Mediterranean farming systems, the usual approach used to be a multifunctional one. Various forms of multiple cropping were used, including alley cropping, agroforestry or silvopastoral systems including the simultaneous occurrence on the same territory of different plants, with or without the presence of animals. The shift towards the intensive farming methods has caused the interruption of such activities, and monocropping and specialized production methods have prevailed. Nowadays, a new impulse towards the diversification of farming systems has been given, and the traditional production methods are addressed to a new interest. MAPs may fit very well in diversified production patterns. Besides having *per se* the potentiality to be addressed to a number of utilizations, MAPs may enhance the multifunctional aptitude of the agricultural system as a whole. Such an issue is especially true in agroforestry, where the role of MAPs is well established by a plentiful literature.

### **Constraints**

The first constraint in the exploitation of Mediterranean grown MAPs is productivity: to improve all aspects of productivity of such crops is the first step in order to strengthen their competitiveness, also enhancing their suitability to transformation. In such sense, much work is still to be done in order to set at optimum level the cropping technique to be applied, also under organic management, by setting out the best practices for fertilization, weeds management and mechanization, especially for the more time-consuming operations such as harvest. Some other constraints are still to be solved, such as the scarce availability of specific equipments to be used on-farm, the low market transparency (that makes difficult the establishment of market channels), the high investment costs, the still low level of integration between the various steps from herbs production to marketing, and the rapid expansion of competitive production above all from developing countries.

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# Lowering Nitrate Content of Flue-Cured Tobacco: Reducing Harmfulness by Tuning Nitrogen Fertilization

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In tobacco products and smoke, nitrate reacts with alkaloids to form tobacco specific N-nitrosamines (TSNA), compounds with special relevance in carcinogenesis. Here we show that increasing nitrogen fertilization resulted in substantial increase of nitrate content without gain in productivity. Our findings suggest that a “low-harmful-tobacco” can be obtained by tuning N fertilization.

Supplementing crops with N fertilizers is a key to increasing crop yields. Most of the N absorbed by the plants is incorporated into protein, but a fraction remains in the form of non-protein N, mainly amino acids, and nitrate. In both plants and animals, nitrate can be reduced to nitrite and nitrosation reactions produce carcinogenic N-nitrose compounds (Wolfe and Patz, 2002). Among N-nitrose compounds, the so called tobacco specific N-nitrosamines (TSNA) are recognized to have special significance among the class of carcinogenic compounds identified in tobacco and in the smoke of tobacco (Hoffmann and Hoffmann, 1997). Green and freshly harvested tobacco leaves contain nitrate but are virtually free of TSNA. It is during the post-harvest processing, and also during tobacco combustion, that nitrate is reduced to nitrite which then reacts with tobacco alkaloids to form TSNA (Fisher et al., 1989). The scope of this study was to evaluate the effects of N fertilization on both leaf nitrate content and productivity of flue-cured tobacco.

## Methodology

A field experiment was conducted during the years 1997, 1998 and 1999 in Bovolone (Verona), Italy (Lat. 45°16' N, Long. 11°07' E). The soil of the site was classified as coarse-loamy, mixed, mesic, superactive Fluventic Haplustept. Five N rates were applied to flue-cured tobacco (cv. K326) in a randomised block design with 2 replications. The rates of N supply were 0, 15, 30, 45 and 60 kg N ha<sup>-1</sup> in 1997 and 0, 20, 40, 60 and 80 kg N ha<sup>-1</sup> in both 1998 and 1999. Nitrogen was supplied as calcium nitrate, two weeks after crop transplantation. The size of individual plots was 200 m<sup>2</sup>. Following the traditional practice of the region, tobacco leaves were harvested, when ripe, in four primings per year. On each harvest date, a fresh leave sample was collected from 50 plants (i.e. one entire row), corresponding to 25 m<sup>2</sup>, on the inner part of each plot. Each sample of fresh leaves was cured in barns wherein humidity, temperature and air flux are constantly regulated to obtain a proper tobacco quality. Subsequently, sub-samples of the flue-cured product were collected for laboratory analysis of nitrate content. Nitrates were analyzed by a colorimetric method after reduction with hydrazine sulphate and Cu<sup>2+</sup>.

## Results

Overall, the level of nitrate concentration on the one hand, and crop productivity on the other, were profoundly influenced by the year of cultivation (Fig.1a-b). The nitrate concentration of the unfertilized treatment 0 kg N ha<sup>-1</sup> was 42 mg kg<sup>-1</sup> in 1997, 22 mg kg<sup>-1</sup> in 1998 and 37 mg kg<sup>-1</sup> in 1999. The rate of N supply 40 kg N ha<sup>-1</sup> increased nitrate content significantly (P<0.001) in 1999 but not in the previous years. Annual rates of N supply equal to or higher than 60 kg N ha<sup>-1</sup> increased significantly (P<0.01 in

1997 and 1998;  $P < 0.001$  in 1999) the nitrate content compared to 0 rate. In particular, the N supply 60 kg N ha<sup>-1</sup> increased nitrate concentration by +71% in 1998 and +333% in 1999. The highest N supply 80 kg N ha<sup>-1</sup> increased nitrate content by +106% in 1998 and +421% in 1999. It is worth noting that such increases in nitrate content were never accompanied by a substantial gain on biomass yield. Under our experimental conditions, the N released by the soil was sufficient to sustain the needs of tobacco growth while N fertilization, in most of the cases, undesirably raised nitrate concentration in the leaves.

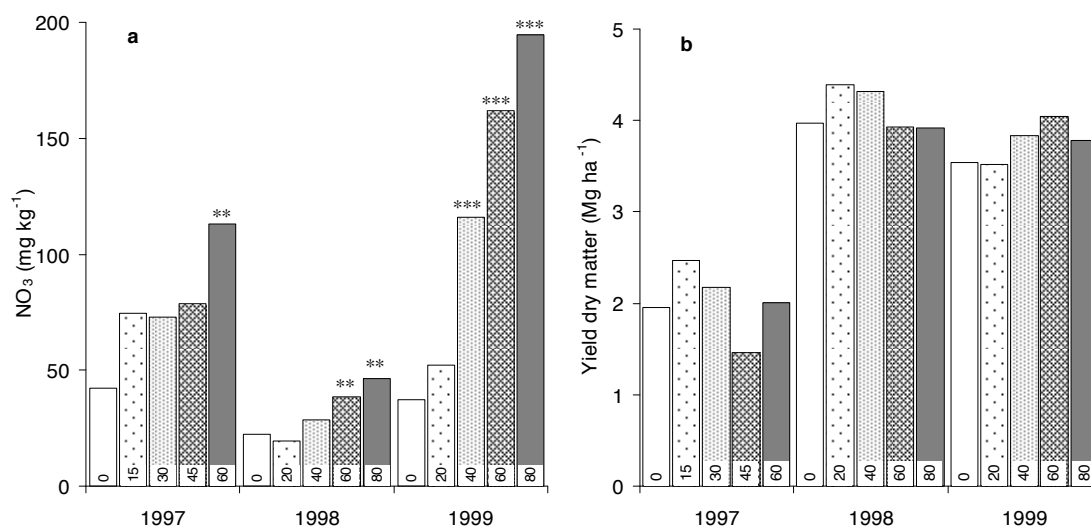


Figure 1. Leaf nitrate content (a) and yield dry matter (b) of flue-cured tobacco in response to N supply for the years 1997, 1998 and 1999. N rates of application are indicated within bars. Asterisks indicate that treatments are significantly different to 0 N supply within individual years, for  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*), respectively.

## Conclusions

Since nitrate content is related to TSNA content, we postulate that a strategy of “low-N-fertilization” could play a role in alleviating the social costs of tobacco smoking by reducing the incidence of tobacco-related diseases. A healthy tobacco does not exist and will not exist. On the other hand, tobacco addiction and exposure is likely to persist, despite to the increasing prohibition rules devised by governments and judicious information campaigns promoted by Medical Associations. Therefore, it is an ethical task for enlightened societies to adopt adequate policy actions in order to mitigate the incidence of tobacco-related diseases. In this context, we suggest that tobacco growers should be encouraged to “low-harmful-tobacco” by economic incentives. If a higher price have to be accorded to farmers producing low nitrate tobacco, then such a strategy is likely to decrease, in the long term, the annual number of deaths and economic losses attributable to tobacco smoke.

## Acknowledgments

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# The Stinging Nettle (*Urtica dioica* L.): a Neglected, Multifunctional Species for a Low-impact Land Use

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The perennial stinging nettle (*Urtica dioica* L.) is a well known world-wide spread herbaceous species growing on ruderal sites, at the edges of forests and in wooded areas of riverline floodplains. Even if considered by the contemporary agriculture as a weed to be eliminated, stinging nettle has great potentials as multi-purpose crop under a low input cultivation.

The entire plant or specific organs of nettle have specific uses in several areas: in medicine and in herbal medicine the dried leaves (used as infusion) or the juice obtained from the whole plant demonstrated anti-inflammatory and diuretic properties; in bio-dynamic agriculture stinging nettle is used as pest control and growth stimulator. Nettle is also employed for chlorophyll production, for fodder and in human alimentation (soups and spinach) (Vogl and Hartl, 2003).

A historically important aspect of nettle exploitation is the high content of bast fibre (similarly to hemp and flax), used in textile industry in Europe until the Second World War. The introduction of cotton and other cheaper fibres caused a decline in fibre nettle production and consequentially of its cultivation. Nevertheless, due to the growing demand of natural, healthy and hypoallergenic products, processors and traders of organic textiles have sought to diversify their products by introducing nettle fibers into the organic textile market. Furthermore, thanks to the limited number of pest and disease and to low agronomical care requirements, fibre nettle has a great feasibility to be cultivated by organic farming (Vogl and Hartl, 2003).

Coupled to the commercial exploitation, the cultivation of nettle can also lead to several environmental benefits, such as increasing biodiversity of traditional farming, soil erosion reduction as a perennial crop, recovery of overfertilized soils as nitrophylic species.

In this frame the development of a sustainable production chain, coupled with the necessity of an high and stable quality of the fibre, must be supported by the research for the selection of suitable clones and the development of specific agronomical practices.

## Methodology

Under this positive perspective of the cultivation of fibre nettle, our Institute has been involved in several activities related to the definition of the cultivation and quality aspects, in the framework of the LaMMA-TEST project “Technologies for the Textile Chain”, founded by the Tuscany Region in collaboration with the National Research Council and the Province of Prato. The aim of the project is to supply supporting tools for the environmental sustainable development of the textile chain.

Practical activities have been conducted by our Institute related to the assessment of the nettle cultivation. Due to the high level of heterozygosity, the homogeneity in nettle fibre production of selected plants is not guaranteed by seed propagation; then pure material and a homogeneous crop can only be achieved by vegetative propagation. In this frame researches on clonal propagation were conducted with the aim to obtain both *in vivo* and *in vitro* propagation protocols. At the same time the assessment of low input agronomic techniques for stinging nettle cultivation were pursued.

Genetic improvement researches on stinging nettle were conducted aimed to the selection of stable enhanced clones with high quality fibre. Fibre clones have been first selected by Bredemann (1959), obtaining bark fibre content and quality comparable to hemp and flax. A recovered Bredemann clone was cultured *in vitro* and treated with increasing doses of gamma radiations (25, 30, 35, 40 Gy), in

order to induce mutations with improved multifunctional characteristics. The doses were chosen based on the previously assessed lethal dose. Surviving plants were propagated then transferred in an open field for a selection based on morphological and physicochemical properties.

## Results

Regarding the propagation techniques, stinging nettle demonstrated a good attitude for the *in vivo* propagation systems by cutting, with and without hormonal treatment for root induction.

The *in vitro* assessment of cultures and the following steps of propagation, rooting and acclimatization well suited on stinging nettle. A propagation medium and a rooting medium were defined (Table 1).

Tab. 1: Components and concentration of the defined medium for propagation and radication of microcuttings.

Component	Concentration in the propagation medium	Concentration in the rooting medium	Unit
Sucrose	30	30	g l <sup>-1</sup>
Agar	5.5	5.5	g l <sup>-1</sup>
MS salts	4.3	2.15	g l <sup>-1</sup>
Thiamine	0.4	0.4	mg l <sup>-1</sup>
Myo-inositol	0.1	0.1	g l <sup>-1</sup>
BAP	3.08	0	μM
IBA	0.5	1.5	μM

Surviving plants deriving from irradiation after an *in vitro* selection were transferred in open field for a selection based on morphological and physicochemical properties linked to the practical and commercial uses of the crop.

Field trial has been established in order to study the effects of agronomic factors such as sowing season, plant density, harvest time. As a perennial crop, showing the highest performances from the second year of cultivation, deeper investigation and multiple year assessment are in progress for the evaluation of the yields of nettle as a commercial crop. In the second year of trials plants reached a height of 170.8 cm, while the fresh stalk and leaves biomass was 4225.9 g·m<sup>-2</sup> and 375 g m<sup>-2</sup>, respectively. Dry biomass resulted 1542.1 g·m<sup>-2</sup> and 108.8 g·m<sup>-2</sup> for stalk and leaves, respectively.

## Conclusions

The multi-purpose strategy, coupled with the low environmental impact of its cultivation, could help to promote a neglected species as a newly emerging crop.

## Acknowledgements

This work was carried out in the framework of LaMMa-TEST project “Technologies for the Textile Chain”, founded by the Tuscany Region in collaboration with the National Research Council and the Province of Prato.

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# Legume Crops as Sources of Functional Compounds: Flavonoids in Seeds and Sprouts

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The recent development of the nutraceutical market is bringing up an increased demand of crops rich in compounds with beneficial effects on human health. Among these flavonoids, and more generally polyphenols, are an important class of plant secondary metabolites with proven bioactivity. The most appreciable sources of flavonoids in crops are grain legumes (particularly beans, chickpeas, peas), forage legumes (clover and alfalfa) and soybean. Numerous studies about flavonoid content have been performed on different genotypes of soybean, whereas available information about other legume species are quite scarce. In this context a scientific effort aimed at finding a “Mediterranean” alternative to soybean as source of functional compounds is of particular interest.

The objective of the present research was to screen a wide germplasm collection, composed of several accessions of grain legumes, for their phytochemical profiles in seeds and seedlings.

## Methodology

35 grain legume accessions were investigated for their flavonoid content in both seeds and seedlings. For comparison 8 soybean (*Glycine max* L.) cultivars, known for their high flavonoid (isoflavones) content, were also included in the analysis. The investigated plant material is part of a wider germplasm collection composed of several accessions of forage and grain legumes available at the Department of Agroenvironmental Science and Technology, University of Bologna, Italy.

Flavonoids were extracted following the procedure described by Romani et al. (2005). Aglycone forms of glycoside flavonoids were obtained by acid hydrolysis of the extracts as previously described by Dinelli et al. (2006). The solutions were filtered through a 0.45 µm nylon filter prior to HPLC-DAD analyses.

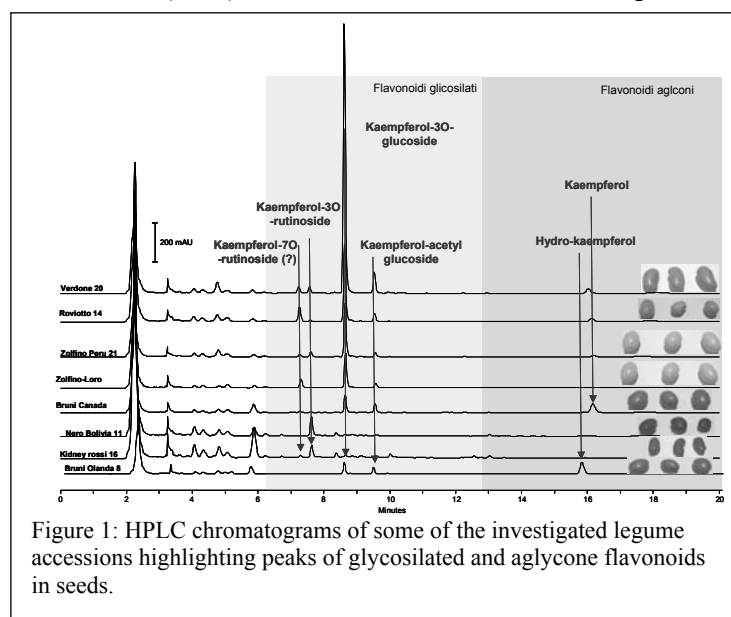


Figure 1: HPLC chromatograms of some of the investigated legume accessions highlighting peaks of glycosylated and aglycone flavonoids in seeds.

total flavonoid content.

## Results

Results highlighted a broad range of variability in bioactive compounds under both qualitative and quantitative composition. In particular, most of the seeds of investigated accessions contained conjugated forms of the flavonoid kaempferol (Figure 1). The compound kaempferol 3-O-glucoside was the main flavonoid found in all samples with a percentage ranging between 47% and 68% of total flavonoid content. The second most abundant flavonoid was kaempferol 3-O-xylosylglucoside with a percentage ranging between 16% and 33% of

Other bioactive compounds, such as vitexin, were also detected in seeds of adzuki bean accession (*Vigna angularis*). Beside one common bean accession (ie Zolfino del Perù) with a flavonoid content ( $1713.4 \pm 11.6$  µg/g seed weight) comparable with that detected in soybean seeds, the total flavonoid content of all other investigated accessions varied from  $1.5 \pm 0.5$  (Bianco di Spagna) to  $569.8 \pm 2.6$  (Roviotto) µg/g seed weight.

All accessions were also analyzed to identify and quantify flavonoids in sprouts. Daidzein, glycitein

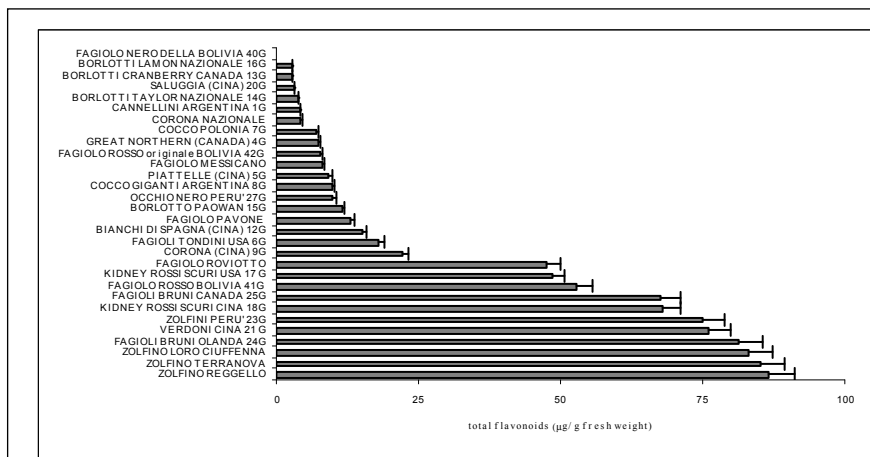


Figure 2. Total flavonoid content (µg/g fresh weight) detected in sprouts of some of the investigated legume accessions.

and genistein, along with kaempferol and quercetin were detected in most of analyzed accessions with amounts ranging from 2.8 to 86.7 µg/g fresh weight (Figure 2) (approximately 3-10 times lower than isoflavones in soybean sprouts). The great variability detected in the investigated accessions

highlighted the marked incidence of the genotype in determining the functional compound content of sprouts. The highest value were detected in the accessions Zolfino Reggello ( $86.79 \pm 5.65$  µg/g fresh weight), Zolfino Terranova Braccioloni ( $85.2 \pm 4.6$  µg/g fresh weight), Zolfino Loro Ciuffenna ( $83.1 \pm 5.6$  µg/g fresh weight), Bruno Olanda ( $81.4 \pm 3.6$  µg/g fresh weight) and Verdone Cina ( $75.9 \pm 3.6$  µg/g fresh weight). In these genotypes the flavonoid content is almost comparable to the total flavonoid content detected in soybean accessions ( $104.4 \pm 5.8$  µg/g fresh weight).

## Conclusions

Despite many phytochemicals investigations on constituents of several leguminous species have been reported, studies focused mainly on the aerial parts (leaves, stems, flowers) of the plant (Williams et al., 1995). Data from the present study provided new insight on the flavonoid composition of several grain legume accessions in seeds. The identification of a bioactive compound, such as kaempferol, that may have health-promoting effects in humans, resulted to be of great interest in a perspective of source of functional compounds for nutraceutical application. In addition, to the best of our knowledge, this is the first time that sprouts of grain legumes other than soybean are proposed as sources of health-promoting phytochemicals (flavonoids).

To summarize, the present research highlighted the possibility of considering some of the investigated legume accessions as a valuable and "Mediterranean" alternative to soybean to obtain plant-derived products with health benefits.

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# Woad (*Isatis Tinctoria* L.): a New Use for a Multifunctional Crop

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Since ancient times woad (*Isatis tinctoria* L.) has been cultivated in Europe to produce a blue pigment from the leaves. The cultivation lost importance at the beginning of the 1900s, due to the production of synthetic indigo. Recently, the increasing demand for natural dyes has encouraged woad cultivation once again. The plant is also widespread as a weed throughout Europe. Woad is a multifunctional crop: the seeds contain essential oils used to produce moisturizing cosmetics. Woad is also considered a medicinal plant, due to its anti-inflammatory properties, but it is also a noticeable source of indole-type glucosinolates, bioactive molecules exploitable as fine chemicals. The most interesting one is glucobrassicin (GBS), the natural precursor of indole-3-carbyinol, an anti-tumoural compound, active particularly against estrogen-responsive tumours (Michnowicz and Bradlow, 1990; Kim and Milner, 2005). Epidemiological studies suggested a protective effect against breast cancer associated with a high intake of broccoli, due to a shift in estrogen metabolism (Fowke et al., 2000). Broccoli was considered a good source of GBS, until a recent study reported that chemically treated woad leaves contained much higher GBS levels than mature broccoli (Galletti et al., 2006). A purification method was thus assessed which made it possible to produce pure GBS (Iori et al., 2003) and to start studies regarding its anti-tumoural properties (Iori et al., 2008). Although woad is not considered as an edible vegetable worldwide, rural people living in Sicily around Mount Etna are used to eating the flower buds collected from the spontaneous plants, commonly found on abandoned land (Branca, 1992). This study reports data on the GBS content of the flower buds of some woad populations, sampled around Mount Etna over two years, in order to determine the GBS level ingested with the diet.

## Methodology

In March/April 2007 and 2008 large samples (about 500 g) of floral buds of spontaneous populations of woad were collected in 4 localities around Mount Etna in Sicily, Italy, as reported in the table. Samples were freeze-dried and ground to powder prior to HPLC analysis of glucosinolates, performed according to the ISO 9167-1 method. Each sample was analysed four times and sinigrin was used as the internal standard. The glucosinolates were identified by standard desulfoglucosinolates available in our laboratory and then quantified considering the amount of the internal standard and the response factor of the identified glucosinolates.

Data were submitted to analysis of variance by Statgraphics plus 5.1 statistical program and means were separated by l.s.d. test at  $p \leq 0.05$  significance level.

## Results

HPLC chromatograms reported two main peaks in all the samples, corresponding to GBS and gluconapin (GNA) with values ranging from 33 to 69  $\mu\text{mol g}^{-1}$  dw for GBS and from 33 to 65  $\mu\text{mol g}^{-1}$  dw for GNA, as reported in the table. The statistical analysis showed a significant interaction for year x locality. Nevertheless, in both years the Pedara population showed the highest GBS values while the one sampled at Linguaglossa showed the lowest values. The other two populations instead showed a lower GBS value in the second year and, conversely, higher GNA values. However, the GBS mean value of the Mount Etna populations sampled in this study is 10-50-fold higher than that of mature broccoli or Brussel sprouts (1-3.2  $\mu\text{mol g}^{-1}$  dw) (Kushad et al., 1999). This finding highlights that the floral buds of woad represent an extremely interesting source of dietary GBS, which should have protective effect against breast cancer, as suggested by epidemiological studies on GBS-containing

vegetables (Fowke et al, 2000). Since the availability of the fresh product is restricted to a short period of the year, dried extracts could be produced to be proposed as diet integrators.

Finally, concerning the purification of GBS for fine chemical purposes, the values of floral buds reported here are suitable for purification of the compound. These amounts of GBS are as high as those obtained in a previous study concerning young leaves of the Italian accession Casolavalsenio, after jasmonic acid treatment (Galletti et al., 2006). Thus floral buds of Mount Etna populations do represent a rich source for the purification of GBS in high yield at low cost, avoiding any stimulation practices to enhance GBS content.

Glucobrassicin and gluconapin content ( $\mu\text{mol g}^{-1}$  dw) of floral buds of woad sampled in 4 Mount Etna localities (Sicily, Italy) in 2007 and 2008.

Localities	Slope	Altitude (m a.s.l.)	Glucobrassicin ( $\mu\text{mol g}^{-1}$ dw $\pm$ sd)		Gluconapin ( $\mu\text{mol g}^{-1}$ dw $\pm$ sd)	
			2007	2008	2007	2008
Mascali	East	870	68.8 $\pm$ 3.3 a*	35.8 $\pm$ 0.3 b	33.5 $\pm$ 2.1 c	55.4 $\pm$ 1.3 b
Linguaglossa	North	550	34.1 $\pm$ 3.0 c	35.3 $\pm$ 1.7 b	45.4 $\pm$ 2.9 a	47.2 $\pm$ 4.0 c
Maletto	West	960	56.1 $\pm$ 6.4 b	33.1 $\pm$ 2.6 b	43.4 $\pm$ 3.3 b	65.1 $\pm$ 4.6 a
Pedara	South	700	68.7 $\pm$ 6.0 a	60.8 $\pm$ 0.5 a	34.6 $\pm$ 1.9 c	40.8 $\pm$ 2.7d
mean		-	56.9 $\pm$ 16.4	41.3 $\pm$ 13.1	39.2 $\pm$ 6.1	52.1 $\pm$ 10.5

\*Values in columns followed by the same letter are not different for  $p \leq 0.05$  after ANOVA (l.s.d. test).

## Conclusions

Woad is a multifunctional crop which can be exploited as a dye plant and, maybe more important, as functional food, in the form of fresh product or dried extracts, with chemopreventive properties. Floral buds also represent a rich source of GBS, an extremely interesting compound for fine chemical purposes.

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# Influence of Application 24-Epibrassinolide During Seed Wheat Development at the Stress Conditions

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## Introduction

Brassinosteroids are endogenous hormones that have a structure very similar to steroids. The first molecule of brassinosteroid was identified by Grove in 1979, who named it brassinolide (Grove et al., 1979). Brassinosteroids are also capable of diminishing the effects of negative factors of habitats, such as low or high temperatures (Jiang, Wang, 1996; Kulajeva et al., 1991), lack of water, soil salinity, effects of pesticides and phytopathogens (Takematsu, Takeuchi, 1999; Khripach et al., 1997). They may also influence the uptake of certain minerals. The final effects of brassinosteroids include increased yield and improvement in quality of field crops (Khripach et al., 2000). Structural analogues of brassinosteroids are registered in several countries as growth regulators for several plants (Khripach et al., 1997). Brassinosteroids have been recommended by several research projects as perspective substances, improving crop yield, where application of 24-epibrassinolide during the period of flowering usually has positive effects on development of seeds and development and growth of sprouts germinating from these seeds in the next generation.

## Methodology

The analysis of the influence of abiotic stresses and possibilities of reduction their negative effect by application of 24-epibrassinolide was provided. The pot experiments with standard volume of homogenized soil in greenhouse experiments were used with the three winter wheat cultivars Ebi, Estica and Samanta and spring wheat cultivars AC Helena, Lucia and Mollera. The pots were divided into three treatments of experiment alternatives, see Table I.

Table I: The schene of trial

Variant	Watering	Temperature	Light regime	Stress induced
Control	70 % of field water capacity	23 °C/ 15 °C	16/8 h	-
Stress	37 % of field water capacity	33 °C/ 20 °C	16/8 h	40.DC
Stress + 24 - epibrassinolide	37 % of field water capacity	33 °C/ 20 °C	16/8 h	40.DC

The watering was regulated in accordance with results from the VIRIB device (firm Litchman, Czech Republic). The spraying of 24 -epibrassinolide took place at the begin of flowering (61.DC), using 24-epibrassinolide solution with concentration of ( $10^{-9}$  M). After harvest of the seeds, the analysis of crude

proteins content, lipids content, total starch content, damaged starch content, dry matters (DM) weight and net energy content were provided. A statistical programme Statistica, version 6.1. Cz, statistical methods ANOVA was used for processing of statistical data, at significance level  $\alpha=0.05$ .

## Results

The stress treatment with application of 24-epibrassinolide decreased the starch content, as in the case of protein or lipids content, while different varieties respond to treatment differently. Statistically significant differences in starch content among treatments were obtained (Table IIa).

Table II: The HSD test by Tukey; marked. differences (\*) are significant on significance level  $p < 0.05$

a) Variable: Content of starch			b) Variable: Energy content		
Variant	{1}	{2}	{1}	{2}	{3}
Control {1}		0.000112 *		0.000112 *	0.000249 *
Stress {2}	0.000112 *		0.000112 *		0.061862
Stress and spray {3}	0.000442 *	0.011921 *	0.000249 *	0.061862	

The largest starch content was at control (66.40 g.100 g<sup>-1</sup> DM), the lowest content was at stress version (61.42 g.100 g<sup>-1</sup> DM). The application of 24-epibrassinolide increases starch content of stress experiment to the 63.51 g.100 g<sup>-1</sup> DM. Statistical analysis of the influence of variety on the damaged starch ratio shows that the lowest ratio was found in the variety Samanta (average value of all three experiment alternatives 1.79 g.100 g<sup>-1</sup> DM), while the highest in the varieties Lucia and AC Helena (average value of all three experiment alternatives 4.91 and 4.85 g.100 g<sup>-1</sup> DM). The experimental results clearly indicate that the use of 24-epibrassinolide demonstrably decreased the effects of non-biotic stress factors on the energy content, as shown in Table IIb. In the case of wheat plant, both spring and winter forms, the stressed plants were treated with 24-epibrassinolide, expected to have a similar effect as abscis acid (ABA).

## Conclusions

It follows from the obtained results that the stress abiotic factors i.e. combination of drought and high temperatures significantly affect decrease of measured traits of the the grain. There is possibility to conclude that differences among measured traits at cultivars and at different type of environments exist. The application of 24-epibrassinolide at stress conditions has positive effect on the traits of the stressed plants but level of values of the control treatment weren't obtained. In case of analysis of influence of above named phytohormone on the stress tolerant cultivars any effect was obtained. There is possibility to use this effect in seed production at selected plant species.

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## Acknowledgement

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# The Effect of 24 – Epibrassinolide on Gases Exchange of Wheat

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## Introduction

The current changes in weather bring with them a evident fluctuation in temperatures and also a relatively irregular and random distribution of precipitation during the vegetation period of field crops. Therefore a study of plants' adaptation to a water deficit is ever more topical, as the water deficit leads to a fall in the uptake of nutrients, a restriction on photosynthesis, dry matter formation, the amount and quality of the yield. The negative effect of a water deficit can be alleviated by the application of certain natural and synthetic compounds. Of the natural compounds, the brassinosteroids have such effect. Brassinosteroids were first identified by Grove in 1979, who labelled them as brassinolides (Grove *et al.*, 1979). Brassinosteroids can reduce the negative impact of the external environment such as low or high temperatures (Jiang and Wang, 1996), a lack of water, saline soil, the influence of pesticides and phytopathogenic organisms (Takematsu and Takeuchi, 1999). The final consequence is an increase in yield (Khripach *et al.*, 2000 and Sakurai, 1999). The aims of trial was acknowledge of influence 24-epibrassinolide on rate of photosynthesis and rate of transpiration by wheat's plants under drought and high temperature.

## Methodology

The analysis of the influence of abiotic stresses and possibilities of reduction their negative effect by application of 24-epibrassinolide was provided. The pot experiments with standard volume of homogenized soil in greenhouse experiments were used with the three winter wheat cultivars Ebi, Estica and Samanta and spring wheat cultivars AC Helena, Lucia and Mollera. The pots were divided into three treatments of experiment alternatives. The first treatment was the standard (control) set with 70 % of the field water capacity, day temperature from 20 to 25 °C and at night temperature 15 °C. The second treatment represented a combination of stress by drought and high temperatures. For this alternative, the daily average temperature was 33 °C and at night 20 °C, soil water content 35 to 40 %. The watering was regulated in accordance with results from the VIRIB device (firm Litchman, Czech Republic). The third treatment of experiments were the stress versions with application of 24-epibrassinolide as spraying. The spraying took place at the begin of flowering (61.DC), using 24-epibrassinolide solution with concentration of ( $10^{-9}$  M). The photosynthesis and transpiration rates were measured gasometrically in the plants in two-intervals. The said characteristics were measured by a portable infrared analyser LCpro+ (ADC Bio Scientific Ltd.). A statistical programme Statistica, version 6.1. Cz, statistical methods ANOVA was used for processing of statistical data, at significance level  $\alpha = 0.05$ .

## Results

Tab 1a shows that the photosynthesis and transpiration rate was affected not only by the version of the experiment but also by the time of measurement. In the stressed plants, it is possible to see a gradual decrease of photosynthesis rate, depending on the duration of water deficit. It is apparent from the obtained results that the photosynthesis rate is provably lower in the stressed plants ( $8.01 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) in comparison with the control plants ( $9.48 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The rate of photosynthesis in the stressed plants

with 24-epibrassinolide was taller by 15.73% than photosynthesis by stressed plants. The genotype Mollera had the average photosynthesis rate  $9.18 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . On the contrary, the cultivar Samanta reduced the photosynthesis rate because the average photosynthesis was  $8.72 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Table I: The HSD test by Tukey; marked. differences (\*) are significant on significance level  $p < 0.05000$

a) Variable: Rate of photosynthesis							b) Variable: Rate of photosynthesis					
Cv.	{1}	{2}	{3}	{4}	{5}	{6}	{1}	{2}	{3}	{4}	{5}	{6}
AC Helena {1}		9.15*	9.15	9.15*	9.15*	9.15*		1.09	1.09*	1.09*	1.09*	1.09*
Lucia {2}	8.93*		8.93*	8.93*	8.93*	8.93*	1.08		1.08*	1.08*	1.08*	1.08*
Mollera {3}	9.18	9.18*		9.18*	9.18*	9.18*	0.85*	0.85*		0.85*	0.85*	0.85*
Samanta{4}	8.72*	8.72*	8.72*		8.72*	8.72*	0.80*	0.80*	0.80*		0.80*	0.80*
Ebi {5}	8.82*	8.82*	8.82*	8.82*		8.82	1.03*	1.03*	1.03*	1.03*		1.03*
Estica {6}	8.83*	8.83*	8.83*	8.83*	8.83		1.06*	1.06*	1.06*	1.06*	1.06*	

Another measured characteristic was the transpiration rate. Similarly like with photosynthesis, transpiration, too, was provably affected by the version of the experiment where stressed maize plants had a lower transpiration rate ( $0.95 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) as compared with the plants in the control group ( $1.03 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The application of 24-epibrassinolide increased the rate of transpiration by the stressed plants rather 1.0%. Transpiration rate was the lowest in the cultivar Samanta ( $0.80 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and the highest average transpiration rate was in the Lucia ( $1.08 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), see tab. Ib.

## Conclusions

The results show that the photosynthesis rate was the lowest in the stressed plants ( $8.01 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and was the highest in the control group ( $9.48 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The lowest transpiration rate was identified in the stressed plants:  $0.95 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , while the highest one was identified in the control plants ( $1.03 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The application of 24-epibrassinolide at stress conditions has positive effect on the photosynthesis of the stressed plants but level of values of the control treatment weren't obtained. The cultivars AC Helena, Samanta are tolerant to the drought and high temperature in comparison with Estica a Lucia which have low level of tolerance. The best reaction of cultivar to the application of phytohormone has at cv. Mollera and on the other hand without any influence was obtained at cultivar Samanta (stress tolerant cultivar).

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# The Influence of the Application of 24-Epibrassinolide on the Formation of Dry Matter and Yield in Wheat

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## Introduction

The current changes in weather bring with them a evident fluctuation in temperatures and also a relatively irregular and random distribution of precipitation during the vegetation period of field crops. Therefore a study of plants' adaptation to a water deficit is ever more topical, as the water deficit leads to a fall in the uptake of nutrients, a restriction on photosynthesis, dry matter formation, the amount and quality of the yield. The negative effect of a water deficit can be alleviated by the application of certain natural and synthetic compounds. Of the natural compounds, the brassinosteroids have such effect. Brassinosteroids were first identified by Grov in 1979, who labelled them as brassinolides. Brassinosteroids can reduce the negative impact of the external environment such as low or high temperatures (Jiang and Wang, 1996), a lack of water, saline soil, the influence of pesticides and phytopathogenic organisms (Takematsu and Takeuchi, 1999). The final consequence is an increase in yield (Khripach et al., 2000).

## Methodology

The analysis of the influence of abiotic stresses and possibilities of reduction their negative effect by application of 24-epibrassinolide was provided. The pot experiments with standard volume of homogenized soil in greenhouse experiments were used with the three winter wheat cultivars Ebi, Estica and Samanta and spring wheat cultivars AC Helena, Lucia and Mollera. The pots were divided into three treatments of experiment alternatives, see Tab. I.

Tab I: The scheme of trial

Variant	Watering	Temperature	Light regime	Stress induced
Control	70 % of field water capacity	23 °C/ 15 °C	16/8 h	-
Stress	37 % of field water capacity	33 °C/ 20 °C	16/8 h	40.DC
Stress and spray	37 % of field water capacity	33 °C/ 20 °C	16/8 h	40.DC

The watering was regulated in accordance with results from the VIRIB device (firm Litchman, Czech Republic). The spraying took place at the begin of flowering (61.DC), using 24-epibrassinolide solution with concentration of ( $10^{-9}$  M). In selected growth phases: 49.DC, 51.DC, 55.DC, 61.DC, 65.DC, 69.DC, 71.DC, 83.DC, 87.DC and 91.DC the weight of dry matter (DM) from the straw and ears was determined. In the phase 91.DC the grain and straw yields were determined. The data obtained were treated using the STATISTICA computer program using a multifactorial Anova with a level of significance level  $\alpha = 0.05$ .

## Results

The results show that the abiotic stressors have a noticeable negative effect on the amount of dry matter of the above-ground biomass in wheat. For this variant the average weight of the straw was 2.35 g and ears 2.80 g. In the control plants the weight of straw was 2.85 and that of the grain 3.40 g. The application of the phytohormone significantly reduced the negative effect of the stressors, as, in comparison with the stressed plants, the dry matter of the straw was higher by 4.26 % and that of the ears by 4.32 %. The amount of dry matter was influenced by the genotype of the variety, where the lowest average weight of dry matter for straw and ears for a average plant was found in the variety Ebi. For this variety the weight of the straw and

ears was 2.43 g and 2.91 g respectively. The highest weights for straw (2.64 g) and ears (3.21 g) were obtained for the variety Samanta. Straw weight ranged from 2.03 g (49.DC) to 2.84 g (69.DC). At the time of full ripeness the weight was 2.50 g. The greatest weight of straw was during flowering. The weight of the ears grew from the start of their formation (49.DC) – 0.68 g up to full ripeness 4.5 g.

The yield of straw and grain following the effect of drought stress and high temperatures was also monitored. The straw yield ranged from 812.50 g.m<sup>-2</sup> (Ebi, stress) to 969.31 g.m<sup>-2</sup> (Samanta, control), as supported by Figure 1. The Ebi variety showed the lowest average straw yield (843.02 g.m<sup>-2</sup>) and Samanta had the highest yield (937.15 g.m<sup>-2</sup>). When evaluating the effect of the variants on the straw yield it can be stated that the stressed plants showed the lowest yield (842.08 g.m<sup>-2</sup>) and the control the highest – 920.18 g.m<sup>-2</sup>. The application of 24-epibrassinolide increased straw yield at average by 6.11 % in comparison with the stressed plants. Of the varieties the lowest increase showed Samanta and the highest Estica. The lowest yield has been found in the variety Ebi (484.14 g.m<sup>-2</sup>) and the highest in Samanta (553.50 g.m<sup>-2</sup>), Fig. 3. Grain yield was distinctly influenced by the experimental treatment, as the stressed variants had a fall in yield of 15.25%. For straw the fall was 8.46%. The most dramatic fall in grain yield due to stress was shown by the variety Ebi and the least by Samanta. For stressed plants of all varieties the grain yield was increased after the application of the phytohormone. The variety Samanta reacted the least to the spray (increase of 1.98%) and Estica reacted the most (increase of 6.16%) in comparison with the stress.

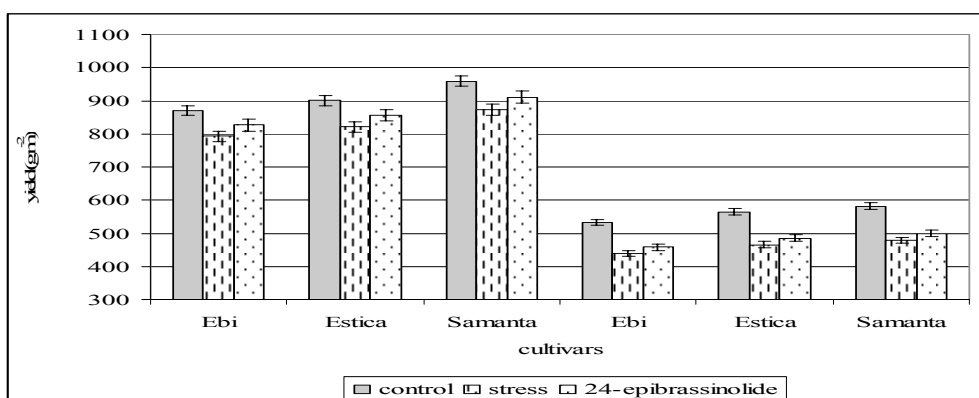


Figure 1 Grain and straw yields (g.m<sup>-2</sup>)

## Conclusions

The results show a clear negative effect from abiotic stressors on the amount of dry matter in the above-ground biomass of wheat and on the size of the grain and straw yield. The abiotic stressors significantly reduced the amount of dry matter produced by the plants of all varieties. After the application of 24-epibrassinolide the negative effect of the stressors was reduced for all of the varieties under investigation. The variety Estica had the best reaction to the application of 24-epibrassinolide, Samanta had the smallest reaction. Of the varieties under investigation Samanta is tolerant to drought and high temperatures and Ebi is sensitive.

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# A Nutraceutical Lesson from Brassica Vegetables: Cardioprotection by Sulforaphane

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Cruciferous vegetables, of which the most commonly consumed come from the Brassica genus, are rich sources of sulfur-containing compounds called glucosinolates; their hydrolysis by a class of plant enzymes called myrosinases results in the formation of biologically active compounds, such as indoles and isothiocyanates. Myrosinase transforms glucoraphanin, a glucosinolate from which the enzyme is physically separated in intact plant cells, into the isothiocyanate sulforaphane (SF) upon damage to the plant such as from chopping or chewing. SF demonstrated a strong chemopreventive effect both in vitro and in vivo (1) and one of the possible mechanism is thought to be related to the induction of phase 2 enzymes of xenobiotic transformation. Most of these enzymes have antioxidant properties as they detoxify reactive electrophiles, oxidised compound, etc. Thus, SF could be considered to act as an indirect antioxidant because it boosts the antioxidant defence system capacity. The up-regulation of endogenous antioxidant systems may represent a promising strategy for protecting cells against oxidative damage. No data are available to support a similar role of SF in cardioprotection.

In this study, using primary cultures of neonatal rat cardiomyocytes, we examined if SF treatment can induce phase 2 enzymes and if this role of chemical inducer can lead to cardioprotection against oxidative stress. In particular, we have characterized the time dependent SF effect on gene expression, induction, and activity of a series of endogenous antioxidants and phase 2 enzymes. We have also investigated the protective effects of the SF-induced cellular defences on cardiac cell injury elicited by H<sub>2</sub>O<sub>2</sub>.

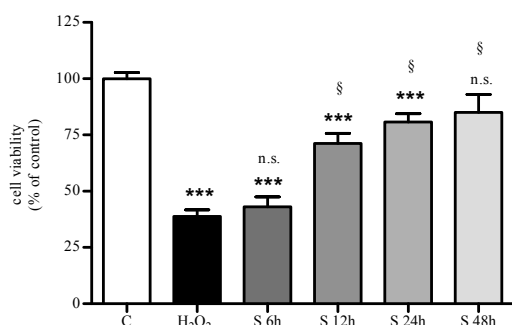
## Methodology

Cultured rat cardiomyocytes were isolated and grown until complete confluence as reported in (2). Cells were treated with 5 µM SF for different times (0.5-48 h). Gene induction of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), NAD(P)H-quinone reductase 1 (NQO1), thioredoxine reductase (TR) was determined by RT-PCR. Western blot analyses of GPx, GR, GST, TR, NQO1 expression were performed using specific antibodies following manufacturer's recommended protocols. The corresponding enzyme activities were measured as reported in (3). The content of intracellular reduced glutathione (GSH) was measured by incubating cells with the fluorescent indicator monochlorobimane (MCB) for 30 minutes at 25µM final concentration. ROS formation was evaluated using 2',7'-dichlorofluorescein diacetate (DCFH-DA) and cell viability by the MTT assay.

## Results

GSH and GSH-related enzymes play a crucial role in the detoxification of ROS and electrophiles, and have been suggested to protect cardiac cells against various forms of oxidative injuries. Intracellular GSH levels significantly increased after 12, 24 and 48 h SF treatment, with the highest increase at the longest exposure times. SF treatment caused a significant increase in both gene and protein expression

of the GSH-related enzymes. Incubation with 5 $\mu$ M SF led to a marked induction of both GR and GSTa1 mRNA levels at 3-48 h treatments. Accordingly, GR and GST protein levels, and the corresponding enzyme activities were significantly increased in respect to control cells after 12 and 24 h, respectively, and this effect was observed also at late incubation times. In contrast, GPX mRNA, protein levels and activity were not influenced by SF treatment at any exposure times. Both TR and NQO1, critically involved in the cellular defense against oxygen radicals, were modulated by SF treatment. TR mRNA and protein levels were significantly increased in respect to control cells after 6 h SF treatment, and NQO1 mRNA and protein expression exhibited a significant induction after 3 and 12 h, respectively. TR and NQO1 activities were significantly increased after 12 h SF treatment.



ROS production following oxidative stress (H<sub>2</sub>O<sub>2</sub>) was significantly lower in SF treated cells only at longer times (12h) confirming the role of indirect antioxidant of this compound.

Incubation of cardiomyocytes with peroxide led to a significant decrease in cell viability, as detected by the MTT reduction assay. Treatment of cells with SF caused a marked protection against oxidative damage. In particular, 48h treatment resulted in a complete protection against peroxide induced-injury (Fig.1).

Fig.1. Effect of SF on cell viability in cells exposed to H<sub>2</sub>O<sub>2</sub>.

\*\*\* p<0.01 vs control; § p<0.001 vs H<sub>2</sub>O<sub>2</sub>.

## Conclusions

Phase 2 enzymes catalyze diverse reactions that collectively result in a broad protection against electrophiles and oxidants, share common transcription regulation, and their gene expression can be coordinately induced by a variety of synthetic and natural occurring agents (4). In this study we demonstrated that treatment of cardiac cells with micromolar concentration of SF results in a significant induction of mRNA and protein expression of a panel of key cellular antioxidants and phase 2 enzymes, including GSH, GR, GST, TR and NQO1 at short exposure times. Enzyme activities were also significantly increased and a delay in respect to the corresponding time course of mRNA induction profile was observed. SF treatment also influences GSH, the major non enzymatic regulator of intracellular redox homeostasis. This drug-mediated elevation of cellular defences is accompanied by a markedly increased resistance to ROS induced cardiac cell injury. Efficient detoxification of ROS requires the coordinate actions of various cellular antioxidants and phase 2 enzymes. Accordingly, simultaneous induction of a scope of key cellular antioxidants and phase 2 enzymes by SF in cardiomyocytes may be an important mechanism underlying the protective effect of this nutraceutical from Cruciferous vegetables against oxidative stress, that characterizes many cardiovascular diseases. These results could justify the assumption of phytochemicals as SF and the dietary consumption of functional foods as Brassica vegetables in the general population or specific subgroups or individuals.

## Acknowledgments

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# Effects of Thymol, Carvacrol and Some Weak Organic Acids on Growth and Ochratoxin A Production by the Food Spoilage *Aspergillus ochraceus*

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## Introduction

Contamination of foods and feeds with mycotoxins represents a high risk for human and animal health. One of the toxins of growing importance in the last decade is ochratoxin A (OTA), a nephrotoxic mycotoxin with carcinogenic and teratogenic properties in rats and possibly in humans (group 2B) (1). *Aspergillus ochraceus* (Ao) is an important contaminant of various substrates, such as cereals, and produce OTA (2). In humans, dietary exposure to OTA has been associated with Balkan endemic nephropathy, a chronic kidney disease linked to tumours of the renal system (3). OTA is highly nephrotoxic and may cause both acute and chronic lesions of kidneys. Similarly, neoplasia in kidney have been reported in the Bulgarian cases of mycotoxic porcine nephropathy. OTA is a moderately stable molecule that remains unaltered during most processes of food transformation and may undergo bio-concentration in some animal tissues/organs reaching concentrations in meat products that are not acceptable for human consumption (4). Moreover, this mycotoxin has been detected frequently in cereal products (5). The consumer demand for the reduced use of chemical preservatives in food has prompted the search for alternatives to fungicides in the control of postharvest diseases. Likewise, chemicals usually applied during cereal storage are becoming less favoured (6). New strategies to reduce and prevent the spoilage of cereals during storage include the use of antifungal plant extracts or weak organic acids (7). The aim of this work was to evaluate the inhibitory effects of phenolic components and weak acids on the growth and OTA production by Ao.

## Methodology

**Fungal inoculum.** The ochratoxin producer strains Ao 5137 (from Dr. Perrone, Bari, Italy) was grown on YES medium (pH 7.0) at 27°C in the dark. The spores were obtained *in vitro* after 7 d of incubation. To determine the mycelial dry weight, the cultures were filtered at the end of each incubation time. The mat was dried at 80°C for 6 h and weighted.

**Antimicrobial agents (AM).** Aliquots of stock solutions (1 M) of carvacrol (98%), thymol (98%), citric, sorbic or propionic acids were singularly added to YES prior to sterilization to give a specific final concentration. The pH of the substrate agar prior to inoculating the culture and after the completion of growth was measured using a solid state pH meter and was approximately equal to 5.5.

**Effect on the growth.** *In liquid broth.* Fractions of 50 ml YES medium with streptomycin (SM) were added to 250 ml Pyrex bottles. The AM were aseptically added to each bottle to give different final concentrations (0–100 ppm). Thereafter, they were inoculated with a spore suspension ( $10^3$  spore/ml). Cultures were incubated statically at 27°C  $\pm$  0.5 °C for 6 to 26 d. Three bottles per AM and concentration were analyzed for mycelium and toxin production. *In agar plates.* Triplicate YES agar Petri dishes (Ø 9 cm) with AM at different concentration were centrally inoculated by pouring 2 µl of  $10^3$  spore/ml. Periodically inoculated plates were removed briefly to be observed and to measure colony diameter. The lowest concentration that completely inhibited fungal growth was taken as the minimum inhibitory concentration (MIC; 8).

**Effects on ochratoxin production.** *Estimation of OTA.* After 1 to 18 days of incubation, 1-ml portions of the medium were withdrawn from the flasks cleaned-up by an immunoaffinity column (Ochraprep).

The production of OTA was determined by an HPLC system equipped with a fluorescence detector ( $\lambda_{\text{exc}}$  332 nm;  $\lambda_{\text{em}}$  470 nm) and a RP-C18 column (Supersphere 4  $\mu\text{m}$ , ODS2, 4  $\times$  125 mm) (Merck & Co.). The analysis was performed under isocratic conditions. The following two solvent systems were employed: aqueous buffer with glacial acetic acid 2 % and acetonitrile in isocratic gradient elution (59:41) at a flow rate of 1 ml/min in 13 min. The limit of quantification was 0.04  $\mu\text{mol/l}$ . *Determination of fungal growth.* To determine the mycelial dry weight, the cultures were filtered at the end of the incubation period. The mat was dried in an oven at 80°C for 6 h and weighted.

## Results

The growth in YES broth supplemented with different concentration of thymol and inoculated with  $10^3$  spore/ml was inhibited at concentration  $\geq 100$  ppm. After 9 d the growth of Ao on agar plates was drastically reduced starting by 150 ppm, and was completely inhibited at 300 ppm. After 11 d the fungal growth started slowly even at 300 ppm reaching the maximum after 22 days. Therefore in agar plates the effect of thymol was fungistatic.

At 12 and 15 d of fungal growth in liquid medium, OTA detected in medium supplemented with thymol 75 ppm was lower ( $P < 0.01$ ) than the control (Tab. 1). The same pattern was observed in the medium with citric acid 2,000 ppm where OTA was lower ( $P < 0.01$ ) than that detected in culture medium at 12 and 15 days of growth. As for carvacrol 75 ppm induced a reduction of OTA at levels not detectable with the described method.

**Table 1.** Influence of thymol, carvacrol, and citric acid on OTA concentration (ppm) 12 and 15 days after incubation in YES broth. Data are mean  $\pm$  SD, n = 10. \*N.D., not detectable.

Incubation time	Control	Thymol (75 ppm)	Carvacrol (75 ppm)	Citric Acid (2,000 ppm)
	[OTA]			
12 d	100.90 $\pm$ 42.96	2.60 $\pm$ 0.60	N.D.*	12.20 $\pm$ 8.58
15 d	99.52 $\pm$ 45.13	8.07 $\pm$ 11.04	N.D.	19.55 $\pm$ 16.03

## Conclusions

Carvacrol, thymol and citric acid clearly showed antimicrobial activity against Ao. Our preliminary data indicated that those antimicrobial agents, at critical concentrations, gave a significant reduction in the production OTA by Ao and might be used to prevent mycotoxin contamination in animal feed.

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# Nitrogen Fertilization of Basil: Effects on Physiology, Oil Gland Morphology and on Essential Oils

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Basil (*Ocimum basilicum* L.), is one of the major essential oil producing species. The high economic value of basil oil is attributed to the presence of phenyl propanoids and terpenoids, such as eugenol and linalool. Although nitrogen fertilization is a fundamental component of modern crop production systems, its importance on basil physiology, oil gland morphology, essential oil content and composition is not adequately studied.

## Methodology

A pot experiment was conducted in northern Greece to determine the effect of different nitrogen rates (0, 50, 100, 200 kg N ha<sup>-1</sup>) on basil (cv. lettuce leaf). A split plot experimental design was used. Nitrogen (ammonium sulfate 21% N), was applied through fertigation. In order to estimate the effect of nitrogen fertilization on basil physiology, gas exchange and photosynthetic yield (a major stress indicator), were monitored bimonthly and diurnally.

Plants from each treatment were harvested at full bloom. Total leaf area per plant was measured with a leaf area meter. Essential oil yield was determined by hydrodistillation for 180 min on fresh tissues (leaves and inflorescences). GC-MS analysis of essential oil constituents was performed and retention indices were determined. An optical stereoscope was used to observe oil gland morphology.

## Results

Carbon assimilation over the experimental period was positively affected by nitrogen with significant differences between the 0 and 200 kg N ha<sup>-1</sup> (8 and 14  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively). Upon diurnal monitoring, carbon assimilation was also increasing due to nitrogen treatments, in particular at midday (Figure 1). Stomatal conductance exhibited a comparable response (data not shown).

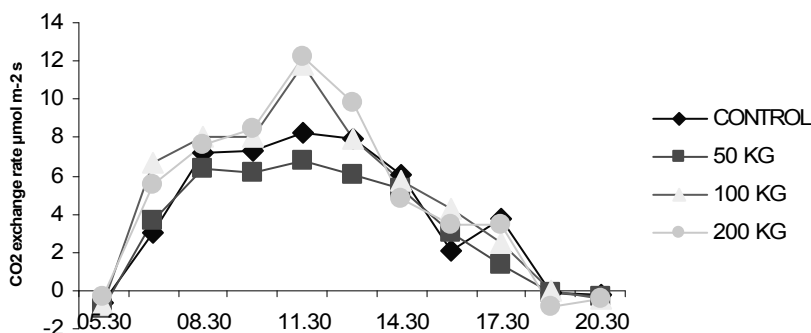


Figure 1. Diurnal course of gas exchange of basil as affected by nitrogen fertilization

Photosynthetic yield suggested that stress was imposed on the 0 and 50 while, on the contrary, it was favored on the 200 kg N ha<sup>-1</sup> nitrogen treatment (0.17 at 0 vs 0.3 at 200, under heat wave). Nitrogen nutrition had a significant effect on essential oil yield of both, leaves and inflorescences (e.g. 0.17 in control, 0.29% in 200 N for inflorescences). On the contrary, oil gland morphology was negatively affected in average diameter and density with the increase of nitrogen nutrition (Table 1).

The positive effect on essential oil yield induced by fertilization can be attributed to a 2.5x increase in total leaf area following nitrogen application (Table 2).

Essential oil profile did not differ among treatments. Linalool, methyl-chavicol, eugenol, cineol, bergamontene and a-cadinol were detected by GC-MS analysis as the main essential oil constituents of both, leaves (Table 3) and inflorescences (data not shown), regardless of the nitrogen treatment applied.

**Table 1.** Oil gland diameter and density of basil as affected by nitrogen fertilization

Treatment (kg N ha <sup>-1</sup> )	0	50	100	200
Oil glands/cm <sup>2</sup>	0.576	0.492	0.217	0.300
Average diameter (µm)	187.2	162.4	143.6	96.2

**Table 2.** Total leaf area of basil as affected by nitrogen fertilization. Different letters indicate least significant differences at  $P < 0.05$ .

Treatment (kg N ha <sup>-1</sup> )	0	50	100	200
Leaf area (cm <sup>2</sup> )	91.54	98.23	162.5	216.1
	a	a	abc	bc

**Table 3.** Relative abundance (%) of main constituents of basil leaf essential oil as affected by nitrogen fertilization

Treatment (kg N ha <sup>-1</sup> )	0	50	100	200
Linalool	49.81	48.13	49.11	52.82
Methyl chavicol	27.37	28.55	28.19	26.75
Eugenol	1.14	0.97	0.90	1.08
Cineol	7.55	7.39	7.55	7.20
Bergamontene	2.37	2.60	2.41	2.02
a-cadinol	2.68	2.69	2.86	2.60

## Conclusions

Although affecting physiological parameters and basil morphology, nitrogen fertilization was not apparently a crucial factor in essential oil composition. However, the increase in total leaf area and consecutively in essential oil yield is of agricultural importance, since it is bound to augment farmer's net income.

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# Activity of *Melia azedarach* L. Extract against Root-Knot Nematodes

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## Introduction

Control of Root Knot nematodes, one of the most destructive crop pests<sup>1</sup>, is currently rather difficult considering the shortage of nematicides still included in Annex I of 91/414/EEC. Focus is attracted on natural origin biopesticides that are relatively safer but equally effective compared to their chemically synthesized predecessors<sup>2</sup>. In the search for plant origin compounds with nematicidal potential, *Melia azedarach* L. (Meliaceae), a plant indigenous in India but distributed also in Greece, was studied.

## Methodology

Grinned, dry, ripe fruits, previously defatted with hexane in Soxlet apparatus, were extracted by sonication, with a methanol volume (ml) equal to 5 times the fruit weight (g), three times. The solution was filtered and evaporated to constant weight, yielding an oily dark viscous extract (35 %, w/w). This methanol fruit extract (MFE) was used in bioassays with *Meloidogyne* spp. to study the activity on both nematodes motility and biological cycle. MFE's activity on nematodes paralysis was tested in 96well plates experiment, exposing coeval second stage juveniles (J2s)<sup>3</sup> to 0.0125 – 3.2 mg/ml for 12 – 192 h. The paralysis experiment was performed twice, as a split plot design, with six replications. The main factor was exposure time and the sub-factor was application rate. Paralysis data were expressed as a percentage increase in the number of paralysed J2s in the water control<sup>4</sup> and they were analyzed (ANOVA) using a 9 by 5 factorial approach (9 application rates by 5 exposure times) combined over time. Since ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Differences among treatment means were compared using the Duncan's test at  $P \leq 0.05$ . Moreover, MFE's activity on nematodes biological cycle was tested in pot experiments, laid out in a completed randomized design with five replications. Soil artificially infested with fresh and coeval J2s, was fortified with 5–30 mg MFE/g, and then used for transplanting tomato plants. The fresh root weight, shoot weight and the total number of female nematodes per gram of root<sup>5</sup> were assessed thirty days later. Efficacy was expressed as a percentage decrease in the number of females per g of root corrected according to the control<sup>6</sup>, and then it was fitted in the log-logistic model<sup>7</sup> to estimate the concentration that caused a 50% decrease in females per g of root ( $ED_{50}$  value). Root weight means were separated using Duncan's test at  $P \leq 0.05$ . In all cases water served as control.

## Results

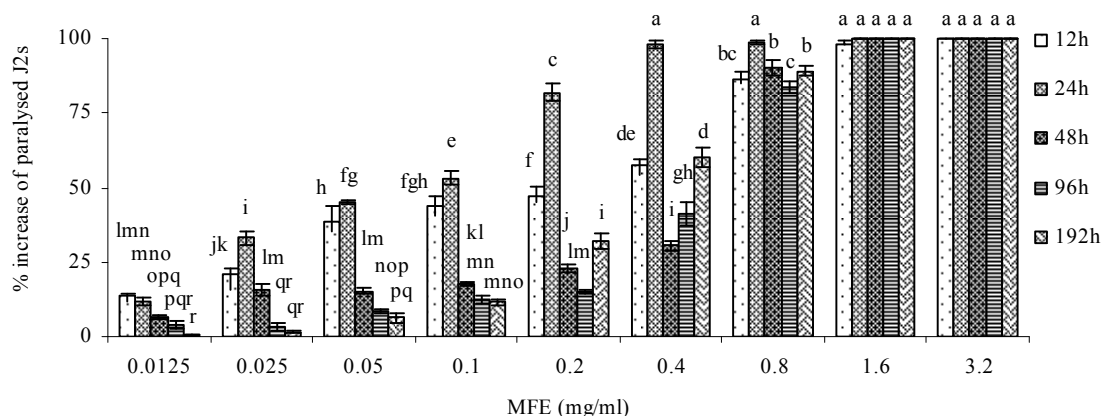


Fig. 1. Effect of MFE, application rate and exposure time, on J2s paralysis. Each bar represents the average % increase of paralysed J2s, of two trials each with six replications per treatment. Percentages are based on paralysed J2s counted in the control. Different lower-case letters between bars indicate significant differences ( $P \leq 0.05$ , Duncan's test). Error bars represent the standard error of the mean values.

The ANOVA indicated significant MFE application rate by exposure time interaction ( $P < 0.05$ ) and therefore the interaction means are presented (Fig. 1). Concentrations less than 0.8 mg/ml paralysed J2s, but the effect was in general reversible indicating a nematostatic activity. However, J2s found paralysed 12 h after treatment with 0.8 to 3.2 mg/ml never regained their capacity to move, indicating a nematocidal activity.

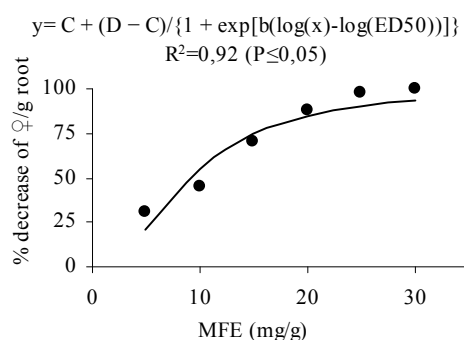


Fig. 2. Dose response curve for % decrease of females per g of root, following exposure to MFE. Each point represents the average % decrease of females per g of root, of five replications per treatment. Percentages are based on female counts in the control. Line represents the predicted function calculated by fitting a log-logistic regression model for non linear regression analysis.

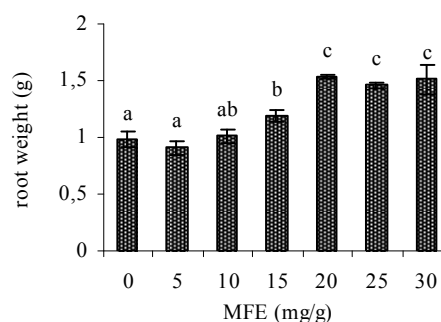


Fig. 3. Effect of application rate of MFE on root weights of tomato plants. Each bar represents the average of five replications per treatment. Different lower-case letters between bars indicate significant differences ( $P \leq 0.05$ , Duncan's test). Error bars represent the standard error of the mean values.

Generally, the MFE showed bioactivity at all doses tested in pot experiment. Clear dose response relationships were established with the highest doses of 25 and 30 mg/g evoking 100 % nematode control, while the  $ED_{50}$  was calculated 9.16 mg/ml (Fig. 2). Additionally, as compared to the untreated control, treatment of tomato plants with MFE resulted to a significant increase in the fresh root weight (Fig. 3). Shoot fresh weights were not significantly different amongst treatments ( $P > 0.05$ ) while phytotoxicity was never observed (data not shown).

## Conclusions

The present study clearly proves the nematode paralysis and development disruptive activity of *M. azedarach* against *Meloidogyne* spp., indicating specifically that at concentrations exceeding a certain threshold (0.8 mg/ml) MFE can act as nematocide. This is the first report of *M. azedarach* activity against *Meloidogyne* spp., posing interesting perspectives for its use within root-knot nematode management and control, in the frame of an environmental-oriented sustainable agriculture.

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# Cruciferae Vegetables: a Source of Phytochemicals for Brain Health

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## Introduction

There is growing interest in nutraceuticals aimed at counteracting selective neuronal death associated with neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Oxidative stress, accumulation of iron and inflammatory processes are the major factors responsible for the dysfunction or death of neuronal cells that contributes to initiation and progression of neurodegeneration in neurological disorders (1). Edible plants belonging to the family Cruciferae and genus Brassica (e.g. broccoli, cauliflower, kale, and Brussels sprouts) contain substantial quantities of isothiocyanates (mostly in the form of their glucosinolate precursors) some of which are very potent inducers of phase 2 enzymes and may play a special role in affording cytoprotection (2). Among the various ITCs, sulforaphane (SF) has recently gained attention as a potential neuroprotective phytochemical. In particular, in vivo studies suggest that SF may reduce acute neurodegenerative events such as the infarct volume and the edema at cerebral level in a rat model of transient middle cerebral artery occlusion (3). To date, there are no data available about the potential preventive role of SF in chronic neurodegenerative diseases such as PD and AD. In this study, we investigated the potential neuroprotective activity of SF in in vitro models of PD. In particular, an experimental approach using a pulse/chase treatment of human dopaminergic SH-SY5Y cells with 6-hydroxydopamine (6-OHDA), a PD specific neurotoxin to determine oxidative stress and neuronal death, was applied.

## Methodology

**Cell culture.** SH-SY5Y cells were grown at 37°C with 5% CO<sub>2</sub> in DMEM 10% FBS. To determine the neurotoxicity, the total antioxidant activity (TAA) in cellular fractions, as well as the cellular level of glutathione (GSH), SH-SY5Y cells were seeded in 96-well plates at 3×10<sup>4</sup> cells/well, in 100 mm culture dishes at 4×10<sup>6</sup> cells/dish, and in 60 mm culture dishes at 2.5×10<sup>6</sup> cells/dish, respectively. All experiments were performed after 24 h of incubation at 37°C in 5% CO<sub>2</sub>. To evaluate the neuroprotective effects of SF, SH-SY5Y cells were treated with various concentrations of SF for 24 h at 37°C with 5% CO<sub>2</sub> before treatment with 6-OHDA (100 µM for 3 h). **Determination of neurotoxicity.** The neuronal viability in terms of mitochondrial metabolic function was evaluated at 21 h post-treatment with 6-OHDA by the reduction of MTT to formazan as previously described by us (4). The values are expressed as percentages of control cells. **Determination of TAA in cellular fractions.** TAA, a marker of cellular antioxidant status, was measured on the membrane and cytosolic enriched fractions as previously reported (4). TAA values are expressed as µmol of Trolox Equivalent Antioxidant Activity per mg of protein (TEAA µmol/mg protein). **Determination of GSH cellular level.** The GSH level was determined with a standard colorimetric method in terms of DTNB reduction (5). The values are expressed as nM of GSH per µg of protein.

## Results

Preliminary experiments showed that in the absence of 6-OHDA, treatment of SH-SY5Y cells with SF concentrations up to 5 µM for 24 h did not affect the neuronal viability (data not shown). Treatment of

SH-SY5Y cells with SF (0,6-5  $\mu$ M) showed a concentration-dependent decrease of 6-OHDA-induced neurotoxicity (Figure 1).

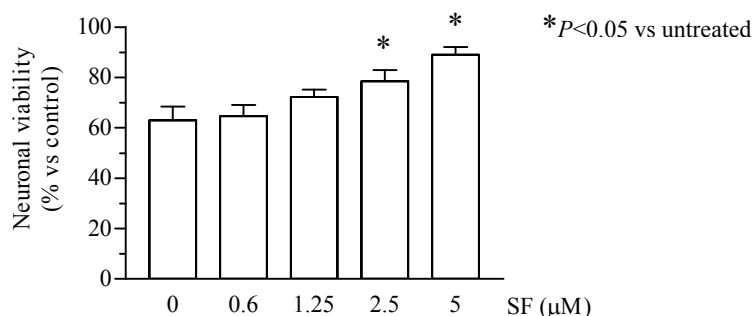


Figure 1. SF protects SH-SY5Y cells against 6-OHDA induced neurotoxicity.

To evaluate at which subcellular level the SF counteracts oxidative damage, we measured TEAA at membrane and cytosolic levels. As reported in Figure 2, the treatment with SF for 24 h induced a significant increases of TEAA in the cytosolic fraction. By contrast, similar treatment did not modify the TEAA level of the membrane fraction (data not shown).

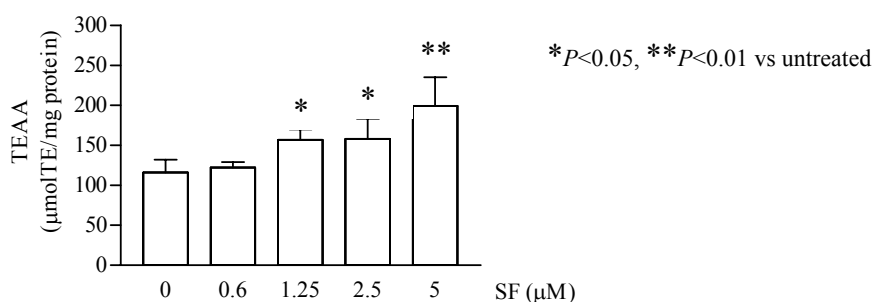


Figure 2. SF increases the TEAA in the cytosolic fraction of SH-SY5Y cells

To confirm the antioxidant activity of SF at cytosolic level, we then evaluated the GSH level in the same cytosolic fraction. SH-SY5Y treated with 5  $\mu$ M of SF showed a significant increases of GSH level in comparison to untreated cells ( $120.00 \pm 20.54$  vs  $46.12 \pm 14.70$  nM/ $\mu$ g protein,  $p < 0.05$ ).

## Conclusions

These results show that SF can protect neurons against toxicity induced by 6-OHDA and confirm its indirect antioxidant activity. In particular, these neuroprotective effects could be ascribed to the ability of SF to increase the TAA and GSH at cytoplasm level of neurons. These findings support further research into SF and other isothiocyanates as nutraceutical components for the prevention of neurodegenerative diseases such as PD.

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# Evaluation of Antioxidant Activity of Grains From Graminaceae, Pseudocereals and Leguminosae

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Regular consumption of fruits, vegetables and whole grains provide a wide range of nutrients and biologically active compounds which may reduce the incidence of various disease (1). Whole grains are rich sources of fibre, vitamins, minerals, and phytochemicals including phenolics, carotenoids, vitamin E, lignans,  $\beta$ -glucan, inulin, resistant starch and sterols (2). Nutritional guidelines put grains and grain products at the base of the food guide pyramid to emphasise grains or grain product consumption as part of normal diet for optimal health (3). While nutritional and technological properties of grain products are widely investigated, few information are available for health implications of grain flour and by-products consumption. Therefore, a great interest is addressed to the determination of antioxidant activity (AA) of grain flour. In this study the AA of seven herbaceous crop species was evaluated. The species under study were the Graminaceae oat (*Avena sativa* L.), teff (*Eragrostis tef* (Zucc.) Trotter) and finger millet (*Eleusine coracana* (L.) Gaertn); the pseudocereals quinoa (*Chenopodium quinoa* Willd; Chenopodiaceae) and amaranth (*Amaranthus* spp., Amarantaceae) and, finally, the Leguminosae faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.). These crops, belonging to several botanical families are widely spread under different climatic environments. Some evidences are reported in literature in relation to their functional quality and antioxidant activity. The antioxidant capacity of oat is largely due to the presence of tocopherols, tocotrienols, phytic acid, flavonoids, and non-flavonoid phenolic compounds such as avenanthramides (4). Finger millet contains phenolic acids, flavones and is the only millet species containing condensed tannins. Teff is reported to have tannins (5,6). Quinoa and amaranth are rich in several phytochemicals that act as powerful dietary antioxidants (7). Leguminosae provide micronutrients, vitamins, carotenoids (8) and phenols, all of which are considered to be bioactive compounds (9,10). The objective of this investigation was the evaluation of AA of hydrophilic and lipophilic antioxidant components from grain samples. AA of the different species under study was evaluated both on hydrophilic and lipophilic flour extracts.

## Methodology

All reagents were purchased from Sigma Chemical Co. (St Louise Mo) or from Fluka Chemie GmbH. Whole grains (5 g) of the investigated species were milled by means of a Cyclotec 1093 Sample Mill (1 mm sieve). To remove external saponin, quinoa grains were previously washed vigorously. Two solvent systems were used to extract hydrophilic and lipophilic antioxidants components from whole grain flours i.e. water and exane/ethyl acetate (9:1 v/v) solution, respectively (11,12). *Extraction with water.* Flour samples were weighted and suspended in distilled water at a w/v ratio equal to 1 g/3 mL. The suspensions were placed in an ice-water bath for 1 h, stirred for 1 min at 15 min intervals; then, they were centrifuged twice at 18700 x g for 20 min at 4°C and the final supernatants (water extracts) were stored in an ice-water bath and daily used. *Extraction with exane/ethyl acetate solution.* 2 g of whole grains flour was saponified under nitrogen by adding ethanolic pyrogallol (60 g/L), ethanol (96%), sodium chloride (1%) and potassium hydroxide (600 g/L). After alkaline digestion at 70°C for 45 min, the samples were cooled in an ice bath and sodium chloride was added. The suspension was then extracted twice with n-hexane/ethyl acetate (9:1 v/v). The organic layer was collected and evaporated to dryness. The dry residue was dissolved in ethanol, stored in an ice-water bath and daily used. To determine the AA of the water and exane/ethyl acetate extracts we used ABTS method (13), with water and ethanol as solvent respectively. ABTS method is based on the ability of some antioxidants to reduce the cationic radical ABTS<sup>+</sup>. This redox reaction results in a decrease of absorbance of ABTS<sup>+</sup> at 734 nm. AA of the hydrophilic and lipophilic components of the flour from

the different species was calculated by means of proper calibration with Trolox. Always, the AA was expressed as  $\mu\text{mol}$  Trolox equivalents/g of whole flour.

### Results

In Fig. 1 the AA of hydrophilic (A) and lipophilic (B) antioxidant components of the whole flour from the investigated species is reported. Always, the hydrophilic antioxidants were more active than the lipophilic ones. Two different ranking lists were obtained for hydrophilic and lipophilic antioxidants, thus showing a different contribution of these components in determining AA of different grains. In A, ABTS method shows a very high AA value in faba bean, high in chickpea and lower in the other species. In B the highest AA value was observed in chickpea, followed by faba bean, quinoa, teff and amaranth, and the lowest ones in finger millet and oat.

### Conclusions

The comparison among the different species under study shows the highest antioxidant activity for leguminosae species and quinoa both in hydrophilic and lipophilic extracts and the lowest activity for oat and finger millet. Further studies will be necessary to evaluate both genetic and environmental influence on AA of the different species, as well as to enlarge evaluation of AA to other methods besides the ABTS one.

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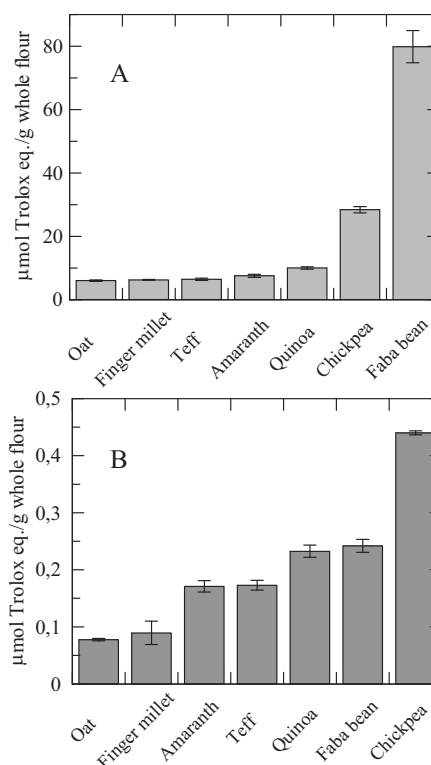


Figure 1. Determination of AA of hydrophilic (A) and lipophilic (B) antioxidant components in the investigated species measured by using ABTS method.



# Effects of Highly Diluted and Mineral Treatments on the Nutraceutical Properties and Phytopathological Status of Cauliflower

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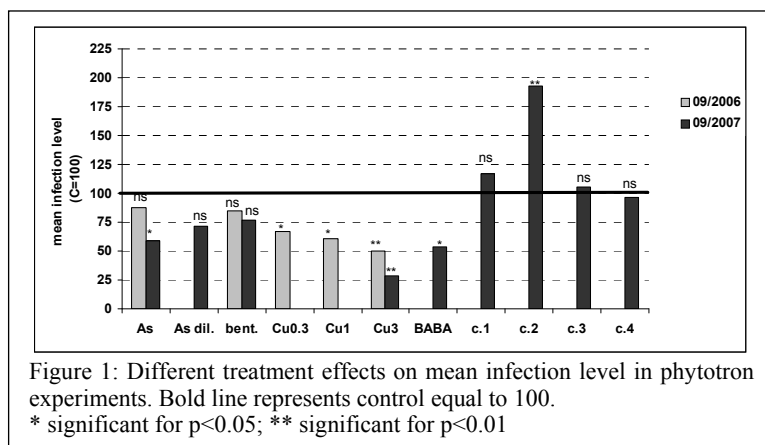
Dark leaf spot caused by *Alternaria brassicicola* (Schw.) Wilts. is a very common disease of *Brassica* crops world-wide. It often results in yield losses and reduced seed quality. This disease appears as small dark spots at all growth stages of the plant. In organic agriculture the control of dark leaf spot, as well as of most fungal diseases, is based on the use of mineral products such copper, that has a high efficacy and a long-lasting action. Unfortunately, copper use presents some disadvantages: it can be phytotoxic and it can accumulate in the ground with negative consequences on microflora and microfauna. For these reasons, European Union delivered a directive (Commission Regulation EC no. 473/2002) that imposes the reduction of copper use in organic agriculture. In this context, highly diluted preparations could represent suitable treatments, complementary or alternative to copper, in organic agricultural protocols. The literature on the effects of highly diluted treatments on plants provides a limited number of papers: besides germination and growth tests on different plant species, some experiments have been performed on phytopathological models, whereas very few and outdated descriptions are available as far as field trials are concerned (Betti *et al.*, 2007). The aim of this work is to give a contribution on the effects of highly diluted treatments on dark leaf spot caused by *A. brassicicola* on cauliflower.

## Methodology

Plants of *Brassica oleracea* L. cultivar clx 33247 were used both for experiments in phytotron and for a field trial. Plants were artificially inoculated by spraying a fungal suspension ( $1 \times 10^7$  conidia ml<sup>-1</sup>) on the leaves. In phytotron (25±1 °C, photoperiod of 8 h) a preliminary screening of different highly diluted treatments, at decimal (dH) and/or centesimal (cH) Hannemanian potencies (Masci, 1997), was carried out by foliar spray either before or after fungal inoculation (As<sub>2</sub>O<sub>3</sub> 35 and 45 dH, AgNO<sub>3</sub> 35, 36, 45 and 46 dH; Sulfur 6 dH, 5 and 201 cH; Cuprum 5 dH; isopathic 4 cH). In the first experiment (09/2006), As<sub>2</sub>O<sub>3</sub> 35 dH (As) and a bentonite treatment (bent.) at 10 g/l were compared with copper oxichlorure (Cu) at 0.3, 1, and 3 g/l, the control being water. In the second experiment (09/2007), the treatments with As, bent. and Cu 3 g/l (as positive control) were repeated and compared with As diluted 1:5000 (As dil.), β-aminobutyric acid (BABA, 5 mM) and four complex treatments (c.1- c.4) composed, in different combinations, by *Silicea* 200 cH, Mn-Cu, Mg, I micronutrients, *Silicea* dH6, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 35 dH and *Pulsatilla* 30 cH. In the field trial, performed in the experimental farm of Bologna University (09-12/2006), the same treatments of the first phytotron experiment were tested. The field was divided in plots consisting of 6 plants/treatment, and each treatment was replicated four times in a randomized complete block design. Treatments were sprayed weekly on the leaves 3 times before and 4 times after artificial fungal inoculation. The evaluation of infection level on leaves (phytotron experiments) or corymb (field trial) was recorded by making a visual assessment of the necrotic area on each plant on the basis of an infection scale, previously defined. Data were subjected to analysis of variance (ANOVA), followed by Dunnett post-hoc test.

## Results

In the preliminary screening of highly diluted treatments the best disease control was obtained by As,



which induced an infection level reduction of about 20%. Phytotron experiment results, shown in Figure 1, confirmed the disease control effect of both As and As dil. (infection level reduction vs. control of about 15-40%, significant at  $p < 0.05$  for As in the second experiment). A similar reduction (15-25%), but not significant, was obtained with bent. Cu at all concentrations and BABA significantly reduced disease severity

( $p < 0.05$  and  $p < 0.01$  for Cu 3g/l). As far as complex treatments are concerned, only c.2 (without *Silicea* 200 cH) gave significant results ( $p < 0.01$ ), inducing an increase of the infection level. In field trials, disease assessments on cauliflower heads, performed in 3 successive times (Figure 2), showed in the last measurement a similar disease symptom reduction for As (46%), bent. (42%) and Cu 3 g/l (45%), significant vs. control at  $p < 0.05$ .

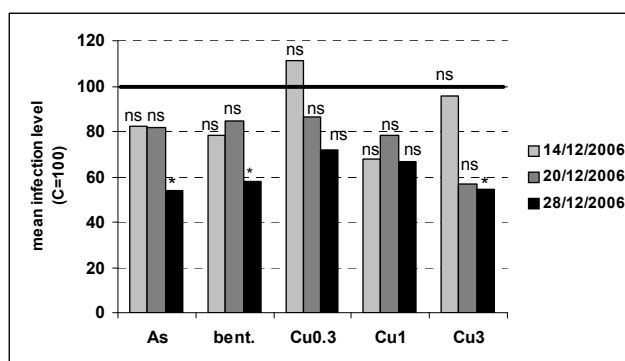


Figure 2: Different treatment effects on mean infection level in field trial. Bold line represents control equal to 100.  
\* significant for  $p < 0.05$

## Conclusions

The obtained results need further investigations but they seem to support the possibility of an agricultural application of highly diluted treatments.

Our experimentation is still in progress with other field trials. The aim is to check the effects, against a natural infection of *A. brassicicola*, of the same treatments used in the second phytotron experiment. Besides phytopathological analyses, an evaluation of organoleptic characteristics and nutraceutical properties of differently treated plants will be also carried out at the end of the crop life cycle. In particular, glucosinolates, a class of plant secondary metabolites typical of *Brassicaceae*, will be analysed: these organic compounds seem to participate in the plant resistance mechanisms (Ménard *et al.*, 1999) and research indicates that they may have potential in fighting human cancers (Talalay *et al.*, 2001). One privileged target could be small farms (and in particular, those of nutraceutical and herbalist sectors) practicing organic farming that strive to be environmental-friendly and economically viable.

## Acknowledgements

Authors would like to thank Ce.M.O.N., Italy for the financial support in glucosinolate analyses.

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# Performance Of *Crambe Abyssinica* As New Crop For Non-Food Uses In North-East Italy

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High erucic acid oils are a potential source of raw material for some oleochemical transformations as well as for direct use in the production of erucamide – a slip agent for plastic films (Walker, 2004). High erucic acid rapeseed (HEAR) is the common “green” source of erucic acid and it is mainly cultivated in central Europe.

*Crambe abyssinica* Hochst ex R.E. Fries is another interesting crop for such industrial uses, capable of yielding even higher amounts of erucic acid than HEAR (Bondioli et al., 1998; Temple-Heald, 2004). *Crambe* has been studied for many years (Meijer et al., 1999), but its spread is still limited in our environment, because of its poor adaptability to the European continental climate (Fontana et al., 1998). This crop, native to the Mediterranean regions of North Africa, has positive agronomic traits, such as tolerance to drought and good adaptability to poor sandy soil. Conversely, it does not tolerate cold or waterlogging, so that solely spring sowing must be adopted in central and northern Europe.

Aiming at testing the possibility of introducing *Crambe abyssinica* in the typical crop rotations of North-East Italy and establishing its yield potential, a two-year field trial was set up at the experimental farm of the University of Padova.

## Methodology

The trial was carried out at Legnaro (45°21'N, 11°58'E, NE Italy), following a completely randomised block design, with 3 replicates. Three commercial varieties of *Crambe abyssinica*, i.e., Mario (Triumph, Italy), Nebula, and Galactica (Springdale Farm, UK) were compared during 2006 and 2007. In both years, sowing took place in spring (March 30 2006, March 15 2007) and harvesting at the beginning of summer (July 5 2006, June 30 2007). Only a limited amount of fertiliser was applied before sowing (30, 90 and 90 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively). Inter-row distance was 0.23 m and the amount of seed 18 kg ha<sup>-1</sup>. The high amount of seed was necessary to compensate for its low degree of germination. There was no need to control weeds or pests during the crop cycle.

## Results

The growing season (April-June) in the two years was characterised by different weather conditions. In the 2006 crop cycle, the mean minimum and maximum temperatures were 9.8 and 19.8 °C, respectively, and precipitation was 193 mm. In 2007, mean temperatures were much higher than in 2006 (minimum: 11.8 °C; maximum: 22.8 °C), and were associated with very little rainfall in April (flowering stage) – only 5 mm – and a surplus (almost double that of the historical average for the location) in May-June (capsule filling-maturation) (a total of 276 mm, during the crop cycle).

Yield (capsulated seeds) was much higher in 2006 than in 2007 (mean of varieties: 3.1 vs. 2.3 t DM ha<sup>-1</sup>), probably due to the more favourable climatic conditions, i.e., warmer climate and higher rainfall at flowering. No significant differences emerged among genotypes in terms of yield, although the Mario variety, which was selected in the Italian environment, reached slightly better performances in both years (Fig. 1).

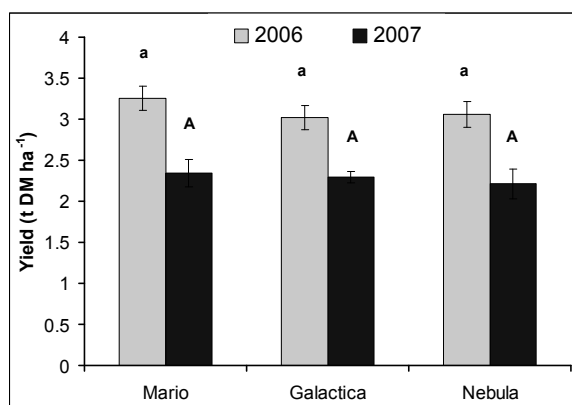


Fig. 1. Capsulated seed yield in varieties of crambe in two-year trial. Letters: significant differences among varieties within same year (LSD test,  $P \leq 0.05$ ). Vertical bars: standard error.

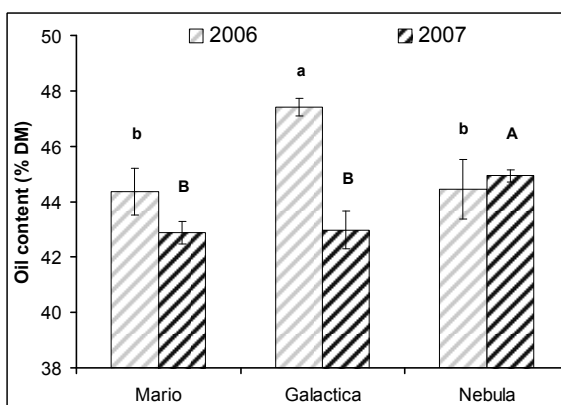


Fig. 2. Oil content in varieties of crambe in two-year trial. Letters: significant differences among varieties within same year (LSD test,  $P \leq 0.05$ ). Vertical bars: standard error.

The mean oil content of crambe was comparable to that of HEAR when de-capsulated seeds were analysed. For example, in 2006, the Galactica variety reached a maximum of 47% of oil, a value commonly found in high erucic rapeseed in the same environment (Mosca et al., 2007). In 2007, the high temperatures during the crop cycle very probably caused a reduction in the final oil percentage of Mario (-2%) and Galactica (-5%), but not Nebula, which showed very stable behaviour across years.

As expected, the content of erucic acid in the oil was very high in all varieties, and in 2006 reached a mean fraction of 58.4%, much higher than in HEAR varieties (~50%). These results indicate that, in our environment, crambe has lower productivity than HEAR in terms of erucic acid ( $0.61$  vs.  $0.89$  t ha<sup>-1</sup>), although the higher tenor of this fatty acid may facilitate the extraction process.

Considering the short cycle of this crop in spring sowing, the total amounts of both oil and erucic acid produced were considerable, although significant variations may be expected across years due to changing climatic conditions. In this regard, greater precocity may allow some genotypes to show better performance in terms of oil content but not of seed yield (e.g., Nebula).

## Conclusions

The possibility of introducing *Crambe abyssinica* into crop rotation in NE Italy seems feasible, with good yield results ( $>2$  t DM ha<sup>-1</sup>) even in conditions of little rainfall at flowering and high temperature, as happened in 2007. The growing cycle is positively short, about 3 months, and these results also suggest the possibility of adopting a low-input management, although more precise agronomic techniques (e.g., sowing date and density, inter-row distance, fertilisation) must be improved in order to maximise yield in each environment.

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## **SUB SESSION 2.2**

### **BIOMASS AND ENERGY PRODUCTION**

Chairman: Melvyn Askew



# 4F CROPS

## Future Crops for Food, Feed, Fibre and Fuel

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### Introduction

As different sectors – food, feed, fiber, and fuels – compete for land, the yielding potential of the future non-food crops has to be as efficient as possible in order to minimize the competition for land. In the long term, bioenergy crops provide the largest potential. This development will be driven by: additional productivity increases, further liberalization of agricultural markets and the introduction of high-yield bioenergy crops (EEA, 2006). The *main aim* of the project is to survey and analyze all the parameters that will play an important role in successful non-food cropping systems in the agriculture of EU27 alongside the existing food crop systems.

**Table 1** The consortium of the 4F CROPS project

Participants	Participant organisation name	Country
1 (CO)	Centre for Renewable Energy Sources - CRES	Greece
2	University of Catania –UNI.CT	Italy
3	Agriculture University of Athens – AUA.eco	Greece
4	Institute for Energy and Environmental Research -IFEU	Germany
5	Agro technology & Food - A&F	Netherlands
6	University of Bologna - UNIBO	Italy
7	National Institute for Agriculture Research - INIA	Spain
8	University of Lisbon - UniNOVA	Portugal
9	Institute of Natural Fibres - INF	Poland
10	University of Agricultural Science in Bucharest - UASB	Romania
11	Agriculture University of Athens - AUA.bio	Greece
12	National Agricultural Research Foundation - NAGREF	Greece
13	Baltic Renewable Energy Centre - EC BREC	Poland

### Methodology

The project will be carried through eight work packages (starting 1/7/08). The work will start (**WP1**) with a review of the current land use in EU27. This review will be used as a base for the prediction of the future land use in short term (2020) and long term (2030). For the prediction of the future land use, a number of parameters will be carefully taken under consideration, the most important being the existing intensive agricultural systems, climate change, regional differences and market demands for food, feed, fiber and fuel.

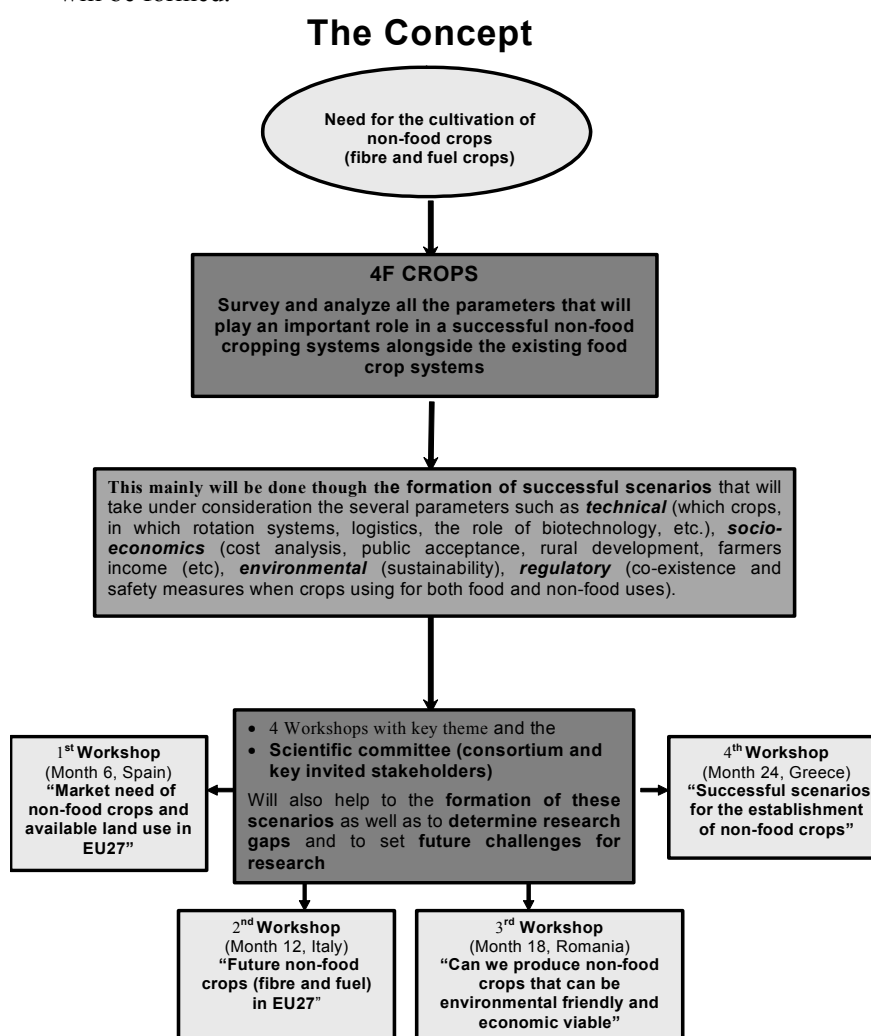
The work will continue (**WP2**) with the mapping of cropping possibilities that will be based on regional potential levels, ecology and climate. The future crops will be categorized in four major categories, namely crops for **food**, animal **feed**, **fiber** and **fuel**. In this work package several parameters will be critically addressed, like crop domestication and genetics, physiology, input requirements (water, fertilizers, chemicals) and crop management possibilities (double cropping / parallel cropping). A group of favorable non-food crops which allow parallel cropping with food crops will be defined for the present, a short time frame (2020) and a longer one (2030).

This group of crops will be then subjected to a comparative cost analysis (**WP3**) for the same time framework. For the economic appraisal of crops, monitoring of economic parameters, such as commodity prices, interdependency of crops and the new CAP amendments will also be included.

Economic analysis of few and conventional crops will look into the economic and financial details of production, and distribution of agricultural products from the point of view of the producer. Computerized analytic methods will be developed and used for this reason. Based this analysis, the economic viability and performance of the new crops will be demonstrated and estimates of future land use changes will be made. Socio-economic impacts, like farmers' income, rural development, public development, public acceptance, safety measures, etc. will also be recorded and critically analyzed.

For the selected non-food crops and the bio-products (bioenergy, bio-based material) the environmental implications will be assessed compared to their respective conventional products (fossil energy, conversional materials) (**WP4**). For this, several environmental impacts will be assessed like soil quality and soil erosion, air quality and climate change, water issues, biodiversity and landscape by using life cycle assessment and environmental impact assessment methods.

Through **WP5** the existing policies will be recorded in a national context and will be critically evaluated. Emerging best practices and the key factors that led to the success stories will be pointed out. Barriers that prevented the development of non-food cropping systems will be analyzed and reviewed. In addition co-existence and safety measures when crops as used for both food and non-food uses will be addressed and recommendations will be sought. A framework of strategic policies options will be formed.



The work accomplished in WP1 – WP5 will be used as a base to elaborate scenarios for successful non-food cropping alongside food cropping systems (**WP6**), which will provide answers whether a complete bioeconomy is a viable option for EU 27.

A whole dissemination plan has been designed (**WP7**) and the main elements of this plan are: the development of the web-site (intranet), the project workshops (*with key themes*) dissemination activities such as articles, leaflet, conferences, fact sheets, links, etc. **WP8** is a general work package aiming at the coordination, management and reporting of the project.

## References

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Figure 1. The concept of the project



# Testing Resource Use-Efficiency in Annual Energy Crops

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Annual and multi-annual energy crops have reciprocal advantages and disadvantages, whose balance in specific cases ensures either group a competitive edge over the other. Annual species do not constrain crop rotation, allowing each year freedom of choice. More to that, they are often represented by major species already grown for food uses. In the present scenario of rapid development of bio-energy, a field comparison of annual crops may contribute to assess their potential in terms of resource use-efficiency (energy, water, nitrogen), in view of the multiple energy products they are potentially suited for.

## Methodology

In the year 2006/07, 11 annual crops were grown in a completely-randomized block design in Cadriano (BO; 44°33' N, 11°21' E, 32m a.s.l.), on a silty-loam soil. They included four cereals: winter wheat (W), barley (B), maize (M) and grain sorghum (GS); four oilseeds: sunflower (SF), the oilseed rape *Brassica napus* (BN) and the two less-common species *B. carinata* (BC) and *B. juncea* (BJ); three biomass species: sugar beet (SB), fibre sorghum (FS) and sweet sorghum (SS). For each species a single genotype suited for non-food uses was grown according to the principles of integrated farming issued by the Emilia-Romagna Region ([www.ermesagricoltura.it](http://www.ermesagricoltura.it)). All crops were in rainfed conditions. At harvest, the dry yield of product and by-product was determined. Samples of both components were analyzed (oil, sugars, fibre, total nitrogen), in order to assess the potential yields of thermal energy, 1<sup>st</sup>- and 2<sup>nd</sup>-generation ethanol (Badger, 2002), diesel fuel and biogas (Amon et al., 2007), showing for each crop the combination with the highest energy output (GJ ha<sup>-1</sup>). According to energy inputs, crop net energy (NE; output - input) and energy efficiency (EE; output/input) were also determined.

Soil samples (0-0.9 m depth) were taken at the beginning and at the end of crop cycle, oven-dried, weighted and analyzed (N-CaCl<sub>2</sub>; 7 crops out of 11), in order to assess water use efficiency (WUE; Passioura, 1977) and nitrogen use efficiency (NUE; Moll et al., 1982) for both dry biomass and energy. All data were subjected to ANOVA and to SNK test for the separation of significant ( $P \leq 0.05$ ) means.

## Results

Both winter- and, especially, summer crops experienced a serious drought (-30% precipitation than average, quite-evenly distributed around the year). Accordingly, in grain crops the harvest index was low, varying from 0.21 (BJ) to 0.47 (M), although yields were not always below the average for the area. Differences in dry yield especially arose among the three crop groups (Fig. 1): biomass crops achieved 30 t ha<sup>-1</sup> as average, vs. 14 and 8 t ha<sup>-1</sup> of cereals and oilseeds, respectively.

Applying different conversion processes to the same plant organ led to contrasting results in terms of energy output (data not shown); thermal energy was always higher than 2<sup>nd</sup>-generation ethanol (crop residues, biomasses) and biogas (cereal whole plants), although heat is not so an efficient and manageable source of energy as the liquid biofuels. The potential energy output of the 11 crops reflects their dry matter yield (Fig. 1): 400 GJ ha<sup>-1</sup> were achieved by FS and SS; about 200 by SB and the cereals as average; 120 by the oilseed crops. In grain crops, the contribution from ethanol or diesel fuel was modest with respect to heat from crop residues.

The amount of energy spent in cropping varied from 19 (BJ) to 25 (M) GJ ha<sup>-1</sup>. All crops exhibited a positive energy contribution, although much variable from crop to crop (Table 1): NE, expressing the surplus from the energy balance, varied between 60 (BJ) and 440 (FS) GJ ha<sup>-1</sup>; EE, representing the multiplying factor of subsidiary energy, ranged from 4 to 21 for the same two crops.

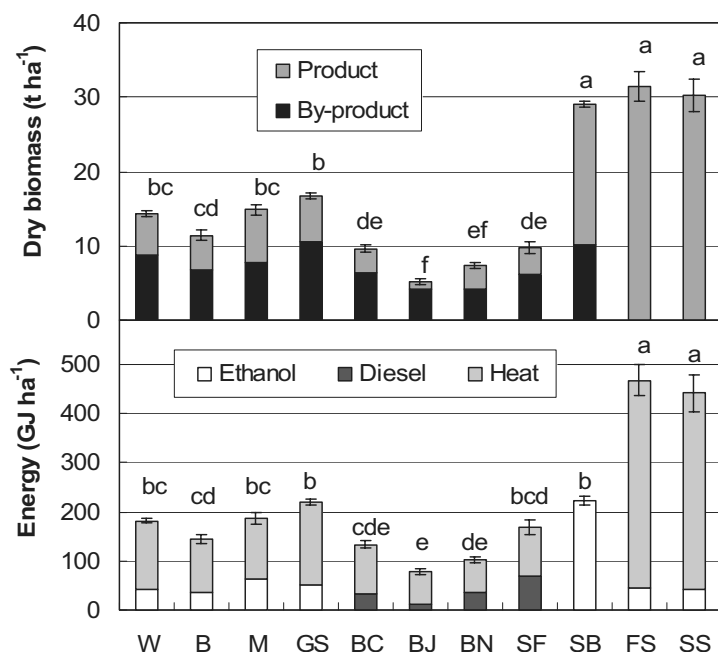


Fig. 1 - Dry biomass (top) and energy yield (bottom) of 11 annual crops.

since a high efficiency in tapping soil nitrogen pool (data not shown) was not associated with an equally-high ability to convert the nutrient into biomass and energy.

Table 1 - NE (MJ ha<sup>-1</sup>) and EE (adim.); WUE (kg m<sup>-3</sup>; MJ m<sup>-3</sup>) and NUE (kg kg<sup>-1</sup>; MJ kg<sup>-1</sup>).

Trait	W	B	M	GS	BC	BJ	BN	SF	SB	FS	SS
NE	159 bc	124 cd	162 bc	195b	115 ce	58 e	84 de	148 bd	201 b	443 a	421 a
EE	7.9 bc	7.4 bc	7.4 bc	9.0 b	7.0 bc	4.0 d	5.4 cd	8.1 bc	9.9 b	20.8 a	19.8 a
WUE {	5.2 cd	4.5 de	6.3 c	7.9 b	3.3 ef	1.9 g	2.6 fg	4.0 def	7.7 b	11.8a	11.9 a
	66 cd	57 cde	80 c	104b	46 def	28 f	37 ef	70 cd	59 cde	174 a	174 a
NUE {	28 bc		33 bc				19 c	24 bc	39 b	59 a	70 a
	357 b		416 b				265 b	394 b	287 b	872 a	1022 a

## Conclusions

The four oilseeds turned out to be the least-favourable energy crops under all the considered aspects; the fact is consistent with the low yield of highly energy-demanding grains committed, in practice, to a single conversion process (bio-diesel). Biomass crops, at the opposite, showed the best option: especially fibre and sweet sorghum featured high yields despite summer drought, peaking in the efficiency of all crop inputs. It is perceived as intrinsically-expensive productions, such as bio-diesel, need to be carefully evaluated in the planning phase, or involve that the co-products (e.g., residual seed meals) or other benefits (e.g., urban quality of the air) be taken into account, aside from the above results, for a successful development of the specific energy chain.

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WUE of dry biomass peaked in the two biomass sorghums (Table 1), quite surprisingly followed by SB, which in turn equalled the other two C<sub>4</sub> species (M and GS). Conversely, oilseed crops, and especially BJ, were outperformed. In terms of energy, water achieved less than 30 MJ per unit cubic meter in BJ; about six-times as much in FS and SS.

At last, NUE of the seven selected crops showed a productivity of about 30 kg dry weight kg<sup>-1</sup> of soil-available N in cereals; about 20 in oilseed crops; from 40 to 70 in biomass crops (Table 1). In terms of energy, one kg of N approximately made for a minimum of 250 MJ in BN, up to four-times as much in SS. With respect to NUE, SB did not perform so well as in other traits,

# Influence of Substrate Hormonal Composition on *in vitro* Multiplication of *Arundo donax* L.

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*Arundo Donax* L., or Giant Reed has a high biomass productivity and it is considered, even by EU programmes, as a promising crop for energy but also for cellulose for paper and rayon industry. It may be also useful for soil erosion control.

Traditional propagation of giant reed is by rhizomes division. However, this type of propagation, is time consuming and requires a considerable economical effort for explanting, division of rhizomes, planting and successful establishment of the crop. Such disadvantages could be overcome by *in vitro* propagation. However, regeneration and large scale propagation have not been reported so far for this species. In order to set up a protocol for *in vitro* multiplication of giant reed a series of research has been carried out. First results on *in vitro* initiation of the crop has been described previously (Cavallaro et al., 2007). In this paper, results of a research aiming at studying the influence of substrate hormonal composition on shoot multiplication are discussed.

In this research, the effects of two different hormones (6-benzylaminopurine-BA and Kinetin-K) and two different concentrations (2, 3 mg L<sup>-1</sup>), were evaluated.

## Methodology

The genetic material used, clone “Fondachello”, was collected during October 2006 in the experimental field of the Catania University situated in the Catania plain (10 m a.s.l.). It belongs to a collection of 39 genotypes (Cosentino et al., 2006) collected in Sicily and Calabria (South Italy).

In order to begin the *in vitro* cultivation, the dormant buds situated on the nodes of the central part of the stem were excised and disinfected in a solution of 5 g L<sup>-1</sup> of mercury dichloride for 5 min, in a solution of NaClO (1,5% of active Cl) x 20min and then three times rinsed with sterile distilled water. Cultures were incubated at 23 °C with 16 h photoperiod. After 20 days the developed shoots were carefully excised from the node and transferred on the multiplication medium.

The experiments were carried out using a completely randomized block design with four replications, including the following factors:

- 2 different hormones added to the basic medium (6-benzylaminopurine-BA, Kinetin-K)
- 2 concentrations for each hormone (2 and 3 mg L<sup>-1</sup>)

Basic medium included MS macro and micro nutrients (Murashige T., Skoog F., 1962), Morel's vitamins, 100 mg L<sup>-1</sup> myo-inositol, 30 g L<sup>-1</sup> saccharose, 1 mg L<sup>-1</sup> indole -3-acetic acid-IAA, 0.05 mg L<sup>-1</sup> gibberellic acid -GA and 8 g L<sup>-1</sup> agar.

After two subculture, the percentage of adventitious shoots with normal growth were evaluated.

Four explants were used per treatment. Each treatment was three times replicated.

## Results

BA addition to the basic medium determined a significant greater multiplication rate (3.1 normal shoots per plant) as compared to K (2.5 normal shoots per plant). Among the concentrations studied, 3 mg L<sup>-1</sup> was more effective in stimulating proliferation of adventitious shoots than 2 mg L<sup>-1</sup>. However the highest level of K determined a significant higher number of dead shoots (11.7%) and rooted plantlets (29,0%) as compared to BA at the same concentration (3,0% dead plants and no rooted plantlets). The appearance of roots in this phase in fact is a negative feature since it may determine a decrease in the number of new secondary shoots differentiated. Moreover, at 3 mg L<sup>-1</sup> (Fig.1) concentration BA determined a 40% increment in the mean unitary fresh weight of adventitious shoots as compared to K.

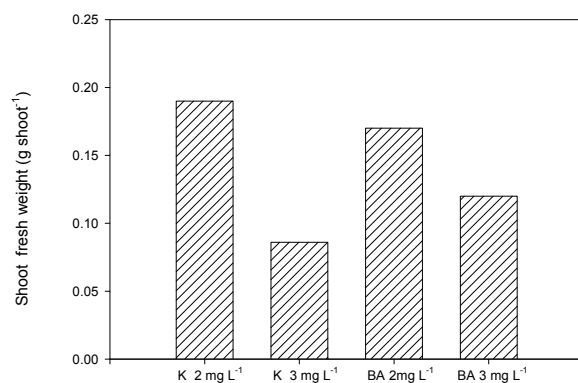


Fig. 1. Mean shoot fresh weight in relation to hormonal composition of the medium

### Conclusions

First results obtained showed that among the two hormones tested, BA, at a concentration of 3 mg L<sup>-1</sup>, resulted the most effective leading to the highest number of well developed secondary shoots, and no rooting. However, further research including more multiplication subcultures must be carried out in order to assess if this multiplication rate is a stable feature.

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# Identification of Biomass Crop Production Area and Genetic Algorithm Application for Optimal Location of Processing Plants

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When planning fiber and biomass production (hemp, *Miscanthus*, *Arundo*, *Sorghum*, etc.) at regional scale, some common problems arise, due to the distance of the production sites to the processing plant and to the very low density of the material to be transported (0.1-0.3 t/m<sup>3</sup>). These facts generate sustainability issues related to the needed minimization of costs and the evaluation of the feasibility of the supply chain, in interaction with the yielding possibilities of the areas. Defining optimal production areas within a region is often complicated by aspect related to transport costs, land productivity and its distribution, climate and soil variability. To improve the managing and planning of crop areas, the use of GIS has an important role, particularly when used in connection with simulation models and optimization algorithms. This stress the need to settle appropriate tools for this aim, able to be used in a GIS software framework.

## Methodology

To this aims, a methodology has been developed to: a) determine the optimal area in which it is convenient to grow crops for biomass, given a number of existing processing plants, their processing capacity and locations; b) identify of the optimal locations of a given number of processing plants and determine the related production areas in order to minimize total transport cost. The case b) can falls within the classical category of “facility-location problem” that has typically been solved using mathematical integer-programming techniques (Drezner & Hamacher, 2002). More specifically this problem consists of determining the location of  $p$  production plants in a given region which satisfy  $n$  demand points (in this case, the crop production locations) in such a way that the total sum of transport costs between each production location point and its nearest facility ( $C_{trasp}$ ) is minimised: so the problem is minimize  $\sum \min(C_{trasp_i})$  under some given constrains. Literature on optimal facility location problems and modelling logistics is very extensive (for a review see ReVelle and Eiselt 2005). Notwithstanding, to date there are few specific application to the biomass and bioenergy sector, in particular for planning purposes. In this case, we propose a solution to this optimisation problem with an application of a genetic algorithm (GA; Goldberg, 1989) in a procedure (*croparea*) that has been integrated within SemGrid, a raster freeware GIS software developed at the University of Udine (Danuso and Sandra, 2006). The integration within SemGrid framework allows the user to easily manage georeferenced data and perform various spatial analysis operations also by scripts. The procedure performs simultaneously the location-allocation problem and the determination of each resulting production area, heightened as new raster layer in output. Cropping areas and optimal plant locations are determined on the base of the yield production map (previously constructed in SemGrid), distance to the plants and transport costs. In our case, in the genetic algorithm, each

Tab. 1. Inputs for the command *croparea*.  
The output is a layer with the cropping areas.

layer with crop yield
code and coordinates of the processing plant
processing capacity of the plant (t/year)
Land cropping intensity coefficient
mean/standard deviation of cropping module areas
Fixed and variable transport costs (tractor and trucks)
Tortuosity factor
Total land production to be allocated to the plants
Layer with allowed areas for plant location
Genetic algorithm parameters

The integration within SemGrid framework allows the user to easily manage georeferenced data and perform various spatial analysis operations also by scripts. The procedure performs simultaneously the location-allocation problem and the determination of each resulting production area, heightened as new raster layer in output. Cropping areas and optimal plant locations are determined on the base of the yield production map (previously constructed in SemGrid), distance to the plants and transport costs. In our case, in the genetic algorithm, each

chromosome consists of sets of coordinate of all processing plants ( $2p$  genes where  $p$  = number of plants to locate) and the fitness function corresponds to  $\sum_i \min(C_{transp_i})$ . The procedure allows the user to manipulate the four typical parameters of a GA: number of chromosomes in the population, crossing-over probability, mutation probability, maximum number of generations. Regarding transport assumption, we have considered the possibility of transport by truck and tractor; critical travel distance factor (TDx) determines the profitability of the tractor vs. truck transportation. Regarding source locations, where biomass are cultivated and stored in balls, it is supposed that these are uniformly and random distributed through the region of analysis, grouped in nodes of a given number of hectares, randomly assigned. We also introduced the constrain that plant locations fall within cropping area region.

### Applications

In figure 1, we present an application to the determination of four hemp production areas in the plain of the Italian region Friuli Venezia Giulia, using a grid layer of crop yield with a cell of 100x100 m. Gray



Fig. 1. Friulian plain hemp production areas

zones are the production areas connected to processing plants indicated with black dots. In this example it was hypothesised a total production of 80.000 t/year and four plants each with 20.000 t/year of capacity. In this case the GA parameters were: number of chromosomes = 60; crossing-over probability = 0.15; mutation probability = 0.01; maximum number of generations=100.

### Conclusions and prospects

Further improvements are needed and will include: the possible refinement and addition of more parameters to better estimate production yields and travel costs; a

better automatization of the procedure and the construction of a user interface that allows for a easier application in case studies; the refinement of the genetic algorithm.

### Acknowledgement

This work was developed within the project “NO FOOD: Sviluppo di nuove filiere per le produzioni no-food (oli industriali, fibra, cellulosa ed amidi) con studi e ricerche sulle tecnologie e sulla razionalizzazione dei processi e dei sistemi produttivi” funded by Regione Friuli Venezia Giulia (legge regionale 26/2005 – B. U. R. del 4/8/2004, n° RAF/7/4146)

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# Comparison of 7 Ligno-Cellulosic Biomass Feedstock Species: 6-Years Results in the Low Po Valley

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Ligno-cellulosic biomass is a renewable resource with the potential to be a significant contributor for sustainable land use. In fact, an increased reliance on ligno-cellulosic biomass for energy generation may fulfil the following needs: i) curbing the rise of atmospheric carbon dioxide via displaced use of fossil energy and enhanced carbon sinks; ii) reducing the dependence on imported fossil fuels with rising market prices; iii) sustaining the income of rural populations; iv) for some species: exploiting marginal lands, unsuited for arable crops; v) for other species: increasing biodiversity in crop rotations. Several plant species has been proposed as a potential source of ligno-cellulosic biomass. The scope of this study was to compare the performances of seven plant species cultivated for ligno-cellulosic biomass in the environmental conditions of the Low Po Valley.

## Methodology

A field experiment was conducted from 2002 to 2007 at the Station of CRA-CIN, located in Anzola dell'Emilia (Bologna), Low Po Valley, Northern Italy (Lat. 44°32'N, Long. 11°80'E, 38 m a.s.l.). The location is characterized by an annual average precipitation of about 600 mm. The following plant species has been compared: the herbaceous annuals fiber sorghum (*Sorghum bicolor* L. Moench) and hemp (*Cannabis sativa* L.); the herbaceous perennials giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus x giganteus*); and three woody perennials: short rotation coppice of hybrid poplar (*Populus x canadensis*), willow (*Salix alba* L.) and black locust (*Robinia pseudoacacia* L.). The experimental design was a randomized block with four replications. The dimension of individual plots was 40 m<sup>2</sup>. The field experiment was not irrigated. Herbaceous crops were fertilized with 120 kg nitrogen (N) ha<sup>-1</sup> year<sup>-1</sup>, applied in form of urea. Woody perennials were fertilized with the same N amount at time intervals of two years, after every harvest. Insects were controlled chemically in hybrid poplar and willow. Weeds were controlled mechanically during the first year. Annual and herbaceous perennials were harvested every year, whilst woody perennials were harvested every two years.

## Results

As far as herbaceous crops are concerned, giant reed showed a superior productivity compared to other crops (figure 1, left). In first year after transplant both giant reed and miscanthus provided modest yield, amply compensated in the subsequent years. In the period 2003-2007 giant reed has always overweighted 40 t DM ha<sup>-1</sup> of aboveground biomass. Overall, sorghum and miscanthus showed inferior but still good yields, whilst hemp showed the lowest productivity. Among the woody species, black locust showed the highest productivity in the second and third harvest (figure 1, right). Conversely, the yields of hybrid poplar and willow appears to decline over time. This is likely due to the fact that hybrid poplar and willow have a low tolerance to frequent, biennial cuttings. The comparison of average annual yield performances for the 7 species is shown in figure 2. It is quite evident that giant reed has no contenders in terms of productivity. However, also sorghum and miscanthus showed good performances. When qualitative aspects of harvested biomass are considered, hemp, black locust and

hybrid poplar have better calorific power ( $16.4\text{--}15.9 \text{ MJ kg}^{-1}$ ) and lower ash content ( $2.1\text{--}2.5 \%$ ) compared to the highly productive species (Di Candilo et al., 2004).

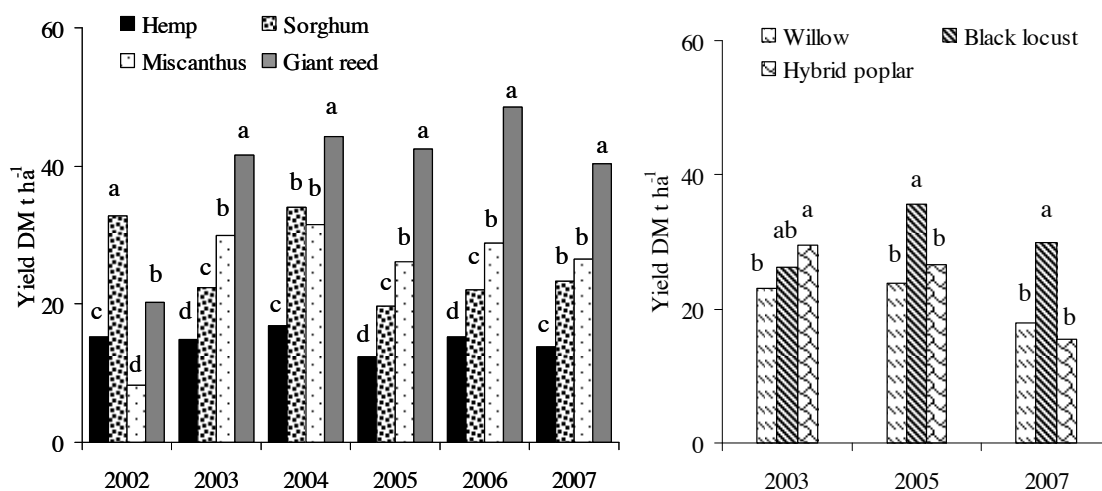


Figure 1. Patterns of productivity for herbaceous (left) and woody (right) plant species throughout the experiment. Means sharing common letters within years are not significantly different at  $p < 0.05$ . REGWQ multiple range test.

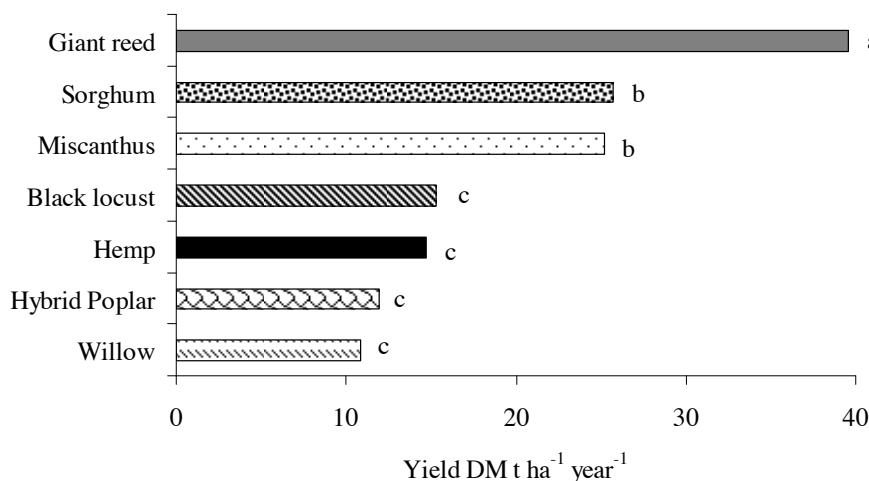


Figure 2. Average annual productivity for the 7 species in comparison. Means sharing common letters are not significantly different at  $p < 0.05$ . REGWQ multiple range test.

## Conclusions

Under our experimental conditions there is compelling evidence that giant reed is a superior crop in terms of productivity, despite to its C3 photosynthetic pathway. The explanation of its extraordinary performance probably lies on a combination of extended canopy cover, good adaptation of every type of soils and the capability to take up deep soil water. Yet the yield of sorghum and miscanthus can be deemed as quite good in the light of rainfed conditions. However, it is important to consider that biomass produced by hemp, black locust and hybrid poplar has better calorific power and lower ash content compared to highly productive species.

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# Assessment of Mediterranean Pastures Biomass and Nitrogen Status Using Field Spectrometry

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Timely assessment of pasture biomass and nitrogen status is required for livestock management and natural resources conservation (Starks et al. 2006). Traditional assessment methods based on destructive sampling and laboratory analysis are expensive and time consuming; this limits their potential use for monitoring purposes. Remote sensing techniques are a cost-effective alternative to traditional methods, allowing a fast and non-destructive estimation of pasture biophysical and biochemical variables from local to regional scale. Despite several studies have addressed the potential use of remote sensing as a tool for quantitative estimation of vegetation biophysical properties, still few results are published where hyperspectral field measurements have been acquired in complex ecosystems such as Mediterranean pastures, characterized by mixed species composition, high degree of non-photosynthetic vegetation and variable grazing pressure.

The objective of the proposed research is to retrieve Mediterranean pasture biomass and nitrogen status by means of reflectance data acquired with high resolution field spectrometers. Moreover, we evaluated the possibility to apply the predictive models developed at field level with high resolution sensors also at landscape level with airborne or satellite sensors.

## Methodology

The study was carried out in 2006-2007 in a mixed grassland (10 ha) located at Foresta Burgos, in central Sardinia (40°23'N, 8°54'E). Three campaigns were conducted in different vegetation growth stages: the autumn production peak, spring maximum growth rate and spring production peak. Measurements were acquired along 12 linear transects (10m), 6 located in grazed areas and 6 positioned in exclusion fences. Overall, 35 plots were monitored because one plot was lost during the last campaign due to technical reasons. In the grazed area the grazing pressure was kept constant during the experiment to 0.5 cattle unit ha<sup>-1</sup>.

Reflectance in the 325-1075 nm spectral range was measured every 2 m along the transect with an ASD Fieldspec HH spectrometer. Vegetation fractional cover (Fc) was determined from hemispherical digital photograph taken with a Nikon Coolpix 8400 digital camera equipped with a fish-eye lens (Nikon FE-E8 8mm). Biomass was harvested along the transect in 10 cm wide stripes (1m<sup>2</sup>). Above-ground green biomass (AGGB, t ha<sup>-1</sup>) and non photosynthetic vegetation (NPV, t ha<sup>-1</sup>) were manually separated and weighted. Sub-samples were then used for leaf area index (LAI, m<sup>2</sup>m<sup>-2</sup>) calculation, using a Leaf Area Scanner (Li-cor 3100 C), and laboratory measurement (NIRS) of nitrogen concentration (N<sub>C</sub>, %) and nitrogen availability (N, kg ha<sup>-1</sup>).

Different vegetation indexes were calculated from reflectance data: narrow-bands (3.5 nm) simple ratios (SR= [R<sub>i</sub>/R<sub>j</sub>]) involving all the possible combination of reflectance (R) in the wavelength i and j between 400 and 1000 nm; short-band (10 nm) SR based on AVIRIS (NASA-JPL)-like spectra; broad-band (>50 nm) SR derived from TM (Landsat Thematic Mapper)-like bands.

The predictive power of all these indices has been tested by ordinary least square (OLS) linear regression for estimating AGGB, LAI, N<sub>C</sub> and N. Model performances were evaluated with a Leave One Out (LOO) cross-validation in terms of cross-validated coefficient of determination (Q<sup>2</sup>) and root mean square error (RMSE<sub>CV</sub>).

## Results

An overview of the results obtained is presented in Table 1. Narrow band SR[i,j] involving bands in the near-infrared (NIR) plateau (i=800-930 nm) and in the red edge (j=720-740 nm) yielded the best performance for AGGB ( $Q^2 = 0.73$ ,  $RMSE_{CV} = 2.35$ ), LAI ( $Q^2 = 0.73$ ,  $RMSE_{CV} = 0.37$ ) and N ( $Q^2 = 0.74$ ,  $RMSE_{CV} = 7.36$ ). Concerning  $N_C$ , best predictive models ( $Q^2 = 0.54$ ,  $RMSE_{CV} = 0.36$ ) were obtained using SR[i,j] involving NIR (i=780-820 nm) and longer wavelengths of the red edge (j=750-770 nm). Compared with the traditionally used SR [i,j], calculated with NIR (i =780-900) and red bands (j=650-680 nm), the proposed indices are better suited for pasture agronomic variables estimation. These results are in agreement with recent studies which reported the red edge as a key spectral region for assessing crop agronomic variables (Hansen & Schjoerring 2003).

Hyperspectral indices, either derived from narrow-band (3.5 nm e.g. ASD Fieldspec HH) or short-band (10 nm e.g. AVIRIS) sensors, allowed significant improvements in the predictive ability of vegetation indices compared to indices based on multispectral sensors characterized by non-contiguous broad bands (>50 nm e.g. Landsat TM). Major improvements are observed for prediction of nitrogen concentration, which resulted poorly related to all the broad-band indices tested. Indeed, the use of hyperspectral sensors is recommended for timely assessment of Mediterranean pasture biomass and nitrogen status.

Table 1. Cross validated coefficient of determination ( $Q^2$ ) of regression models derived for AGGB, LAI, N and  $N_C$  prediction. Narrow-band and short-band (AVIRIS) hyperspectral SR [i,j] were selected for each variable. Broad-band (TM) multispectral SR [i,j] were calculated with all the TM channels combinations in the VIS-NIR range.

Index type	Band center [i, j] (nm)	Band width (FWHM) (nm)	Coefficient of determination ( $Q^2$ )			
			AGGB	LAI	N	$N_C$
<i>Narrow Band SR (3.5 nm)</i>	[920,729]	3.5	0.73	-	-	-
	[895,730]	3.5	-	0.73	-	-
	[905,730]	3.5	-	-	0.73	-
	[788,769]	3.5	-	-	-	0.54
<i>Traditional SR (3.5 nm)</i>	[780,680]	3.5	0.26	0.30	0.31	-0.11
<i>Short-band (AVIRIS) SR (10 nm)</i>	[923,731]	8.5	0.71	-	-	-
	[894,731]	8.5	-	0.72	-	-
	[904,731]	8.5	-	-	0.72	-
	[787,765]	8.5	-	-	-	0.48
<i>Broadband (TM) SR (&gt;50 nm)</i>	[830,660]	140-60	0.35	0.34	0.35	-0.06
	[830,550]	140-60	0.55	0.54	0.56	-0.01
	[830,485]	140-70	0.37	0.37	0.36	-0.10
	[550-660]	60	-0.01	-0.01	-0.02	-0.01
	[485-550]	70-60	0.24	0.19	0.14	-0.07
	[485-830]	70-60	-0.04	-0.07	-0.06	0.00

## Conclusions

Remote sensing techniques offer interesting potential for non-destructive assessment of forage quantity and nitrogen status in Mediterranean pastures. For applications at landscape scale, the use of airborne or satellite hyperspectral sensors (e.g. CASI, AVIRIS, HYPERION) is recommended to increase predictive models accuracy.

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# Protecting Water Resources in Biofuels Production

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Countries worldwide are setting ambitious goals for biofuel production. For example, the new U.S. federal energy law mandates a fivefold increase in current production to 36 billion gallons by 2022. China's goal is to increase current production fourfold to 15 billion liters by 2020. India also is setting similarly aggressive production goals. Countries face serious environmental limitations in meeting these goals in a sustainable manner, with water availability being among the most pressing. The U.S. National Academy of Sciences (NAS) recently investigated the impacts of biofuels production on water availability, and identified "agricultural practices and technologies" to reduce negative impacts (National Research Council, 2007). While the NAS report considers only impacts on U.S. water supplies, it has international importance both because it is the first report of its kind, and because its findings and recommendations may well serve as benchmarks for other countries attempting to protect their water resources in biofuels production.

The NAS report finds that expanded ethanol production could increasingly stress water resources by expanding biofuel crops into dryer regions requiring new irrigation, or substituting biofuel crops for others requiring less irrigation. It identifies "[e]fficient application of irrigation water [as] one of the most important ways to mitigate any effects that increased biofuels [crop] production may have on water resources," noting that "[t]here are several irrigation techniques that reduce the amount of water applied per unit of biomass produced, thus improving irrigation efficiency regardless of crop type" (p. 28). The report effectively applies a 'rule of thumb' that measures conserved water as a reduction in water application. The report also identifies water recycling within biorefineries as a means of reducing "consumptive use of water." (p. 5).

In a classic case of unintended consequences, these measures may decrease the sustainability of biofuels production by increasingly stressing water supplies. The paper asks the following questions: Will reductions in applied water resulting from improved on-farm irrigation efficiency conserve water in biofuels crop production? Will water recycling conserve water by reducing consumptive use in biorefineries?

## Methodology

The paper appeals to first principles of irrigation hydrology to determine the impacts of improved on-farm irrigation efficiency on applied and consumptive water use in crop production. These impacts are embedded into a series of hypothetical stream-flow diagrams to test the accuracy of the rule-of-thumb equating reduced on-farm application with water conservation on a broader geographic scale. A similar approach is used to investigate the impact of water recycling on water use in biorefineries and on basin-wide water availability.

## Results

The rule-of-thumb in the NAS report is an inaccurate measure of basinwide water conservation because it neglects the fate of applied water not converted to evapotranspiration (ET). Water that is not converted to ET recharges basin-wide freshwater surface and groundwater sources if it is not severely degraded in quality, or does flow into a saline water body. Consequently, improvements in on-farm irrigation efficiency that increase ET—even while reducing on-farm water application—fail to conserve water on a broader geographic scale when irrigation return flows are an important component of basinwide hydrology. Following similar logic, water recycling in biorefineries increases evaporative

losses, and thus consumptive water use. This reduces downstream water supplies when treated wastewater is discharged back to the stream.

### **Conclusions**

Policymakers attempting to conserve water in biofuels production may unintentionally increasingly stress surface and ground water supplies if policies target increases in on-farm irrigation efficiency and water recycling in biorefineries. Improvements in on-farm irrigation efficiency that increase *ET* fail to conserve water on a broader geographic scale when irrigation return flows are an important component of basinwide hydrology. In this case, a rule-of-thumb equating reductions in withdrawals with water conservation is inaccurate. Agricultural water conservation policies should target reduced *ET*, not simply reduced water withdrawals. Following similar logic, water recycling increases evaporative losses in biorefineries. This reduces downstream water supplies when treated wastewater is discharged back to the stream. Policies attempting to conserve water in biofuel processing should promote the adoption of new technologies that reduce evaporative losses.

Although policies targeting increases in on-farm irrigation efficiency and water recycling in biorefineries may not reliably conserve agricultural water on a basinwide scale, they can mitigate water quality impacts of biofuels production. Both measures mitigate water pollution by reducing the throughput of water into farm and biorefinery production.

These results stress the need for countries to understand the true benefits and costs of adopting proposed water conservation measures in biofuel production so that policies achieve desired outcomes and minimize unintended consequences.

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# Physiological Background of Biomass Accumulation by Perennial C<sub>4</sub> grasses in Poland

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One of the reduction ways of CO<sub>2</sub> emitted into the atmosphere is the greater use of renewable energy sources such as biomass. The most promising plants for these purposes seem to be the perennial rhizomatous grasses with the C<sub>4</sub> photosynthetic pathway. One of them is the genus *Miscanthus* Anderss. originating from Southeast Asia. This genus includes *Miscanthus x giganteus* Greef et Deuter, the most cultivated species with respect to biomass production across Europe. The genus *Miscanthus* Anderss. includes the two other genotypes such as *Miscanthus sacchariflorus* (Maxim.) Benth. et Hook. and *Miscanthus sinensis* Anderss. Very important advantage of *M. sinensis* genotypes over *M. x giganteus* is their improved winter hardiness. The data from the field trials in Denmark [5] indicate that *M. sinensis* clones can out-yield *M. x giganteus* genotypes. Switchgrass (*Panicum virgatum* L.) is a warm season perennial herbaceous C<sub>4</sub> grass that is indigenous to North America and is found from Mexico to Canada. Switchgrass is established from seed and for that reason the start cost of cultivation is less expensive and involves less risk than that of *Miscanthus*, which is propagated by rhizomes. The general objective of this work is the evaluation of potential biomass productivity of *M. sinensis*, *M. sacchariflorus* and *P. virgatum* throughout Poland on the basis of empirical model and the regional meteorological data. In this paper the results concerning *RUE* (radiation use efficiency) have been shown.

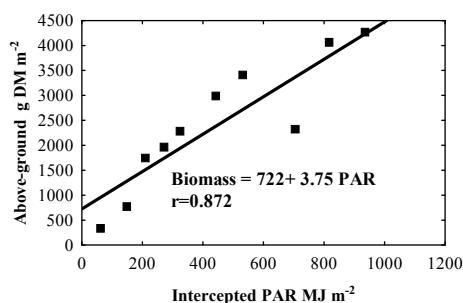
## Methodology

The research was conducted at the Dolna Odra Power Plant in Nowe Czarnowo (53°12'N, 14°27'E) on the experimental model consisting of the separated plots (10 m<sup>2</sup>). The seedlings of *M. sinensis* and *M. sacchariflorus* have been planted in 2004 at the density of two plants·m<sup>-2</sup>. In the same year, *Panicum virgatum* v. Cave-in-Rock has been sown at the seeding rate 15 kg·ha<sup>-1</sup>. At the beginning of each growing season mineral fertilizers have been applied at 80 kg N·ha<sup>-1</sup>, 40 kg P·ha<sup>-1</sup>, 60 kg K·ha<sup>-1</sup>. The weeding was controlled manually. Starting from the beginning of the vegetation season (last spring frost) at two week intervals 20 shoots of each species were harvested. Plants were separated into leaves, stems and inflorescences. The assimilation area was measured using the DIAS (Delta-T Devices, Cambridge, UK); then plants are oven-dried at 80 °C and weighted.

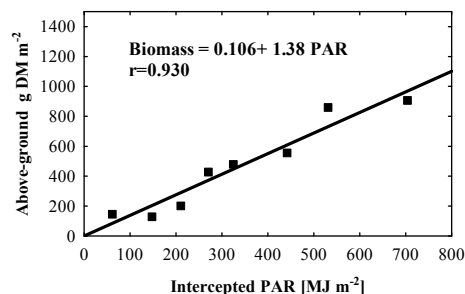
## Results

There is a lack of data describing growth and yielding of C<sub>4</sub> perennial grasses in different growing regions in Poland. The existing data are mainly derived from microplot experiments, observations from botanical gardens or collections that have been done on single plants. The comparative study of *M. sinensis* clones and *M. x giganteus* indicates that in the Middle West region of Poland *M. sinensis* clones yielded lower than clones of *M. x giganteus*, in the third year of vegetation (Jeżowski 2008). In our study the maximal autumn dry matter yields of *M. sinensis*, *M. sacchariflorus*. and switchgrass were 19.7, 9.1 and 30 t·ha<sup>-1</sup>, respectively. Compared to genus *Miscanthus*, switchgrass is smaller, thinner and generally leafier but produces higher amount of shoots per area. *RUE* is an effective and efficient approach to quantifying plant biomass accumulation. The *RUE* was highest for the

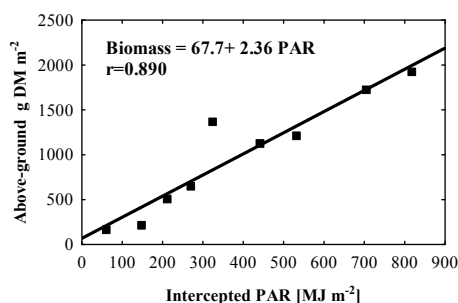
switchgrass ( $3.75 \text{ g} \cdot \text{MJ}^{-1} \text{ PAR}$ ). This is comparable to the value of  $3.3 \text{ g} \cdot \text{MJ}^{-1} \text{ PAR}$  determined for *M. x giganteus* growing in the Netherlands (Vleeshouwers. 1998). On the other hand, the *RUE* for *M. sinensis* was almost the same as calculated for the Ireland conditions by Clifton-Brown et al. (2000).



Switchgrass (*Panicum virgatum*)



*Miscanthus sacchariflorus*



*Miscanthus sinensis*

Relationship between above ground dry matter DM and intercepted PAR. The slope of the regression is the *RUE*.

## Conclusions

Experiments conducted in this study were designed specifically to provide near-optimum growing conditions for *C*<sub>4</sub> genotypes biomass production. The main factor limiting biomass accumulation in Poland is water availability, hence the 2007 year as exceptionally wet at May-June period resulting in great dynamics of the leaf area development and dry matter accumulation. Further work is necessary to confirm presented results.

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# Energy Balance of Sweet Sorghum Genotypes (*Sorghum Bicolor* L. Moench) in a Semi-arid Environment of South of Italy

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European Council sets a 20% share of renewable energies in EU energy consumption by 2020 (CEC, 2008). This target will be supplemented by a minimum 10% obligation for biofuel and it would require 17.5 million hectares or 15% of the total arable land of EU-27 (EU, 2007). Sweet sorghum (*Sorghum bicolor* (L.) Moench) may produce bioethanol from sucrose contained in the stems and also contribute to the production of bioethanol from the lignocellulosic biomass as a second generation biofuel. This C4 crop shows an high yield potential in non limiting water conditions (Cosentino et al., 1996), but in Southern Europe the high temperature requirements for seed germination which impose its growing season during summertime, when rains are almost absent, makes necessary the use of irrigation. On this basis a field experiment was carried out in order to study the energy indices of two sweet sorghum genotypes in relation to different energy inputs.

## Methodology

The field experiment was carried out in 2002 in the South of Italy (Enna, 450 m a.s.l., 37°23' N Lat, 14°21' E Long). Two sweet sorghum genotypes (Roce and M81E) with two different sowing times (April and July) and with four different input levels: I<sub>1</sub>= 100% ETM restoration and N<sub>120</sub>=120 kg ha<sup>-1</sup> I<sub>2</sub>) irrigation until seedling establishment and N<sub>30</sub> = 30 kg ha<sup>-1</sup>; I<sub>3</sub>) irrigation until seedling establishment and at blooming and nitrogen supply = 60 kg ha<sup>-1</sup>; I<sub>4</sub>) 100% ETM restoration and supply of manure were studied in a split plot design with three replicates. Harvest occurred in October the 9<sup>th</sup> and in December the 18<sup>th</sup> respectively for Roce and M81E (physiological maturity) in first sowing time. Both the genotypes in the second sowing time were harvested in January the 15<sup>th</sup>.

Energy inputs during the cultivation were calculated according to Mantineo et al., 2007. The energetic evaluation of production processes was based on the protocols of the experiment, determining energy costs for machinery fabrication and repairs, for fuel consumption for the several mechanical operations, for irrigation, for fertilizers, herbicides and planting materials.

Energy outputs were calculated separately for different biomass components by means of energetic equivalents (Odum, 1988). The data of productive characteristics and energy parameters were analysed by a three-way ANOVA, using CoStat Version 6.003 (Cohort Software) related to a split-plot experimental design in field.

## Results

On the average of the studied factors, aboveground dry biomass decreased going from the first sowing time to the second one (28 t ha<sup>-1</sup> against 16.7 t ha<sup>-1</sup>), but the late sowing time allowed the saving of 63% of irrigation water because plants made use of autumnal rainfalls (tab.1).

The late genotype M81E, in the average of the other factors, showed to be significantly more productive than Roce genotype (26 against 18.7 t ha<sup>-1</sup> respectively); moreover a significant interaction “variety x sowing time” was observed because late variety showed a yield reduction more marked in the first sowing time than in the second one. The reduction of input level, in the average of the other factors, decreased yield from 25.7 t ha<sup>-1</sup> to 19.4 t ha<sup>-1</sup> respectively for I<sub>1</sub> and I<sub>2</sub>.

Tab. 1- Above ground dry biomass (t ha<sup>-1</sup>) in relation to the studied treatments

	First sowing time (April 24 <sup>th</sup> )			Second sowing time (July 28 <sup>th</sup> )			Average		
	M81 E	Roce	average	M81 E	Roce	average	Input	M81 E	Roce
I1	41.4	24.8	33.1	20.8	15.7	18.2	<b>25.7 a</b>	31.1	20.3
I4	38.2	24.7	31.5	19.1	15.1	17.1	<b>24.3 a</b>	28.6	19.9
I3	27.6	21.5	24.6	17.3	13.9	15.6	<b>20.1 b</b>	22.4	17.7
I2	26.2	19.8	23.0	17.7	13.7	15.7	<b>19.4 b</b>	22.0	16.8
average	33.4	22.7	<b>28.0 a</b>	18.7	14.6	<b>16.7 b</b>	22.4	<b>26.0 a</b>	<b>18.7 b</b>

Int. genotype x sowing time DMS 3.22 (p ≤ 0.05) and 4.44 (p ≤ 0.01)

Net energy yield recorded in the first sowing time was higher than the second one (438.6 against 258.2 GJ ha<sup>-1</sup>) (tab. 2). M81E, late genotype showed a net energy yield significantly higher compared to the early genotype Roce (408.8 against 88.6 GJ ha<sup>-1</sup>). As far as energy input is concerned, net energy yield decreased with the increase of energy input level from 393.90 GJ ha<sup>-1</sup> (I2) to 304.7 GJ ha<sup>-1</sup> (I1).

Tab. 2- Net energy yield (GJ ha<sup>-1</sup>) in relation to the studied treatments

	First sowing time (April 24 <sup>th</sup> )			Second sowing time (July 28 <sup>th</sup> )			Average		
	M81	Roce	average	M81	Roce	average	M81 E	Roce	Input
I1	644.7	376.5	510.7	321.8	232.4	277.1	484.4	304.4	<b>393.9 a</b>
I4	597.4	380.8	489.1	299.5	235.8	267.7	448.4	308.2	<b>378.4 a</b>
I3	435.3	344.7	390.0	272.6	213.7	243.2	354.0	279.2	<b>316.6 b</b>
I2	418.9	310.7	364.8	275.5	213.9	244.7	347.2	262.3	<b>304.7 b</b>
average	524.1	353.1	<b>438.6 a</b>	18.7	14.6	<b>258.2 b</b>	<b>408.2 a</b>	<b>288.6 b</b>	384.4

Energy ratio (output/input) showed the same trend of net energy yield (tab. 3). The reduction of energy input level, in the average of the genotypes, determined in both sowing times an increase of the energy ratio from I1 to I2 treatment more marked in the first sowing time than in the second one (46.7 % against 33.6%).

Tab. 3- Energy ratio (output/input) in relation to the studied treatments

	First sowing time (April 24 <sup>th</sup> )			Second sowing time (July 28 <sup>th</sup> )			Average		
	M81 E	Roce	average	M81 E	Roce	average	Input	M81 E	Roce
I1	14.59	9.58	12.08	13.08	9.72	11.40	<b>11.74 c</b>	13.83	9.65
I4	17.22	11.87	14.54	17.87	14.29	16.08	<b>15.31 b</b>	17.54	13.08
I3	18.43	16.66	17.55	16.73	13.33	15.03	<b>16.29 b</b>	17.58	15.00
I2	25.79	19.50	22.65	19.22	15.15	17.18	<b>19.92 a</b>	22.50	17.33
average	33.4	22.7	<b>28.0 a</b>	16.73	13.12	<b>14.92 b</b>	15.81	<b>17.87 a</b>	<b>13.76 b</b>

## Conclusions

The late variety M81E yielded more than the early variety Roce in both sowing dates. The first sowing date determined a higher production than the second. The energy ratio resulted highest in the I2 low input treatment and lowest in I1 high input treatment.

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# Biomass Production in Hilly Areas of Central Italy

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The agricultural sector can provide an important contribution to reduce the fossil fuels consumption and GHG emissions through agroenergetic production from dedicated energy crops and biomass residuals. To evaluate these topics, a research project was started in 2006 with the contribution of Tuscany Region administration. The aim was to promote a short supply chain in hilly areas of central Italy (Tuscany), furthering the local energetic biomass production and the reduction of transport cost. In particular, this paper describes a study about potential local production of energetic biomass from two traditional crops suitable to biofuel production. The considered crops were maize (*Zea mays* L.) and sunflower (*Helianthus annuus*, L.) used respectively to bioethanol and biodiesel production. This study was conducted in Sinalunga rural district (Siena – Italy). The evaluations were carried out taking into account the impacts of climate and meteorological variability through the application of simulation models of growth and production. The results were discussed to evaluate the variation in energetic biomass potential production in relation to the climate change and global warming scenarios.

## Methodology

Collection and control of data about study area: the farm “Fattoria Belvedere” is located in hilly area of central Tuscany. Daily minimum and maximum air temperatures (°C) and daily precipitations (mm), were recorded from 1995 to 2003. The solar global radiation daily data were calculated through RadEst v. 3.0 software, that is able to estimate global radiation in relation to a daily thermal range on the base of Bristow and Campbell model (1986). The main soil characteristics were extracted from the geological map of Tuscany. Maize and sunflower yields ( $\text{Kg ha}^{-1}$ ), agricultural management conditions, such as modality and time table of irrigation and fertilization, sowing and harvest dates were provided by the farmer. All data collected were controlled to detect and adjust for the possible unaccounted or out-range data.

Calibration and validation of the model: the crop growth model adopted to simulate yields was CropSyst (Stockle and Nelson, 1999). CropSyst is able to simulate the crops growth and production depending on cultivar specific parameters (photosynthetic sensibility, maximum LAI, phenological phase time etc.), management parameters (modality and time table of irrigation and fertilization, sowing dates, etc.) and soil parameters (texture, soil depth, nitrogen and organic matter content, etc.). From meteorological input-data (daily minimum and maximum air temperatures, precipitations, solar global radiation) CropSyst calculates the crop yield on the base of Photosynthetically Active Radiation (PAR) and coefficients of biomass distribution and allocation. For the maize and sunflower CropSyst was calibrated for the study area through sensitivity analysis in relation to thermal time necessary to the maturation phase. The soil was 1.10 m depth, with texture (sand 24%, clay 37%, silt 39%) and an organic matter content of 1.5%, were considered. A rainfed system for the sunflower was considered, while for the maize only emergency irrigations, when water field capacity fallen down 35%, were considered. The fertilization is that actually used by farmer: the quantitative for hectare and the fertilizer type change for each years. The fertilizer used at seeding are: 11-22-16 (200-300 kg /ha for sunflower and 400-500 kg/ha for maize) or 18-46-0 (200 kg/ha for sunflower). Urea was used at seeding or in cover (100-200 kg/ha for sunflower and 200-300 kg/ha for maize). Pearson's correlation coefficient ( $r$ ) and Root Mean Square Error (RMSE) between observed and simulated yields were calculated.

Impacts of meteorological climate variability on productions and plant phenology: the model was used to predict the annual yields and the onset of grain filling (GF) and maturation (M) dates, consequential to daily mean temperatures increases by 0.5 °C and 1.0 °C respect to daily temperatures recorded from 1995-2003. The climate impacts were evaluated calculating the variation of yields (%), grain filling and maturation time (Days Of the Year; DOY).

## Results

For the maize and sunflower meteorological simulation model of growth and production was calibrated and validated on a study area (Fig. 1).

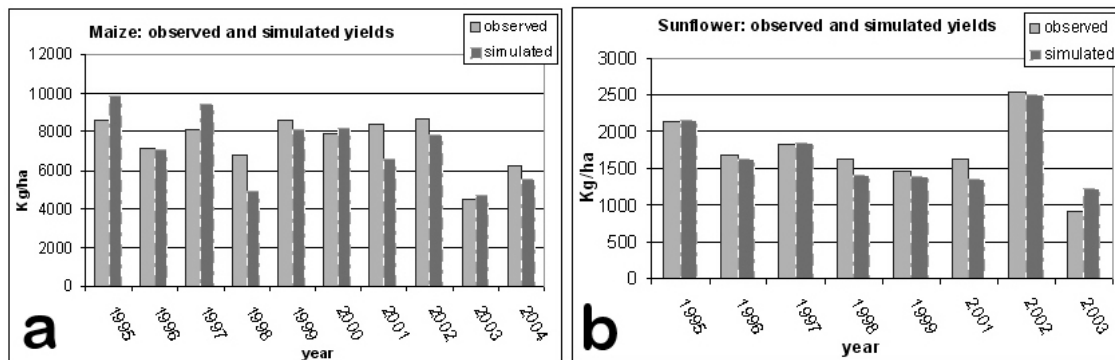


Fig. 1: Comparison between observed and simulated yields for maize (a) and sunflower (b). With a mean temperature increasing by 0.5 °C, the yield showed a decrease of 6% and 4% respectively for maize and sunflower. For the same crops it fallen down by 12% and 11%, respectively, when considering 1.0°C of rising temperatures (Fig. 2).

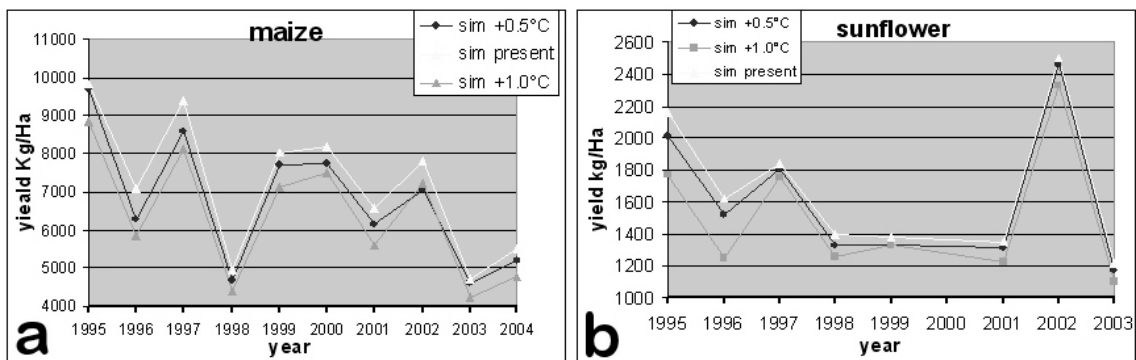


Fig. 2: Simulated yields for maize (a) and sunflower (b). As regard crops phenology, the rising temperatures caused the duration of phenological phases to be reduced as shown in table 1 and, as a consequence, an advancing of the ripeness.

	Time reduction between GF and M (Days)		GF anticipation (Days)		M anticipation (Days)	
	0.5° C	0.1° C	0.5° C	0.1° C	0.5° C	0.1° C
<b>T° increase</b>						
<b>Maize</b>	1.4	2.7	2.1	3.8	3.5	6.5
<b>Sunflower</b>	0.9	2.2	2.4	4.6	3.3	6.8

Tab. 1: Impacts of temperature increases on the phenological phases of maize and sunflower. Legend: GF = rain; M = maturation.

### Conclusions

The study results provide an estimation of the impacts of increasing temperatures, as resulting from global warming, on growth and production of crops traditionally cultivated in the Tuscany and suitable to biofuels production. The simulation models predicted a negative impacts of the temperature increase on yield levels for sunflower and maize. The temperature increase showed effects on phenological phases with an anticipation of grain filling and maturation dates for both the crops. The validated model can be used to carry out the same evaluation in analogous agroecosystems; it can represent a instrument particularly useful to supply information about the crop response to predicted meteorological variability.

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# Effects of an Intensified Biomass Production of Agricultural Enterprises on Ecological and Economic Indices

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Studies for the production of bio energy from agricultural products predominantly deal with issues like conversion, the technology, the energy output and the efficiency on the process level. Effects on the land use change on the farm or the regional level are often not adequately considered.

The production of biomass for energy use has to follow the same principles and regulations as the production of food and fibers. Given this background a study was initiated by the Institute for agriculture and environment (ilu) at the FNL in 2007 to estimate and calculate the effects of various levels of bioenergy production in Germany.

## Methodology

Unlike most studies which have previously tried to estimate the potential of bioenergy from crop production, we have used different farms in various areas of Germany and tried to maximize their energy production in the context of the farm operation. This approach leads to a number of restrictions based on environmental and crop rotation limits. Such limits have not been considered in previous studies.

As a tool for this study we used the model REPRO. This is a computer model developed at the Martin Luther University of Halle-Wittenberg, which can analyze energy and material flows on field or farm level. With REPRO, it is possible to assess and to analyze different environmental indicators, however, for this investigation we focus on the parameter nitrogen balance and TGE, which is the basis of the evaluation of the nitrogen management for the farm operation. A special feature which has to be mentioned in this context is the fact that in the model REPRO the calculation for the indicator nitrogen and TGE balance is closely related to the balance of soil organic matter (SOM) thus positive or negative changes in the content of SOM have an effect on the nitrogen balances and thus also effect the TGE due to changes in the carbon stocks. The calculation of the erosion risk of the different sites was based on detailed information of the geography and soil conditions.

The different farms included in this study represent major environmental areas in Germany as well as different structures with respect to farm size and operation focus. This will allow for a comparison with other parts of Germany.

## **Results and discussion**

In this short abstract we will only briefly highlight some of the major results, which differ in our study compared with other studies that have tried to calculate the potential of energy production for Germany.

Our results show that limitations exist for various environmental reasons, which do not comply with current environmental legislation or the regulations included in cross compliance (CC). Important was the limitation on the acceptable level of erosion. A strong increase in the acreage of corn for biomass production will cause an increase in the erosion potential in most sites in Germany. This stands in stark contrast to CC and the soil protection legislation. Especially in areas with loess soils and strong seasonal precipitation, such limitations apply.

Additionally important is the effect of the slurry from biogas plants on soil fertility and especially on SOM stocks. In cases with a focus on biogas produced on the basis of corn, it is, according to our calculations, not possible to maintain the level of SOM in the long term. This result stands in contrast to CC and again the soil protection legislation. In our calculation, we have assumed that all slurry from biogas plants is recycled in the very soil where the corn was grown. In reality this will not be the case, since large biogas plants collect corn from large areas and the slurry is only redistributed in a much smaller area. On top of that it contradicts the idea of bioenergy production itself, since such a decrease in SOM stocks would cause a release of CO<sub>2</sub> and affect the TGE potential.

The increase on acreage for oilseed rape (OSR) is mostly limited for reasons of rotations design. Most areas in Germany are now growing OSR every third year. A further shortening of the rotations is not feasible. This clearly limits the potential to increase the OSR production.

On the other hand, specific potentials, which will be important in the future due to environmental legislations have mostly not been considered in other studies. This applies especially to the use and potential meadows and grassland. The proportion of grassland has to increase substantially in most regions of Germany in the future due to the effects of the EU water initiative. Large areas will experience much wetter conditions as today, because drainage will be limited in order to establish nature like waterways. This restricts the potential arable crops. And with the perspective of energy production this is important for a future increase in corn production, however, from a technological point of view the options to use grass and grassland for energy production are not fully elaborated yet, this we recommend to focus on the development of more options to use grass for the production of energy.

## **Conclusions**

Most studies of the potential of energy production from crops have not considered limitations from environmental or rotational effects. If such limitations are included in the calculations, the potential of bioenergy production is substantially lower. This is mainly due to the effects of limitations in ground water pollution and difficulties to maintain SOM balances with an intensive biogas production.

# Resource Use for Food and Energy Crops

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## Introduction

Global demand of crop produce has been projected to double in the 21<sup>st</sup> century to meet the basic needs of a growing population for food and feed. On top of this big challenge, the current political support for renewable energy sources accelerates strongly the commercial use of biomass, including crop produce, for biofuel and power plants. This recent development increases the pressure on the resource base (land, water, biodiversity). There are big gaps in understanding the global capacity for sustainable plant-based bioenergy production, while maintaining food security (Hill *et al.*, 2006). It is evident that commercial biomass production will increasingly compete with food crops for arable land and fresh water resources (Varis, 2007). First effects on prices and availability of commodities, used as staple food and feed, can already be observed. Those trends can be illustrated by the developments in the USA, where multinationals and retailers play a dominant role in the food chain and trading of agricultural commodities. It is still the major exporting country of crop commodities, such as maize, wheat and soybean. However, the booming ethanol production from maize grain since the last 5 years caused an unbalance between demand and supply of maize resulting in an abrupt increase in maize prices (Cassman & Liska, 2007). To meet the growing demand for maize without expanding the acreage of agricultural land, the current trend in yield increase of about 110 kg ha<sup>-1</sup>y<sup>-1</sup> (a relative rate of 1.2%) have to be doubled.

The paper aims to identify opportunities and limits of resource availability and use for growing energy crops in different regions of the world.

## Bioenergy: the past, present and future

Shifting society's dependence away from fossil energy to renewable biomass resources is generally viewed as an important contributor to providing sustainable energy supply for developing and developed countries and effective management of greenhouse gas emissions (Hill *et al.*, 2006). In 2001 the global use of energy amounted to 10.2 Gigaton oil-equivalents and an average use per capita of 1.67 ton. The division of energy sources over oil, coal, gas, traditional biomass (wood, etc.), nuclear power, hydroelectric power, modern biomass (energy crops) and other renewable sources (e.g.: wind, solar) amounted to: 35, 23, 22, 9, 7, >2, >1 and <1%, respectively (Wilkinson *et al.*, 2007). Bioenergy consumption has been important in many countries, e.g. Brazil, China and Scandinavian countries, for a long time. Wright (2006) reviewed the energy consumption in 2002 for some countries and regions. The contribution of biomass to the total energy consumption in countries ranged from 2.8 (USA) to 27.2% (Brazil) and in Europe from 1.3 (The Netherlands) to 20.0% (Finland).

Since the mid-1970s many research initiatives were taken to increase the biomass resource base for production of bioenergy. Perennial crops (e.g.: *Eucalyptus*, willow, palm oil, sugarcane, switch grass) as well as annual crops (cereals, rape seed) were considered. It was concluded that energy crops have been most successful in penetrating the energy market where subsidies or tax incentives have been applied by governments. However, the energy market has dramatically changed since 2002; fossil fuel prices rose from less than 50 \$ to over \$ 100 per barrel. So, the economic incentives to grow crops for the production of biofuel are becoming stronger.

Presently, bioenergy production is expanding, especially in Brazil, the United States and South-East Asia, where sugar cane, corn and palm oil are converted into ethanol or biodiesel. Also, the European Union set directives to increase the use of biofuels. Europe does have ample resources (land, climate, infrastructure, processing industry, etc.) to continue to play an important role to support a growing world population with food, feed and biofuel. Van Dam et al. (2007) concluded that in Central and Eastern Europe a vast acreage of agricultural land can become available for the production of biofuel when high-technology cropping systems securing a high crop productivity would be introduced. Rounsevell et al. (2006) project for the European Union (EU15) an increase in land use for bioenergy crops of up to 8 % of the total land area by 2080. These land use changes require that productivity of food and feed crops continues to increase. Consideration of secondary biofuels will be less competitive for food production but may have implications for other resources which is not well understood.

### **Resource use of land, water and biodiversity**

The acreage of land needed for producing sufficient food and feed and feedstocks for industrial use (e.g. cotton) and biofuel depends on the productivity per unit of land. Currently, the most common strategy for food, feed and biofuel production is based on high-input low-diversity agricultural systems. Ecologists tend to characterize these systems by large-scale monocultures subject to large inputs of fertilizer, irrigation water, and pesticides (Foley *et al.*, 2005). However, in reality there is huge variation in cropping systems determined by agroecological growing conditions (e.g.: rainfed or irrigated, temperate or tropical, lowland or highland), soil traits (biological, chemical and physical), cropping systems (monoculture, intercropping, plant-animal systems). Therefore, the production potential of crops and cropping systems have to be assessed at a regional scale (Ewert et al., 2005), taking into account the diversity in soil traits and climatic conditions.

The prospects for further boosting net primary productivity of crops are mainly related to capturing more light per unit of land, to a higher water productivity and nitrogen-use-efficiency. Comparative modelling and solid field experimentation are needed to explore to what extent crop yields can be improved under given climatic and management conditions

### **Conclusions**

A quantum leap in crop productivity and resource use efficiencies is needed to meet the demands for food, feed and fuel of a growing global population in an environmentally sound and socio-economically beneficial way. Understanding of the impacts on other resources is limited and needs further attention to identify region-specific strategies for biofuel production.

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# Site Adapted Choice of Energy Crops for Methane Production – Model Based Analysis of Drought Limitations on Maize Yield Potential

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Since the European and German energy policy has focused on the support of renewable energy production (Renewable Energy Sources Act; amendment 2004), the acreage of crops grown for biomass to be used in biogas plants increased dramatically. Because of the high yield potential of forage maize, energy crop rotations often tend to maize monocultures or rotations with very high percentage of maize. To increase biomass yield of energy cropping systems, especially for maize, the choice or breeding of late maturing cultivars is currently considered as an option. Due to increased leaf area duration and therefore a higher water demand of these cultivars, this strategy may fail in some regions of Germany because of low water availability.

The objective of this work was to explore options for a site specific optimised energy cropping system with focus on water demand and water use efficiency (WUE) of different maize cultivars.

## Methodology

A field experiment was carried out near Braunschweig (629 mm annual rainfall, average air temperature of 9.1°C, Lamellic Luvisol with 96mm usable field capacity). Three maize cultivars differing in maturity group (Flavi: mid early / PR36K67: mid late / Mikado: late) were tested under two water supply levels (“intensive”: soil water held >50% useable field capacity by artificial irrigation / “extensive”: rain-fed, “emergency” irrigation only in one year) during three years (2005 – 2007). Growth curves describing leaf area index and crop height were fitted to experimental data and coupled with model modules for evapotranspiration and soil water budget. Using this approach, there is no feedback of drought stress on plant parameters. But as leaf area index was fitted directly to the experimental data and therefore leaf area duration are very accurately described, changes in plant growth caused by drought stress are indirectly included in the model results.

## Results

Dry matter yield of the intensive water treatment was in the normal range for this site. The latest maturing cultivar Mikado had a higher yield than Flavi and PR36K67. The soil water model was able to simulate the frequently measured soil water contents within the depths of 0-30 and 30-60 cm sufficiently well with RMSE values for single soil layers between 0.02 and 0.08 (cm<sup>3</sup>/cm<sup>3</sup>) (data not shown). The cumulative actual transpiration increased with maturity group and leaf area duration. The difference between the mid early cultivar Flavi and the mid late cultivar PR36K67 was twice as great as the difference between PR36K67 and Mikado. Both, the ranking and the range of differences was found for the cumulative potential transpiration as well, but on a higher level. Leaf area duration was much greater for Mikado than for PR36K67 and Flavi which is similar to the ranking of yields and similar to cumulative actual and potential transpiration. Water use efficiency ranked similar to the dry matter yield. Transpiration use efficiency on the contrary was highest for Flavi and equal for PR36K67 and Mikado.

**Tab.1:** Dry matter yields (experimental) and soil water and water use efficiency parameters from a three year experiment (2005-2007) with three maize cultivars with contrasting maturity grown under two levels of water supply.

Water supply level	Cultivar (maturity group)	Dry matter yield [g/m <sup>2</sup> ]	Cum. actual transpiration [mm]	Cum. potential transpiration [mm]	Leaf area duration [m <sup>2</sup> leaf * day / m <sup>2</sup> soil]	Water use efficiency [g/l]	Transpiration use efficiency [g/l]
Intensive	Flavi (S250)	2350.0	272.8	380.5	550.1	4.6	8.6
	PR36K67 (S370)	2326.7	295.5	442.1	612.4	4.5	7.9
	Mikado (approx. S500)	2416.7	305.8	476.8	855.9	4.8	7.9
Extensive	Flavi (S250)	1903.3	212.5	333.6	420.9	4.4	9.0
	PR36K67 (S370)	1816.7	218.8	353.5	608.7	4.2	8.3
	Mikado (approx. S500)	2113.3	226.1	385.3	661.6	4.9	9.3

For the extensive irrigation treatment the ranking of dry matter yield, cumulative potential transpiration, leaf area duration and water use efficiency was similar to the intensive treatments, but on a lower level. The cumulative actual transpiration of the three cultivars was still lower than for the intensive water treatment, but the ranking of the cultivars was the same. Transpiration use efficiency was different from the intensive water treatment. In the extensive treatment, the late cultivar Mikado had a higher value than Flavi (mid early), which had a higher value than PR36K67 (mid late) cultivar.

## Conclusions

The superior leaf area duration of the later maturing cultivars caused an increased transpiration but lowered the proportion of evaporation of the total evapotranspiration. Therefore the whole water use (evaporation plus transpiration) was only slightly affected by the higher leaf area duration of the cultivars with later maturity. Consequently, because of a higher dry matter yield, water use efficiency was higher for the late cultivar than for the mid late or mid early cultivar.

Initially, it was hypothesized that cultivars with a high leaf area duration and therefore a high transpiration may exhibit greater yield depression under drought stress (provided that the reaction on water limitation is quantitative and qualitative the same for all cultivars). We could not observe this even in the dry year 2006. Late maize cultivars for methane production therefore may not suffer from an increased risk of yield failure under drought stress conditions. But this conclusion is based on experimental data from one site only.

To get a more confident conclusion about the interaction between maturity group and drought stress it is necessary to use a plant growth model which is able to estimate growth depression caused by limited water supply. This task is currently carried out. After completion, simulation studies (different maturity groups, different sites) will be carried out to transfer local experimental results to other regions and to determine site specific limitations of different maize genotypes. Further research is needed to quantify quality aspects under drought conditions and for late maturing cultivars. The dry matter content of late cultivars occasionally was lower than 30%, leading to an increased risk for proper ensiling.

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# Usage of Fodder Galega Biomass for the Biogas Production

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Galega (*Galega orientalis* Lam.) is recently introduced in Latvia, due to its persistency and high yielding ability. Longlived legume survives in pure stands for 25 and more years and provides annual DM yields from 9.56 to 11.0 t ha<sup>-1</sup> (Adamovich, 2000, 2006, 2007). Successful treatment of galega seeds with nodule bacteria results in fixation of atmospheric nitrogen from 200 to 453 kg ha<sup>-1</sup> (Adamovich and Klasens, 2002), thus can to decrease the need for commercial nitrogen fertilizers. Production of methane rich biogas through anaerobic digestion of organic materials can to provide clean form of energy, due to low emissions into environment from burning of biogas. Biogas can be used for heat and power generation or as a vehicle fuel, thus reducing the need for fossil fuels and slowing down the climate change (Dubrovskis, 2007).

Aim of this study was estimation of productivity of fodder galega/grass swards and investigation of biogas output from fodder galega and galega haylage in anaerobic treatment process.

## Methodology

Field experiments were conducted during a 21 years period (1986-2007), aiming to estimate continuous green forage production from fodder galega-grass swards in the stage of intensive growth.

The 35 mixed (13 binary and 22 multi – species) swards were developed on stagnic – luvisol soils. Pure swards, binary- and multi-species seed mixtures were composed of fodder galega cv. ‘Gale’ and 13 grass species. Stands were sown in early May in 1986, 1990 and 1997. The total seeding rate was 1000 germinating seeds on 1 m<sup>2</sup>. In all experiment series the mixture contained 40% fodder galega and 60% grass seeds.

The biogas yield was investigated on laboratory scale equipment. Original laboratory equipment B4 consists 6 digesters, were working in batchwise mode. Each digester is of volume of 5 l and equipped with heating devices for automated regulation of temperature inside of the digesters at 37±1.0°C or 54±1.0°C. Digesters are equipped with sensors for automated registering of pH and gas volume data in computer. The substrates used for anaerobic fermentation in digesters F1 – F4 were galega mixtures with cow manure at different proportions (Table). Galega was chopped and mixed with water previously. The substrates used for anaerobic fermentation in digesters F5 and F6 were galega haylage mixtures with inoculum (fermented cow manure). Additional water was added in digester F6 to increase moisture (Table). Substrates were analysed using approved methods for organic matter, volatile solids and moisture content before filling in digesters. Accuracy of measurement was ±0.02 for pH value or ±0.0025 l for gas volume. Results were fixed in computer and by hand in notebook daily.

## Results

Fodder galega significantly surpasses other forage legumes in respect to productive longevity, and fluctuations in DM yield were insignificant between years of use. Inclusion of a grass species in a mixture resulted in yield increase by 28 to 36 % already in the first production year. Split application of the 90 kg N fertiliser affected negatively the proportion of galega in a sward, resulting in the decrease of DM yields by 1.04 t ha<sup>-1</sup> at two cutting management, compared to unfertilized plots.

Average yields from pure galega stands were 9.50 t ha<sup>-1</sup> DM or 6.16 t ha<sup>-1</sup> DM at two-cutting or four-cutting management in 9 production years respectively. Average yields from three species galega-grass swards with no N fertilizer were 9.80 t ha<sup>-1</sup> or 6.56 t ha<sup>-1</sup> DM at two-cutting or four-cutting management respectively.

In all digesters according to temperature creating warm gas (air with CO<sub>2</sub>) filled gasholders. When hydrolysis and acidification processes were starting, pH decline and start to rise again after some period in digester 1, where adaptation period was longer. It is explained with too high organic load in this digester. Results are shown in Table .

Table. Substrate and biogas parameters

Parameter	Unit	digester 1	digester 2	digester 3	digester 4	digester 5	digester 6
Substrate composition	%	100 cm	25 cm 75 g+w	50 cm 50 g+w	75 cm 25 g+w	55 in 45 gh	32 in 68 gh+w
Total substrate weight	kg	4.120	3.294	3.593	3.624	1.142	2.144
Total solids	%	14.7	5.83	9.41	12.6	20.9	11.9
Organic solids	%	12.8	3.4	7.2	10.5	19.4	11.1
Biogas yield	l/kg <sub>VSD</sub>	411	627.8	535	436		
Average methane content	%	53.2	61.2	57.8	56.1	40.1	49.0
Methane yield	l/kg <sub>VSD</sub>	218.6	384.2	309.2	244.2		
Conversion rate	%	62.5	68.3	64.6	63.8		

Remarks: cm - cow manure, gh – galega haylage, in – inoculum (fermented cow manure), , g+w - galega plus water, gh+w - galega haylage plus water, VSD - volatile solids degraded.

It is obviously, that methane yield is higher for substrate having low concentration of organic solids. Average methane content in biogas was 41% or 49%, released from digester F5 or F6 respectively, during 64-day anaerobic fermentation period. Carbon dioxide concentration in biogas released from galega haylage lowers from 45 -55% at the beginning of fermentation period to 23-25% at the end of anaerobic treatment process. Maximum daily volume of biogas from galega haylage was 1.42 l·day<sup>-1</sup> at 13<sup>th</sup> day, or was 1.82 l·day<sup>-1</sup> at 38<sup>th</sup> day in digesters F5 or F6 respectively. Minimal ph value was 6.35 at 4<sup>th</sup> day or 6.38 at 8<sup>th</sup> day in digesters F5 or F6 respectively. Anaerobic fermentation of galega haylage is ongoing more intensively in substrate with less total solids content. Estimated specific volume of biogas was 116 l·kg<sup>-1</sup> VS for digester F5 and 244 l·kg<sup>-1</sup> VS for digester F6, or by 52% higher in digester F6 after 64-day fermentation period.

## Conclusions

Fodder galega in pure stands or in mixtures with grasses is productive and persists for long periods, and three species mixtures proved to be most productive. Competitive grasses in the mixtures reduce productive longevity of swards compared to pure galega stands.

Galega can to produce high (up to 384.2 l/kg<sub>VSD</sub>) biogas yield obtained despite to organic overloading in two digesters and working without mixing of substrate. Biogas can be successfully obtained from chopped galega haylage with inoculum of fermented cow manure.

Estimated cumulative volume of biogas released after 64-day fermentation period from galega haylage, having volatile solids content in digesters 19.4% or 11.1%, was 116 l·kg<sup>-1</sup><sub>VS</sub> or 244 l·kg<sup>-1</sup><sub>VS</sub> respectively, or by 52% higher in digester having low content of volatile solids. Cumulative volume of gases, released from galega haylage in fermentation period, approximates help by 2<sup>nd</sup> order equations.

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# Age Effect on Switchgrass Biomass Yields Based on One Decade Results

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## Introduction

Switchgrass (*Panicum virgatum* L.) is an erect warm-season (C<sub>4</sub>) perennial grass that grows naturally from 55° N latitude to deep into Mexico, mostly as a prairie grass. Over the last two decades it has become an important warm-season pasture grass for fodder production when cool season C<sub>3</sub> grasses are less productive in summer [1]. The main purpose of this work was to test the adaptability and biomass productivity of ten switchgrass varieties (upland and lowland ones) that established in Greece in 1998. This work presents the age affect on growth and yields of switchgrass in Greece for a decade.

## Methodology

In the framework of the European funded project FAIR5 CT97 3701 several switchgrass trials were established and one of them is presented in Table 1. During all growing periods (1998 - 2007), a series of measurements was carried out including canopy height and number of tillers per square meter at the end of the growing season. At the end of each growing season the final harvest was signaled (2 m<sup>2</sup> per plot) by the first killing frost, and the fresh and dry matter yields and yield components were determined. For each experimental trial, the effect of varieties on growth characteristics and yields was tested by a standard analysis of variance (ANOVA). LSD multiple range tests were used in partitioning the means (statistical significance at the  $P=0.05$ ).

**Table 1.** Site and treatments of the switchgrass trial in Greece.

Sites	Site coordinates	Irrigation	Fertilization	Tested varieties
Greece (Aliartos)	latitude 38° 22, longitude 23° 10 altitude 114 m	Through a drip irrigation system a total quantity from 250-300mm were added every year.	Each year, at the early stages of growth nitrogen fertilization rate of 75 kg N/ha was applied. Basic fertilization before sowing was applied every five years.	Caddo, Cathage, CIR, Forestburg, Kanlow, SL 93-2, SL 93-3, SL 94-1, SU 94-1 and Summer

## Results

**Height:** All varieties gave the highest plants in the second growing period (185 cm mean of all varieties). A great decline was recorded in fifth growing period (120 cm, mean plant height) and plant height until the eighth growing period did not exceed 134 cm, while in the last two growing periods the plants were higher (between 154 and 162 cm) (Figure 1). In all years the lowland varieties were the ones that gave always higher tillers compared to the upland ones.

**Tiller density:** The experimental field that the highest tiller density in years 2 and 3 when the mean tiller density was 1456 and 1392 tillers per square meter, respectively. Thereafter, in years 4 and 5 the tiller density was decline and was 757 tillers per square meter in year 5, while between year 6 and 10 the tiller density did not exceed 560 tiller/m<sup>2</sup> (Figure 1).

**Yields:** The dry matter yields (averaged overall varieties) were maximized in years 2 and 3 with dry biomass yields of 18t/ha, while in year 4 was reduced and was 15 t/ha. From the year 5 to year 10 the dry biomass yields were further declined and varied from 9 to 10.4 t/ha (Figure 1). Its worth to mention that the mean dry biomass yields of the decade was 12 t/ha.

**Table 2.** Dry matter yields (t/ha) for the ten tested varieties and for the period 1998-2007.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Caddo	10.7	20.1	20.1	18.2	9.2	7.9	6.5	6.7	7.6	8.1
Cathage	15.2	18.9	17.5	14.8	8.9	9.1	9.2	9.2	8.8	8.9
CIR	11.8	15.8	17.9	13.1	8.1	6.8	7.1	8.2	8.4	8.7
Forestburg	13.2	19	18.4	11.7	7.4	10.8	10.7	8.6	7.9	8.1
Kanlow	10.4	17.1	21.3	16.9	9.8	10.4	12.3	12.2	8.9	9
SL 93-2	11.6	16.8	16.7	17	9.2	9.1	10.4	9.7	8.6	8.3
SL 93-3	12.4	19	17.8	16.4	11.5	10.3	9.5	8.8	8.7	9.3
SL 94-1	9.4	15.8	18.8	16.2	10.7	11.5	12.5	9.9	9.3	9.4
SU 94-1	8.5	14	11.3	14.8	11	9.4	13.5	12.8	10.7	10.4
Summer	12.1	21.1	19.3	12.9	7.1	12.8	11.9	10.2	9.3	9.5

### Conclusions

All varieties (upland and lowland ones) were their higher values for both growth characteristics (plant height and tiller density) and yields in the second and the third growing period. Thereafter, a decline was recorded and this decline was quite bigger in year 5. Between the years 5 and 10 the values for growth characteristics and yields were remained low and no further decline was recorded (Table 2). The lowland varieties (Cathage, Kanlow, SL 93-3, SL93-2 and SL 94-1) were the ones that in all years gave higher yields compared to the upland varieties (Caddo, CIR, Forestburg, SU 94-1 and Summer). This superiority of the lowland over the upland varieties reached its peak value in the fifth growing period that came up to 15%.

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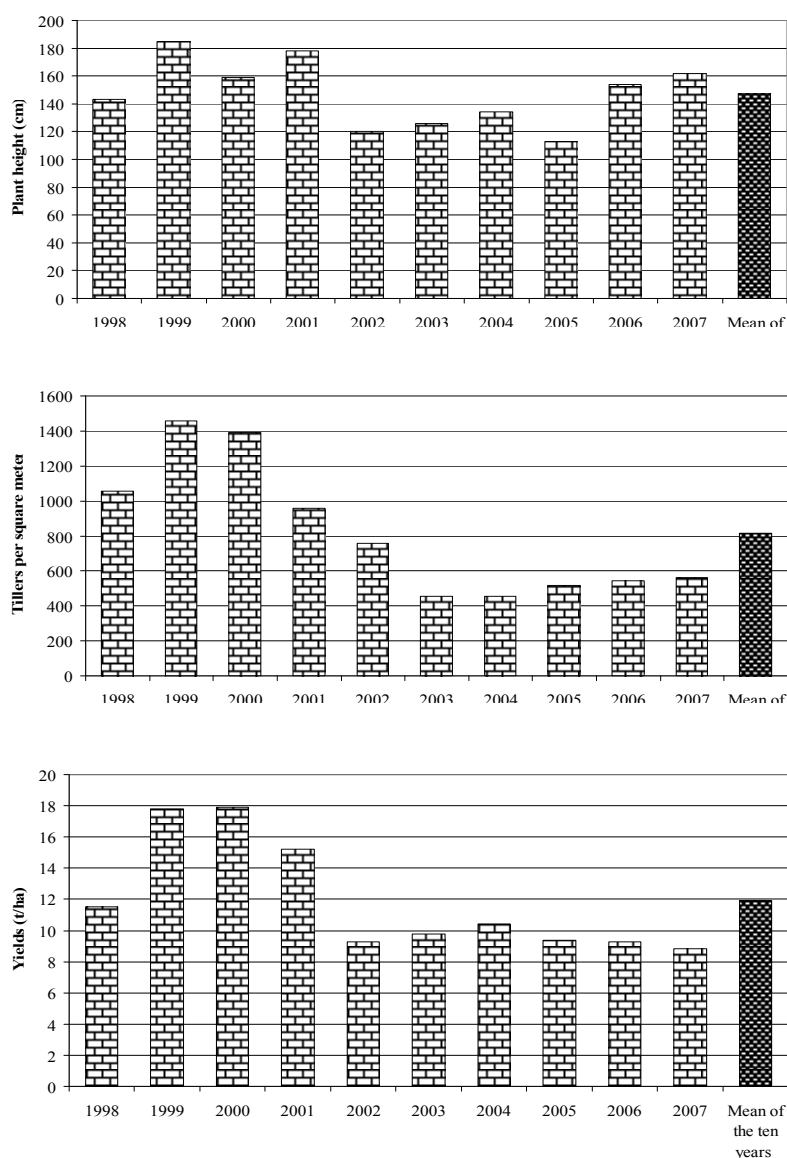


Figure 2. Plant height, tiller density and dry biomass yields for a decade.

# Development of Winter Oilseed-Rape Growing Experience in Central Latvia Farms

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## Introduction

Sowing area under oil-seed rape (*Brassica napus* ssp. *oleifera*) has grown dramatically in Latvia: from 400 ha in 1997 up to 100 000 ha in 2007. Approximately 51% of the produced rape is being processed in bio-fuel, but the remaining part – in the food oil and is exported. There is still huge potential to increase rape production capacity to reach 5.75% from the total amount of transport fuel put into circulation, which is the target for 2010 set by EU. To reach this goal, there are necessity to increase area under oilseed rape and productivity of oilseed rape in Latvia. Main part of the area under winter oil-seed rape is cultivated in Central Latvia farms because of more suitable wintering conditions. Although area sown with spring rape is still dominating (67% from the total rape sowing area), winter rape is preferred due to possibility to obtain higher seed yield.

The aim of our research was to clarify experience of winter rape growing in production conditions in Central Latvia farms (2006-2007).

## Methodology

According to the promoted aim, nine farms in 2006 and 15 in 2007 were randomly chosen in Central Latvia to clarify experience of winter rape growing.

Farmers were questioned to find out different information about winter rape growing experiences like: field recordings, winter rape growing manner, total area of the farm, crop rotation etc. We were allowed to analyze one randomly chosen field in each farm to carry out different observations. Diseases *Alternaria* blight (*Alternaria* spp.) and *Phoma* leaf spot (*Leptosphaeria* spp.) incidence and severity in the autumn was evaluated. At the same time visual situation of the fields were evaluated in 9 point scale (9- visual situation very good, plants well developed; 1- 75% plants were not germinated). In early spring situation of the fields were evaluated again to define wintering of the field in 9 point scale (9- visual situation very good, all plants vegetating; 1- all plants dead). During summer incidence of *Alternaria* pod spot was evaluated. Fields were inspected to register incidence of *Phoma* stem cancer and *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) directly after harvesting.

## Results

The most effective way to control diseases in oilseed rape is to use crop rotation accurately. It is advised to have a 3 year long interval before sowing winter rape in the same field. Rape proportion was more than 25% (up to 50%) (Table 1) in eight from 15 farms total, it can become a threat to oilseed rape disease incidence increase in central Latvia farms. Farmers are mostly choosing oilseed rape line varieties over hybrids. The most suitable time for winter rape sowing is 15<sup>th</sup>-20<sup>th</sup> of August for line varieties and until 25<sup>th</sup> of August for hybrids in Latvia. Sowing dates in farms are different and later than the ones advised (Table 1), for example, in farm 'Dzenisi' winter rape was sowed on 3rd of September in 2006. The most common previous crop in winter oilseed rape fields is winter wheat (Table 1), which is a typical situation in central Latvia farms which are mainly cereal grower farms. Farmer's recognize that late wheat harvesting is probably the reason for late sowing dates. Experience in Europe is that winter rape can be cultivated using minimal soil tillage (without ploughing) or classical tillage system (with ploughing). Minimal soil tillage is becoming very popular in winter rape growing technologies in Latvia. In year 2007 minimal soil tillage was used in six farms from 15

inspected, but that could become a serious problem because there is a lack of research in this area. Soil chemical analyses were not used seriously for choosing rape fertilizers almost in all inspected farms. Also foliar fertilizers were applied without any reasonable motivation almost in all farms except Dobeles Agra where data of plant analyses was used to choose foliar fertilizers.

Table 1  
Oilseed rape proportion, sowing dates, previous crop and seed yield from the inspected field in farms

Farm	Winter rape proportion, %		Sowing date		Previous crop		Seed yield, t ha <sup>-1</sup>	
	2006.	2007.	2006.	2007.	2006.	2007.	2006.	2007.
Krisjani	21	19	26.08.05.	22.08.06.	Winter wheat	Winter wheat	X	1.9
Cerini	28	<b>29</b>	26.08.05.	17.08.06.	Winter wheat	Winter wheat	2.9	2.7
Vangali	33	<b>49</b>	25.08.05.	24.08.06.	Winter wheat	W.wheat/rape	2.4	3.7
Dzenisi	X	<b>46</b>	X	03.09.06.	X	Winter wheat	X	2.3
Uzvara Lauks	X	17	X	28.08.06.	X	Spring barley	X	3.4
Peterlauki	20	13	18.08.05.	20.08.06.	Fallow	Fallow	4.2	2.9
Abelites	29	<b>32</b>	22.08.05.	25.08.06.	Winter wheat	Spring barley	2.2	2.3
Azaidi	25	22	22.08.05.	17.08.06.	Winter wheat	Winter wheat	2.0	2.3
Deloferma	X	22	X	28.08.06.	X	Winter wheat	X	1.5
Lielauzeles	X	<b>50</b>	X	28.08.06.	X	Winter wheat	X	2.5
Strazdi	21	<b>36</b>	25.08.05.	22.08.06.	Winter wheat	Winter wheat	3.3	3.9
Vecaue	9	12	19.08.05.	22.08.06.	Grassland	Winter wheat	3.7	2.0
Dobeles Agra	38	<b>40</b>	26.08.05.	27.08.06.	Winter barley	Winter wheat	2.7	2.2
Ziedi JP	X	24	X	25.08.06.	X	Winter wheat	X	2.3
Zelta Druva	X	<b>26</b>	X	20.08.06.	X	Grassland	X	4.2

Problematical rape wintering was noted in winter 2005/2006 when one field was totally damaged by adverse winter conditions. Winter 2006/2007 was very good for rape wintering in central Latvia. *Alternaria* blight and *Phoma* leaf spot was found in sowing autumn at both of years. Incidence of these diseases was increasing (*Alternaria* blight 77% in 2005 and 79% in 2006; *Phoma* leaf spot 1.1% in 2005 and 18.5% in 2006 on average), but severity was still insignificant (Treikale. 2006, Balodis et al. 2008). Incidence of *Alternaria* pod spot increased sharply during both years (2% in season 2006 and 73% in 2007 on average), also the severity was still insignificant. Similarly with *Phoma* stem cancer, incidence increased from 42% in 2006 to 91% in 2007. Incidence of *Sclerotinia* stem rot was very low at all in 2006, but in 2007 incidence increased up to 30%, due to very suitable weather conditions for *Sclerotinia* stem rot infection. Fungicides application against *Sclerotinia* stem rot were not used in farms in 2006, but were used in seven farms during season 2007. In years 2007 higher yields were gained in fields where fungicide application was used against *Sclerotinia* stem rot (on average – 2.5-4.2 t ha<sup>-1</sup> with fungicide, 1.5-3.7 t ha<sup>-1</sup> without fungicide). In general winter oilseed rapeseed yields were very different (1.9 – 4.2 t ha<sup>-1</sup>) depending on conditions in field (Table 1).

## Conclusions

Main benefit of our first two research year's investigations was getting off rather a lot of data to clarify experience of winter rape growing in production conditions in Central Latvia farms. Data range is nearly enough to clarify some of further research directions. Research should be continued for collecting long-term data about winter rape growing tendencies in Central Latvia.

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# Legal Regulations Liquid Fuels' Biocomponents and Production Possibilities in Poland

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## Methods

The main purpose of this study were: 1) to present data on the progress towards the achievement of the national targets specified in Directive 2003/30/EC and to analyze the legal regulations regarding the biofuels market in Poland, 2) to determine the local potential for the production of liquid biofuels (bioethanol, biodiesel) and raw materials of agricultural origin used for biofuels. The results are based on literature sources and own calculations.

## Results

### Legal regulations pertaining to the biofuels sector in Poland

*The Long-term Programme for Promotion of Biofuels or Other Renewable Fuels for 2008 - 2014* (Polish Monitor no. 53 item 607) was adopted under Article 37 of *Act on Biocomponents and Liquid Biofuels* of 25 August 2006 (Journal of Laws of 2006 no. 169 item 1199). It supports the meeting of the objective of 5.75% and 10% substitution of conventional fuels by alternative fuels in the road transport sector by the year 2010 and 2020 respectively, set in Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 *on the Promotion of the Use of Biofuels or Other Renewable Fuels for Transport*.

Directive 2003/30/EC was transposed into national legislation in Poland with *Act on Biocomponents and Liquid Biofuels* of 25 August 2006 (Journal of Laws of 2006 no. 169 item 1199) and *Act on Fuel Quality Monitoring and Control* of 25 August 2006 (Journal of Laws of 2006 no. 169 item 1200). Those two Acts introduced numerous changes into the Polish legal system, aimed at creating favorable conditions for the development of biocomponents and liquid biofuels market.

The Regulation of the Minister of Finance *amending the regulation on excise duty exemption* of 22 December 2006 (Journal of Laws of 2006 no. 243 item 1766), which entered into force on 1 January 2007 to bring national provisions into line with EU law, resulted in a reduction in agricultural support regimes for biofuels. Excise tax exemption and compensation for the producers of liquid fuels containing biocomponents and liquid biofuels decreased, while the costs of biofuel blending increased.

### Raw materials for the production of biocomponents used in liquid fuels

Oils from various crops can be used for fuel. In Poland oilseed rape provides the highest oil yield per unit of land area – 750 to 1.200 kg·ha<sup>-1</sup> (Budzyński and Ojczyk 1996), which makes it the principal raw material for the production of fatty acid esters. Rapeseed oil fuel, compared to hydrocarbon fuel, is ecologically safer, more readily biodegradable, produces less smoke, CO, HC and S, and does not contain heavy metals, but it may have a slightly higher content of nitrogen compounds (Bocheński 2003). Only high-quality rape seeds of low-erucic acid (double zero) varieties are processed for energy purposes (Budzyński and Jankowski 2003). Due to the introduction of the general principles of Directive 2003/30/EC, providing for a minimum percentage of biofuels to be marketed

and distributed, the demand for oilseed rape as an energy crop is expected to increase and reach around 1.2 mln tons by the year 2010 (Jankowski and Budzyński 2003, Kuś 2004).

Bioethanol may be used as a petrol substitute for flexible fuel vehicles in the form of 95% alcohol or as a petrol additive in the form of dewatered 100% alcohol (Kupczyk and Ekielski 2002). Adding oxygenates, such as ethanol, to high-octane fuels can provide a number of environmental benefits (Bocheński 2003). Bioalcohol production relies on various crops, including cereals, potatoes, topinambour and sugar beets, as well as on molasses and other by-products rich in saccharides (Kupczyk and Ekielski 2002, Gradziuk 2003, Ostrowska and Cieśliński 2003, Kuś 2004). Maize is the most efficient cereal crop with respect to energy and alcohol yield per unit of land area (Mystkowski 2003). However, sugar beets are characterized by the highest total productivity per unit area. Molasses - a rest product formed during sugar beets processing - may be also used as a sugar source in the bioethanol production process (Łabętowicz et al. 1999).

The results of own research indicate that covering raw material needs and meeting the objectives set forth in Directive 2003/30/EC by the year 2010 will require a total yield obtained from approximately 521 000 ha of rye production land or 155 000 ha of maize production land or 166 000 ha of potato production land.

Production surplus of potatoes, cereal grains or other agricultural raw materials, particularly those which are not harvested for food or feed use, may contribute to an increase in water-free alcohol production. High-level bioethanol production may offer new market opportunities, thus raising demand for agricultural products and contributing to an increase in farmers' income (Pawlak 2000).

## Conclusions

Biofuel production is expected to increase gradually in the coming years, primarily due to the global upward trend in oil prices, the need to reduce toxic substance emissions, the aspiration towards energy self-sufficiency and limited crude oil reserves. Therefore, the role of the ethanol will increase in the fuel industry. Poland is on its way towards the achievement of objectives relating to the use of biofuels, defined in Directive 2003/30/EC, and national indicative targets. The biodiesel sector is expected to grow and develop dynamically on condition that certain tax incentives are introduced.

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# Oilseed Brassica Species for Fuel Production in Mediterranean Environments: First Field Results and Technical Evaluations

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Energy is a major constraint of both developed and under-development countries, and the sustainable production of biofuels for use in industrial and domestic sectors has been implemented successfully in many parts of the world. For a number of reasons, some *Brassica* species, such as *B. napus*, *B. juncea* or *B. carinata*, seem especially interesting as biofuel crops for Mediterranean environments, and many studies and researches have been carried out in order to ensure a successful introduction of them inside the current farming systems. In this work we report some of the data obtained from a more complex experiment, carried out in 2006/07 on different sites in Sicily, that involved three experimental fields and many genotypes of rapeseed (*B.napus* var. *oleifera*) and *B. carinata*. The research was not only aimed to point out some aspects of the cropping technique to be applied to such crops into these special environments, but also to evaluate the effects eventually exerted by the selected agronomic variables on the technical aspects of the obtained biofuel.

## Methodology

Three diverse experimental fields managed by the DAAT of Palermo University, respectively termed “A”, “B” and “C”, were set out in 2006-07, the first two being located in the experimental farm “Sparacia” (Cammarata – AG - Sicily), and the third one in a private farm not far from the above (Xirbi – CL – Sicily).

Table 1. Oilseeds Brassicas - Comparative traits about the provenance of the tested seeds.

	Experimental design	Genotypes	Sowing date	Harvest date
Field A	RCB (3 repls)	9 ( <i>B. napus</i> )	Jan 5 <sup>th</sup> , 2007	Jun 13 <sup>th</sup> to 19 <sup>th</sup> , 2007
Field B	RCB (3 repls)	3 (2 <i>B. napus</i> , 1 <i>B. carinata</i> )	Dec 30 <sup>th</sup> , 2006	Jun 13 <sup>th</sup> , 2007
Field C	Open field	1 ( <i>B. carinata</i> )	Dec 19 <sup>th</sup> , 2006	June 10 <sup>th</sup> , 2007

This work refers only about the results obtained from the three genotypes in common to all fields (*B. napus* var. *oleifera*, cvs. Lilian and Licolly, and *B. carinata* cv. Sincron). On samples of seeds coming from all fields the oil was extracted by mixing the seeds flour (obtained with mechanical grinding) with hexane (250 ml hexane/100 g of milled seeds), and thereafter removing the solvent (to be recycled) by means of a rotatory evaporator. Oil content was determined by means of Soxhlet extraction. The obtained oils were finally analyzed by the Laboratories of the Customs Agency of Catania, in order to evaluate the suitability of the crude extracted oils for fuel use in heating domestic plants. A commercial rapeseed oil sample (for food use) was used as a lab test.

All data were submitted to statistical analysis by means of the statistical package SAS for Windows v. 9.0 (SAS Institute Inc., Cary, NC, USA). The differences among mean values, as ANOVA was offering a significant result, were detected by means of the HSD Tukey's test at  $P \leq 0.05$ .

## Results

Although seed yields (table 1) exhibited a high variability, *B. carinata* showed a generally good performance – probably related to the high unitary seed mass – being on average as productive as the high-yielding rapeseed variety Licolly.

Generally low was the oil content in *B. carinata* seeds but the satisfactory yield performance allowed to determine an oil yield not statistically different along genotypes.

Table 2. Oilseeds Brassicas - Major agronomical results

Genotype	Field	Seeds yield (kg ha <sup>-1</sup> at 9% moist.)	100 seeds mass (g at 9% moist.)	Oil content (% on d.m.)	Oil yield (kg ha <sup>-1</sup> )
Rapeseed 1 (Lilian)	A	1,618.1 AB	0.35 B	44.5 AC	607.5
	B	772.8 B	0.34 BC	46.9 A	372.3
Rapeseed 2 (Licolly)	A	2,119.3 AB	0.27 C	41.0 BC	911.2
	B	2,155.2 A	0.31 BC	45.6 AB	957.0
<i>B. carinata</i> (Sincron)	B	1,940.5 AB	0.46 A	39.7 C	805.2
	C	2,341.5 A	0.47 A	41.2 AC	905.6

n.s.

Means followed by the same letters are significantly not diverse at  $P \leq 0.05$  (HSD Tukey's test)

Table 3. Oilseeds Brassicas - Major technical characters of the tested oils.

		Viscosity at 50 °C (mm <sup>2</sup> s <sup>-1</sup> )	Sulphur <sup>(2)</sup> (% w w <sup>-1</sup> )	Ash (% w w <sup>-1</sup> )	Calorific value (MJ kg <sup>-1</sup> )	C (%)	H (%)	N (%)	Density (g l <sup>-1</sup> )
Test:year	UNI EN ISO 3104:2000	EN ISO 20884:2004			ASTM D 240:2002	ASTM D5291:2002			
U.M. <sup>(1)</sup>	± 1.6	± 0.22·10 <sup>-3</sup>			± 0.28	± 1.66	± 0.56	± 0.32	
Genotype	Field								
Licolly	A	15.6	0.52·10 <sup>-3</sup>	0	40.27	78.28	12.51	1.25	826.23
	B	12.1	<0.20·10 <sup>-3</sup>	0.74·10 <sup>-2</sup>	40.20	78.37	12.45	1.38	879.00
Lilian	A	7.0	0.36·10 <sup>-3</sup>	0.43·10 <sup>-2</sup>	39.73	78.67	13.04	0.83	865.87
	B	7.9	0.37·10 <sup>-3</sup>	0.82·10 <sup>-2</sup>	39.91	79.35	13.22	1.08	856.50
Sincron	B	15.7	0.66·10 <sup>-3</sup>	0.38·10 <sup>-2</sup>	40.36	79.88	12.93	1.20	841.53
	C	18.5	0.91·10 <sup>-3</sup>	0.34·10 <sup>-2</sup>	40.74	79.34	13.16	0.69	843.17
Rapeseed oil (food)		26.1	<0.20·10 <sup>-3</sup>	0.71·10 <sup>-2</sup>	40.16	79.19	12.68	1.21	881.80
Gasohol <sup>(3)</sup>		(at 40°C) 2.0-7.4	< 0.02	0	42.00	87.00	13.00	0	852.00
Heavy petroleum distilled oils	Very fluid	< 21.2	≤ 2.5	≤ 0.05	39.79 to 40.60				
	Fluid	21.2-37.4	≤ 3.0	≤ 0.10					
	Semi-fluid	37.5-91.0	≤ 4.0	≤ 0.15					
	Dense	> 91.0	≤ 4.0	≤ 0.20					

<sup>(1)</sup> Uncertainty of the method at 95% confidence; <sup>(2)</sup> In the EU, maximum allowed S content from 2009 will be 10 mg kg<sup>-1</sup> or 0.001 % (EU Reg. 2003/17/CE, in Italy D.L. 21/3/05 n° 66); <sup>(3)</sup> for thermal industrial and civic uses (UNI 6579)

As shown in table 3, viscosity value was low for all extracted oils (classable as “very fluid”), an advisable trait for fuel use in heating domestic plants. Sulphur and ash content were far below the mandatory law limits and calorific value, close to the one of gasohol, was the same of heavy petroleum distilled oils.

## Conclusions

Oils from Brassicas evidenced similar traits to common fossil oil fuels, hence showing a good suitability as an environmentally-friendly fuel in domestic and industrial heating plants.

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# A Simulation Study of the Effects of Field Patterns on Cross-Pollination Rates in Oilseed Rape (*Brassica napus* L.)

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Gene flow between cultivars within a landscape may lead to impurities that reduce harvest value. In oilseed rape (OSR), as for most crops, harvest impurity rates are expected to depend on the spatial distribution of crops over the landscape. However, as OSR crops leave seed banks in European agro-ecosystems, gene flow is also a temporal process interacting with cropping systems. The objective of the present paper was to use a mechanistic, spatially-explicit gene flow model to analyse gene flow between OSR varieties and volunteers in different landscapes and to identify the pertinent landscape variables determining local and regional harvest impurities.

## Material and methods

The GENESYS model was used to simulate OSR volunteer dynamics and gene flow as a function of cropping systems, in agricultural landscape over time (Colbach et al., 2001). The input variables are the (1) regional field pattern, (2) the crop succession in each field, (3) the cultivation techniques used to manage each crop; (4) the genotype of the OSR varieties (e.g. GM vs. non-GM, male sterility genes). These input variables influence the annual life-cycles of cultivated, volunteer and feral OSR populations. The life-cycle is simulated each year in each field and in the uncultivated areas of the simulated region. Pollen flow is calculated daily during flowering, seed dispersal once during seed shed; dispersal proportions between fields depend on field areas, shapes and distances.

The simulation plan resulted from combining the following factors: (1) the regional field pattern (six existing French patterns + five virtual patterns simulated with the GenExp software (Adamczyk et al., 2007); (2) the proportion of OSR grown in the region (either 15% or 30% of the fields), (3) the proportion of GM OSR grown in the region (10% or 50% of the OSR fields), and (4) the regional cropping system (high, medium and low-gene flow systems). At the onset of each simulation, the crops are distributed randomly in the field pattern with respect to the probabilities determined by OSR and GM proportions and cropping system rotations. During the subsequent years, the crops depended on the rotation. A field once grown with a GM variety was never cultivated with a non-GM variety and vice versa. Each scenario resulting from the four simulation factors was run twice, with a different initial crop allocation. The simulations started with an empty seed bank and lasted seven years. The first and the last years were used for analysis, the former being a volunteer-free situation, the latter a situation with both GM and non-GM volunteers.

## Results

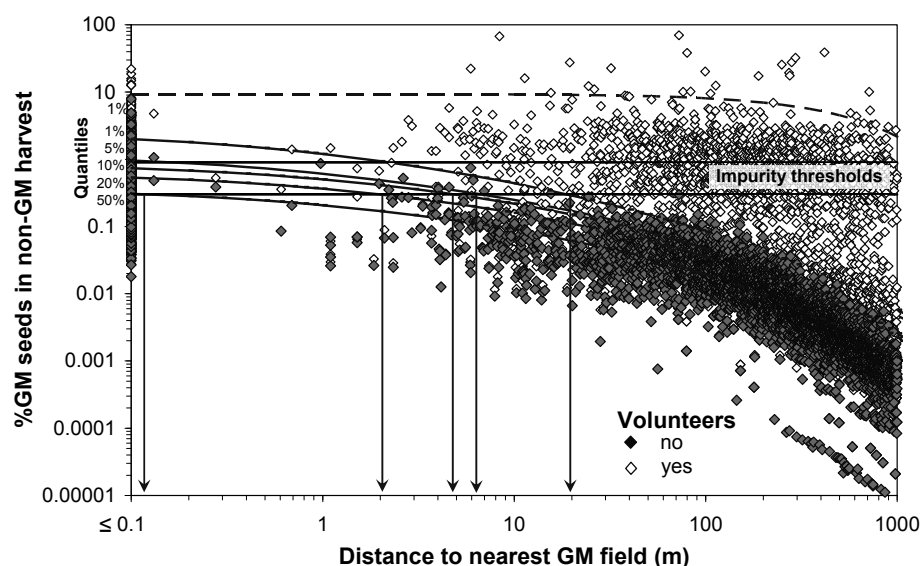
Through regression analyses, we determined spatial and agronomic factors that most affected regional and local harvest impurity rates of non-GM OSR (Table 1). At the local (field) level, harvest impurity was mostly explained by the distance to the closest GM field during the first year of OSR cultivation (Fig. 1). After six years, impurity was mostly explained by the density of GM volunteers in the analysed field and in neighbour non-OSR fields (results not shown). Quantile regressions were fitted to the simulated data to determine the maximum regional area of GM OSR and isolation distances between

GM and non-GM crops as a function of the risk accepted by the decision-maker (i.e. the % of situations exceeding harvest impurity thresholds), the cropping system and the volunteer infestation (Fig. 1).

Table 1. Analysis of covariance of regional harvest impurity (% GM seeds in non-GM harvests averaged over all non-GM OSR fields). Partial  $R^2$  and parameter estimates resulting from linear models of log10-transformed output variables

Factors	Year 1 (noOSR volunteers)				Year 7 (with OSR volunteers)			
	$R^2$	Estimates			$R^2$	Estimates		
		low	medium	high		low	medium	high
Cropping system	0.00	-2	-2	-2	0.26	-2.30	-2.59	2.00
Regional crop distribution								
% area with OSR	0.10	0.008	0.017	0.024	0.01	0.004	0.005	0
% area with OSR the previous year		not tested			0.32	0.003	0.010	0
% area with OSR 2 years ago		not tested			0.04	0.003	0.013	0
% OSR fields with GM	0.69	0.008	0.022	0.020	0.63	0.007	0.017	0
Field pattern characteristics								
mean area (ha)	0.01	-0.04	-0.09	-0.02	0.00	-0.02	-0.04	0
mean shape (m/m)	0.01	0.01	0.00	0.23	0.04	0.03	0.23	0
Total $R^2$	0.81				0.94			

Figure 1. Harvest impurity of non-GM OSR fields with the distance to the nearest GM OSR field depending on volunteer infestation in the case of the medium-flow cropping system. Determination of isolation distances for different risk levels with quantile regressions and impurity thresholds for year 1.



## Conclusions

Cropping systems were most important in determining impurity rates and the way impurity rates related to regional or local factors. Determination of isolation distances and other regional rules (e.g. the maximum regional area of GM OSR) to ensure harvest purity should thus consider past history of OSR cultivation in the area and, in particular, how current or future cropping systems will manage volunteers. We were able to determine such regional rules as a function of the risk accepted by the decision-maker (i.e. the % of situations exceeding harvest impurity thresholds), the cropping system and the volunteer infestation.

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# Propagation of *Arundo Donax* L. by Means of Rhizome and Stem Cuttings

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Perennial lignocellulosic crops are considered suitable for energy production from an agronomic, economic and environmental issues. The possibility of obtaining 2<sup>nd</sup> generation bioethanol makes them very interesting for bioenergy production. Among these, Giant reed (*Arundo donax* L.), is particularly interesting to the Italian environments (Cosentino et al., 2005).

Propagation of this species occurs exclusively asexually, due to its sterility out its native area (Asia) (Polunin and Huxley, 1978). Therefore the introduction of this species among the agricultural crops requires solutions throughout to the agamic propagation (stem cuttings or rhizomes) and related issues (size of the rhizome with relative presence of buds ready or dormant, utilisation of the stem, whole or fractions, rooting capacity of the different fractions, apical dominance). In order to provide useful indications to carry out large-scale facilities, different ways of *Arundo donax* propagation were studied.

## Methodology

The research was carried out in spring 2007-winter 2008 at the experimental farm of the Faculty of Agriculture, University of Catania (Catania plain, 10 m above sea level, 37° 25' Lat. N, 15° 30' Long. E). The following two methods of propagation were carried out:

1<sup>st</sup> trial: rhizome cuttings: Rhizome cutting with three buds ready (R1), one bud ready (R2), rhizome cutting of 10 cm length (R3) and 5 cm length with only latent buds(R4);

2<sup>nd</sup> trial: stem or part of this: whole stems (S1), the base portion (S2), the median portion (S3), the apical portion (S4). Stem cuttings, were cut a length of around 100 cm, and putted in the field on April the 17<sup>th</sup> at depth of 10 cm, overlapping them for a length of 50 cm. Its were transplanted on April 17<sup>th</sup> 2007 in row 100 cm apart. Rhizomes were transplanted on April the 26<sup>th</sup> with a distance row of 50 cm, corresponding to a plant density of 2 rhizomes m<sup>-2</sup>.

Plant material was collected from “Fondachello” clone previously selected in Sicily (Cosentino et al., 2006). Both trials were carried according to a randomised blocks experimental design with three replications. Elementary plots were 24 m<sup>2</sup> (6 x 4 m). Soil was ploughed and harrowed before stem cuttings burying. At transplanting, soil was irrigated and other two irrigations were carried out every 30 days, giving a total irrigation volume of 230 mm and 250 mm, respectively in the two trials.

In both trials, during the growing season stem emission were recorded. At harvest that occurred on March the 5<sup>th</sup>, stem height was measured (on a sample of 6 canes per plot), stem base diameter, biomass and humidity were measured.

Data recorded were analysed by one-way ANOVA, using CoStat Version 6.003 (Cohort Software) related to a experimental design in field.

## Results

1<sup>st</sup> trial – Sprouting was observed between 14 (R1) and 20 days (R4) from transplanting (Tab. 1). Sprouting rate resulted highest in R1 treatment (100%) and lowest in R4 (75%). Stem density at the end

of cycle (November) was equal to 6.6, 5.6 and 6.3 stems  $m^{-2}$ , respectively in R1, R2 and R3, with a significant advantage in comparison to R4 treatment (3.9 stem  $m^{-2}$ ). At harvest yield components did not show significant differences among treatments. Yield resulted also not significant difference among treatment, with an average value of 2.6 t  $ha^{-1}$ .

Table 1- Rhizome cuttings. Morphological and biological characteristics in relation to studied treatments

Treatments	Establishment (% )	Emergence <sup>1</sup> (d)	Stem density (n $m^{-2}$ ) on 5/11/07	Stem density (n $m^{-2}$ ) on 11/12/07	Height (cm)	Base diameter (mm)	Stem weight (g)	Dry biomass (t $ha^{-1}$ )
R1	100a	14.0a	3.8a	6.6a	176.5a	11.8a	360.7a	2.7a
R2	81.3c	17.0ab	2.5b	5.6a	145.6a	11.9a	270.9a	2.0a
R3	93.8b	17.0ab	3.3a	6.3a	163.0a	11.8a	312.1a	2.6a
R4	75.0d	20.5a	1.8c	3.9b	168.8a	12.0a	372.9a	2.9a
<b>Average</b>	<b>88.8</b>	<b>17.8</b>	<b>2.8</b>	<b>5.6</b>	<b>163.5</b>	<b>11.7</b>	<b>329.2</b>	<b>2.6</b>

<sup>1</sup>Days after transplant

2<sup>nd</sup> trial – Number of buried nodes per  $m^2$  resulted different in studied treatments, since the distance of nodes on the stem decreased from the base to the top. In whole stem (S1) buried nodes were equal to 38.1 per  $m^2$ , on the average, instead 10.1 and 12.9 nodes  $m^{-2}$  in S2 and S3 treatments, but 32.2 nodes  $m^{-2}$  in S4 (tab. 2). Sprouting was observed in all treatments since 37 days from transplant. At the end of autumn 2007 stem density did not show significant differences among treatments (4.3 stems  $m^2$  in the average) (tab. 2), even if sprouting rate resulted different in relation to the number of buried nodes. Stem height (114.1 cm in the average), base diameter (9.0 mm in the average) and dry biomass (0.4 t  $ha^{-1}$  in the average) did no show significant differences among treatments.

Table 2 – Stem cuttings. Morphological and biological characteristics in relation to studied treatments

Treatment s	Buried nodes (n $m^{-2}$ )	Emergence <sup>1</sup> (d)	Stem density (n $m^{-2}$ ) on 11/12/07	Sprouting rate (%)	Height (cm)	Base diameter (mm)	Stem weight (g)	Dry biomass (t $ha^{-1}$ )
S1	38.1a	35a	3.9a	10.3b	104.7b	9.0a	181.3a	0.57a
S2	10.1b	38a	3.9a	38.8a	128.4a	8.8a	215.0a	0.42a
S3	12.9b	37a	5.2a	40.0a	118.2ab	9.0a	201.0a	0.34a
S4	32.2a	37a	4.3a	13.2b	105.1b	8.9a	204.9a	0.24a
<b>Average</b>	<b>23.3</b>	<b>36.8</b>	<b>4.3</b>	<b>25.6</b>	<b>114.1</b>	<b>9.0</b>	<b>329.2</b>	<b>0.39</b>

<sup>1</sup>Days after transplant

## Conclusions

Both of the experiments showed a good production of a new shoots and consequently of stems in relation to all studied treatments. Even the smaller portions of rhizome were able to root and sprout Rhizomes produced a number of new stems twice and also heavier and higher than the stems produced by the stem cuttings. Stem cuttings or whole stem seems to be suitable in the same way to produce a good stand.

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# Sustainable Crop Rotations for the Production of Biodiesel from Rapeseed (*Brassica napus* L. var. *oleifera* D.C.) in the Semi-Arid Mediterranean Environment

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The production of biofuels represents one of the strategies followed by the European Union to face the strong dependence from the fossil fuels (Com, 2006). The species of the *Brassicaceae* family can be utilised to this purpose, given also their widespread cultivation in Europe, provided the setting up of low input crop management techniques. In the semiarid condition of the Mediterranean environments many species of this family are native (Kumar and Tsunoda, 1978). Therefore, their cultivation is feasible as they grow during the cold and rainy winter period (Copani *et al.*, 2007). Bearing this in mind, a research was carried out in order to evaluate the behaviour of rapeseed (*Brassica napus* L. var. *oleifera* DC.) in crop rotation with local widespread crop: durum wheat (*Triticum durum* Desf.) and chickpea (*Cicer arietinum* L.).

## Methodology

The research was carried out in the two years 2002-2003 in Sicily (South of Italy, Enna, 550 m a.s.l., 37°21' N Lat, 14°16' E Long).

The following factors were studied:

- Preceding crops: wheat, chickpea, rapeseed;
- Crops: wheat (*Triticum durum* Desf., cv. Simeto), chickpea (*Cicer arietinum* L., cv. Sultano), rapeseed (*Brassica napus* L. var. *oleifera* D.C., cv Kabel, spring variety);
- Input level: high (conventional tillage, fertilisation using 80 kg ha<sup>-1</sup> of N in durum wheat and rapeseed, 0 kg ha<sup>-1</sup> in chickpea; 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>); low (minimum tillage, fertilisation with 40 kg ha<sup>-1</sup> of N in durum wheat and rapeseed, 0 kg ha<sup>-1</sup> in chickpea; 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>).

The crops were sown on December the 10<sup>th</sup> 2002. Weeding was carried out manually.

A split-plot experimental design was applied with three replication. Single plot of 12 square meter. Data were analysed by means of two-way ANOVA within each studied crop, using CoStat version 6.003 (Cohort Software) related to a experimental design in field.

## Results

In the average of “inputs” and in relation to the preceding crop, rapeseed gave the highest yield after chickpea (1.99 t ha<sup>-1</sup>) and the lowest yield after rapeseed itself (1.34 t ha<sup>-1</sup>) whilst after wheat produced 1.5 t ha<sup>-1</sup> (Fig.1).

The rapeseed as preceding crop determined in chickpea yield equal to 1.67 t ha<sup>-1</sup> lower than what found after wheat (1.85 t ha<sup>-1</sup>) whilst the lowest production was after chickpea itself (1.4 t ha<sup>-1</sup>) (Fig.2). Wheat after rapeseed gave a yield equal to 2.22 t ha<sup>-1</sup>, higher than those of wheat after wheat (2.05 t ha<sup>-1</sup>) and lower than those of wheat after chickpea (2.68 t ha<sup>-1</sup>) (Fig.3).

In relation to the levels of input, rapeseed showed the highest differences among the two levels (1.39 t ha<sup>-1</sup> against 1.85 t ha<sup>-1</sup>, respectively for the low and high input), with a gap of 25%. The lowest difference (4.8%) was obtained in chickpea (1.59 against 1.67 t ha<sup>-1</sup>, respectively for the low and high input).

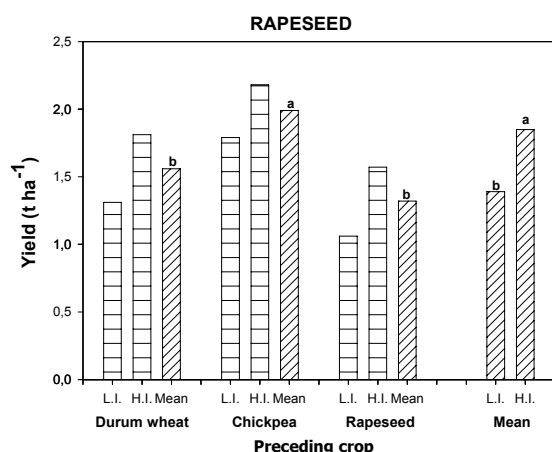


Fig.1 - Rapessed yield ( $t\ ha^{-1}$ ) in relation to crop rotation. Bar with different letter are significantly different.

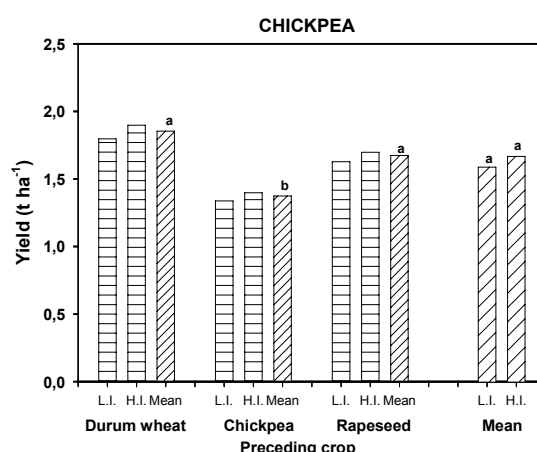


Fig. 2 - Chickpea yield ( $t\ ha^{-1}$ ) in relation to crop rotation. Bar with different letter are significantly different.

## Conclusions

The research carried out in a semi-arid environment of Sicily showed the good possibility of introducing rapeseed within the present Mediterranean cropping systems where durum wheat is often a mono-crop, taking into account also the improvement of soil fertility determined by the high crop residues and the crop rotation. As far as low input techniques are concerned, rapeseed seems to be less tolerant to the reduction of inputs compared to the other two crops. This is probably due to the soil tillage seedbed preparation, considering the small dimension of the seeds. Moreover as well know, the legume crop resulted the best preceding crop for wheat and rapeseed. In fact, chickpea to a certain extent allowed to balance the reduction of inputs.

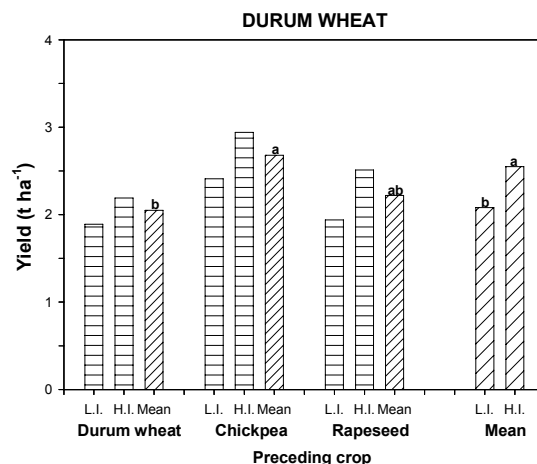


Fig. 3 - Durum wheat yield ( $t\ ha^{-1}$ ) in relation to crop rotation. Bar with different letter are significantly different.

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# Energy Balance of Durum Wheat (*Triticum Durum* Desf.) Cropping as Related to Different Agronomic Inputs in Mediterranean Environment

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In recent years there has been a sudden increasing of energy cost which is affecting all production activities; this aspect is dramatically influencing the extensive production of durum wheat, the main field crop in Sicily (South Italy). Energetic parameters like as net energy yield and energy efficiency, may be used as indicators of the sustainability of production systems in these environments. The aim of this study is to evaluate the energy balance of durum wheat in relation to different agronomic inputs (fertilizer and soil tillage techniques).

## Methodology

The field experiment was carried out in two years (1999-2000) at Enna (Sicily, 550 m a.s.l., 37°23' N Lat, 14°21' E Long). “Mongibello” durum wheat variety was sown on February the 3<sup>rd</sup> and on November the 24<sup>th</sup>, respectively for the first and the second year. In comparison to an ‘organic’ treatment, the factorial combination of the following factors was studied:

- Tillage (Conventional, Minimum and No tillage).
- Fertilizers levels (high input: 80 kg ha<sup>-1</sup> N and 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>; low input: 40 kg ha<sup>-1</sup> N and 45 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>).

Energy evaluation of production processes used energy costs for machinery fabrication and repairs, fuel consumption for the several mechanical operations, fertilizers, herbicides and seed production. Human labour and solar energy were not considered (Hülsbergen et al., 2001).

In order to analyse the energy inputs for each treatment, farming operations were considered (technical means, materials used and execution time. Production means were converted into energy terms. Energy input of mechanization included two types of costs: direct costs for fuel and lubricant consumption of tractor, and indirect costs, considering primary energy content of the materials that compound farm machines, energy cost due to their assembly and fabrication and energy cost for their maintenance and repair (Doering, 1980). Outputs were determined by means of the biomass composition and energy conversion factors (Odum, 1988). Nitrogen content in samples of each plant part was determined by means of Kjeldahl method (AOAC, 1990). The amount of protein was calculated as follow: protein % of dry matter = N% x 5.75. Ash content (dry weight basis) was measured after 15 hours in muffle furnace at 550°C until constant weight. Finally, the dry matter content of each sample was detected using a precision thermo-balance and the ash content was determined on dry matter basis. The productive data and energy parameters were analysed by ANOVA, using CoStat Version 6.003 (Cohort Software).

## Results

In the first year grain yield was lower than yield obtained in the second year with a reduction of 34% (1.41 t ha<sup>-1</sup> against to 2.12 t ha<sup>-1</sup>, respectively) because of the late sowing. In relation to this fact, ‘no tillage’ treatment determined a bad result in the yield of the first year (1.08 t ha<sup>-1</sup>), but not in the second year (2.22 t ha<sup>-1</sup>) with respect to ‘conventional’ and ‘minimum’ tillage. In the first year ‘low input’

yield (1.22 t ha<sup>-1</sup>) was significantly different than 'high input' yield (1.63 t ha<sup>-1</sup>), especially because of the best results obtained in 'conventional' tillage.

In the second year the reduction from 'high' and 'low input' yield was equal to 31% (2.57 t ha<sup>-1</sup> against to 1.67 t ha<sup>-1</sup>). In both years yield obtained in 'organic' treatment resulted lower than 'conventional' tillage, as 30% less (Tab. 1).

Tab. 1 – Grain yield in relation to different studied treatments (t ha<sup>-1</sup>)

Treatment	Conventional	Minimum	No tillage	Average	Organic
1 <sup>st</sup> year					
High input	2.00 a	1.74 ab	1.15c	1.63 a	
Low input	1.36 bc	1.51 b	1.01 c	1.29 b	
Average	1.68 a	1.63 a	1.08 b	1.41	0.87
2 <sup>nd</sup> year					
High input	2.33 a	2.63 a	2.74 a	2.57 a	
Low input	1.78 b	1.55 b	1.69 b	1.67 b	
Average	2.06 a	2.09 a	2.22 a	2.12	1.38
<b>Average</b>	<b>1.87</b>	<b>1.86</b>	<b>1.65</b>	<b>1.77</b>	<b>1.13</b>

The highest energy yield was obtained in the second year in 'no tillage high input' treatment (32.10 GJ ha<sup>-1</sup>), while the lowest in 'conventional low input' (13.6 GJ ha<sup>-1</sup>). 'Organic' treatment was lower than all conventional treatments (12.65 GJ ha<sup>-1</sup>). Energy efficiency was also significantly higher in the second year and in 'no tillage' treatment, both in 'high input' condition (3.02) and 'low input' condition (3.23).

Tab. 2 – Net energy yield in relation to different studied treatments (GJ ha<sup>-1</sup>)

Treatment	Conventional	Minimum	No tillage	Average	Organic
1 <sup>st</sup> year					
High input	11.06 a	11.21 a	4.24 b	9.08 a	
Low input	6.28 b	13.67 a	8.02 b	9.32 a	
Average	8.67 b	12.44 a	6.13 b	9.20	3.68
2 <sup>nd</sup> year					
High input	16.88 b	28.88 a	32.10 a	20.70 a	
Low input	13.60 b	14.13 b	20.45 a	16.12 b	
Average	15.24 b	20.60 a	26.27 a	18.41	12.65
<b>Average</b>	<b>11.96</b>	<b>16.52</b>	<b>16.20</b>	<b>13.81</b>	<b>8.17</b>

Tab. 3 – Energy efficiency in relation to different studied treatments

Treatment	Conventional	Minimum	No tillage	Average	Organic
1 <sup>st</sup> year					
High input	1.46 a	1.58 a	1.27 b	1.44 a	
Low input	1.36 b	2.06 a	1.83 a	1.75 a	
Average	1.41 a	1.82 a	1.55 a	1.60	1.32
2 <sup>nd</sup> year					
High input	1.70 b	2.40 a	3.02 a	2.37 a	
Low input	1.77 b	2.11 b	3.23 a	2.37 a	
Average	1.74 b	2.26 a	3.13 a	2.37	2.10
<b>Average</b>	<b>1.58</b>	<b>2.04</b>	<b>2.34</b>	<b>1.99</b>	<b>1.71</b>

## Conclusions

Results suggest that no-till systems had positive impact on biomass and energy yields, overall in the favourable years, moreover, it can improve the carbon sequestration potential and enhance soil fertility and energy yield production targets.

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# Crop Physiology of Different Genotypes of Sorghum (*Sorghum Bicolor* (L.) Moench) in South of Italy

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Sorghum can be grown in semiarid temperate and tropical environments an energy crop to produce heat and electricity and also first and second generation bioethanol. This plant is native of Central Africa and is characterised by a C4 photosynthetic pathway and thus could use high temperatures and high radiation intensity of Mediterranean environment (Cosentino, 1996).

## Methodology

This research aimed at comparing the crop physiology of nine genotypes of sorghum: six sweet sorghum genotypes (Cowley, Korall, Roce, Sofra, MN 15000 and NK 506), three grain sorghum genotypes (Arblan, Aralba and Arminia) and three fiber sorghum genotypes (ABF 306, H 128 and H132).

Field experiment was carried out in Enna (Sicily, 550 m a.s.l., 37°23' N Lat, 14°21' E Long) in 2001. A randomised block experimental design with 3 replicates with a single plot of 33.6 m<sup>2</sup> (5.6 x 6 m) was applied. Sowing was carried out on May the 20<sup>th</sup> adopting a row distance of 70 cm and a plant density of 11 plant m<sup>-2</sup>. At sowing, 60 kg ha<sup>-1</sup> of N (as ammonium sulphate) and 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (as mineral perphosphate) were supplied; further 60 kg ha<sup>-1</sup> of N (as ammonium nitrate) were given to the crop as top dressing.

Aiming at studying crop physiology of the studied genotypes, phenology, growth analysis, thermal time and leaf gas exchange were studied along the growing season. Stomatal conductance and leaf transpiration, by means of a steady state porometer (LI-1600, Li-Cor Inc., Lincoln, Nebraska, USA) and leaf photosynthesis by means of the open system IRGA of the Analytical Development Com. LTD (LCA4 model) on the last fully expanded leaf were measured at noon, on six dates during vegetative phase.

## Results

Aboveground dry biomass was in the average equal to 18.9 t ha<sup>-1</sup> and varied significantly among genotypes studied. The most productive genotypes were the sweet genotypes Sofra (26.3 t ha<sup>-1</sup>) and the fiber genotype H132 (26.1 t ha<sup>-1</sup>) followed by sweet genotype Cowley (23.6 t ha<sup>-1</sup>). Less productive were the grain genotypes (14.9, 13.9 and 10.7 t ha<sup>-1</sup>, respectively for Armonia, Aralba and Arblan) and the fibre genotype H128 (15.3 t ha<sup>-1</sup>) (Tab. 1).

Net photosynthesis varied from 36.1 to 28.3  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; stomatal conductance varied from 0.55 to 0.30 cm s<sup>-1</sup> and leaf transpiration ranged between 14.0 and 8.7  $\mu\text{g H}_2\text{O cm}^{-2} \text{ s}^{-1}$ .

About grain genotypes, a wide share of biomass is accumulated in panicles starting from the beginning of July (40 days from sowing). Since the end of July, moreover, stems tends to decline after reaching maximum value and a large share of biomass accumulated moved, probably, to panicles (around 2-3 t ha<sup>-1</sup>).

The early genotypes (Arblan, Aralba, Armonia, H 128, Roce, Korall, ABF 306) pointed out a steady accumulation of biomass arrested around the second half of August. During July the later genotypes (H 132, Sofra, Cowley, MN 1500) reached half of the total biomass produced, but stopped their growth

because of a water lack occurred in that period. The following water restoration led to the growth resumption up to the end of October.

LAI showed a decreasing at first, until reaching a maximum value corresponding to different phases of the cycle in relation to genotype: at boot phase in grain genotypes, in sugar genotypes (except MN 1500) and in earlier fibre those (ABF 306 and H 128), at the beginning of flowering in late genotypes MN 1500 and H 132. In the latter ones, the resumption of vegetative in response to rains occurred in early September, resulted in an issue of new leaves and, therefore, an increase of the value of LAI.

Tab. 1 – Productive, biological and physiological parameters in studied genotypes.

Genotypes	Yield (t ha <sup>-1</sup> )	Cycle length (d)	LAI max	CGR max (g m <sup>-2</sup> d <sup>-1</sup> )	WUE (g l <sup>-1</sup> )	RUE (g MJ <sup>-1</sup> )	NUE (g g <sup>-1</sup> )	Phyllochron (°C d)	Thermal time to flowering (°C)
<i>Arblan</i>	10.66 f	100 a	3.6	29.3	2.87	0.80	61.1	50.96	780.5
<i>Aralba</i>	13.29 e	104 a	5.2	27.3	3.58	0.91	54.7	45.05	920.7
<i>Armonia</i>	14.92 e	105 a	4.7	44.5	4.02	1.07	51.3	51.93	949.5
<i>Cowley</i>	23.57 b	168 d	8.4	38.6	3.71	0.82	140.0	49.05	1717.5
<i>Korall</i>	18.81 cd	124 c	7.5	42.0	3.31	1.09	113.4	47.73	1293.0
<i>Roce</i>	17.76 d	123 c	7.4	23.9	3.10	0.92	97.5	47.39	1293.0
<i>Sofra</i>	26.25 a	163 d	7.9	40.6	4.14	1.05	146.2	49.02	1795.9
<i>MN 1500</i>	19.73 cd	167 d	8.9	21.6	3.11	0.78	141.4	50.35	1919.3
<i>NK 506</i>	21.31 c	161 d	6.8	24.2	3.36	0.93	103.6	46.45	1705.9
<i>ABF 306</i>	19.70 cd	126 c	7.0	59.7	3.47	1.10	89.5	45.39	1338.8
<i>H 128</i>	15.31 e	114 ab	6.8	44.8	3.17	0.90	94.9	46.25	1338.8
<i>H 132</i>	26.13 a	168 d	7.3	45.4	4.12	1.04	140.6	48.58	1958.0
<b>Average</b>	<b>18.95</b>	<b>135</b>	<b>6.8</b>	<b>48.2</b>	<b>3.50</b>	<b>0.95</b>	<b>102.9</b>	<b>48.18</b>	<b>1417.6</b>

Water use efficiency (WUE) was variable; the sweet genotypes Sofra and Cowley, the fiber genotype H132 and the grain genotype Armonia showed the highest values (4.14, 3.71, 4.12 and 4.02 g l<sup>-1</sup>, respectively). The highest nitrogen use efficiency (NUE) values were obtained in Sofra (146.2 g g<sup>-1</sup>), MN1500 (141.4 g g<sup>-1</sup>), Cowley (140.0 g g<sup>-1</sup>) among sweet genotypes, and in H132 (140.6 g g<sup>-1</sup>) among fiber genotypes (tab .1).

Phyllochron (thermal interval between the appearances of successive leaf tips) varied between 51.9°C (Armonia) and 45.1°C (Aralba). Heat sums, with base temperature as 9° C, from sowing to the stage for the beginning of flowering, for each genotype were calculated. Grain genotypes requested a lesser amount of thermal degree days to reach the flowering stage (from 780.5 to 949.5°C, respectively Arblan and Armonia). By contrast, higher values of sums thermal were recorded in MN 1500 (1919.3°C) and H 132 (1958.0°C) (tab. 1).

## Conclusions

Results showed a different behaviour among the studied genotypes with reference to thermal time of phenological events, LAI, dry biomass accumulation and dry biomass partitioning. Among the high yielding varieties (H132 and Sofra) the sugar genotypes Sofra resulted earlier (1745.9 against 1958.0 °C thermal time to flowering) and with a higher NUE (146.2 g g<sup>-1</sup> against 140.2 g g<sup>-1</sup>) and RUE (1.05 g MJ<sup>-1</sup>).

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# Integration of Oil-Seed Crops in Mediterranean Agro-Pastoral Systems to Supply Bio-Fuels to Local Power Industry

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The growing of rapeseed (*Brassica napus* var. *oleifera* D.C.) and Ethiopian mustard (*Brassica carinata* A. Braun) as oilseed crop for biodiesel production in southern Europe has gained new interest, following the implementation of policies aimed at increasing the production of locally produced bio-fuels. The study reported in this paper is part of a feasibility study designed to provide a scientific assessment on the introduction of oil seed crops in the context of the Mediterranean agro-pastoral systems of central Sardinia. Locally, the oilseed demand is from a 34 MW electric power station recently installed by Biopower Sardegna Spa, who funded the study, to supply the local industrial site. The overall objective of the experiments is also to build a dataset to adapt CROPGRO model of DSSAT (Jones *et al.*, 2003) to rapeseed. In this paper, we will provide preliminary data from the field experiments on rapeseed and an overview of the research design.

## Methodology

The field study was started in autumn 2007 in three private farms at Ottana, in central Sardinia, Italy (39°25'47.38'' N, 9°31'59.28'' E). All farms are located in an ancient alluvial soil characterized by low pH and fertility, but also by deep clay soil layers that are responsible for diffuse waterlogging in depressed areas. The experiment was arranged in unbalanced incomplete block design. Plot sizes ranged from 600 m<sup>2</sup> to 5418 m<sup>2</sup>. Four varieties of rapeseed (Kabel, PR46W31, PR46W10, PR46W14) and two of Ethiopian mustard (ISCI 7, BRK 147) were sown in the first week of November 2007 at the three sites using 0.17 m row spacing. A seeding rate of 8 kg ha<sup>-1</sup> (rapeseed) and 10 kg ha<sup>-1</sup> (Ethiopian mustard) was adopted using a conventional seed drill. Fertilization was set up to 132 kg ha<sup>-1</sup> N and 92 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> in order to prevent nutritional stress. Soils had never been planted with oil seed crops before. Weed and pest control was not performed. After emergence, three permanent sampling areas per plot (10 plants each) were chosen for systematic phenological observations according to the BBCH scale. The development stages recorded were emergence (10), flowering (65) and ripening period (89). Plant height was also measured in the sampling areas. A systematic sampling of plant phenology, growth and biomass partitioning was designed for rapeseed cv Kabel in order to develop a specific module for rapeseed of DSSAT but is not reported in this paper. Residual biomass samples were fresh weighed and then oven dried for 48 h at 80°C to determine dry matter production. A few days before the crop harvest a final hand-harvested sample was collected from each plot, from which a random sub-sample of 15 plants was taken to measure yield components (number of pods per plant, number of seeds per plant and 1000-seed weight). Three samplings areas (0.50 m<sup>2</sup> each) per plot were also randomly chosen and harvested to measure harvest index (HI) and population density. Data were submitted to GLM procedure (SAS Institute Inc., Cary, NC, USA; 2002).

## Results

For seek of brevity, only phenology of rapeseed varieties and yield components results of Kabel are reported in this paper. There were no appreciable differences between varieties in the first developmental stages (Table 1). Development of the three PR rapeseed was significantly later than

Kabel, with no significant differences among them. Seed harvest of all PR hybrids occurred 27 days after Kabel.

Table 1 – Development stages recorded according to BBCH scale (Means values  $\pm$  standard error)

Varieties/Hybrids	07/01/2008	17/04/2008	Harvesting date (BBCH -89)
Kabel	14 $\pm$ 0.05	74 $\pm$ 0.2	28/05/08
PR 46W31	14 $\pm$ 0.06	64 $\pm$ 0.2	16/06/08
PR 46W10	14 $\pm$ 0.07	65 $\pm$ 0.2	16/06/08
PR 46W14	14 $\pm$ 0.06	65 $\pm$ 0.2	16/06/08

At the end of flowering period, plant height (Table 2) ranged from 103 to 112 cm. Plant height growth and development were constrained in waterlogged areas, that were excluded from the sampling. At harvest, the number of pods and seeds per plant averaged from 44 to 67 and from 767 to 1128 respectively. Only in Farm 3, seeds moisture content was higher than that in other farms because of green weeds presence at harvest. The lowest harvest index observed was due to seed shattering, related to a very dry and hot weather soon before harvest.

Table 2 – Plant characters and yield components of rapeseed cv ‘Kabel’

Character	Farm 1	Farm 2	Farm 3	Mean	C.V.%
Plant density (plants m <sup>-2</sup> )	110 a	75 a	103 a	96	47
Plant height (cm)	112 a	111 a	103 b	109	18
Number of pods plant <sup>-1</sup>	44 b	63 a	67 a	58	67
Number of seeds plant <sup>-1</sup>	767 b	1101 a	1128 a	999	75
1000-seed dry weight (g)	3 a	3 a	3 a	3	19
Seeds moisture content at harvest (%)	13 b	14 b	25 a	17	13
Seed yield (t ha <sup>-1</sup> )	1.3 a	1.2 a	1.4 a	1.3	43
Harvest Index	0.20 a	0.17 b	0.22 a	0.20	10

Means values followed by different letters in each row are significant different at  $P \leq 0.05$  probability level according to LSD test

## Discussion

The four cultivars evaluated in this experiment represented a wide range of rapeseed types available on markets (early to late maturing, semi-dwarf to tall). In the tested group of cultivars, there were three late maturing hybrids, and an early cultivar with slow initial growth. However, in terms of phenology, the three PR hybrids were very similar, while Kabel was much earlier and hence suitable for better performance in the specific context of Sardinia. Plant height and yield components as number of pods per plant and number of seeds per plant did not affect rapeseed yield. The mean harvest indices for dry matter in our study were lower than reported for rapeseed (Hocking *et al.*, 1997a) because of seed shattering at harvest, which may be a serious limiting factor for rapeseed production in Mediterranean conditions. Further studies are being conducted in the area to extend the inference of these experimental results through simulation modelling and to assess the feasibility of integrating Ethiopian mustard and rapeseed in the context of Mediterranean agro-pastoral systems.

## Acknowledgements

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# Inter-relations and Multiple Regressions among Plant Traits and Environmental Parameters in a Three-year Kenaf (*Hibiscus cannabinus* L.) Field Trial

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Kenaf is a fast-growing annual herbaceous C<sub>3</sub> plant gaining interest in the latest years as a multipurpose crop providing material for the bio-based fibre industry and energy uses, with high productivity and low agronomic requirements. In the USA recent research has demonstrated several potential uses for each of the two stem components (bark and core), generally separated from each other (Taylor, 1992; Fuller, 1994).

Although kenaf has been widely investigated, no much data are reported for relatively high latitudes of Mediterranean Europe. In the framework of the “BIOKENAF” project supported by EU, under the rising interest for kenaf in recent years, agronomic field trials have been established in a rainfed location of Northern Italy which may represent an upper boundary for kenaf diffusion (45° N).

In parallel to the assessment of the growing patterns, the whole dataset from three years of agronomic field experiments was used to evaluate the degree of inter-relation among plant aspects and the ability of some traits and environmental factors to predict kenaf growth. Significant relations may give the opportunity to estimate growth through the measure of non-destructive parameters of easier assessment, thus avoiding the burden of destructive samplings and associated operations, or even the use of allometric measurements as a tool of potential support for yield forecasts in commercial fields, along with few environmental factors.

## Methodology

Field trials were carried out in the years 2003, 2004 and 2005 at the Experimental Farm of Bologna University located in Cadriano, near Bologna, Italy (44°03' N, 11°02' E, 33 m a.s.l.), in a flat area of the Po Valley. Two seeding times (S1: early seeding on may; S2: late seeding on June), two genotypes (Everglades 41; Taining 2) and two plant densities (D1: 200000 pl ha<sup>-1</sup>; D2: 400000 pl ha<sup>-1</sup>) were cross-tested in a completely-randomized block design at three replications.

Several destructive harvests were performed on 0.75 m<sup>2</sup> in each plot during the cycle. At each harvest the following traits were assessed: stem height (m); base stem diameter (mm); plant fresh biomass (FB; g m<sup>-2</sup>); dry matter content (DM; g kg<sup>-1</sup>); dry biomass (DB; g m<sup>-2</sup>); dry stem biomass (DS; g m<sup>-2</sup>) and leaf area index (LAI; m<sup>2</sup> m<sup>-2</sup>; LI-3001, LI-COR, Lincoln, NE). Typical daily meteorological data were recorded at the farm weather station. The thermal sums from emergence to the end of the cycle (GDD; °C) were calculated assuming a basal temperature of 12°C, according to Carberry and Abrecht (1990). The evaluation of the degree of inter-relation among plant traits by means of the Pearson's correlation (r) and the multiple linear regression (stepwise forward procedure) were carried out according to the software SigmaStat 2.03 (SPSS, inc.). An allometric approach was applied to the whole three-year dataset, i.e. including the two genotypes, the two densities and for all the harvests (n = 660).

## Results

Air temperatures and the amount of precipitation received during crop cycle exhibit a different picture in the three years, which gave the possibility to test an large range of climatic conditions. GDD from

emergence as a mean between S1 and S2 was 1509°C in 2003, 1434 °C in 2004 and 1343°C in 2005, while the accumulation of rainfall was 101 mm, 189 mm and 484 mm in 2003, 2004 and 2005, respectively.

The cross correlations among plant traits are reported in Table 1. Almost all the traits have resulted highly inter-related. DB and DS, in particular, have exhibited high associations with plant height and base stem diameter (r ranging from 0.65 to 0.90). FB, the simplest of destructive parameters since it does not require oven-drying, has equally proved to be well associated to DB and DS (r = 0.85 and 0.72, respectively).

Table 1. Correlations (r) between plant traits during the three growing seasons. FB = fresh biomass; DM = dry matter content; DB = dry biomass; DS = dry stem. ns,\* and \*\* means insignificant, significant at  $P \leq 0.05$  and at  $P \leq 0.01$ , respectively.

Plant trait	Height	Diameter	FB	DM	DB	DS
<b>Diameter</b>	0.75 **					
<b>FB</b>	0.68 **	0.66 **				
<b>DM</b>	0.62 **	0.35 **	0.03 ns			
<b>DB</b>	0.86 **	0.70 **	0.85 **	0.48 **		
<b>DS</b>	0.90 **	0.65 **	0.72 **	0.62 **	0.96 **	
<b>LAI</b>	0.56 **	0.63 **	0.62 **	0.37 **	0.63 **	0.56 **

A multiple linear regression (forward stepwise selection procedure) of the three potential regressors Height, Diameter and FB on DB and DS has equally proved successful: for DB, adj.  $R^2$  rises from 0.740 of the simple relation with Height to 0.878 of the multiple one; for DS, from 0.804 to 0.836. In both cases Diameter is the last parameter to be picked by the procedure, i.e. that delivering the weakest contribution. Multiple linear regression based on the association with heat and moisture, leads to the following regressions:

$$DB = -41.3 + 0.689 * GDD (^{\circ}C) + 1.063 * Precipitation (mm); \text{adj. } R^2 = 0.76^{**};$$

$$DS = -110.6 + 0.480 * GDD (^{\circ}C) + 1.563 * Precipitation (mm); \text{adj. } R^2 = 0.86^{**}.$$

Conversely, the correlations, although highly significant, do not seem reliable enough for the prediction of LAI. It is perceived as the growth of leaf canopy is a more-complex occurrence than the build-up of plant/stem biomass (Carberry and Muchow, 1992).

## Conclusions

The good correlations and multiple-linear regressions among plant traits represent valuable tools of potential support for yield forecasts, giving the possibility of estimating growth through the measure of non-destructive parameters of easier assessment, and encourages their adoption as well as that of simple climatic factors (temperatures, precipitation) for yield forecasts.

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# Effects of Mycorrhiza and Nitrogen Fertilizer on the Biomass Production of Chickpea

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Chickpea (*Cicer arietinum* L.) is unique among pulse crops due to its seed protein content and wide adaptability in ecologically diverse environments. It plays a significant role in farming systems as a substitute for fallow in cereal rotations and reduces the need for N fertilization through fixing atmospheric nitrogen and breaking disease cycles of gramineous crop.

Arbuscular mycorrhizal fungi (AMF) are obligate mutualistic symbionts which colonize the roots of the vast majority of crops. Mycorrhizal external hyphae provide a larger absorptive surface than root hairs and thus help with the uptake of relatively immobile ions from soil. However, it has also been reported that AMF hyphae are capable of taking up and transporting  $^{15}\text{NO}_3$  to the host plant (Bago et al. 1996). Additional N uptake due to AMF infection often found in nodulated legumes (Biró et al. 2000) can result from increased rhizobial  $\text{N}_2$  fixation (due to the improved phosphorus supply) or increased N uptake from soil by the AMF hyphal network.

The objective of the present study was to evaluate the effects of inoculating AMF on growth of chickpea in absence or presence of rhizobia or after application of additional fertilizer N.

## Methodology

Experiments in Mitscherlich pots were carried out in a fence house in the city of Vienna, Austria. The experiments were arranged in a randomized complete block design with five replications. The factorial design included the following factors:

- Year (2006 or 2007)
- AMF inoculation (M+ or M-, i.e. with or without inoculation)
- Nitrogen nutrition (N- R-, N+ R- or N- R+, i.e. only soil supply, with mineral fertilizer at 100 kg N ha<sup>-1</sup> or with inoculation of rhizobia)
- Harvest date (H1 or H2, i.e. at flowering or physiological maturity).

Chickpea seeds were sown in pots filled with a mixture (1:1) of sterilized soil and sand and manually thinned out to three seedlings per pot. The soil was a chernozem of silty loam taken from the experimental farm Gross-Enzersdorf of BOKU University. The soil-sand substrate (2006 or 2007: 25 or 41 kg  $\text{NO}_3\text{-N ha}^{-1}$ , respectively) was sterilized before sowing using a soil heater (105 °C, 24 h).

Inoculation of the AMF treatments (M+) was done by adding the AMF product “Symbivit®” to the pots at planting. Pots with fertilizer application (N+) received an amount of 314 mg N per pot as a calcium ammonium nitrate (27% N) solution one week after emergence. Rhizobia (R+) were inoculated by using the water suspension “Radicin®” one week after emergence.

Harvested plants were divided into shoots and roots, at maturity shoots were further divided into straw and pods for dry matter determination. The mycorrhizal colonization was evaluated according to Vierheilig et al. (1998).

The statistical analysis of all observations was done by the procedure MIXED of the SAS software. With significant factorial effects ( $p=0.05$ ), least significant differences (lsd) were calculated.

## Results

The inoculation with AMF was successful, because all inoculated plant samples were substantially infected. The root infection level was higher in 2006 (54.5%) than in 2007 (24.9%). Without inoculation no infected roots were found at all. Similarly, rhizobia inoculation resulted in nodule

production (2006 or 2007: 46.1 or 3.2 mg pot<sup>-1</sup>) while without rhizobia addition no nodules were observed.

Mycorrhiza increased total dry matter in both years, but this enhancing effect was significant only at the first harvest in 2006 and at the second harvest in 2007 (Fig. 1). However, we found no consistent effects of nitrogen nutrition on chickpea growth, neither due to fertilizer N nor due to rhizobial infection (Fig. 2). Only root dry matter was significantly affected by nitrogen nutrition. On average across years and harvest dates, mineral N fertilizer application reduced root growth compared to the unfertilized crops with and without rhizobia (main effect, data not shown). Interactions between AMF and nitrogen nutrition were also not significant.

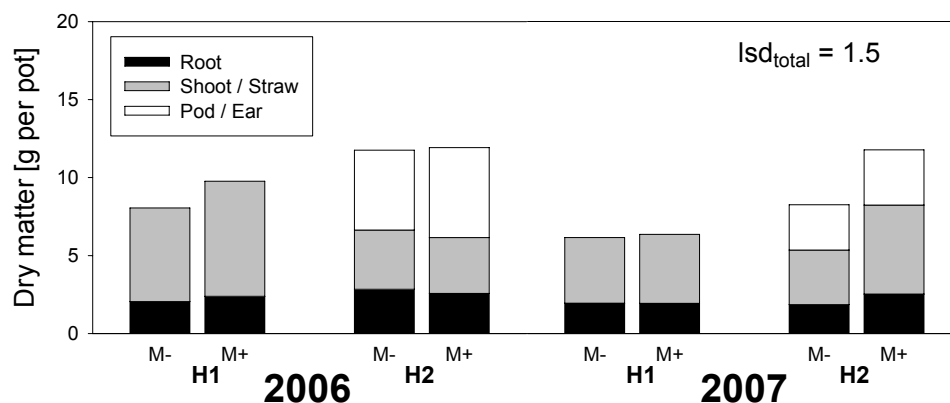


Fig. 1. Dry matter of crops and their fractions as affected by AMF inoculation, year and harvest date

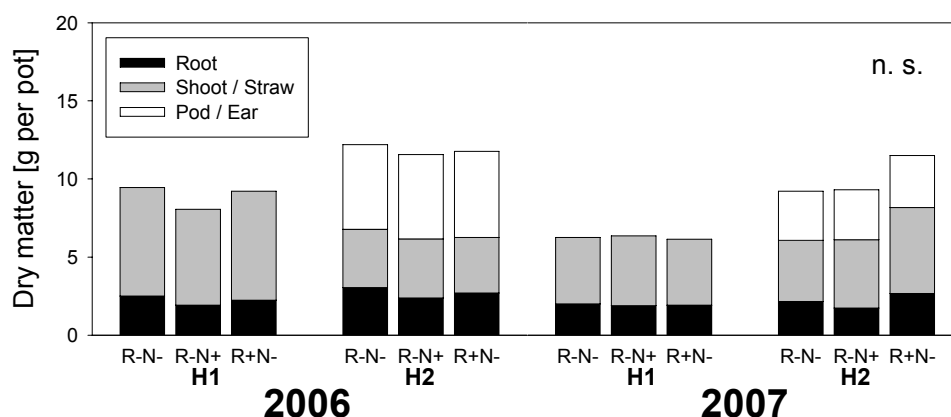


Fig. 2. Dry matter of crops and their fractions as affected by nitrogen nutrition, year and harvest date

## Conclusions

AMF inoculation led to chickpea infection and subsequently enhanced dry matter production. Presumably, this growth stimulation was not related to nitrogen nutrition, and nitrogen was not a growth limiting factor in our experiments.

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# Possible Utilisation of Maize for Biomass Production in Latvia

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## Introduction

Maize (*Zea mays* L.) has been grown for forage production since 1950-ties in Latvia. Although maize is not the dominating crop for forage in Latvia, area under it is growing with every year. Presently in the European countries maize has become important as biomass crop for biogas production. Utilization of bio-energy is very typical nowadays also in Latvia and the greatest potential of biogas production in the future is related to agricultural sector. The first biogas production project in agriculture is getting to be realized in Research and Study farm “Vecauce” of Latvia University of Agriculture (LLU) in 2008, and dairy cow manure together with plant biomass (mainly maize) are planned to be used as substrate. As maize has not been investigated before especially as energy crop, we should use available data from forage trials. Researchers highlighted importance of maize variety and harvesting time for maximum maize yield and methane yield from ha (Amon et al., 2007). The aim of this paper is to describe maize yield potential for biomass production depending on used variety and harvesting time in Latvia.

## Methodology

Three factor field trial were carried out in 2005-2007 in the Research and Study farm “Vecauce” (latitude: N 56° 28', longitude: E 22° 53') of LLU. Trials were arranged in four replication randomised blocks with plot size 16.8 m<sup>2</sup>. Planted population density was 82,000 plants per ha. Original seed of four maize varieties (Factor A) with different maturity rating defined by FAO number (Earlstar (FAO 160), RM-20 (FAO 180), Tango (standard, FAO 210), Cefran (FAO 340)) was used. Soil at the site was sod podzolic sand loam with pH<sub>KCl</sub> - 7.0-7.1, available for plants content of P – 198-263 mg kg<sup>-1</sup>; K – 191-196 mg kg<sup>-1</sup>, humus content – 21-25 g kg<sup>-1</sup>. Maize was sown at four different sowing times (factor B – data are not presented) starting with April 25<sup>th</sup> at ten day intervals till May 25<sup>th</sup> in all years. Fertilization was as follows: 34 kg ha<sup>-1</sup> P, 75 kg ha<sup>-1</sup> K and 148 kg ha<sup>-1</sup> N (18+70+60). Harvesting was done at four different times (factor C), beginning with September 1 at ten day intervals. Another single-factor trial to investigate the yield potential of different maize varieties (see Table 1) was carried out in the same field conditions and with the same management practices. The sowing of this trial was done on May 5<sup>th</sup>, but harvesting on September 20<sup>th</sup>. Different observations were carried out during vegetation period, but in this paper only results of dry matter (DM) yield, t ha<sup>-1</sup> and DM concentration at harvest are analysed. Analyses of DM content (g kg<sup>-1</sup>) of whole plant were carried out using standard method (Forage analyses met 2.2.1.1.). Meteorological conditions were variable in the research years, but all three on average were suitable for maize growing. Average day and night temperature from 25 April to 30 September was 14.4 °C in 2005, 15.7 °C in 2006, and 14.8 °C in 2007. Sum of precipitation during the same years and the same period was 298, 267 mm, and 339 mm, respectively. Results were statistically analysed using standard analysis of variance.

## Results

The choice of the optimum genotype is one of the key factors in biogas production. Amon et al. (2007) found that late ripening varieties (characterized by FAO 600) produce more biomass than medium or early ripening varieties (FAO 240 – 390). According to our previous research (Gaile, 2004) in Latvia

wax ripeness (dry matter content at least 300 – 350 g kg<sup>-1</sup>) declared as the best harvesting time also for biogas production (Amon et al., 2007) could be reached only using varieties characterised by FAO numbers up to 220 and even so not always (Table 1). Obtained DM yield was substantially ( $p<0.05$ ) dependent on used variety in specific year (Table 1), but looking on average yield per year during trial period – meteorological conditions in specific year affected yield greatly. The same was observed from three factors trial: influence of meteorological situation in harvest year was substantial ( $p<0.05$ ; by 21%), but variety influence was not substantial ( $p>0.05$ ).

Table 1. Maize biomass yield and dry matter content at harvest

Variety	FAO	DM yield, t ha <sup>-1</sup>			DM content, g kg <sup>-1</sup>		
		2005, n=19	2006, n=25	2007, n=28	2005, n=19	2006, n=25	2007, n=28
<b>Tango- check</b>	<b>210</b>	<b>16.75</b>	<b>13.97</b>	<b>17.93</b>	<b>279.2</b>	<b>313.4</b>	<b>305.6</b>
Nancis	120	14.09	13.30	19.75	318.0	409.0	342.2
Earlstar	160	16.96	13.65	-	281.2	316.4	-
Algans	190	16.09	14.12	19.06	294.8	369.8	303.5
Estelle	200	-	15.12	20.45	-	342.3	315.1
Laurelis	210	15.20	15.08	19.74	257.0	312.8	286.1
Cemilk	220	16.41	13.89	20.63	266.8	313.6	281.8
Celux	225	15.35	13.27	17.87	262.8	301.5	271.6
Cester	230	15.08	13.62	19.84	247.0	298.6	279.4
LG-2229	230	-	15.01	20.55	-	328.6	285.9
Celive	245	-	13.78	19.20	-	286.1	256.4
Cefran	340	16.04	13.58	-	226.4	262.1	-
<b>Average for all included hybrids</b>	<b>X</b>	<b>15.62</b>	<b>14.21</b>	<b>19.25</b>	<b>273.5</b>	<b>331.6</b>	<b>303.5</b>
LSD <sub>0.05</sub>	X	0.90	1.23	1.58	X	X	X

Harvesting time of maize is recognized as other important factor influencing maize biomass yield along with used hybrid (Amon et al., 2007). On average per three years from four varieties and four sowing times (three factor trial) obtained DM yield was 11.41 t ha<sup>-1</sup> on 1 September, 13.30 t ha<sup>-1</sup> on 10 September, 14.51 t ha<sup>-1</sup> on 20 September and 15.43 t ha<sup>-1</sup> on 30 September. Harvesting time in our three year research affected DM yield by 36.7% ( $p<0.05$ ). Dry matter content of the whole plant yield was even more affected by delayed harvest in September (by 48.2%). Although the highest average yield with the best DM content was reached when maize was harvested on 30 September not always such late harvesting is reasonable due to possible fall frosts before this date. Such frosts occur on 17, 18 September 2005 when maize was frozen. We observed slight yield and drastic quality decrease from 20 till 30 September in 2005.

## Conclusions

Used variety or genotype affected maize DM yield as well as DM content substantially, but looking in three year research period superior impact on these parameters had agro-meteorological conditions of specific year. Future demand is to investigate special biogas varieties' influence on dry matter yield, yield chemical composition and specific methane yield per hectare. Our results similarly to results in other European countries showed strong harvest time effect on maize yield and further research is needed to clarify harvest time effect on specific methane yield per hectare, outcome of biogas when maize is frostbitten and in cases when maize with low DM content (below 25%) is harvested.

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# Effects of NH<sub>3</sub>-Volatilization from Biogas Residues on the Environmental Protection Potential of Energy Cropping in Northern Germany

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The German government supports renewable energy production by fermentation of crops in biogas plants to significantly decrease national greenhouse gases emissions. According to EU Directive 2001/81/EC Germany has also to fulfil a reduction of ammonia emissions in the next years. Due to high ammonia contents biogas wastes are retransferred to agro-ecosystems as agriculture fertilizer. However, the knowledge of NH<sub>3</sub>-losses after field application of these slurries, in particular from mono-fermentation of crop biomass, is up to now rudimentary.

## Methodology

In 2007 and 2008 two factorial field trials were set up at two locations in northern Germany (loamy soil and sandy soil). In addition to the typical continuous maize cropping alternative biogas crop rotations with maize, wheat and rye grass were established. Treatments were applied in four replicates including four organic N-fertilizers (co-fermented and mono-fermented biogas slurry, pig and cattle slurry) in four application levels.

Three measurement techniques were used to determine ammonia losses. Appropriate for factorial field trials, the Standard-Comparison-Method (Vandre and Kaupenjohann 1998) allows simultaneous and comparative single plot measurements of relative losses. Quantitative ammonia losses were then derived from the scaling of the relative losses by comparison with results of a variant of open dynamic chamber method (Pacholski et al. 2006). The micrometeorological „backwards Lagrangian stochastic dispersion technique” (Sommer et al. 2005) was used as reference method to validate NH<sub>3</sub>-losses by the other two methods. The field measurements were supported by laboratory studies on e.g. infiltration of the slurries.

## Results

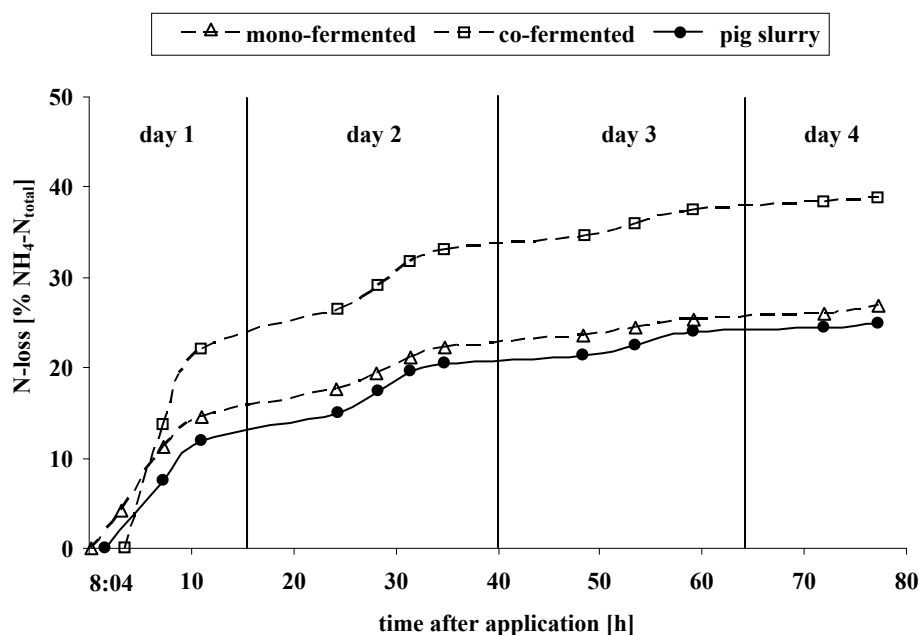
The fermentation process in biogas plants runs at pH-values just under 8.0. The storage (closed tanks) of the biogas waste as well as short transportation to the fields have no significant effects on the pH. Therefore at application the pH of biogas waste used in our field trials was significantly higher than the pH of animal slurries (Tab.1). It is well known that high pH-values are a basic requirement for an intense NH<sub>3</sub>-volatilization process. Other drivers of the NH<sub>3</sub>-volatilization from the mono-fermented biogas waste, e.g. pH buffer capacity, were similar compared to the pig slurry. In contrast, the co-fermented waste had a higher pH buffer capacity as well as a higher viscosity. Due to the biogas waste characteristics the NH<sub>3</sub>-emissions of mono-fermented biogas waste were in the range of pig slurry.

**Tab.1:** Average organic fertilizer qualities measured during the field trials in 2007

fertilizer	pH	NH <sub>4</sub> <sup>+</sup> -N [kg/m <sup>3</sup> ]	NH <sub>4</sub> <sup>+</sup> -N [%]	viscosity [mPas]	pH buffer capacity [mol H <sup>+</sup> /l]
mono-ferment (n = 14)	7.6	1.81	61	28	0.071
co-ferment (n = 14)	7.6	1.77	54	257	0.102
pig slurry (n = 12)	7.2	2.80	72	33	0.068

NH<sub>3</sub>-emissions from the co-fermented biogas waste were up to 50% higher, the greatest differences normally occurring on the first day (Fig.1). During the following days the ammonia volatilization kinetics were analogue. Field trial ammonia measurements were in good agreement with results of the reference method.

The results of the measurement campaign 2007 showed that the average NH<sub>3</sub>-losses from mono-fermented biogas waste were with round about 15% of total ammoniacal nitrogen (TAN) similar as those from pig slurries. The average NH<sub>3</sub>-losses from co-fermentation waste were with 25% of TAN considerably higher.



**Fig.1:** Average cumulative N-losses, n=4, 120kg N/ha, june 2007, wheat, loamy soil, field trial method

## Conclusions

Though the NH<sub>3</sub>-losses of co-fermented biogas waste were higher than losses from mono-fermented waste, in general losses of both wastes should be in the same range. The mono-fermented biogas waste had an atypical pre-treatment before the fermentation process. This led to a very low viscosity and a faster infiltration. Preliminary results of other untreated mono-fermented biogas wastes, measured in 2008, did not show such low viscosities, but higher NH<sub>3</sub>-losses.

Nevertheless, estimates for Germany based on the data set of 2007 indicate a significant effect of field applied biogas waste on the national NH<sub>3</sub> emission budget. Thus reduction of CO<sub>2</sub>-emissions by expansion of the biogas sector complicates the compliance of national emission ceilings for other atmospheric pollutants like ammonia. According to our findings a separation of dry matter and liquid phase (better infiltration properties) before field application could be a good measure to reduce NH<sub>3</sub>-emissions efficiently.

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# Bark : Core Ratio Relationships in Kenaf

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Currently, the main interest for kenaf (*Hibiscus cannabinus* L.) is due to the peculiar characteristics of the stem and of its fibres in particular. The two main components of the stem are the external part, containing the longer fibres (bark), and the inner part containing shorter fibres (core). The outer bark fibres are very appreciated for several industrial products, as high grade pulps for paper, composite boards and textiles (Mc Millin et al., 1998). Conversely, the inner core fibres have a smaller market value and are suitable for products such as pulps for packaging, animal bedding, sorbents and horticultural mixes (Muzzarelli, 1994). Therefore, for defining the industrial quality of stalks, the bark : core ratio is one of the most important parameters to evaluate. In this work data from a research carried out in southern Italy are reported, to assess the pattern and some relationships of the bark : core ratio during the whole crop cycle of the kenaf.

## Methodology

The trial was carried out in 2004 in Basilicata region (Metaponto plane, 40° 02' N; 16° 55' E) on silty-clay soil with good fertility. Kenaf was irrigated by the restitution of three levels of evapotranspiration (ETc 100%, 66%, 33%); two genotypes were tested: Tainung 2 and Guatemala 4, the latter only fully irrigated. The split-plot experimental design with three replicates was adopted. The sowing, with rows at the distance of 0.5 m, was performed on late April adopting a single-row pneumatic driller and a seed rate of 70 seeds m<sup>-2</sup>. Four plants samples were collected every two weeks, starting from 48 days from sowing (dfs), until early January 2005 (265 dfs). Morphological (height, basal and medium stem diameter) and productive (bark and core fresh and dry matter) parameters were measured.

## Results

In general, the bark : core ratio showed a linear decreasing during the crop cycle, starting from values ranging from 0.8 to 1.0 during the second month after sowing, to values ranging from 0.5 to 0.6 at the end of the cycle, for all the compared treatments (Fig. 1). The latter values are similar to those reported by Mambelli and Grandi, 1995, Losavio et al., 1997, McMillin et al., 1998, Alexopoulou et al., 2000, Bañuelos et al., 2002, nevertheless only few information is available about the bark : core ratio pattern during the crop cycle.

The bark : core ratio vs. plant height and vs. stem diameters relationships showed for all treatments values decreasing with increasing of the single morphological parameter. No significant difference was observed between genotypes at the same irrigation level. On the contrary, among irrigation treatments, restitution of 33% ETc showed a different pattern as compared with the other treatments, not different each other, both with reference to plant height (Fig. 2) and to stem diameter, basal (data not showed) and medium (Fig. 3). This is due to the strong effect of the fall down in water availability on all traits of the plant and on the reduction of the biological cycle length that stops vegetative growth.

Considering the linear relationship between stem dry matter production and plant height (Fig. 4), consistent with prior results (Weng et al., 1988; Mambelli and Grandi, 1995; Webber and Bledsoe, 2002) and also stem diameter (data not showed), the bark and core yields could be simply estimating by measuring only one of the above mentioned parameters during the whole crop cycle.

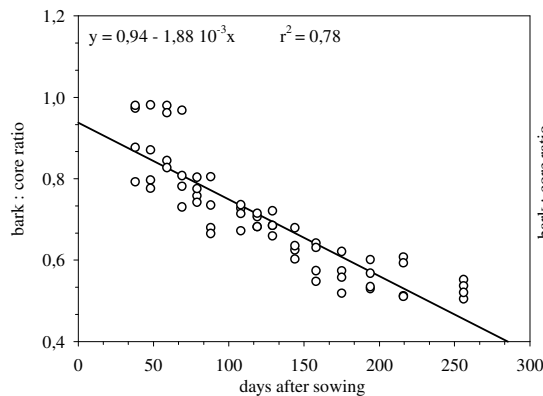


Fig. 1 - Relationship between bark : core ratio and age of the plant.

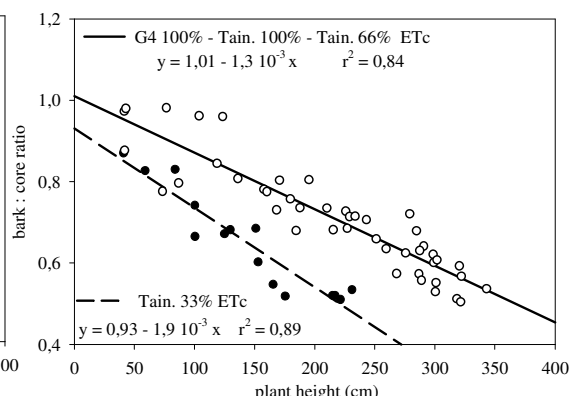


Fig. 2 - Relationships between bark : core ratio and plant height for the compared treatments.

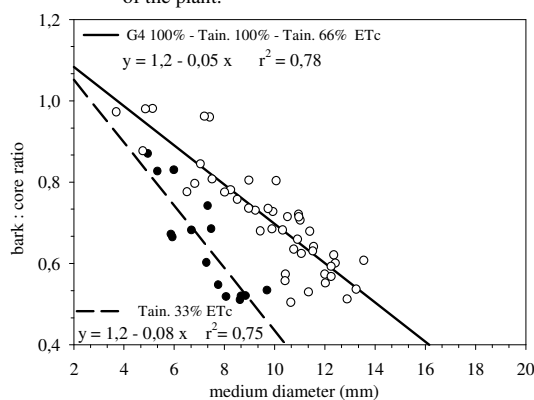


Fig. 3 - Relationships between bark : core ratio and plant medium diameter for the compared treatments.

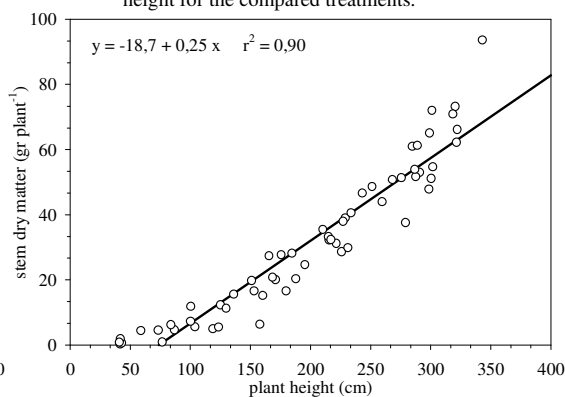


Fig. 4 - Relationship between plant height and stem dry matter.

## Conclusions

Results demonstrate that:

- the bark : core ratio decreased as kenaf increased in size;
- only very low irrigation level affected relationships between bark : core ratio and plant height or diameter;
- according to the above mentioned relationships, stem, bark and core dry matter yields could be simply estimating by measuring, during the whole crop cycle, one of the morphological traits considered.

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# Some Aspects of Fertilisation Practices in Oilseed Rape in Romania

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In order to accomplish the national commitments to produce biofuels, oilseed rape is the major option in Romania for the time being. More than 300.000 t biodiesel could be produced in Romania using actual facilities.

Even though the surfaces harvested have increased from 300 ha in 1996 to 349.000 ha in 2007, the yields remain small, almost always less than 2 t/ha.

There are many factors that influence the current state of affairs, but one of the most important of those is the fact that the Romanian farmers lack the required information regarding the specific influences that each fertiliser might have on oilseed rape yield. We have performed soil analysis for samples collected from different locations in the South of Romania. In all the situations the sulphur content of the soil was less than 5 ppm which is considered a level that could lead to sulphur deficiencies in plants (Borlan et al., 1992).

The aim of our study was to provide the farmers from the SE of Romania some information regarding the nitrogen, phosphorus and sulphur rates effects on the oil seed rape yield.

## Methodology

The first experiment was conducted under the field conditions at Braila Research Station (SE of Romania), between 2005-2007. The cultivar used was Elvis (hybrid) and the previous crop was wheat. The crop was two times irrigated with 450 mc/ha, before sowing and before flowering. Phosphorus fertilisers were applied before sowing and the nitrogen was spread before stem elongation (BBCH growth stage 30).

The second experiment was conducted in a plot from a farmer field. The cultivar used was Nelson (hybrid) and the previous crop was wheat. We have used the following treatments:

- Control: 50 kg P<sub>2</sub>O<sub>5</sub>/ha + 100 kg N/ha.
- T1: 50 kg P<sub>2</sub>O<sub>5</sub>/ha + 100 kg N/ha + 60 kg S/ha. Sulphur fertiliser was applied as elementary sulphur.
- T2: 50 kg P<sub>2</sub>O<sub>5</sub>/ha + 100 kg N/ha + 60 kg S/ha. Sulphur fertiliser was applied as complex fertiliser.
- T3: 50 kg P<sub>2</sub>O<sub>5</sub>/ha + 100 kg N/ha + 60 kg S/ha. Nitrogen and sulphur fertiliser have been spread as ammonium sulphate (foliar application).

Phosphorus fertiliser and 12 kg N/ha were applied in the autumn, before sowing. 50 kg N/ha and 60 kg S/ha were spread in February spring before stem elongation (BBCH growth stage 30). The remaining nitrogen fertiliser (up to 100 kg N/ha) were applied 3 weeks later as ammonium nitrate.

All the experiments were conducted in randomised blocks, with 3 replications of 20 m<sup>2</sup>.

We have carried out the following soil analysis: pH value, mineral nitrogen content, mobile phosphorus and potassium content, sulphur content.

## Results

The results are shown in tables 1 and 2.

The highest yields, with no significant differences between treatments, were obtained using 80 kg P<sub>2</sub>O<sub>5</sub>/ha and 100, 150 and 200 kg N/ha. For that reason, the farmers have to use the cheapest treatment, 80 kg P<sub>2</sub>O<sub>5</sub>/ha and only 100 N/ha. These results are in accordance with the fertiliser practices in Romania, where no more than 150 kg of nitrogen/ha are applied at oilseed rape.

The lowest yields were noticed when no phosphorus fertiliser were used, emphasising the fact that oil sees rape has a high requirement for this nutrient.

Table 1. Nitrogen and phosphorus fertilisers influence on seed yield in oilseed rape at Braila (2005-2007)

Treatment	Yield (kg/ha)	Treatment	Yield (kg/ha)
P80 N150	3,828 A	P0 N100	2,921 E
P80 N100	3,760 A	P0 N50	2,819 E
P80 N200	3,612 AB	P0 N200	2,309 F
P40 N150	3,465 BC	P0 N0	2,018 G

Duncan's Multiple Range Test: Mean values with the same letter are not significantly different at the 5% probability level

Despite the fact that sulphur content in soil is low (2.5 ppm), the sulphur fertilisers increased significantly the seed yield only in one treatment, when complex fertiliser was used (table 2). The small decrease in seed yield in the ammonium sulphate treatment is due to the foliar damages caused by the concentration of the fertiliser that was sprayed on the leaves.

The yield obtained in the field production was 2800 kg/ha, because the farmer did not use sulphur fertiliser.

Table 2. Sulphur influence on oilseed rape yield

Treatment	Seed yield (kg/ha)
Control (nitrogen)	2775 B
T1 (elementary sulphur)	3292 AB
T2 (complex fertiliser containing S)	3800 A
T3 (ammonium sulphate)	2500 B

Duncan's Multiple Range Test: Mean values with the same letter are not significantly different at the 5% probability level

## Conclusions

- We have to conduct other experiments regarding the timing of nitrogen application, due to its influence depending on the chemical properties of soils, such as pH, mineral nitrogen content etc. In high pH soils, with low nitrogen content, the largest yields were obtained using more than 200 kg ammonium sulphate per hectare applied before sowing.
- The role of sulphur in oilseed rape fertilisation practices has to be reconsidered by the Romanian farmers. The most appropriate and profitable sources of sulphur fertilisers have to be found.

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# Biogas Production in Northern Germany – Status Quo and Potential Environmental Effects

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Energy crop production is promoted in several European member states by additional subsidies, which may lead to a food-fuel competition for productive land. Energy efficiency (EE) therefore represents a crucial determinant of sustainable bioenergy production systems. In Germany, biodiesel from rapeseed represents a main pathway of biofuel production from arable land. With respect to EE, however, it is outcompeted by biogas. Anaerobic digestion of slurry and crops has evolved as an energy efficient alternative, especially when equipped with power-heat coupling, and in 2007 the number of plants has increased to over 3,700. As a consequence, the acreage of maize, which is the dominating co-substrate for fermentation increased substantially. Recently, some criticism was voiced concerning the sustainability of energy crop production. The objective of the present study therefore was (i) to monitor the status quo of biogas production in the state of Schleswig-Holstein (Northern Germany) in terms of substrate acreage and use of digestion residues and (ii) to evaluate the situation with respect to its environmental effects.

## Methodology

The study is based on a monitoring of 59 biogas plants, assumed representative with respect to spatial distribution, size, and management for the total of 110 plants in Schleswig-Holstein in the year 2006. Biogas farms were characterized by data on technical aspects, acreage of crops for substrate supply, crop management and biogas residue use, obtained from interviews with plant operators.

## Results

Biogas production in Germany is characterized by a substantial spatial concentration with high plant densities in northwestern and southern parts of the country, where we also find a high animal density. Likewise, a heterogeneous distribution in the state of Schleswig-Holstein was detected, with hot spots of biogas production in the northern part on light sandy soils of high groundwater vulnerability. Concerning substrate use, the monitoring revealed that 77% of the biogas plants were co-digesting slurry and crops, which is beneficial with respect to the mitigation of greenhouse gas (GHG) emissions from slurry application. The usage of slurry ( $2\text{--}60 \text{ t kWel.}^{-1} \text{ yr}^{-1}$ ) and co-substrates ( $0.26\text{--}0.83 \text{ ha kWel.}^{-1} \text{ yr}^{-1}$ ; average:  $0.45 \text{ ha kWel.}^{-1} \text{ yr}^{-1}$ ) varied substantially, and showed no close relation to plant size. All biogas plants used maize, while grass (14%), small grain cereals grown for grain (25%) or silage (5%) were of minor importance. The predominance of maize was reflected in the corresponding acreages of 11,829 ha maize, 383 ha small grain cereals, and 315 ha grassland. The expansion of maize led to the displacement of various crops, winter rape seed being most affected, followed by small grain cereals, grassland and sugar beet. Extrapolating from 59 to 134 biogas plants (current number in 2007) would result in about 30,600 ha devoted to substrate production (4.8% of the arable land in Schleswig-Holstein). Together with silage maize and grain maize, the total maize acreage comprised 122,500 ha, i.e. 19% of the arable land. Furthermore, a trend for growing maize in monoculture was observed. Out of 45 conventional agricultural farms with biogas production, 14 grew maize in pure monoculture, on 9 farms the cereal acreage was too low for complete crop rotation systems, and the remaining 22 farms practiced an irregular crop change rather than true crop rotations. The high proportion of maize mono-

culture seems harmful not only to biodiversity, but also to soil organic matter (SOM) conservation. A long-term study by Vertès et al. (2005) from Brittany, France, investigating grass/maize rotations found a close relationship between the grass/maize ratio and the decrease in SOM. The introduction of grass-land into bioenergy crop rotations thus provides an efficient measure for SOM conservation. However, grass as substrate is depreceated by many biogas plant operators because of technical difficulties in the digestion process. Another aspect of substrate production, which influences energy efficiency, but also profitability is the transport distance between field and fermenter (Gerin et al., 2008). Average transport distances of 4.8 km seemed comparably low, and no significant relationship between plant size and transport distance was detected. The amount of biogas residues produced during anaerobic digestion varied substantially among plants, with average values of 20 m<sup>3</sup> biogas slurry (kW<sub>el</sub>, yr)<sup>-1</sup>, 37 m<sup>3</sup> ha<sup>-1</sup> farm area (without purchased substrates), or 56 m<sup>3</sup> ha<sup>-1</sup> of substrate production. In terms of nutrient supply from biogas residues, the monitoring revealed that a substantial proportion of biogas farms, especially those located on light sandy soils and focusing on forage production, are characterized by N- and P-oversupply (Tab. 1), which may result in an increased risk of N-leaching (Herrmann et al., 2005). A similar situation was found for maize nutrient supply, where N- and P-fertilization exceeded the crop demand (average yield: 14 t DM ha<sup>-1</sup>) on a considerable number of farms, while potassium availability was rather low (Tab. 2).

Table 1. Frequency distribution of nitrogen and phosphorus supply (kg ha<sup>-1</sup>) from biogas residues for the interviewed biogas plants (n = 25), where nutrient availability is calculated for the whole farm acreage (without purchase) or including the acreage of purchased substrates (with purchase).

	Nitrogen supply		Phosphorus supply	
	without purchase	with purchase	without purchase	with purchase
< 50 kg ha <sup>-1</sup>	1	3	4	7
50-100 kg ha <sup>-1</sup>	3	6	12	15
100-150 kg ha <sup>-1</sup>	6	9	5	0
150-200 kg ha <sup>-1</sup>	7	4	1	0
> 200 kg ha <sup>-1</sup>	8	3	0	0

Table 2. Nitrogen, phosphorus and potassium fertilization (kg ha<sup>-1</sup>) of maize grown for biogas production, provided as minimum, maximum and average values (n=28).

	Biogas residue			Total		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Min.	90	33	75	115	65	75
Max.	215	160	264	248	210	264
Average	145	61	128	185	103	128

## Conclusions

Biogas production in Schleswig-Holstein (Northern Germany) developed rapidly during the last years, with hot spots located in water protection sensitive areas, maize as the dominating substrate, and a substantial proportion of farms characterized by N- and P-oversupply. This may cause problems with respect to nutrient leaching, humus degradation, and a loss of biodiversity. The impact of biogas production on GHG balance and system sustainability is currently under investigation ([www.biogas-expert.uni-kiel.de](http://www.biogas-expert.uni-kiel.de)).

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# Potential Utilisation of Early Potato (*Solanum Tuberosum* L.) Crop Residues: Biomass for Energy and Waste Tuber for Starch Production

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The Italian early potato cultivation covered a land area of about 20,000 ha, about half of this is concentrated in Sicily (ISTAT, 2008), with an high tuber production for fresh consumption.

In the early potato crops usually seed-tubers are imported from foreign country, and high input (above all fertilization and irrigation) are utilized with consequent high costs of crop management (Mauromicale and Ierna, 1999). So it could be important using field crop residues and waste tubers for other utilisations, that could also help to solve problems for disposal of residual crops.

The possibility of using crop residues and above all waste tubers to produce bio-energy in potato seem to be reliable, thanks to the high starch content of tubers.

Field crop residues (leaves, stems, roots) could be used for biohumus production (Manukovsky et al. 2001), for heat and power generation. Waste tubers could be used for starch production with no-food application, comprising hydrogen (Xie et al. 2007), ethanol and biogas production (Mitch 1984, Weiland 1993, Kaparaju and Rintala, 2005, Parawira et al., 2008).

Until now a study on determining field potato crop residues of early potato cultivation in the Mediterranean environment has been lacking.

The aim of the present study was to evaluate in a early potato crop for marketable tubers, residual biomass production, waste tuber and starch yield.

## Methodology

The research was carried out at the experimental field of the U.O.S. of Catania, ISAFOM - CNR (National Research Council of Italy) on the coastal plain, south of Siracusa (37°03' N, 15° 18' E, 10 m a.s.l.) which is a typical area for early potato cultivation in Sicily during two years (1998 and 1999) period.

Two cultivars: Spunta and "Sieglinde", which are widely cultivated in the Mediterranean region (Ierna and Mauromicale, 2006) were utilized.

Plantings with whole seed tubers were made on 20 January and 30 January in the first and second year respectively. In each year a randomised block design with three replications was used. Plot size was 6.0 by 5.6 m, with plants spaced 0.3 m apart, in rows separated from one another by 0.7 m (equivalent to a plant density of 4.76 plants m<sup>-2</sup>). Typical crop management used in this area for tuber production was adopted.

Marketable tubers were harvested by hand at 116 and 121 days after planting, respectively in the first and second year, when about 70% of above-ground biomass was dry. After harvest of marketable tubers (diameter 40-75 mm) the residual biomass: aboveground part, roots and waste tubers (diameter lower or higher than marketable tubers, deformed, split, damaged or green) were also harvested and weighed.

In laboratory samples of all plant parts were dried at 105 °C and weighed for determining dry matter content. On a representative waste tuber sample starch content was determined (AOAC, 2005). Data were analyzed with ANOVA and means were separated on the basis of an *LSD* test. The percentage data was arcsin √% transformed before analysis. Data without transformation is reported (Snedecor and Cochran, 1989).

## Results

On average of the two years, residual biomass yield was significantly higher in “Spunta” (2.58 t ha<sup>-1</sup> DM) than in “Sieglinde” (1.76 t ha<sup>-1</sup> DM). Concerning biomass partitioning, “Spunta” showed the highest incidence of above-ground part than “Sieglinde” (36 % and 28 %, respectively), and lower incidence of waste tubers (60% and 69%, respectively); roots incidence on total biomass was 3.5% on average of the cultivars (Tab. 1).

Table 1 - Residual biomass yield and contribution (% on total) of different parts, in relation to the cultivar (mean data 1998-1999). Different letters within each column indicate significant differences for  $P < 0.05$ .

Cultivar	Total biomass yield (t ha <sup>-1</sup> DM)	Incidence		
		Aboveground biomass	Waste tubers	Roots
		(% on total)		
Spunta	2.58 a	36 a	60 b	4 a
Sieglinde	1.76 b	28 b	69 a	3 a
Means	2.17	32	64.5	3.5

“Sieglinde” showed an higher tuber dry matter content (19.4%) than “Spunta” (17.9%) and starch amount (13.6 vs 13.1 % f.w.), but starch yield was lower in “Sieglinde” than in “Spunta” (0.85 vs 1.13 t ha<sup>-1</sup> of starch) (Tab. 2).

Table 2 - Tuber dry matter content, starch amount and starch yield in relation to the cultivar (mean data 1998-1999). Different letters within each column indicate significant differences for  $P < 0.05$ .

Cultivar	Dry matter content	Starch amount	Starch yield
	(%)	(% f.w.)	(t ha <sup>-1</sup> )
Spunta	17.9 b	13.1 b	1.13 a
Sieglinde	19.4 a	13.6 a	0.85 b
Means	18.65	13.35	0.99

## Conclusions

These preliminary results showed that early potato crop at harvest of marketable tubers left a residual biomass of about 2.2 t ha<sup>-1</sup> DM; tubers represented 64% of total biomass and yielded about 1 t ha<sup>-1</sup> of starch.

Considering new early potato varieties are spreading in the last years, it should be useful to evaluate the variability in relation to biomass crop residues, waste tubers and starch amount. Research investment is required taking into account also the effects of crop management, such as irrigation water regimes and sowing date.

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# The Recovery of $^{15}\text{N}$ -labelled Fertilizer Applied to Kenaf (*Hibiscus cannabinus* L.)

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Kenaf (*Hibiscus cannabinus* L.) represents an interesting crop for fibre production, which recently has been proved to be a good source of biomass for cellulose pulp, to be used in the paper industry in Mediterranean countries. The remaining part of the plant, rich of cellulose and hemicellulose, may be a source for the production of bio-ethanol of second generation. The climatic conditions of Mediterranean environment allow this tropical crop to produce seeds (Foti et al., 1998). Recently, the response of the crop to N fertilizer and to the water availability has been investigated (Patanè et al., 2007), but in order to determine the correct crop nitrogen fertilization, a clearer understanding of the uptake of the applied N and its storage in the various plant organs is necessary.

On this basis a field experiment was carried out in order to study the destiny of the nitrogen from mineral fertilizer on kenaf under different water levels and nitrogen doses by means of  $^{15}\text{N}$  labelled nitrogen application.

## Methodology

The 'Agronomic Section' of DACPA of the University of Catania (Italy), in the framework of the project "BIOKENAF - Biomass Production Chain and Growth Simulation Model for kenaf" supported by EU, carried out a field experiment in 2005 in a location of the eastern coast of Sicily, South Italy (10 m a.s.l., 37°25' N Lat, 15°30' E Long) using cv. 'Tainung 2'. The following two experimental factors were studied: water supply ( $I_{25}$ ,  $I_{50}$ ,  $I_{100}$  = 25, 50, 100% of evapotranspiration-ETc, respectively) and nitrogen level:  $N_{75}$  and  $N_{150}$  = 75 and 150 kg ha<sup>-1</sup> of N, respectively). The sowing was carried out on May the 26<sup>th</sup>, adopting a 200,000 plant ha<sup>-1</sup> plant population. Isotopically labelled nitrogen (N) was distributed in top dressing fertilisation (2/3 of total nitrogen fertilizer) as ammonium sulphate ( $^{15}\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> (2% atomic excess) dissolved in 3 l of distilled water in a subplot of 4 m<sup>2</sup> (2m x 2m). After application, each plot was watered with 2 l of distilled water to wash labelled fertilizer from the leaves on the soil. At harvest, occurred on October the 26<sup>th</sup>, leaves, stems, flowers and root were analysed for N labelled at the ISO ANALYTICAL Laboratory (Cheshire, UK). Nitrogen derived from fertilizer index (Ndff) was determined according to Recous et al. (1988):  $\text{Ndff (\%)} = 100 (c-b)/(a-b)$ , where: a = atom%  $^{15}\text{N}$  abundance of fertilizer; b = atom%  $^{15}\text{N}$  abundance of control plant that did not receive labelled fertilizer; c = atom%  $^{15}\text{N}$  abundance of labelled plant sample. Nitrogen recovered from labelled fertiliser (% N rec.) was calculated according to Hauck e Bremner (1976):  $\% \text{ N rec.} = 100 P(c-b)/f(a-b)$ , where P = meq of N in the sample; f = meq of N in the fertiliser.

## Results

On the average of the studied factors, nitrogen derived from fertilizer in the plant (Ndff %) and the nitrogen recovered in the plant from labelled fertiliser (% N rec.) resulted equal to 21.43% and 55.29%, respectively (Tab. 1).

The percentage of nitrogen derived from fertilizer (Ndff %), in the average of the studied treatments, found in the roots (30.38 %) resulted highest compared to stems, leaves and flowers and capsules (23.38%, 20.21% and 20.32%, respectively).

The percentage of nitrogen derived from fertilizer in the plant (Ndff %) increased with the nitrogen fertilisation levels (15.08% against 27.78%, respectively for N<sub>75</sub> and N<sub>150</sub>). With low availability of water in the soil, Ndff% is equal to 21.27% (25% of Etc restoration) and 18.51% (50% of Etc restoration), whereas with good water condition the Ndff% attained at 30.97%. Irrigation levels (from 25% to 100% of Etc restoration) increased the nitrogen recovered from labelled fertiliser (% N rec.) from 38.74% to 72.08% (tab.1), whilst the level of N application determined slight differences of N recovery (59.61 and 50.98%, respectively for N<sub>1</sub> and N<sub>2</sub>).

Tab. 1 – Percentage of N derived from the fertilizer (%Ndff) out to the total plant nitrogen and percentage of N in the plant recovered from the fertilizer (% N recovered) out to the doses of applied fertiliser.

Treatment	Stems		Leaves		Flowers and capsules		Roots		Plant	
	Ndff %	N% rec.	Ndff %	N% rec.	Ndff %	N% rec.	Ndff %	N% rec.	Ndff %	N% rec.
I <sub>25</sub> N <sub>75</sub>	14.36	9.42	18.63	27.18	18.78	3.03	27.65	2.39	17.65	42.02
(St. error)	(2.1)	(3.8)	(4.7)	(6.6)	(2.3)	(0.7)	(0.78)	(0.1)	(2.5)	(9.9)
I <sub>25</sub> N <sub>150</sub>	22.41	10.72	26.90	20.92	26.59	2.07	31.55	1.71	24.88	35.47
(St. error)	(3.3)	(2.3)	(2.0)	(0.6)	(3.15)	(1.0)	(1.7)	(0.1)	(2.54)	(2.6)
<b>Average I<sub>1</sub></b>	<b>18.39</b>	<b>10.09</b>	<b>22.77</b>	<b>24.05</b>	<b>22.68</b>	<b>2.55</b>	<b>29.60</b>	<b>2.05</b>	<b>21.27</b>	<b>38.74</b>
I <sub>50</sub> N <sub>75</sub>	11.91	20.08	12.66	27.17	13.02	3.17	25.05	3.93	13.51	54.35
(St. error)	(2.8)	(1.7)	(5.2)	(10.7)	(3.5)	(0.1)	(1.8)	(0.9)	(3.3)	(11.5)
I <sub>50</sub> N <sub>150</sub>	21.67	17.50	24.27	33.00	24.69	2.95	32.5	2.33	23.51	55.77
(St. error)	(5.8)	(3.3)	(9.7)	(14.1)	(8.3)	(0.9)	(1.9)	(0.12)	(6.4)	(18.1)
<b>Average I<sub>2</sub></b>	<b>16.79</b>	<b>18.79</b>	<b>18.46</b>	<b>30.08</b>	<b>18.85</b>	<b>3.06</b>	<b>28.76</b>	<b>3.13</b>	<b>18.51</b>	<b>55.06</b>
I <sub>100</sub> N <sub>75</sub>	30.48	45.48	12.29	29.29	13.78	4.2	28.6	3.5	25.29	82.47
(St. error)	(7.04)	(12.25)	(3.1)	(4.6)	(2.9)	(1.4)	(0.4)	(1.1)	(5.2)	(18.0)
I <sub>100</sub> N <sub>150</sub>	39.48	30.16	26.51	24.57	25.09	3.10	36.90	3.86	36.65	61.69
(St. error)	(3.5)	(1.2)	(0.1)	(4.5)	(2.3)	(0.8)	(1.5)	(0.93)	(1.9)	(3.1)
<b>Average I<sub>3</sub></b>	<b>34.98</b>	<b>37.82</b>	<b>19.40</b>	<b>26.93</b>	<b>19.43</b>	<b>3.10</b>	<b>32.73</b>	<b>3.68</b>	<b>30.97</b>	<b>72.08</b>
<b>Average N<sub>1</sub></b>	<b>18.92</b>	<b>24.99</b>	<b>14.53</b>	<b>27.88</b>	<b>15.19</b>	<b>3.47</b>	<b>27.11</b>	<b>3.27</b>	<b>15.08</b>	<b>59.61</b>
<b>Average N<sub>2</sub></b>	<b>27.85</b>	<b>19.47</b>	<b>25.89</b>	<b>26.16</b>	<b>25.45</b>	<b>2.71</b>	<b>33.64</b>	<b>2.63</b>	<b>27.78</b>	<b>50.98</b>
<b>Average</b>	<b>23.38</b>	<b>22.23</b>	<b>20.21</b>	<b>27.02</b>	<b>20.32</b>	<b>3.09</b>	<b>30.38</b>	<b>2.95</b>	<b>21.43</b>	<b>55.29</b>

## Conclusions

The percentage of nitrogen derived from fertilizer (Ndff %) is almost 24%, while the other nitrogen derives from organic matter in the soil, nitrogen in irrigation water and dry deposition. The plant uptakes almost 55% of the fertilizer applied. The remaining part of fertilizer was probably immobilized by microorganisms and, partially, was lost by volatilization or leaching.

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# Effect of the Cycle Length on the Energetic Use of Barley (*Hordeum vulgare* L.)

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In Spain, the growing and excessive dependence of external energy, around 80% in the last years, and the need to preserve the environment and to ensure a sustainable development, make us promote an efficient use of energy and the employment of green resources. Therefore, the expansion of renewable resources is the result of different economic, social and environmental reasons, also being fundamental to reach the international commitments in environmental subjects. Many traditional crops, including cereals and oilseeds, could be used as biomass inputs. The agrarian economy of Castilla-La Mancha (Central Spain) is based on the production of olive, vineyard and cereal crops, mainly wheat and barley. The use of barley as an energetic resource would allow to grow in marginal soils, establishing population and favouring the economic development in rural areas. In addition to that, this crop is characterized by a punctual and moderate water consumption, not coinciding with the summer period, which allows the crop to develop under rainfed conditions. In opinion of Lafarga and Goñi (2007), a demand of 70,000 ha of barley-wheat for energetic uses is predicted by 2010 in Spain. Numerous previous works realized by this and other research groups have studied the differences in grain yield among different barley varieties of long and short cycle; however, the total biomass of the crops is not usually taken into account due to the fact that the straw is normally considered as an agrarian by-product with no economic value. The aim of this work was to analyse the biomass production of 16 short-cycle and 16 long-cycle genotypes of barley.

## Methodology

A field experiment was conducted in Ciudad Real, Central Spain (3°16' W, 39°10' N, altitude 640 m). A design of subdivided plots with four replications was adopted. The main treatment was the length of the cycle, with two levels (short and long-cycle), and the second treatment was the chosen variety, resulting in a total of 128 plots of 1.2x15 m (18 m<sup>2</sup>). Field operations were the same and developed at the same time for all the trial. Sowing was performed on 1 December by using a special experimental sower. The crop was grown under rainfed conditions. Fertilization consisted of 300 kg ha<sup>-1</sup> of 8-24-8 (N-P-K) at pre-sowing and 100 kg ha<sup>-1</sup> of N as a top-dressing. Before harvesting with an experimental harvester, two samples of 0.20 m<sup>2</sup> were randomly cut to ground level in each basic plot. The length of the ears was measured without considering the beards. The analysis of variance and correlations among the parameters measured were determined. A path coefficient analysis, as described by García del Moral et al. (1991), was performed with the purpose of providing a suitable representation of the relative magnitude of each total biomass component, by separating the direct effects from the indirect ones exerted through the compensations induced on the others. The program SPSS 13.0 for Windows and InfoStat 2006d.2 were used for the statistical study.

## Results

The long-cycle genotypes reached the highest stem ( $P<0.01$ ) and ear ( $P<0.05$ ) length, and the highest amounts of straw ( $P<0.01$ ) and total biomass ( $P<0.01$ ). No differences in grain and ear biomass were found between both types of barley (Table 1). The correlation study (Table 2) shows a significant ( $P<0.01$ ) positive relationship between yield and biomass, and a higher effect of the stem production on the total biomass development. The path coefficient analysis (Table 3) supports these results, although

the direct effect of both components is lower due to the positive correlation between them. Hence, the indirect effect that each component exerts on total biomass through the other is positive.

Table 1. Summary of the analysis of variance (n=128; total degrees of freedom, 127).

Variable	Source of variation	Degrees of freedom	Mean square	F	p-value
Stem length	Model	31	76223.42	33.12	<0.0001
	Cycle	1	1984629.65	862.34	<0.0001
	Variety	30	12609.88	5.48	<0.0001
	Error	96	2301.45		
Ear length	Model	31	128.73	3.80	<0.0001
	Cycle	1	149.00	4.40	0.0386
	Variety	30	128.05	3.78	<0.0001
	Error	96	33.86		
Grain yield	Model	31	2544042.18	1.40	0.1086
	Cycle	1	902496.13	0.50	0.4823
	Variety	30	2598760.38	1.43	0.0972
	Error	96	1814078.00		
Stem biomass	Model	31	7784542.89	3.68	<0.0001
	Cycle	1	187845498.78	88.75	<0.0001
	Variety	30	1782511.03	0.84	0.6975
	Error	96	2116485.97		
Ear biomass	Model	31	3303876.02	1.50	0.0684
	Cycle	1	656371.53	0.30	0.5859
	Variety	30	3392126.17	1.54	0.0585
	Error	96	2196238.45		
Total biomass	Model	31	13843265.46	1.77	0.0182
	Cycle	1	210709656.13	27.01	<0.0001
	Variety	30	7281052.44	0.93	0.5710
	Error	96	7800798.42		

Table 2. Correlation matrix among biomass parameters (n=128).

	1	2	3	4	5	6
1. Stem length		0.202*	0.041	0.710***	0.020	0.447**
2. Ear length			0.081	0.117	0.068	0.107
3. Grain yield				0.561**	0.972***	0.846***
4. Stem biomass					0.563**	0.905***
5. Ear biomass						0.862***
6. Total biomass						

. \*, \*\*, \*\*\*: Significant at 0.05, 0.01 and 0.001 probability level, respectively

Table 3. Path coefficient analysis. Dependent variable: Total biomass (n=128).

Effect	Via	Coefficients	p-value
Stem biomass	Direct	0.62	
Stem biomass	Ear biomass	0.29	
Total R		0.91	<0.0001
Ear biomass	Direct	0.52	
Ear biomass	Stem biomass	0.34	
Total R		0.86	<0.0001

## Conclusions

The results indicate that short-cycle barleys sown in winter produce grain yields similar to those obtained in long-cycle barleys. For this reason, if grain yield is the goal of the crop, the choice of the genotype could be done according to another criteria. However, if the purpose is to get biomass production, thus a long-cycle genotype would be chosen.

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# Biomass Production Potential of Long-Term Switchgrass Stands, an Environmental Friendly Bio-Energy Crop for Future

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## Abstract

The present work reports the results of the experiment on long term evaluation of 18 genotypes of Switchgrass at field level under the climatic conditions of South Italy. The main purpose of this work was to study the cultivation techniques and evaluate other than the biomass productivity also the adaptability of Switchgrass varieties (upland and lowland ones) for a long period of time under Mediterranean climate.

## Introduction

Biomass, alongwith other renewable energies, allows reducing dependence on external energy, as well as reducing the greenhouse effect of gas emissions. It has some additional advantages too, as it safeguards the maintenance and diversification of agricultural sector, and contributes the development of employment especially on the marginal lands and rural areas. Amongst the different paths for biofuel industry development, use of dedicated energy feedstock, i.e. biomass grown deliberately for biofuel applications, could represent a promising solution for the security of supply issues for future biofuel production systems. With a view to it, research has been conducted on herbaceous biomass crops at ENEA Research Centre, Trisaia. The present report is prepared on the basis of a long term evaluation of switchgrass, a perennial crop, to identify the varieties that require low inputs and give relatively high yields even under less favourable conditions in the Mediterranean Italy.

## Methodology

Experimental fields of Switchgrass (*Panicum virgatum* L.) were established in Trisaia in view of the work started in 1998 in the framework of European network. The trials were established in June in a total area of about 1hectare [1]. The biomass yield was evaluated for 18 genotypes (Alamo, Blackwell, Forestburg, Caddo, Cathage, CIR, Kanlow, NU 94-2, Pangburn, SL 93-2, SL 93-3, SL 94-1, SU 94-1, Summer, Sunburst, Trailblazer, 9005439 and 9005438). During the establishment year, various measurements were taken at several intervals. After a killing frost when the latest maturing variety had completely senesced, the final harvest was made.

## Results and discussion

Evaluation of 18 varieties (upland and lowland type) in terms of growth and productivity has been carried out. Biomass yield was determined by one single winter harvest every year when the moisture content was less than 20%. Production potential recorded for all the tested varieties was quite high except for the genotypes 9005439 and 9005438. Pick yields for all varieties increased to an average of 12.1 t/ha in the third growing season. The lowland varieties resulted in higher dry yields compared to the upland group. This superiority was quite larger and it led to 29% higher dry matter yields over the upland varieties (2000). It's noteworthy that significantly higher yields were recorded especially for the variety SL 93-3 that performed 26.1 t/ha in the third year and resulted the best yielding variety in Italy. In the whole period (1998 to 2007), the same variety gave the highest (SL 93-3, lowland variety) and the lowest yields (9005439, upland variety).

As regards the ageing, nitrogen and irrigation effect on the growth and dry yields of switchgrass, it has been observed that the yields were not found to be much affected in most of the years by different applied nitrogen rates as has already been reported earlier [2,3]. It is important to mention here that from the year 2001 onwards, no irrigation was done and only one medium dose of Nitrogen fertilizer (75 kg/ha) was applied. Excellent stand with good establishment has been observed in almost all the genotypes.

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**Table 1.** Dry matter yields (t/ha) for the tested varieties in Basilicata (Italy), 1998-2007.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Caddo	2.3	7.6	10.1	8.1	7.3	3.3	7.7	4.9	4.1	2.4
Cathage	2.5	7.6	9.4	9.7	6.8	2.8	5.3	3.1	3.1	1.4
CIR	2.5	7.1	11.4	7.6	4.9	1.8	4.7	2.4	1.6	1.8
Kanlow	1.4	4.7	15.3	12.1	7.9	3.7	5.8	4.0	5.9	4.2
NU 94-2	2.9	8.3	10.8	11.9	7.7	4.1	7.3	3.5	2.9	1.9
Pangburn	2.4	8.5	11.9	12.9	9.0	4.7	7.0	6.2	5.1	2.4
SL 93-2	2.2	8.5	20.2	13.4	9.9	5.2	9.7	8.5	8.0	7.0
SL 93-3	2.1	14.6	26.1	21.8	16.9	9.6	11.5	10.4	9.0	5.8
SL 94-1	2.4	9.9	14.9	17.0	9.5	5.2	9.4	7.1	7.8	6.4
SU 94-1	3.0	10.4	15.8	13.0	10.9	5.0	8.9	7.4	4.7	2.8
Summer	1.3	6.3	7.8	7.8	7.8	3.5	7.1	5.9	2.6	1.4
Sunburst	0.9	3.5	8.2	5.5	5.5	2.0	5.8	2.8	2.0	0.4
Trailblazer	2.8	5.8	10.7	6.1	5.6	3.3	7.6	4.2	3.1	1.6
9005439	-	1.7	5.6	3.3	3.8	1.3	5.2	2.8	2.2	0.7
9005438	-	1.8	6.9	4.1	6.7	1.2	5.0	3.2	2.1	1.6
Mean	2.2	7.1	12.3	10.3	8.0	3.8	7.2	5.1	4.3	2.8

Switchgrass, with its perennial life form, has been evaluated as an alternative energy crop in Europe. It has been reported that the cost price differs in each country according to yield and input parameters (www.switchgrass.nl). Economic analyses of switchgrass under current studies is based on the ten years data of 18 different switchgrass varieties cultivated for biomass production. The cost price based on inputs and yields, was calculated taking into account the expected local farmer conditions. Total costs are separated in direct costs (consumable goods) and labour and machine costs. The cost for different switchgrass varieties varies according to their yields. Among all the varieties, only Alamo and SL 93-3 proved to be the high yielding varieties in Italy. By comparing switchgrass with other perennial biomass crops, for example miscanthus, switchgrass seems to perform a bit better concerning the environment, because of its better performance with low irrigation, low input for establishment and lower fertilisation. Nevertheless, in order to establish the best cultivation methods and the best performing varieties further studies on large scale production with only high yielding varieties are required to establish switchgrass as an economic biomass crop. Environmental evaluation of switchgrass indicates that switchgrass can be considered as an environmental friendly crop that can contribute to agricultural diversification as well as to sustainable development in the agricultural, energy and industrial sectors. It can improve the soil fertility, the biodiversity (serve as habitat for many animals) and can fulfil different land use functions like erosion control and improvement of the landscape quality. The biomass can be used as CO<sub>2</sub> neutral renewable resource that once established offers a labour extensive long term production alternative. In addition, industrial approach, coupled with substantial government subsidies, can make the production cheap, reducing degradation of soil and water and our dependence on imported biomass, increasing thereby utilisation of marginal lands as well as protecting and conserving the soils .

### Conclusions

- Present work reports the evaluation of the adaptability, growth, production potential, cost price and environmental aspects of Switchgrass cultivation in Southern Italy.
- The crop under trial is with deep rooting system, and is relatively high yielding one even under more marginal conditions, without high input of fertilizers or pesticides that might contribute to achieve environment goals.
- Economic analyses of switchgrass cultivation reveals that high initial cost of the perennial crop plantation is depreciable during the subsequent period of crop life time as compared to the annual crops. Moreover, the environmental effects and social costs associated with energy resources should be sustained and valued.

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# Weed-poplar Competition in the First Season of Crop Growth

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In recent years there has been rapidly increasing interest in short rotation poplar in northern Italy because of the many economic advantages. A disadvantage is that weed management strategies are still in progress and there are no commonly-accepted guidelines for management of weeds during the first season of poplar growth. There is therefore a need to know the effect of weed competition on crop yield for appropriate definition and timing of weed control tactics, based on the understanding of the competitive relationship between crop and weeds. An experiment was conducted in Padova (north-east Italy) in 2007 with a series of treatments increasing either duration of interference or length of weed-free period in order to find the critical point (CP), and critical period of weed control (CPWC).

## Methodology

The study was done at the Padova University Experimental Farm in the Po Valley, north-east Italy. The soil is silty-loam textured (11.8% clay, 44.9% silt, 43.3% sand), rich in limestone, with sub-basic pH (pH = 8.11) and relatively low organic carbon content (0.92%). In the 2007 growing season there was 510 mm of rainfall. Spring and summer were very hot and two irrigations of 35 mm each were necessary in May and in July. Cattle slurry was applied before planting, for a total of 120-45-45 kg/ha of N-P-K. Cuttings of *Populus nigra* L. clone AF2 were planted 3.0 x 0.6 m (5,555 plants/ha) on 14th April. The experimental design was completely random with ten treatments and three replicates, each plot was 12 m long with 20 plants. Two types of weed interference treatments were implemented. One set, increasing the duration of weed interference in order to evaluate the duration of tolerated competition (DTC), was established by delaying weed control from the time of crop planting until predetermined crop growth stages. At this time weeds were removed and the plots were kept weed-free by weekly manual hoeing throughout the rest of the season. The other set of treatments, increasing the length of the weed-free period (WFP), was conducted by maintaining weed control from the time of planting until a specific crop growth stage, then subsequently allowing emerging weeds to remain for the rest of the season. In December, at the end of the growing season, the entire plot was harvested by cutting trees 5-10 cm from the soil surface, and the dry wood yield per plot was determined. A logistic model (Onofri 2001) was fitted to yield data (mean of three replicates) representing the duration of tolerated competition (DTC) as follows:

$$Y = b + \frac{(a - b)}{(1 + \exp(c * (\ln(T_R + 0.0000001) - \ln(d))))} \quad [1]$$

To describe the effect of the increasing length of the weed-free period (WFP) on the relative yield and to determine the end of the CPWC, the following Gompertz model was used:

$$Y = b + ((a - b) * \exp(-\exp(-c * (\ln(T_R + 0.0000001) - \ln(d)))))) \quad [2]$$

In both equations, Y is the yield (% season-long weed-free period), a is the upper asymptote, b is the lower asymptote, T<sub>R</sub> is the duration of weed interference or the duration of the weed-free period measured from sowing and expressed as Growing Degree Days, c is the slope, and d is the point of

inflexion. Data were fitted using the Non Linear Regression module of Statistica 7.0 (Statsoft Inc., 2005). The intersection point between DTC and WFP curves defines the CP.

## Results

Weed flora density was high (600-1200 plants/m<sup>2</sup>), with competitive species, such as *Echinochloa crus-galli*, *Sorghum halepense*, *Chenopodium album*. The length of the competition affected the crop moisture content at harvest ( $F(22, 50)=2.93$ ;  $p<0.01$ ), being lower where the competition was shorter, even if this result is of scarce practical importance. Mean yield of the weed-free plot was 4.0 t/ha of dry matter, while the season-long infested plot was practically zero. Intermediate treatments provided intermediate yield, also indicating that the plot size and treatment timings were chosen properly. For the first month after planting the cuttings weed interference is negligible, but poplar growth is slow and after 30-40 days the effect of competition increases very quickly, and yield is reduced to zero after 80 days of continuous competition. Regression analysis shows the good fitting of both models ( $R^2=0.99$ ). The CP occurs 45 days after planting, corresponding to a yield loss of 29%. For a CPWC of 5% weeds must be controlled from 30 to 66 days after planting (Figure 1).

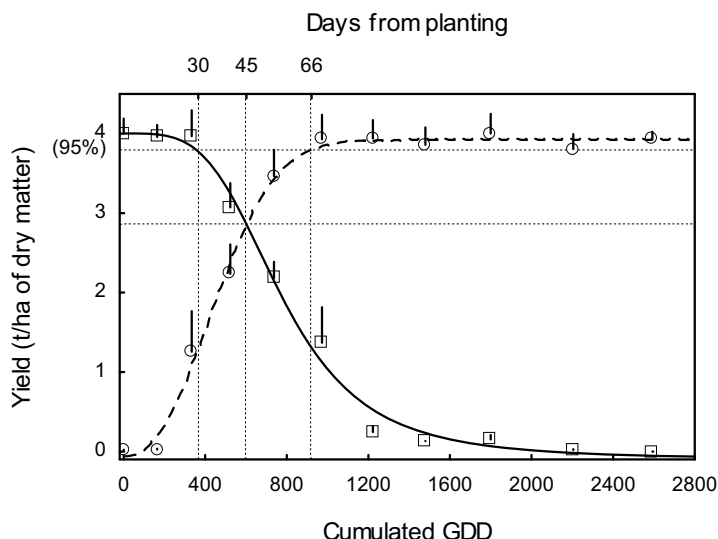


Figure 1. Effect of weed interference on dry yield. Experimental data and fitted curves of: increasing duration of weed interference (DTC,  $\square$ ), curves (solid) calculated with the logistic model (Eqn. 1); increasing weed-free period (WFP,  $\circ$ ), curves (dotted) calculated with the Gompertz model (Eqn. 2). Each yield value represents the mean yield of three replicates, and standard errors are indicated (vertical bars). Horizontal dotted lines indicate the 5% acceptable yield loss level used to determine the critical period of weed control (CPWC), and the yield loss when DTC=WFP; vertical dotted lines indicate the beginning and end of CPWC, and the CP.

## Conclusions

The results show that when weed density is high the continuous competition can annul yield. The 95% CPWC is quite a long interval, also given that accepted pre-emergence herbicides are effective for about 30 days. Weed control should thus also include post-emergence treatments, but fully selective herbicides for dicotyledonous weed species are not yet available. Effective management should therefore rely on a combination of chemical and mechanical treatments within an IWMS framework.

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# Valorization of Biomass Crop Residues of Globe Artichoke [*Cynara cardunculus* L. subsp. *scolymus* (L.) Hegi] to Yield Bio-Energy in Italy

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Producing bio-energy is one of the focus of the agriculture and using the residual biomass of the different crops, minimising also the problems for disposal.

Globe artichoke is a Mediterranean crop actually cultivated only for capitula production for human diet. The Italian land area involved in this cultivation is about 52,000 ha, among which about 90% is concentrated in only three regions, Apulia, Sicily and Sardinia (ISTAT, 2008).

In previous studies it was shown globe artichoke crop residues (leaves and stalks) could be used to obtain lignocellulosic biomass for pellets production, while roots for inulin production (Raccuia et al., 2004) to obtain fructose to yield HMF (5-HydroxyMethylFurfural) (Yuriy Romàn-Leshkov et al., 2007). The aim was to quantify the potential globe artichoke residual biomass production in order to obtain bio-energy and estimate the potential yields in Italy.

## Methodology

Considering the varieties adopted in Italy belongs mainly to “Violetto di Sicilia” and “Romanesco”, while others varieties, including “Spinoso di Palermo”, “Violetto di Toscana” and “Violet of Provence”, are less spread, the evaluation of biomass crop residues was conducted in two years field trial, taking into account three groups of varieties (“Violetto di Sicilia”, “Romanesco” and “Others”).

As regards the cultural practices (irrigation, fertilisation, weed and pest control) the globe artichoke usual crop management for head production was used.

Both years heads (capitula) were harvested at the marketing stage regardless of size. Harvests were made once a week from November to February and every 3 - 4 days from the beginning of March, (data non reported). After heads harvest, field fresh residual biomass (leaves, stalks the first year and leaves, stalks and roots the second year) were weighted. In laboratory on leaves and stalks samples the dry matter (DM) content, crude proteins, ashes and NFE (Nitrogen Free Extract) were evaluated (AOAC, 2005). The values obtained from chemical caracherization of aboveground biomass were used to calculate the energy obtainable for biomass combustion, using the following conversion coefficients: 17 MJ Kg<sup>-1</sup> DM for NFE, 23 MJ Kg<sup>-1</sup> DM for crude proteins (Bittante et al., 1990). Root carbohydrates were characterised by HPAEC-PAD ‘Dionex 500’ (Baert, 1997). All analyses were performed in duplicate for each agronomic replicate and are reported on a dry matter (DM) basis.

Data was submitted to the Barlett’s test for the homogeneity of variance and then analysed using the analysis of variance (ANOVA). Means were statistically separated on the basis of Student - Newman - Kewls test, when the “F” test of ANOVA for treatment was significant at least at 0.05 probability level. Significance was accepted at  $P < 0.05$  level (Snedecor and Cochran, 1989).

## Results

On average of genotypes and years of cultivation dry aboveground biomass yield resulted 11.7 t ha<sup>-1</sup>. “Romanesco” on average of the year of cultivation, attained the highest yield (15.7 t ha<sup>-1</sup>) (Tab. 1).

On the basis of chemical characterization of biomass, the caloric value resulted 16,000 kJ kg<sup>-1</sup> of biomass. Considering the yields attained the bio-energy expressed as MJ ha<sup>-1</sup> resulted 187.

Tab.1 – Residual dry aboveground biomass yield and its partitioning in the types studies during the two year of trial

	Violetto di Sicilia			Romanesco			Others			General Means
	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	Mean	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	Mean	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	Mean	
Stalks	3.6	3.6	3.6	7.5	9.0	8.3	6.3	5.1	5.7	<b>5.9</b>
Leaves	6.0	2.8	4.4	7.5	7.3	7.4	7.0	4.3	5.7	<b>5.8</b>
Aboveground biomass	9.6	6.4	8.0	15.0	16.31	15.7	13.3	9.40	11.3	<b>11.7</b>
Mean squares of treatment										
	Stalks			Leaves			Aboveground Biomass			
	Absolute Value	% of total		Absolute Value	% of total		Absolute Value	% of total		
Type (T)	21.8***	92		9.0***	38		58.9***	76		
Year (Y)	ns	0		12.5***	52		11.2**	14		
T X Y	1.9*	8		2.5**	10		8.0*	10		

\* Significant at 0.05 probability level.; \*\* Significant at 0.01 probability level; \*\*\* Significant at 0.001 probability level; ns not significant

Roots biomass, collected on the second year of cultivation, resulted on average of types 5.0 t ha<sup>-1</sup> DM, with a production of 1.7 t ha<sup>-1</sup> of inulin. “Romanesco showed the highest inulin yield (2.7 t ha<sup>-1</sup>) (Fig 2).



Fig. 1 – Bioenergy yield (MJ ha<sup>-1</sup>) in the types studied. Different letters within the same trait indicate differences at  $P \leq 0.05$ .

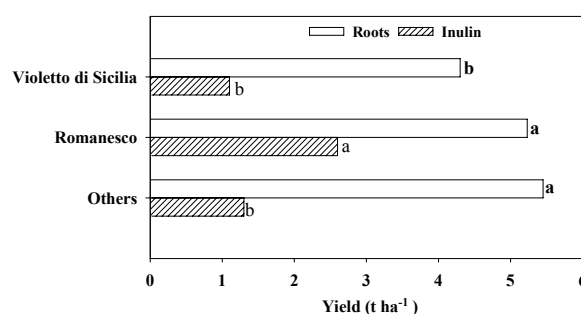


Fig. 2 – Roots biomass (t ha<sup>-1</sup> DM) and inulin (t ha<sup>-1</sup>) yield in the types studied. Different letters within the same trait indicate differences at  $P \leq 0.05$ .

## Conclusions

The obtained results showed, on average of the studied factors, that crop for head production left 11.7 t ha<sup>-1</sup> of DM of residual aboveground biomass and 5.0 t ha<sup>-1</sup> DM of roots. Inulin production was 1.7 t ha<sup>-1</sup>, it could be used to yield fructose that can be transformed in HMF. Considering the areas involved in globe artichoke cultivation and the varieties adopted, Italy could produce about 8,000 GJ year<sup>-1</sup> of bio-energy. Because of the land involved in this cultivation is concentrated mainly in restricted areas of Apulia, Sicily and Sardinia, using globe artichoke biomass crop residues to yield bioenergy could have the advantage to reduce also the costs of trasport. Moreover these productions could contribute to the eco-sustainable development of the local agriculture.

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# Biomass Yield in Different Genotypes of Cardoon Cultivated in Marginal Areas of the Mediterranean Environment

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Cardoon (*Cynara cardunculus* L.) is a perennial plant native of the Mediterranean regions, comprising two botanical varieties, *altilis* DC. (domestic cardoon) and *sylvestris* Lam. (wild cardoon). Recent studies were conducted on cardoon for biomass production, in order to assess the possibility as an alternative low input management crop in Mediterranean environment (Fernandez et al. 2006; Raccuia and Melilli, 2007). In addition, studies were conducted on intraspecific variability for seed germination under salt and moisture stresses of wild cardoon Sicilian populations (Raccuia et al. 2004).

The aim of this work was the evaluation of biomass productions in different genotypes of cardoon in order to valorize marginal areas of the Mediterranean environment, characterized by unfavorable environmental conditions such as low rainfall during the grain filling period and high air temperature.

## Methodology

This trial was carried out at Modica in South Sicily, during 2006-07 growing season. Eighteen cardoon genotypes (2 *C. cardunculus* L. var. *altilis*, and 16 *C. cardunculus* L. var. *sylvestris*) were grown. The adopted experimental design was randomized block with three replications. The crop is two years old, it was grown under low inputs management, without any irrigation and fertilization. The biomass was harvested on July. At harvest on a large number of plants, for each agronomic replication were determined the number and weight of heads, the fresh biomass weight and its partitioning. Achenes were obtained trashing the capitulum. In laboratory dry matter content of each part of the plant were calculated in thermoventilated oven at 105°C. The grain oil content was determined (AOAC, 2005). All analyses were performed in duplicate for each agronomic replicate and are reported on a dry matter (DM) basis.

Data was submitted to the Barlett's test for the homogeneity of variance and then analyzed using the analysis of variance (ANOVA). Means were statistically separated on the basis of Student - Newmann - Kewls test, when the "F" test of ANOVA for treatment was significant at least at 0.05 probability level. Significance was accepted at  $P < 0.05$  level. Coefficient of Variation (CV%) was calculated following the statistical standard procedures (Snedecor and Cochran, 1989).

## Results

The obtained results showed, on average of the genotypes, an aboveground biomass yield of 8.94 t ha<sup>-1</sup> DM, and grain yield of 0.83 ha<sup>-1</sup> DM. The highest dry biomass and grain yields were 21.9 and 2.2 t ha<sup>-1</sup>, recorded in wild cardoon "S16". An high range of variability was observed, with CV of 64.2 (biomass yield) and 77.6 % (grain yield) (Tab. 1).

Grain oil content is reported in figure 1. On average of genotypes it resulted 234 g kg<sup>-1</sup> DM, with values ranging from 197 ("S10") to 259 g kg<sup>-1</sup> DM ("S16").

Averaged for genotype, grain oil yield resulted 0,20 t ha<sup>-1</sup>, with a good range of variability from 0,03 ("S 18") to 0.56 t ha<sup>-1</sup> ("C5") (Tab. 1).

Tab. 1 Aboveground biomass, grain yield (t ha<sup>-1</sup> DM) and grain oil yield (t ha<sup>-1</sup>) in *C. cardunculus* genotypes

Genotypes	Biomass yield	Grain yield	Grain oil yield
	(t ha <sup>-1</sup> DM)		(t ha <sup>-1</sup> )
C4	12.52	1.25	0.32
C5	16.67	2.15	0.56
S 1	15.03	1.31	0.31
S 2	14.12	1.20	0.30
S 3	16.82	1.81	0.46
S 4	7.58	0.61	0.14
S 5	6.12	0.71	0.17
S 6	7.38	0.39	0.09
S 7	5.69	0.39	0.08
S 10	5.09	0.53	0.10
S 11	4.82	0.36	0.08
S 14	10.85	1.17	0.27
S 16	20.79	1.89	0.49
S 17	4.63	0.25	0.06
S 18	1.82	0.13	0.03
S 21	2.98	0.18	0.04
S 23	5.29	0.37	0.09
S 24	2.65	0.25	0.05
Mean	8.94	0.83	0.21
CV (%)	64.2	77.6	11.3
LSD $P \leq 0.05$	1.35	0.13	0.047

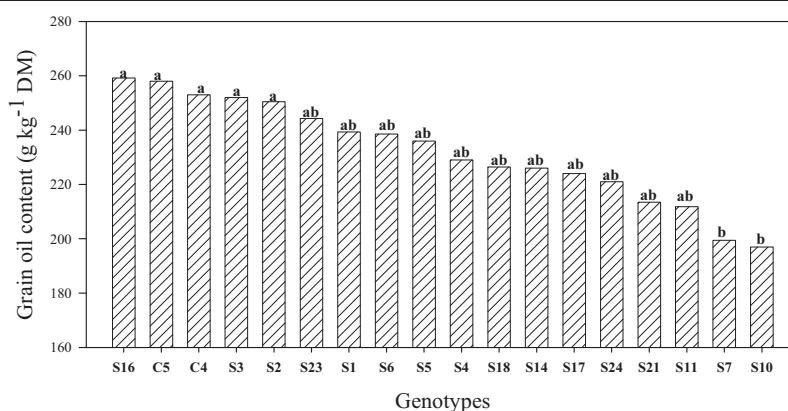


Fig. 1 – Grain oil content (g kg<sup>-1</sup> DM) in *C. cardunculus* genotypes. Different letters indicate differences at  $P \leq 0.05$ .

## Conclusions

The obtained results make *C. cardunculus* a promising energy and oil crop for cultivation in marginal areas of southern Italy, where it is more difficult to achieve the productivity potentials of other biomass crop and could offer the possibility of exploiting the Mediterranean marginal areas for energetic purposes.

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# Effect of Plant Density on Crop Growth and Biomass Productivity of *Miscanthus x Giganteus* Greef et Deu

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*Miscanthus* is a genus of C<sub>4</sub> perennial, identified as potential biomass crops for renewable energy in the northern part of Europe. Some investigation carried out in Mediterranean environment where showed a high adaptation capacity, however water stress can seriously compromise the biomass production during growing season (Foti et al. 2003; Cosentino et al. 2007).

## 2. Materials and methods

Field trials were conducted in three years 2005/06, 2006/07 and 2007/08, at Modica, locality of South East of Sicily, Italy, (280 m a.s.l., 36° 49' N). In a randomized complete block design with three replicates, under water supply, three plant density (4, 6 and 8 plants per square meter) were studied. Plantlets of *Miscanthus giganteus* provided by rhizomes of the Catania stand which was established in 1996, were transplanted in the field on May 2005, in a low deep soil with a high amount of soil skeleton. In this year, soil water content were maintained in optimal conditions, in order to guarantee seedling establishment. During the growing season, air temperature and rainfall were recorded using a meteorological station (CR10, Campbell-USA). At harvest, carried out on at the end of March for all three years, plant samples were dried at 60 °C in a thermo-ventilated environment for dry matter determination. Plant height and number stems, were measured on a sampling area of 2 m<sup>2</sup>. The data of morphological and productive characteristics were statistically analyzed by ANOVA.

## 3. Results

### 3.1. Climatic factors

The mean air temperatures, were recorded during the active growth phase (June - August); higher values were observed in the second and third years than first year, around 19 - 20 °C and 31 °C, respectively for the minimum and maximum temperatures (Tab 1).

In the three years of experimental field, the amount

of rainfall, recorded from January to April, has determined soil hydrological conditions optimal for the crop emergence. In particular, values around to 309, 312 and 292 mm were registered respectively in the first, second and third year.

The total summer rainfall (June – August) in the three years was not sufficient to sustain crop growth (Tab. 1) and water for irrigation was supplied.

Tab. 1 – Rainfall (mm) and mean air temperature (°C) were recorder during the growing season (June – August) in the three years of research

Years	Rainfall (mm)	Mean max T (°C)	Mean min T (°C)
2005/06	23.5	28.3	18.6
2006/07	68.5	30.8	19.2
2007/08	30.0	31.6	20.2
Average	40.7	30.2	19.3

### 3.2. Morphological and productive traits

The studied morphological parameters were affected by the different crop density levels, the higher the crop density, the lower the plant density. In particular, crop height reached highest values in the second year (188, 181 and 178 cm, respectively for 4, 6 and 8 plants  $m^{-2}$ ). Plant height showed a slight reduction due to the lacking of water for irrigation in the 2008.

The number of stems showed an increase from lower to higher plant density: 4, 6 and 8 plants  $m^{-2}$  in all years, reaching high values statistically significant difference in the second year (69, 105 and 125 stems per  $m^{-2}$ , respectively). A significant reduction of the number of stems was observed in the third year.

After transplanting in the first year, stem dry weight decrease in the higher crop density levels, but whit remarkable effects on the 8 plant density per square meter.

A significant effect of plant density on growth and biomass productivity was found, with 8 plants  $m^{-2}$ , to the major stems per unit area. A greater aboveground dry biomass production was observed in the years after the first. In particular in the second year where, due to better soil water availability, were found production values of 9.7, 14.2 and 16.3  $t\ ha^{-1}$ , respectively for 4, 6 and 8 plants  $m^{-2}$ . In the third year water stress caused by shortage of water for irrigation accelerated leaves senescence; which reduced the amount of stem dry matter accumulated in the summer season (Fig. 1).

### 4. Conclusion

The plant density affected plant growth reducing plant height, stem dry weight, and increasing number of stems and total aboveground biomass yield. Water availability limits plant growth and biomass production of the *Miscanthus* in the Mediterranean regions. Therefore, its introduction as an alternative energy crop is possible in areas with good water resources availability.

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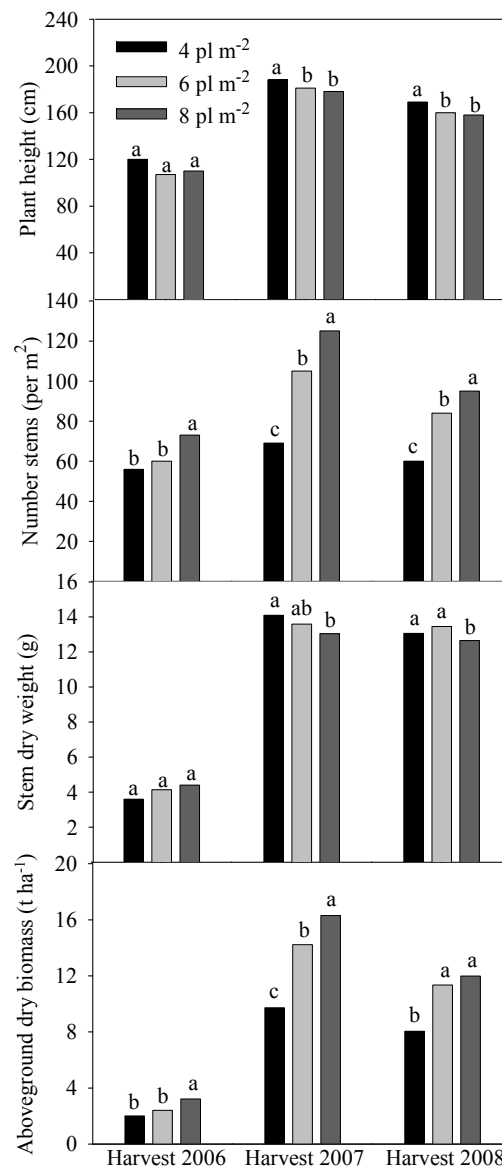


Fig. 1 – Morphological and productive characteristics were measured in the three years trial

# Effect of Plant Density on Oil Yield in *Cynara Cardunculus* L. to Produce Biodiesel

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Different studies on cardoon (*Cynara cardunculus* L.) about biomass productions for different crop utilization showed the potential of this crop to be suitable to different biomass application, such as lignocellulosic biomass for energy, seed for oil extraction to obtain biodiesel in Mediterranean environment (Raccuia and Melilli, 2007).

Aiming to maximize the biomass and oil yields researches about the influence of genotypes and plant densities were carried out.

## Methodology

This trial was carried out at Ragusa (South Sicily), in 2006-07 growing season. Two cardoon genotypes (one *C. cardunculus* L. var. *altilis*, “C” and *C. cardunculus* L. var. *sylvestris* “S3”) were grown at four different plant density (1, 2, 4 and 8 plant m<sup>-2</sup>). The adopted experimental design was set as randomized blocks with three replications, including 4 different plant densities:

- 1 plant m<sup>-2</sup>: 1.0 m apart in rows - 1.0 long row (20 plants per plot);
- 2 plants m<sup>-2</sup>: 1.0 m apart in rows - 0.5 long row (40 plants per plot);
- 4 plants m<sup>-2</sup>: 0.5 apart in rows - 0.5 long row (80 plants per plot);
- 8 plants m<sup>-2</sup>: 0.5 apart in rows - 0.25 long row (160 plants per plot).

Each plot was 20 m<sup>2</sup>. The crop was grown under low inputs management. The biomass was harvested on July. At harvest on a large number of plants, for each agronomic replication were determined the number and weight of heads, the fresh biomass weight and its partitioning. Achenes were obtained trashing the capitulum. In laboratory dry matter content of each part of the plant were calculated in thermoventilated oven at 105°C. The grain oil content was determined (AOAC, 2005). All analyses were performed in duplicate for each agronomic replicate and are reported on a dry matter (DM) basis.

Data was submitted to the Barlett’s test for the homogeneity of variance and then analysed using the analysis of variance (ANOVA). Means were statistically separated on the basis of Student - Newmann - Kewls test, when the “F” test of ANOVA for treatment was significant at least at 0.05 probability level. Significance was accepted at  $P < 0.05$  level (Snedecor and Cochran, 1989).

## Results

From the analyses of variance (Tab. 1) resulted the interaction “Genotype X Densities” was not significant, the “genotype” factor affected only the total biomass yield, while “density” factor was significant at 0.001 probability level for all the traits.

On average of all the studied factors biomass yield was 16.2 t ha<sup>-1</sup> DM varying from 10.4 (1 plant m<sup>-2</sup>) to 26.0 t ha<sup>-1</sup> (8 plant m<sup>-2</sup>). Grain yield resulted 1.04 t ha<sup>-1</sup>, with an oil production of 0.3 t ha<sup>-1</sup>. Passing from 1 to 8 plant m<sup>-2</sup>, grain yield varied from 0.71 to 1.83 t ha<sup>-1</sup>, while oil yield from 0.18 to 0.48 t ha<sup>-1</sup> (Tab. 2).

The figures 1 and 2 reported the obtained results in relation to genotype and plant densities. The two genotypes showed the same behavior to the effect of plant densities. In particular for all the traits significant differences were observed only using densities of 4 and 8 plants m<sup>-2</sup>.

The linear regression between the total oil yield and number of plant used has been shown in Figure 3. High correlation coefficient has been obtained between the two traits for both the genotypes.

Tab. 1 – Analyses of variance for the studied characteristics in *C. cardunculus* L.

Traits	Font of variation		
	Genotype (G)	Density (D)	G X D
Biomass Yield (t ha <sup>-1</sup> s.s.)	*	***	ns
Grain Yield(t ha-1)	ns	***	ns
Oil Yield (t ha-1)	ns	***	ns

\* Significant at 0.05 probability level.; \*\*Significant at 0.001 probability level; ns not significant

Tab. 2 Effect of plant densities on biomass, grain, oil and biodiesel yields, on average of the two genotypes. Different letters within the column indicate differences at  $P \leq 0.05$ .

Plant	Biomass (t ha <sup>-1</sup> DM)	Grain (t ha <sup>-1</sup> )	Grain oil (t ha <sup>-1</sup> )
1	10.4 c	0.71 c	0.18 c
2	11.7 c	0.81 c	0.21 c
4	16.5 b	1.15 b	0.30 b
8	25.9 a	1.83 a	0.48 a
<b>Means</b>	<b>16.2</b>	<b>1.13</b>	<b>0.29</b>

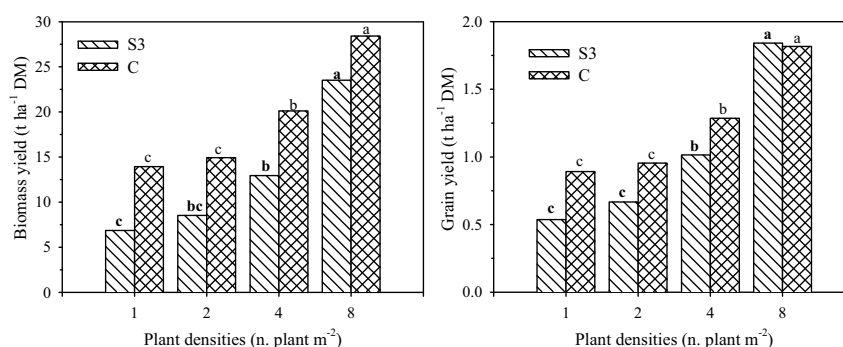


Fig. 1 –Biomass and grain yield (t ha<sup>-1</sup> DM) in *C. cardunculus* genotypes in relation to plant densities. Different letters within the same genotype indicate differences at  $P \leq 0.05$ .

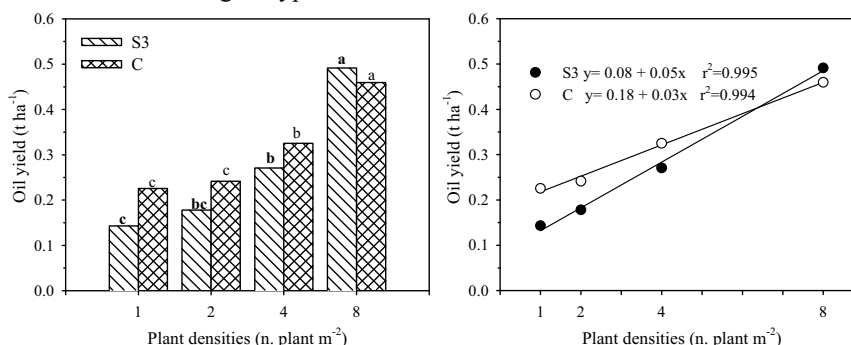


Fig. 2 –Oil yield (t ha<sup>-1</sup>) in *C. cardunculus* genotypes in relation to plant densities. Different letters within the same genotype indicate differences at  $P \leq 0.05$ .

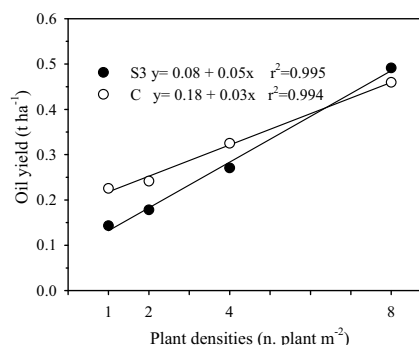


Fig. 3 Relationship between oil yield and plant density

## Conclusions

On the basis of these results it is clear the plant densities affected all the studied traits. It is worthwhile to note that the oil yield reached high values in consideration the crop was grown under low input management. Contrary to other oil crops, whole *C. cardunculus* plants can be used for different purposes (Fernandez et al. 2006). However, research investment is required to increase and optimize yield, taking into account the effects of other crop managements.

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# Development of New Lines of Brassica Carinata for Energy Production

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The objective of the work is to reduce the cost of bioenergetics crops cultivation. The sustainable supply and use of energy for transportation represents a real challenge. Terrestrial as well as aerial mobility are strongly affected by petrol shortage. 84% of all petroleum extracted is processed as fuels, including gasoline, diesel, fuel oils and liquefied petroleum gas [1]. Burning oil releases carbon dioxide into the atmosphere contributing to global warming. Consumption of oil is currently around  $30 \cdot 10^9$  barrels per year. The accepted estimation of oil reserves amount to  $1200 \cdot 10^9$  barrels [2]. At current consumption levels, world oil supply would be gone in about 40 years, around 2050. The growing social, economical and political interest for the development of alternative fuel sources is not only due to general concerns of sustainability but also related to human development and geopolitical stability. The possibility to obtain internal combustion liquid engine fuels starting from vegetable products represents an attractive challenge. Biodiesel as well as bioethanol can be mixed with fossil fuels in varying percentages, without changing physiochemical properties of the fuel. The manufacture of biofuel requires that suitable quantities of biomass be grown, harvested and transported to the conversion plant site. Many questions must be studied in great detail to select the proper species or mixture of species for operation of the system. These questions concern such matters as growth cycle, fertilization, sun availability, temperature, precipitation, propagation and planting procedure, soil and water needs, harvesting methods, diseases resistance, growth area competition with food, feed and fiber, growth area availability, simultaneous or sequential growth of biomass for biofuel and foodstuff or other applications. In the ideal case, biomass chosen for energy application should be high-yield, low-cash-value species that have short growth cycles and that grow well in the area and climate chosen for biomass production. Fertilization requirement should be minimized. The species grown should have low water needs and be able to utilize efficiently available precipitation. For land-based biomass, the requirements should be such that the crops can grow well on low-grade soils and do not need the best classes of agriculture land.

## Methodology

*Brassica carinata* was selected as oleaginous, arid-resistant species capable to produce oil and biomass and live in condition of arid-culture. Previous works showed the possibility to use *Brassica carinata* as alternative oil crop for the production of biodiesel [3,4]. About 600 plants have been cultivated and 102 have been selected for specific characters such as number of primary branches, height of the plant, color of the flower and pods resistant to shattering. The amount of seed per plant was determined gravimetrically. The oil in the seeds was determined after extraction with chloroform. The seeds were manually grinded and extracted with chloroform (about 20 ml of solvent per gram of seed). The extraction was performed in close vessels at 40°C for 16 h. After cooling the mixture was filtered on paper filter under vacuum and the residue washed with 10 ml of chloroform. The solvent was removed under vacuum and the oil was weighed. The residue on the filter was heated at 110°C for 6 h and weighed to determine the dry weight. The water content was determined by weight difference.

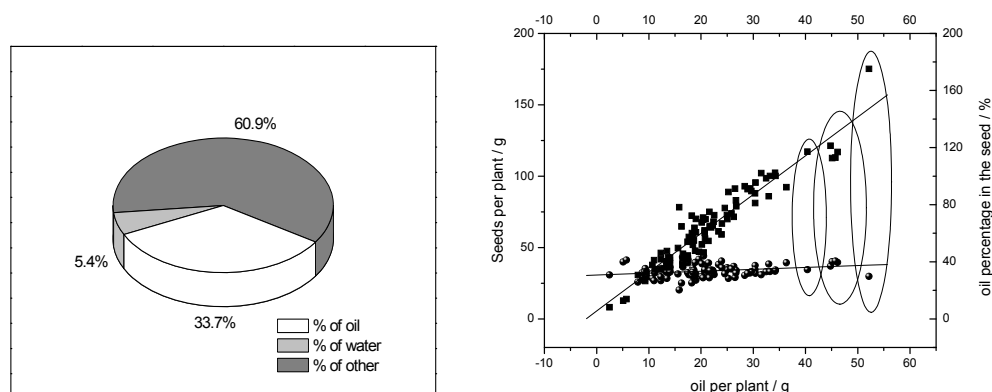


Fig.1 Left) Average content of the seeds. Right) Seeds per plant (square) and oil percentage in the seeds (circle) as a function of the oil content in the plant.

## Results

Fig.1 shows the average composition of the seeds of the selected plants. The oil content is about 33.7% while the humidity is 5.4%. The remaining are mainly proteins. The total oil content of the selected plants has been evaluated and fig. 2 reports the seeds per plants and the oil percentage in the seeds as a function of the total oil content. It is easy to see that the plants with the highest oil content (more than 50 g per plant) has a high number of seed per plant (more than 175 g of seed per plant) while the percentage of oil in the seed is low (30%, about 3.7% lower than the average oil content). From these result 6 plants with the highest number of seeds and 5 plant with highest oil content have been selected for cultivation. To increase the productivity we will try to transport by hybridization the characters that specify for high oil content into plants with high number of seeds.

## Conclusions

11 plants with well defined characteristics have been selected and cultivate. Genetic improvement, carried out with interspecific hybridizations and selection techniques, will allow to choose the best cultivars in terms of high seed number and high percentage of oil in the seed. The best plant could have an oil content per plant up to 45% with a productivity of 180 g per plant with an estimated amount of oil of about 80 g per plant.

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# Sustainable Options for Biomass Energy Cropping

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Bioenergy is intended to use renewable instead of non-renewable resources for energy production, in the process not adding fossil CO<sub>2</sub> to the air but using what is already there. Nevertheless, both the energy balance and the agricultural sustainability of the various forms of bioenergy vary, and are widely seen as poor in the case of first-generation bioethanol from cereal starch (Hill et al., 2006), becoming more attractive as more of the plant is used and efficiency of production increases. Although energy cropping can contribute to farm economy when otherwise unproductive land is taken into use, the use of food or feed, and of the land normally used for food or feed, for energy production strikes many as unsustainable even while the need to reduce the release of greenhouse gases is clear.

In many cropping systems, the largest single energy input, and the largest single source of greenhouse-gas release, is synthetic nitrogen fertilizer. This represents 15% of the energy input of the crop production cycle (Hill et al., 2006) or even more, and has been associated with the release of considerable amounts of N<sub>2</sub>O, a potent greenhouse gas that also destroys ozone (Crutzen et al., 2007). Shrewd usage of biological nitrogen fixation can displace the need for synthetic nitrogen fertilizer in the production phase. Ploughing and other land preparations represent another key energy input, as well as an opportunity for soil erosion and degradation, reduced by the use of perennial species or minimum tillage. Perennial crops offer the further advantage of carbon sequestration into the perennating organ.

In industrial agriculture, a clean monocrop is sought, for good reasons of ease of maintenance and harvest of uniform grain, but these criteria are no longer central in biomass cropping. Monocrops seldom harvest the available natural resources as efficiently as communities (e.g., Tilman et al., 2006). Thus we are investigating legume-grass perennial blends for maximizing dry matter production. Legume under-crops or co-crops have potential for use in tree-based energy cropping, oilseed biodiesel and annual grasses. Non-legume components of the blends maximize uptake of mineralized nitrogen, minimizing nitrate runoff.

In many regions there are areas of land that are considered inappropriate for food, feed and fibre cropping due to pollution, contamination or difficult management. These include acid-sulphate soils that are highly reduced, contain a high concentration of sulphides and are widespread at high latitudes as well as in the tropics. Drainage of these soils leads to oxidation of the sulphides to sulphates, a massive drop in pH, solubilization of many minerals, and a downstream kill of fish (Yli-Halla et al., 1999). Management of these soils requires a high water table, greatly restricting cropping options. Reed canarygrass (*Phalaris arundinacea*) has already shown potential as an energy crop on these soils in the Nordic-Baltic region (Pahkala and Pihala, 2000) and we are seeking to add sustainability to this system by incorporating biological nitrogen fixation from a legume intercrop.

## Methodology

Monocrops and mixed crops of annual and perennial species were sown in southern Finland in May - June 2007 and May 2008. In 2007, plots were 2.5 x 6 m, and dry matter yield was determined in 1 m<sup>2</sup> subplots at the end of the growing season. Annual crops included maize cv. Campesino, faba bean cv

Kontu, fodder vetch (*Vicia sativa*), Persian clover, white lupin, blue lupin, yellow lupin, oilseed hemp, and annual ryegrass, in 54 combinations in four replicates in a randomized complete block design. Perennial crops included reed canarygrass, lacy phacelia (*Phacelia tanacetifolia*), Washington lupin (*Lupinus polyphyllus*), fodder goat's rue (*Galega orientalis*), alsike clover, white clover, birdsfoot trefoil, and a fodder grass blend, in 61 combinations, again in four replicates and a randomized complete block. All species were sown at 100% normal density and half of the plots without legumes received an additional 80 kg N ha<sup>-1</sup>. Sample carbon and nitrogen contents were determined by the Dumas method in an Elementar VarioMax CN analyser. Subplots of 50 plants were marked in the perennials in November 2007 and survivorship determined in May 2008. In 2008, plots are 2.5 x 15 m and the annual crops are maize, fibre hemp and faba bean while the perennial crops are reed canarygrass, fodder goat's rue, white clover, white sweet clover, and brownray knapweed (*Centaurea jacea*), with sowing densities adjusted in the mixed plots. Two sites are used, one with acid-sulphate soil and a relatively high water table, and the other with conventional soil conditions.

## Results

In 2007, the maize yielded 27 t/ha dry matter without additional N fertilizer and 28 t/ha with the additional N (SE 1.7 t/ha). This result indicates that the overall level of fertility in the field was very high. Grains had just started to fill at the time of normal harvest, in the middle of September. Intercropping with Persian clover, faba bean and fodder vetch significantly reduced not only the maize yield but the whole plot yield. Faba bean on its own produced 10 t/ha dry matter at physiological maturity, significantly better than any of the lupins (SE 0.5 t/ha). Hemp, with additional N, produced 8.8 t/ha dry matter and it also reduced the growth of the lupins in the mixed plots.

In the perennial plots, the grasses and legumes established well together but the lacy phacelia dominated and smothered the other species in its mixtures. Mildew infected alsike clover and perennial lupin. More than 98% of the fodder grass plants survived the winter, 87% of the reed canary grass, 90% of the white clover and 76% of the alsike clover and galega. Survivorship of the perennial lupin was only 48% and of the trefoil only 12%.

## Conclusions

In this season the outstandingly high production of the maize indicates its potential as a biomass crop, even at these high latitudes, but sustainable management systems have to be developed. A small parallel experiment with a non-adapted faba bean cultivar indicated its potential for higher dry matter production than the adapted cultivar and similarly, fibre hemp showed greater potential than oilseed hemp.

Reed canarygrass blends with both white clover and galega showed good potential for interspecific compatibility and low incidence of disease. The high productivity of reed canarygrass-galega blends for methane production has been shown elsewhere in the Baltic region (e.g., Kryzeviciene et al., 2007). We will also be testing whether the hypoxia of the saturated zone of the soils slows the rate of degradation of dead roots, contributing further to carbon sequestration.

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# Study of Reed Canary Grass – Possible Source for Energy Utilization

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## Introduction

Reed canary grass belongs to the group of alternative plants intended for further industrial and energy utilization. Reed canary grass (*Phalaris arundinacea* L.) is a perennial stolonate *Poaceae* family plant. This plant is supposed to be predominantly utilized as a raw material for cellulose production and potential energy resource. Aim of this study is to determine the influence of site and N fertilization on yields and level of quality of reed canary grass phytomass for combustion.

## Methodology

In the period 1996 – 2007 the field experiments with reed canary grass were performed at two different sites with three levels of nitrogen fertilization and two terms of harvest (in autumn and in spring). Before establishment of plant stand 50 kg/ha K and 26.5 kg/ha P was applied in all sites. P and K fertilizers had not been applied in the following years. Synthetic nitrogen fertilizers were annually applied in three different rates: N0 = without nitrogen fertilization, N1 = pre-sowing application of 30 kg/ha of ammonium sulphate early in spring, N2 = annual split application of 60 kg/ha - pre-sowing application of 30 kg/ha of ammonium sulphate and 30 kg/ha of ammonium nitrate in the shooting period. Effects of nitrogen fertilization and site conditions on above ground biomass production were studied. It was evaluated the dependence of the harvest terms on water content in harvested material, on losses of phytomass during the winter period and on content of main elements in plants.

## Results and discussion

In Table 1 there are available average yields expressed in dry matter amount, together with soil and climatic characteristics of individual sites. At the site Ruzyně the phytomass yields harvested in autumn varied in average from 5.47 t/ha of dry matter (2005) to 12.9 t/ha (2002). At the site Lukavec the yields of phytomass expressed in dry matter varied from 3.90 t/ha (2000) to 13.8 t/ha (2004). The highest yield (15.6 t/ha) was reached at the site Lukavec in 1998 after adding 60 kg/ha N. The average yield in the whole testing period was 8.63 t/ha in Ruzyně and 8.23 t/ha in Lukavec.

Yields of phytomass produced by reed canary grass were positively influenced by the split graduated nitrogen fertilizer application (N2). Annual pre-sowing nitrogen application of 30 kg/ha increased phytomass production by 1.3 t/ha (15.1 %) at the site Ruzyně and by 1.5 t/ha (18.1 %) at the site Lukavec, when compared with N0 variant. Application of 30 kg/ha of N (N1) led to phytomass yield increase by 2.7 t/ha (27 %) at the site Ruzyně and by 2.8 t/ha (29.2 %) at the site in comparison with N0 (Table 1). It is evident that reed canary grass yield was highly affected above all by nitrogen fertilization and weather conditions. Phytomass yield was predominantly influenced by the distribution of precipitation during vegetation season in each year.

Landstrom et al. (1996) reported about obtaining in Sweden average yields of 7.5 t/ha in spring and 9 t/ha at the end of vegetation season in 5 year period (evaluation beginning in the second year) when using nitrogen fertilizing rates of 100 kg/ha. Dry matter losses during the winter season reached about 25 %. In neighbouring countries average yields of dry matter ranged between 4.5 and 9.0 t/ha.

For energy and economic purposes there is important the term of harvest. Table 2 shows the reed canary grass yield and phytomass moisture differences between different terms of harvest. When harvested in the last decade of November the reed phytomass is not suitable to be burned without

drying. In the mentioned time we recorded phytomass moisture of 47 %. When harvested in spring with water content under 20 % the material can be used for pressed briquettes and pellets production, storage or to be directly burned. From the Table 2 is evident phytomass dry matter losses during the winter season 22.5 %.

Spring harvest is preferable because of higher contents of elements in reed canary grass phytomass when compared to later harvests. Our results of such comparison are presented in Table 3. Also Landstrom et al. (1996) or Hadders and Olsson (1997) gave evidence of obtaining better parameters for burning utilization when harvesting in spring time.

Table 1. Influence of site and N fertilization on yields of dry matter phytomass reed canary grass (t/ha) harvested in late autumn (average of 1996-2007)

Site	N0	N1	N2	Average
<b>Ruzyně</b> (altitude above sea level - 350 m, great soil group - orthic luvisol, average annual air temperature - 8,2 °C, average annual precipitation sum - 477 mm)				
Yields – range of determined values	3.9 – 11.3	4.2 – 13.9	6.0 – 14.6	5.5 – 12.9
Yields – average of 1996-2007	7.3	8.6	10.0	8.63
<b>Lukavec</b> (altitude above sea level - 620 m, great soil group - eutric cambisol, average annual air temperature - 6,8 °C, average annual precipitation sum - 686 mm)				
Yields – range of determined values	3.4 – 11.5	3.9 – 14.3	4.4 – 15.6	3.9 – 13.8
Yields – average of 1996-2007	6.8	8.3	9.6	8.23

Table 2. Yields of fresh matter, dry matter (t/ha) and moisture content at harvest time (%) reed canary grass in various terms of harvest (average of 1996-2003)

Harvest in autumn			Harvest in spring		
Yield			Yield		
Fresh matter	Dry matter	Moisture content	Fresh matter	Dry matter	Moisture content
15.04	8.00	46.8	7.61	6.20	18.5

Table 3. Elements content in plants of reed canary grass in various terms of harvest

Harvest term	Elements content (% of dry matter)					
	N	P	K	Ca	Mg	S
In autumn	0.995	0.170	0.569	0.401	0.119	0.222
In spring	0.923	0.143	0.343	0.245	0.055	0.106

## Conclusions

According to our results there is evident that reed canary grass prefers conditions with higher humidity and nitrogen fertilizing. Provided the plant stand is well established, reed grass could last on one site for many years and provide stable yields of phytomass. Among the main preferences of reed canary grass can be mentioned low cost of the crop establishment, minimal biocides use and low other direct cost during the vegetation season. Reed canary grass can be grown in areas with both low and high altitude above sea level which is also beneficial for its broader utilization.

## Acknowledgements

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# Preliminary Zoning of Agricultural Land for *Miscanthus* (*Miscanthus* × *Giganteus*) For The Czech Republic

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## Introduction

The goal of the study was to create a zoning of agriculture land for *Miscanthus* (*Miscanthus* × *giganteus*) for biomass production which can be used as a tool to assess its regional production and potential, to balance different cropping systems (food, forage, material, energy) and to prevent some of the environmental risks of biomass production on a practical level as well as in strategic planning.

## Methodology

Two main sources were used to create the zoning methodology: *i*) results of field testing of *Miscanthus* × *giganteus* on more sites and Czech agricultural land valuation or more precisely its production-ecological soil units (BPEJ in Czech), which contains information on climatic, soil and site characteristics. Each unit should comprise sites with similar production conditions for agricultural (plant) production. BPEJ are expressed using in our case 3-numeric code that stands for: 1. Climatic region (10 classes of KR in Czech), 2.-3. Main soil units (78 classes of HPJ in Czech). Digits 1, 2 and 3 of BPEJ create a main soil-climatic unit (HPKJ in Czech). Over 500 HPKJ units were identified as a base for the zoning of *Miscanthus*. HPKJ units with similar results were used to create four groups of land suitability sites for *Miscanthus*.

## Results

Based on results of long-term field research of *Miscanthus*, on its biological characteristics and agronomic requirements and using of above mentioned methodology the basic zoning of agricultural land was carried out for *Miscanthus* × *giganteus*. Dry matter yields of above ground biomass from spring harvests on 4 sites are shown in Table 1. Preliminary agricultural land suitability types for *Miscanthus* × *giganteus* for conditions of the Czech Republic are shown in Table 2 which includes also expected yields of dry biomass in spring harvests. Both results may be corrected according to results of current field testing. Given yields are achievable under average climatic conditions and if *Miscanthus* is grown in accordance with recommended methodologies. Our results are comparable to those published for other countries – Ireland (Clifton-Brown et al., 2000), Netherlands (Werf et al., 1993) and whole Europe (Clifton-Brown et al., 2004).

## Conclusions

The zoning methodology of Czech agriculture land *Miscanthus* was created including preliminary agricultural land suitability types.

The zoning can now be used in the strategic planning to determine ecologically or economically unsuitable sites or some environmentally conflicting areas for biomass production using *Miscanthus* in the Czech Republic.

Table 1. Spring dry matter yields of *Misacanthus* × *giganteus* from 4 testing plots under different doses of N-fertilisers (average from 9 years)

Site / N fertilization:	N0	N1	N2	Average
Lukavec (Lu)	7.857	8.203	10.988	9.132
Průhonice* (Pr)	9.910	-	-	9.910
Ruzyně (Ru)	13.001	14.614	16.414	14.017
Troubsko (Tr)	10.644	13.868	18.073	14.195

Notes: \* average from 4 years (2002-2005)

N0 = 0 kg N/ha/year, N1 = 50 kg N/ha/year, N2 = 100 kg N/ha/year

Table 2. Preliminary agricultural land suitability types for *Miscanthus* × *giganteus* in Czech Republic



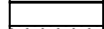

KR/HPJ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
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KR/HPJ	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
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Notes: KR – climatic region, HPJ – main soil units

Preliminary agricultural land suitability types:

	Very suitable and optima sites (spring yield over 14 t (DM)/ha/year)
	Suitable sites (spring yield 11 t (DM)/ha/year ± 2 t)
	Unsuitable sites (spring yield below 7 t (DM)/ha/year)
	Not existing HPKJ

## Acknowledgement

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# Biomass of Grasses for Energy: Possibilities and Expectations

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Bio-energy has a role to fulfil the main expectations of the politics and the society: ensuring security of supply, tackling climate change and making economy more competitive.

Assessment of the potential biomass supply in Europe showed that the largest biomass potentials lie in energy crops, which have long lead times (Ericsson, Nilsson, 2006).

It is forecasted that in the Lithuania energy crops could be grown on 22 % (740 thousand ha) of the total agricultural land, from which 42% for biofuels and 52% for biomass. Grasses are expected to be grown on a 129 thousand ha area of the total arable land (Šateikis, 2006). The grassland in Lithuania accounts for over 40% of the agroecosystem: meadows and natural pastures – 448 thousand ha, perennial legumes and grasses (up to 5 years old) - 416 thousand ha and permanent grasslands – 573.9 thousand ha. The reduction in dairy and beef cattle stock has brought a decrease in the utilization of grassland as feed source. Therefore there is a need to estimate the potential of grassland use for alternative purposes, such as biomass for energy or for other feedstock for non-food.

The use of grassland for energy is one of the potential renewable resources and a good possibility of promoting multifunctional, sustainable agricultural sector. Grassland has a number of positive effects on the environment and biodiversity. Using pasture or landscape management grass as a substrate for biomethanisation method is generally possible, however yields were found lower compared to other crops (Prochnow et al., 2005). Therefore other considerations also exist, as due to a higher productivity of arable crops, there is a trend for converting grassland to arable land (Taube et al., 2007).

The anaerobic digestion of energy crops and agricultural residues can contribute considerably to national energy balances (Plockl, Heerman, 2006) and is promising means of achieving multiple environmental benefits.

## Methodology

Field experiments were carried out at the Lithuanian Institute of Agriculture in Dotnuva (55° 24'N). The soil of experimental site is characterised as *Apicalcari - Endohypogleyic Cambisol, light loam*. Swards of different species composition were grown. The swards were cut two or three times per year. Dry matter (DM) yield were determined on the basis of total dry matter amount per plot and calculated as t DM yield ha<sup>-1</sup>. The data of experiments were statistically processed using analysis of variance.

## Results

Scarce research on mixtures of *Trifolium repens*/*Lolium perenne*, *Medicago sativa*/L. *perenne* and *P. pratensis*/*T. repens* and pure sward of *L. perenne* in Lithuania showed that dry matter yields of fodder grass swards, cut three times per year, were primarily affected by the climatic conditions, sward composition and growing technology. The main difference in dry matter yield of grasses swards was determined by growing them in different field conditions and DM yield was higher when grasses had been grown in leys compared with pastures (table 1).

The results, obtained in the experiment in growing *Phalaroides arundinacea* L. and mixtures with *Melilotus officinalis* (L.) Lam., *Lupinus polyphyllus*, *Galega orientalis* Lam., grown as energy crops,

showed, that pure sward of *Phalaroides arundinacea* L. produced higher DM yield, than mixtures with legumes (table 2).

**Table 1.** Total dry matter yield of different swards, t ha<sup>-1</sup>

Swards	Total dry matter yield, t ha <sup>-1</sup> per year					
	In pastures			In leys		
	2004	2005	2006	2004	2005	2006
<i>Trifolium repens/Lolium perenne</i>	5.26	4.74	2.74	7.97	6.49	2.01
<i>Medicago sativa/L. perenne/P. pratensis</i>	8.63	8.24	4.52	9.23	8.85	6.74
<i>T. repens/M. Sativa/L.perenne</i>	7.04	7.03	4.77	8.84	9.21	7.13
<i>L.perenne</i> (N <sub>240</sub> )	7.72	5.70	4.26	9.13	5.63	3.67
LSD <sub>.05</sub>	0.497	0.466	0.591	0.356	0.429	0.518

Research on biomass utilization of pure *Phalaroides arundinacea* L. and mixtures with *Melilotus officinalis* (L.) Lam., *Lupinus polyphyllus*, *Galega orientalis* Lam. for biogas generation, estimated on laboratory scale biogas digesters and investigations showed that the energy potential of swards is greatly dependent on the productivity of grasses (table 2) (Navickas et al., 2003)

**Table 2.** Energy potential of the biomass of perennial grasses used for biogas production (Navickas et al., 2003)

Sward composition	1 <sup>st</sup> cut			2 <sup>nd</sup> cut			Total
	DM, kg ha <sup>-1</sup>	CH <sub>4</sub> , %	EA, GJ ha <sup>-1</sup>	DM, kg ha <sup>-1</sup>	CH <sub>4</sub> , %	EA, GJha <sup>-1</sup>	EA, GJ ha <sup>-1</sup>
Phalalaris arundacea							
purse sward	6,405	68.0	88.97	2,905	65.0	40.90	129.87
mixture with <i>M. officinalis</i>	4,527	62.1	32.33	1,570	64.4	19.65	51.99
mixture with <i>L. polyphyllus</i>	5,139	65.3	56.00	1,753	63.1	20.57	76.57
mixture with <i>G. orientalis</i>	5,892	70.2	98.94	2,174	63.8	38.66	137.59

## Conclusions

Grassland or grasses in arable land in our climate and soil conditions could be grown in mixtures with legumes and used for energy purposes. We need more research on energy balance of different grassland-based or ley/arable crops systems, impact of biofuel development from crop and grassland on soil quality, pesticides in water and other variables.

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# Differences of Yield and Grain Energy Content of Spring Barley Cultivars Growing Under Water Stress

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## Introduction

Processes connected with water regime of plants corresponding with plants genotype disposition – from point of view requirements of water and abilities of water management, adjustment ability, growth and yield formation (Samarah, 2005). This work was focused on evaluation of six barley cultivars growing in low water availability conditions. Response of cultivars in yield and grain energy content to water shortage was studied.

## Methodology

Six barley cultivars – two historical varieties Norimberk (Nor) and Valticky (Val) and four modern cultivars Amulet (Amu), Jersey (Jer), Krona (Kro) and Malz (Mal) were used for experiments. The experiments were conducted under the shelter in the pots with soil during spring of the years 2006 and 2007. Two treatments of water regime were maintained during the whole time of growth – the full watered (W) and water stressed (S) defined by volumetric soil moisture. The water potential in the mature leaves was measured by psychrometric method using Wescor PSYPRO instrument. The leaves length was measured at their full leaf area. Energy content of grains was measured by combustion calorimeter IKA C200 calorimeter. All the measurements were accomplished on the main stalks.

## Results

The Fig. 1 showed the reduction of leaf water potential in stressed plants. The results are an average from the all measurements on the full expanded leaves during the plant development. Wide differences in leaves water potential depending on fully controlled water supply were found because the external conditions temperatures and air humidity was uncontrolled. These differences and responses may be associated with different osmotic adjustment of cultivars as Gunasekera et al. (2004) found.

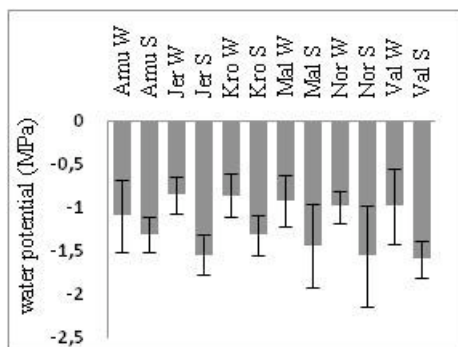


Fig.1 Water potential of leaves during growth

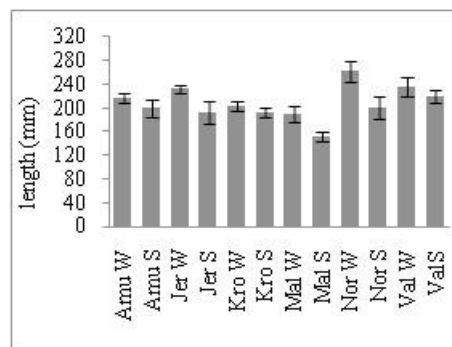


Fig.2 Length of the leaves

For the assessment of length of leaves (Fig. 2) we used the third, fourth and fifth leaves because influenced mostly the grain yield formation. The differences in the leaves length were found between watered and

stressed treatments in cultivars Jersey, Malz and Norimberk. The evaluation of productivity of main grains (Fig 3) proved the reduction of dry matter mature spikes in all cultivars. The greater differences were found in Amulet, Jersey, Krona. The historical cultivars reduced yield of main spikes but there were found greater imbalance between separated spikes. The distribution of assimilates was monitored by using the combustion calorimetry as energy content (Fig 4). We found significant differences in the content of energy increased in stressed treatments previously, except in Norimberk.

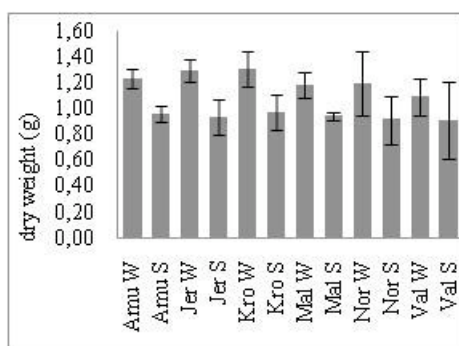


Fig. 3 Dry weight of main spikes

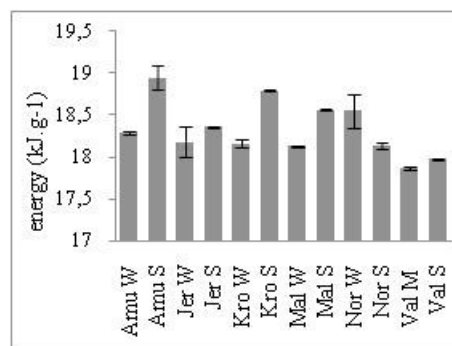


Fig 4 Brutto energy of main spike grains

These results are hardly to be discussed because another influence affected distribution of assimilates. In the case of Norimberk disposition to mildew, long-term water stress can to change metabolism. First of all the photosynthesis or damage was affected the sink and to changing of the capacity and rate of assimilate accumulation (Griffiths et Parry, 2002).

## Conclusions

The six of spring barley cultivars were studied. The physiological responses of water potential, growth of leaves, dry matter and amount of storage energy in grains in conditions of full watering and water stressed were chosen for this study. Differences in water potential were found probably because the genotypes differ in ability of osmotic adjustment. The productivity of main spikes and energy content shown the different ability distribution of assimilates. The reduction of leaf growth was connected with energy accumulation in grains. The lowest ability of grains energy accumulation in water stress conditions was found in historical varieties.

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## Acknowledgements

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# Evaluation of Stem and Fibre Yield of Monoecious and Dioecious Variety of Hemp in Northern Italy

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Hemp (*Cannabis sativa* L.) is a multipurpose crop which is grown both for its fibres and seeds. Recent research has shown that the cultivation technique (i.e. plant population, genotype, harvest time, harvesting system, environment of cultivation) can influence the production of hemp fibre in terms of quantity and quality (Amaducci et al., 2002; Amaducci et al., 2005; Amaducci et al., 2008). In our work a selection of monoecious and dioecious genotypes commercially available and present on the European variety list were cultivated for 2 years in the North of Italy; stem yield and fibre quality were determined at beginning and end of flowering.

## Methodology

Field experiments were carried out in 2003 and 2004 in Cadriano (32 m a.s.l.; 44°33' latitude). The experiment was a two factors (genotype and harvest) split-plot design combined over 2 years. The varieties were 11: Carmagnola, Dioica88, Epsilon68, Fedora17, Felina34, Ferimon, Fibranova, Futura75, Lovrin and Tiborszallasi and Chamaleon only in 2004. In both years nitrogen fertilization was applied before sowing at a rate of 60 kg ha<sup>-1</sup> (Amaducci et al., 2002). Sowing took place on 16th April 2003 and 8th April 2004, row distance was set at 13 cm and seeds were sown 3-4 cm deep, target plant density was 240 plants m<sup>-2</sup>. Two harvests were carried out at specific phenological stages: beginning of flowering and end of flowering. Harvesting was carried out when 50 % of plants had reached a predetermined stage of growth (Mediavilla et al., 2001). 15 representative plants per plot were selected and dried at 105 °C for further processing and quality determination. Fibre extraction was carried out with NaOH according to the Bredemann method (1942); bast fibre yield was calculated by multiplying the stem yield by the bast fibre content (Sankari, 2000). Thin cross sections were hand cut from the middle part of the 3<sup>rd</sup> internode immediately after harvest in both years for Carmagnola, Epsilon68, Felina34, Ferimon, Fibranova. Stem cross sections were coloured with Green Methyl and Red Congo and observed and photographed under Leitz Orthoplan light photomicroscope (Amaducci et al., 2005).

## Results

The length of the vegetative cycle varied greatly among varieties, which were consequently classified as “early” (Fedora, Felina, Ferimon), “intermediate” (Chamaleon, Lovrin, Futura 75, Tiborszallasi, Epsilon 68) and “late” (Carmagnola, Fibranova, Dioica 88). Considering that stem biomass is mainly accumulated during the vegetative phase the higher yields (15.8 t ha<sup>-1</sup>) were obtained with late genotypes, the lower yields (4.6 t ha<sup>-1</sup>) with early ones. Fibre content, determined in 2004 after chemical extraction (Bredemann method), was on average 18.5%. The highest percentage of fibre was found in the Chamaleon (20.1%) the lowest in the Tiborszallasi (15%). Fibre yield, calculated on the base of stem yield and fibre content, was higher for Fibranova (3.2 t ha<sup>-1</sup>) and lower for Fedora and Ferimon (0.9 t ha<sup>-1</sup>). Fibre quality determination was based on the mean diameter of single bast fibre cells and fibre maturity (degree of filling of the fibre cell lumen). Finer fibres were obtained for all varieties in 2004, and in both years Carmagnola had the fine fibres (23.8 µm), while Felina (26.6 µm)

the coarser. Fibre maturity was highly affected by harvest time and it increased in both years during flowering, showing limited variation among varieties.

### Conclusions

Dioecious varieties confirmed to out-yield the early monoecious genotypes in a traditional area for hemp cultivation in the North of Italy. Maximisation of fibre yield was achieved at the end of flowering when fibre cells are mature.

### Acknowledgments

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Table 1. Stem production ( $\text{t ha}^{-1}$ ) in two years and two harvest time. LSD ( $P < 0.01$ ) = 2.7. Pure fibre content (%) in two harvest times. LSD ( $P < 0.01$ ) = 12.0

Genotype	Stem production ( $\text{t ha}^{-1}$ )					Fibre content (%)		
	2003		2004		Mean's	2004		Mean
	Harvest I	Harvest II	Harvest I	Harvest II		Harvest I	Harvest II	
Chamaleon						20.4	21.2	20.8
Carmagnola	15,9	15,1	15,6	16,6	15,8	15,9	15,4	15,6
Dioica88	13,3	13,7	14,6	16,7	14,6	19,7	20,4	20,0
Epsylon68	9,4	11,1	6,3	12,3	9,8	18,9	19,2	19,0
Fedora17	3,7	7,7	3,8	5,9	5,3	17,8	18,9	18,4
Felina34	4,2	8,9	4,5	6,3	6,0	18,5	20,4	19,4
Ferimon	3,3	5,8	3,8	5,6	4,6	17,7	20,7	19,2
Fibranova	13,7	12,9	15,0	16,5	14,5	20,4	20,4	20,4
Futura75	9,5	11,8	9,4	11,0	10,4	19,4	18,5	19,0
Lovrin	8,8	10,7	13,5	13,5	11,6	16,9	16,7	16,8
Tiborszallasi	6,2	10,8	12,2	12,0	10,3	15,0	15,0	15,0
Mean	8,8	10,8	9,9	11,6	10,3	18,2	18,8	18,5

Table 2. Average diameter ( $\mu\text{m}$ ) of primary fibre cells measured in the 3<sup>rd</sup> internodes in five genotypes and in two years. LSD ( $P < 0.01$ ) = 2.2

Variety	2003	2004	mean's
Felina34	26.6	26.6	26.6
Ferimon	25.7	23.8	24.8
Epsylon68	28.6	24.2	26.4
Carmagnola	25.1	23.8	24.5
Fibranova	29.2	25.7	27.4
mean's	27.1	24.8	25.9

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## **SUB SESSION 2.3**

GENOMICS FOR A MORE SUSTAINABLE AGRICULTURE

Chairman: Roberto Tuberosa



# Tailoring Plants to Environmental Constraints by Scaling up From Molecular Biology to Crop Physiology

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Plants will face large changes in environmental conditions during the next decades, in particular in terms of temperature and of water deficit. This may have particularly serious consequences for the distributions and biomass accumulation of crops in Europe during the next century (IPCC 2007). Paradoxically, plants undergo almost the whole range of conditions observed in their ecological area in one location over short periods of time. Differences in leaf temperature by 20°C are commonly observed over 12 h during a day, and differences in mean air temperature between successive days can be as high as 10°C. In the same way, water deficit is usually rapidly fluctuating in European fields. Plants are subjected to alternations of wet conditions, e.g. after a rain or an irrigation, and dry conditions during which they cope with a temporary water stress. As a consequence, "water stress" or "temperature stress" have no straightforward definition because, in addition to their severity, they are defined by their timing. Stresses which last a few hours or several days have different consequences on the plant physiology. Stresses sensed at different periods of the crop cycle have markedly different consequences on biomass accumulation and yield.

This presentation aims at reviewing the strategies for improving the ability of plants to cope with the fluctuating stresses presented above. It focuses on temperature, evaporative demand and soil water deficit, and on the combinations of methods at different scales of organisation, from cell to field.

## **I How to deal with fluctuating temperatures in natural conditions ?**

Temperature determines most physiological processes but fluctuates rapidly within a day and between days. It is difficult to compare genotypes or environmental scenarios in such fluctuating conditions except if rates are compensated for temperature in an unequivocal way. If a well-conserved law relates physiological rates to temperature, it can serve as a baseline for expressing rates in a temperature-independent way. This idea is used by crop models for correcting the duration of the phases of crop cycle with simple linear equations (Keating et al. 2003). We have developed it for mechanisms involved in plant growth with a linear response in a limited range of temperature (14 - 30°C Sadok et al 2007). Leaf elongation rate of maize, expressed per unit thermal time, was stable during the night even in naturally fluctuating temperature, and remained unchanged for several nights and experiments. This provides a genotype-dependent rate for each genotype which can be analysed genetically and used for studying the effects of other stresses. Recently, we have explored a larger temperature range (5-40°C) and more mechanisms involved in plant development, with a modified Arrhenius equation widely accepted in Biology. Common responses were observed in the range 5-40°C for the rates of maize leaf expansion, cell division, germination, leaf appearance and leaf initiation; thereby providing a baseline law which can serve to calculate temperature-compensated rates for developmental processes. Responses of 300 genotypes with diverse origins (tropical or temperate) were common. These results have two important consequences (i) The genetic variability in response to temperature may be less than expected, and restricted to the tolerance to high and low temperatures. (ii) Temperature compensation, widely used in crop models for predicting the rate of progress of phenological stages, can apply to most developmental processes in a wide range of temperature with a biologically-based and parsimonious model (3 parameters). This may have large implications in crop modelling.

### **Tolerance to water deficit**

Photosynthesis and transpiration have essentially the same determinisms, namely leaf area and stomatal control. As a consequence, one cannot reduce transpiration without reducing biomass accumulation. There is an opposition between the avoidance of stress risk, which requires reducing transpiration or invest more assimilates in roots, and a maximum production under mild water deficit which requires maintaining photosynthesis, expansive growth and carbon partitioning to grains, at the cost of a higher risk of crop failure. Tolerance to water deficit is therefore an optimisation process, rather than a tolerance per se (Collins et al. 2008).

We have attempted to identify gene alleles which confer either a plastic response which saves water or a "flat" response which maintains growth under water deficit. For that, we characterise the response curves of genotypes to soil water status and to evaporative demand. For each genotype, common responses were observed in field, greenhouse and controlled conditions. Responses have been mostly established in a phenotyping platform (*Phenodyn* Sadok et al 2007) which allows measurement of leaf growth rate and transpiration in 400 plants simultaneously, with a time definition of some minutes over a large range of environmental conditions. Sensitivities to soil water deficit and to evaporative demand were determined for all genotypes of mapping populations, by the use of response curves whose parameters are valid in several experiments. They can therefore be considered as stable characteristics of each genotype. We then identified QTLs of these sensitivities which were partly common to three mapping populations (temperate or tropical material). These QTLs were confirmed in an analysis of three families of near isogenic lines. The mechanisms driven by genetic responses, in term of cell wall properties, turgor maintenance, root hydraulic conductivity and ABA signalling, have been analysed and will be discussed.

In one mapping population, QTLs of leaf growth maintenance have been compared to QTLs of Anthesis - Silking Interval (ASI) which is strongly linked to silk growth and to yield maintenance under water deficit (Fuad-Hassan A et al. 2008). About half of QTLs were common between leaf and silk growths (Welcker et al. 2007), thereby raising the possibility that source and sink strengths are linked genetically.

A combined genetic - ecophysiological model based on the measured QTL effects on model parameters predicts the time courses of leaf growth under any climatic scenario in genotypes known by their alleles only. This model has been inserted in a whole-plant model which simulates total leaf area and biomass accumulation in the field as a function of environmental conditions (Chenu et al 2008). This opens the way to the use of "virtual genotypes" in breeding programmes, for the evaluation of the appropriate alleles for each climatic scenario.

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# Plant Cell Wall Biology; Solving Old Problems with Functional Genomics

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## Introduction

Primary cell walls of higher plants are comprised predominantly of a framework of cellulose microfibrils, which are embedded in a matrix phase that consists of a range of non-cellulosic polysaccharides, proteins and phenolic compounds. These components form covalently and non-covalently linked molecular networks that have the strength, flexibility and porosity necessary for the varied functions of the cell wall during growth and development. Furthermore, the overall composition of walls, together with the molecular sizes and fine structures of wall polysaccharides, can be altered to accommodate changing requirements for wall properties as cells grow and differentiate. Primary walls of dicotyledonous plants consist of a cellulosic network embedded in a matrix of complex polysaccharides, of which xyloglucans and pectic polysaccharides are most abundant. Walls of the Poales, including grasses and important cereals such as wheat and barley, are constructed in essentially the same way, although glucuronoarabinoxylans and (1,3;1,4)- $\beta$ -D-glucans predominate in the matrix phase of these species, while levels of pectic polysaccharides, glucomannans and xyloglucans are relatively low. During secondary wall development, lignin can be deposited across the wall, but lignin biology will not be addressed here. Cell walls are central determinants of plant form, growth and development, and change in response to environmental and pathogen-induced stresses. Walls play important roles in the quality of plant-based foods for both human and animal consumption, and in the production of fibres during pulp and paper manufacture. In the future, wall remnants in crop residues could be used as a source of renewable fuel. A thorough understanding of wall synthesis, polysaccharide re-modeling and wall degradation will provide essential background information for the manipulation of wall properties and for the improvement of crop quality and processing in the future. Biochemical approaches

The chemical structures of most wall polysaccharides have been defined in detail, but the enzymes involved in their synthesis and re-modeling remain largely undefined. Earlier biochemical studies did however suggest that type I polysaccharide synthases include enzymes that catalyse the iterative incorporation of glycosyl residues from sugar nucleotide donors into molecular backbones that constitute the main chains of wall polysaccharides. These were shown to be integral membrane proteins with multiple transmembrane helices, and have been difficult, if not impossible, to purify by traditional biochemical methods. They are believed to be involved in the biosynthesis of homopolysaccharides such as cellulose, (1,3)- $\beta$ -D-glucan, and possibly (1,3;1,4)- $\beta$ -D-glucan. The other putative function for integral membrane proteins is the synthesis of the backbones of matrix phase heteropolysaccharides, including xyloglucans, arabinoxylans, and glucomannans. A second class of enzymes involved in wall polysaccharide synthesis contains the glycosyltransferases, which are defined in a more restricted sense insofar as they transfer glycosyl residues from the donor to a polysaccharide backbone, in a single event. They have a single transmembrane helix that spans the membrane and functions as an anchor, together with a short cytosolic NH<sub>2</sub>-terminus, an extended luminal hydrophilic stem region and a globular catalytic domain within the lumen of the Golgi, towards the COOH-terminus of the protein. Examples of this second, type II class of enzymes would be the  $\alpha$ -L-arabinosyltransferases, which are assumed to add single  $\alpha$ -L-arabinofuranosyl substituents to the (1,4)- $\beta$ -D-xylan backbone of arabinoxylans, and the  $\alpha$ -D-xylosyltransferases, which add single  $\alpha$ -D-xylosyl residues to the (1,4)- $\beta$ -D-glucan backbone of xyloglucans. Despite the concerted efforts of many biochemical groups around the world, very little progress was made with the purification of these

membrane enzymes from plant extracts. As a result, amino acid sequence information was not generated and it was not possible in the early days of molecular biology to take this route to the identification of the genes encoding plant cell wall polysaccharide synthases. Functional genomics, emerging functional genomics, molecular genetics and X-ray crystallographic technologies are now allowing us to re-examine the central questions related to wall biosynthesis. The availability of the rice, *Populus* and *Arabidopsis* genome sequences, various mutant populations, high density genetic maps for cereals, high throughput genome and transcript analysis systems, extensive publicly available genomics resources, and an increasing array of analytical systems for the definition of candidate gene function, are allowing us to take a much broader, non-biased approach to the description of wall biosynthesis in plants. For plant cell wall biosynthesis, the polysaccharide synthases are now believed to be encoded by multi-gene families of the cellulose synthase (CesA) and cellulose synthase-like (Csl) groups, possibly in concert with other genes. The CesA and Csl enzymes are large proteins, with about 1000 amino acid residues, and contain three to six transmembrane helices towards the COOH-terminus and one to two towards the NH<sub>2</sub>-terminus. They share a common DDDQxxRW motif, which is believed to be involved in sugar binding and catalytic activity of the enzyme. However, reverse genetics approaches based on the identification of candidate genes have not been particularly successful, partly because transcript levels of key genes are often low and are difficult to correlate with wall composition. The biosynthesis of (1,3;1,4)- $\beta$ -D-glucans In the case of the biosynthesis of the cereal (1,3;1,4)- $\beta$ -D-glucans, which are found almost exclusively in the walls of the Poaceae, early interest in QTL mapping of (1,3;1,4)- $\beta$ -D-glucan levels in barley grain enabled a forward genetics approach to be adopted. In this way it was shown that a cluster of the monocot-specific CslF genes in rice is located in a genomic region corresponding to one containing a major quantitative trait locus (QTL) for grain (1,3;1,4)- $\beta$ -D-glucan content in barley. When rice CslF genes were expressed in *Arabidopsis* and (1,3;1,4)- $\beta$ -D-glucan was detected in the walls of transgenic *Arabidopsis* lines, it was concluded that the rice CslF genes encode polysaccharide synthases that are essential for the synthesis of the (1,3;1,4)- $\beta$ -D-glucans of monocot cell walls, although the participation of other enzymes or ancillary proteins could not be precluded (Burton *et al.*, 2006; 2008). Genetics of (1,3;1,4)- $\beta$ -D-glucan biosynthesis Examination of the CslF gene subfamily of barley revealed the presence of seven HvCslF genes and their corresponding cDNAs have been isolated and sequenced. The genes have been assigned positions on genetic maps from a Clipper x Sahara mapping population and the rates of transcription of individual members of the gene subfamily have been quantitated in a number of organ and tissue extracts from barley. In some cases the genes are collocated with QTL for (1,3;1,4)- $\beta$ -D-glucan levels in barley grain, and new association mapping techniques have enabled the identification of other loci that appear to be involved in the regulation of (1,3;1,4)- $\beta$ -D-glucan biosynthesis. Manipulation of (1,3;1,4)- $\beta$ -D-glucan levels in barley grain In order to manipulate (1,3;1,4)- $\beta$ -D-glucan levels in barley grain, over-expression studies have been undertaken in transgenic barley lines. The results of these experiments suggest that levels of (1,3;1,4)- $\beta$ -D-glucan levels in barley grain can be altered in this way, but that many genes are likely to be involved in the process.

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# Integrated Approaches To Wheat Improvement Under Drought

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## Introduction

Water is essential to sustaining human and environmental health but is at scarcity level in many countries. The availability of water is also a major determinant of world-wide crop yield. Wheat is the most widely grown crop both in the UK and worldwide. Even in the UK wheat yield losses due to drought average 1-2 t ha<sup>-1</sup> but the losses are much greater in other countries with higher temperatures and lower rainfall. The predicted changes in climate patterns are projected to increase the losses. Although there is genetic variability for drought tolerance and yield in wheat germplasm, drought tolerance is a complex and multigenic trait which makes it difficult to breed for.

Durum wheat is one of the most widely cultivated crops in the Mediterranean basin. It is mainly grown under rain-fed conditions characterized by drought and thus water is a major determinant of final yield. Molecular genetics and genomic tools offer new opportunities to identify allelic variation, study candidate genes and select loci defining responses and yield adaptation to drought. A number of EU projects funded in FP5 and FP6 have exploited multidisciplinary approaches to investigate the genetic basis of drought resistance and stability of yield in durum wheat.

## Methodology

The OPTIWHEAT and TRITIMED projects have integrated crop physiology and genomics approaches (forward- and reverse-genetics) to identify loci controlling wheat responses and growth under drought conditions, while integration of research knowledge has been promoted by the EU projects OPTIMISE and WUEMED. In order to better understand the relationship between genotype, component traits, and environment over time we are adopting a multidisciplinary approach. By integrating genetics, genomics, soil science, crop physiology, biochemistry and biomathematics and agronomy we aim to both understand the drought response and identify candidate genes, QTLs and component traits that can be used to develop crops with high stable yield under drought. Candidate are being evaluated by TILLING an EMS-mutagenised durum wheat lines, ECOTILLING panels of accessions or production of transgenic plants.

## Results

A large TILLING population of Durum wheat (4500+ unique lines at M2,) has been produced from EMS mutagenised seed. M2 DNA stocks and M3 seed for the TILLING

population are archived at two sites. This material will be made available to interested researchers and breeding companies. The population is being taken to M4 by single seed descent. We have identified more than 20 lines for two target genes, *GA20ox1* and Rubisco activase, by TILLING within the mutant population. We have defined natural variation in cultivars in the IDUWUE and TRITIMED panels by ECOTILLING. Datasets for field trials were generated to determine the agronomic performance of genotypes grown with and without drought. We have identified QTLs associated with yield and yield stability under drought. A list of candidate gene sets associated with Durum wheat stability of yield under drought stress has been created.

### **Conclusions**

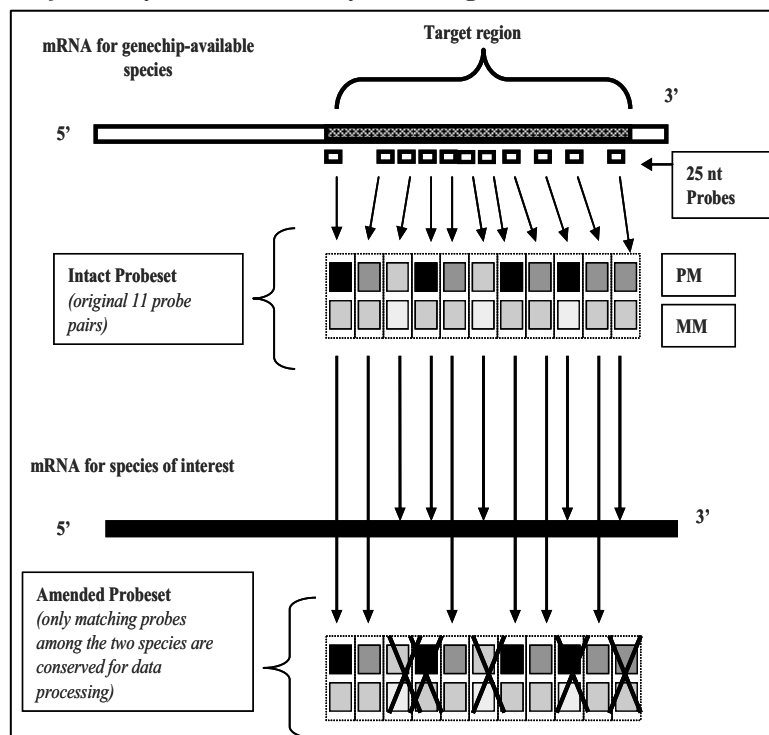
Employing an approach that integrates genetics, genomics, soil science, crop physiology, biochemistry and biomathematics and agronomy can increase our understanding of the drought response and identify candidate genes, QTLs and traits that can be used to develop crops with high stable yield under drought.

# Development of Bioinformatic Tools Aimed at Spreading Microarray Profiling Techniques in Wild and Non-Model Crops

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Identification of plant ‘autonomous’ strategies aimed at counteracting environmental stresses helps, upon introgression in commercially-valuable crops, reduction of external inputs, and therefore is a key aspect for development of sustainable agriculture. However, a major constraint toward identification of those wild defence strategies and associated genes in various germplasm is the lack of high-throughput expression profiling platforms for many less-studied (including wild relatives of model species) crop species. In fact, high-quality, standardized microarray platforms as Affymetrix oligonucleotide arrays (GeneChip) are only available for a dozen of species, namely *Arabidopsis*, sugarcane, tomato, *Vitis vinifera*, soybean, rice, barley, *Medicago*, cotton, *Citrus*, maize, and poplar. On the other hand, a



conspicuous number of expressed sequences are available for a variety of plants (including wild relatives of crop species). In fact, more than 1,000 ESTs or full-length cDNA are available for 254 plant species (e.g. see Childs et al., 2007). Finally, a wide range of further species presents several hundreds sequences and a GeneChip-based profiling approach would be extremely advantageous over traditional methods.

However, no high quality, commercially available and standardized chip platform is likely to be developed for those above mentioned species unless a relevant number of customers are interested in its purchase.

**Figure 1.** Outline of probe selection procedure for heterologous hybridizations

## Methodology

We have developed several bioinformatic strategies allowing to conduct accurate microarray expression profiling experiments for species which, despite availability of some sequences, lack an associated microarray platform. A previously tested approach consisted in production of a “Global Match File” (Bagnaresi et al., 2008). This file was created by blasting all available sequences from two species (the one of interest, potato, and the “GeneChip available” one, tomato). This allowed retrieval of best matching orthologs and annotation, alignment quality evaluation, keyword searches and establishment of a core set of highly homologous genes. Here we report on a further strategy being currently developed. This novel strategy improves the Global Match File approach and exploits some

peculiar features of Affymetrix GeneChips. In fact, in GeneChip design each gene is represented by a cluster of oligos (usually 11 oligos called “PM probes”, 25 nt long). These are designed towards the 3’ end of mRNA region called “target” region (few hundreds up to 500 nucleotides long, see Fig. 1). The PM probes are flanked by MM probes, identical to the PM counterpart but with a mismatch in the middle of the sequence (13<sup>th</sup> nucleotide). MM probes are conceived to capture sequence-independent noise to be subtracted from genuine (sequence-specific) one. Each of the 11 PM-MM couples are scattered throughout the chip, but are virtually grouped in an entity called “probeset” which assembles the 11 PM and MM probes referring to the same gene. The probes are tracked by their physical coordinates that are stored in dedicated organizer files (Chip Description File, CDF). Thus, each gene can rely on 11 independent data sources for hybridization intensity calculation. This redundancy in some cases helps reducing errors in signal estimates which may arise due to cross-hybridization and noise. However, in most cases, 4 to 3 probes have been shown to be sufficient for an accurate estimate of signal (Lu et al., 2007). In extreme cases (e.g. just one probe left), signal intensities can still provide a rough data to be validated with additional techniques as Real-Time PCR.

## Results

To allow for heterologous hybridizations to be conducted as homologous ones in the highly-standardized GeneChip platform, we developed a probe level masking procedure as follows:

- i) A brute-force algorithm consisting of a sliding window analyzes ESTs from species of interest (window size can be arbitrarily set; suggested values are from 17 to 25 nt to ensure hybridization) by matching to Affymetrix probe sequences (obtained from closest, GeneChip-available species). Hits (17-25 contiguous nucleotides in the EST to the probe of a specific probeset) are recorded
- ii) Probes recorded as hits are ordered based on their correspondence from 5’ to 3’ in the target
- iii) Two equivalent outputs are generated: a probe mask list (to be used with dCHIP software) and a custom CDF file which eliminates unrecognized probes from probesets (see also Table 1)

**Table 1**

Procedure	Output	Compatibility
Probe set masking for dCHIP	Small text file containing probe masks as: Probeset_ID 1 3 4 5 6 7 10. The numbers refer to probe number (from 1 to 11) within probeset to be excluded from computation.	dCHIP software
Generation of custom CDF	Custom CDF (text file) where probe coordinates from non-matching probes are eliminated.	Various software

The above methods are being incorporated in a VB6 script (“Easymask”) to facilitate unassisted masking procedures.

## Conclusions

The probe masking procedure here described allows conducting high-quality microarray experimentation even for species devoid of a dedicated GeneChip. In particular, an heterologous hybridization is turned to an homologous one as the mismatching sequences are excluded from data analysis. Availability of high-throughput expression profiling techniques will help pinpointing resistance mechanisms of less-studied species (e.g. wild relatives of crops) and therefore may reveal critical for introgression of resistance traits, a key aspect for development of sustainable agriculture.

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# The Genetic Improvement of Pyrethrum (*Chrysanthemum Cinerariaefolium* L.): a Biotechnological Approach

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Pyrethrum (*Chrysanthemum cinerariaefolium* L. = *Tanacetum cinerariaefolium* (Trev.) Schultz-Bip.) is a perennial herbaceous plant belonging to the family *Asteraceae*, native to Albania and the area of former Yugoslavia. Pyrethrum is the only species in the genus *Tanacetum* having an agronomic importance, although the genus consists of several species producing similar types of bioactive metabolites. The species is grown in order to obtain the insecticidal compounds collectively termed pyrethrins, which are found primarily in the flower head. Pyrethrum may be easily propagated by seeds, vegetative splits, stem cuttings (rooted or not under mist), and tissue culture. The first attempts to introduce its cultivation into the semi-arid Mediterranean environments have brought to satisfactory results, and the species has shown a good response in terms of biomass and flowers yield, even when technical inputs were applied in a reduced amount. Much work must still be done, however, in order to set a properly detailed management protocol for the genetic improvement of the species by means of biotechnology. In this work we discuss the first results of a specific experimental activity aimed to point out a micropropagation protocol for *Pyrethrum*, with the purpose to optimize the *in vitro* culture conditions, using explants sources as tissue donors for protoplast isolation and setting preliminary experiments in protoplast fusion methods to improve the flowers yield per plant and to increase the pyrethrins level.

## Methodology

*Pyrethrum* plants were collected at the beginning of 2008 from a two-year collection field already set in the experimental farm Sparacia (Cammarata, AG – Sicily) and transplanted at the Institute of Plants Genetic, division of Palermo.

Establishment of *in vitro* culture. In order to establish a proper *in vitro* culture technique, several plant materials (leaves and shoots) and sterilization protocols have been tested (table 1). The explants were put in a MS medium (Murashige and Skoog, 1962), supplemented with 30 g L<sup>-1</sup> sucrose, 3 mg L<sup>-1</sup> BAP and 0.5 mg L<sup>-1</sup> NAA. The pH of the medium was adjusted to 5.7, gelled with 0.8% agar and autoclaved (20 min., 120 °C).

Germination tests. Preliminary germination tests were performed on mature *Pyrethrum* seeds, by means of two different protocols: in the first case, after a pre-treatment of seeds at 4 °C for 48 hours, sterilization was attained by means of NaClO 15% for 10 min and 3 rinsings with sterile distilled water. 15 seeds for each plate were put to germinate on sterile filter paper. In the second case, seeds were pre-treated at 4 °C in a 50 ppm gibberellic acid solution for 24 hours. Seeds sterilization was performed as above, and seeds were further transferred for germination on a MS medium, supplemented with 50 mg L<sup>-1</sup> sucrose, 8 g L<sup>-1</sup> agar and 3 mg L<sup>-1</sup> BAP. In both cases, seeds started germinating after about 10 days; after 1 month approx., the new plantlets were transferred on Magenta boxes, with the same growing medium. All tests concerning establishment *in vitro* and germination were carried out in rooms at controlled temperature set at 25 ± 1 °C, under a photoperiod of 16 hrs light with an intensity of 30 μmol m<sup>-2</sup> s<sup>-1</sup>.

Isolation of protoplasts. Leaf tissue was used for protoplast isolation, by means of two different enzyme solutions (table 2).

Table 1 – Protocols used for the disinfection of *Pyrethrum* explants for *in vitro* establishment.

Steps	I	II	III	IV	V	VI
1°	Washing (water)	Washing (soap and water) (Tween 20)	Washing (soap and water) (Tween 20)	Washing (soap and water) (Tween 20)	Washing (soap and water) (Tween 20)	Washing (soap and water) (Tween 20)
2°	Ethanol 75% (3 min)	HCl 1mol L <sup>-1</sup> (few sec.)	Ethanol 75% (3 min) + PVP at 1%	Ethanol 75% (40 sec)	Ethanol 75% (40 sec)	Ethanol 75% (40 sec)
3°	15% NaClO (15 min)	15% NaClO (20 min)	20% NaClO (25 min) + PVP at 1%	20% NaClO (20 min) + PVP at 1%	20% NaClO (15 min) + PVP at 1%	15% NaClO (15 min) + PVP at 2%
4°	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet	3 rinsings (5 min each) with sterile distilled water, under a Laminar Flow Cabinet

Table 2. Composition of the enzyme solution used for *Pyrethrum* protoplast isolation

	Protocol 1	Protocol 2
Macerozyme	1.00 %	0.20 %
Cellulase	1.00 %	0.25 %
Pectolyase	0.20 %	0.08 %
Sucrose	0.70 M	0.50 M
CaCl <sub>2</sub> · 2H <sub>2</sub> O	12.00 mM	5.00 mM
MES	6.00 mM	-----

## Results

The methodology for surface sterilization of explants has undergone, as shown in table 1, a progressive amelioration, as an effect of the results obtained both in the count of contaminations and in browning of tissues. In the first attempts for the *in vitro* establishment, many difficulties have been met, due to the high level of pollution on all kinds of explants, especially on leaves. By modifying times and concentrations of the solutions used for sterilization we came to the final protocol, that allowed, besides a marked reduction of contaminations, also a qualitative improvement of the obtained material. The tissue browning, detected on leaves and above all on shoots, was a major obstacle to the *in vitro* establishment of *Pyrethrum*, that was overpassed starting from the application of the sterilization protocol III by means of the addition of PVP (polyvinyl pyrrolidone; Housti et al. 1992). With the aim to improve such a result, we decided to raise its concentration up to 2%. In germination trials, the two tested protocols did not show any significant difference in germination time and percentages, whereas the growing of plantlets was markedly faster on MS medium. Protoplasts isolation is still under study, in order to evaluate the response to different concentrations of enzyme solutions.

## Conclusions

Although many initial difficulties in pointing out the sterilization protocols, the *in vitro* establishment of *Pyrethrum* was stated to be a practical possibility. Our results may represent the starting point for a further oriented research activity.

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# Genetic and Agronomic Aspects of Heterosis in Maize: QTL Analysis, Development and Evaluation of Near Isogenic Lines for Heterotic QTL

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Heterosis indicates the hybrid superiority over its parental lines and is often found for traits of agronomic importance. Heterosis is sizable for allogamous species, especially maize (*Zea mays* L.), and is crucial in crop sustainability through the optimization of yield physiology. Hybrid superiority has been extensively exploited but, despite a century of investigations (Shull, 1908), its genetic basis is not completely understood, yet. On the other hand, there is today a renewal in the attempt to understand the mechanisms of heterosis, owing to the availability of new genetic tools. In this connection, we undertook a three-step research with the objective to gain information on agronomic and genetic aspects of heterosis in maize. We first conducted a study to detect Quantitative Trait Loci (QTL) affecting heterosis. A set of 142 Recombinant Inbred Lines (RILs), derived from the single cross B73 H99, was crossed to the three testers B73, H99 and B73 H99; then testcrosses were evaluated, together with the RILs, in three environments. Several QTL for agronomic traits were detected and most of them were characterized by dominant or overdominant gene action (Frascaroli et al. 2007). The most important QTL for grain yield overlapped with QTL for other traits like plant height and yield components, suggesting that QTL affected first these traits and then grain yield, with a pleiotropic gene action. As it has been recently reviewed (Holland 2007), the ultimate objective of QTL mapping is to identify the causal genes that underlie them. For this purpose, as a second step, we conducted a Marker Assisted Selection (MAS) aimed at developing pairs of Near Isogenic Lines (NILs) for six QTL, i.e., lines sharing the same genetic background but differing for the parental alleles at one QTL.

In agronomic evaluations of maize breeding programs, it has been observed that, despite the increased hybrids' productivity, the level of heterosis has not substantially changed in the last decades, unless under stress conditions (Duvick, 2004). An intriguing area of research might be thus to look at changes that have accompanied the increase of heterosis under stressful conditions. The objective of this third step of the study was to investigate the role of QTL affecting heterosis in coping with stress, by evaluating NIL pairs crossed with related testers at low and high plant densities.

## Methodology

Six pairs of NILs, corresponding to six QTL, were crossed with the two parental inbred lines B73 and H99. The 24 testcrosses are now being evaluated in a multi-year research conducted over several environments and at low and high plant densities (4.5 and 9.0 plants m<sup>-2</sup>, respectively). Traits for which strong heterosis was detected in our previous studies were evaluated and results obtained in the first year trial are herein presented.

## Results

ANOVA, not shown, revealed significance of density and QTL effects for most QTL and traits, but only of few interactions density QTL effect. In particular, mean values over testcrosses and QTL genetic parameters (i.e., additive and dominance effects) are shown in Table 1 for an important QTL located on chromosome 3, in the bin 5. As expected, the high plant density lead to a higher plant height and to lower grain yield per plant and kernel weight as compared with the low plant density.

Table 1. Testcross mean values and genetic parameters for QTL 3.05

	Plant height (cm)		Yield per plant (g)		Kernel weight (mg)	
	Low density	High density	Low density	High density	Low density	High density
Mean	193.6	205.2** <sup>(1)</sup>	194.4	115.1**	262.4	257.2*
Additive effect <sup>(2)</sup>	1.3	0.8	1.9	2.0	0.9	0.2**
Dominance effect <sup>(3)</sup>	0.2	2.1**	10.3	8.4	4.4	8.5*
Additive effect (%) <sup>(4)</sup>	0.7	0.4	1.0	1.7	0.3	0.1
Dominance effect (%) <sup>(4)</sup>	0.1	1.0	5.3	7.3	1.7	3.3

<sup>(1)</sup> \*, \*\*: comparison between low and high density significant at P 0.05 and at P 0.01, respectively;

<sup>(2)</sup> as the difference between mean values of the two NILs across the combination with the two testers;

<sup>(3)</sup> as the difference between the mean of the two heterozygotes and the mean of the two homozygotes;

<sup>(4)</sup> additive or dominance effects as percentage of the mean.

Additive effect showed a decline with high density (significant for kernel weight only), while dominance showed a significant increase for plant height and kernel weight. As for yield per plant, no significant variation was observed for dominance, likely due to scale effect. In fact, yield per plant mean value largely decreased due to stress conditions, and the contribution of dominance expressed as percentage of the mean shows an increase from 5.3% in non-stress to 7.3% in stress condition.

The role of heterosis in less-than-optimal conditions is in accordance to what observed by Duvick (2004), i.e., that the relative importance of heterosis was greater at the higher plant density. The relevance of heterosis has been reported also for other stresses, e.g., drought (Betran et al., 2003) and excessive moisture (Zaidi et al., 2007), suggesting that common adaptive mechanisms are involved and/or hybrid vigour can confer greater plasticity to adapt to unfavourable environmental conditions (Echarte and Tollenaar, 2006).

## Conclusions

These preliminary findings confirmed the importance of heterosis in coping with stress, stimulating us to continue this research aimed at gaining a better understanding of heterosis, with emphasis on its role in enhancing crop sustainability.

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# Genomic Tools for the Study of the Adaptation of Barley Crop to Climatic Constraints

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Apart from being an important agricultural crop for food and feed, barley has been largely exploited worldwide as a model species for biological research, due to its inbreeding habit and the diploid system, which make the inheritance studies easy to perform (Hayes et al. 2003).

Abiotic stresses are responsible for significant yield losses in barley on a worldwide scale, and yet under severe stress conditions, barley is one of the most important sources of energy for human food and animal feed. Recent advances in the genetic and molecular understanding of abiotic-stress responses in barley resulted in the identification of single loci, Quantitative Trait Loci (QTL) and genes related to adaptation of the crop to the agricultural environment (Cattivelli et al. 2002).

A new resource for the study of abiotic stress tolerance in barley, the 'Nure' (winter) x 'Tremois' (spring) mapping population, has been exploited in the present work to map QTLs of adaptation of barley to climatic constraints.

## Methodology

One hundred and eighteen doubled-haploid (DH) lines were derived by anther culture from the F<sub>1</sub> of the cross of 'Nure' x 'Tremois' and used for linkage and QTL analyses (Francia et al. 2004). The winter parent – 'Nure' – is a modern, high yielding, two-rowed feed-barley cultivar with a wide range of adaptability and high cold tolerance. The spring parent – 'Tremois' – is a modern, high yielding, French two-rowed malting variety adapted to fertile environments and susceptible to low temperatures. SSR, AFLP and DArT molecular markers were screened for polymorphism and selected for mapping. Candidate Genes known to be involved in barley abiotic stress response and plant development were re-sequenced in the two parental genotypes in order to find Single Nucleotide Polymorphisms and develop PCR-based markers for their mapping (Tondelli et al. 2006). Linkage map was constructed using JoinMap v4.0.

Multilocal irrigated vs. non-irrigated field trials of the NT population have been carried out during two years (2003/04 and 2004/05) in Mediterranean Europe, North Africa and West Asia to map QTLs of yield and drought adaptation traits, under the frame of the European MABDE project. Moreover, three independent measures of low-temperature tolerance were scored on the NT segregating population: winter survival under field conditions, frost resistance in a controlled freezing test, and evaluation of the functionality of the PSII reaction center after freezing at -12°C (F<sub>v</sub>/F<sub>m</sub> value). Finally,

the DH lines were grown under different controlled conditions (phytotron) of photoperiod and vernalization, and heading dates were scored. The software MapQTL v5.0 was used to determine the genomic regions controlling the described traits.

## Results

The final ‘Nure’ x ‘Tremois’ linkage map is composed of more than 500 molecular markers, mainly DArT markers. Candidate genes (CGs) involved in barley response to abiotic stresses (e.g CBF-DREBs and MYBs transcription factors) and plant development (e.g. MADS-Box and FT-like transcription factors) have been placed on the NT map, as well.

Yield performance of the NT population differed markedly in the irrigated vs. non-irrigated field trials, ranging from just under 0.5 t/ha (in Spain and Jordan not irrigated sites) to over 5.5 t/ha (in Syrian irrigated field trial). QTL analyses of yield and yield components from 16 independent field trials is under way and could reveal the importance of loci other than the familiar major developmental genes present in barley. The NT population is also a good genomic model for frost tolerance, since two major loci of tolerance, *Fr-H1* and *Fr-H2* on chromosome 5H segregate, each one with a given Candidate Gene. This makes possible to dissect the population into discrete phenotypic classes of tolerance to frost.

In a further experiment, QTLs controlling heading date under different photoperiod and vernalization conditions were placed on chromosomes 5H (coincident with *Vrn-H1*, a major locus for vernalization requirement), 4H (*Vrn-H2*) 1H, and 2H. The expression levels of a MADS-Box transcription factor co-segregating with the QTL of 2H, were evaluated in the ‘Nure’ and ‘Tremois’ parents in order to confirm its involvement in the control of barley flowering time. Finally, the agronomic behaviour of the groups of DH lines differentiated for alleles carried at the QTL markers was surveyed.

## Conclusions

The ‘Nure’ x ‘Tremois’ DH population represents a valuable tool to study in a unique genetic system several agronomic traits, in particular the adaptation of the crop to climatic constraints. Candidate genes (CGs) involved in barley response to abiotic stresses and plant development have been showed to co-segregate with QTLs related to plant adaptation to such constraints, and their functional characterization has been undertaken.

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# Promising Genetic Resources for Mediterranean Durum Wheat Yield and Quality Improvement

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**Key-words:** durum wheat, water stress, adaptation, grain yield, grain quality

In Morocco, the area planted annually with cereals varies around 5.3 million hectares, i.e., 60% of total agricultural lands. The production constitutes 43% of total consumption (Bartali E., 1995). About 1.2 millions hectares of durum wheat (*Triticum durum* L. var. durum) are sown annually with an average yield quite low (1,1 t/ha; ONICL 2004). Morocco is ranked third in the Mediterranean region and first in the North Africa and Middle East region in term of durum wheat acreage. The average durum wheat consumption is about 90 kg/person/year. Arid and semi arid regions (60% of the Moroccan lands) are characterized mainly by drought and poor rainfall distribution within the durum growing season (El Mourid et Karrou, 1996). Due to these reasons, the average yield is variable and ranges from 0.5 to 1.2 t/ha (Jouve, 1988). Additionally, grain quality of the new cultivars is not high and needs to be improved. The objective of this study was to identify new genetic resources to be used in breeding programs aimed at improving the Mediterranean local varieties.

## Methodology

Seventy six Mediterranean durum accessions including old local genotypes were considered. These genetic materials were evaluated in field trials conducted at Sidi El Aidi (SEA) and Marchouch (MCH), two INRA's research stations representing semi-arid and favorable rainfall regions, respectively. The field trials were carried out according to an augmented design with five replicated (six plots) checks (Haurani, Waha, Kourifla, Gidara2 and Mrb5).

The following morpho-physiological traits were recorded: plant height (PH), days to heading (DH), days to maturity (DM), number of spikes per square meter (NSM), specific weight (SW), number of grains per spike (NGS), thousand kernel weight (TKW) and grain yield (GY). In the laboratory, quality parameters were measured on 21 accessions characterized by different yield potential (high, medium and low). The quality parameters evaluated were: Sodium Dodecyl Sulphate Sedimentation test (SDS), vitrousness (VR), yellow index (YI) and ash content (AC). The accessions were also examined for gliadin and glutenin subunits composition.

## Results

The ANOVA showed significant differences among genotypes for all the morpho-physiological traits and for the grain quality parameters measured; genotype by environment interaction was also significant.

Principal component analysis (PCA) was also performed using the data of each location. The first axis of PCA explained 38.8 and 39.9% while the second axis explained 20.2 and 19.0%, respectively for MCH and SEA. In the two environments, the correlation pattern between traits was quite different.

More in details, DH and DM were correlated with plant height in both locations. In SEA site, NSM, GW, TKW, NGS and SW were positively correlated with yield, while in MCH, NSM and NGS were the only traits significantly correlated with grain yield. These results suggest that different morpho-physiological mechanisms contributed to determine the variability in yield among accessions in the different locations. Cluster analysis grouped the accessions independently from their origin.

For grain quality, the results of electrophoretic analysis shows that 58% of the accessions carried  $\gamma$ -45-gliadin subunits correlated with strong durum wheat gluten band. In general, as evidenced also by Nachit et al. (1995), the accessions with high SDS have a  $\gamma$ -45-gliadin. Indeed, 77% of the accessions with  $\gamma$ -45 gliadin have an SDS quite high, i.e. greater or equal to 37 ml. For the checks, Waha and Mrb5 have the  $\gamma$ -45-gliadin, and the others have  $\gamma$ -43-gliadin. For SDS, VR, YI and AC, high genetic variability was identified among the investigated accessions. Therefore, this germplasm represents a promising gene pool for durum wheat crop improvement. Principal component analysis explained 67.2% of the variability. VR and YI were correlated positively, but YI and SDS were moderately negatively correlated. No significant correlation was detected between the other traits. The accession clustering shows that the origin of accessions was not correlated with the quality traits.

Relationships between quality parameters and yield appear to be influenced, by climatic conditions during grain filling. Thus, depending on temperatures and water input during this phase, correlation coefficients between some quality traits and grain yield, can be either positive, negative or close to zero. Consequently, breeders may need to take into account these climatic variables during grain filling in order to identify the best genotypes with acceptable technological quality.

## Conclusions

The evaluation of local materials and improved lines shows the presence of a sizeable variability for the morpho-physiological and grain quality traits herein investigated. The origin of accessions did not show any particular association with the morpho-physiological or the quality traits, thus indicating the feasibility of using them in breeding programs across the Mediterranean Basin aimed to improve durum wheat yield and quality. Additionally, grain quality was found to be independent from adaptation to different environments.

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# Temporal Expression of Enzymes Involved in Amino Acids Metabolism in Developing Quality Protein Maize Seeds

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The content of essential amino acids such as lysine and threonine is limited in cereals resulting in low nutritional quality of storage proteins in mature seeds. These amino acids and also methionine share a common precursor, aspartate, in a branched and complex pathway (Azevedo et al. 2006). Six enzymes are required for the final steps of lysine synthesis following the formation of dihydrodipicolinate. *LL-DAP aminotransferase* (LL-DAP-AT, EC 2.6.1.17) enzyme is involved in the step from tetrahydrodipicolinate to diaminopimelate in higher plants but at the present time the precise route is not known. On the other hand, *O*-phosphohomoserine produced by the action of homoserine kinase (HK, EC 2.7.1.39), is an important intermediate of the aspartate pathway since it is the branch-point where threonine and methionine synthesis diverge. *O*-Phosphohomoserine can be converted to threonine by the action of threonine synthase (TS, EC 4.2.99.2), the fifth enzyme involved in the synthesis of threonine from aspartic acid. In order to better understand the regulation of some of the key enzymes controlling lysine and threonine metabolism in the maize endosperm during development, we performed relative quantification gene expression of LL-DAP-AT and TS. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected as reference gene.

## Methodology

Gene expression studies were carried out using immature seeds by harvesting maize ears directly into liquid nitrogen at 14, 20 and 24 days after pollination (DAP). The plant material used were a wild type maize line (L161) and two Quality Protein Maize (QPM) lines (L161o and L161q). Total RNA extraction was performed using Trizol reagent based on the single-step RNA isolation method developed by Chomczynski and Sacchi (1987). The cDNA amplification was carried out according to Invitrogen SUPERScript<sup>TM</sup> Kit. Highly purified primers for target gene *LL-DAP-AT* (forward primer AAA TAC GTC GGG TTC ACA GG; reverse primer CAC GTT GAA TCC GAG TGA TG), *TS* (forward primer CGG TAT GGA GGA GGC TA; reverse primer CAC TGA TCG GTG GAT TAG CA) and for the reference gene *GADPH* (forward primer TCT GAA TGG CAA GCT CAC TG; reverse primer AGC TTG ACG AAG TGG TCG TT) were generated commercially (IDT, Integrated DNA Technologies, Inc) for real-time PCR-based quantitative assay. Real Time RT-PCR were done according to Kit Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). The mathematical model used is based on the PCR efficiencies and the mean crossing point deviation between the sample and control group (Pfaffl et al. 2002).

## Results

Figure 1 shows the melting curve, fluorescence over cycling data and standard curve for each gene: *TS*, *LL-DAP-AT* and *GAPDH*. Real time PCR efficiencies (*E*) (Table 1) were calculated from the slopes given in LightCycler software (BioRad iQ5 Optical System software) and showed rates for *LL-DAP-AT* ( $E_{LL-DAP-AT} = 0.96$ ), *TS* ( $E_{TS} = 1.18$ ) and *GAPDH* ( $E_{GAPDH} = 1$ ). In developing grains, *LL-DAP-AT* was induced in both QPM lines at 14 days after pollination (DAP), and an enhanced expression was observed at 20 DAP in L161o. At 24 DAP, the line L161q exhibited up-regulation for both analyzed genes and *LL-DAP-AT* was three times more expressed than *TS*.

## Conclusions

These results are an important part of a broader picture to understand the mechanisms responsible for increased protein quality in the QPM varieties that would enable more rapid and significant improvement of maize nutritional quality, particularly when lysine, threonine and methionine are concerned, since different expression of these key regulatory steps may regulate the balance between the different branches of the pathway leading to the increased synthesis of one or another amino acid.

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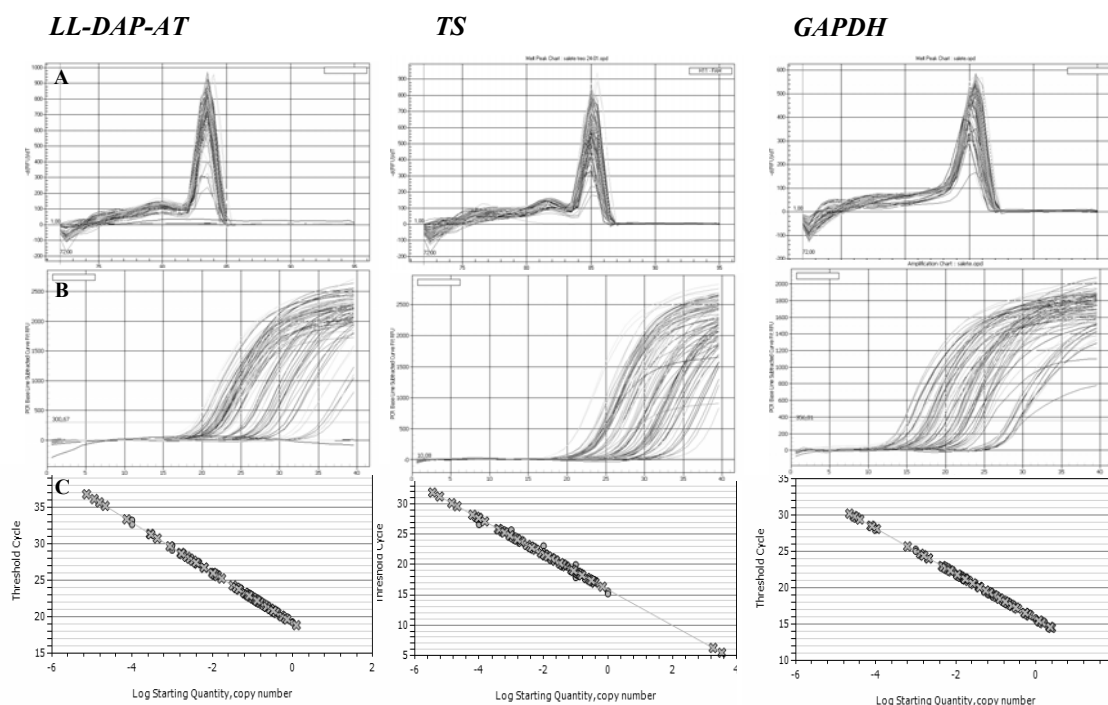


Fig. 1. LL-DAP-AT, TS and GAPDH genes. (A) melting curve, (B) dF/dC and (C) Standard Curve.

Table 1. LL-DAP-AT, TS and GAPDH Relative Expression Report from opaque (OP) and QPM (Q) maize lines.

Gene	Type	Reaction Efficiency (%)	14DAP	20DAP	24DAP
TS OP	Target	0.962	2.0	3.2	8.3
AT OP	Target	0.997	<b>2.9*</b>	<b>12.8*</b>	6.3
TS Q	Target	0.962	2.1	0.1	<b>8.7*</b>
AT Q	Target	0.997	<b>2.8*</b>	0.1	<b>23.2*</b>
GAPDH	Reference	0.999	1	1	1



# Study of Genetic Diversity of the Wheat Leaf Rust Fungus *Puccinia triticina* in Morocco Using AFLP

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## Introduction

Leaf rust is a widespread and persistent problem through the world. Much of breeding effort against leaf rust in wheat has been concentrated on genes for race-specific resistance. Race specific resistance to leaf rust typically remains effective for only a few years, and recent studies suggest that the available resistance genes in hexaploid cultivated wheat, *Triticum aestivum*, have been nearly exhausted (Anikster *et al.*, 2005; Bai *et al.*, 1994; Moseman *et al.*, 1985).

The commonly found alternate host of *P. triticina* in the temperate zones is *Thalictrum speciosissimum* L. (in the Ranunculaceae). In the Mediterranean region, *Anchusa italica* Retz. (in the Boraginaceae) was described as the alternate host for leaf rust of wheat. (D'Oliveira *et al.*, 1966; Ezzahiri *et al.*, 1992). Virulence markers may represent only a small portion of the total genetic variation present in the population. In plant pathogenic fungi, DNA-based molecular markers such random amplified polymorphic DNA (RAPD) have been assessed for usefulness in characterizing genetic variation in cereal rust populations and are generally assumed to be independent of host selection (Kolmer *et al.*, 1995). Use of a more powerful technique such as amplified fragment length polymorphism (AFLP) technique (Vos *et al.*, 1995) may allow molecular differentiation of phenotypes that differ for virulence yet have identical RAPD phenotypes. (Kolmer *et al.*, 1999).

The objective of this study was to examine the molecular genetic diversity of *P. triticina* collections in Morocco using the amplified fragment length polymorphism (AFLP) technique and to determine how the variation is partitioned within and between different geographic locations, using Abda-Doukala, Chaouia-Tadla, Gharb-Saïss, and tangérois as a case study.

## Methodology

Sampling and multiplication of the fungus:

Wheat leaves bearing uredinia of wheat leaf rust (*P. triticina*) were collected from 2005 to 2006 throughout commercial fields of wheat in Morocco.

38 isolates of Urediniospores of *Puccinia triticina* were multiplied in a local sensible variety "Fertas" and were collected with a cyclone spore collector into a size-00 gelatin capsule and stored in desiccators at 4°C.

DNA extraction:

DNA was extracted from urediniospores using a cetyltrimethyl ammonium bromide (CTAB) protocol with one chloroform step. (Villaréal *et al.*, 2002).

AFLP protocol, electrophoresis and gel staining:

The DNA of each isolate underwent a restriction digest with Pst I and Tru 91, the total mixture was incubated at 37°C for one hour. For the ligation, the restricted DNA was incubated in presence of PstI adapter and Mse I adapter for 3 hours at 37°C.

After a pre-amplification with non selective AFLP primers, the final amplification was done with five combinations of selective primers.

Final amplification products were separated on a 6% polyacrylamide denaturing gel for 1h 40min at constant power of 50w.

The DNA bands in the gels were developed using a silver nitrate stain.

## Results

A total of 253 loci was scored for the five AFLP primer combinations used and ranged in size from 150 to 400 bp.

Genetic diversity in terms of percent polymorphic loci was found to be the lowest for Tangérois isolates (55.34%) and the highest for Chaouia-Tadla and Gharb-Saïss (88.14%). Nei's diversity index and Shannon index were found highest for Gharb-Saïss isolates and lowest for Tangérois isolates.

Genetic Nei's Distances were ranged from 0.038 for the most closely related collections to 0.109 for the most distantly related collections. Chaouia-Tadla and Gharb-Saïss collections were the most closely related while Tangérois collection quite distinct from the other populations.

The proportion of the total genetic diversity due to the collection differentiation ( $G_{ST}$ ) averaged 0.1207 over all loci, the gene flow was found to be superior to 1,  $N_m=3.6426$ .

## Conclusions

In the present study AFLP markers were used to estimate the extent of genetic diversity of *Puccinia triticina* in Morocco, we found that isolates from Abda-Doukkala and Chaouia-Tadla were the most genetically closed to each other, this seems consistent with the fact that the two regions are geographically close.

The collection differentiation was found to be low, showing that in Moroccan collections of *Puccinia triticina* little genetic differentiation is taking place, this is explained by the high gene flow.

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# TILLING in Barley: the TILLMore Resource

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## Introduction

TILLING (Targeting Induced Local Lesion IN Genomes) is a reverse-genetics approach combining chemical mutagenesis with a sensitive DNA screening-technique to identify point mutations in target genes (McCallum et al. 2000). We developed TILLMore, a TILLING resource in barley (cv. Morex) including 4,906 M<sub>4</sub> families derived from a sodium azide treatment. Molecular screening for mutation at agronomical important genes was based on the analysis of 8- to 12-fold DNA pools produced from M<sub>2</sub> or M<sub>3</sub> DNA samples. The mutagenized population, although developed for reverse-genetics purposes, was also analysed for forward-genetics studies.

## Methodology

### ***Mutation screening***

A protocol based on DNA heteroduplex detection was successfully implemented using a LICOR4200 gel analyzer (LI-COR Inc., USA) as described by Colbert et al. (2001) or an ABI3730 capillary DNA Sequencer (ABI, USA). TILLMore was screened with assays designed on several target genes involved in different aspects of the development and metabolism of barley. For the first set of assays, the molecular screening was carried out on 3,148 samples using a *Ce/I*-based heteroduplex assay coupled with gel-electrophoretic detection on LI-COR analyzer screened for mutations in 8-fold sample pools. For the molecular screening of other target genes, we used an ABI3730 sequencer system on the entire population of 4,906 samples screened for mutations in 8- and 12-fold sample pools.

### ***Forward-genetics screening***

The entire TILLMore M<sub>3</sub> population was grown in the field and scored for visible phenotypes in reference to untreated Morex. Phenotypic information was collected as to habitus, heading data, leaf appearance, presence of necrotic spots, plant colour, plant height, plant morphology, spike appearance and tillering. To detect root morphology alterations, a preliminary analysis on ca. 1,000 M<sub>3</sub> families was carried out using a paper-roll approach (Woll *et al.*, 2005). Ca. 12 seeds/family/paper-roll replicated were grown under controlled conditions (16/8 h photoperiod and 24/22 °C day and night, respectively). Seminal roots observations and measurements in reference to untreated Morex were performed on eight-day old seedlings.

## Results

### ***TILLMore reverse-genetics screening***

We screened TILLMore with assays designed on several target genes involved in different aspects of the barley development and metabolism. For each tilled gene, the molecular screening yielded an allelic series of mutants with an average of ca. seven alleles per gene corresponding to a mutation density of one mutation every 404 kb. Almost all the mutations detected were CG-TA transitions and several (ca. 70%) implied a change in amino acid sequence and therefore possible effects on phenotype.

In order to predict the impact of mutations on protein function we utilised bioinformatic methods like SIFT and PARSESNP (Ng and Henikoff, 2001; Taylor and Greene, 2003). It is expected that values of SIFT or PSSM (for PARSESNP) scores above specific thresholds indicate missense mutations which are more likely to have a deleterious effect on protein function. In our case, three mutations showed significant PSSM score values while the application of the SIFT algorithm predicted a possible deleterious effect only for one mutation.

### ***Forward-genetics screening***

The main purpose of our barley-mutagenized population was the implementation of a TILLING resource facility to be used for reverse genetics. However, the same population can also be used for forward-genetics studies. For this purpose, the 4,906 M<sub>3</sub> families were grown in the field and visible phenotypes were scored during the growing season in reference to untreated Morex plants. A visible variant phenotype was recorded for 32.7% of the M<sub>3</sub> families (1,605/4,906) either fixed or segregating within the family.

A change in plant colour, including families showing segregation for albino seedlings, was the phenotype most frequently observed (27% of mutated families; 12% of total families). Furthermore, a preliminary morphological analysis of 1,000 M<sub>4</sub> families by means of a paper-roll approach allowed for the detection of putative root mutants showing for instance a reduction of root length and severe modifications in root appearance and growth. Ca. 7% of the tested families showed a clearly altered root phenotype. In order to confirm the observed phenotype the paper-roll screening will be repeated for the putative mutants identified during this preliminary screening.

An on-line database with further information on phenotypes observed in the forward-genetics screening is publicly accessible at [www.distagenomics.unibo.it/TILLMore/](http://www.distagenomics.unibo.it/TILLMore/) and seed is available upon request.

## **Conclusions**

Our report describes TILLMore, a new TILLING resource in barley based on a mutagenized population derived from the cv. Morex. The use of NaN<sub>3</sub> provided an efficient alternative to more commonly used mutagenic agents to obtain a high mutation density suitable for TILLING. Additionally, the high frequency of visible phenotypes will make this population useful for forward-genetics screening. The TILLMore resource is available as a reverse-genetics platform to the research community on a cost-recovery basis and/or through collaborations (for details, see [www.distagenomics.unibo.it/TILLMore/](http://www.distagenomics.unibo.it/TILLMore/)). TILLMore will efficiently complement existing functional genomic resources within the cereal research community and help moving closer to a more tangible impact of TILLING on breeding programs.

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# QTLs Associated to Yield under Different Water and Nitrogen Availability in Durum Wheat

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**Key-words:** durum wheat, water stress, low nitrogen, adaptation, grain yield, Quantitative Trait Locus,

The future sustainability of agriculture and food production at a global level will largely depend on the capacity to release improved cultivars (cvs.) more tolerant to abiotic stresses, and able to use more efficiently natural resources (e.g. nutrients and water) while preserving food quality and enhancing nutritional value. Utilization of genomic tools can greatly facilitate breeding procedures aimed at enhancing crop sustainability, yield potential and yield stability. The purpose of this research was to investigate the genetic basis of yield and other agronomic traits under different water and N regimes in a durum wheat (*T. durum* Desf.) population of recombinant inbred lines (RILs).

## Methodology

A population of 181 RILs was developed by Società Produttori Sementi (Bologna, Italy) from the cross between the Italian elite durum cvs. Meridiano and Claudio, both characterized by high yield potential and stability. During 2007, the RILs were tested in five field trials according to an augmented design including three replicated checks: three trials were conducted in northern Mexico (Obregon, CIMMYT's primary yield testing location, 27° 24' N, 109° 56' W) under full irrigation (OB-FI), reduced irrigation (OB-RI) and low N supply (OB-LN); one in southern Italy (Lucera, Foggia, 41° 33' N, 15° 22' E) and one in northern Italy (Cadriano, BO, 44° 39' N, 11° 29' E) under rainfed conditions adopting normal rates of N fertilizer. The following traits were considered: heading date (HD), maturity date (MD), plant height (PH), grain yield (GY), number of spikes per m<sup>2</sup> (SPM2), number of grains per m<sup>2</sup> (GRM2), grain weight (TKW), test weight (TWT), flag leaf greenness (SPAD) and NDVI spectro-radiometrical index, both measured at the end of flowering.

The linkage map, based on wheat Simple Sequence Repeat (SSR) and DArT (Triticarte Pty Ltd, Yarralumla, Australia) markers (98 SSRs and 316 DArT markers), was obtained using JoinMap 4.0 (Stam, 1993). The map covered a total of 1856 cM. A subset of 213 selected markers (intermarker distance ≥ 3 cM) was used to identify the Quantitative Trait Loci (QTLs) controlling the traits of interest. Composite interval mapping was carried out for each environment using the adjusted field data. Windows QTL Cartographer v. 2.5 was used (<http://statgen.ncsu.edu>). The results of a preliminary analysis using the "model 6 standard analysis" with up to 5 control markers and a window size of 10 cM are reported. A LOD threshold of 2.5 was used for QTL declaration.

## Results

RILs were tested under environments widely different in photoperiod and temperature conditions. The RILs showed a wide range of variation for all the considered agronomic traits (Table 1), with the mean length of the crop cycle (see HD and MD) ranging from ca. 120 days in Obregon to ca 200 days in northern Italy. Mean yield was between a minimum of 1.71 t/ha in OB-RI and a maximum of 6.28 t/ha in northern Italy. A substantial transgressive segregation was observed for most traits, particularly for HD and MD in Obregon.

Table 1. Mean values and range of variation of the 181 durum wheat RILs across the five environments tested in 2007.

	OB-FI <sup>a</sup>		OB-RI		OB-LN		LU		CD	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
HD (d)	90	75-106	79	71-104	83	58 - 99	133	125-140	168	159-172
MD (d)	129	117-139	115	100-130	119	103 - 133	-	-	208	201-214
PH (cm)	112	90 - 129	75	51 - 99	100	84 - 118	90	73 - 113	92	78-111
GY (t/ha)	5.35	2.14 - 8.00	1.71	0.08-3.39	4.04	2.39 - 5.48	5.50	2.93 - 7.52	6.28	2.12-8.37
SPM2 (no/m <sup>2</sup> )	420	277-601	156	50-270	287	152 - 389	471	243 - 698	420	290-632
TKW (g)	40	21 - 55	41	25 - 49	51	42 - 60	45	32 - 59	39.3	30 - 48
GRM2 (no x 1000/m <sup>2</sup> )	13.2	6.4-22.8	4.1	0.03-9.7	7.9	4.9 - 10.7	12.5	5.4 - 17.2	16.1	5.6-21.9
TWT (kg/hl)	79.5	70 - 84.4	78.3	76- 82	80.8	77- 84	81.0	75 - 85	75.0	68 - 80
SPAD (units)	55	44 - 63	60.1	56 - 67	40.2	28 - 49	-	-	49.5	41 - 56
NDVI (units)	0.90	0.77 - 0.98	0.45	0.82-1.08	0.84	0.11-0.99	0.89	0.85 - 0.92	0.88	0.82-0.93

a) OB-FI, OB-RI and OB-LN: Obregon full irrigation, reduced irrigation and low nitrogen, respectively; LU: Lucera, Italy; CD: Cadriano, Italy.

Major QTLs ( $\text{LOD} \geq 3$  and  $R^2 \geq 10\%$ ) for HD and MD were detected only on chrs. 4B and 5A. Both QTLs showed a large interaction with the environment. Additionally, minor QTLs were found on chrs. 3B and 7A. Several QTLs were found for PH (chrs. 2B, 3B, 4B, 5A, 6A, 6B and 7B), TKW (2A, 2B, 3A, 4B, 5A and 6A) and TWT. Most of the QTLs for TWT overlapped those for TKW. GY was controlled by the major QTL for adaptation on chr. 5A. However, the second main QTL cluster (chr. 4B) for adaptation and agronomic traits (TKW and TWT) did not affect GY. Additional QTLs for GY were identified on chrs. 2A, 2B, 3A, 5B, 6A and 7B. Chr. 5A harboured two regions carrying QTLs for PH, GY, TKW and TWT. The first QTL region was detected on the 5AL centromeric region, in an 11-cM interval between *Xcfa2121*, *Xbarc40* and *Xgwm156*. Within this interval, a QTL for GY was found in LU ( $R^2 = 8.5\%$ ) and two QTLs for TKW and TWT in CD. The second 5A QTL cluster for HD, GY and related traits was located in the central region of the chr. (near *Xwmc388.2* and *Xgwm1570*) with the LOD peaks within a 10-cM interval. This region strongly influenced the crop cycle and GY in all the field trials carried out in Obregon. The Claudio allele considerably delayed HD (difference between the homozygous alleles from 10 to 18 days) in Obregon and negatively impacted GY, TKW and TWT. The position of both 5AL QTL clusters is mostly overlapping with the two main QTLs affecting response to photo-thermal conditions and thus earliness in wheat (Hanocq et al., 2007). In particular, the putative genetic position of the major QTL cluster on the 5AL central region overlaps with the location of *VRN-1*, a major gene controlling vernalization requirement in wheat (Yan et al., 2003).

## Conclusions

These results highlight that variation at key chr. regions controlling phenology largely influence broad adaptability of tetraploid wheat, similarly to what has been observed in hexaploid wheat (Cockram et al., 2007), and that large genetic variation for adaptation, yield potential and yield components is present in the elite durum wheat germplasm.

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# The Potential of a Proteomic Approach to Optimize Durum Wheat Technological Quality

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Proteomics has the potential to contribute to our understanding of grain quality by elucidating the way in which the genes are expressed under different environmental conditions. In particular grain filling is a critical growth stage in determining technological quality of durum wheat, involving the folding of storage proteins and their assembly by disulphide cross-links to form the gluten matrix of dough. The production of grain specifically suited to market requirements may be achieved both by using suitable genotypes and by optimizing growth conditions. In the framework of the project FAR-AGROGEN of the Italian Ministry of University, our research group aims at evaluating by a proteomic approach the change in protein composition of durum wheat cultivars during grain filling, also due to environmental variables. A preliminary evaluation of different durum wheat cultivars has been necessary in order to choose the ones to be used in the project. In this report the characterization of grain proteome of three durum wheat cultivars characterized by different technological quality, is reported.

## Methodology

Grain samples were obtained by three durum wheat cultivars (Latino, Ofanto and Simeto), grown in a pot experiment carried out at Agricultural Faculty in Foggia during 2005-2006 crop season. At harvest, the technological parameter gluten index was evaluated by Glutomatic 2200, according to ICC 155 official method. Two dimensional gel electrophoresis was performed on four replicates for each grain sample. Gluten proteins fraction was extracted from whole grain flour according to Hurkman e Tanaka (2004), separated by 2D PAGE (Ferrante et al., 2006) and analyzed by ImageMaster 2D Patinum 6.0 software (Amersham).

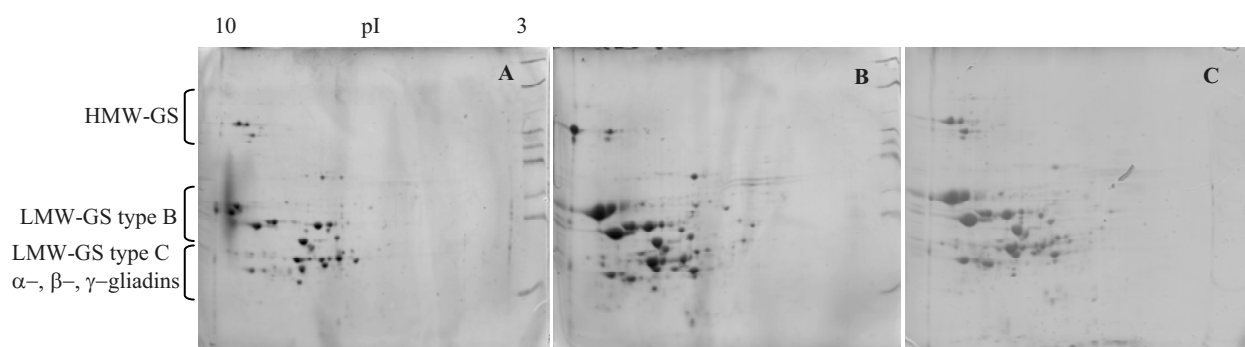
Protein spots were excised from gel and digested with chymotrypsin using an optimized digestion protocol reported in Palermo (2008). Digested peptides were analyzed in a nano-HPLC-ESI-MS/MS. The use of a C<sub>18</sub> column (75µm particle size, PepMap, LC-Packings) and a proper mobile phase gradient containing ACN/ water, FA 0.1% (v/v). Spectra were evaluated by Data Analysis software (Bruker Daltonics). Protein identification was performed by searching the NCBI using MASCOT ([www.matrixscience.com](http://www.matrixscience.com)). Blast local alignment search tool (BLAST) was used to find regions of local similarity between sequences.

## Results

As for technological quality, cultivars were significantly different ( $P \leq 0.01$ ), Simeto showing the highest gluten index value (91.8), followed by Ofanto (70.6) and Latino (9.2).

By comparison of protein patterns significant differences were observed among cultivars. In particular, with respect to Ofanto and Simeto, 29 protein spots were not present in Latino, most of them belonging to the low molecular weight region. On the contrary Latino showed 7 specific spots, one belonging to the high molecular weight region and 6 located in the low molecular weight region. Among the latter spots, only 2 were analyzed using nano-HPLC-ESI-MS/MS and identified as a LMW-GS type-1 and an

$\alpha$ -gliadin, respectively. In Ofanto main differences were observed within the high molecular weight region that shows the absence of 4 spots and the presence of 2 specific protein spots. Finally, Simeto showed 13 specific spots, 3 belonging to the high molecular weight region and 10 to the low molecular weight region; within the latter protein spots, 7 were analyzed and identified as LMW-GS (3),  $\alpha$ -gliadins (2) and  $\gamma$ -gliadins (2).



**Figure 1.** Two-dimensional electrophoresis of Latino (A), Ofanto (B) and Simeto (C) gluten proteins.

By MS/MS analysis the cyclization of the glutamine residue was evidenced in pyroglutamic acid in some HMW-GS. In the same gel some proteins were identified in more than one spot (different PM or PI) suggesting the presence of different isoforms of the same protein. An interesting result was obtained in some gliadins from Simeto and Ofanto: a greater number of cysteine residues was observed with respect to the usual ones, caused by aminoacidic substitutions in the protein sequence.

## Conclusions

The proteomic approach appears to be a powerful tool to characterize durum wheat gluten composition. In this paper some cultivar-specific spots were identified for the varieties under study. Furthermore, the existence of different isoforms of some gluten proteins was observed. Further investigations are in progress to evaluate the effects of environmental conditions in protein expression of grains from different durum wheat genotypes.

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# Genetic Analysis of the Cultivar Creso Durable Resistance to Leaf Rust

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**Key-words:** durum wheat, leaf rust, Quantitative Trait Locus, adult plant resistance, seedling resistance

Leaf rust is one of the most damaging wheat fungal pathogens worldwide. Nevertheless, genetic and molecular mapping studies aimed at characterizing leaf rust resistance genes in durum wheat have been undertaken only recently (Herrera-Foessel et al., 2005; Martinez et al., 2007). Resistance to *Puccinia triticina* conferred by the durum wheat cultivar (cv.) Creso (released in Italy in 1974 and derived from CIMMYT's and Italian materials) has remained effective since 1975 in cultivation environments characterised by recurrent leaf rust epidemics (Pasquini and Casulli, 1993; Martinez et al., 2007).

The objectives of this study were: (i) to map the genetic determinants of the resistance to leaf rust from the cv. Creso and (ii) to identify SSRs linked to the resistance gene for marker-assisted selection.

## Methodology

A recombinant inbred population of 176 lines from Colosseo (C) × Lloyd (L) was characterized for leaf rust response and genotyped with microsatellite (SSR) markers. Colosseo is a direct derivative of Creso, the main source of leaf rust resistance. Lloyd is a North American cv. with susceptible response under field conditions in Italy. A panel of 62 F<sub>6,8</sub> lines with diverse pedigrees related to Creso was also used to validate the major gene for leaf rust resistance inherited from Creso. Artificially inoculated field trials (three reps) were set up in Argelato, Bologna (Po Valley), Italy. The RILs were evaluated in 2006, while the panel of advanced lines was evaluated in 2006 and 2007. Inoculation was carried out with a mixture of 16 leaf rust isolates collected in Italy. Percentage of infected leaf area (LRS, according to the modified Cobb scale, Peterson et al., 1948) was used to evaluate the leaf rust response in the field at three stages of the disease cycle. The infection type (IT) response at the seedling stage was evaluated using a selection of isolates according to the decimal 0-9 McNeal's scale (McNeal et al., 1971). A genetic map based on 554 SSR and DArT markers covering 2022 cM was obtained. Quantitative Trait Locus (QTL) analysis was carried out on a map with 213 evenly spaced markers. The panel of Creso-related advanced lines was molecularly characterized with 43 evenly distributed SSRs (used to evaluate the familial relationships among the lineages) and with 14 SSRs selected from the chromosome (chr.) region found to harbour the major leaf rust resistance QTL.

## Results

The frequency distribution of the RILs tested under open field in 2006 for LRS and AUDPC indicated the presence of a major gene/QTL accounting for most of the phenotypic variation. Heritability values for LRS ranged from 72 to 82%. Colosseo showed a substantially resistant response throughout the disease cycle, while Lloyd confirmed its susceptible response, with LRS values up to 46.9% in the latest infection stage. The QTL analysis evidenced the presence of only one major QTL (*Q<sub>Lr.ubo-7B.2</sub>*) that was mapped in the distal region of 7BL, within a 5 cM interval (LOD - 2 supporting interval) and flanked by microsatellite markers *Xbarc340.2* and *Xgwm146* in the upper part and by *Xgwm344.2* in the distal part of the region. This QTL was detectable across the complete infection cycle, with *R*<sup>2</sup> values ranging from 49.8% at the early stage of disease development (kernel milk stage) to 76.9% in the late phase (end of grain-filling). The additive effects (computed as half of the phenotypic difference between the means of the RIL groups homozygous for Lloyd and Colosseo, respectively) ranged from

6.6 to 18.4% for LRS at the early and late stage of the disease cycle, respectively. The effect of the QTL was also large on both kernel weight and test weight, with a gain equal to 1.8 g per 1,000 kernels and 0.8 kg hl<sup>-1</sup> in favour of the RILs carrying the resistance allele from Colosseo, respectively. The QTL did not overlap with QTLs controlling heading date. Three additional minor QTLs were identified by means of selective CIM analysis carried out on a subset of 76 lines with the molecular haplotype homogeneous to Lloyd at the *QLr.ubo-7B.2* significance (LOD 3.0) chr. interval. At two of the three QTLs, the resistance allele was contributed by Lloyd (*QLr.ubo-2A* in the distal region of chr. arm 2AL and *QLr.ubo-3A* in the proximal region of chr. 3AS), while at the third QTL (*QLr.ubo-7B.1* in the proximal region of chr. 7BS) the resistance allele was contributed by Colosseo. Each of the three QTLs showed an additive effect for LRS equal to ca. 5%.

The resistance of Colosseo was also expressed at the seedling stage. Colosseo showed an average IT value = 3 (resistance) with 14 out of the 16 Italian leaf rust isolates tested. Two isolates were clearly virulent to Colosseo/Creso (IT response = 8, similar to that showed by Lloyd). One isolate avirulent to Colosseo was used to characterize the complete set of RILs at the seedling stage. Heritability of the IT score was equal to 91.9%. Similarly to what found in the open field experiment, the resistance at the seedling stage was controlled by a major QTL explaining 58.9% of the phenotypic variation (LOD peak value = 32.8); the QTL significance interval and LOD peak position were coincident with the *QLr.ubo-7B.2* ones. The effect of *QLr.ubo-7B.2* was validated in the association study. Out of 14 SSRs spanning the 7BL distal chr. region, only those located within the *QLr.ubo-7B.2* significance interval were associated to leaf rust response (*Xwmc526.1*, *Xwmc526.2*, *Xbarc340.2*, *Xgwm146* and *Xgwm344.2*). At these markers, the alleles inherited from Creso or its resistant derivatives were associated to the lower leaf rust infection level.

Three important leaf rust resistance genes have already been mapped in this region: *Lr19*, from *Lophopyrum ponticum* (Sharma and Knott, 1966), and the two closely linked genes *Lr14a* and *Lr14b* (distal portion of chr. 7BL; Dyck and Samborski, 1970). Moreover, the mapping location (Herrera-Foessel et al., 2007) of *Lr14a*, one of the few designated resistance genes that originated from *Triticum turgidum*, is coincident with that of *QLr.ubo-7B.2*.

## Conclusions

We have ascertained that the leaf rust resistance carried by the durum cv. Creso is mainly controlled by a major hypersensitive gene located in the 7BL distal chromosome region. The resistance haplotype inherited by Creso can now be selected through marker-assisted selection. The availability of precise genetic stocks for this gene in a homogeneous genetic background will facilitate testing for allelism and gene postulation studies.

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# Restriction Analysis of the *dspE* Gene of Virulent Strains of *Erwinia amylovora* from Different Host Plants in the Po Valley

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## Introduction

In the Po Valley (Northern Italy) the primary fire blight *foci* caused by *Erwinia amylovora* (Ea) were certified on pear plants in 1994. In the following years Ea was progressively spread in that valley. In 1997, a severe epidemic of fire blight occurred in the Emilia-Romagna region. The AFLP genomic profiles of Ea populations associated with this epidemic revealed that the isolates belong to the same clone [1]. From 1994 to 2001 systematic monitoring showed that the infection frequency was high and prevalent on pear plants (87.2%) whereas it was low in hawthorn shrubs (9.14%) and very low in apple trees (1.68%) [2]. Recently, an increasing infection frequency on apple trees was evidenced beside a widespread presence of the pathogen in hawthorn. The Phytosanitary Services assumed the occurrence of strains with different virulence. The pear clonal populations might have adapted to different host plants or new strains might have been introduced in the Po Valley. These hypotheses were tested studying the genomic profiles and the virulence either of the wild populations currently present in the area and of the experimental populations obtained through successive passages on different host plants. Here we report the results of the restriction fragment analysis of the virulence *dspE* gene in wild Ea populations from different host plants using the 1994 clone as reference strain.

## Methodology

**Bacterial cultures.** 7 Ea isolates from apple (4386), pear (3605, 3963), hawthorn (4378, 4366, 4368/1) and photinia (4395) were used. The strains were isolated in the Emilia-Romagna and Veneto regions during 2000-2001. Ea OMP-BO 1077/7 virulent strain, isolated from pear in 1994, kept lyophilized at 4°C, was used as reference strain. The cultures were grown on YDC agar for 24 h at 27 °C.

**Virulence assessment.** This was carried out on pear fruitlets [1] and apple leaves of clonal M9 plantlets grown in single pots in the greenhouse. A standard inoculum dose was inoculated through transversal cuts at the leaf apex. After 21 days, the percentage of the infected area was evaluated using the APS Assess software [3]. For each strain the mean of the percentage infected areas of 10 leaves was used to calculate the virulence index (I).

***dspE* gene analysis.** Oligonucleotides EF<sub>2</sub> (forward primer: 5'-CGG TTG CAG AGA ATT GCA-3') and ER<sub>1</sub> (reverse primer: 5'-TTC ATT TCC AGC CCT TCC TT-3') based on the *dspE* gene of Ea321 strain [4] were designed [5] and used as primers in the PCR with the total DNA of Ea 1077/7 as template. The PCR product, analyzed on an 0,9% agarose gel, was digested overnight with *EcoRI*, *AluI*, *HaeII*, *MspI*, *HhaI*, *MseI* and *RsaI*. The fragments were separated in a 1.5 % agarose gel.

## Results

In all the 7 Ea strains, the virulence index was higher (1.8) than that of 1077/7 (0). On apple leaves the infected area was highly variable even within the same plantlets inoculated with the same strain. The virulence index of the 7 strains varied between -0,464 and 0,847. The amplicon (≈ 5.5kb) obtained in the reference strain by PCR using primer EF<sub>2</sub> and ER<sub>1</sub> was similar in size to *dspE* gene [4]. In the reference strain, the restriction fragment profiles of the *dspE* gene showed distinct and reproducible fragments within the range of 5,000 and 100 bp showed in Fig.1: *EcoRI* (3 bands); *MseI* (13); *RsaI* (13); *HhaI* (11); *HaeII* (14); *MspI* (14) and *AluI* (12). The same profile was obtained by each enzyme in the 7 Ea isolates.

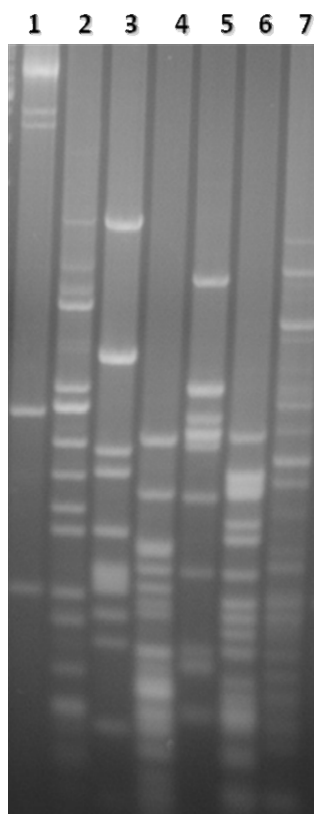


Figure 1. Restriction fragment analysis of the *dspE* gene of the Ea reference strain. Lanes 1 to 7: restriction fragment profiles by *EcoRI*; *MseI*; *RsaI*; *HhaI*; *HaeII*; *MspI* and *AluI* respectively.

## Conclusions

The Ea virulent strains chosen for the restriction fragments analysis of the *dspE* gene were initially selected for their high virulence index on immature pear fruits within a wide collection of strains isolated from different host plants and locations in the Po Valley [1]. Therefore, they represented the more virulent strains of Ea existing in the Po Valley in 2001-2002. The virulence analysis on M9 apple leaves evidenced a high intra-strain variability in the percentage of infected areas and a high inter-strain variability in the virulence indexes. As a consequence, the statistical analysis did not revealed any significant differences in the virulence between the 7 Ea strains and the reference one. These more virulent strains had restriction profiles of *dspE* gene indistinguishable from those of the reference strain. These results agree with the hypothesis according to which the Padanian Ea strains belong to the same clone evidenced in the previous AFLP genomic analysis [1]. The product encoded by the *dspE* gene is injected into the plant host cells [6, 7] and can be subjected to a selective pressure from the host species becoming a key factor in the Ea adaptation to a plant species other than that of origin. The above restriction analysis of the *dspE* gene did not support the hypothesis on the adaptation of the pear original clone to new hosts in the Po Valley. Our results concur with those of Giorgi and Scortichini [8] even if they analyzed only a portion of the *dspE* gene taken from a wide collection of strains isolated from different host plants around the world.

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# Genetic Dissection of Resistance to *Soil-Borne Cereal Mosaic Virus* (SBCMV) in Durum Wheat

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**Key-words:** durum wheat, *Soil-borne cereal mosaic virus* resistance, Quantitative Trait Locus, ELISA

*Soil-borne cereal mosaic virus* (SBCMV), a Furovirus transmitted by *Polymyxa graminis* Led., is responsible for the wheat mosaic, a disease that is widespread in the main wheat growing areas of the world. Most of the durum wheat cultivars (cvs.) grown in Italy and in the Mediterranean region are characterized by susceptibility. However, valuable sources of resistance have been identified in the cultivated durum germplasm (Ratti et al., 2006). To date, no specific genetic analysis of SBCMV resistance has been reported in durum wheat.

Our objective was to map the genetic determinants of SBCMV resistance in a durum wheat segregating population of recombinant inbred lines (RILs) obtained from the cross between two elite cvs.

## Methodology

A population of 181 RILs from the cross between Meridiano and Claudio, two elite Italian cvs., was considered. Meridiano is moderately resistant to SBCMV, with mild symptoms even under high infection levels, while Claudio is moderately susceptible (Vallega et al, 2002). The RILs were tested for SBCMV-response during 2007 and 2008 in Cadriano, Northern Italy (44°35'N 11°27'E, Po valley), in a field showing a severe and uniform SBCMV infection. A randomized complete block design (two reps) was adopted, with plots of 2.4 m<sup>2</sup>. Replicated plots of the two parents as well as of the highly susceptible cv. Grazia were also considered. Symptom severity (SS) was scored at different plant growth stages, using a 0 (no symptoms) to 4 (severe mottling and stunting) scale (Vallega and Rubies-Autonell, 1985). We report the results for the mid-end of tillering and first-node stages (SS1 and SS2, respectively) only. For the 2007 experiment, the following traits were also available: virus concentration determined twice (ELISA1 and ELISA2) on bulk samples of leaves collected at the same time of SS1 and SS2, grain yield (GY, q/ha) and test weight (TWT, kg/hl). Virus concentration was measured using DAS (double antibody sandwich) ELISA according to Clark and Adams (1977).

The molecular genetic map was assembled using microsatellite (SSR) and DArT markers. A provisional linkage map was constructed with 414 markers (98 SSRs and 316 DArT markers), grouped at high LOD threshold ( $\geq 6$ ) into 41 linkage groups and covering a total of 1856 cM. A subset of selected markers with an intermarker distance  $\geq 3$  cM was used for QTL analysis.

## Results

In both seasons, cv. Grazia reached a high symptom scores (equal to 4), indicating that the disease pressure was sufficiently high to discriminate the materials; moreover, the infection level throughout the field experiments was quite uniform (limited variability among the replicated plots of Grazia). In both years, the RILs exhibited transgressive segregation in both directions, indicating complex inheritance of SBCMV-response with resistance alleles contributed by both parents. Grain yield of the RILs (2007 data) ranged from 2.8 to 69.8 q/ha and was severely affected by infection as evidenced by the high correlations between GY and SS1 and SS2 ( $r$  -0.83 and -0.93, respectively,  $P$  0.001) as well as ELISA1 and ELISA2 values ( $r$  -0.72 and -0.76, respectively,  $P$  0.001). The negative effect of SBCMV was also noticed on TWT.

Single-marker analysis and composite interval mapping analysis using all 414 markers segregating among the RILs showed that a relatively limited number of markers, grouped in a few chromosome (chr.) regions, were involved in the SBCMV-response.

The most relevant region significantly associated to SS1 and SS2 in both years and ELISA1 and ELISA2 in 2007 was identified in a 30 cM wide interval located in the distal end of the 2BS linkage group (*QSBm.ubo-2BS*). Five markers encompassed this region: four DArT markers (117438, wPt-5738, wPt-2106 and 381522) located in the 2BS telomeric region within a 5 cM distance and one SSR marker (*Xwmc243*) located at a 30.8 cM distance. The map position of the DArT markers was validated after inspection of co-linearity with the hexaploid-wheat DArT-based genetic map (<http://www.triticarte.com.au/content/publications.html>) and with a proprietary DArT- and SSR-based durum linkage map (Mantovani et al., 2008). The LOD values at the QTL peak (wPt-2106) were very high ( $> 40$ ) for most of the SBCMV-response traits. The favourable allele conferring the resistant response was inherited from Meridiano. The same chr. region significantly affected also GY and TWT. The location of *QSBm.ubo-2BS* is coincident with that of the recently reported *Sbm2* locus, detected in the hexaploid wheat background (cv. Cadenza) in the 2BS chr. end (Bayles et al., 2007).

Seven additional chr. regions showed significant marker-trait associations ( $P \leq 0.01$ ) across SBCMV-response traits (i.e. SS and ELISA values) and years. They were located on chr. 1BL (378539 and wPt-6142), 3BS centromeric (wPt-8686, *Xwmc43*, wPt-1159, *Xgwm685* and wPt-5390), 4AL proximal region (*Xwmc161*, 379716, wPt-7289), 5AS (*Xgwm443.1*), 5AL (348667, 349142, *Xwmc524*), 5BL (*Xbarc243*) and 7BL (wPt-3533, wPt-1330, 117233, *Xgwm132.2*). The favourable alleles were contributed by both parents (i.e. by Meridiano at 1BL, 3BS, 4AL and 5AL, and by Claudio at 5AS, 5BL and 7BL), thus supporting the observed transgressive segregation. The QTLs identified in the distal regions of chrs. 5AL and 5BL in our durum population could represent the homoeologous copies of *Sbm1* detected by Bass et al (2006) in bread wheat.

## Conclusions

Our results evidenced that the genetic control of SBCMV resistance in the durum mapping population Meridiano  $\times$  Claudio is oligogenic, with a major QTL (*QSBm.ubo-2BS*) explaining most of the variability for the SBCMV-response traits. However, it should be noted that the favourable allele carried by Meridiano at this major QTL does not provide complete resistance, as demonstrated by the mean symptom severity and ELISA values of the parental cultivar. This work integrates the results obtained up to now in hexaploid wheat, and highlights the importance of the 2BS telomeric chr. region in the control of resistance to SBCMV in wheat and its relevance for marker-assisted selection strategies.

Acknowledgements. Research carried out with the financial contribution of the Emilia-Romagna Region (CEREALAB - SITEIA Project).

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**SESSION 3**

**CLIMATE CHANGE AND AGRICULTURE**





## **SUB SESSION 3.1**

### **ASSESSMENT OF THE VULNERABILITY OF AGRICULTURAL SYSTEMS AND AREAS**

Chairman: Giampiero Maracchi



# Vulnerability of Fruit Growers to Climate Change

## Observed Impacts and Assessments

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The vulnerability of economic sectors to climate change depends on both the expected regional climate change and the sectors' ability to adapt. In Germany, 80% of the total land area is used by agriculture and forestry sectors which are the most climate-sensitive branches. The adaptation of fruit plantations to climate change takes time and requires long-term investments. Returns are not realised until 3 to 6 years after the initial investments and capital recovery can take as long as 25 years. Serious problems facing fruit growers are: shifts in the timing of blossom and fruit ripeness, changes in late frost and hail risk, shortage of soil water, and a higher pest infestation possibility in the future. Therefore, detailed investigation on the impacts of climate change relative to fruit growing is necessary. In this paper we would like to present the first results in the shift of blossoming dates due to climate change and possible changes in frost hazard. The paper emphasises the importance of phenological networks and observations in Europe.

### Methodology

Phenological models are important tools to estimate the impact of climate change on plant development. In phenology the use of heat and chilling units to model plant development is common. It is known that trees in mid latitudes require a certain period with chilling temperatures, usually low temperatures between 0 and 10 °C, before they can react to higher temperatures which force the development of buds, induce leaf unfolding, and blossom in spring. Since dormancy release cannot be directly observed or measured, it is only possible to describe this stage using statistical model-fitting techniques, which makes the phenological model more complex.

The simplest phenological model is the thermal time model, which does not take dormancy into account. It assumes that the release of dormancy is usually reached at the end of the year. In this model the daily forcing rate  $R_f(T_i)$  is added up until the state of forcing  $S_f(t)$  is equal or greater than a critical value  $F^*$ . This value is the temperature requirement which is necessary to facilitate blossoming.  $F^*$  and  $T_{Bf}$  are both model parameters (Eq. 1, 2) which must be optimized for each fruit crop in each fruit growing region so that the root mean square error (RMSE) between modelled and observed data is minimal. In this study the starting date ( $t_1$ ) was fixed on 1 January.

$$S_f(t) = \sum_{i=t_1}^t R_f(T_i), \text{ whereas } S_f(t_2) := F^*, \text{ with } t_2 = \text{smallest number, for that: } S_f(t_2) \geq F^* \quad (1)$$

In order to calculate the daily forcing rates, different equations exist. Sarvas (1974) suggested the following logistic function:

$$R_f(T_i) = 0, \quad \text{if } T_i \leq T_{Bf}$$

$$R_f(T_i) = \frac{28.4}{1 + \exp(-0.185(T_i - T_{Bf} - 18.4))}, \quad \text{if } T_i > T_{Bf} \quad (2)$$

In order to project changes in the timing of plant development we have used two regional climate change scenarios for Germany (REMO, WETTREG) both of which are based on the ECHAM5/MPIOM GCM run. We have also used further phenological models (chilling/forcing models as sequential models, parallel models, etc.) to estimate the impact of climate change on blossom and fruit ripeness. Hereby, we were able to present an ensemble of possible changes in phenology, induced by the different model assumptions.

## Results

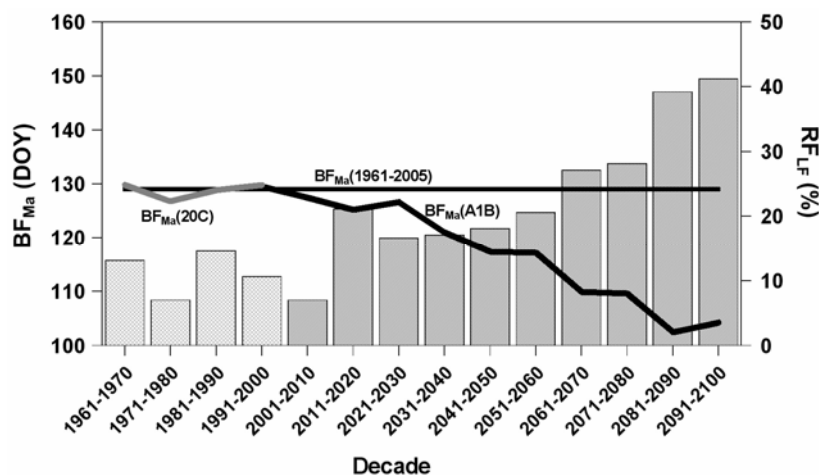
Phenological models for eight fruit crops were developed. The model parameters ( $F^*$ ,  $T_{Bf}$ ) were optimised at the 3,672 10x10 km pixels (height<1000 m) in Germany. The phenological models were used to estimate shifts in the timing of blossom due to climate change. Figure 1 shows possible changes in the timing of apple tree blossom in the fruit growing area, 'Altes Land', according to the projected temperature change in the climate scenario WETTREG, A1B.

In the period 1961-2005, the average beginning of apple blossom was observed on 9 May. In this time the blossoming date has already advanced by 14 days ( $P \leq 0.01$ ), mainly due to rising temperatures in spring. In the future this trend could continue. The beginning of blossom can shift by another 23 days, up to the period 2071-2100 (Fig. 1). Currently, frost after the beginning of tree blossom is relatively seldom (1961-2000:  $RF_{LF} = 11.3\%$ ). To the end of this century the late frost risk can increase up to 36.2 % in the period 2071-2100. This can lead to frost damages if the frost event is strong enough to harm the opened flower buds. Usually minimum temperatures between  $<-2.0$  and  $\geq -4.0$  °C can cause visible frost damages, in this temperature range the frost risk also increases from 3% (1961-2000) to 11% (2071-2100).

Fig. 1:

Calculated changes in the beginning of apple blossom ( $BF_{Ma}$ ) in the region 'Altes Land' (station Jork) according to the WETTREG scenario A1B (thermal time model:  $t_1=1$  January,  $T_{Bf}=3.3$  °C,  $F^*=157$  FU), and possible changes in the relative frequency of late frost ( $RF_{LF}$ ) up to 10 days after the beginning of apple blossom.

DOY: day of the year  
20C: Control run  
A1B: Scenario



## Conclusions

Frost during the blossoming time can harm the fruit yield much more than changes in the ripening period or even drought. This investigation showed that small changes in the beginning of flowering could increase the late-frost risk, even if the frost-free season generally is extended in the future. The result could be economic losses for fruit growers if no adequate frost protection is introduced. In Italy (Emilia-Romagna region) an increase in frost risk damage has already been observed in the last decades (Zinoni et al. 2002, Anconelli et al. 2004). Investigations by Sušnik and Žust (2001) for Slovenia also point to an increase of spring-frost frequency in 1990's and in 2001. In Germany strong frost damages on fruit trees were recently observed in 2007, a year with a very early blossom for fruit trees.

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# Adaptation of Maize Phenology to Climate Change in Veneto Region

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Climate change has led to shifts in phenology in many species distributed widely across taxonomic groups. For this reason long-term datasets of phenology and meteorological data are important for understanding how vegetation responded to past climate and predicting the response of plants to future climate change. Most studies focus on changes in the natural vegetation, whereas, despite their potential economic importance, only a few deal with trends in agricultural and horticultural varieties (Chmielewsky et al., 2004).

Obviously, the ongoing warming trend has had measurable impacts on the growth and yield of field crops, but the size and extent of the impacts have differed spatially and temporally. There is a clear need to synthesize crop yield and climate data from different areas to provide critically needed observational constraints on projections of the impacts of both climate change and management practices on future food production (Lobell and Asner, 2003).

Starting from a meteorological dataset (1956-2007) and 3 years (2005-2007) of phenological observations on maize crop in 4 representative stations across Veneto region, in north-eastern Italy, an analysis of the effect of climate change was conducted to investigate whether the changes have had significant impact on the growth and yield of maize, which is one of the staple crops in the region.

## Methodology

The observed data on maize used in this work were from the 2005-2007 Veneto phenological network. This network is formed by volunteer partners who collaborate by collecting the data using the BBCH scale (BBCH, 1997), every 10 days during the main stages of the vegetative cycle.

Two maize FAO classes were used: 500 and 600. A dummy variable analysis was used on these 3 years of observations to examine group and time effects in regression.

These data were the starting point to reconstruct the phenological trend in maize during the long-term period using Growing Degree Days (GDD) and 8 °C as base temperature. A historical dataset (1956-2007) of daily mean temperatures was available for the considered meteorological stations near the crop sites. Meteorological data came from automatic ARPAV stations ([www.arpav.it](http://www.arpav.it)). Planting date was assumed as 1<sup>st</sup> of April.

An analysis was then conducted of the break point on the reconstructed stages (emergence, flowering and maturity), utilizing the Strucchange library of R software.

## Results

The typical behaviour of the two FAO classes of maize was shown by dummy variables: 500 reached almost all the phenological stages earlier (Figure1). This is due to the genetic characteristics of the varieties used in the network.

Break point analysis applied to each FAO class and main growth stage showed a change between 1979 and 1980. After the break point, all stages were reached earlier in both FAO 500 and FAO 600; in particular the differences became greater at the end of the vegetative cycle (34 days in FAO 500 and 38 in 600 ). (Table 1)

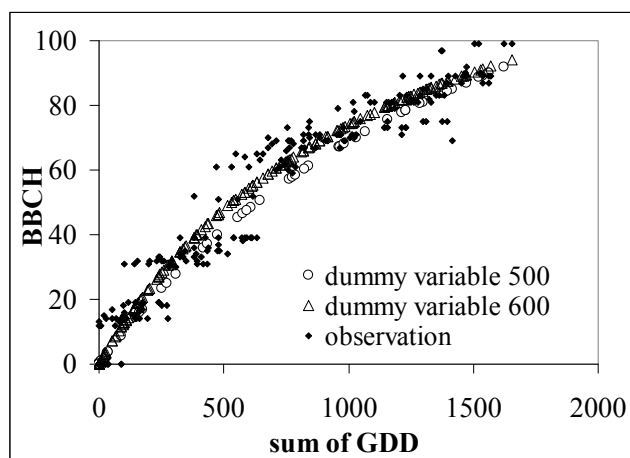


Figure 1. Dummy variables analysis in FAO class 500 and 600 in 2005-2007.

FAO class	emergence		flowering		maturity	
	ante	post	ante	post	ante	post
500	111	102	180	159	244	210
600	130	115	196	172	261	224

Table 1. Julian day of the main growth stages ante and post break point

### Conclusions

Break points in the main phenological stages in maize were observed during the long-term period of 1956-2007 in both FAO 500 and FAO 600 maize classes in Veneto region, north-eastern Italy.

Significant changes occurred for each growth stage. All phenological stages were reached earlier.

Increasing temperatures shortened the reproductive growth period and reduced the length of the growing season. These results indicated that ongoing climate warming trends are having ~~some~~ measurable impacts on the growth of field maize crop.

This study highlights the need for further investigations into the combined impact of temperature and phenology on management practices, suggesting a new potential role of management for adaptation.

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# Expert Knowledge-Based Methodology for Land Degradation Risk Evaluation

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In the framework of European IMAGE project, a methodology for land degradation risk assessment has been defined in order to evaluate both the current risks and, on the basis of hypothetical scenarios, the expected risks for the year 2020. The methodology has been applied to the Ofanto river basin which has an extension of about 2700 square km, evaluating four main risk typologies: land desertification, soil sealing, water degradation and soil erosion.

The pilot area is a representative site for the Mediterranean conditions. In particular, during the last fifty years, the area has undergone relevant land use changes with land abandonment in the marginal areas and agricultural intensifications of plain zones. Taking into account the pressure of agricultural activities and the conflict for water exploitation between the different irrigated districts, the assessment of the main risks affecting the area is particularly valuable; it can represent a starting point for supporting policy makers throughout the decision process both about the allocation of water resources and about conservative land resources management.

## Methodology

The aim of this study was to define a suitable methodology able to produce risk maps for the Ofanto river basin, considering both the existing situation and the expected conditions generated by different possible scenarios. The methodology was differentiated for the evaluation of current (M1) and the expected risk (M2).

The applied approach is expert-based and therefore qualitative, and the results obtained depend heavily on expert judgment. Nevertheless, it can be considered a good way to derive information about land degradation phenomena for the scale of the produced maps, and whenever the absence of suitable quantitative data prevents any analytical risk modelling.

The core of M1 (Fig. 1) is an integrated analysis of the entities: Land Systems, Land Transitions (1960-2000) and Slope, which are interrelated through a three-entry cross-matrix called EMIA (Environmental Matrix for Impact Evaluation) used for the expert-based definition of risks.

Land Systems (FAO 1995), set up in scale 1:100.000, have been delineated detecting homogeneous geographic units, in terms of the environmental factors and agro-forestry resources capable of influencing their potential use and the dynamics of degradation processes. The result is a Land Systems map made up of different permanent environmental structures, linked to the integrated long-term action of climate, lithology, morphology, biotic community and permanent human changes (e.g. reclaimed land, terraces, soil erosion). To obtain the Land Transitions map, a land use change analysis has been performed using 1960 and 2000 land cover maps with a common legend nomenclature. The maps have been intersected using GIS to produce a base layer that represents the grid of polygons used to render the risk maps for the investigated area.

The EMIA matrix has been processed by experts using a spreadsheet, associating the risk levels intensity for each Land Transition and for each Land System using the slope values as intensity modulator. The association procedure for risk level intensity is based on the judgment of experts who have a deep knowledge of the processes active inside each Land System of the study area. The risk intensity is ordered into four levels: no, low, medium and high risk.

The EMIA matrix, processed separately for each risk type, has been subsequently rendered as risk maps, using the base layer, by GIS processing.

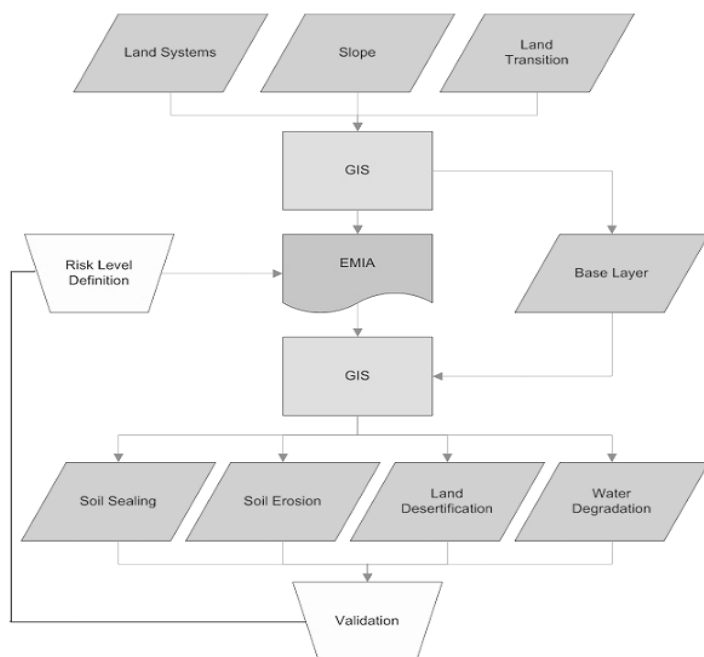


Figura 1. Flow chart of the methodology for the current risk level assessment (M1)

The M2 methodology has been developed in order to produce expected risk maps according to different scenarios. Similarly to M1, it is an expert-based approach for risk evaluation. Two types of scenarios have been considered: infrastructural and water management. The former mainly focused on improving water availability by infrastructural interventions such as dam rehabilitation and the restoration and enlargement of water distribution network, the latter focuses on decreasing water needs by the introduction of appropriate agronomic techniques or microirrigation systems.

Essentially M2 is based on a transformation of the current risk maps obtained by increasing or decreasing differentially the risk intensity at a global scale (for example considering a Climate Change Scenario) or locally (for example in an Irrigation Network Expansion Scenario).

M2 is fully implemented in GIS environment using geoprocessing functions and different geographical data. The main required input is represented by the current risk maps, obtained applying M1.

Scenarios statements are used for defining adequate rules for the transformation of risk intensity. Such rules are implemented in the GIS environment by Visual Basic code and they drives the risk intensity transformation differentially in relation to the values of Land System, Land Transformation and Slope coupled with an additional layer.

## Results

The results produced by this methodology may be shared among the actors involved in the decision making process and may represent a focal step for facilitating active participation and discussion about land degradation phenomena affecting a given area. The qualitative results can be expressed as percentages of the area affected by a risk and by comparing a scenario risk with the current situation.

## Conclusions

The simplified approach for risk identification and classification carried out in this study seems to be a useful tool whenever work is conducted at high map scale and when an analytical risk evaluation is made difficult by the absence or the low-quality of available data.

Both parts of the methodology (M1 and M2) produce results heavily dependent on the opinion and knowledge of experts.

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# Statistical Analysis of the Night Weather Conditions Favouring the Epidemic of the Brown Rust in the Grand Duchy of Luxemburg

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## Introduction.

Brown rust of wheat caused by *Puccinia triticina* Rob. and Des. f.sp. cause significant yield loss in all wheat-growing areas of the world when proper control methods, i.e. combination of resistant cultivars and fungicide sprays, are not available or incorrectly applied (Sache 2000; Bancal *et al.* 2007). In the Grand Duchy (G.D) of Luxemburg, fungal management strategies in winter wheat are mostly based on the control of *Septoria tritici*. However, incidence of brown rust (*Puccinia triticina*), another important widely distributed wheat disease, has been observed to increase in severity over several growing seasons within the past decade. A reason for these sudden and unpredictable brown rust occurrences in the region could be traced to recent global climate changes.

Before 2000, this disease was observed only when the growth stage of wheat was between GS79 and GS87 of the decimal code (Zadoks *et al.*, 1974).

However, since 2002, the brown rust disease is observed regularly much earlier into the crop and year 2007 has to be considered as a very particular year with an explosion of the brown rust that clearly affected the plant health. Brown rust settled very quickly and maintained during all the season and in almost all our observation sites on the sensitive cultivars in the Grand Duchy of Luxemburg, in Belgium and bordering countries.

The aim of this paper is a critical evaluation of meteorological parameters which favour the epidemic of the brown rust in the Grand Duchy of Luxemburg. The assessed parameters will be used in epidemiological and forecasting models of the yellow rust in the Grand Duchy of Luxemburg.

## Methodology

Replicated field experiments were established in the sites of Everlange and Reuland, G.D. of Luxemburg, for the three growing seasons between 2000 and 2003.

Weather conditions change in the Grand Duchy of Luxemburg were monitored because they are suspected to be mainly responsible of this new trend of the disease. Between 2000 and 2003, we studied the impact of the night temperature variations and of night wet periods duration to determine the range of temperatures and wet periods which favoured the infection of wheat by *Puccinia triticina*. The night temperature data are taken in a random way in intervals [0°C=<t°C=<33°C] during spring and early summer. The night data of relative humidity are spread between 60 and 100%. Rain is often considered as a conducive factor for the spread of the disease because rain events are followed by an extended period of leaf wetness, which is critical for rust infection (germination and penetration processes) (Sache, 2000). The effect of rain is here indirect.

In parallel with this analysis, we studied correlation between proportion (%) of meteorological parameters in 2000 and 2003 and the disease incidence and severity data collected by visual estimations and recorded as a percentage leaf area of L1, L2 and L3 (flag leaf is L1) recorded on susceptible winter wheat at Everlange, Reuland and Emerange. Proportion of meteorological parameters is

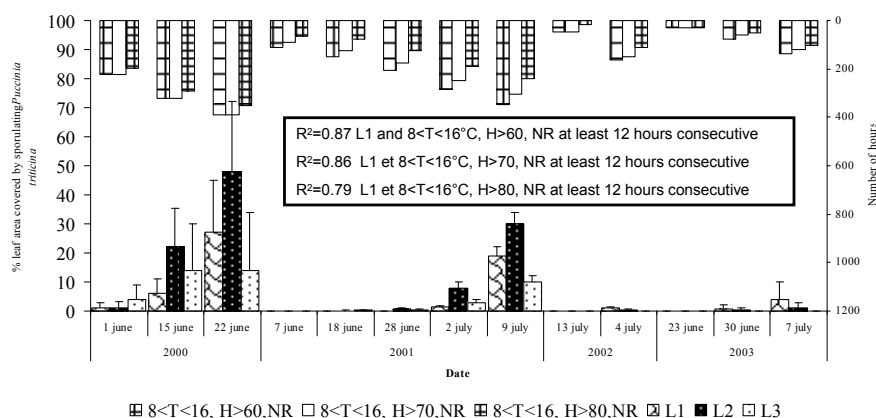
calculated over 4 periods (in winter, spring, all year long, from May 10 to July 10). Main meteorological parameters classes tested are: **i)** presence of rain and three intervals of relative humidity (RH > 60 %, RH > 70 %, RH > 80 %). **ii)** Four intervals of temperatures (0°C < t°C < 4°C , 4°C < t°C < 8°C , 8°C < t°C < 16°C, t°C > 16°C); **iii)** These same intervals of temperatures with relative air humidity ranging between 80 and 100%. Above and below this temperature range, the disease evolution is nearly stopped. Rainfall is also compulsory but only in the start of the infection process for laying down spores on leaves. This analyse is carried out by using the daily meteo data of Everlange, Reuland and Obercorn.

## Results

The analysis of the night weather and brown rust incidence data gives the following results:

- a strong correlation between the number of hours with particular weather conditions and the percentage leaf area covered by brown rust lesions for the two upper and youngest leaves, which are mostly responsible for assimilating the nutrients for filling the grains, in the G.D. of Luxembourg (fig.1).
- Such weather conditions include a period of at least twelve consecutive hours with temperatures between 8 and 16°C and a relative humidity (RH) greater than 60%, with optimal values lying between 12 and 16°C and greater than 80% RH.

**Figure 1: Evaluation of meteorological parameters.** Correlations between the night meteorological parameters and the % of leaf area covered by uredospores *Puccinia tritici* of leaves L1, L2 and L3 recorded in Everlange, Reuland and Emerange in year 2000 and 2001. N.B: NR = No rain.



## Conclusions

The best correlation between the plant disease and the weather conditions is obtained when the following favorable conditions are met during 12 consecutive hours at least:

Night Temperature between 12°C and 16°C and night relative humidity higher than 80% without rain.

This correlation is positive and significant between this class and the rate of disease recorded on the flag leaf L1 ( $R=0.93$ ;  $R^2=0.87$ ;  $P < 0.05$ ) and L2 ( $R=0.87$ ;  $R^2=0.76$ ;  $P < 0.05$ ). Moschini and Perez (1999) found similar conditions for the development of brown rust epidemics in Argentina, reporting optimum weather values of daily mean temperatures between 12 and 18°C and RH greater than 49%.

This study aims to improve wheat management with respect to disease control methods, and by improving such strategies, it could contribute in generating better profits for farmers through both the achievement of better yields and the reduction of costs, as well as benefiting the overall environmental health by reducing the number of unnecessary chemical applications.

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# Climate Change and CAP Reform: Opportunities and Risks for Mediterranean Agriculture

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## Introduction

Climate is changing: not only scientists, but also governments and the general public have got enough evidences of the recent variations in terms of averages and frequency of extreme events, such as the recurrence of drought, floods, hurricanes, etc. Much less certain is the interpretation of these recent evolutions (e.g. the distinction between anthropogenic and natural components) and the projections into the future.

Agriculture plays – or could play – multiple roles, for instance in terms of contribution toward the mitigation of anthropogenic emissions of greenhouse gasses through carbon sequestration, but it has also to face a varied combination of emerging risks and opportunities, very much diversified around the Globe. The Mediterranean area is one of the most critical, for several reasons: greater difficulties in the analysis and projection of the phenomena, evidences of higher probabilities of dramatic impacts, possible exacerbation of socio-economic, cultural and environmental conflicts.

The agricultural sector is facing new challenges, requiring the integration of different policies, objectives, etc. within an innovative and comprehensive approach toward sustainable production systems and management of natural resources.

The work reported here stipulates the need for designing an innovative package of production strategies and technologies, tailored on the specific characteristics of socio-ecosystems, in which the need for adaptation to climate changes and the opportunities to participate to mitigation strategies are embedded within the current and future evolution of the Common Agricultural Policy (CAP) and of the markets of agricultural commodities.

The main aim of this work is to examine risks and opportunities for Mediterranean agriculture, focusing in particular on the adaptation measures relevant within the contexts of the evolving EU policies: the Health Check of the CAP (the current process of revision of the policy tools) and the strategies targeting water scarcity and droughts.

## Methodology

This paper builds upon the outcomes of the EU project Meacap (Impact of Environmental Agreements on the Common Agricultural Policy) and integrates some of the outcomes with an *ad hoc* review of a series of recent papers and official documents related to the fields of climate change research and policy and the ongoing reform of the Common Agricultural Policy (so called Health Check of CAP).

## Results

Recent reports (EEA, 2006) evidenced a general tendency towards reductions of GHG emission of agricultural origin for the combined effects of the evolution of the market, of production techniques and policies (not only the CAP, but also the indirect effects of environmental policies for water and air).

Concerning mitigation policies the Meacap project has recently analysed the Fourth National Communications (NC) of EU Member States, finding 113 policy measures in the agro-forestry field, testifying the potential contribution of those sectors to mitigation strategies. On the other hand, an

accurate analysis of NC's shows that usually only measures already "traditionally" included in agri-environmental schemes are mentioned and thus only indirect and uncertain effects should be expected. Moreover, notwithstanding the efforts in the development and implementation of mitigation policies, it is clear that climate will continue to change in the short and medium term, at least because of the accumulated effects of past phenomena. As a consequence, a stronger attention should be paid adaptation strategies, in order to cope with the expected impacts.

Regarding adaptation risks and opportunities, a recent study (AEA, 2007) reported that in the Mediterranean, opportunities will be very limited, while the main risks could come from declining yields and increasing conflicts for water resources. Strategies have thus to be developed for the introduction of new cultivars or crops better suited to water and heat stresses. Problems from new pests and diseases are also considered a high risk in these zones.

One positive aspect is that, in general, there seems to be greater awareness and greater consideration of adaptation measures in the Mediterranean area than in northern EU, and this is again because Mediterranean farmers are more used to deal with climate variability and adverse events, such as droughts. Farmers have always adapted to changes in climate, in terms of adaptive management to cope with climate variability. The challenge now is to adapt within very short periods of time to potentially extreme impacts and new risks and opportunities. This has to be achieved through a combination of managerial, infrastructural and technical measures.

## Conclusions

Uncertainty and diversity of methods and results seem to be the only elements in common in the scientific literature and in the official reports. This exacerbates the substantial dichotomy between the varied contents of the scientific literature and the simplified/standardised approaches suggested in the guidance documents at international level (see for instance the IPCC guidances) and implemented in the official national communications and reports. The former analysing heterogeneity of phenomena, uncertainty, spatial variability and raising doubts about the effectiveness of the various policy measures, the latter proposing standards to be adopted in the diversified contexts of the globe. This is a typical problem in environmental policy in general, but it appears to be exacerbated in this context. Difficulties are to be expected when the assessment of policy effectiveness has to be considered, because theoretically cost-effective measures could end up by providing only episodic quantifiable benefits.

CAP mechanisms can be used to stimulate and facilitate adaptation and other mechanisms must also be utilised, such as insurance, capacity building, networks and partnerships.

Significant methodological progresses are needed in particular with respect to the treatment of:

- a) the complex dynamics of characterising agro-forestry socio-ecosystems evolutions, discontinuities and the perspectives for building more robust risk management approaches capable to exploit or reinforce their resilience;
- b) the spatial and temporal variability of phenomena and consequent need for site specific strategies;
- c) the limited availability of systematic knowledge on cause-effect chains and feedback effects of the various measures;
- d) the difficulties in managing the various scales (from field measurements to national communications), in particular in terms of model validation possibilities;
- e) the difficulties in monitoring and verifying the long-term phenomena involved, in particular for what concerns carbon accumulations in soils;
- f) the relationships between sciences and policy/decision making and in particular the development of adequate "adaptation assessment frameworks";

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# Interaction of Free Air Carbon Dioxide Enrichment and Controlled Summer Drought on Soil and Plant Water Relations and on Canopy Growth in a Maize Field

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## Introduction

Concomitant to a further increase of the atmospheric CO<sub>2</sub> concentration the recent IPCC report (IPCC, 2007) predicts higher temperatures and increased summer drought also for European growth conditions. Growing demand for energy crops and warmer temperatures will favour maize cultivation. However, the predicted increase in water shortage could be a problem. Therefore, a biennial research project has been started in 2007, in which the interaction of increase in atmospheric CO<sub>2</sub> concentration and summer drought on maize is investigated under field conditions. The objective of the study is to quantify the effect of elevated CO<sub>2</sub> concentration (ca. 170 ppm above ambient) on water use and plant growth of maize and to examine the extent to which drought stress is mitigated by CO<sub>2</sub> enrichment due its water saving effect.

## Methodology

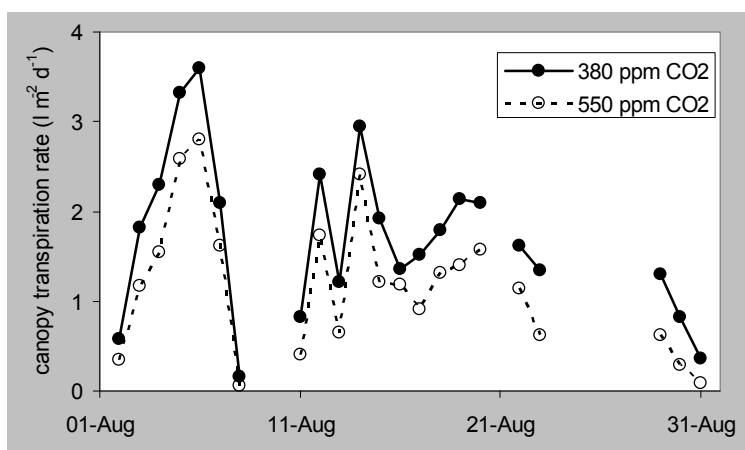
Maize is grown in 2007-2008 on an experimental field site according to local farm practice. The soil is a luvisol of a loamy sand texture with a low plant available water content (PAWC) in 0-60 cm depth (maximum PAWC is ca. 100 mm). At the site severe drought stress, i.e. more than 20 days with PACW<30 mm during July and August, occurred every 2<sup>nd</sup> year during the past 40 years. The field is equipped with three FACE-rings (FACE = Free Air CO<sub>2</sub> Enrichment, 20 m diameter), in which the atmospheric CO<sub>2</sub> concentration is elevated to 550 ppm during daylight hours. Three circular field plots are used as ambient CO<sub>2</sub> controls. Each ring is split into two semicircles with different water supply (1: with irrigation = well watered (PAWC>50 mm); 2: without irrigation = drought stress). In order to ensure drought also in a rain-laden summer, a facility for rain exclusion has been designed, which can be operated in combination with the FACE-system. The rain exclusion system was introduced in the 2<sup>nd</sup> year. It consists of an aluminium frame system (20 m x 12 m ground area) installed over each of the 6 semicircles of the drought stress treatment. A transparent PVC cover (tarpaulin) is assembled temporary during heavy rainfall in July and August if necessary for ensuring drought stress.

Treatment effects on plant water relations are measured at the leaf (gas exchange measurements) and the whole plant level (sap flow measurements with Dynamax sensors). Soil water content is followed with TDR-Sensors and canopy climate is recorded with psychrometers. Plant growth is analysed by destructive biomass sampling. The applicability of the rain exclusion system is tested by measuring its effects on the performance of the CO<sub>2</sub> enrichment and on the climatic conditions.

## Results and Discussion

In 2007, an extraordinary rain-laden August prevented the occurrence of severe drought conditions. At the end of July PAWC decreased to 50-60 mm for ca. 2 weeks in the non irrigated plots, and the well watered plots were irrigated to keep PAWC above 60 mm. During this period soil water content was higher under maize grown in elevated than ambient CO<sub>2</sub>. FACE produced a warmer and drier canopy

climate, which was observed especially on sunny days. Thermal imaging demonstrated an increase of up to 2°C of the canopy surface temperature due to CO<sub>2</sub> elevation. However, the sum of the canopy air temperature from July until September was hardly affected by the CO<sub>2</sub> treatment. Elevated CO<sub>2</sub> decreased stomatal conductance and transpiration at the whole plant level (Figure 1). The sap flow measurements indicated that whole plant transpiration was reduced by more than 30% during summer months. Final above ground biomass of the ambient CO<sub>2</sub> plots was identical among the two water treatments (2139 g m<sup>-2</sup>) and unaffected by CO<sub>2</sub> enrichment. The same applied to green leaf area index. Consequently, our data show that maize growth is unaffected by CO<sub>2</sub>-elevation when there is no restriction in water supply. However, CO<sub>2</sub> elevation causes an appreciable decrease in transpiration. This is consistent with recent findings in another FACE study with maize (Leakey et al., 2006).



**Figure 1:** Temporal changes in whole plant transpiration measured with sap flow sensors for plants grown under ambient (380 ppm) and elevated CO<sub>2</sub> level (550 ppm).

In 2008, the rain exclusion system was operated for the first time. Figure 2 shows a FACE-ring equipped with the rain exclusion system installed (without tarpaulin) over one of

the CO<sub>2</sub> exposed semicircles in May. The tarpaulin reduces incident PAR by approx. 10-20%. First results on the performance of the rain exclusion system, its influence on CO<sub>2</sub> distribution and climatic conditions together with effects on maize growth will be shown.

**Figure 2:** View of a FACE ring within the maize field in May 2008 after installation of the rain exclusion system, which will be used to prevent unwanted heavy rainfall during summer.



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# The Effects of Climatic Drought on the Sugar Beet Yield in England– Now and in the Future

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## Introduction

In England, the sugar beet crop is spring-sown and mostly rain-fed. The crop performance is subject to annual weather variability. Consequently, annual sugar yield fluctuates because drilling, crop growth and development depend on the prevailing weather conditions. Temperature, coupled with the soil condition in spring, primarily controls the start and the duration of sowing date and so determines the length of grand growing season which dictates the total solar radiation the canopy is able to intercept. Then it is the amount of rainfall relative to the amount of potential evapotranspiration (PET) that can create the severity and the frequency of soil moisture deficit during the summer months of June, July and August. We refer to the insufficient water from rainfall relative to the PET as climatic drought. Climatic drought that limits sugar yields is commonly observed at some point in summer in every growing season in England. The objectives of the paper are to quantify the relative importance of climatic drought in each of June, July and August; to test the Broom's Barn sugar beet growth model for its use to combat weather variability; and to assess the effects of climatic drought under future climate change on the sugar yield in England.

## Methodology

Sugar yields plus sowing and harvesting dates were obtained in rain-fed and irrigated experiments from 1976 to 2007 at Broom's Barn Research, which is located in and resembles the principal sugar beet growing area of England. In all experiments, prophylactic pest and disease control measures were adopted and the crops were grown and managed in accordance with the prevailing agronomic guidelines. Daily values of maximum and minimum temperatures, rainfall, solar radiation and potential evapotranspiration (PET) were retrieved from the Broom's Barn weather station (52°16N 0°34'E, 75masl). The annual sugar yield loss due to drought was calculated as the difference between the sugar yield from irrigated and that from the rain-fed crop. The climatic shortage of rainfall amount during the summer growing months was calculated as the difference between the accumulative PET and the accumulative rainfall amount. Regression analysis was performed to apportion the effects of climatic drought on the loss of sugar yield in June, July and August. The observed sugar yields were compared with simulated sugar yields using the Broom's Barn sugar beet growth model (Qi et al., 2005) in order to validate its use to account for weather variability. The impact of the future weather change was assessed for the national sugar yield in the current sugar beet growing area with the daily weather generated at ten representative weather locations for 150 years under the high CO<sub>2</sub> emission scenario in the time segment of 2020, 2050 and 2080 against the baseline weather scenario covering weather from 1961-1990 (Semenov, 2007).

## Results

Fig. 1 shows the accumulated rainfall and PET in June to August since 1976. Except for 1987 and 2007, the rainfall was less than the PET to various extents in all other years. Furthermore, the annual variability was much larger in the summer rainfall than in the summer PET. Fig. 2 shows the sugar yields for the rain-fed and irrigated crops. The sugar yields ranged from 5.1 to 16.5 t/ha in the rain-fed crops whereas they ranged from 8.1 to 16.8 t/ha in the irrigated crops.

Jaggard et al (1998) estimated sugar yield losses due to climatic drought in summer in the UK. Throughout the period prior to 1995, they estimated a sugar yield loss of 1.57 t/ha per 100 mm of summer rainfall shortage for sandy loam soils. Our estimation after inclusion of observations after 1995 was 1.44 t/ha per 100 mm of summer rainfall shortage. The estimated relation is:  $Y_L = 0.236 + 0.1438R_S$  where  $Y_L$  is the sugar yield loss and  $R_S$  is the summer rainfall shortage in mm, and the variance

accounted for is 41.4%. However, when taking into account the separate effects of climatic drought in June, July and August, the estimated relation is:  $Y_L = 0.01636R_{SJun} + 0.02712R_{SJul} + 0.0091R_{SAug} - 0.496$  where  $Y_L$  is the sugar yield loss,  $R_{SJun}$ ,  $R_{SJul}$  and  $R_{SAug}$  are the rainfall shortage in mm in June, July and August, respectively., and the variance accounted for is 55.2%. Analyses of standard partial regression coefficients showed that climatic drought was responsible for 31, 52 and 17% of the variance accounted for the total yield loss in June, July and August, respectively.

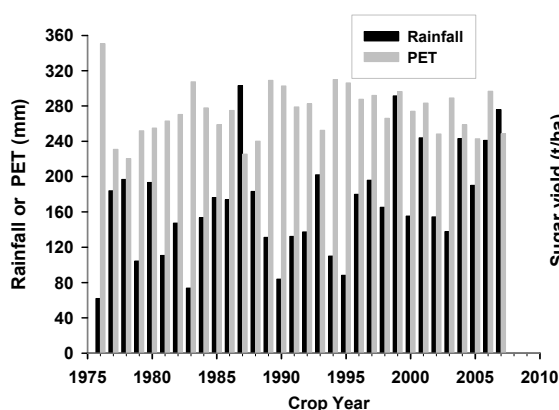


Fig. 1 the total rainfall and PET in the period of June to August

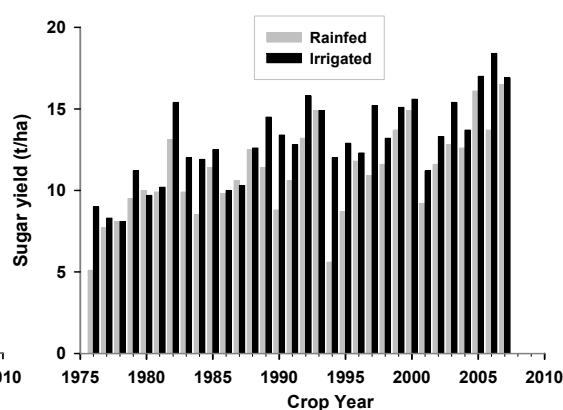


Fig. 2 the sugar yields from rain-fed and irrigated crops

Any process-based and weather-driven crop growth models should be capable of prediction and of taking account of weather variability so that the prediction can be accurate enough for any intended purposes. Analyses of model goodness-of-fit showed that 83% of the variation in the observed sugar yields can be accounted for by the simulated sugar yields using the Broom's Barn sugar beet growth model.

Table 1 shows that the projection of future weather under the high CO<sub>2</sub> emission scenario will have positive impacts on the sugar beet yield in England. However, because of drought the annual yield variability measured as the difference between 5- and 95- percentile sugar yield will also increase.

Table 1 Changes in 5-, 50- and 95-percentiles of simulated sugar yield (t/ha) when crops are harvested on 31 October in England for different time segments under the high CO<sub>2</sub> emission scenario.

Time segment	5-percentile	50-percentile	95-percentile
Baseline	7.2	10.1	12.6
2020	7.9	11.6	14.3
2050	9.3	13.6	17.2
2080	10.6	15.5	20.2

## Conclusions

The sugar beet crop has frequently suffered from climatic drought during summer months in England. The rainfall shortage in July was the most influential and responsible for 52% of the total sugar yield loss caused by drought stress. The Broom's Barn sugar beet growth model was capable of taking the weather variability into account since the model simulated sugar yields accounted for 83% of the variation in the observed sugar yields. The main impact of future weather change will be positive for sugar beet yield in England. However, the annual yield variation will also increase such that it will become difficult for the process chain to manage.

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# Important Factors to model Climate Change Effects on Evapotranspiration

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Although growers have considerable control over crop production, a major concern is the anticipated increase in evapotranspiration (ET) due to global warming. ET rates, however, are also affected by radiation, humidity, wind speed, crop morphology, and crop physiology in addition to temperature. Crop ET ( $ET_c$ ) is commonly estimated as the product of reference ET ( $ET_o$ ) and a crop coefficient ( $K_c$ ), and the main factors affecting  $K_c$  values are net radiation, aerodynamic resistance, and canopy resistance differences between the reference and crop surfaces. The standardized  $ET_o$  equation has fixed values for the canopy resistance ( $r_c$ ), and different values are likely for other crops. The  $r_c$  values might also adjust with increasing  $CO_2$  and higher temperature. Aerodynamic resistance ( $r_a$ ) depends on atmospheric stability, wind speed, and surface roughness. The relative aerodynamic contributions of sensible heat to  $ET_o$  and  $ET_c$  could change if the canopy development or the wind speed climatology are modified by global warming. In this paper, we will discuss how the  $ET_o$  and  $K_c$  values vary with microclimate and how  $K_c$  values and  $ET_o$  rates might react to global warming.

## Methodology

The standardized reference evapotranspiration equation was developed to estimate the ET from a “short”, 0.12 m height, dense canopy ( $ET_o$ ) or a “tall”, 0.5 m height, dense canopy ( $ET_r$ ), where both surfaces have known albedo, roughness, and canopy characteristics. Since management factors affect real ET, an equation that perfectly matches the ET of a reference surface will never be found for all management conditions, thereby, justifying the standardized equation for reference ET. A slightly modified version of the standardized daily reference evapotranspiration equation (Allen et al., 2005) using net radiation ( $R_n$ ) in  $MJ\ m^{-2}\ d^{-1}$ , mean daily temperature ( $T$ ) in  $^{\circ}C$ , saturation vapour pressure ( $e_s$ ) in kPa at the mean air temperature, mean daily actual ( $e$ ) vapour pressure in kPa, slope of the saturation vapour pressure curve ( $\Delta$ ) in  $kPa\ ^{\circ}C^{-1}$  at the mean daily air temperature, and the psychrometric constant ( $\gamma$ ) in  $kPa\ ^{\circ}C^{-1}$  is:

$$ET_o = \frac{0.408(R_n) + \gamma \left( \frac{C_n}{T + 273} \right) \left( \frac{e_s - e}{r_a} \right)}{\Delta + \gamma \left( 1 + \frac{r_c}{r_a} \right)} \quad (1)$$

where  $C_n=187200$  combines the air density, specific heat, and latent heat of vaporization. The respective canopy resistance values are  $r_c=70$  and  $r_c=45\ s\ m^{-1}$  for short and tall canopies, and the aerodynamic resistances are  $r_a=208/u_2$  and  $r_a=118.3/u_2$  for short and tall canopies, respectively, using wind speed measured at 2 m height ( $m\ s^{-1}$ ). Because of rounding, Eq. 1 is slightly different from the daily equation in Allen et al. (2005).

Equation 1 was used to compute  $ET_o$  and  $ET_r$  values using one year of daily data from 17 California Irrigation Management Information System (CIMIS) stations having widely different climates. Linear regressions of  $ET_r$  versus  $ET_o$  through the origin were computed to estimate the “local”  $K_c$  for 0.5 m height alfalfa. In all cases, the slope of the  $ET_r$  versus  $ET_o$  regression line closely matched the slope of a line from the origin to the mean July values for  $ET_r$  and  $ET_o$ . Therefore, the

alfalfa  $K_c$  values should be closely related to the mean July weather data. A comparison was made by calculating correlation coefficient values between each weather variable and the estimated  $K_c$  factors.

## Results

Table 1 shows estimated  $K_c$  values for alfalfa using all daily data from 2003 for 17 CIMIS stations. The correlation matrix between weather variables and the  $K_c$  values is shown in Table 2. Assuming that the slopes are indicative of  $K_c = ET_r/ET_o$  at the various locations, Table 2 shows that the  $K_c$  is clearly related to  $ET_o$ . The poor correlation with  $R_s$ , indicates that  $K_c$  values differ mainly because of aerodynamic factors. There are high correlations between  $T_x$  and  $K_c$  and between  $e_s-e$  and  $K_c$ , but there is also a high correlation between  $T_x$  and  $e_s-e$ . Consequently, spatial differences in  $K_c$  factor seem to be mainly related to variations in  $e_s-e$ . While  $e_s$  will rise with global warming, recent decades show no global increase in  $e_s-e$  (Roderick & Farquhar, 2000) presumably because the vapour pressure is also rising. Therefore, we expect that alfalfa  $K_c$  values will remain relatively constant. Increased stomatal resistance in response to higher  $CO_2$  concentration could increase canopy resistance and it might counteract temperature-induced ET increases to a small extent. Changes in wind speed could impact on ET rates, but little information is available on global warming effects on wind speed.

Station	$K_c$
Torrey Pines	1.16
Oxnard	1.19
Otay Lake	1.20
SLO west	1.23
Pt.SanPedro	1.24
Oakville	1.26
Tulelake	1.28
Escondido2	1.28
Concord	1.29
Browns Valley	1.30
Belridge	1.31
Denair	1.31
Madera	1.34
Buntingville	1.35
Stratford	1.37
Oasis	1.40
Indio	1.47

Table 1. Crop coefficient ( $K_c$ ) estimated as the slope of daily  $ET_r$  vs  $ET_o$  through the origin using data from 17 CIMIS stations in 2003.

	$ET_o$	$R_s$	$T_x$	$T_n$	$u_2$	$T_d$	$e_s-e$	$K_c$
$ET_o$	1.00	0.54	0.96	0.59	0.74	-0.45	0.98	0.94
$R_s$	0.54	1.00	0.50	-0.27	0.34	-0.75	0.49	0.37
$T_x$	0.96	0.50	1.00	0.62	0.56	-0.34	0.99	0.87
$T_n$	0.59	-0.27	0.62	1.00	0.40	0.32	0.63	0.63
$U_2$	0.74	0.34	0.56	0.40	1.00	-0.34	0.61	0.78
$T_d$	-0.45	-0.75	-0.34	0.32	-0.34	1.00	-0.41	-0.41
$e_s-e$	0.98	0.49	0.99	0.63	0.61	-0.41	1.00	0.91
$K_c$	0.94	0.37	0.87	0.63	0.78	-0.41	0.91	1.00

Table 2. Correlation matrix for mean July  $ET_o$ , solar radiation ( $R_s$ ), high ( $T_x$ ) and low ( $T_n$ ) temperature, 2-m wind speed ( $u_2$ ), dew point ( $T_d$ ), vapour pressure deficit ( $e_s-e$ ), and estimated  $K_c$  (i.e., slope of  $ET_r$  Vs.  $ET_o$  using daily data for the year 2003 from 17 CIMIS stations). Note that  $e_s=f(T_x)$  and  $e=f(T_d)$ .

## Conclusions

Our analysis and recent trends in temperature and humidity indicate that the impact of global warming on crop coefficients are likely to be insignificant if the vapour pressure deficit remains relatively constant and the wind speeds are unaffected.

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# Seasonal Weather Predictions and Crop Modelling for Wheat Yield Forecasting in Northern Italy

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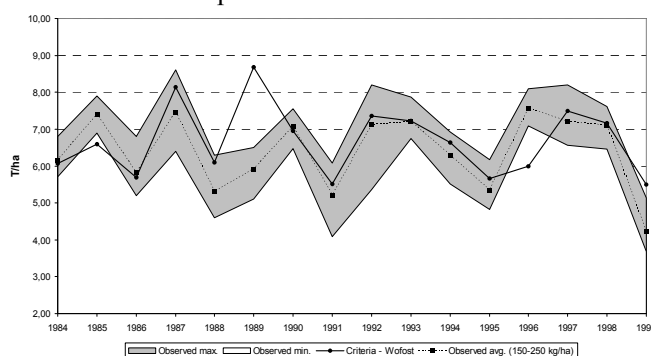
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A coupled numerical soil water balance and crop growth model (Criteria/Wofost – C/W) developed within the European R&D projects DEMETER and ENSEMBLES (Marletto et al., 2007), calibrated with field data from the Bologna university experimental farm of Cadriano, is used to predict wheat yield up to three months before harvest. The prediction of meteorological fields needed as input by the model are obtained by applying the statistical downscaling scheme developed at the regional weather service of Emilia-Romagna to the global operational multi-model seasonal predictions produced at the European weather centre (Ecmwf) within the EURO-SIP Project. This paper presents the methodology used and the preliminary results obtained in view of developing an operational wheat yield prediction chain.

## Methodology

The high resolution seasonal forecasts needed as input for the crop model are obtained by calibrating and downscaling the EUROSIP multi-model seasonal predictions, using a statistical scheme developed at ARPA-SIM. The scheme is the MOS version of the 'Perfect Prog' calibration and downscaling scheme described in Pavan et al. (2005). The final high resolution calibrated prediction consists always of an ensemble of 22 3-month averages map values over Italy for a group of local climate indices including cumulated precipitation, the wet day frequency, averaged minimum and maximum temperature, minimum and maximum temperature standard deviation over the 3 months, and the mean difference of temperature between dry and wet days.

The EUROSIP forecasts consist of multi-model global ensemble forecasts obtained running two separate coupled models: the ECMWF (Anderson et al., 2006) and the GloSea UK Met Office (Gordon et al., 2000; Pope et al., 2000) models. For both models ensemble seasonal forecasts are available for the period from 1987 to present. For application purposes only Z500 and T850 fields have been considered over a regular 2.5°x2.5° horizontal grid and, for each model and each year, 11 ensemble members have been used. The operational EURO-SIP forecasts are run starting on the first day of each month and extend up to 6 months into the future.



**Figure 1: Comparison between wheat yield simulated using measured weather data (Criteria-Wofost, thin line,) and observed yield range (grey band, Cadriano, 1984-1999).**

The observational data used to define the local climate predictands are obtained starting from the daily analysis of precipitation and minimum and maximum temperature produced operationally by UCEA (Girolamo and Libertà, 1990) covering the whole Italian territory from 1987 to present, with an approximate resolution of 35 km.

The calibrated predictions that will be used as input of the operational agronomic application here described will be the May-June-July averaged predictions obtained from the EURO-SIP forecasts initialised on the 1<sup>st</sup> of April.

The MOS calibration and downscaling technique has been shown to improve substantially the quality of the original EURO-SIP system forecasts by removing the model biases and errors not only on mean values, but also on the interannual large-scale variability. Seasonal predictions feed a weather generator (WG) based on the Richardson scheme (1984) and modified by G. Campbell. The WG produces ten replicates of daily series of temperature and precipitation used as input for the C/W model.

The fundamental role played by watertable depth (wd, m) and capillary rise in the water balance and crop growth induced to devise an empirical statistical formula to assess wd from climatology and the observed and forecast precipitation anomaly over a period of nine months. The formula was calibrated with observed wd data from Cadriano.

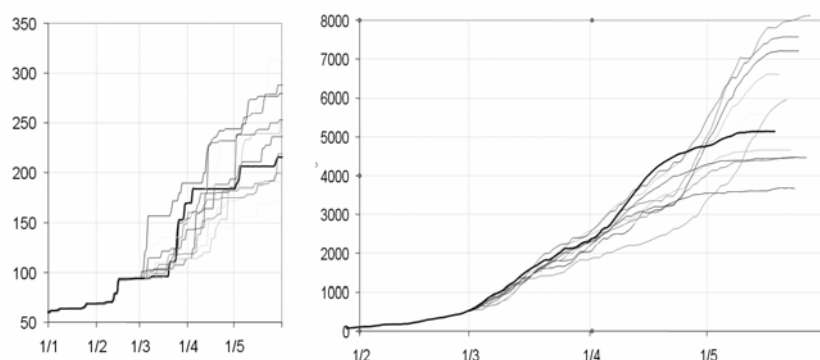


Figure 2: Example of simulation for year 2007. Left: observed cumulated rainfall from Oct. 2006 (black solid line) and 10 runs of weather generator using as input seasonal predictions (grey thin lines). Right: wheat yield simulation from observed daily data (black solid line) and wheat yield simulations obtained using WG data (grey thin lines).

## Results

Figure 1 shows preliminary results of wheat yield simulated with the C/W coupled model in years 1984-1999 at the experimental farm of Cadriano, compared with yield data from the most fertilized plots (150, 200 and 250 kg N /ha). In three years (1986, 1988, 1990) simulated yields were lowered by 20% because of known crop damages and diseases not simulated by the model while no clear explanation can be found at the moment for the 1989 and 1996 simulation anomalies. Figure 2 shows the springtime (MAM) seasonal forecast ensemble of 2007 and an ensemble of growth simulations obtained from the forecast, in a year with precipitation considerably lower and wheat phenology considerably earlier than usual. Interestingly both the observed precipitation and the growth simulated with observed data fall within the forecast ensembles.

## Conclusions

Results obtained up to now clearly indicate the possibility to set up an operational wheat yield forecasting chain starting with the downscaled MJJ seasonal forecast, followed by daily series generation, watertable level assessment, and finally growth simulation with the coupled C/W model. The procedure is going to be tested with 1987-1999 data from the Cadriano experiment.

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# Options for the management of water use in energy crop production in areas prone to summer drought

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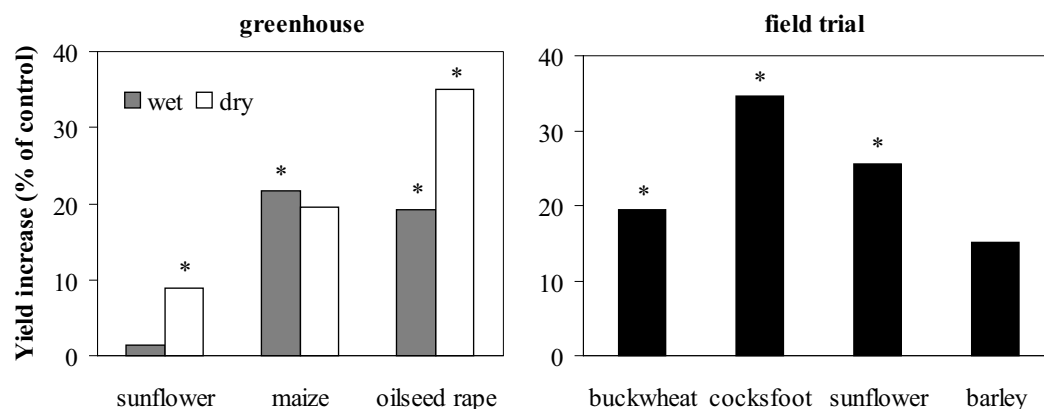
Prolonged lack of rainfall in summer is one of the major production constraints for energy crops grown on sandy soils in south-east Germany. Capturing residual moisture from the spring rains by using soil ameliorants capable of storing water for extended periods may decrease the risk of crop failure. A field trial was established on former coal mining areas near Cottbus, Brandenburg, and a greenhouse pot experiment was conducted at the Institute for Plant Nutrition in Bonn using soil from the field site. The effect of the soil ameliorants on soil moisture, plant growth, nutrient uptake, water use and water use efficiency was determined.

## Methodology

Water use (WU) and water use efficiency (WUE) were investigated for sunflower, maize, oil seed rape in a greenhouse experiment and for buckwheat, cocksfoot, sunflower and barley in the field trial. Crops were supplied with different organic and synthetic ameliorants mixed into the top 0.3m of the soil. The sandy soil from the field site was poor in nutrients. Mineral fertiliser was added before sowing and incorporation of the soil ameliorants. Soil moisture was monitored in 5 depths in the field and transpiration was monitored on a daily basis in the greenhouse. Whereas in the field trial plants were kept under natural moisture supply, in the greenhouse trial plants were either fully watered (wet) or subjected to a controlled drought stress (dry). Biomass development was measured at regular intervals and yield was determined at the end of the vegetation period. The results of a synthetic ameliorant based on a super absorbent (polyacrylate) combined with volcanic rock material (Geohumus International GmbH, Frankfurt, Germany) are presented here.

## Results

In comparison to the untreated only mineral fertilised control the synthetic ameliorant increased the yield of all crops between 2 and 35 % (Fig. 1).



**Fig. 1:** Yield effects of the soil ameliorant in percent of untreated controls in the greenhouse and the field trial. Marked bars show significant differences ( $p < 0.05$ ) between ameliorated and untreated soil.

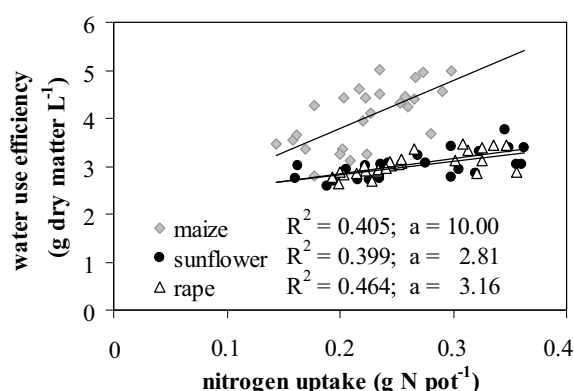
Water was not a limiting factor in the field trial due to the wet climatic conditions in 2007. Therefore, no correlation between the yield and the water use was observed. Soil ameliorants did not produce significant effects on WUE of the tested plant species relative to untreated controls.

Whereas ameliorated soils increased plant WUE in all treatments and crops in the greenhouse trial, no additional effects were observed with varying water supply (Tab. 1). Thus it can be argued that the increase of WUE by the ameliorant is not an effect of a better availability of water. The ameliorant enhanced plant nitrogen uptake despite the fact that the available nitrogen was the same in all treatments as the nutrient content of the ameliorants was determined and all treatments were equalized with appropriate amounts of mineral fertiliser before the experiment.

As shown in Fig. 2 the WUE was significantly correlated with the increased nitrogen uptake of the different crops used in the greenhouse experiment. As expected, maize had a higher WUE than sunflower or rape, but maize as well reacts more intensive on the increase of nitrogen uptake as shown by the higher coefficient of correlation (a) in Fig. 2.

**Tab. 1:** Water use efficiency (g dry matter L<sup>-1</sup>) of the treatments with synthetic ameliorant in comparison to the control in the pot experiment under dry and wet soil moisture conditions. The stars (\*) mark the moisture scenario with significant differences between ameliorated and untreated soil.

	sunflower		maize		rape	
	wet	dry*	wet	dry	wet*	dry*
control	2.94	3.05	4.18	4.57	2.99	2.88
ameliorant	2.96	3.33	4.66	4.67	3.28	3.45



**Fig. 2:** Correlation between the water use efficiency and nitrogen uptake of different crops used in greenhouse experiment ( $R^2$  = coefficient of determination;  $a$  = coefficient of correlation;  $n = 27$ ).

## Conclusions

The synthetic ameliorant increased the yield not only under conditions of drought stress, it also had an effect under the rainfed conditions in the field trial and the fully irrigated (wet) treatment in greenhouse experiment. The water use efficiency was increased, probably due to an effect of enhanced nitrogen uptake. The effect of nitrogen supply on water-use efficiency is well known now (Brück 2008), but we do not yet know why the ameliorant increased the nitrogen uptake and so the nitrogen use efficiency. No differences were observed in the content of mineral nitrogen between the ameliorated and the untreated soil after harvest implying that less nitrogen was recovered in the untreated soils. This may be an effect of a change in the root/stem ratio or in the root-length density in the ameliorated soil, or the ameliorant may have reduced the immobilisation of nitrogen by the microbial biomass. These questions will be addressed in the future studies of the ongoing project.

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# Starting a Bottom-Up Strategy to Introduce Climate-Change Adaptation Options in Castilla y Leon Agriculture

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## Introduction

Climate Change effects will possess new constraints on Castilla y Leon agriculture, as have been estimated from several assessments (Mínguez et al., 2005; Alcamo et al., 2007). Several adaptation options have been recommended as well from the available assessments, which are comprised in the Spanish Climate-Change Adaptation Strategy (OECC, 2006).

However, those up-bottom assessments are not enough to implement such options within the farmer's community. A bottom-up approach must be followed as well, according to figure 1 (OECC, 2006).

Therefore, a bottom up approach was started in the framework the EU proposal ADAGIO (Eitzinger et al., 2006). The farmer opinions about the Climate-Change vulnerability of Castilla y Leon agriculture was pointed out through a survey. Present paper shows the survey results.

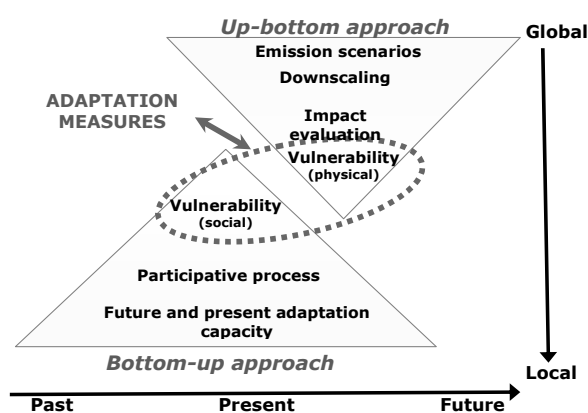


Fig. 1. The two approaches for developing Climate-Change adaptation options in agriculture

## Methodology

URCACyL, the most important Farmer Union of Castilla y León, was used as platform to farmer consultation. URCACyL comprises more than 50 000 farmers, which yielded a total production of 1270 million of euros in 2006. The survey was designed to know directly from farmers the following items:

- Knowledge level regarding Climate Change from both, global and local point of views
- Already-observed Climate risks on local agricultural production.
- Agricultural risks of the expected Climate Change for Castilla y Leon
- Willingness to adopt the already-identified Climate-Change adaptation measures.
- European, National and regional regulations that could encourage or limit the adoption of Climate-Change adaptation options.

## Results

Utset et al. (2007) provide all the survey results. Almost 50% of farmers answering the survey are in vineyards and irrigated fields. Moreover, younger and educated farmers were majority in survey. These farmers are only small percent of Castilla y Leon rural population.

The increment of pests and diseases has been identified as the most important climate risk now and in the future, although it can be related to the current invasion of *Microtus arvalis*, one of the worsens pests in Castilla y Leon history. More than 95% percent of farmers would change their current management of pests and diseases, following professional indications.

A 0.95 probability statistically-significant percent of farmers think that less precipitations and more frequent drought will very influence the regional agriculture, but mainly in rainfed crops. Future heat waves would also affect significantly, but in a lesser extent. Besides, vineyards are not included in their perception of risky crops regarding temperature increments. Their answers about unexpected freezes and storms are diverse.

The 42% of farmers are expecting to quit the farm, if climate conditions become extremely worse in the future. A 0.95 probability-significant correlation of 0.74 was found between aged farmers (more than 60) and those willing to quit the farm. Moreover, a 0.41 correlation coefficient was found between farmers willing to quit the farms and those younger than 35 years.

On the other hand, only 62.5% of farmers are willing to change land use while 95% would introduce new cultivars. The 75% percent of the consulted farmers are willing to change irrigation management, including new irrigations in currently rainfed cropping.

Castilla y Leon farmers seem to be not very aware about the potential influence of current policies on Climate-Change adaptation options, as CAP and Rural Development Policy. Most of them reject to include in the CAP cross-compliance measures involving eventual large investments, as changes in irrigation. However, they would agree to include in cross-compliance measures related to pest and diseases control.

Furthermore, 81% of the consulted farmers are willing to participate in demonstration proposals aimed to evaluate/introduce Climate-Change adaptation options in their farms.

## Conclusions

The increment of pests and diseases is the worse climate risk identified by the regional farmers. Droughts and less precipitation are important constraints, but mainly in rainfed crops. However, irrigated agriculture in the region could be very vulnerable to Climate Change, as has been pointed out in most of the up-bottom assessments.

Castilla y León farmers are more willing to introduce new varieties, able to cope with Climate Change risks, than to change land use or seeding and harvest dates. Consequently, a participatory process aimed to introduce Climate-change adaptation options in Castilla y Leon agriculture must take in account current varieties and the potential breeding tasks, instead of only simple management changes.

The current policies regarding Climate Change and agriculture must be divulgated adequately within the farmer community. Besides, demonstration activities in the framework of the Rural Development subventions must be conducted in the farms willing to adopt some Climate-Change adaptation measures.

A relative large percent of farmers would quit their land if the climate risks increment in the future. They are mainly aged people, but some young people agree also to abandon the farm. This could be related to the Castilla y Leon depopulation, particularly in the countryside. It means that any action aimed to introduce Climate-Change adaptation option in the regional agriculture must deal with the actual social issues, through a participative process. Such process must drive hope to farmers regarding the future of the regional agriculture, instead of creating alarm or fears.

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# Towards an Ideotype of Teff for Temperate Climates

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Teff (*Eragrostis tef* (Zucc.)Trotter) is a short day C4 cereal crop species, originating from Ethiopia. Teff grains and flour do not contain gluten. Gluten is a multi-protein complex in seeds that can cause coeliac disease in genetically predisposed humans. For this group, but also for other consumers, teff would be a valuable alternative. Therefore, there is an interest in growing the crop elsewhere for specialty food markets.

Teff can be grown in the temperate climates of NW Europe, such as in The Netherlands. The yields, however, are modest (1000 – 1500 kg ha<sup>-1</sup>), the harvest is (too) late in the season (September) and the crop is sensitive to lodging. The harvest index, HI, rarely exceeds 0.25. The economic feasibility of teff production in temperate climates would be reinforced if the production level could be raised to at least 2500 kg ha<sup>-1</sup>, and harvest time advanced to ca mid-August, in order to avoid the more wet and humid conditions later in the season. The objective of ongoing research is to underpin an 'ideotype' of high-yielding teff. To this end analyses are made of the developmental pattern, including the phenological response to day length and temperature. Lodging resistance will be analyzed too, adopting approaches that Ennos (1991) and Crook and Ennos (2000) applied to wheat.

## Methodology

Day length sensitivity of several genotypes was studied in pot experiments, using a glasshouse facility providing similar exposure to natural day light for all treatments. A combination of incandescent and fluorescent lamps was used to create several day length treatments. Reciprocal transfer treatments (Ellis et al., 1992) were used to determine the durations of the basic vegetative phase (BVP or juvenile phase), the photoperiod sensitive phases (PSP) and the post photoperiod sensitive phase (PPP). During the BVP the rate of development of the plant is not responsive to photoperiod; during the PSP it is. By consequence, the length of the PSP depends on the day length whereas the BVP and PPP are independent from day length conditions. The PPP is the time between the end of the PSP and heading. Across experiments the minimum day length was 9 hours and the maximum 18 hours. Day temperature was set at 23°C and coincided with the day light period of 9 hours; the night temperature to 15 °C (transition time 2h). Leaf appearance rates were monitored. The dates of heading, tiller numbers, grain yield and harvest index were also monitored in a field experiment near Wageningen (lat. 52 °N).

## Results

Teff showed quantitative short day behaviour. Fig 1 shows an example of the type of results obtained in reciprocal transfer experiments. There was clear genetic variation among genotypes in the duration of each phase of photoperiod sensitivity (Table 1). Hence, there is sufficient genetic variation to breed for

**Table 1. Ranges in durations (d) of photoperiod sensitivity phases and ranges durations to heading under constant day length (9 and 16 h) across five teff genotypes**

Basic vegetative phase (BVP)	5 to 10
Photoperiod sensitive phase for short day conditions (PSP <sub>s</sub> )	6 to 15
Photoperiod sensitive phase for long days (PSP <sub>l</sub> )	17 to 62
Photoperiod insensitive phase (PPP)	18 to 23
Sowing to heading 9 h day length	36 to 49
Sowing to heading 18 h day length	40 to 96

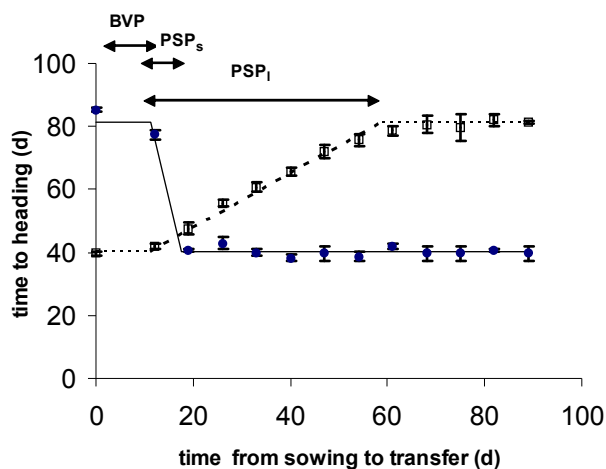


Figure 1. Example of results of reciprocal transfer experiments, exchanging pots with teff plants between a long day environment (LD, 16 hour day length) and a short day environment (SD, 9 hours). Error bars represent standard deviations. See Table 1 for explanation of BVP, PSPs and PSP<sub>i</sub>

Cereals and grasses show variable degree of tillering. Teff belongs to the PACCAD clade (Doust, 2007) showing ‘real’ tillers, i.e. lateral shoots with elongated internodes, generally resembling the main shoot in characteristics and axillary shoots, i.e. laterals emerging from phytomers with extended internodes. Teff branches very prolificously and keeps on producing axillary shoots even after flowering and even up to the top node of the plant. For example, our earliest cultivar counted three times more tillers at harvest than at flowering. There is clear genetic variation and hence hope to reduce investment of dry matter in these shoots to the benefit of seeds, analogous to crops domesticated earlier.

Under controlled conditions our highest yielding variety showed a HI of 0.35 at 9 hour day length versus 0.26 at 18 hour day length. Extrapolated to the field these data indicate that the crop could yield substantially more than the current ca 2 ton per ha (with HI of 0.1), provided the crop is supported against lodging and seed losses by rain and wind are prevented. Current lodging research in cooperation with Dr Ennos (Manchester University) will help to define the ideotype to breed for in more detail.

## Conclusions

Today, teff production at northern latitudes faces the risk of yield losses in quantity and quality by lodging and late maturity. Nonetheless, the tremendous variation within the species germplasm and the superior grain properties create a potential to grow teff as a reliable and profitable commercial crop in these regions. This on condition that extensive breeding efforts are made so as to realize the ideotype as emergent from critical assessment of the plant’s characteristics under different environments.

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a genotype of which the phenology is best adapted to European latitudes. The time from sowing to heading in the field (ever changing day length) correlated with durations observed in the greenhouse. Current experiments with reciprocal transfer treatments, including day length and temperature regimes, will provide information to design a phenological model to explain the date heading in the field for different genotypes in different climates.

The number of main stem leaves (Y) versus time (X; days after sowing) could be described with one single equation (Eq 1) across five genotypes and three constant day length treatments (9, 12 and 16 h):

$$Y = 13.35 - 14.91 \cdot 0.979^X \quad (R^2 = 0.98) \quad (\text{Eq 1})$$

At 9 h day length 6-8 main stem leaves were formed across cultivars and at 16 h day length 8-12 leaves. Vegetative development is similar across genotypes and photoperiods until it is interrupted by panicle initiation.

# Phenology Of Maize And Temperature Changes In The Last 40 Years

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Increases of temperature, already observed in northern Italy and at the global scale, are expected to have an impact on agriculture. In particular such a change may have effects on crop growth and final yield, since these are largely determined by weather conditions during the growing season. The science studying the relationships between climatic factors, seasonal changes and developments cycles is plant phenology. Phases are reached when plant cumulate a determined amount of heat, that can be calculated using the method of Growing Degree Days (GDD). Aim of this study was to calculate the cumulative GDD for maize using daily temperature and sowing date for maize recorded in the experimental farm of the University of Bologna for the period 1967 – 2007. Moreover experimental phenological data are available for the period 2003 – 2007. Growing season length and phenological main phases appearances trends will be evaluated, together with air temperature and number of hot days.

## Methodology

An agro-phenological station was installed in 2003 in Cadriano (Bologna, Italy, 44° 33' 03" N, 11° 24' 36" E, 33 m a.s.l.), next to the University of Bologna agrometeorological station.

The soil texture is sand 37%, silt 45%, clay 18%, with a shallow groundwater. The crops were selected on the base of the most common field varieties in Emilia-Romagna (Ventura et al., 2006). For every crop several rows were sowed, the number depending on the species, and the samples were selected only on the central rows, to minimize edge effects. The cultivation of the crops was conducted under optimal water conditions especially at the beginning of the crop cycle. The phenological surveys were performed every week during the biological cycle, according to the operational protocol defined by the Italian Phenagri project (Botarelli et al., 1999). Next to the agrophenological station is the agrometeorological station (Ventura et al., 2002), with mechanical and electronic instruments, including hygrothermograph, rainfall recorder, phreatimeters (2.5 m depth) as well as anemometers and radiometers. Both the mechanical and electronic instruments are in use and periodically checked and calibrated.

To determine the phenological stages of flowering and physiological maturity of maize the method of the Growing Degree Days (GDD) was used. The five years of experimental data were compared to calculated GDD to confirm the thermal amount needed to reach the two stages.

Sowing date for maize came from a long term experiment held in the same farm during the period 1967-2007, with different cultivars all FAO class 500.

All the meteorological and phenological data time series were evaluated with the non-parametric Mann-Kendall test (Sneyers, 1990).

## Results

The climate of the experimental sites is temperate-continental with a mean annual air temperature ( $T_{med}$ ) of 13.1°C and precipitation annual amount of 709 mm, almost constant in time (don't shows a significant trend with Mann-Kendall test), with picks in spring and autumn.  $T_{med}$  in Cadriano during the period 1967-2007 increased of about 1.4°C.

One aspect of climate change that has received limited attention is the potential difference between changes for daily maximum and minimum temperatures,  $T_{min}$  and  $T_{max}$ , and resulting changes in the

diurnal temperature range (DTR). We observed a more significant increase in  $T_{\max}$  than in  $T_{\min}$ . The former increased from 17.4°C at the end of the sixties to the actual 19.1°C, while the latter increased from 7.5°C to 8.7°C in the same period. As a result DTR increased of about 0.5°C, with a negative trend in September, October, November and December and a positive trend in the other months. All temperature data trends are significative with 99% confidence limit.

As a result of increased temperatures, mainly during winter, in the last decades the sowing date was anticipate, therefore flowering stage anticipated, namely 18 days in the period from 1967 to 2007 (Fig. 1). During the study period the crop cycle, in particular the time from sowing till flowering, lengthened of 13 days. Since the crop cycle start earlier with respect to Julian days, the physiological maturity anticipated 25 days (99% significant with Man-Kendall) but the length of cycle to maturity was constant (trend not significant with Man-Kendall). Both these results were confirmed by phenological observations (empty dots in Fig. 1), that were coincident with stages as calculated by GDD.

Another effect of increasing temperatures on maize is related to high temperatures. They influence the maize growth and maturation, because when the maximum air temperature exceeds the higher threshold (30°C) the plant slows growing. During the period from 1967 to 2007 in the study area the number of days with temperature over 30°C increased significantly (Fig. 2). Considering the slope of the regression line, the hot days went from about 25 to about 43 per year.

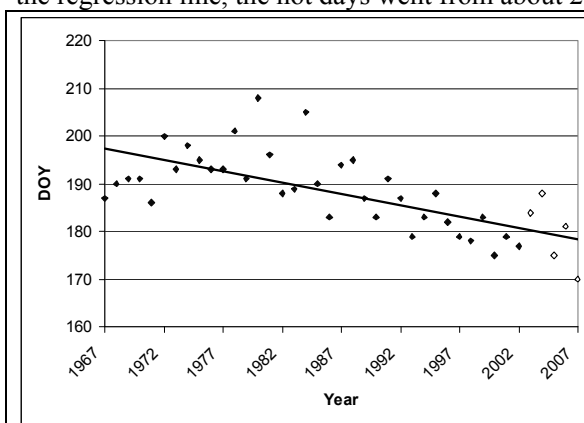


Fig. 1 Flowering stage appearance. Empty dots are observations, black dots are calculated by GDD. (confirmed by Mann Kendall test with 99% confidence).

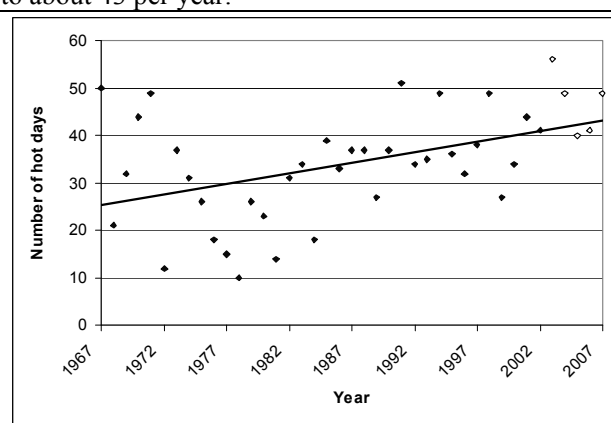


Fig. 2 - Number of days per year with maximum air temperature higher than 30°C. (confirmed by Mann Kendall test with 99% confidence)

## Conclusions

Increasing temperatures, mainly during cold months, caused an advance of vegetative renewal and sowing date, and consequently an anticipate flowering, accompanied by longer duration of the flowering stage. This is probably due to the lower temperatures experienced by the crop during the early stages. Moreover, an early sowing can increase the risk of low temperatures and precipitations, and the exposition to diseases. On the other hand the growing number of hot days causes an increase of the demand of irrigation and of the risk of lower yield, as found by other authors (Wang et al., 2008).

## Aknowledgments

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# Potato Root Growth and Distribution under Three Soil Types and Full, Deficit and Partial Root Zone Drying Irrigations

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## Introduction

A good and basic knowledge of crop root distributions and water uptake patterns is a critical issue in soil and water management and it is becoming more important since we are going to develop modern agricultural practices to use water and nutrients efficiently (Coelho and Or, 1999).

Deficit irrigation has been adopted for a long time to cope with fresh water shortage. Recently a novel water saving irrigation technique called partial root zone drying (PRD) has been introduced, which has no or little negative effect on yield (Kang and Zhang, 2004). Kang and Zhang (2004) and Shahnazari et al. (2007) argued that under PRD, new root growth may be induced due to different soil water pattern in the soil, which increases the efficiency of water and nutrient uptake. However, since PRD is a new irrigation technique, few studies have been done to explore its effect on different physiological aspects.

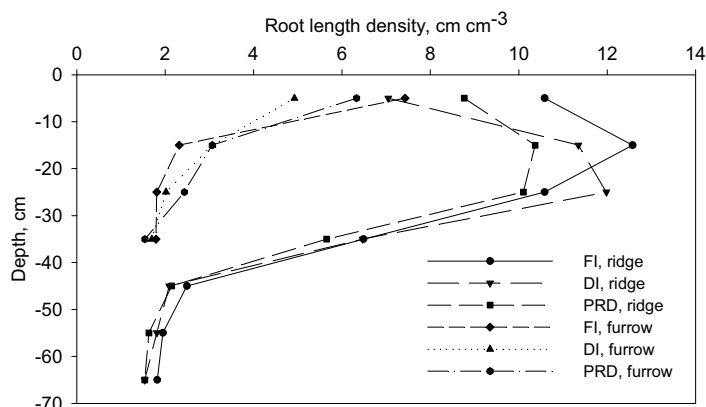
Root studies are generally very time and labour consuming and to the best of our knowledge, no study has been done to investigate the effect of different irrigation techniques in different soil types under field conditions. Such studies are helpful in elucidating how root architecture is affected by these environmental conditions and will enable modellers to develop root distribution models to describe root system function in SPAC models. The present study has focused on potato root system reactions to environmental conditions.

## Methodology

Potatoes (cv. Folva) were planted with 30 cm plant distance and 75 cm between rows, in semi-field (lysimeter) conditions in summer 2007 at Research Center Foulum, Aarhus University, Denmark. Three irrigation methods were applied in three soil types: sandy loam, loamy sand, and coarse sand with c. 20, 10 and 5% clay respectively. The irrigation treatments were full, deficit (DI), and partial root-zone drying (PRD), with three replications. The plants were fully irrigated till tuber bulking (13<sup>th</sup> June) and then irrigation treatments started as DI and PRD where plants received 65% of fully irrigated plants. On 30<sup>th</sup> July root samples were taken on top of the ridge down to 70 cm and in the furrow down to 40 cm at 10 cm intervals with a 5 cm diameter auger. In FI and DI treatments, samples were taken below a plant and at the halfway between two plants (15 cm from the plant) just in one side; but in PRD on both sides in order to represent the changes in root distribution under wetting and drying pattern of PRD. Exactly the same sampling pattern was also done in the furrows. Overall, four positions were considered for FI and DI and six positions were considered for PRD. Samples were washed gently and roots were collected in sieves with 250  $\mu$  m mesh sizes and root lengths were determined by the Newman method and then converted to root length density (RLD) by considering the soil volume. Data were subjected to ANOVA analysis and fitted to the Gerwitz and Page (1974) decaying exponential model,  $l = a \exp(-b \times d)$ , in which the fitted parameter,  $b$ , considers the soil moisture and other environmental factors.  $l$  and  $d$  are root length density ( $\text{cm cm}^{-3}$ ) and sampling depth (cm), respectively, and  $a$  is a fitted parameter representing root length density at soil surface.

## Results

ANOVA showed that there was no significant difference between the irrigation treatments on RLD, which indeed revealed that potatoes under limited water supply have produced the same amount of root as FI. This finding confirms that drought conditions may induce relatively more root production. However, the order of the values of RLD was FI>DI>PRD. While loamy sand had higher RLD than coarse sand (but not significantly), sandy loam had significantly lower RLD than the other two soil types across all the depths. The Figure shows the root length density distribution for all the sampling positions in the loamy sand.



Treatment	Position	Sandy loam	Loamy sand	Coarse sand
FI	Ridge	0.21	0.11	0.14
	Furrow	0.52	1.4	0.17
DI	Ridge	0.24	0.15	0.14
	Furrow	0.38	0.62	0.24
PRD	Ridge	0.18	0.12	0.14
	Furrow	0.55	0.47	0.28

ANOVA revealed that  $b$  values were not significantly different between treatments. However with regard to soil types, loamy sand and coarse sand had significantly lower  $b$  values ( $p=0.01$ ) than sandy loam.

## Conclusions

In this study we found deficit and partial root zone drying irrigations on potato produced the same amount of roots as full irrigation and the differences between treatments were not significant. Moreover, we found that root penetration was higher in loamy sand and coarse sand than sandy loam and more roots were found in deeper soil layers of the loamy sand. Root length densities fitted well ( $R^2>0.9$ ) to a decaying exponential model and we found that on top of the ridge and in the furrows the fitted parameter of the model was minimum for the loamy sand and coarse sand, respectively.

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It is seen that on top of the ridge RLD decreases gradually from -25 cm to -45 cm and then became constant below -45 cm. The same root growth pattern was observed in the other two soil types too. It is noteworthy that around 90% of the root system, in all irrigation treatments, is found in the top 35 cm of the ridge.

Since the height of ridge was ca. 35 cm from bottom of the furrow, then RLD at depth of -5 cm in the furrow represents RLD at depth -35 cm measured from top of the ridge but between two ridges.

The Table shows the values of fitted parameter,  $b$ , of the Gerwitz and Page (1974) model. It is seen that loamy sand and coarse sand have the minimum values of the fitted parameter on the ridge and the furrow, respectively. Low value of the fitted parameter,  $b$ , reflects more even and deeper root penetration in the soil.

# Climate Effects on Ampelometric Characters of *Vitis vinífera* L. cv. CENCIBEL

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Castilla-La Mancha extends over a territory of about 80.000 square kilometres located on the central plateau of the Iberian Peninsula. This region hosts the greatest concentration of vineyards in the world. Many varieties of wine grapes are cultivated but Cencibel is the autochthonous red variety that provides the best wines. This variety has many synonyms (Chomé, 2003) but "Tempranillo" in La Rioja is the most widespread. In a study presented recently (Martins, 2004) on surveys conducted in La Rioja, Douro, Alentejo and Valdepeñas, outlined the assumption that the possible area of origin of the variety is Valdepeñas.

## Methodology

We have been working in the characterization of different clones of Cencibel from old vineyards in the West of La Mancha since 2003. In 2006 and 2007 we took ampelometric measures from 15 possible different clones and we found out some differences that might be due to the climate, very different the two consecutive years. In 2006, the measures were taken with rule and hand angle meter. In 2007, we improved the method using software for shape analysis WINFOLIA V2006 ©Regents Instruments. The systematic of leaves collection was the same in 2006 and 2007 except in the date, which was later in 2007 (climatic reasons). Using Multivariate Analysis Techniques (software XLSTAT\_Pro version 7.5 © 1995 -2006 Addinsoft y XLSTAT\_3DPlot version 3.0 © 1995 – 2006 Addinsoft.) we try to explain the main differences found out and give instructions to validate data from different years independently from climatic events.

## Results

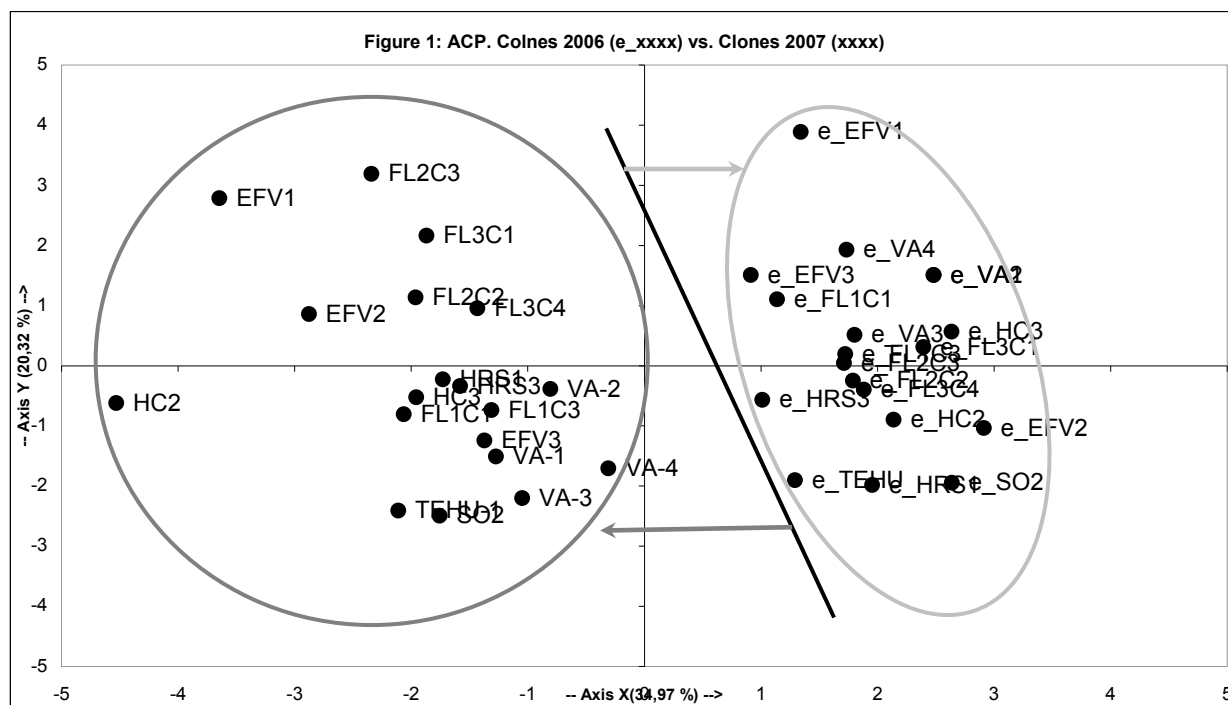
The ampelometric characterization is very important to classify different varieties taking measures of length and angles from adult leaves. But this procedure supposes that the ampelometric data does not change from year to year. The 2007 results remain substantially groups of 2006, except Tempranillo clones (that appear more mixed with the rest). The groups of clones are the same obtained in earlier studies (Amorós, 2004): Group HC+HRS, group TE, group FL, group SO and group VA (data provided on the poster).

Year	Tm. may	Tm. June	R. April	R. May	GDD (Apr.-Oct.)
2006	19°C	22.5°C	45 mm	19 mm	2065°
2007	15°C	20°C	132 mm	55 mm	1702°

Year 2007 was more rainy (Rainfall April and May in the table) than 2006 and we found that the leaves have shown a significant increase in size (Carrión, 2006). This fact is perfectly eliminated by taking measures always respect N1 (Main leave nerve).

Temperatures were higher in 2006 than in 2007 (see in table mean temperature in May and in June) and the delay in development remains all over the season as we can see in the total GDD (Winkler, 1962). This is the reason why the difference in the leaves development is not the same even the delay in the recollection from 2007 respect 2006. When practicing PCA set at two years of study we find this surprising Figure 1 in which we can warn the clear separation between 2006 (notation e-XXX) with the same clones in 2007 (XXX notation).

The image is nearly symmetrical respect to the vertical axis of the graph. We look for the reasons in the graph of the variables (see poster) checking that the separation between the two years was due to the size of the angles between the nerves leading a1, a2, a3 and petiole sinus size (SA/N1) and petiole length (P/N1). To confirm the validity of the ampelometric analysis we made FDA to the data from 2006, 2007 and 2006-2007 together.



## Conclusions

It is important therefore to take the leaves sample in the same phenological state every year to be measured, mainly for the characters of angles between the major nerves that are opening along the season, and petiole sinus, which is closing.

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# Partial Root-zone Drying Increases Water Use Efficiency and Marketable Yield of Potato

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Potatoes rank fourth in production volume among the world's agricultural products after wheat, rice and corn (FAO, 1995). Potato is relatively sensitive to soil water deficits (Doorenbos and Kassam, 1979), and studies to introduce water saving techniques such as deficit irrigation (DI) have often yielded discouraging results (e.g. Shock et al., 1998). As a shallow-rooted crop, irrigation remains crucial for growing potato plants even in humid areas, and this consumes large quantities of fresh water. During the last decade a novel irrigation strategy, partial root-zone drying (PRD), has been developed (Dry and Loveys, 2000). The PRD approach is to use irrigation to alternately wet and dry two spatially distinct parts of the plant root system. This could potentially be superior to DI because *inter alia* PRD maintain easily available water in the main root-zone (Shahnazari et al., 2007; Shahnazari et al., 2008). In this study we analysed the effect of PRD versus full (FI) or DI irrigation in different plant growth stages on water use, plant growth and product quality.

## Methodology

We conducted experiments on potato (*Solanum tuberosum* L. cv. Folva) response to PRD-irrigation during three years 2005-2007 in outdoor rain-protected lysimeter facilities. The potatoes were planted with 30 cm plant distance and 75 cm between rows. Three irrigation methods were applied in three soil types: loam, sand loam, and coarse sand with c. 20, 10 and 5% clay respectively. The irrigation treatments were full (FI), deficit (DI), and partial root-zone drying (PRD), with four replications. The plants were fully irrigated till tuber-bulking and then irrigation treatments started as DI and PRD where plants received 70% of fully irrigated plants. On coarse sand also PRD and DI throughout the growing season and PRD during tuber-initiation was tested.

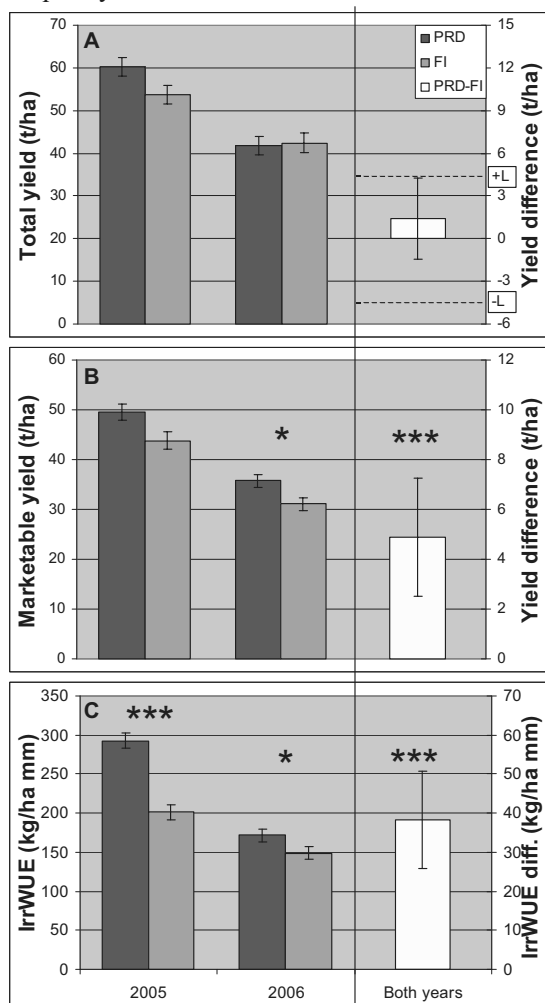
## Results

PRD increased the concentration of abscisic acid in xylem sap expressed from stems, which in turn decreased stomatal conductance. The lysimeter experiments allowed a full evaluation of the water balance components. Evapotranspiration was reduced by c. 30 mm or 10-15% by PRD during the tuber-bulking phase of growth compared to FI (Table 1).

**Table 1.** Water balance in FI and PRD treatments on three soils as average of 2006 and 2007

Soil	Treatment	Irrigation (mm)	$\Delta$ Soil water (mm)	Drainage (mm)	Total ET (mm)
Coarse sand	FI	245	11	24 $\pm$ 4.5	232
	PRD	207	23	23 $\pm$ 3.5	207
Sand loam	FI	220	20	8 $\pm$ 0.6	232
	PRD	177	40	8 $\pm$ 0.4	209
Loam	FI	245	23	26 $\pm$ 1.4	242
	PRD	196	34	12 $\pm$ 1.6	218

A meta-analysis using bioequivalence tests on data from coarse sand during 2005 and 2006 showed that the total yield was essentially the same in PRD and FI, while marketable yield was increased by 14% in PRD due to a larger fraction of tubers with sizes between 40-60 mm. The occurrence of quality defects: scab and malformed tubers was essentially the same in PRD and FI. Consequently, the irrigation water use efficiency was significantly increased by over 20% by PRD during the tuber-bulking phase compared to FI (Fig. 1). PRD during the tuber-initiation stage of growth, however, decreased the yield and quality of tubers.



**Figure 1.** A-left: Total yield (Y) of potatoes in the PRD and FI treatments during two years 2005 and 2006, bars indicate  $\pm$ SEM (n=4).

A-right: Bio-equivalence test of weighted average yield difference between PRD and FI for the two years, bars indicate  $\pm$ CI90. As threshold limit ( $\pm$ L) in the test we used 4.5 t/ha.

B-left: Marketable yield of potatoes in the size classes from 40-60 mm in the two treatments.

B-right: Difference in marketable yield between PRD and FI as average of the two years. PRD increased marketable yield with 4.9 t/ha.

C-left: Irrigation water-use-efficiency in the two treatments.

C-right: Difference in irrigation water use efficiency between PRD and FI as a weighted average of the two years. PRD increased irrigation water use efficiency with 38.3 kg/(ha mm).

## Conclusions

Collectively, the results indicate that intelligent irrigation management, even in a drought sensitive crop as potatoes, can achieve considerable water savings. However, during the early, most sensitive potato growth-stages evaporative demands have to be met. This corresponds well with recent results obtained by Saeed et al. (2008). PRD is a new and relatively unexplored biological water saving technique, which still can be optimised with respect to ABA-signalling effects. We have demonstrated here that

PRD can induce an increase of middle-sized tubers and marketable yield, and this effect also need further investigation, in order to be optimised and used in potato crop management.

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# Is Durum Wheat-Winter Pea Intercropping Efficient To Reduce Pests And Diseases?

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Intercropping (IC) is known as an agricultural practice which can improve the use of environmental resources (light, nutrients and water) resulting in yield advantages compared to sole cropping (SC) (Willey, 1979) particularly in low input systems. But, diseases and pests can strongly affect both yield and grain quality in such systems. Now, numerous studies have shown significant reductions in harmful insects and on diseases in IC compared to SC of the same species (Vandermeer, 1989; Kinane and Lyngkjaer, 2002) even if others studies did not confirmed these findings. The aim of our study was to evaluate the assumption that IC can reduce pea pests (green aphids and weevils), pea ascochyta and main durum wheat diseases (mildew, brown rust, fusarium and septoria).

## Methodology

An experiment was carried out in Auzeville (SW France) in 2006-2007. Three main treatments were compared: *i*) durum wheat (cv. Neodur) sown at 280 plants.m<sup>-2</sup> (W-SC), *ii*) winter pea (cv. Lucy) sown at 60 plants.m<sup>-2</sup> (P-SC), *iii*) durum wheat-winter pea IC, each specie sown at half of normal density (IC). Two fungi managements have been evaluated: *i*) no fungicide treatment (NT) and *ii*) two applications (T) of metconazole fungicide (90 g.ha<sup>-1</sup>). Two fertiliser-N sub-treatments were applied on W-SC and IC as following: *i*) no fertilizer (N0), *ii*) moderate fertilization (N1) splitted in 2 applications of 80 kg N.ha<sup>-1</sup> at wheat tillering and 60 kg N.ha<sup>-1</sup> at stage 'flag leaf visible'. P-SC was only evaluated without fertilization. The two species were sown in row-intercropping on Nov. 9, 2006. The experiment was a two replicates split-split-plot. Each sub-plot (21 m<sup>2</sup>) consisted of 11 rows of length 12 m spaced 14.5 cm. Pea aphids have been counted every week on 10 plants. During pea flowering, the number of nodules of 5 plants has been evaluated considering five classes (1 to 5) from no nodules to more than 20 nodules per plant. Pea ascochyta has been quantified separately for stem, leaves and pods, considering a note of attack (0 to 100), respectively from 'no symptom' to more than 80% of the surface covered by the disease. For each wheat diseases (mildew, brown rust, fusarium and septoria), 10 plants have been observed and the percentage of attack has been calculated considering the percentage of plants attacked multiplied by the percentage of the surface covered by each of the four diseases. The note was divided by 4 in order to represent a pondered mean effect. Analyses of variance were performed and means were compared using the LSD test at the 0.05 probability level.

## Results

As hypothesised, the number of green aphids per pea plant was significantly higher in SC than in IC (Figure 1). Moreover, no difference was observed between N0 and N1. The number of green aphids increased from the beginning of March to the middle of May and then decreased until the beginning of June. Focusing on weevils (Figure 2), the percentage of nodules drilled was higher than 85% and we did not observed any difference between IC and SC neither between N0 and N1. Moreover, the number of nodules seems not to be different between IC and SC and thus even if the amount of nitrogen coming from N<sub>2</sub> fixation was higher in SC compared to IC (97 kg N.ha<sup>-1</sup> and 61 kg N.ha<sup>-1</sup> respectively corresponding to 56% and 89% of all the N acquired by pea).

Figure 1: Number of aphids per plant of pea at different sampling dates for sole crop (SC) and Intercrop (IC). Values are the means (n=2).

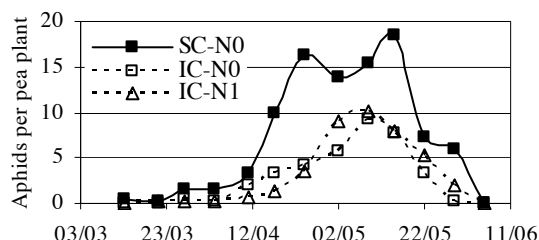
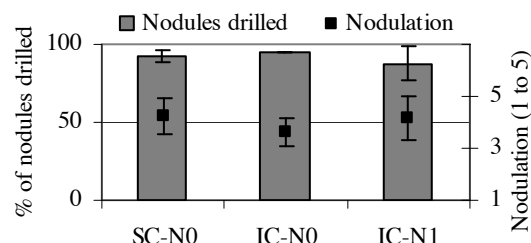


Figure 2: Note of nodulation on pea roots (scale 1 to 5) and percentage of nodules drilled. Values are the means (n=2 ± S.E.).



For all treatments, pea ascochyta notes (Figure 3) were higher on stems and leaves compared to pods. The fungicide protection (T) allowed a significant reduction of ascochyta in IC but not in SC. Nevertheless, the levels of attack were similar in IC and SC for NT but ascochyta was slightly reduced in IC with fungicide application. Considering wheat diseases, no difference was observed between IC and SC and higher values were found for N1 compared to N0. Moreover, the increase of Fusarium and Brown rust with fertilization was greater than for Septoria. The fungicide application has been very efficient for both IC and SC reducing total diseases by 88% and 47% for N0 and N1 respectively.

Figure 3: Pea Ascochyta notes in IC and SC for stems, leaves and pods and for the different treatments. Values are the means (n=2 ± S.E.).

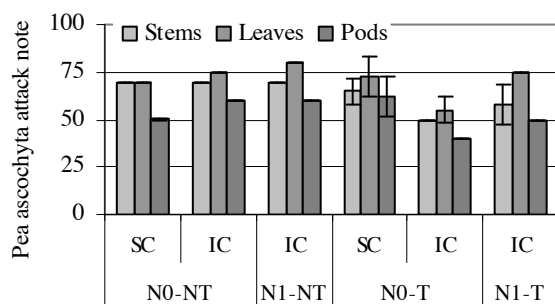
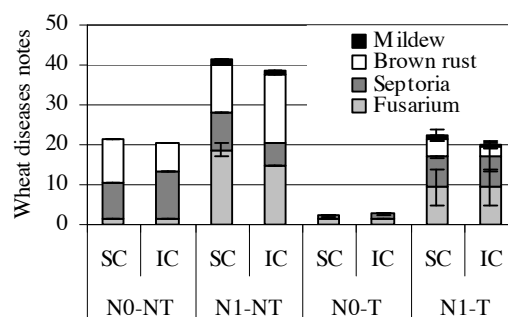


Figure 4: Wheat diseases notes in IC and SC for Fusarium, Septoria, Brown rust and Mildew. Values are the means (n=2 ± S.E.).



## Conclusions

Our results showed that IC seems not very efficient to reduce wheat and pea diseases, excepted for pea ascochyta which has been significantly reduced in IC with fungicide application. However, we observed that some diseases were reduced in IC while others were increased indicating that fungi diseases were specifically dependant on interactions between plant architecture, disease dispersion and farming practices. Concerning pests, the effect of IC on the reduction of pea aphids but not weevils can be attributed to the greatest mobility of weevils. Moreover, it can be suggested that the effect of IC depends on plant environmental conditions (resources dilution, physical barrier, microclimate, chemical...). As a consequence, because IC involves functional complementary groups of plants, such systems could be optimized in order to reduce the use of pesticides but this needs further studies in order to better understand interactions between plants, diseases, pests and farming practices in IC.

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# Leaf Senescence Response to Changes in the Source-Sink Ratio during Grain-filling in Maize

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## Introduction

Maize grain weight response to changes in assimilates availability per grain after flowering had noticeable differences depending on whether assimilates are decreased or increased. Borrás et al. (2004) showed that while decreases in assimilates during grain filling significantly and proportionally reduced grain weight, increases in assimilates per grain did not result in a clear increase in final grain weight. Thus, it seems that grain growth is more or less co-limited by both source- and sink-strength during post-silking. If this interpretation is right, grain growth demands are just satisfied by the source strength and therefore senescence would be at least partially governed by the strength of the sink-demand. Very little is known about the effect of sink size on leaf senescence and detail studies are required to determine the degree of dependence of leaf senescence on grain growth demands.

Leaf senescence pattern in maize is characterized by two phases: a first phase with a lower senescence rate and a second phase in which senescence rate is increased (Borrás et al., 2003). The first phase of slow senescence is in parallel with the slow phase of grain biomass accumulation (lag phase), while the advance of the second senescence phase is concurrent with the active grain biomass accumulation phase. Consequently, green leaf area loss in post-flowering is the mirror image pattern of grain biomass accumulation. Sinclair and de Wit (1975) suggested that green leaf area loss in post-flowering is associated with grains nutrients demand; as a result, senescence rate would be directly related to the magnitude of assimilate demanded by the grains. Only limited information is available on how changes in source-sink ratios throughout grain filling may alter the dynamics of leaf senescence in maize (e.g. Sadras et al., 2000). Therefore, we aimed to analyze the effect of source-sink ratio manipulations after silking on leaf senescence rate during grain growth in maize in order to (i) characterize the leaf senescence pattern (i.e. evolution of senesced area by leaf), and (ii) study photosynthesis rate and leaf chlorophyll content during grain filling period.

## Methodology

A maize hybrid commonly grown in the region (Helen) was sown under semi-controlled conditions in large rectangular containers (1 m length x 1 m width x 1 m height) situated at ETSEA Campus, University of Lleida (41°35'S, 58°29'W). Crop was sown at a density of 14 plants per m<sup>2</sup>. The crop was fertilized with 100 kg N ha<sup>-1</sup> at sowing, and another application with 14 l ha<sup>-1</sup> of macro (P, K) and micro (B, Fe, Mn, Zn, Cu) at the stage of c. 4-5 leaves. The crop was watered throughout the growing season in order to avoid water stress. Treatments consisted of (i) a control, (ii) ears bagged at silking to prevent pollination, (iii) ear removal 10 days after silking, (iv) ear removal 20 days after silking, and (v) removal of all leaves (defoliation) except three (the penultimate, the ear leaf and one intermediate leaf between them) at silking. The experimental was set in a randomized complete block design with three replications.

Crop phenology was determined for each individual plant. The accumulated thermal time (TT) from silking was calculated (base temperature 8 °C; Ritchie and NeSimth, 1991). Measurements of senesced leaf area, photosynthetic rate (Model Lci, ADC Bioscientific, UK) and leaf greenness (SPAD readings) were taken in all treatments twice a week.

## Results

Leaf area at silking was  $2,40 \cdot 10^{-2}$ ,  $4,34 \cdot 10^{-2}$  and  $5,35 \cdot 10^{-2} \text{ m}^2$  in the penultimate, ear and intermediate leaves, respectively. The relative loss of green area (%) was bi-linear in the penultimate and intermediate leaves; and was linear in the ear leaf. Differences in senescence rate between treatments were slight and only noticeable in the penultimate and the ear leaves. As a result, no significant ( $P>0.10$ ) differences were found between treatments in leaf green area duration at the canopy level (Fig. 1).

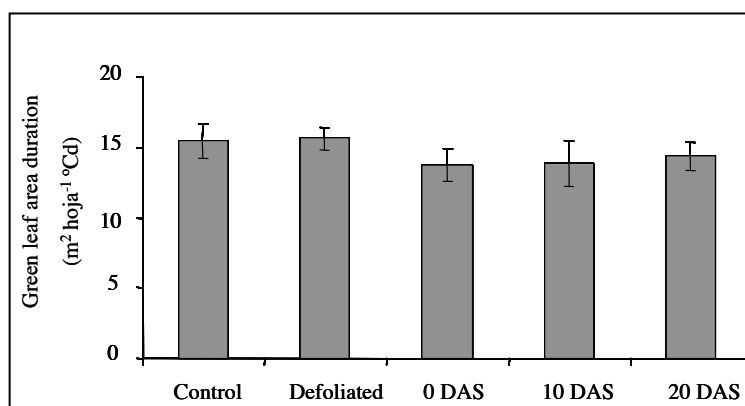


Fig. 1. Green leaf area duration in grain-filling period in maize for different source-sink ratios treatments. Control, defoliated at silking and ear removal at 0, 10 and 20 days after silking (0, 10, 20 DAS).

The evolution of leaf chlorophyll content decreased linearly throughout the grain filling period. In addition, higher source-sink ratios did not result in higher capacity of the leaves to maintain high photosynthetic rates. For all the three leaves, the decreased in photosynthesis rate was partially related to chlorophyll loss and therefore there was a positive relationship between photosynthesis and SPAD readings ( $R^2 = 0.46$ ,  $P<0.001$ ).

## Conclusions

The higher source-sink ratio treatments (ear removal at 0, 10, 20 days after silking) presented higher senescence rate than the control and the removal of all leaves treatments. The SPAD readings and photosynthesis rate per leaf area decreased linearly along the post-silking period, but without significant differences between the control and the other treatments. Higher source-sink ratios did not result in higher capacity of the leaves to maintain high photosynthetic rates or leaf greenness. Thus, leaf senescence in maize was not directly related to sink size and the hypothesis that grain growth would be strongly source-limited should be tested more thoroughly. At least the assumption that leaf senescence would be dependant on sink-strength that is compatible with a source-limited grain growth was not fulfilled in the present study. This is relevant as it might mean that further rising yield in maize could be achieved by further increasing sink strength (either more grains set or greater grain weight potential) without penalties in the leaf area duration and assimilative capacity during grain filling.

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# Derivation of Plant Parameters of Winter Wheat from Reflection Spectrometry

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For applications in precision agriculture fast, easy and non-destructive methods to measure site specifically parameters characterising the crop are an important prerequisite. For winter wheat it is an established method to derive information about the nutrition status and fertilisation demand from the reflection of the canopy (Yara N-Sensor). Besides the common indices like infrared-to-red ratio, infrared-to-green ratio or red edge inflection point (REIP), high resolution narrow band spectrometers like the hand held tec5 HandySpec Field and the tractor based Yara FieldScan allow for the calculation of a multitude of different indices based on a large number of wave bands.

The aim of this study was to identify reflection indices suited for these measurements and to derive equations for the estimation of the plant parameters leaf area index (LAI), above ground dry matter (DM) and nitrogen amount in the plant biomass (Nshoot).

## Methodology

Canopy reflection was measured at several dates during the growth period using a HandySpec Field (tec5) hand held spectrometer in different plot trials with winter wheat on the experimental farm Hohenschulen of the University of Kiel, Germany, during the years 2005 to 2007. At the same time LAI was measured with the Licor LAI-2000 and at some dates also destructive measurements of LAI, DM and Nshoot were carried out.

A special software was developed to calculate indices from the measured reflection in a simple ratio form ( $SR = \lambda_1 / \lambda_2$ ) and in a normalized difference form ( $NDI = (\lambda_1 - \lambda_2) / (\lambda_1 + \lambda_2)$ ). These indices were calculated for all possible combinations of wavebands and correlated to the plant parameters. The software provides the correlation coefficients in a matrix as a criterion to discriminate suited indices. The combinations of wavebands with the highest correlation to plant parameters were chosen and for these newly identified indices and some commonly used reflection indices estimation equations were determined by linear regression. Independent data from a different subset of trials were used for validation to compare the estimation accuracy..

## Results

For all plant parameters the highest correlation was achieved with indices consisting of one waveband in the near infrared wavelengths and one waveband in the transition from red to infrared. The correlation with measured data for these indices was high and generally considerably better than commonly used indices like the normalized difference vegetation index NDVI (Tab.1 shows the relations exemplarily for Nshoot). The REIP performed best of the indices described in literature and almost as good as the best newly derived index which is the reflection at 740nm divided by the reflection at 800nm (R740/R800).

The prediction accuracy of the estimations derived from R740/R800 for the validation data set was good with root mean squared errors (RMSE) of 0.43 m<sup>2</sup>/m<sup>2</sup> for LAI, 152 g/m<sup>2</sup> for DM and 3.03 g/m<sup>2</sup> for Nshoot. The estimation equations are valid for all development stages from BBCH 30 to BBCH 59 (Fig.1).

Table 1: Correlation coefficient  $r^2$ , estimated parameters  $m$  and  $b$  of the linear regression  $y = m \text{ Index} + b$  and root mean squared error RMSE of selected vegetation indices for the amount of N in the aboveground biomass

Index	r <sup>2</sup>	m		b		RMSE [g N/m <sup>2</sup> ]
R740/R800	0.91	-48.0	± 1.3	45.7	± 1.0	1.47
NDI(740,800)	0.91	73.1	± 1.9	-1.04	± 0.28	1.45
R800/R550	0.89	1.24	± 0.04	-0.266	± 0.295	1.60
NDVI	0.66	27.5	± 1.6	-13.0	± 1.3	2.81
SAVI	0.55	32.0	± 2.3	-7.03	± 1.20	3.21
REIP	0.89	0.948	± 0.026	-678	± 19	1.55

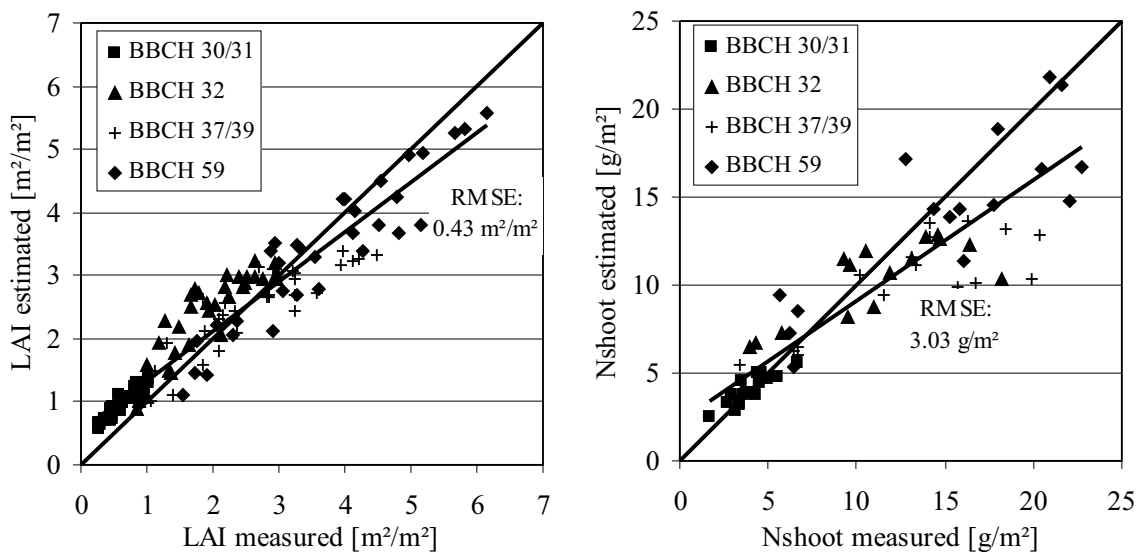


Figure 1: Prediction accuracy of the derived equations to estimate LAI (left) and Nshoot (right) from the newly derived reflection index R740/R800 for an independent validation data set containing data from BBCH 30 to BBCH 59.

## Conclusions

The presented method is well suited to identify reflection indices which give more accurate fast and easy estimations of crop parameters than indices commonly used in remote sensing. Wavebands in the transition zone from red to near infrared and in the near infrared part of the spectrum contain the most information about the crop status which is in good accordance with the findings of Reusch (2003). The relations found are valid for the development stages from BBCH 30 to BBCH 59 which makes them useful for practical applications in site specific management.

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# An Innovative Way to Handle Residues in a No-Tillage Maize-based System Under Sprinkler Irrigation in Southern Spain

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Residues management is a major limitation for no-tillage adoption in irrigated maize-based cropping systems in southern Spain. Average maize yield for the common 700 cycles in the region are higher than 12 t ha<sup>-1</sup> (Aguilar et al., 2007). This implies that more than 20 tn ha<sup>-1</sup> of residues may be left in the field after harvest. The residues are necessary for improving soil quality as well as for limiting soil erosion, particularly in the slopping lands in this region (Boulal et al., 2007). However, there is a drawback to maintaining them as they affect negatively the establishment of the following crop, typically cotton, because of the reduced soil heating during seedling germination and emergence and the increased damages by armadillo bugs and slugs (Griffith et al., 1977). The main objective of this work is to present and characterize an innovative system developed in a commercial farm that overcomes these problems.

## Methodology

Data were collected from a commercial farm in Fuente Palmera (Córdoba, Spain) in which the innovative system was introduced in 2001 in two paddocks, and from an experiment that compares conventional and permanent bed systems established in 2006 in Córdoba (Spain). Measurements included: crop establishment, soil temperature at emergence, yield and components, residues evolution as well as soil chemical and physical characterization and soil hydro-physic characterization using a portable rainfall simulator.

The crop is cultivated on the top of permanent beds with 95 cm inter-beds distance (given by cotton harvester) in Fuente Palmera and 85 cm distance in Córdoba. Two weeks before sowing, the residues from the top of the beds are shoved to the lower part of the furrow leaving a clean band of 20 cm approximately. A single row of crop is established in this band, 5 cm apart from the centre, which is the distance at which drill's boots are deviated. The following year, the drill starts sowing at the other side of the field so the row is established 10 cm apart from preceding crop. The beds facilitate the respect of control traffic resulting in 20 % of furrows with no traffic in any operation (sowing, treatment application, fertilization or harvest). Irrigation was applied with a pivot or sprinklers.

## Results

Figure 1 shows that maize yield or irrigation needs were not significantly affected by the introduction of the permanent bed system and left of residues on surface (Figure 1). These results agree with the limited literature on permanent bed systems (Limón-Ortega et al. 2000). The new system requires more dedication from the farmer, particularly for crop establishment. Soil temperature during emergence in the permanent bed system (PB) when residues covered the surface was 7.5 and 5 °C lower at 2 and 5 cm deep, respectively, compared to uncovered soil in a conventional system, in agreement with Benjamin et al. (1990) and Murdock et al. (1992). Pest damages in seedlings were reduced although a treatment was required for good control of slugs (metaldehyd) in two out of six years.

In December 2008, PB had 210 g m<sup>-2</sup> of dry residues covering the surface after 5 years of no tillage whereas CT has none. In PB, the residues and the reduction in tillage resulted in an increased soil organic matter (top 10 cm), from 1,5% to 2.4%; this is a higher increment than the one obtained in rainfed cereal-based agriculture in the same region (Ordoñez-Fernández R. et al. 2007). The residues also helped to control soil losses by protecting it from the rain drops and by reducing soil movement in the water runoff. When compared with the conventional system, the PB system improved the saturated hydraulic conductivity, the soil infiltration rate and reduced significantly soil losses (Sánchez-Domínguez, 2004; Boulal et al. 2008). Similar results were obtained in a permanent bed planting system with crop residue retention (Govaert et al., 2007).

## Conclusions

Conservation agriculture of irrigated annual crops is possible in commercial farms with no yield penalty. In order to fit cotton or maize cycle in the region, residues must be removed from the sowing row well in advance if appropriate temperatures for plants establishment must be reached. The deposition of residues on the furrows helps to limit pest damages at seedling stage, however, chemical controls may be necessary occasionally. The amount of residues is enough to protect the soil from water erosion.

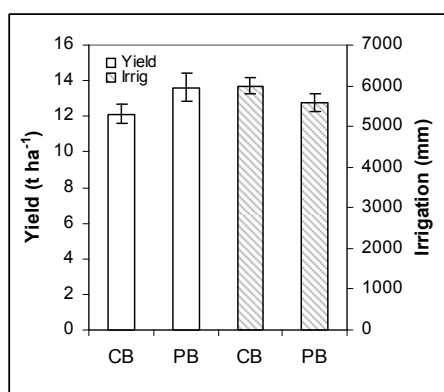


Figure 1. Average paddock maize yield and applied irrigation before (CB, conventional beds; 3 years) and after the introduction of permanent bed system (PB; 6 years).

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# Plant Production and Growth in Mycorrhiza Inoculated Plants of *Spartium Junceum* L.

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In semi-arid Mediterranean ecosystems, scarce and irregular rainfall, a long dry and hot summer and damaging human activity may synergistically act as a driving force which can accelerate the desertification processes. Desertification has a negative environmental impact.

In particular, desertification reduces the inoculum potential of mutualistic microbial symbionts that are key ecological factors in governing the cycle of major plant nutrients and hence in sustaining the vegetation cover in natural habitats.

Previous studies (Salamanca et al. 1992) have shown that *Spartium junceum* L., a native shrub legume, could be a good candidate species for a reclamation strategy in the Mediterranean region.

The aim of the present research was to produce plants enriched with mycorrhizal inoculum; to evaluate the effectiveness of mycorrhizal inoculum on various types of propagation material; to define an *in vitro* propagation protocol of *Spartium junceum* L.; to assess the capability of rooted plantlets to establish an effective mycorrhizal symbiosis; to determine the influence of mycorrhizal inoculation on growth and development of different propagation material.

## Methodology

The effect of inoculation of the arbuscular mycorrhizal fungus *Glomus viscosum* spp. on micropropagated plantlets, on seedlings produced *in vitro* and *in vivo* was investigated.

To obtain **seedlings *in vivo***, the seeds were pregerminated in polystyrene plug flats (40 plugs density 240 plants m<sup>2</sup>) with one seed per plug, each with a capacity of 50 cm<sup>3</sup>. Culture substrate was constituted by commercial peat mixture (organic carbon 46 %, organic nitrogen 1–2 %, organic matter 80 %) added with perlite (2:1 v/v). The plug flats were kept in a greenhouse under controlled conditions with day–night temperature ranging from 20–22 °C and relative humidity of 60 %, with automatic irrigation.

To obtain **seedlings *in vitro***, the seeds were germinated in sealed glass flasks (500 cm<sup>3</sup>) (25 seeds in each flasks) containing 50 cm<sup>3</sup> of basal medium (BM: Morone-Fortunato and Avato 2008), without sucrose, after autoclaving. These sealed glass flasks were kept in a culture chamber at 23°C ± 1°C, 16 h photoperiod, 60 µE s<sup>-1</sup> m<sup>-2</sup> light intensity.

**Microplants:** apical buds ≈ 6-7 mm long were excised from seedlings *in vitro*. These initial explants were transferred to the basal medium (BM) with sucrose (20 g dm<sup>-3</sup>) and supplemented with benzylaminopurine (BAP 0,2 mg dm<sup>-3</sup>) for five weeks and were subcultured at intervals of 4 weeks.

Subsequently, shoots were transferred to the same basal medium supplemented with gibberellic acid (GA<sub>3</sub> 0,5 mg dm<sup>-3</sup>) for shoot extension. The explants were kept for 4 weeks in this substrate. Root induction was performed by culturing the shoots, for 8 weeks, in the basal medium (BM) supplemented with indole acetic acid (IAA 6mgdm<sup>-3</sup>). Inoculation of AM fungi was obtained incorporating 10 g of inoculum-quartz sand mix under the root system of *Spartium junceum* L. at the moment of transplanting in plastic plots 200 cm<sup>3</sup>.

In greenhouse, seedlings and microplants were inoculated. Five plants per treatment were harvested 30 and 150 days after inoculation and morphological parameters were assessed.

The analysis of mycorrhizal roots of inoculated plants was carried out following the protocol of Phillips and Hayman (1970) and the samples observed using an optical microscope (Zeiss). The

percentage of root colonization was determined by slide method (Giovanetti and Mosse 1980) 30 and 150 days after mycorrhizal inoculation.

## Results

Mycorrhizal fungus application in microplants and seedlings produced *in vitro* increased growth, improve plant survival and biomass development; mycorrhizal colonization was high (60 – 70 %). After five months, mycorrhizal colonization of seedlings produced *in vivo* was not very high (30%) and did not produce stimulation of plant growth.

**Table 1.** Morphological parameters of *S. Junceum* L. (different propagation material) at harvest (150 days after mycorrhizal inoculation). Values represent means  $\pm$  standard error. Different letters indicate statistically significant differences ( $p \leq 0,01$ ) comparing treatments across the lines of the table.

Morphological Parameters	Microplants		Seedlings <i>in vitro</i>		Seedlings <i>in vivo</i>	
	M+	M-	M+	M-	M+	M-
Shoot lenght (cm)	44.8 $\pm$ 0.58 a	26.6 $\pm$ 1.91 c	36.3 $\pm$ 1.76 b	28 $\pm$ 1.15 c	18.6 $\pm$ 1.12 d	19.2 $\pm$ 0.42 d
Number of nodes (n)	33.2 $\pm$ 4.66 a	25.2 $\pm$ 2.87 ab	28 $\pm$ 1.15 ab	20 $\pm$ 1.15 bc	9.2 $\pm$ 1.46 c	9.6 $\pm$ 0.68 c
Shoot fresh weight (g)	2.1 $\pm$ 0.17 a	0.6 $\pm$ 0.1 bc	0.9 $\pm$ 0.03 b	0.6 $\pm$ 0.07 bc	0.4 $\pm$ 0.006 c	0.36 $\pm$ 0.03 c
Shoot dry weight (g)	0.4 $\pm$ 0.05 a	0.1 $\pm$ 0.02 c	0.2 $\pm$ 0.02 b	0.14 $\pm$ 0.03 bc	0.06 $\pm$ 0.003 c	0.06 $\pm$ 0.003 c
Total root lenght (cm)	18 $\pm$ 3.3 ab	8.2 $\pm$ 1.1 c	16.7 $\pm$ 2.52 ab	12.3 $\pm$ 2.02 bc	15.9 $\pm$ 0.86 ab	18.1 $\pm$ 0.67 ab
Number of roots (n)	20.4 $\pm$ 2.06 a	8 $\pm$ 0.7 b	15.3 $\pm$ 2.33 ab	12 $\pm$ 2 b	11 $\pm$ 1.74 b	11,8 $\pm$ 1.56 b
Root fresh weight (g)	0.21 $\pm$ 0.08 a	0.07 $\pm$ 0.01 d	0.1 $\pm$ 0.005 cd	0.08 $\pm$ 0.006 d	0.14 $\pm$ 0.02 bc	0.17 $\pm$ 0.02 ab
Root/shoot fresh weight	0.1 $\pm$ 0.07 c	0.12 $\pm$ 0.03 c	0.11 $\pm$ 0.007 c	0.13 $\pm$ 0.006 c	0.36 $\pm$ 0.05 b	0.47 $\pm$ 0.07 a

**Table 2.** Mycorrhizal colonization (*Glomus viscosum* strains A6) in the root systems of *S.junceum* L. different propagation material. Values represent means  $\pm$  standard error. Different letters indicate statistically significant differences ( $p \leq 0,01$ ) comparing treatments along the columns of the table.

	Mycorrhizal colonization (%)	
	30th day	150th day
Seedlings <i>in vivo</i>	29.17 $\pm$ 4.17 b	33.3 $\pm$ 3.33 b
Seedlings <i>in vitro</i>	41.7 $\pm$ 4.17 b	59.3 $\pm$ 3.7 a
Microplants	59.7 $\pm$ 4.97 a	70.4 $\pm$ 3.7 a

## Conclusion

Our results demonstrate that there was an effective co-operation between micropropagation and mycorrhizal inoculation. Greater growth of microplants and *in vitro* seedlings was due to a rapid establishment of mycorrhizal infection confirming the effectiveness of mycorrhizal symbiosis on these materials. The effectiveness of *Glomus viscosum* inoculation in improving performance of *Spartium junceum* L. was evident and so this biotechnology (mycorrhizal inoculation) can be used in revegetation strategies for desertified lands.

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# Nitrogen and TGE Balances in Sugar Beet Rotations

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Nitrogen and total greenhouse gas emissions (TGE) balances are important indicators to assess the sustainability of agricultural production. This project deals with the assessment and analysis of sugar beet production on crop rotation and the farm level. The environmental effects of sugar beet cultivation have previously been mainly analyzed on the crop level, however, in this project we especially focus on the crop rotation level and the entire farming system.

This project is part of a collaborative project, coordinated by the Institute for sugar beet research (IFZ) in Goettingen. Other projects focus on the economic effects of different production systems as well as parameters as erosion.

## Methodology

There were 12 farms evaluated over a 3 year period. As a tool for assessing we used the model REPRO. This is a computer model developed at the Martin Luther University of Halle-Wittenberg which can analyze energy and material flows on field or farm level. With REPRO, it is possible to assess and to analyze different environmental indicators, however, for this investigation we focus on the parameter nitrogen balance and TGE, which is the basis of the evaluation of the nitrogen management for the farm operation. A special feature which has to be mentioned in this context is the fact that in the model REPRO the calculation for the indicator nitrogen and TGE balance is closely related to the balance of soil organic matter (SOM) thus positive or negative changes in the content of SOM have an effect on the nitrogen balances and thus also effect the TGE due to changes in the carbon stocks.

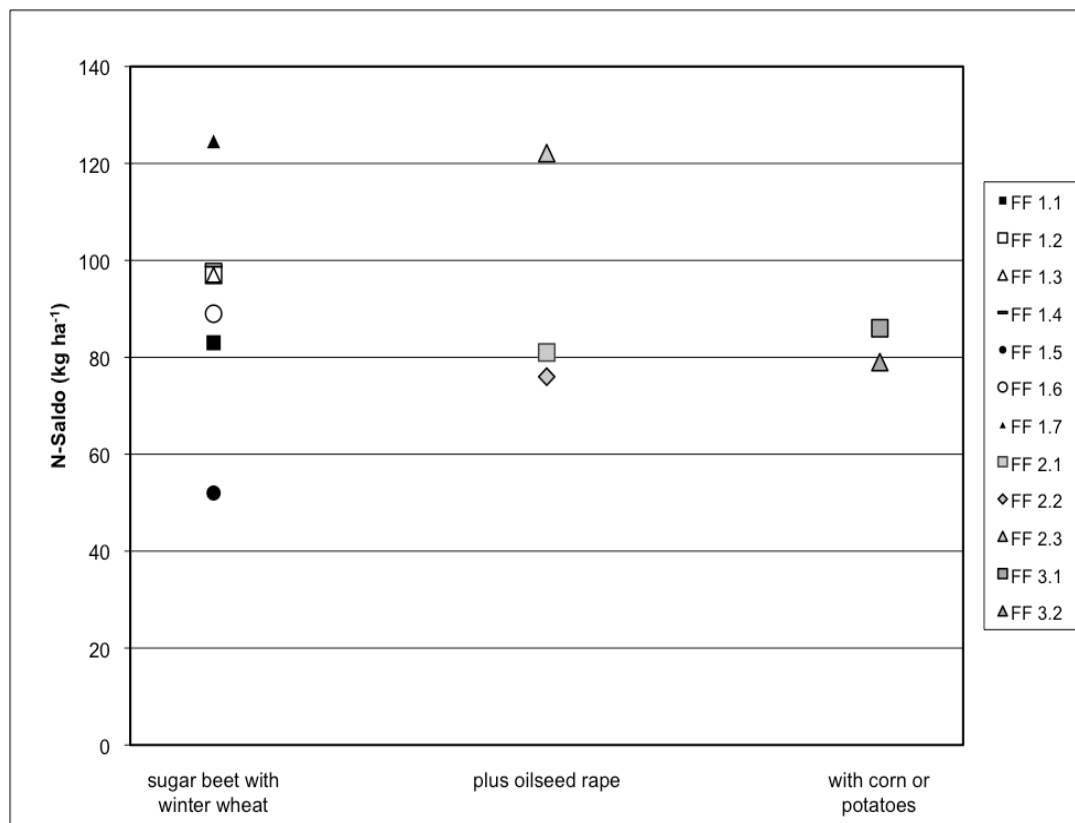
The different crop rotations on the farms were differentiated into (1) rotations with only sugar-beet and winter wheat, (2) rotations with sugar-beet, oilseed rape and winter wheat and (3) rotations with sugar-beet, winter wheat and either corn or potatoes. For all calculations changes in the SOM were considered. The calculations of the total greenhouse gas emission potential (TGE) of nitrogen fertilizers we have used the IPCC approach of a proportion of 1,25 percent of the applied nitrogen fertilizers.

## Results and discussion

The nitrogen-withdrawal, as a significant part of the N-balance, reflects parameters like farms size, husbandry, crop yields and nitrogen contents. Those parameters are balances against the N-supply. A special feature of the nitrogen balance in the model REPRO are changes in soil-nitrogen stocks and the consequences for the calculations. A negative balance of soil organic matter (SOM) therefore requires a mineralization of the soil organic nitrogen pool, whereas a positive balance of contributes to an increase in soil organic matter. Thus, the positive influence of organic fertilization within the crop rotation can be assessed.

In our results of the three different types of crop rotations on average produced a nitrogen surplus of 80 kgN/ha a, ranging from 50 to 120 kgN/ha a. This demonstrates the great influences of different sites and husbandry on such balances. There was a tendency of the crop rotations “3” including corn or potatoes to produce slightly lower nitrogen surpluses, whereas the highest balances were found in the rotations with only sugar-beet and winter wheat (see figure).

The green house gas emission potential ranges from 2000 to more than 4000 kg CO<sub>2</sub>eq/ha a. The lowest figures were calculated for the rotations with sugar-beet and winter wheat only. The highest figures occurred in the rotations with either corn or potatoes. A major factor in the explanations of such large differences was the effect of different husbandry treatments on the soil organic mater stocks, since nitrogen fertilization was on a fairly similar level in all rotations compared. Some crop rotations should a considerably decrease in SOM whereas for other rotations we calculated substantial increase in SOM. The differences ranges from a surplus in the SOM stocks equivalent to 400 kg CO<sub>2</sub> eq/ha a to a decrease of 2000 kg CO<sub>2</sub> eq/ha a.



## Conclusions

It is of great relevance that all calculations of nitrogen and energy balances are done the rotational level to avoid misconceptions about the effect single crops. Only an approach, which takes into account the complete rotations will correctly include the effect of the different preceding-following crop combinations. This is especially true for the calculation of greenhouse gas emissions. Additionally, changes in the SOM have to be included in the calculation, which are of great relevance for both, nitrogen balances and greenhouse gas emissions.

# Germination and Emergence response of Pea (*Pisum sativum* L.) to low Temperature: Comparison of Winter and Spring genotypes

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In northern Europe, many spring crops are sown earlier and earlier and some species are now sown in autumn to escape high temperatures and drought during seed filling. These crops are bred to be tolerant to frost damages during winter, but changes in their cropping cycle also lead to changes in sowing conditions. Our aims were, in the case of pea, to determine if winter and spring genotypes differed in their germination and shoot elongation when submitted to various temperatures and if a generic model of crop emergence, SIMPLE (Dürr et al., 2001), could be further used to choose the best sowing dates in a given area by numeric simulations.

## Methodology

Four pea genotypes were studied: two winter genotypes, Champagne and Cheyenne (2007 only), and two spring genotypes, Térèse and Baccara. To test the stability of genotypic response to varying production conditions, seeds were collected from two production sites in 2006 and one in 2007. Each lot was germinated at 5, 10, 15, 20 and 25°C and grown in the dark after germination to mimic elongation before emergence at 7, 10, 15, 17, 20 and 25°C. Base temperature (T<sub>b</sub>) and thermal time for germination and elongation were calculated for each lot having data which showing a linear increase of germination or elongation with temperature. Seed lots produced in 2006 were sown at two sites in northern (Mons) and western France (Angers) on March 13 and 15<sup>th</sup>, 2007 while 2007 seed lots were sown at one site (Angers) on November 13<sup>th</sup>. Soil water content, temperature and soil surface structure were regularly assessed throughout the experiments. Germination and emergence field results were used to compare observed germination and emergence with those simulated by the crop emergence model.

## Results

Under controlled conditions, germination final percentage was always very high even at 5°C (FP5, Table) but winter genotypes germinated faster whatever the temperature. Germination base temperatures (T<sub>b</sub>) did not differ significantly and were very low (-1°C). Cumulative thermal times to reach 50% germination (Tt50) were lower for the winter genotypes. Elongation base temperature was higher than germination base temperature (3-6°C) and showed a high interaction between genotypes and seed production conditions. Mean elongation rates to reach 5 cm were lower whatever the temperature for Cheyenne and at temperatures higher than 10°C for Champagne and cumulative thermal times (Tt5cm) were higher for Champagne. Maximal epicotyl length was higher only for Champagne. (Tt5cm)

On experimental plots, emergence percentages above 95% were observed except for the autumn experiment with Cheyenne (89%) and Baccara (73%). Champagne always emerged faster than Baccara (Figure) while Térèse emergence rate was intermediate. Germination percentages were very high (98-

100%) and germination period was short (3-4 days in spring and 6-8 in autumn). Soil water content was always higher than base water potential of each lot and no dry crusts or big clods blocked seedlings at the soil surface. However, during the autumn trial, heavy rainfalls at the end of the germination phase caused soil compaction and waterlogging. The reduced emergence of Baccara and Cheyenne could therefore be due to differences in either soil conditions between plots and/or in genotype behaviours under these soil conditions. With the exception of these two plots, field observations and simulated data fitted well for germination and emergence. Finally, during these field trials, short periods of low temperature only delayed emergence.

	Germination			Elongation		
	FP5	Tb	Tt50	Tb	Tt5cm	
Champagne	96	-0.82 ns	26.8	4.14 bc	51.1	
	100	-0.82 ns	25.7	3.31 ab	50.4	
	100	-0.52 ns	17.6	1.92 a	69	
Cheyenne	100	0.99 ns	24	4.98 cd	44.3	
Baccara	99	-0.94 ns	39.6	3.74 bc	42.8	
	100	-0.35 ns	34.8	3.78 bc	45	
	98	-0.04 ns	37.72	5.88 d	35.2	
Térèse	98	-2.79 ns	36	5.94 d	31	
	100	-1.86 ns	33.4	3.37 bc	43.6	
	99	-1.86 ns	33	4.88 cd	46.7	

Table - Seed lot characteristics under controlled assays

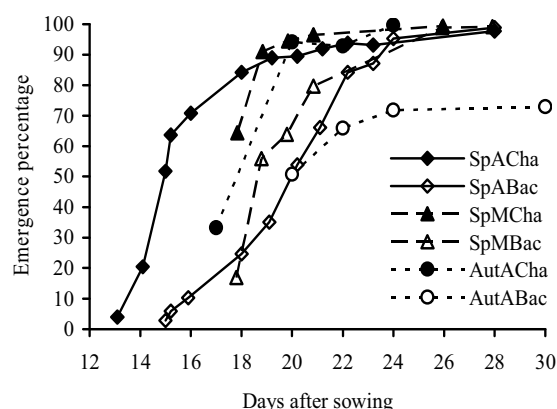


Figure – Emergence of one winter (Champagne) and one spring genotype (Baccara) in the three field trials (Sp, Spring - Aut, Autumn - A, Angers - M, Mons)

## Conclusions

Field emergence was quicker for Champagne, one winter genotype, but as shown by laboratory observations, this was only due to faster germination while elongation was slower. Breeding for frost tolerance during the vegetative phase could hence be correlated with faster germination. Moreover, simulation data from the SIMPLE model fitted observations well. Hence, the model could be used further to simulate pea emergence under of wide range of sowing dates and years to estimate risks of poor emergence rates or emergence delays as has already been carried out on other crops (Dorsainvil et al., 2005; Moreau-Valancogne et al., 2008). However, further work is necessary to test the effects of soil compaction and excess water which are frequent at the end of autumn in the loamy soils of western France. Low emergence percentages (25-58%) were also observed on a farm plot where, two days after sowing a long cold period was recorded (-2 to +2°C for two weeks). Because sown seed lots showed the same behaviour under controlled conditions as Champagne and Cheyenne and because no other emergence-limiting factor was observed, these results suggested that the effects of a long cold period should be further explored.

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# Regulated Deficit Irrigation in Peach and Nectarine at Farm Level

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## Introduction

Regulated Deficit Irrigation (RDI) consists on the application of water below the full crop-water requirements during periods of crop growth that are less sensitive to water deficit (Chalmers *et al.*, 1986), and is a viable strategy during periods of water shortage (Feres and Soriano, 2007). For stone fruits, such as peach and nectarine, the least sensitive stage to water stress is stage II of fruit growth, characterized by a slow fruit growth rate and rapid vegetative growth. On-farm experiments in peach have shown that RDI scheduling based on stem water potential was a viable strategy that reduced applied irrigation water by 33%, relative to full requirements, without reducing yield or crop value. At present, there is a need for upscaling these results from experimental plots to commercial farms. This leap in space scale must be accompanied by adapting new tools that enable the early detection of water stress with higher spatial resolution thermal and narrow-band multispectral remote sensing imagery. Airborne thermal and multispectral imagery can provide pre-visual indicators of water stress (Berni *et al.*, 2008; Suarez *et al.*, 2008). In order to do this upscaling, a 5-years program (RIDECON-CONSOLIDER) started on 2006, funded by the Spanish Ministry of Education and Science, to study several aspects of the deficit irrigation practices in Spanish horticulture. In this work, full irrigation and RDI scheduling in two demonstration plots are compared, in peach and nectarine, and focus is done on the early assessment of water stress with airborne thermal and narrow-band multispectral imagery.

## Methodology

Two demonstration plots were established in a peach (*Prunus persica* L. cv. BabyGold 8) and nectarine (*Prunus persica* L. cv. SwinLady) orchards in a commercial farm on deep alluvial soil located near Cordoba (Spain), with the same experimental design for both plots. Two large subplots (3150 m<sup>2</sup>) with 6 lines each and about 30 trees/line (3.5 \* 5 m grid) were chosen to compare the two irrigation schedules. In well-irrigated subplot (R), water was applied according to the crop water requirements in every stage. In RDI subplot, water was withheld from the beginning of the irrigation season until the beginning of the stage III according to Fereres and Soriano (2007). To determine water status, midday stem water potential was measured weekly in 6 trees/treatment. At harvest, water applied, yield, fruit diameter and fruit number was determined. Thermal and multispectral cameras were installed in an unmanned aerial vehicle (UAV) flying at 150 m altitude. The images yielded 40 cm spatial resolution (320x240 pixels with 16-bits) in the thermal, and 20 cm pixel size in the visible and near-infrared multispectral camera. For more information concerning calibration, image capture and analysis, see Berni *et al.* (2008) and Suarez *et al.* (2008).

## Results and discussion

As soil was near field capacity, stem water potential did not show any difference for the first 20 days in both, peaches and nectarines (Fig. 1). Differences were maxima just before rewatering, reaching 0.3 and 0.2 MPa, for peach and nectarine, respectively. The day before the rewatering period, the temperature map showed differences near 10°C between both treatments (Fig. 2). After rewatering, both treatments followed a similar evolution until harvest. At the end of the season, the total water saved in RDI was equal to 63 and 108 mm for peach and nectarine respectively (Table 1). No differences were found in yield (Table 1) or quality parameters, such as total soluble sugars or total

acidity (*data not shown*). RDI treatments resulted in a large number of smaller fruits (Table 1; Fig. 3). No difference in fruit size or number was found in nectarine, and both treatments exhibited similar distribution (*data not shown*).

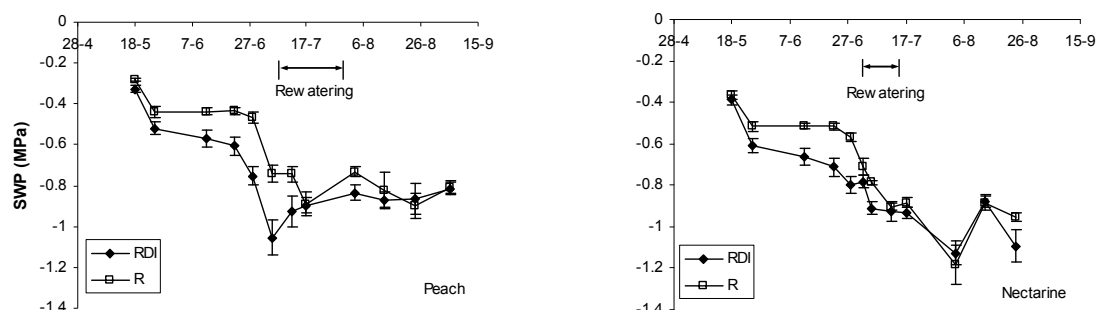


Fig. 1: Evolution of stem water potential (MPa) for peach and nectarine trees under regulated deficit irrigation (RDI) and control (R) treatments.

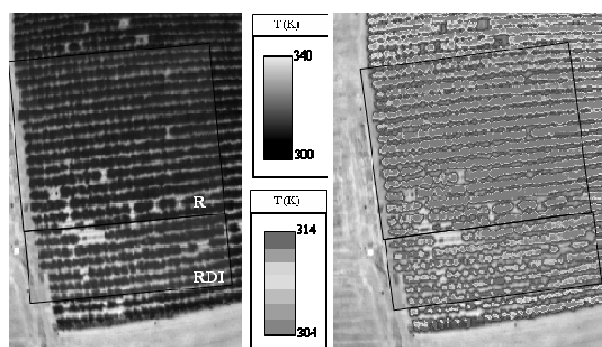


Fig. 2: Original image and map of temperature of the experiment plots, acquired with the thermal camera.

Species	Treatment	Water applied (mm)	Fruit number (n/tree)	Yield (kg/tree)
Peach	R	465	170	42,4
Peach	RDI	402	195	40,8
Nectarine	R	312	256	44,5
Nectarine	RDI	204	253	45,2

Table 1: Water applied (mm), fruit number (n/tree) and yield (kg/tree)

## Acknowledgements

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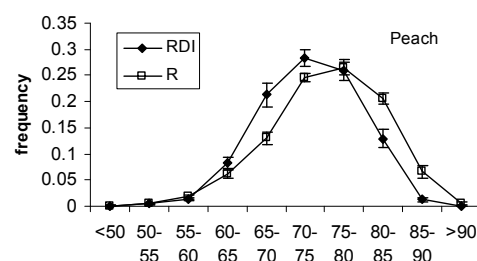


Fig 3: Distribution of fruit diameter at harvest.

## Conclusion

RDI techniques are suitable for irrigation scheduling in commercial farms. Water savings will depend on the contribution from soil water storage. Thermal imagery is a valuable tool for farmers to quantitatively monitor the plant water status in order to control water deficit levels and schedule the irrigation events.

# Growth and yield performance of grain amaranth *Amaranthus cruentus* L. in response to different drought conditions

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## Introduction

Prospects for future expansion of grain amaranth (*Amaranthus* spp.) as an ancient under-utilised pseudocereal are encouraging. In addition to its nutritional qualities (Bavec and Bavec, 2006), grain amaranth is also reported as a promising C<sub>4</sub> crop adapted to be produced in unfavourable semiarid conditions (Kigel, 1994). Putative drought tolerance of grain amaranth was proved by water use efficiency comparable to other drought adapted crops and apparent ability to increase rooting depth in response to water stress and thus efficiency of soil water extraction (Johnson and Henderson, 2002). Although development responsiveness to soil water has been reported in pot experiments on vegetable amaranth (Liu and Stützel, 2002; Liu and Stützel, 2004; Ommami and Hammes 2006), the growth performance pattern and yield formation of grain amaranth under drought conditions is still unclear. Thus, the objective of the study was to quantify the effects of soil water as the limiting factor imposed at different physiological stages on performance and productivity of *Amaranthus cruentus* 'G6'.

## Methodology

A pot experiment was conducted in conditions of plastic greenhouse at the University Agricultural Centre of Faculty of Agriculture, Maribor in 2007. Grain amaranth *A. cruentus* 'G6' was over sown into the pots (75 cm in high, 14 cm in diameter) filled with native top soil of sandy loam texture. After sowing, pots were subjected to two different soil water tensions: – 0.4 MPa (adequate moisture) and – 0.7 MPa (drought condition). After emergence thinning was carried out to one plant per pot and tensiometer tubes (30 cm long) were placed randomly into four pots per treatment. Seedlings were exposed to different soil water treatments: constant adequate moisture (W1) and drought (W2) throughout the growing period, and drought initiated at crop inflorescence formation (W3). Soil water tension was monitored every second day during the course of the experiment using the puncture tensiometer instrument (SDEC France, SMS 2500S) and water was added according to defined soil water retention curve. Crop samples were taken before inflorescence formation (S1), at the beginning of flowering (S2) and at maturity (S3). The stated phenological stages were considered to begin when inflorescence was visible and when pollen started to shed on 75% of the plants. Six plants per treatment were harvested at S1 and S2 and nine for final grain yield determination at S3. The performance of amaranth was assessed by evaluating number of leaves, leaf area, fresh and dry weight (dried at 70 °C) of plant parts and grain yield. Harvest indexes HI<sub>r</sub> and HI<sub>l</sub> were calculated as a ratio of grain to biomass yield in dry matter with and without root mass, respectively. The experiment was arranged in randomised block design with three replications. Obtained data were subjected to analysis of variance (ANOVA) procedure (Statgraphic, 2005) to ascertain significant differences between treatments ( $P \leq 0.05$ ). Significant differences among treatments were determined with Duncan's multiple range test ( $\alpha = 0.05$ ).

## Results

Although results obtained at the time of first sampling already indicated diverge in amaranth growth performance, the treatments affected only the leaf fresh weight (Table 1).

During the second set of plant sampling, taken at the beginning of flowering (S2), the measured parameters were influenced by soil water regime and decreased according to duration of drought exposure (Table 1). Sampling at maturity (S3) showed significant influence of soil water regime on all evaluated parameters with the exception of harvest indexes. Grain yield was reduced for 28% and 51%, and biomass yield for 20% and 50% in W3 and W2, respectively. Constant drought throughout the growing period did not cause significant reduction of most other measured parameters in comparison to drought initiated at crop inflorescence formation (Table 1).

Table 1 The effect of water regime on growth and yield performance of grain amaranth *A. cruentus* 'G6' as evaluated before inflorescence formation (S1), at the beginning of flowering (S2) and at maturity (S3)

Parameter	Treatments mean (cm, g, cm <sup>2</sup> per plant)							
	1. sampling (S1)		2. sampling (S2)			3. sampling (S3)		
	W1	W2	W1	W2	W3	W1	W2	W3
Root length	12.8	9.2	65.6	55.5	66.2	47.4	50.2	50.6
Stem height	44.7	35.3	88.7 <b>a</b>	58.0 <b>c</b>	75.5 <b>b</b>	103.8 <b>a</b>	65.7 <b>b</b>	82.1 <b>b</b>
Inflorescence lenght	--	--	17.8 <b>a</b>	9.7 <b>c</b>	11.2 <b>b</b>	29.4 <b>a</b>	15.4 <b>b</b>	17.6 <b>b</b>
No. of leaves	17.5	14.2	27 <b>a</b>	19 <b>c</b>	22 <b>b</b>	21 <b>a</b>	18 <b>b</b>	24 <b>a</b>
Root weight (fresh)	5.79	3.0	18.7 <b>a</b>	6.1 <b>c</b>	13.4 <b>b</b>	13.0 <b>a</b>	5.6 <b>b</b>	10.2 <b>a</b>
Stem weight (fresh)	21.9	11.9	48.9 <b>a</b>	16.0 <b>c</b>	36.3 <b>b</b>	32.7 <b>a</b>	13.7 <b>b</b>	23.2 <b>ab</b>
Inflorescence weight (fresh)	--	--	12.7 <b>a</b>	4.4 <b>b</b>	7.4 <b>b</b>	25.3 <b>a</b>	10.3 <b>b</b>	15.3 <b>b</b>
Leaves weight (fresh)	19.5 <b>a</b>	11.1 <b>b</b>	34.6 <b>a</b>	14.1 <b>c</b>	23.1 <b>b</b>	18.0 <b>a</b>	7.5 <b>b</b>	10.3 <b>b</b>
Grain weight (fresh)	--	--	--	--	--	6.5 <b>a</b>	3.2 <b>b</b>	4.6 <b>b</b>
Biomass (fresh)	47.2	25.9	114.9 <b>a</b>	40.6 <b>c</b>	80.1 <b>b</b>	95.4 <b>a</b>	40.4 <b>b</b>	63.6 <b>b</b>
Leaf area	636	394	1009 <b>a</b>	721 <b>b</b>	846 <b>ab</b>	--	--	--
Root weight (dry)	1.1	0.8	4.2 <b>a</b>	1.9 <b>b</b>	4.2 <b>a</b>	5.3 <b>a</b>	2.3 <b>b</b>	4.3 <b>a</b>
Stem weight (dry)	2.2	2.1	9.5 <b>a</b>	3.4 <b>b</b>	8.9 <b>a</b>	8.8 <b>a</b>	4.2 <b>b</b>	6.9 <b>ab</b>
Inflorescence weight (dry)	--	--	2.0 <b>a</b>	1.0 <b>b</b>	1.7 <b>a</b>	5.6 <b>a</b>	2.3 <b>b</b>	3.4 <b>b</b>
Leaves weight (dry)	2.7	2.3	9.3 <b>b</b>	7.4 <b>b</b>	12.8 <b>a</b>	5.0 <b>a</b>	3.6 <b>b</b>	5.3 <b>a</b>
Grain weight (dry)	--	--	--	--	--	3.9 <b>a</b>	1.9 <b>c</b>	2.8 <b>b</b>
Biomass (dry)	6.0	5.2	25.0 <b>a</b>	19.1 <b>b</b>	22.1 <b>ab</b>	28.5 <b>a</b>	14.2 <b>b</b>	22.8 <b>a</b>
HI <sub>r</sub>	--	--	--	--	--	0.15	0.14	0.13
HI	--	--	--	--	--	0.19	0.16	0.16

Evaluated parameter means without ascribed letter within a particular sampling is not significantly affected ( $P > 0.05$ )

Means within a row and particular sampling (S1-S3) followed by different letter are significantly different (Duncan,  $\alpha = 0.05$ )

## Conclusion

Tested grain amaranth, *A. cruentus* 'G6', exhibited reduction in growth and yield formation when plants were exposed to drought stress at different phenological stages, while harvest index was not influenced. Water shortage during inflorescence formation appears to be critical growing stage influencing growth performance and grain yield.

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# Stomatal conductance and gas exchange of grain amaranth *Amaranthus cruentus* L. in response to different drought conditions

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## Introduction

Stomata closure in response to water deficit is the major physiological control for the reduction of transpiratory water loss and prevents deleterious dehydration of leaf tissue. As a plant possesses  $C_4$  photosynthesis pathway, the grain amaranth (*Amaranthus* spp.) is characterised by the ability to use water efficiently by its carbon concentrating mechanism which counteracts the limitation of photosynthesis  $CO_2$  diffusion. This, in relation to  $C_3$  plants, allows high net photosynthesis at a lower stomatal conductance, lowering transpiration and conserving water, especially in conditions of high temperature, irradiance and drought (Kigel, 1994; Bavec and Bavec, 2006; Ripley et al., 2007). Omami and Hammes (2006) reported reduction of stomatal conductance and net photosynthesis of vegetable amaranth *A. cruentus*, but a remarkable ability of crop to recover after re-watering. Liu and Stützel (2002) reported vegetable amaranth to have a high capacity of osmotic adjustment that may sustain turgor maintenance and thus assimilation during drought stress.

Due to the lack of information on grain amaranth photosynthesis as affected by drought occurred at different phenological stages, a greenhouse pot experiment was conducted to study the physiological responses of *Amaranthus cruentus* 'G6' to different soil moisture regimes.

## Methodology

A pot experiment was conducted in conditions of plastic greenhouse at the University Agricultural Centre of Faculty of Agriculture, Maribor in 2007. Grain amaranth *A. cruentus* 'G6' was over sown into the pots (75 cm in high, 14 cm in diameter) filled with native top soil of sandy loam texture. After sowing, pots were subjected to two different soil water tensions:  $-0.4$  MPa (adequate moisture) and  $-0.7$  MPa (drought condition). After emergence thinning was carried out to one plant per pot and tensiometer tubes (30 cm long) were placed randomly into four pots per treatment. Seedlings were exposed to different soil water treatments: constant adequate moisture (W1) and drought (W2) throughout the growing period, and drought initiated at crop inflorescence formation (W3). Soil water tension was monitored every second day during the course of the experiment using the puncture tensiometer instrument (SDEC France, SMS 2500S) and water was added according to defined soil water retention curve. The stated phenological stages were considered to begin when inflorescence was visible and when pollen started to shed on 75% of the plants. Stomatal conductance, net photosynthesis and leaf transpiration were measured with a LCpro+, portable photosynthesis system (ADC BioScientific Ltd., United Kingdom). Measurements were taken twice: at the end of vegetative phase, before inflorescence formation (M1) and at the beginning of flowering (M2). Measures were made on the upper fully expanded leaf on five to six randomly chosen plants per treatment and a set of five measures were taken per leaf. Environmental conditions in the broad leaf chamber ( $6.25\text{ cm}^2$ ) were: photosynthetically active photon flux density  $783\text{ }\mu\text{mol photon m}^{-2}\text{ s}^{-1}$  and leaf temperature at  $25\text{ }^\circ\text{C}$ . Photosynthetic water use efficiency (WUEp) was calculated as the ratio of measured net photosynthesis to stomatal conductance.

The obtained data were subjected to analysis of variance (ANOVA) procedure (Statgraphic, 2005) to ascertain significant differences between treatments ( $P \leq 0.05$ ). Significant differences among treatments were determined with Duncan's multiple range test ( $\alpha = 0.05$ ).

## Results

Net photosynthesis and transpiration rate measured before inflorescence formation were strongly reduced in plants exposed to water stress. Reductions were attributed to decrease in stomatal conductance for 79% in comparison to well watered plants with conductance of  $286.6 \text{ mmol m}^{-2} \text{ s}^{-1}$  (Table 1).

Table 1. Stomatal conductance and gas exchange rate of *A. cruentus* 'G6' in response to soil water regimes conducted until the first measurement (M1)

Water regime	Stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	Net photosynthesis ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	Transpiration rate ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	WUEp ( $\text{mmol mol}^{-1}$ )
	**	**	**	ns
Treatments mean $\pm$ SEM				
W1 (control)	$286.6 \pm 5.39\text{a}$	$15.1 \pm 0.16\text{a}$	$2.4 \pm 0.02\text{a}$	$6.2 \pm 0.09$
W2	$59.6 \pm 4.25\text{b}$	$5.2 \pm 0.49\text{b}$ (32%)	$0.8 \pm 0.05\text{b}$ (33%)	$5.9 \pm 0.24$

Means within each column followed by different letters are significantly different (Duncan,  $\alpha = 0.05$ )

Values in parenthesis indicated relative reduction of measured parameter (in %) to control

Among three water regime treatments to which amaranth was exposed until the second set of measurement, the gas exchange values were highest in W1, followed by W3 and regime of continuous drought (W2), although the stomatal conductance in W2 was significantly the same as in W3. In this manner the net photosynthesis was reduced for 32% and 47%, and transpiration rate for 17% and 25% in W3 and W2, respectively. Photosynthetic water use efficiency (WUEp) was not affected by water stress at first measurement (Table 1), while evaluation at the beginning of crop flowering showed a gradual decrease in WUE from 7.7 (W1) to 6.7 (W3) and  $5.4 \text{ mmol mol}^{-1}$  in plants subjected to constant water stress (Table 2).

Table 2. Stomatal conductance and gas exchange rate of *A. cruentus* 'G6' in response to soil water regimes conducted until the second measurement (M2)

Water regime	Stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	Net photosynthesis ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	Transpiration rate ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	WUEp ( $\text{mmol mol}^{-1}$ )
	**	**	**	**
Treatments mean $\pm$ SEM				
W1 (control)	$169.2 \pm 2.9\text{a}$	$18.8 \pm 0.18\text{a}$	$2.4 \pm 0.02\text{a}$	$7.7 \pm 0.03\text{a}$
W2	$119.3 \pm 3.6\text{b}$ (29%)	$9.9 \pm 0.7\text{c}$ (47%)	$1.8 \pm 0.02\text{c}$ (25%)	$5.4 \pm 0.36\text{c}$
W3	$109.8 \pm 3.6\text{b}$ (35%)	$12.7 \pm 0.30\text{b}$ (32%)	$2.0 \pm 0.05\text{b}$ (17%)	$6.5 \pm 0.09\text{b}$

Means within each column followed by different letters are significantly different (Duncan,  $\alpha = 0.05$ )

Values in parenthesis indicated relative reduction of measure (in %) to control

## Conclusion

Although the grain amaranth has been considered as drought tolerant, stomatal conductance and thus the net photosynthesis of tested *A. cruentus* 'G6' was affected and reduced, especially when crop is grown under drought condition throughout the growing period. Photosynthetic water use efficiency was reduced in response to water stress duration.

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# Estimating Radiation Interception Using High Resolution Multispectral Imagery In Open-Tree Orchards

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## Introduction

Solar radiation is the main source of energy for physiological processes conducted by plants. It drives fundamental plant and biophysical processes, such as photosynthesis, stomatal conductance, transpiration, leaf temperature, respiration and other secondary plant processes. The supply of radiation is a limiting factor to potential production, determined by the incident radiation condition as well as by the optical and architectural properties of the stand (Ross, 1981).

The objective of this work is the development of methodologies for remote measurement of radiation absorption in photosynthetically active radiation (PAR) and near infrared (NIR) bands in heterogeneous canopies. The study was conducted using high resolution multispectral imagery acquired in diurnal flight campaigns over peach and orange orchards. A leaf-canopy radiative transfer model linked to a radiation interception model was validated against field-measured radiation interception data acquired with a ceptometer over the course of the day. The 3D radiative transfer modelling approach enabled accounting for orchard architecture, planting grid, crown dimensions and background effects on the Normalized Difference Vegetation Index (NDVI) used as an indicator for canopy radiation interception. Estimates for radiation interception using the modelling approach yielded errors below 13% RMSE.

## Methodology

### *Study sites*

The ground truth datasets and airborne images were acquired in two commercial peach and orange orchards located in Cordoba (37° 48'N, 4° 48'W) and Seville (37° 20', 5°50'W), respectively. The peach orchard was planted in 5 x 3 m pattern and the orange trees in 7 x 3 m pattern.

### *Field data collection*

A ceptometer (SunScan Canopy Analysis System, Delta-T Devices Ltd, Cambridge, UK) was used for the ground truth data of intercepted radiation. The measurements were taken beneath the four central trees of the orchard. The multispectral sensor used in this study was a 6-band multispectral camera (MCA-6, Tetracam, Inc., California, USA), with centre wavelengths at 490, 550, 670, 700, 750, and 800 nm. Bands centered at 670 and 800 nm were used for computing the *Normalized Difference Vegetation Index* (NDVI).

### *Simulations with a leaf-canopy radiative transfer and radiation interception models*

The leaf-canopy radiative transfer model, FLIGHT 5.5 (North, 1996) linked to a radiation interception model (Mariscal et al., 2000) were validated against field-measured radiation interception data acquired with the ceptometer over the course of the day. The FLIGHT 5.5 3D canopy model enabled us to simulate the effects of different input parameters on NDVI, such as the orchard architecture, planting grid and background effects.

## Results

A first approximation to estimate intercepted radiation by using remote sensing was conducted assessing the relationship between vegetation indices calculated from the airborne imagery (NDVI) and field-measured intercepted radiation measured with the ceptometer (Fig.1). The aggregated image reflectance from the four central trees of the orchard, including soil and shadows, was used to compute NDVI. The diurnal variation of NDVI over the course of the day (Figure 1) was driven by soil and shadows variation as function of the sun geometries. To evaluate the influence of these parameters in the vegetation index, the 3D canopy model, FLIGHT5.5, was used. An example is illustrated in Fig 2, showing the changes in canopy NDVI as a function of the planting pattern. The canopy model shows the sensitivity of NDVI to planting grids and leaf area densities, therefore suggesting the need for understanding NDVI as a function of canopy structure in open canopies. These results suggest the need for assessing NDVI as function of ground cover, leaf area index and soil background. The relationship between NDVI and intercepted radiation in open canopies conducted linking the canopy reflectance model with the interception model developed by Mariscal et al (2000) yielded estimates with errors below 13% (Figure 3). These results were obtained relating modelled NDVI with radiation interception as function of canopy and structural parameters, viewing geometry and leaf optical properties required as inputs for the two models.

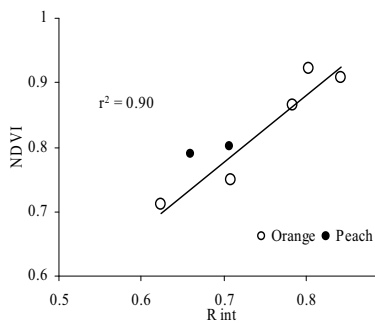


Fig. 1 Relationship between NDVI and fraction of radiation intercepted at different times of

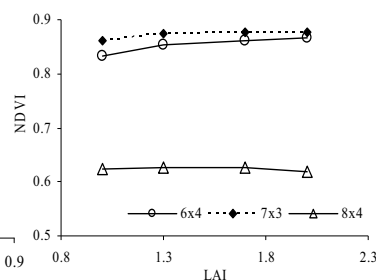


Fig. 2 Effects of different planting patterns on NDVI.

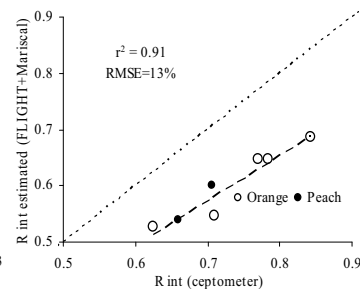


Fig. 3 Relationship between measured and simulated intercepted radiation.

## Conclusions

High-resolution airborne NDVI acquired diurnally is a potential indicator of intercepted radiation in heterogeneous canopies. However, simulation assessments conducted on NDVI as a function of canopy architecture, soil background and sun angle demonstrate that NDVI is highly affected by these parameters. A methodology based in 3D models enabled the simulation of canopy reflectance linked to radiation interception simulations, assessing the effects of leaf, canopy, background and sun geometry inputs on NDVI. Intercepted radiation estimates through the linked models validated against field measurements yielded a relative RMSE of 13% and  $r^2=0.906$ . The goal is to adapt and validate this linked model approach to other open-tree orchards to map radiation interception at the orchard scale.

## Acknowledgements

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# UAV Monitoring of Experimental Fields

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The purpose of this research was an assessment into the usability of a UAV (Unmanned Aerial Vehicle) platform for the monitoring of experimental fields. In this paper, the monitoring of (36x36 m) experimental fields localised in the Wielkopolska Region, belonging to the Institute of Soil Sciences and Plant Cultivation were presented. This region is characterised by a soil mosaic that is easily detected by using remote sensing methods. In the experiment, winter wheat, winter rape, and maize were cultivated at different applied doses of nitrogen. The main aim of using aerial photography based on the UAV platform was the detection into the spatial diversity of the soil's properties. A secondary aim was detection into the plant's condition during the vegetation period. In identifying the variability of the control unit (6x6 m), when compared to the whole plot, allowed the evaluation of the soil's mosaic influence for the experiment. Samples of the raw and detailed geographic information system photos were shown. Comparing multi-temporal data can indicate that the soil mosaic effect is best shown on the pictures taken during the late vegetation period, when the colour (vegetation) indices are relatively low.

## Methodology

Aerial photography is being more often applied in modern agricultural research. The use of alternative sources (obtained by the remote sensing platform), seems to be fully suitable for research held on small areas (Inoue et al., 2000, Jensen et al., 2007, Hunt et al., 2005, Kozyra and Pudełko 2006, Sugiura et al., 2005). The UAV used for remote sensing is based on the paraplane's construction (powered parachute), which was composed of a mounted RGB digital camera (Sony DSC-F828), GPS receiver and video camera with a radio modem connection. Selected images that were obtained and based on the UAV were converted to the orthophoto RGB index map (Rydberg A. et al., 2007). In this study, the index  $G/(R+G+B)$  was used to recalculate the UAV images (Fig. 1). All analyses were conducted in the geographical information system (Erdas Imagine 8.4).

## Results

A remote controlled flying model allows obtaining aerial photography from very low altitudes (20 – 500 m a.g.l.). The paraplane's construction enables the UAV to fly at low speeds. The radio modem connection transmits the video signal directly to the operator's eye, facilitating navigation and making easier photography of small experimental fields. The initial analysis of aerial photographs and the received (base on the maps) index of the vegetation, showed the differential of the spectral reflection from crops within the experimental plots. Photographs performed in the following periods confirmed the consistency/dependability of this differential. The observed differences could not be a result of the established experiment, because they appeared in an irregular manner, and had no relationship with the amount of nitrogen fertilization dosage that was applied for each manured area. A ground inspection confirmed the hypothesis into the influences of mosaic soil, regarding the appearance of these types of differences in the strength of vegetation. Based on the orthofoto map, it was possible to evaluate

percentage areas within a field, where the soil conditions are significantly uncharacteristic. On average, this percentage was approx. 20% of all experimental fields.

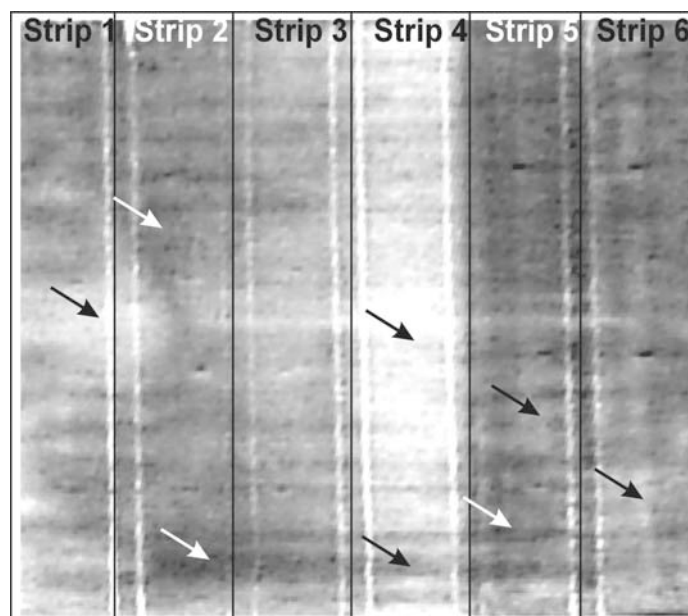


Fig. 1. An example of the orthophoto RGB index map. The black arrows indicate where the soil influenced the experiment.

### Conclusions

This research confirms that this alternative method of the obtaining aerial photography is very efficient in its usage. The characteristics of this remote sensing platform allow easy usage in the monitoring of experimental plots and small fields. The photographs are of a high resolution, and because of this, it is possible to assess the spatial variability of different attributes for the field and strength of crops. In the case of experimental plots, low altitude aerial photography allows estimation into the influence of the soil's mosaic based on the results, along with estimation into the variation of vegetation index for each investigated area, as well as allowing the selection of areas that have the smallest measuring error within their borders.

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# Variability of the Dates of the Beginnings and the Ends of Agricultural Periods in the Polish Coastal Zone of the Baltic Sea

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## **Introduction**

Progressing changes in the climate cause an increase in the climatic risk to plants growing due to an increased frequency and extremity of unfavourable to farming atmospheric phenomena. In the climatic conditions of Poland several unfavourable atmospheric phenomena (such as, for example, floods, droughts, light frosts, hail falls etc.) are observed. They cause a decrease in field crops ranging annually from 10 to 20 % on average (Atlas ... 2001). A characteristic feature of these phenomena is large time and spatial variability that makes it difficult to forecast them (Skowera, Kopec 2008, Atlas... 2004). A large threat to plants production are also very early or very late dates of occurrence of the economic period (the mean daily air temperature  $>3^{\circ}\text{C}$ ), the vegetation period ( $>5^{\circ}\text{C}$ ), the period of active plants growing ( $>10^{\circ}\text{C}$ ) and the plants ripening period ( $>15^{\circ}\text{C}$ ).

## **Methodology**

Decade and monthly air temperatures recorded in 1956 - 2005 at 9 IMGW meteorological stations situated along the coastal zone were used in the study. Trends of occurrence of the beginning and the end of 4 farming periods and probability of their occurrence (particularly of those extremely late in spring) were determined, and using the method of regression the risk to plants growing was assessed. Coastal zones of a differentiated potential threat to the plants plantation posed by very early or very late dates of the beginnings or the ends of occurrence of agricultural periods were determined. The relationship between the date of occurrence of the beginning of temperature  $5^{\circ}\text{C}$  and  $10^{\circ}\text{C}$  and the dates of the beginning of  $3^{\circ}\text{C}$  and the beginning of  $15^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  was computed.

## **Results**

In the Polish coastal zone of the Baltic Sea the beginning of field works falls generally on the middle of March; in the western part of the coast - before 10 March and in the eastern part - after 15 March. After 1980 the field works were carried out even in January in some years or as late as the middle of April in others. Similarly, the beginning of vegetation falls, on average, on 4 April and in extreme years it starts in February or after 20 April. Whereas, the beginning of active growth of plants (at temperature  $>10^{\circ}\text{C}$ ) is generally observed up to 10 May and in extreme years - in the middle of April or at the end of May. It often happens that after a period in which the air temperature is higher than  $10^{\circ}\text{C}$  there are light frosts that cause serious damage in orchards and bushes. The ripening period usually falls after 15 June, and in some years at the end of May or as late as the first half of July. Of the four farming periods mentioned, the beginning of the plants active growth period is characterised by the least variability.

The analysis of the beginnings of farming periods over 1956-2005 shows that there is a negative significant and very significant trend, i.e. considerable acceleration of the beginning of field works in spring (temperature  $>3^{\circ}\text{C}$ ), on average, by 3 weeks, the beginning of vegetation (temperature  $>5^{\circ}\text{C}$ ) by 2 weeks, the beginning of active growth (temperature  $>10^{\circ}\text{C}$ ) by one week and the ripening period by 5 days. An earlier beginning of field works in spring may be hindered by the occurrence of excessive

moistening of the surface layer of soil. A significant and very significant statistical relationship is observed between the dates of the beginning of the periods with threshold temperature of air 5° and 10°C and 3°C, and 10° and 15°C and 5°C.

### **Conclusions**

- A potential decrease in field plants crops in the Polish coastal zone of the Baltic Sea due to excessive delay of the beginning of agricultural periods ranges from 70 to 15%.
- In the coastal zone three areas of a differentiated risk to plants cultivation can be distinguished.

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# The Influence of Climatic Conditions on Seed Yield and Oil Content by Sunflower

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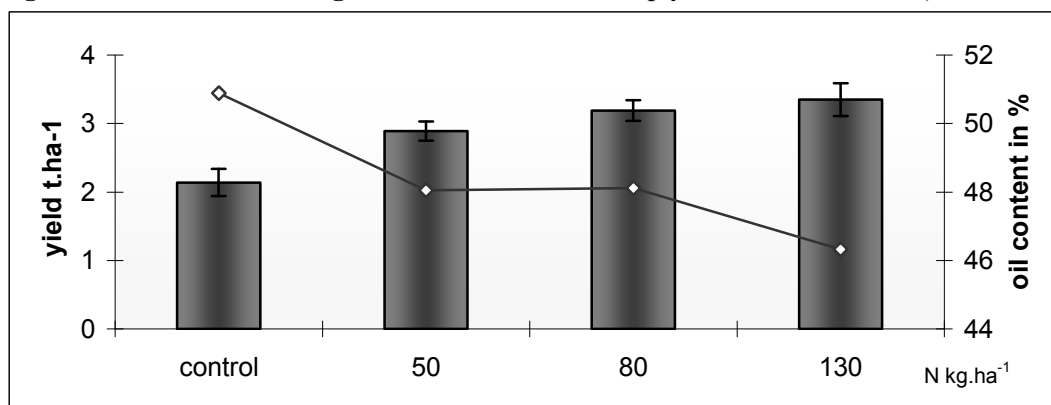
## Methodology

In the field experiment the effect of differential nitrogen nutrition on concentration, uptake and accumulation of nutrients by sunflower plants was studied during the years 2004 -2007. The nitrogen treatments were as follows: 0, 50, 80 and 130 kg.ha<sup>-1</sup>, hybrid Alexandra. The study was carried in Čáslav (altitude 263 m, long term average precipitation and temperature: 590 mm per year and 8,3 °C, resp.) on a degraded chernozemic soil. In the field experiment the effect of differential nitrogen nutrition on concentration, uptake and accumulation of nutrients by sunflower plants was studied during the years 2004 -2007.

## Results

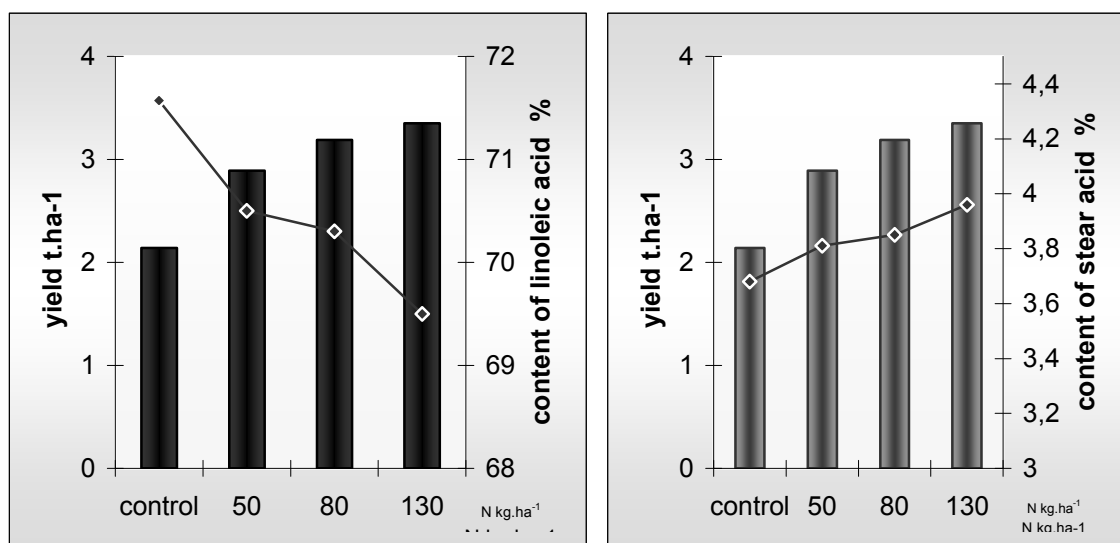
The effect of site conditions and nitrogen fertilisation is conclusively demonstrated in the content of linoleic acid and stear acid in sunflower achenes (the results are shown in Fig 2). Our research shows that between 2004 and 2007 the uptake of nitrogen at the beginning of vegetation ranged from 11 to 98 kg.ha<sup>-1</sup> and in the time of flowering from 53 to 215 kg.ha<sup>-1</sup>. The uptake of phosphorus at the beginning of vegetation was below 5 kg.ha<sup>-1</sup> and in the time of flowering increased to 30 kg.ha<sup>-1</sup>. In addition to site conditions and the effect of weather in the given year, uptake of nitrogen and phosphorus was also affected by the requirements of hybrids. The highest yield in 2007 (4.35 t.ha<sup>-1</sup>) was achieved for variant with 80 kg N.ha<sup>-1</sup>. The oil content in the achenes ranged from 46.31 % to 51.7 % and correlated with the dosage of nitrogen (Fig. 1). The similar results were reached by Blamey at al.(1981), Khokani at al (1993).

**Fig. 1 The influence of nitrogen fertilization on the crop yield and oil content (Čáslav 2004-2007)**



**Fig. 2 The influence of nitrogen fertilization on the content of linoleic and stear acid**

(Čáslav 2004-2007)



## Conclusions

The study of the impact of different temperature and moisture conditions on the development of plants during vegetation, especially in the crucial stages, showed the need for monitoring plant conditions from the beginning of vegetation until the time when it is still possible to carry out corrections in nutrition. If we want to achieve high and good-quality sunflower yields, it is necessary to ensure a continuous supply of nutrients over the entire vegetative period. From the diagnostic perspective, research of nutrient uptake dynamics provides valuable information about the need of nutrients during the sunflower vegetative period.

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## Acknowledgments

To Syngenta for conducting the analyses of the oil content in the achenes.

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# The Influence of Soil – Climatic Conditions and Years on the Yield Silage Maize

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## Methodology

The long-term stationary fertilizer trials were performed at two ecologically different sites to study the subsequent effects of manure and full dressing on the yields of silage maize. The effect of different rates of N fertilizers on the yield of silage maize, N content in plant biomass, and N uptake of above-ground biomass was investigated in the long-term experiment with silage maize. Fertilizers were applied at annual rate 40, 80 and 120 kg N.ha<sup>-1</sup>, and mean annual rate in manure was 167 kg N.ha<sup>-1</sup>. The twelve test groups (repeated 4 times) the impacts of fertilizer use, or of abstaining from the use of N, P, K, Mg and manure, were studied on the two sites Ivanovice (beet production area) and Lukavec (potato production area).

## Results

For the unfertilized test group, representing the basic soil fertility of the location we studied the influence of climatic conditions on the yield of silage maize. In Ivanovice the silage maize yields for this test group ranged as wide as from 13,12 to 22,27 t.ha<sup>-1</sup>. By contrast in the worse quality soil of Lukavec the yields ranged from 7,25 to 18,42 t.ha<sup>-1</sup>, which indicates a lower nutrient content in the soil of the potato-growing region. The highest yield of silage maize in the time period was 28,57 t.ha<sup>-1</sup> in Ivanovice and 28,95 t.ha<sup>-1</sup> in Lukavec (Table 3). Statistically significant difference was also recorded between yield and precipitation in April up to September (Table 1 and 2). The share of factors, which affected significantly the yield, changed in experimental years and entered into interactions, particularly with weather as well as with a fertilization.

Table 1 The climatic conditions at the Ivanovice site (2004-2007)

Precipitation in mm								Temperature in °C						
Year	April	May	June	July	August	Sept	Sum	April	May	June	July	August	Sept	Average
<b>2004</b>	13	16	99	39	29	40	<b>236</b>	11,0	13,7	17,4	19,3	20,1	14,6	<b>16,03</b>
<b>2005</b>	50	108	50	97	87	22	<b>413</b>	10,0	14,3	17,2	19,7	17,6	15,4	<b>15,69</b>
<b>2006</b>	77	70	121	56	157	14	<b>495</b>	10,3	14,5	18,5	22,5	16,9	16,3	<b>16,50</b>
<b>2007</b>	2	96	107	35	55	108	<b>403</b>	11,6	16,0	19,5	20,4	20,0	12,7	<b>16,70</b>

Table 2 The climatic conditions at the Lukavec site (2004-2007)

Precipitation in mm								Temperature in °C						
Year	April	May	June	July	August	Sept	Sum	April	May	June	July	August	Sept	Average
<b>2004</b>	40	48	132	58	64	65	<b>407</b>	8,0	10,6	14,9	16,6	17,6	12,2	<b>13,31</b>
<b>2005</b>	43	75	56	180	116	37	<b>507</b>	8,2	12,8	15,7	17,4	15,1	13,6	<b>13,81</b>
<b>2006</b>	67	100	164	44	123	7	<b>504</b>	7,3	12,3	16,6	20,8	14,2	15,2	<b>14,40</b>
<b>2007</b>	2	90	53	122	76	124	<b>468</b>	9,7	13,7	18,1	18,7	16,9	10,6	<b>14,62</b>

Table 3 Dry matter yield (t.ha<sup>-1</sup>) of silage maize at the Ivanovice and Lukavec (2004 - 2007)

	Ivanovice				Lukavec			
Variant	2004	2005	2006	2007	2004	2005	2006	2007
FYM	13,30	26,40	19,20	15,05	11,23	8,68	16,48	17,84
FYM+PK	14,41	26,93	23,05	15,70	12,76	9,29	16,20	19,52
FYM+N <sub>1</sub> PK	13,93	28,57	22,95	19,12	18,00	12,67	21,35	20,86
FYM+N <sub>2</sub> PK	14,46	27,02	22,70	20,45	23,69	14,63	24,13	24,91
FYM+N <sub>3</sub> PK	17,01	24,94	19,77	18,53	20,48	16,29	28,95	25,20
FYM+N <sub>2</sub> PK+Mg	13,75	26,76	19,07	19,66	20,11	15,35	25,65	22,62
Control	13,12	22,27	18,69	17,01	11,41	7,25	18,42	14,33
FYM+N <sub>2</sub> Mg	12,90	27,12	23,15	18,80	19,46	14,14	22,12	23,76
FYM+N <sub>1</sub>	15,49	25,89	20,22	15,54	17,77	10,70	19,10	20,86
FYM+N <sub>2</sub>	12,37	25,56	22,42	19,67	21,45	12,07	24,31	20,53
FYM+N <sub>2</sub> P	14,43	26,43	21,47	19,36	17,89	16,48	24,04	22,24
FYM+N <sub>2</sub> K	15,05	27,80	21,35	18,61	18,41	13,94	24,40	21,87
Hd <sub>0,05</sub>	1,4161	1,2403	1,1992	1,2513	1,0086	2,2429	1,9025	1,7298
Hd <sub>0,01</sub>	1,9024	1,6662	1,61098	1,6809	1,355	3,0131	2,5557	2,3237

The similar results were reached by Doorenbos at al.(1986), Polley at al (2002), Sinclair at al (2001) and Torriani at al (2007).

## Conclusions

For the maize growth to produce high yields, it is important to ensure the best vegetative conditions. It is basically not possible to influence drought and cold, but it is possible to ensure good nutritional conditions during vegetation.

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# Using Digital Camera Image for Recommending Nitrogen Topdressing Rate at Panicle Initiation Stage of Rice

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## Introduction

Crop growth and N nutritional status at panicle initiation stage (PIS) of rice have been reported to be highly related to the subsequent crop growth rate and, therefore, to the yield, yield components, and grain quality of rice. Model recommending nitrogen topdressing rate at PIS was constructed and validated for its effectiveness through a series of field experiments (Nguyen *et al.* 2007). The model employs shoot fresh weight and SPAD value of Y-leaf at PIS of rice to calculate the nitrogen top dressing rate at PIS. However, the monitoring of shoot fresh weight and SPAD value is destructive, time- consuming, and requiring expensive SPAD meter. We tried to employ digital camera, available universally to farmers, for non-destructive, timely, and cheap monitoring of shoot fresh weight and SPAD value.

## Methodology

**Experiment I:** Field experiments consisting of four rice varieties and five N fertilization levels were conducted in experimental farm, Seoul National University, Korea in 2006. Using the digital camera (DSC F-828; Sony, Japan) with a resolution of 8.0 mega pixels RGB (red, green, blue) color images of paddy field over the experimental plots were taken at a constant height of 2m, resulting in a field of view at the soil surface of 600mm 450m m, between 11:00 and 15:00 every week from early tillering to panicle initiation stage of rice. Just after taking color images, six rice plants were sampled from each plot, weighed for fresh weight, measured for leaf area, dried and weighed for plant dry weight. RGB color component has 256 levels, numbered 0 to 255, and in total,  $256^3$  colors can be represented, with black shown as 0,0,0 and white shown as 255,255,255 (Gonzalez *et al.* 2002). Twelve indices based on the RGB and normalized RGB values were computed for each of the three mosaic digital camera images (Lee *et al.* 2007). In addition, canopy cover was calculated as the ratio of plant pixel to total pixel. Rice plant was segmented from the soil background based on the algorithm of lee *et al.* (2007) using MEGI (modified excessive greenness index).

**Experiment II:** To evaluate a range of color indices for their ability to represent rice leaf color under variable object irradiance condition, five leaves with different SPAD values ranging from 17.6 to 42.3 were collected from the above experimental field and arranged on the black paper and digital camera images were taken under four irradiance levels. Red, Green, and Blue (RGB) features were extracted from the digital images, and calculated the normalized RGB, hue and saturation. Image analyses in experiment I and I were done using a program coded in Visual Basic.

## Results

As shown in Table 1, all the plant variables showed significantly positive correlation with canopy cover, normalized green (g), saturation, and hue values while negative correlation with normalized red (r) and normalized difference index. Leaf area index showed the highest correlation ( $r = -0.76$ ) with normalized difference index. However, canopy cover showed the highest correlation with fresh weight ( $r = 0.89$ ), dry weight ( $r = 0.81$ ), and plant height ( $r = 0.82$ ). Fresh weight of rice canopy showed an exponentially increasing relationship with the increase in fractional canopy cover (Figure 1;  $r^2 = 0.858$ ).

Table 1. Correlation coefficients between plant variables and image analysis indexes

Item	C.C	R	G	B	r	g	b	NDI	Sat.	Hue
Leaf area	0.63	-0.47	0.20	0.26	-0.74	0.43	0.38	-0.76	0.74	0.75
Fresh weight	0.89	-0.43	0.15	-0.07	-0.50	0.64	-0.01	-0.67	0.50	0.53
Dry Weight	0.81	-0.34	0.17	-0.11	-0.38	0.61	-0.09	-0.57	0.38	0.41
Plant height	0.82	-0.24	0.32	-0.06	-0.38	0.65	-0.12	-0.58	0.38	0.41

Note: C.C.; canopy cover, R, G, B and r, g, b; red, green, blue and normalized RGB, NDI; normalized difference index, Sat.; saturation. The threshold values of significance correlation coefficient are 0.256 and 0.332 at probability levels of 0.05 and 0.01., respectively

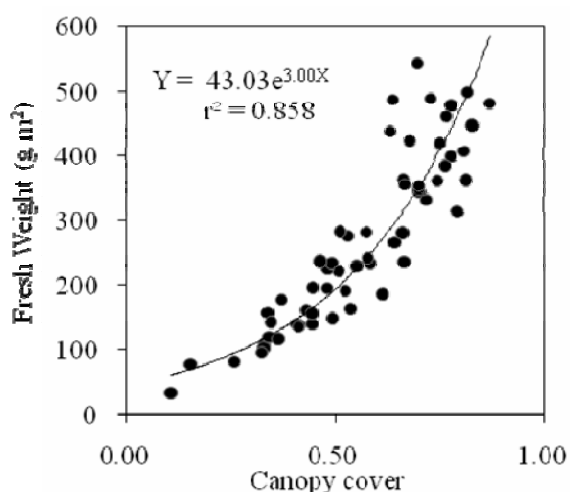


Figure 1. Relationship between plant fresh weight and canopy cover of rice canopy.

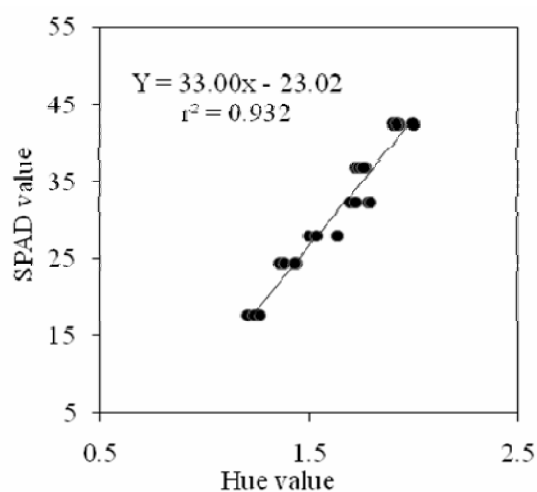


Figure 2. Relationship between hue value and SPAD value of rice leaves.

The SPAD value showed a good linear relationship (Figure 2;  $r^2=0.932$ ) with hue value when pooled across irradiance levels while poor correlation with other color indexes such as normalized RGB values, saturation etc. Hue value can be a good representation of actual leaf greenness (SPAD value) in digital camera images taken under various irradiance conditions.

### Conclusions

The fractional rice canopy that was estimated from digital camera image of rice canopy around PIS, showed a good exponential relationship ( $r^2=0.856$ ) with fresh weight of rice canopy. And also hue values calculated from the digital camera images that were taken for leaves under variable light conditions showed a consistent linear relationship ( $r^2=0.932$ ) with SPAD values. These results imply that digital camera image would be used for N rate recommendation by Nguyen's model (2007) that employs fresh weight of rice canopy and SAD value of Y-leaf as input variables..

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# Effects of deficit irrigation on yield of melon in a mediterranean environment

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In the future, it should become difficult to satisfy food requirements due to declining water resources and limited clean water reservoirs. Strategic options for achieving sustainable agriculture are the cultivation of the low water-demand crops, the water conserving irrigation scheduling and the develop of stress/drought tolerant crop varieties (Qadir *et al.*, 2003).

Improper irrigation management not only contributes to variation in crop yield but also may waste the scarce and expensive water resources (Bandyopadhyay *et al.*, 2005). Conventionally, irrigation is based on optimal crop water supply measured by pan evaporation or calculated by well-described formulas (e.g. FAO Penman-Monteith eq.) multiplied by the crop coefficient (Kc). Field water balance is commonly used to compute actual evapotranspiration (ETa) and its amount could be affected by irrigation schedule (Prihar and Sandhu, 1987; Lubana *et al.*, 2001).

Since Mediterranean areas are often characterized by recurrent water scarcity and along drought, the correct irrigation management is necessary, and therefore the objectives of this research were to evaluate the water requirements and water use efficiency of winter melon. To accomplish these aims the ETa and production were recorded.

## Methodology

The research was carried out during 2006 at Metaponto (MT) in Southern Italy (lat. 40° 24' N; long. 16° 48' E and 8 m a. s. l.) located at the experimental farm of the CRA-Research Unit for the Study of Cropping Systems. The climate is classified as “accentuated thermomediterranean” according to the UNESCO-FAO classification, with mean monthly temperatures of 8.4°C and 24.2°C in the winter and in the summer, respectively. The mean annual precipitation at the experimental farm is 493 mm, with more than 68% of the rainfall occurring during the winter months. The annual potential evaporation rate is high with a mean annual pan evaporation rate of 1548 mm. Evaporation rates are the greatest during the months of June, July and August, with mean monthly rates of 222.3, 262.2 and 229.6 mm, respectively. The soil is classified as a Typic Epiaquerts (according to Soil Taxonomy). Water field capacity (-0.03 MPa) and permanent wilting point (-1.5 MPa) values are 34.5 and 20.1% (percentage of soil dry weight), respectively.

The experimental design was a randomized block with three replications and two irrigation treatments, obtained by irrigating the melon with re-establishing 100 (T1) and 50% (T2) of the calculated maximum evapotranspiration (ETm). ETm was calculated on the basis of evaporation rate from Class A pan (Doorenbos and Pruitt, 1977) and the crop coefficients was applied according to FAO-56 paper (Allen *et al.*, 1998). The evaporation rate from Class A pan and meteorological data were recorded on hourly basis by an automated data-logger located in the experimental area. Irrigation water was supplied by localized irrigation method. The watering was applied when cumulated crop evapotranspiration value reached 19.44 mm (from transplanting to blooming) and 38.88 mm (from full bloom to fruit ripening). The melon (*Cucumis melo* L. var. inodorus cv Rugoso di Cosenza) was transplanted on May 19<sup>th</sup> 2006, after being grown in the greenhouse to the four-leaf stage, with a distance of 1.5 m between rows obtaining a density of 1.1 plants m<sup>-2</sup>. During the melon growing period the actual evapotranspiration (ETa, mm) was measured by using the general water balance equation:

$$ETa = I + ER \pm \Delta S$$

where I is the depth of irrigation, ER is the effective rainfall and  $\Delta S$  is the change in profile water storage. Soil water content was measured the day before and after waterings, with gravimetric method at 0.20 and 0.40 m depth. At harvest, total yield, fruits average weight and number, percentage of pulp, peel, seeds-placenta and dry matter were determined.

## Results

Table 1. Waterings number, seasonal irrigation water and ETa daily during melon growing

Treatments	Waterings (n)	Irrigation water (mm)	ETa daily (mm)
1 <sup>st</sup> -37 <sup>th</sup> Days after transplanting			
T1	5	105.58	3.04
T2	5	52.79	1.81
38 <sup>th</sup> -55 <sup>th</sup> Days after transplanting			
T1	2	84.18	4.85
T2	2	42.09	3.32
56 <sup>th</sup> -75 <sup>th</sup> Days after transplanting			
T1	2	87.35	5.59
T2	2	43.67	3.57

treatment at all stages, owing to water supply for evapotranspiration. The total yield of T1 treatment was significantly higher (36.8%) compared to the T2 due to a higher fruits number (Tab. 2). No significant difference was observed for average fruit weight, percentage of pulp, peel, seeds-placenta and dry matter of pulp. In Figure 1 the third-order polynomial curves of daily ETa of T1 and T2 treatments are showed. The significant polynomial relationship ( $R^2=0.94$  and  $0.91$  for T1 and T2) pointed out the reduction of ETa during the last phases of melon crop. Yield water use efficiency (YWUE, as the ratio of total yield and ETa) of two treatments was constant, around  $14 \text{ kg m}^{-3}$ .

## Conclusions

The research showed that the re-establishing 100% of the calculated maximum evapotranspiration (ETm) in melon crop produced higher ETa and yield, than the reduced irrigation treatment. However, the YWUE and some qualitative parameters of yield did not show significant difference between irrigation treatments.

Table 2. Total yield and its principal qualitative characteristics

Treatments	Total yield (t ha <sup>-1</sup> )	Fruits (n m <sup>-2</sup> )	Fruit weight (g)	Pulp (%)	Peel, seeds and placenta (%)	Dry matter pulp (%)
T1	33.17a	2.89a	1,147.58	50.83	49.17	11.84
T2	20.97b	1.93b	1,086.48	50.44	49.56	11.42

The values followed by different letters are significantly different at  $P<0.05$  (SNK).

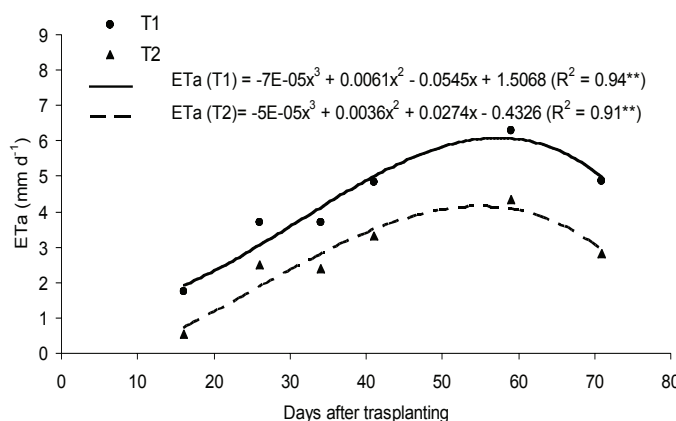


Figure 1. Crop ETa of melon during growing in T1 and T2 treatments.

in water-starved countries: challenges and opportunities, 2003. Agric. Water Manage. 62, 165-185

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During melon growth stages, 9 waterings were effected and water volume applied was  $2771$  and  $1386 \text{ m}^3 \text{ ha}^{-1}$  in T1 and T2, respectively. At these amounts the useful rain must be added (26 mm). Table 1 shows substantially differences in daily ETa between two irrigation treatments, particularly at the maturation of fruits (56<sup>th</sup>-75<sup>th</sup> days after transplanting). The highest daily ETa was observed for T1

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# Green Leaf Area Decline of the Last Three Leaves of Wheat. Determination of the Relationships with Remote Sensing Green Land Cover

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The proportion of green tissue is an important parameter of crop growth models (Bryson et al., 1997 ; Dimmock and Gooding, 2002 ; Bancal et al., 2007). That's why many attempts to estimate the percentage of green cover surface with remote sensing (RS) tools have been explored (Weiss, 2004). The remote sensing of vegetation includes various non-destructive methods from satellites to platforms in the field (Nilsson, 1995). Unlike the direct methods to estimate the proportion of green leaf area, the remote sensing is not too time and money consuming.

The use of short distance RS estimation methods involving in field platforms is an easy step to implement and allows the identification of a first relationship between a remote sensing method and a reference method of green leaf area estimation. In this field, White (2000) shows that the green coverage of soil obtained from the analysis of images taken at 2.8 m above the ground is statistically identical to that obtained from the analysis of images taken along a vertical transect ranging from 0 to 25m high in an arid ecosystem. The objective of this study is to assess the relationship that can be established between the green coverage of soil obtained from aerial photos taken a few meters above the ground and the green leaf area of the last three leaves of a winter wheat crop.

## Methodology

The experiments were conducted in 2007 in experimental plots of winter wheat located in Everlange (Grand Duchy of Luxembourg). The green coverage of soil by the canopy was identified from images taken between 08/05/07 and 03/07/07. The images were taken perpendicular at 3m high above the ground with a Canon PowerShot A620 7.1 Mégapixels. The surface at the height of the canopy covered by the images was 1.10m x 1.40 m. The images were taken once a week on the basis of one photo per plot for each of the 4 replicated plots by variant and the 7 variants (7 variations of variety and fungicide treatment). The image analysis was performed using the software Assess (APS-Press) and Ccover-tool (Guissard, 2005). The results are expressed as a percentage of area covered by green pixels. The green leaf area percentage of the last three leaves was obtained using the leaf area and the green leaf area. The software Assess was used. All the last three leaves were photographed with a non-destructive method using a device allowing a shooting vertical and perpendicular to the leaf at the high of 39 cm. These images were taken 2 to 4 times per month, from the emergence of flag leaf and during 6 to 7 weeks.

A simple regression between the green leaf area percentage and the green soil cover percentage was tested. In a first analysis, all dates for which we have the green leaf area of the last three leaves and the green coverage of soil were used. Next, the same analysis was carried out, but taking into account data acquired at dates when only the last three leaves were still green. Statistical analyses were performed using the Statistica software.

## Results

A simple linear model has been established between the green soil surface cover obtained with the two image analysis software and the green surface percentage of the last three leaves such as : "green surface percentage of the last three leaves" =  $a \times \text{"green soil cover"} - b$ . Each model shows a high significance and a coefficient of determination at around 70% (Table 1).

Logiciel analyse d'images	a	b	R	p	RMSEv
ASSESS	1.618	44.727	0.736	0.00001	19.327
Ccover	1.724	15.818	0.711	0.00001	20.251

Table1 : Statistical characteristics of both simple linear models produced between the percentage of green leaf area and green Cover percentage of soil.

A "Leave-one-out" cross-validation was carried out. The RMSEv (RMSE validation) between the predicted values for each stage of the "Leave-one-out" and observed values give an indication of the robustness of the models obtained (Table1). The RMSEv expressed as a percentage of error of the observed green surface of the last three leaves is predicted by respectively 27% and 28%. This indicates that this type of model can give the percentage of green surface of the last three leaves with a success rate of  $\sim 73\%$ . If we study this time the relationship between the percentage of green area of the last three leaves and the green coverage percentage of soil using only the data obtained from a measurement date, and this for each measurement date, we get a simple linear relation as the previous ones with each software for each date (not shown). The correlation (R) of these relations is improving (R of 80%) from June 19 (pasty maturity). From this date green leaf area remains only in the last three leaves, except for one variety tested.

## Conclusions

There is a linear relationship between the percentage of green soil cover obtained from the analysis of aerial photos and the percentage of green leaf area of winter wheat from the flag leaf emergence to the senescence of the last three leaves. This relationship shows a much better correlation when the number of leaves used (starting from the last appeared) for the percentage of green leaf surface is the number of leaves still green within the crop. The relationship obtained using all available data in the season presents a success of 73% and could most probably be improved with additional data. Improvements in the relationship can also be expected if the following factors are considered: The presence of wind, which increases the values of green soil cover. Second, light intensity which affects the classification of the images pixels. Third, taking into account the leaves that are still green beneath the last three leaves emerged. And Finally, a better discrimination of pixels very shady could afford to exclude these leaves.

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# Estimating Canopy Characteristics of Winter Wheat Using Non-Destructive Devices: Implications for N Fertilisation Advisory

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Non-destructive measurements of canopy parameters can be useful tools in experimental analysis of crop yield formation but also opens new aspects for crop management. During the vegetative growth a close relationship can be observed between aerial dry mass, crop nitrogen (N) amount and leaf area index (LAI) of winter wheat, even under N-limiting conditions, because N shortage reduces leaf growth more than N concentration in the leaves. New non-destructive measurements allow to easily determining LAI during the vegetation period. These measurements may be also suitable to optimise N management in winter wheat and may be advantageous to concentration-related measurements (N-tester, Nitracheck) if reference values for the N uptake for different growth stages are available.

## Methodology

The experiment was carried out in 2003 and 2005 to 2007 on the Hohenschulen Experimental Farm of the University of Kiel, located in NW Germany near Kiel (Schleswig-Holstein) and in 2005 to 2007 on 5 experimental sites in Lower Saxony, Germany. The varieties Cubus, Ritmo, Hybred and Dekan were grown. In spring, nitrogen ( $0\text{--}240\text{ kg N ha}^{-1}$ ) was split-applied at the beginning of spring growth, at the start of stem elongation and at ear emergence. The data collected in 2003, 2005 and 2006 at Hohenschulen were used for calibration of the regression equations. Green area index (GAI) and leaf area index (LAI), the above-ground dry matter (DM) and the N concentrations of the leaf and stem fractions were determined destructively at different stages during the growth periods. At the same sampling dates, GAI and the chlorophyll concentration were estimated using the LAI 2000 (LiCor) and the SPAD Meter (Minolta).

Based on these data, crop N uptake during spring growth was estimated with 3 different linear regression approaches. Approach 1 (A1) correlated directly the measured GAI and the actual N uptake. An improvement of the accuracy of estimation was achieved by implementing the SPAD values (Approach 2 (A2)). In Approach 3 (A3) the leaf DM was estimated by LAI2000 and the leaf N concentration by SPAD. Based on allometric relationships stem DM was calculated. Stem N concentration was related to DM stem using a dilution curve approach similar to the approach Justes et al. 1994 used for  $N_{\text{Shoot}}$  data.

## Results and discussion

In Fig.1 and Table 1 the quality of estimations of the three approaches is shown. A1 revealed a close linear relationship between N uptake and GAI with a highly significant  $r^2$  of 0.76, which is in good agreement with results of Grindlay (1997) and Olesen et al. (2002). The crop N uptake was  $39\text{ kg ha}^{-1}$  per unit GAI, lying clearly above the values of Stokes et al. (1998) who estimated a crop N uptake of  $30\text{ kg N ha}^{-1}$  per unit GAI. Lemaire et al. (2008) observed  $36\text{ kg N ha}^{-1}$  per unit GAI in France and  $21\text{ kg N ha}^{-1}$  per unit GAI in Australia under optimal N supply.

In A1, an accuracy of estimation of 20 kg ha<sup>-1</sup> was achieved on average of all N treatments and as well as for the 240 kg N ha<sup>-1</sup> treatment. Including the SPAD values (A2) reduced the RMSE to 18 kg ha<sup>-1</sup> (0-240 kg N ha<sup>-1</sup>) and 20 kg N ha<sup>-1</sup> for the highest N fertilization. Indeed, A3 showed a higher RMSE than the other approaches (37 kg N ha<sup>-1</sup> for 0-240 and 24 kg N ha<sup>-1</sup> for the 240 kg N ha<sup>-1</sup> treatment). However, due to the underlying allometric relationship it can be used to estimate DM and N uptake for leaf and stem separately, e.g. for calibrating and updating plant growth models. On the other hand, A2 seems to be more suitable to estimate solely N<sub>shoot</sub> of winter wheat. The estimation of N<sub>shoot</sub> of independent validation data (Hohenschulen, 2004, variety Ritmo, N treatment 0-320 kg N ha<sup>-1</sup>) resulted in somewhat lower but still acceptable levels of accuracy. A1 achieved on average of all N treatments a RMSE of 31 kg N ha<sup>-1</sup>, A2 achieved 24 kg N ha<sup>-1</sup> and A3 showed in validation a lower RMSE than A1 (29 kg N ha<sup>-1</sup>).

Tab.1: Root means square error (RMSE) of the approaches for the N application rates

Approach	N application rate [kg ha <sup>-1</sup> ]	RMSE [kg ha <sup>-1</sup> ]
A1	0-240*	20.11
	0	11.30
	80	17.40
	160	20.30
	240	20.10
A2	0-240*	18.10
	0	10.80
	80	17.10
	160	19.60
	240	20.10
A3	0-240*	37.11
	0	16.23
	80	34.65
	160	41.10
	240	23.52

\*0-240 included all N application rates

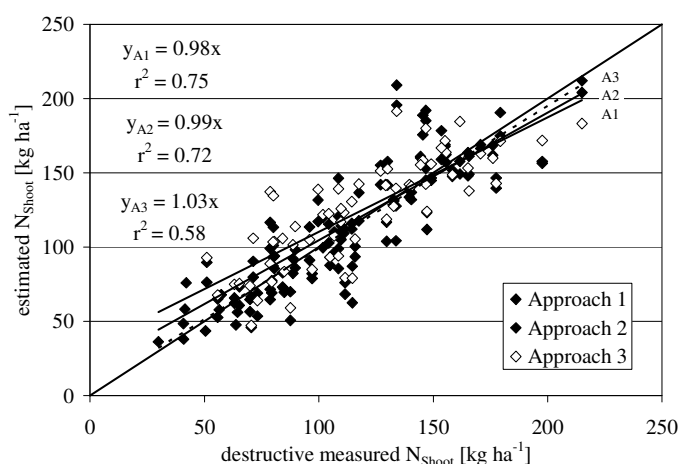


Fig.1: Quality of estimation of A1, A2 and A3 for the N application rate 240 kg ha<sup>-1</sup>

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# Variability of Air Temperature in North Western Poland

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## Introduction

Changes in natural environment of the Earth observed in the last decades originated not only from natural factors but also from human activities. A fast rate of changes measured not by thousands of years, as it had been done earlier, but at the most by decades, is resulted from an increase in population as well as from a civilization progress (Boryczka, Boryczka 2000, Kędziora 1996). The results of the warming of the climate are already clearly seen and a good illustration of that are, among other things, deficiency in water in the soil, series of dry years, drying up of small water courses and bodies of water and, on the other hand, high precipitation causing disastrous floods (Kędziora 1996, Kożuchowski 2004, Atlas...2004, Michalska, Kalbarczyk 2005, Koźmiński i in.2007).

The aim of the study was to determine the rate of increase or decrease in air temperature in the north western part of Poland (Pomerania) during the 55 year period from 1951 to 2005 in regard to months, seasons and a year, and also changes during agricultural seasons

## Methodology

For the realization of the subject matter, data concerning the air temperature have been gathered from 16 IMGW meteorological stations distributed throughout the north-western part of Poland (Pomerania) in 1951-2005. Statistical characteristics involved mean and extreme values, amplitudes, standard deviation in different time intervals: months, seasons, a year, decades. Multiannual variability of temperature was described by means of the linear trend and the rate of the increase or decrease in air temperature in Pomerania was assessed by means of simple direction coefficients of regression in regard to 1951 - 2005. In order to determine monthly air temperature a method of quantiles was applied using 11 classes ranging from extremely cold months (5 % quantile) to extremely warm ones (95 % quantile). On the basis of this classification, calendars of air temperature changes according to months and years over 1951-2005 were worked out for 2 stations. The term 'agricultural periods' means an economic period in which the air temperature is higher than 3°C and a vegetation period – with the temperature higher than 5°C.

## Results

The average annual temperature of air over the examined area varied from 7.1°C to 8.6°C, maximum – from 9.0° to 10.1°C, and minimum – from 5.6° to 7.0°C. In most of the investigated stations the highest annual temperature occurred in 2000 and 1989, and the lowest - in 1956. The average annual temperature of air higher than 8°C occurs in the western part of Pomerania and the lower one - in the eastern part. The standard deviation of the average annual temperature varied from 0.8 to 0.9. The largest seasonal variability of air temperature is observed in winter (December-February), as the values of deviation ranged within the interval 1.8-2.4, and the lowest – in summer (June - August) – values from 0.7 to 0.9. Spring in Pomerania is colder than autumn by about 1°C in the central and southern parts and even by up to 3.5°C along the coast of the Baltic Sea. The analysis of monthly values of temperature shows that the warmest months in Pomerania are July and August. In July the average temperature varied from 16.4° to 18.1°C, and in August it was the same as in July (in the central part of the coast) or from 0.1 to 0.4°C lower in the east and south of Pomerania. It was the coldest in January – from -0.1°C in the western part of the coast to -2.5°C in the central east of Pomerania. The average annual amplitude of air temperature (1951-2005) grows from the north (17.1° – 17.3°C) towards the southern part of the analysed area (19.2 – 19.6°C) showing at the same time a gradual decrease in the value in the successive decades. The course of air temperature in the consecutive years 1951-2005 shows that during the period from February to May a significant statistically increase in temperature of at least  $p=95\%$  level is observed at nearly all meteorological stations. As a result, a growing trend in spring is highly significant ( $p=99\%$ ). Another month which becomes warmer and warmer in Pomerania is August. In the remaining months of the year no statistically significant changes are observed. The values of linear trend coefficient of air temperature in the successive decades of the 50 year period show that it was the coldest over 1971-1980, and the most distinct increase in the average annual temperature of air occurred in the decade of the years 1981-1990, and then in 1991-2000. The

accumulated deviations of annual temperature of air from multiannual average indicate its gradual drop up to 1987, and then from this year onwards, a consequent increase. The analysis of linear regression coefficients defining the relationship between the air temperature and the years shows that the largest increase in temperature varying in different regions of Pomerania from 0.47° to 0.76°C per 10 years occurs in February, and then in March, April and May. A slight decrease in temperature is observed (at some stations) in June and November. The thermal evaluation of the classification of months according to quantile intervals (11 classes), carried out for the station in Chojnice situated in the central part of Pomerania, clearly shows that the months from January to May and also August have become warmer and warmer since the end of 1980s. In 1990-2005 the largest number (17) of extremely warm months (95% quantile) was recorded, and in 1951-1965 the largest number (15) of extremely cold months (5% quantile) was observed.

The heat resources of a given region are reflected in the length of farming periods. The farming period (air temperature  $>3^{\circ}\text{C}$ ) lasts the shortest - below 240 days in the mid-eastern part of Pomerania, and the longest – above 260 days, in the western part. The vegetation period ( $>5^{\circ}\text{C}$ ) lasts from about 210 to about 225 days, respectively. At present the time of duration of the vegetation period is longer than that calculated in the years 1881-1930 by about 15 to 25 days.

### Conclusions

- In north western Poland an increase in air temperature is probable, particularly in spring, and as a result there may be an acceleration of field works ( $t_{sr}>3^{\circ}\text{C}$ ) and of the vegetation period by about 15 to 25 days. In autumn the change in the dates of the end of the periods described is not observed.
- The decrease in the annual amplitude value of air temperature in the successive decades of the analysed 55 year period proves that the impact of the Atlantic Ocean on the climate of the investigated area of the country is larger and larger.
- An increase in air temperature in winter and in spring will result in an increase in evapotranspiration and change of the components of heat balance of the active surface.

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# Effects of Bio and Chemical Fertilizers on Seed Oil of Sunflower under Water Deficit Stress

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## Introduction

Sunflower (*Helianthus annus* L.) is one of the important oil seeds in the world. Sunflower oil has important role in diet due to special characters such as high oxidative stability (Moschner and Biskupek-Korell, 2006). Generally, four types of fatty acids include palmetic acid, stearic acid; omega-9 and omega-6 construct 99.9% of total fatty acids in common sunflower varieties. Many researches show that environmental conditions such as drought and nitrogen fertilizer have excessive effect on fatty acids combination of sunflower seeds. (Steer and Seiler, 2005). Therefore, with regard to plant high requirement to nitrogen fertilization and the role of this element in growth and development of plant and environmental problems resulted from irregular application of N fertilizer, making use of suitable replacing such as bio-fertilizers seems necessary. In deed, interaction between bio-fertilizer and host plant can result to positive effects such as growth and development increase, resistance against illness and improvement of host plant vigour against environmental stresses (Sturz and Nowak, 2000). With regard to role of oil and fatty acids in human healthy and diet, current research was conducted in order to study the effect of bio-fertilizers and drought stress on oil variation, omega-6 and omega-9 and saturated fatty acids in sunflower seeds.

## Methodology

An experiment was conducted during 2006 growing seasons at the Eslam Abad-Gharb Agricultural Research Station, (34° 8' latitude and 46° 26' longitude 1346 altitude) Kermanshah province, Iran. Precipitation rate was 40.5 mm during experiment performance. Soil texture was loamy clay with 7.4 PH. Field capacity and permanent wilting point were 33.7 and 18.3 respectively. Field soil analysis showed that total nitrogen and organic carbon percent were 0.067 and 0.54 respectively. The experimental design was RCBD arrangement in split plot with three replications. The treatments were three levels of irrigation [irrigation at 85% field capacity (I<sub>1</sub>), irrigation at 70% field capacity (I<sub>2</sub>) and irrigation at 55% field capacity (I<sub>3</sub>) in the main plot] and six levels of fertilizer [control (C), nitrogen recommended (N), seed inoculation with *Azotobacter chroococum* & *Azospirillum lipoferum* (AA), AA+100%N recommended (AA<sub>100</sub>), AA+75%N recommended (AA<sub>75</sub>) and AA+50% N recommended (AA<sub>50</sub>)] in sub plot]. Recommended urea fertilizer was 125 kg N/ha in base of soil test. After seed harvest, percent of seed oil was measured and fatty acids composition of oil were determined by gas chromatography. Five different fatty acids include palmitic (7.84 %), stearic (3.04 %); oleic (27.25 %) linoleic (61.72 %) and linolenic (0.1%) were consisted of about 99.95 % of total fatty acids on the average.

## Results

The most and the least oil percent were obtained in I<sub>1</sub>N and I<sub>3</sub>N treatments respectively (table 1). Disturbance in seed metabolic processes and assimilate transfer to seeds can reduce oil percent in seed of sunflower that exposure to drought stress (Bouchereau et al., 1996). In deed, drought stress accelerates plant maturity that resulted to reduction time of oil formation and storage in seed and finally oil reduction in seeds due to drought stress. I<sub>3</sub>C and I<sub>3</sub>AA<sub>100</sub> treatments had the most saturated fatty acids. The least palmetic and stearic acids were observed in I<sub>1</sub>AA treatment (Table 1). The highest rate of omega-9 and omega-6 fatty acid was belonged to I<sub>1</sub>AA<sub>50</sub> and I<sub>1</sub>C treatments, while the amount least was produced in I<sub>3</sub>AA<sub>100</sub> and I<sub>3</sub>AA<sub>75</sub> treatments (Table 1). Result from other researches show that effects of drought stress on sunflower seed oil have contradiction. These contradictions may be due to different conditions in experiments such as time of drought usage, different drought intensity and levels. But the most reports explain that drought stress reduces unsaturated fatty acids (Baldini et al.,

2002). In deed, drought stress affects on oil fatty acid compositions in oil plants seed so that drought stress increment increases saturated fatty acids, while decreases omega-6 and omega-9 fatty acids. The most important of sunflower fatty acids are omega-6 and omega-9 fatty acids and whatever sum of these two acids are more in oil, the oil quality is better for nutrition (Baldini et al., 2000). High omega-9 percent increases oxidation stability in sunflower oil, so that oil resistance increases during to fry something. It is observed that drought stress has not only destructive effect on oil percent, but also affects on oil quality. Investigation of mean variation between bacteria inoculation determined that bio-fertilizer application in integrated with urea fertilizer significantly increased oil percent and omega-9 and in deed, integrated bio-fertilizer with urea could reduce destructive effect of drought stress on quality and percent of sunflower seed oil that led to positive symbiosis relationship between plant and bacteria due to seeds bacteria inoculation. This act increased oil quality yield in sunflower.

Be notice that other researchers have showed that advantages arising of bio-fertilizer application include plant growth stimulation via nitrogen fixation, phytohormones production, mineral and organic acid production, drought resistance increment and delay in leaf senility (Lucy et al., 2004).

## Conclusion

In regards to mentioned advantages of bio-fertilizers, it can be assumption that bio-fertilizer may increase available nitrogen for plant, and quality yield in sunflower via providing suitable condition for plant growth.

Table 1- Means comparisons between interaction effect of drought stress and fertilizer treatments on studied traits in sunflower seeds.

Experimental treatments		Oil percent	Palmetic acid (%)	Stearic acid (%)	Omega-9 (%)	Omega-6 (%)
I <sub>1</sub>	AA	48.76 ab	6.22 e	2.23 h	26.73 de	64.73 ab
	AA <sub>100</sub>	48 abcd	6.29 e	2.38 gh	29.08 ab	62.18 cdef
	AA <sub>75</sub>	48.4 abc	6.38 e	2.25 h	29.48 a	61.70 defg
	AA <sub>50</sub>	48.46 abc	6.8 d	2.37 gh	29.64 a	60.94 fgh
	N	49 a	6.36 e	2.54 fgh	28.07 bc	62.98 cd
I <sub>2</sub>	C	47.16 bcde	6.52 de	2.39 gh	24.95 f	66.02 a
	AA	47.93 abcd	7.39 c	2.65 fg	26.42 de	63.42 bc
	AA <sub>100</sub>	46.30 ef	8.11 b	3.32 cde	28.40 ab	60.03 h
	AA <sub>75</sub>	47.26 bcde	7.96 b	3.10 e	28.59 ab	60.25 gh
	AA <sub>50</sub>	46.66 def	7.9 b	3.24 de	28.97 ab	59.84 h
I <sub>3</sub>	N	45.16 fg	7.79 b	2.73 f	26.27 de	63.13 cd
	C	45.96 ef	7.84 b	3.09 e	26.36 de	62.56 cde
	AA	47.93 abcd	9.44 a	3.70 b	26.47 de	60.31 gh
	AA <sub>100</sub>	45.93 ef	9.41 a	4.55 a	24.88 f	61.12 efgh
	AA <sub>75</sub>	47.30 bcde	9.26 a	3.82 b	27.06 cd	59.74 h
I <sub>3</sub>	AA <sub>50</sub>	46.96 cde	9.2 a	3.53 bcd	26.69 de	60.46 gh
	N	43.83 g	9.19 a	3.3 cde	27.05 cd	60.40 gh
	C	44 g	9.45 a	3.59 bc	25.55 ef	61.33 efgh

In each column means with similar letters are not significantly different at 5% level.

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# Alternative Materials to Black Polyethylene as Mulching in Processing Tomato: Effect on Soil Temperature and Yield

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Historically, vegetable growers have employed plastic films as mulching for providing many benefits such as earlier crop production, higher yields, cleaner and higher quality produce, more efficient use of fertilizer inputs of water resources, reduced soil and wind erosion, etc. (Scarascia-Mugnozza et al., 2006). However, the main negative consequence of the use of plastics in agriculture is related to handling the plastic wastes and the associated environmental impact. To avoid these problems, in the last few years several different degradable materials have been considered, including biopolymer mulches basically composed of polysaccharides, paper or straw.

The objective of this work was to assess the effect of seven mulches of different composition (biodegradable and polyethylene) on soil temperature and yield in an open-air tomato crop.

## Methodology

A field experiment was conducted in a processing tomato crop (*Solanum lycopersicum* L. cv. Perfectpeel) in Ciudad Real, Central Spain (4°2' W, 38°59' N, altitude 640 m). A randomised complete block design was adopted with four replications and nine mulch treatments: black polyethylene mulch (PE, 15µm), two starch-based biodegradable mulches (Mater-Bi®, Novamont, 15µ; Biofilm®, Barbier, 17µm), one oxo-biodegradable mulch (Enviroplast®, Genplast, 15µm), two paper mulches (Mimcord®, Mimgreen, 85 g/m<sup>2</sup>; Saikraft®, Saica, 125 g/m<sup>2</sup>), one barley straw mulch and two different check treatments, consisting of manual and no weed control, respectively. Each basic plot consisted in a single 15 m long row spaced 1.5 m apart, with beds 0,80 m wide. Plants were separated by 0.20 m within a row, which supposes a total population of 75 plants per basic plot. Yield, fruit quality attributes, soil temperature 5 cm depth and air temperature 20 cm above soil were controlled in each treatment during the crop cycle. Air degree-days (DDair) and soil degree-day (DDsoil) were calculated considering a base temperature of 13°C (Nuez, 1995). Data were subjected to analysis of variance and a Duncan's multiple range test ( $P<0.05$ ) was applied to the significant results.

## Results

Marketable and total yield were not affected by the mulch treatments employed, resulting only significantly lower in the no weed control treatment (Table 1). In the same way, the fruit quality attributes analysed such as firmness, solid soluble content or pH were similar in the different treatments (Table 1). In relation to the temperatures reached (Table 2), mean soil temperature was maximum in black PE and minimum in no weed control treatment, both papers and straw mulch. Air temperature, however, followed a different pattern. Soil temperature amplitudes were highest in bare soils than in mulched soils, which confirms the important effect of mulching as a temperature dampener. Among mulches, straw dampened more the soil temperature, whereas PE caused the highest amplitude. Air temperature amplitudes were higher than soil temperature ones, due to the much higher heat capacity of soil relative to air. Marketable yield in relation to mean soil temperature and DDsoil were well fitted by polynomial functions of second degree with maximum values about 23°C and 1,140 degree-days, respectively (Fig. 1).

Table 1. Yield and fruit quality attributes in the mulch treatments in a processing tomato crop.

Treatment	Yield				Fruit quality attributes		
	Marketable <sup>(1)</sup>		Total		Firmness (kg/cm <sup>2</sup> )	°Brix	pH
	kg/5 plants	Rel. to PE	kg/5 plants	Rel. to PE			
Polyethylene	13.4 a	100 a	14.0 a	100 a	4.4 a	4.5 a	4.3 a
Enviroplast®	12.3 a	95 a	13.0 a	95 a	4.8 a	4.5 a	4.2 a
Biofilm®	13.1 a	99 a	13.7 a	99 a	4.7 a	4.5 a	4.2 a
Mater-Bi®	14.0 a	105 a	14.5 a	104 a	4.7 a	4.3 a	4.2 a
Saikraft®	11.4 ab	88 ab	12.3 ab	90 a	4.4 a	4.3 a	4.2 a
Mimcord®	12.7 a	96 a	13.5 a	97 a	4.6 a	4.3 a	4.2 a
Straw	10.7 ab	83 ab	11.2 ab	82 ab	4.5 a	4.4 a	4.2 a
Manual weed control	13.7 a	104 a	14.1 a	102 a	4.4 a	4.5 a	4.2 a
No weed control	8.2 b	63 b	8.7 b	63 b	4.8 a	4.3 a	4.2 a

<sup>(1)</sup> Red + > 2.5 cm-wide green fruits.

For each parameter, treatments followed by different letters differ at  $P < 0.05$ .

Table 2. Soil temperature 5 cm depth and air temperature 20 cm above soil: mean temperature, amplitude (maximum less minimum data) and degree-days (DDair, DDsoil).

Treatment	Soil temperature			Air temperature		
	Mean T (°C)	Amplitude (°C)	DDsoil	Mean T (°C)	Amplitude (°C)	DDair
Polyethylene	23.6	6.3	1.166	23.3	18.7	1,137
Enviroplast®	22.9	5.1	1.090	23.6	20.4	1,165
Biofilm®	22.9	5.2	1.104	24.0	21.9	1,237
Mater-Bi®	22.9	5.1	1.105	24.0	20.6	1,234
Saikraft®	21.9	5.2	1.055	23.7	19.5	1,260
Mimcord®	21.9	4.1	1.051	24.3	22.4	1,330
Straw	22.0	2.4	1.060	23.5	20.9	1,244
Manual weed control	22.3	7.3	1.040	24.3	24.1	1,260
No weed control	21.7	7.3	972	23.6	18.8	1,188

For each parameter, treatments followed by different letters differ at  $P < 0.05$ .

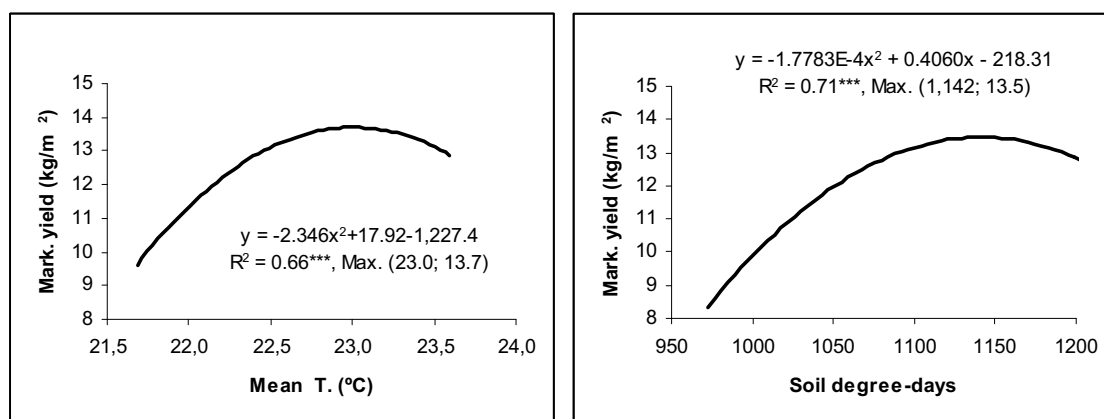


Fig. 1. Relationship between marketable yield - mean soil temperature, -soil degree-days.

## Conclusions

Biodegradable films are a good alternative to polyethylene because do not reduce yield and fruit quality attributes are similar. The straw mulch exerts an important effect as a temperature dampener. Maximum yield is reached with mean soil temperature about 23°C and 1,142 accumulated soil-degrees.

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# Effect of Halo-Priming and Hydropriming on Wheat's Germination Irrigated with Saline and Non-Saline Water and Sowed in Soil at Different Salt Levels

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## Introduction

The effects of salinity (water irrigation and soil) vary with crop pheno-phase and survival in germination phase is essential for crop growth and yield. One the first physiological disorders during seed germination under sal stress is a decrease in water uptake by the seed due to low water potential of the germination medium (Ashraf and Foolad, 2005). In addition to causing various structural changes at different levels of organitation in the seed, slow rate of imbibition may lead to a series of metabolic changes: up-regulation or down-reluation of enzyme activities (Filho and Sodek, 1988; Guerrier, 1988), pertubance in the mobility of inorganic nutrients to devoloping tissues (Ashraf and Wahid, 2000), disturbance in N metabolism (Dell'Aquila and Spada, 1993), etc.

Therefore the aim of this research was to evaluate the germination rate of wheat seeds treated with different methods of seed-priming under saline and non-saline conditions (water irrigation and soil).

## Methodology

The experiment was carried out in Portici-Gussone Park- during winter 2007, under a rainout-shelter ( $116 \text{ m}^2$ ) in 60 plastic pots with  $0.39 \text{ m}^2$  top surface.

Two test have been made: the first test has been a factorial comparison between: 1) irrigation (fresh water and saline water:  $12.5 \text{ dS m}^{-1}$ ) and 2) 6 times of soaking with distilled water (*hydro-priming*) with sowing on non-saline soil; the second test has been a factorial comparison between: 1) irrigation (fresh water and saline water:  $12.5 \text{ dS m}^{-1}$ ), 2) pre-seeding soaking with three saline solutions (*halo-priming*): low (LPS):  $3.9 \text{ dS m}^{-1}$ ; middle (MPS):  $12.5 \text{ dS m}^{-1}$ ; high (HPS):  $21.5 \text{ dS m}^{-1}$ ), 3) soil at four salinity levels: *non-saline* (NS):  $0.56 \text{ mS cm}^{-1}$ ; *low* (LSS):  $1.12 \text{ mS cm}^{-1}$ ; *middle* (MSS):  $2.09 \text{ mS cm}^{-1}$ ; *high* (HSS):  $4.10 \text{ mS cm}^{-1}$ . Two replication per treatment have been made.

During the trials 5 waterings were made about every ten days, with an average volume of  $1.6 \text{ l pot}^{-1}$ . In total for every pot 8 l of water and 64 g of salt were supplied. Every three days, germinated seeds were counted. The test was accomplished 50 days after sowing.

## Results

About hydro-priming experiment, the interaction among irrigation water and soaking times has been significant.

The germination (fig. 1) has increased to the increasing of soaking time; for seeds irrigated with fresh water, germination percentage is increased until 4 hours of soaking, while germination percentage of seeds irrigated with saline water is increased until 8 hours of soaking.

The germination percentage of seeds soaked for 8 hours and irrigated with saline water is not significantly different than the germination percentage of seeds non treated and irrigated with fresh water. About halo-priming test, only the interaction among irrigation water and soil salinity has been significant. The germination rate (fig 2) has always been significantly higher for seeds irrigated with fresh water than seeds irrigated with saline water.

The higher germination percentage (over 80%) was reached with seeds sowed on non-saline soil and irrigated with fresh water, while the seeds irrigated with saline water and sowed on high soil salinity have showed the lesser germination percentage (about 10%).

Finally significant differences were not found for seeds sowed on low and medium soils salinity and irrigated with fresh water but only for seeds irrigated with saline water.

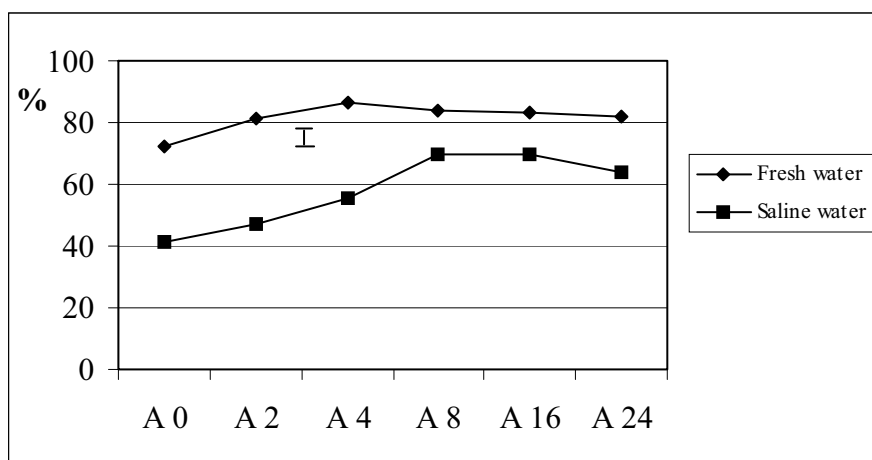


Figure 1. Interaction among irrigation water and soaking time.

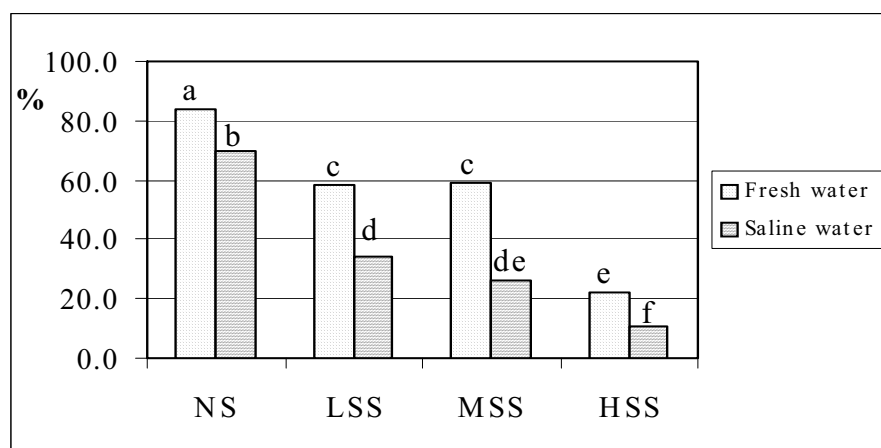


Figure 2. Interaction among irrigation water and soil salinity.

## Conclusions

The hydro-priming has allowed to compensate the effects of lesser germination due to saline irrigations beginning from the 8 hours soaking treatment; besides the halo-priming has not determined favourable effects on germination.

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# Influence of Different Methods of Seed Priming on Maize Germination Irrigated with Saline Water

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## Introduction

About half of the irrigated soil is seriously interested by salinity and/or secondary alkalinity and 10 million hectares of cultivated soil are annually abandoned because of adverse effects of secondary salinization and alkalization due to irrigation (Tanji, 1990). Strogonov, in 1964, proposed that salt tolerance of plants could be improved with a preseeding treatment on seeds with saline solution, soaking seeds and then dehydrating them. This research has evaluated the germination of maize seeds (moderately sensitive) treated with different methods of priming under saline stress conditions (irrigation water and soil).

## Methodology

The experiment was carried out in Portici-Gussone Park-during summer 2007. The cultivation has been made in a rain-out shelter with 116 m<sup>2</sup> surface (14.5 m x 8m), in 60 soil plastic pots with a top area of 0.39 m<sup>2</sup>. Three pre-seeding treatments on maize seeds have been compared: 1) soaking with 3 saline solutions (halo-priming): low (LPS): 1.1 dS m<sup>-1</sup>; middle (MPS): 3.5 dS m<sup>-1</sup>; high (HPS): 6.2 dS m<sup>-1</sup> for 2 times (1 and 6 hours); 2) soaking with distilled water (hydro-priming); 3) thermic treatment (thermo-priming). 76 seeds per pot were sowed. Three levels of soil salinity have been used for halo-priming: low (LSS): 0.34 mS cm<sup>-1</sup>; middle (MSS): 0.72 mS cm<sup>-1</sup>; high (HSS): 3.69 mS cm<sup>-1</sup>. Two replication per treatment have been made. The irrigation water EC<sub>w</sub> was 3.5 dS m<sup>-1</sup>. The 11 watering trial were made twice a week, with an average volume of 1.77 l pot<sup>-1</sup>. In total in every pot 19.5 l of water and 30 g of salt were supplied. Every three days, germinated seeds were counted. The test was accomplished 34 days after sowing.

## Results

About halo-priming, the interaction among saline solution concentration and soaking time and the effect of soil salinity has been significant. About the interaction (fig.1), only the LPS6 treatment was

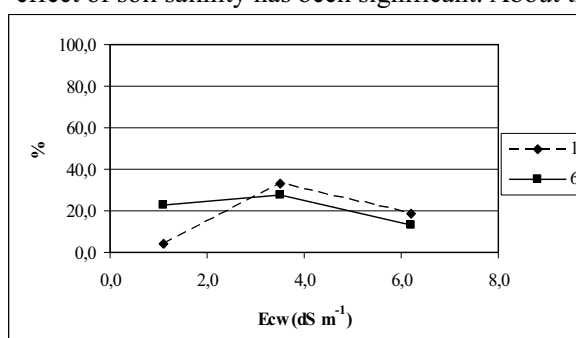


Figure 1 Interaction among saline solution concentration (1.1, 3.5 e 6.2 dS m<sup>-1</sup>) e and soaking time.

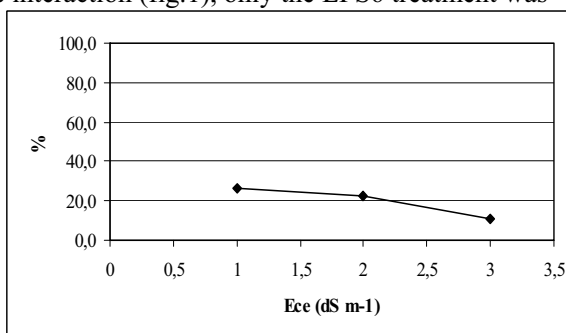


Figure 2 Effect of soil salinity on percentage of seeds germination.

significantly higher than LPS1, on the other side, in the other two points the 1 hour treatment has been higher than the 6 hours treatment, even if there were no statistic differences. Both of soaking times have, initially, increased the seeds germination percentage, reaching the maximum at 3.5 dS m<sup>-1</sup>, then

this percentage has decreased. About the effect of soil salinity (fig. 2), as obvious, the percentage of seeds germination decreased to the increasing soil electric conductivity. Nevertheless, only among soils characterized by extreme salinity values significant differences were displayed. About thermopriming, only the principal effects of temperature and thermo-priming duration has been significant. Particularly,

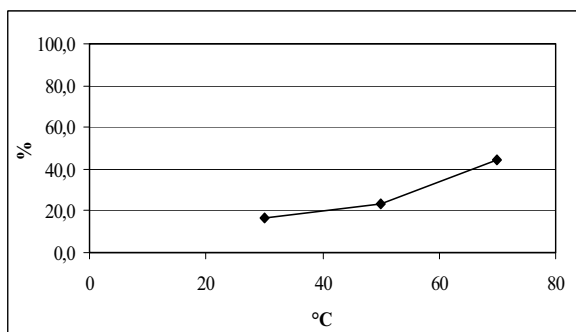


Figure 3 Effect of high temperature (30, 50 e 70°C) on seeds germination percentage.

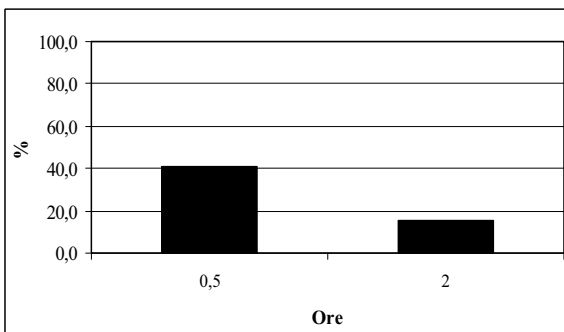


Figure 4 Effect of thermo-priming duration (0.5 e 2 h) on seeds germination percentage.

the germination percentage increased to the increasing of temperature (fig. 3) but only the 70°C treatment has been significantly different than the others two treatments. About treatment duration (fig. 4), the 30 minutes treatment has determined a significant higher germination percentage, about double than the 2 hours treatment. It would seem, therefore, that temperatures over 70°C stimulate the maize

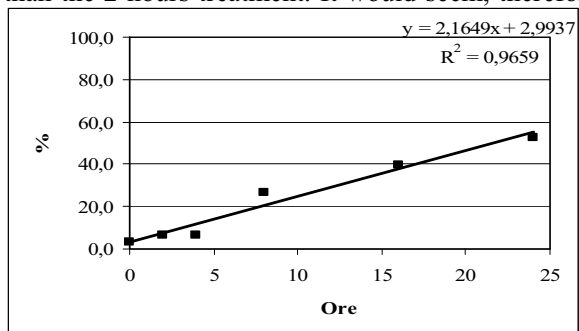


Figure 5 Effect of hydro-priming duration (0, 2, 4, 8, 16 e 24 hours) on seeds germination

seeds germination, even though only for 30 minutes. Probably, a reduction of seeds umidity involves a lowering of seeds water potential, facilitating, subsequently, water uptake from soil under saline conditions. Besides, it is possible that higher temperatures or excessive treatment duration have an opposite effect on seeds germination, due to probable inactivation of enzymes, involved in the germination. Finally about hydro-priming, the longer soaking duration

increased seeds germination percentage (fig. 5). Particularly, the germination percentage is linearly ( $R^2 \cong 0.97$  \*\*) increased to the increasing of treatment duration with an increasing of about 2 points percentage for 1 soaking hour.

## Conclusions

The preseeding treatments don't seem to compensate the lesser germination due to saline water irrigations. Particularly, about seeds soaked in saline solution, the different initial level of soil electric conductivity has further decreased the germination percentage. By the way, the treatment with medium salinity (MPS) has determined the greatest germination. The exposure to higher temperatures has promoted the germination percentage (more than doubled values); the same effect occurred in the shorter duration of thermic treatment. Finally, the duration of soaking in distilled water has always had a positive effects on germination with increased values of more than 2 points percentage for 1 soaking hour.

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# Irrigation Scheduling in Processing Tomato Crop Cultivated in Southern Italy: the Role of Physiological Parameters

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Processing tomato is a largely cultivated plant in Mediterranean area. It is a spring-summer crop very sensitive to water stress; its seasonal water requirement is estimated around 500-600 mm, almost totally supplied by irrigation (Giuliani et al., 2005). Unfortunately, water resources for agricultural use, in arid and semi-arid Mediterranean regions, like Southern Italy, are reducing. So, to save water resources, the adoption of suitable irrigation management strategies is necessary; it may be utilized, for example, a correct irrigation scheduling founded on “plant-based measures”. The aim of this study is to obtain indications on irrigation scheduling in processing tomato through the evaluation of physiological parameters like leaf water potential, osmotic potential and CWSI (“Crop Water Stress Index”).

## Methodology

The trial was conducted during 2005 crop season in Foggia (Southern Italy: 41°46' N and 15°54' E). Four tomato processing hybrids (Ercole, Genius, Tania and Ulisse) were grown in a clay loam soil with a field capacity (-0.03 MPa) of 32% d.w. and a wilting point (-1.5 MPa) of 14% d.w. A drip irrigation system was used and water quantity and time of irrigation were determined using evapotranspiration method. For irrigation were used three different water regimes (restoration of 100%, 75% and 50% of maximum crop evapotranspiration - ET<sub>c</sub>). A three replications split-plot design, with the hybrids in plot and the water regimes in sub-plot, was used.

During water critical stage (f2 - from the beginning of the flowering to the beginning of the fruit color breaking) leaf water status was monitored, before irrigations, in three sampling dates (13/6: T1; 27/6: T2; 11/7: T3), measuring, at 13 a.m., leaf water potential, osmotic potential and crop canopy temperature. Crop canopy temperature was used for empirical CWSI calculation (Idso et al., 1981). At harvest time, marketable tomato yield, water use efficiency (WUE: marketable tomato yield/total water) and °Brix were evaluated. All data were analyzed using analysis of variance (ANOVA); the significant differences among the mean values were calculated following Tukey's Test. For all the parameters under study correlations were also calculated.

## Results

In Table 1 mean values of physiological parameters, averaged across the hybrids, for water regime x sampling date interaction are reported. Physiological parameters showed a negative trend from the beginning to the end of the f2 stage, particularly for leaf water potential and CWSI values as reported also from Mauromicale et al. (1993). About water regimes, 75% ET<sub>c</sub> and 100% ET<sub>c</sub> showed a similar leaf water potential while, the restoration of 50% ET<sub>c</sub> showed the lowest value indicating a higher water stress. In relation to CWSI, in T1 the values were similar among water regimes, while in T2 differences were registered only between 50% and 100% ET<sub>c</sub> and in T3 among all water regimes, showing a delayed effect to water stress.

Table 1. Effect of water regime x sampling date interaction on physiological parameters.

	ETc restoration (%)		
	50	75	100
T1			
Leaf water pot. (MPa)	-0.39 B	-0.19 A	-0.19 A
Osmotic pot. (MPa)	-0.89 a	-0.85 a	-0.88 a
CWSI	0.26 D	0.24 D	0.18 D
T2			
Leaf water pot. (MPa)	-1.02 D	-0.26 AB	-0.28 AB
Osmotic pot. (MPa)	-1.09 a	-1.07 a	-1.04 a
CWSI	0.45 BC	0.31 CD	0.21 D
T3			
Leaf water pot. (MPa)	-1.04 D	-0.88 CD	-0.72 C
Osmotic pot. (MPa)	-1.20 a	-1.05 a	-0.95 a
CWSI	0.74 A	0.57 B	0.25 D

Sampling dates: 13/6 (T1); 27/6 (T2); 11/7 (T3).

Values in a row followed by different letters are significantly different at  $P \leq 0.01$  (capital letters) and at  $P \leq 0.05$  (small letters), following Tukey's Test.

## Conclusions

The results reported are relative to only one growing season so they need to be confirmed. In 2005 crop season quanti-qualitative aspects were strongly influenced from plant water status in central part (T2) of the period from the beginning of the flowering to the beginning of the fruit color breaking (f2 stage). Since in this year physiological parameters in T2 did not show significant differences between the restoration of 75% and 100% ETc, it appears possible to suggest the adoption of a sub-optimal water regime (75% ETc).

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Moreover correlations between physiological, yield and qualitative parameters for each sampling date were done. Highest correlation coefficients were found in T2 (Table 2). This demonstrate that plant water status in the central part of f2 stage may affect more than other periods tomato quanti-qualitative aspects. Marketable yield resulted positively correlated with leaf water potential and negatively with CWSI, while WUE and °Brix were negatively correlated with leaf water potential and positively with CWSI confirming that water stress could improve both WUE and the qualitative parameter (Kirda et al., 2004; Giuliani et al., 2006).

Table 2. Correlation matrix between physiological, yield and quality parameters.

	Mark. yield	WUE	°Brix
T1			
Leaf water potential	0.38 *	-0.29 ns	-0.43 **
Osmotic potential	-0.05 ns	-0.17 ns	-0.34 *
CWSI	-0.18 ns	0.34 *	0.32 ns
T2			
Leaf water potential	0.53 **	-0.61 **	-0.74 **
Osmotic potential	0.03 ns	-0.41 *	-0.23 ns
CWSI	-0.41 *	0.63 **	0.67 **
T3			
Leaf water potential	0.22 ns	-0.43 **	-0.29 ns
Osmotic potential	0.09 ns	-0.19 ns	-0.21 ns
CWSI	-0.39 *	0.69 **	0.72 **

\*\*, \*, ns:  $P \leq 0.01$ ;  $P \leq 0.05$  and not significant, respectively.

# The Use of Remote Sensing Methods in Assessing Cereal Condition After Winter

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The traditional method for estimating the condition of plant vegetation, demands a performance of very labour-intensive biometrical analyses. A new approach of estimating the condition of plant vegetation can be remote estimation based on indicators e.g. NDVI. Today, we have the possibility of obtaining this information by using spectral cameras. This allows the possibility of estimating large areas. The obtained data in the form of photographs, are converted by using geographical information systems (GIS) to the maps e.g. infection of plants, the condition of the vegetation (Kozyra and Pudełko 2006, Bravo et al. 2003). Such methods, are gaining wider use in scientific research as well as in agricultural practice (Kozyra and Pudełko 2006, West et al., 2003 Scotford and Miller 2004). The aim of this research was the estimation into the condition of overwintering winter cereals from aerial low-altitude photography.

## Methodology

The research was held by the Institute of Soil Science and Plant Cultivation - National Research Institute Puławy, in the Experimental Station of Osiny (51°28'N, 22°04'E, 155 m a.s.l.) Poland. In the early spring of 05-05-2006 and 27-04-2007, low altitude aerial photography with a multispectral digital camera, that is registering in the green, red, and near infrared bands was performed. The selection of the registered ranges allows a valuation for the indexes of the vegetation for a photographed plant. In the analysis, the common agricultural research method of indexing NDVI (Normalised Difference Vegetation Index) was used. The photographed plots were the experimental fields with winter wheat and winter triticosecale, on which three different types of cultivation intensity and were set as economical, sustainable, and intensive. The photographed objects were experimental fields with winter wheat and winter triticosecale on which three different levels of the tillage intensity were established: economical (low cost productions, and plant protection), sustainable systems (according to rules of sustainable production) and intensive (maximisation of the production).

## Results

The investigated years were characterised with different conditions for the winter period. In 2006, a cold winter was witnessed with an extended snow cover, which considerably weakened the overwintering plants. Within the investigated fields, an intensity of diseases was observed. The plants were attacked by the snow mould (*Microdochium nivale*), which had an influence on the condition of plants after the winter. However, the mild winter of 2007, with its short snow cover, had a positive influence on the condition of the overwintering of plants.

The performed analyses of aerial photographs allowed the cartographical estimation of the condition of cultivations after the winter. The vegetation map index performed on the photographs from 2006 is characterised with a large spatial variability of the index. The analysis of the dependence between the index and the intensity of diseases allowed defining borders on the map of the infection zones (Fig. 1). Whereas index vegetation maps from 2007 do not show the spatial variability allowing defining borders of such zones.

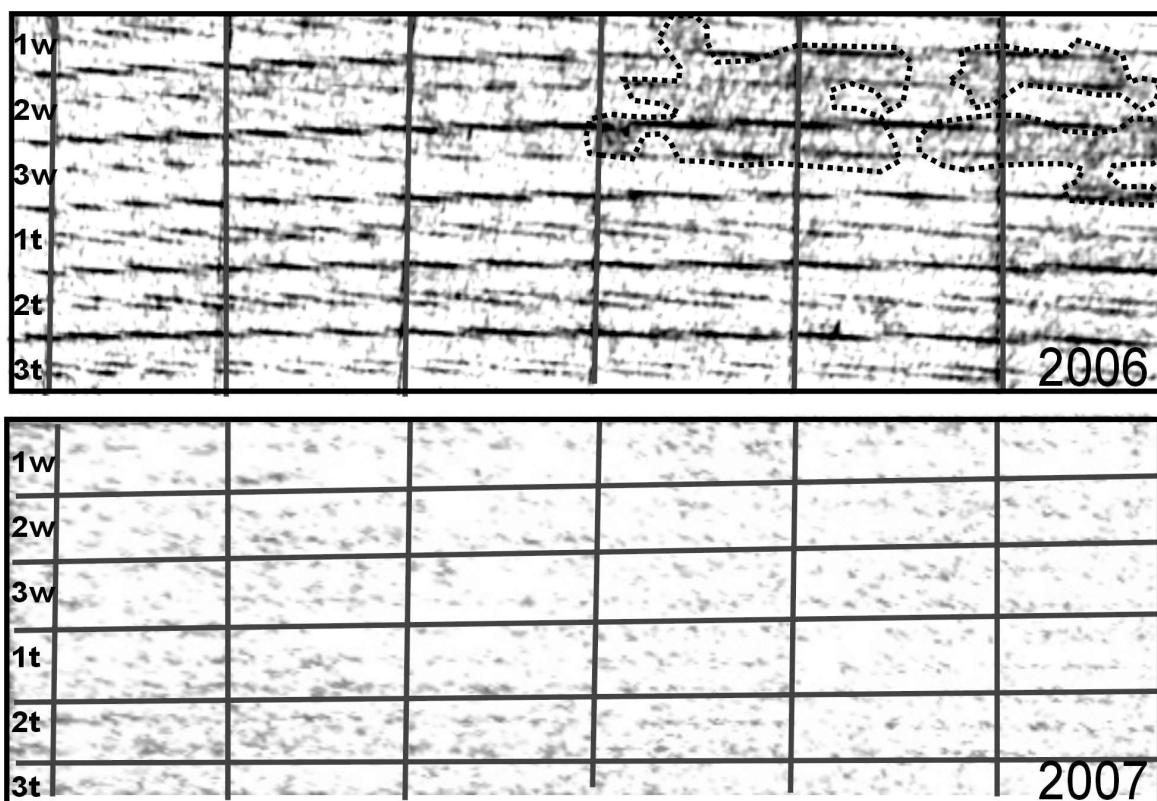


Fig. 1. NDVI maps based on the G-R-NIR camera. The grey intensity of colour indicates a low index value. The highest intensity of diseases was marked with a dotted line. w1 – winter wheat, economical system, w2 – winter wheat sustainable farming system, w3 – winter wheat intensive system

### Conclusions

It was found that the spatial correlation of NDVI maps with intensity of infection, allowed the cartographical estimation of their range.

There was no dependence between the infection and the intensity of the farming system. The location of infected zones (2006) resulted mainly from habitation conditions (soil conditions).

The comparison of maps into the condition of the vegetation period of 2006 and 2007, allows estimation into the influence of meteorological conditions that dominate in winter on the spring-form of winter-corn as well as on the amount of plants that are affected by diseases.

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# A Socio-Ecological Survey in Jalantai Area, Alxa League, Inner Mongolia, China

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Jalantai is an area (38- 42 ° Lat. N; 104 – 108 ° Long. E) located in the west side of the Helan Mountain in the Inner Mongolia Alxa League, Northern China. The climate is arid or extremely arid, with about 7°C as annual mean temperature, even though its extreme values range from - 36.4°C to + 41.7 °C. Precipitations range from 40 to 200 mm per year, occurring mainly during the summer period. The main environmental constraint is represented by the desertification process, characterized by a strong wind erosion and consequent sand storms that affect agricultural activities. Due to its environmental characteristics this area was chosen as extra-European case-study in the ADAM (ADaptation and Mitigation strategies) EU-Project.

This work refers on the preliminary results of the socio-ecological survey carried out in order to evaluate both vulnerability of local farming system to climatic change and the relevant agricultural adaptation strategies to cope with. To this purpose, a socio-ecological approach, matching sociological information with agronomical and climatological data, was set up according to Gunderson & Holling (2002).

## Methodology

A sociological on-field inquire was organized following a non-structured interview methodology as described by Corbetta (1999) and original agronomical information were collected. By this, management practices next to the local farming technological level were used as inputs for the *Cropsyst* simulation model (Stockle et al. 2003). This model is in fact able to simulate crops growth and production depending on crop specific, management and soil parameters. Daily meteorological data-set, needed by the model, were extracted from five neighbouring meteorological stations. Crop yield simulations were focused on the main crops in the region: maize and wheat.

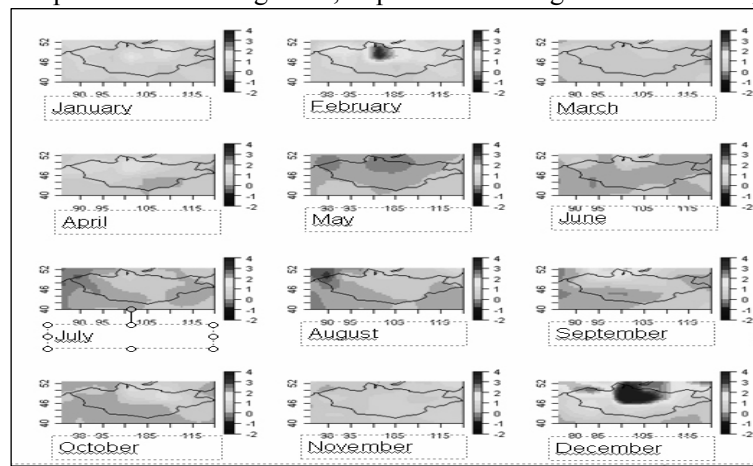
With respect to Mongolian Region (Mongolia State and Inner Mongolia) and in order to describe a climatic change scenario, specific elaborations were carried out to calculate changes between future and present climatic parameters (rainfall and temperature) on monthly base using Hadley-RCM (50 km x 50 km) monthly data (temperature and precipitation). In particular, with regard to IPCC SRES A2 scenario, two time slides were selected: a) baseline period from 1975 to 2005 (present period), b) future period from 2066 to 2099.

## Results

On average, crop yields, as simulated by CropSyst, were 1.93 and 4.18 Mg ha<sup>-1</sup> for wheat and maize, respectively, and resulted, most of the time, lower than the official statistical data regarding Inner Mongolia region. It is reliable to suppose that, such differences were mainly due to a technological gap in the region. Results from the sociological survey confirmed this hypothesis reporting about a real technology backwardness widespread in the rural areas.

Even though technological progress represented, in the past, an important input to developing agriculture in China, nowadays it is important to consider as rural agricultural activities will cope with

a future climatic change. According to Yue et al. (2007) global climate change will cause the northern Regions of China to probably become warmer and drier with respect to present time. Climate change scenarios obtained for this area were coherent with this abovementioned climatological pattern, with a temperature increasing trend, in particular during summer months, and decreasing precipitation trend in



most part of the year. As an exemplification, in Figure 1 is reported the relative variation (delta) in precipitation from the 1<sup>st</sup> to the 12<sup>th</sup> month between two time slices (1975-2005; 2066-2099). In particular, negative values of delta will occur from April to August on the Inner Mongolia. By this, it is reliable to hypothesize that next climatic conditions will affect crop yields causing strong restraints to crop productions.

Figure 1 - Relative variation of monthly precipitation on Mongolian Region with regard to SRES A2 scenario. Baseline period: 1975 –2005; future period: 2066- 2099.

## Conclusions

Local technological gaps still represent an important limitation to reach sub-national or national crop yield levels as well as the application of new political reforms supporting agriculture adaptive strategies in rural areas.

The results highlighted the importance to implement agricultural adaptation strategies to climate change in these regions. Simple strategies such as the shift of the sowing data in order to avoid negative events during growth cycle (such as drought or heat waves) can represent a useful adaptive agricultural strategy for the local peasants. Nevertheless, the main effort should depend on the structural aspects of local agricultural economy such as: introduction of drought-resistant cereal varieties; new breeding techniques for permanent housing; establishing water saving systems; preventing and managing desertification and promoting regional social and economic sustainable development.

An interdisciplinary approach can represent a useful and innovative tool to the agronomic research on climate change impacts where, in particular, the vulnerability of farming system is yet high.

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# Feasibility Study of the Maximum Daily Trunk Shrinkage for Scheduling Mandarin Trees Irrigation

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Irrigated agriculture has dramatically increased over the past 50 years and as a result has exponentially increased food production. This represents one of the pillars of modern life and the welfare state. Water is a vital and scarce resource that must be optimized as irrigation is the largest single consumer of water on the planet, accounting for about 70% of the total water resources. Therefore, it is necessary to obtain useful tools for irrigation scheduling which allow the optimal development of the crop and a good fruit quality. The main objective of this experiment was to obtain suitable reference lines to optimise irrigation scheduling in adult mandarin trees, cv. 'Fortune' grafted on 'Cleopatra', cultivated in the south-east of Spain.

## Methodology

The experiment was carried out during two harvests in a commercial mandarin tree orchard, Fortune/Cleopatra, in Cartagena (Spain). The trees were 15 years old at the beginning of the experiment and spaced at 5m x 3m. The climate of the study area is Mediterranean, with an average annual rainfall of 308 mm concentrated during the autumn-winter season, and an ETo value of 1442 mm. Summers are characterized by extreme drought periods with high temperatures. The very stony soil with clay-loam texture presents a high content of lime and potassium and a medium level of organic matter. The irrigation water is a mixture of water from the Tajo-Segura Transfer and well, reaching an electrical conductivity (EC<sub>25 °C</sub>) value of up to 4.5 dS m<sup>-1</sup> in the second year, due to the scarcity of the former.

The trees were well irrigated to satisfy their water requirements by drip with six emitters per plant (4 l h<sup>-1</sup>). Applied water was determined according to the daily reference evapotranspiration (ETo, Penman-Monteith) obtained from a meteorological station next to the orchard and a crop factor (Kc), obtained by SIAM (Servicio de Información Agraria de Murcia) at the study area. The design of the experiment was completely randomized with four replications.

Midday stem water potential ( $\Psi_s$ ) was measured with a pressure chamber (Hsiao, 1990), with two leaves per replication from the shaded side. Trunk diameter fluctuations (TDF) were monitored using a set of LVDTs installed on the trunks northern side in the same trees as  $\Psi_s$ . Two characterizing TDFs were analyzed: maximum daily shrinkage (MDS) and trunk daily growth rate (TGR) according to Goldhamer and Fereres (2001). Measurements were taken every 30 s and a datalogger CR10X was programmed to report 15 min means. A regression analysis was carried out with respect to the following climatic variables, recorded throughout the whole season: vapor pressure deficit and air temperature at midday (VPD<sub>md</sub>, T<sub>md</sub>), average over 11-16 h local time, maximum and mean (VPD<sub>mx</sub>, T<sub>mx</sub>, VPD<sub>m</sub> and T<sub>m</sub>), reference evapotranspiration (ET<sub>0</sub>), and solar radiation (R<sub>s</sub>).

## Results

The pattern of trunk diameter growth was characterized by a sigmoid curve similar to a fruit growth curve. The relationship between fruit vs. trunk growth presented a high correlation ( $r^2 = 0.99$ ). Trunk growth finished before fruit, approximately when the fruit had grown to 80% of its full size. Trunk growth during stages I and II (March to mid-October),  $\Psi_s$  showed an average value of -1.1 MPa in the

two years studied, similar to those found by Velez et al. (2007) in well irrigated clementine citrus trees. While MDS values ranged between 0.05-0.30 mm in this period, increasing with the water demand. Lower correlation coefficients were observed between  $\Psi_s$  and climate variables compared to MDS vs. climate variables. Annual MDS regressions showed better results with VPD and T being the best predictors of MDS ( $r^2 > 0.50$ ). When these regressions were considered for each phenological period, the determination coefficients improved for the two first stages ( $r^2 \geq 0.70$ ), and decreased in stage III.  $T_{md}$  presented the best relation for both the first year ( $r^2 = 0.76$ ) and the second ( $r^2 = 0.81$ ) (Figure 1). The correlations with  $ETo$  ( $r^2 \approx 0.24$ ) and  $R_s$  ( $r^2 \approx 0.40$ ) were the lowest over the two years of study. A general pattern of lower MDS was observed for a given  $\Psi_s$  as the season progressed, reflected in a slope decrease (-0.28 at stage I and II; -0.07 at stage III). This fact, together with that of the low MDS correlation with variable climates in stage III, coincides with the decrease in trunk elasticity at this stage, similar to findings by other authors in deciduous fruit trees (Marsal *et al.*, 2002; Fereres and Goldhamer, 2003; Intrigliolo and Castel, 2004).

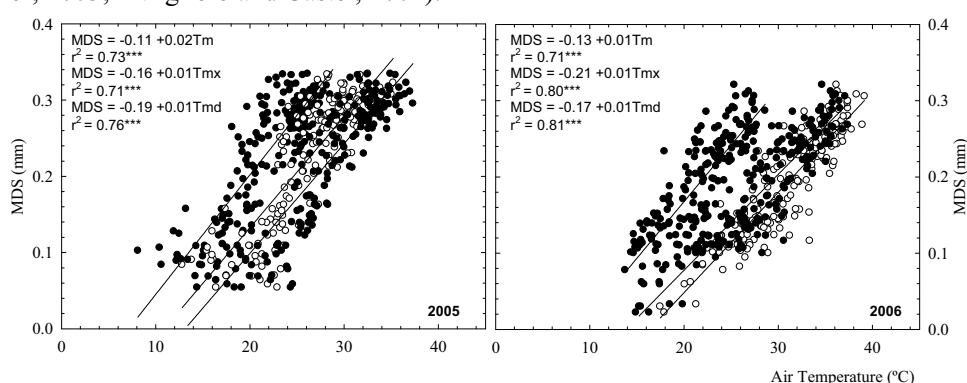


Figure 1. Relationship between MDS and Tm (●), Tmx (○) and Tmd (●) for stages I and II together (from roughly March to October) over the two years. (\*\*\*) indicates significant at  $p < 0.001$ .

## Conclusions

The highest correlations between maximum trunk daily shrinkage (MDS), and air temperature (T), obtained for the two first stages of trunk growth, can be used as a baseline in the irrigation scheduling of adult Fortune mandarins. One must consider that these two stages cover the main plant water requirement period for the whole irrigation season. By only covering the winter months, stage III loses importance in the irrigation scheduling as it is only necessary to apply the fertilized elements during this stage. The increase of  $EC_{25}^{\circ C}$  in the water irrigation, which occurred in the second year, did not affect the baselines obtained.

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## Acknowledgements

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# Climate Change and Irrigation Water Consumption: a Case Study of the Olive and the Tomato in Apulia

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## Introduction

Climate change is ‘perceived’ with growing concern by farmers and society at large. The tools available for verifying the real or presumed variation in climate have not always contributed to uniformity in the interpretation of agro-bioclimatic phenomena. The origin of this controversy may also be found in the approach to the analysis of the historical sequences -when available- which has not been updated from a statistical point of view. Irrigation water consumption by crops can be assumed as a synthetic indicator of the climatic changes in Mediterranean environments with a hot-arid climate.

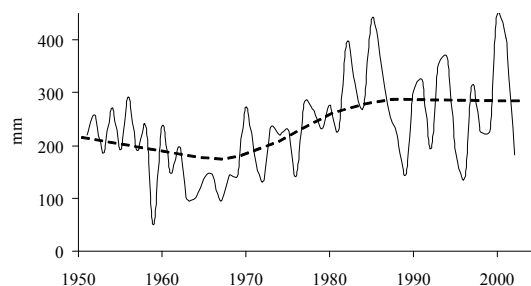
## Methodology

The historical sequences (from 1951 to 2002) of irrigation requirement were estimated for the oil olive and processing tomato, crops identified as case studies in a vast agricultural district of Southern Italy (Apulian ‘Tavoliere’). The data-set registered at the agro-meteorological station ‘Podere 124’ of the CRA-SCA in Foggia (lat. 41°26’49”, long. 15°30’15”, alt. 90m) was used.

The water budgets were estimated on the basis of the ‘Water Balance’ model (Allen et al., 1998). Crop coefficients ( $K_c$ ) were adopted after Allen et al. (lit. cit.) for tomato, and after Caliendo et al. (2001) for olive. The statistical analysis of the data was performed throughout the ‘stochastic’ approach, adopting the ARIMA model for correlating the coefficients between rain (historic data) and irrigation water volume (estimated data), and the Mann-Kendall test for identifying the temporal trends.

## Results

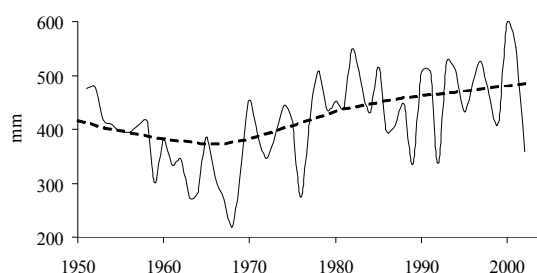
Between 1951 and 1968, the annual reference evapotranspiration ( $ET_0$ ) was, on average, 16% lower than that registered thereafter (952mm vs 1134mm). The irrigation volume estimated for the olive, as per the model, was characterised by: a) Number of Observations = 52; b) Minimum Value (1959) = 49mm/year; c) Average = 233mm/year; d) Maximum Value (2000) = 448mm/year; e) Variance = 8211mm/year. In 1959, during the irrigation season, the crop evapotranspiration ( $ET_c$ ) was 505mm and the rain 436mm; in 2000, the  $ET_c$  was 681mm and the rain 118mm. According to the model, the consumption decreased until about 1968; from this date on, it increased gradually until about 1989, after which it remained (more or less) constant during the following years (Figure 1).



*Mann Kendall Trend Test*  $\tau = 0.318$ , 2-sided  $pvalue = 0.001$

Fig.1 - Temporal trend (dashed line) and irrigation volumes (continuous line) estimated for the olive in the Apulian ‘Tavoliere’

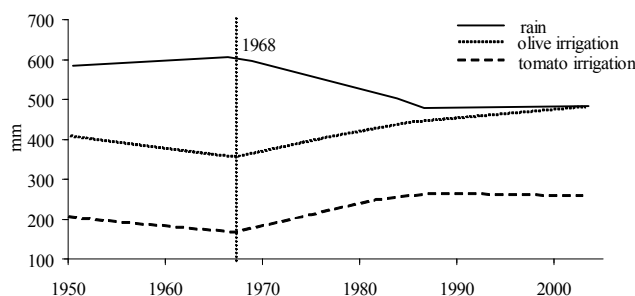
The irrigation consumption of the tomato, as per the model, was characterised by: a) Number of Observations = 52; b) Minimum Value (1968) = 219mm/year; c) Average = 415mm/year; d) Maximum Value (2000) = 599mm/year; e) Variance = 6888mm/year. In 1968, the  $ET_c$  was 376mm and the rain 233mm; in 2000, the  $ET_c$  was 661mm and the rain 118mm. The historical sequence of the irrigation water requirement for tomato was characterised by a lesser variance than that found for the olive due to the different duration of each crop's irrigation season: 3-4 months for the first, 5-6 months for the second (Figure 2).



Mann Kendall Trend Test  $\tau = 0.257$ , 2-sided pvalue = 0.007

Fig. 2 - Temporal trend (dashed line) and irrigation volumes (continuous line) estimated for tomato in the Apulian 'Tavoliere'

The statistical analysis of the historical series led to specify the following ARIMA models: (0-1-1) for olive, (2-1-2) for tomato, and (0-1-1) for the rain. Starting from about 1968, a decreasing trend for the precipitations and, vice versa, a growing trend of the irrigation volumes have been identified for both the olive and the tomato (Fig. 3).



correlation (rain vs olive irrigation) = -0.487; correlation (rain vs tomato irrigation) = -0.261

Fig.3 –Rain vs irrigation volumes estimated for olive and tomato in the Apulian 'Tavoliere'

## Conclusions

The descriptive analysis of the historical sequence confirmed the increase of irrigation water consumption that had been 'perceived' by the farmers. For various years, the increase in  $ET_0$  (even during the winter season) corresponded to lower rainfall. During the fifty-year period considered, a growth trend of the irrigation consumption took place, starting around 1968, for both the olive and the tomato. The integration of the historical sequence, necessary to guarantee statistical stationarity, is further confirmation of the presence of growth trend components in the irrigation requirements of the two crops examined.

In conclusion, regarding the fifty-year period considered, it is possible to affirm that there is a statistically significant negative correlation between rainfall (historic data) and irrigation water consumption (estimated data, as per the adopted model).

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# Effects of Salt Stress and Temperature on Seed Germination of Sweet Sorghum [*Sorghum bicolor* (L.) Moench]

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Salinity stress is a primary limiting environmental condition which restricts successful establishment of crops in many semiarid regions of the Mediterranean Basin. Seed germination may be significantly reduced and delayed by the decreasing rate of water absorption, when saline water is used for irrigation or when soil salinity is high. Salt stress may cause also excessive uptake of ions (Murillo-Amador et al., 2002). In the Mediterranean areas, sweet sorghum can be grown during the arid summer time, making necessary the use of irrigation water (Cosentino et al., 2002). A possibility to avoid the drought period, at least in the first stages of the growing season, could be achieved by the use of early sowings, which however may fail when thermal requirements of the species for germination are not satisfied. Moreover, low temperature and high soil salt concentration may exert a combined adverse effect upon seed germination. In this paper, the effects of salt stress on seed germination of sweet sorghum cv. Roce at two temperatures were examined, in order to estimate the salinity threshold for germination.

## Methodology

Seeds of sweet sorghum (*Sorghum bicolor* (L.) Moench), cv. 'Roce' (S.I.S. Società Italiana Sementi, Bologna, Italy), were used for the experiment. Seeds were surface-sterilised in a 1% sodium hypochlorite solution, rinsed in distilled water and dried before the experiment. Ten salt solutions were used for germination tests, prepared dissolving 0 (control), 25, 50, 100, 150, 200, 250, 300, 350 and 400 mM l<sup>-1</sup> of NaCl in deionized water. These solutions had osmotic potentials ( $\psi$ ) of 0, -0.10, -0.22, -0.42, -0.62, -0.82, -1.03, -1.23, -1.44, -1.64 MPa, respectively.

Germination temperatures (T) of 15° and 25°C, with the last considered the optimum for seed germination of sorghum, were maintained in a thermostatically controlled incubator ( $\pm 1^\circ\text{C}$ ). Samples of 400 seeds (four replicates of 100 seeds each) were placed in covered 9-cm Petri dishes containing a single filter paper moistened with 7 ml of one of NaCl solutions. Petri dishes were hermetically sealed with parafilm to prevent evaporation and then randomised within each temperature and incubated in the dark.

Germination was scored when radicle reached approximately 2 mm length and data were collected daily until no further visible radicle emergence was observed. At the end of the experiments, the final percentage germination and the median response time (actual time to 50% germination or  $t_{50}$ ) were calculated. In order to estimate the base  $\psi$  for germination at each temperature, a linear regression of the reciprocal of median response time ( $1/t_{50} = GR_{50}$ ) vs.  $\psi$ , has been used. The abscissa intercept is an estimate of the theoretical minimum  $\psi$  of germination.

Data of the final percentage germination, previously arcsine transformed, and those of  $t_{50}$ , were statistically analysed by a completely randomised one-way analysis of variance (ANOVA) within each temperature. Means were separated by LSD test ( $p \leq 0.05$ ). Data of  $t_{50}$  at  $\psi$  lower than -1.23 MPa at 25°C and -0.42 MPa at 15°C were excluded from statistical analysis since under these conditions seeds did not achieve 50% germination.

## Results

At optimal T (25°C) seed germination was not affected by the osmotic potential of solution down to -1.03 MPa, being statistically not different within salt treatments (Tab. 1). Germination started to decline significantly at -1.23 MPa but at this  $\psi$  it still exceeded 60%. Afterwards, germination was progressively reduced to less than 50% by the raise of salt stress and at -1.64 MPa it was lower than 20%. At this T, germination time, more than percentage, was affected by the increase of salt solution level, and even at -0.22 MPa, the seeds took significantly longer than control to germinate. At suboptimal T, seed germination of control was close to 90% but it started to decline significantly at the lowest levels of salt stress. Percentage germination kept higher than 50% at -0.42 MPa but at -0.82 it decreased down to 16.7% and at lower  $\psi$ s seeds failed to germinate. At this temperature  $t_{50}$ , longer than 11 days, did not change with salt solutions up to at  $\psi$ s > -0.42 MPa; at this last  $\psi$ ,  $t_{50}$  delayed to 22 days.

Tab. 1. Effect of osmotic potential ( $\Psi$ ) on final percentage germination and  $t_{50}$  at 25° and 15°C in sweet sorghum cv. Roce. Different letters within column indicate significant differences at  $p \leq 0.05$  by L.S.D test.

$\Psi$ (MPa)	Germination temperature			
	25°C		15°C	
	Germination (%)	$t_{50}$ (days)	Germination (%)	$t_{50}$ (days)
0	95.3 a	2.0 e	88.7 a	11.7 b
-0.10	96.0 a	2.7 de	83.7 ab	14.0 b
-0.22	95.3 a	3.0 d	71.0 bc	16.7 b
-0.42	90.0 a	3.0 d	58.7 cd	22.3 a
-0.62	94.0 a	4.0 c	44.3 d	-
-0.82	92.0 a	4.0 c	16.7 e	-
-1.03	89.3 a	5.0 b	-	-
-1.23	62.3 b	6.7 a	-	-
-1.44	34.3 c	-	-	-
-1.64	16.7 d	-	-	-
Significance	***	***	***	**

\*\*, \*\*\* significant at  $p \leq 0.01$  and 0.001 level, respectively

The linear regression, which well fits data of  $GR_{50}$  vs.  $\psi$  ( $r \geq 0.94$ ), allowed to calculating the minimum  $\psi$  at which seeds can germinate at each temperature. A threshold of -1.82 MPa at 25°C and -0.87 MPa at 15°C was estimated (Fig. 1).

## Conclusions

A 25°C, NaCl solution had no inhibitory effects on seed germination down to a certain level, due to an osmotic adjustment which allows the seed to imbibe and germinate (Okçu et al., 2005). At the lowest  $\psi$ s ( $\leq 1.2$  MPa) some seeds failed to germinate probably due to an osmotic barrier against seed coat which prevents radicle emergence. At suboptimal T, other metabolic processes strictly temperature-dependent were involved in germination inhibition under salt stress.

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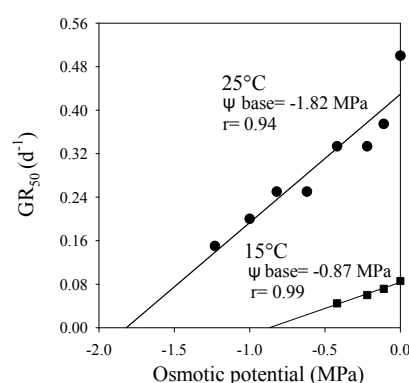


Fig.1. Relationship between  $GR_{50}$  and  $\psi$  at 25° and 15°C.

# Effects of Drought Stress on Germination and Radicle Growth of Sweet Sorghum [*Sorghum bicolor* (L.) Moench]

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Sweet sorghum is a C4 species that is well adapted to temperate and tropical semiarid environments (Cosentino, 1996). In these areas water deficit is one of the main limiting factors of plant production, and it may adversely affect germination and seedling establishment (Murillo-Amador et al., 2002). Indeed, high evaporation rates may occur during the optimal sowing time of sorghum resulting, even when irrigation is applied at sowing, in rapid drying of the medium surrounding the seed and in a marked decrease in water potential.

Drought resistant varieties are under breeding and selection for these environments. In this paper, the effects of increasing levels of water stress on seed germination in two lines of sweet sorghum selected for drought-tolerance, as compared to a commercial cultivar, were studied, in order to also determine their differences in water potential threshold at which germination and radicle growth are inhibited.

## Methodology

Seeds of two lines of sweet sorghum (*Sorghum bicolor* (L.) Moench) provided by the University of S.Cuore, Piacenza (Italy) and selected for drought tolerance (90-5-2 and CC 101), as compared to the commercial cv. Keller, were used for the experiment. Seeds were surface-sterilised in a 1% sodium hypochlorite solution, rinsed in distilled water and dried before the experiment. Water potentials ( $\psi$ ) of 0 (control), -0.2, -0.4, -0.6, -0.8 and -1.0 MPa were induced by adding polyethylene glycol (PEG 6000) to distilled water. The germination tests were carried out in laboratory at 25°C, with this last considered the optimum for germination of sorghum, and maintained in a thermostatically controlled incubator ( $\pm 1^\circ\text{C}$ ). Samples of 400 seeds (four replicates of 100 seeds each) were placed in covered 9-cm Petri dishes containing a single filter paper moistened with 7 ml of one of PEG solutions. Petri dishes were hermetically sealed with parafilm to prevent evaporation and randomised within each temperature and incubated in the dark. Germination was scored when radicle reached approximately 2 mm length and data were collected daily until no further visible radicle emergence was observed. At the end of the experiment, the final percentage germination and the median response time (actual time to 50% germination or  $t_{50}$ ) were calculated. Root length measurements were also carried out for each level of water potential during germination. To this purpose, 10 seeds were chosen randomly, within those germinated firstly per each Petri dish. Radicle was excised from seeds 2 days after initial germination and measured for length. Afterwards, radicles were oven-dried at 60°C until constant weight, for dry weight measurement.

Data of the final percentage germination, previously arcsine transformed, those of  $t_{50}$ , and of radicle length (cm) and dry weight (DW) were statistically analysed by a completely randomised two-way analysis of variance (ANOVA). The theoretical minimum  $\psi$  of germination for each cultivar was calculated using the x-intercept method (Patanè et al., 2008).

## Results

Water stress in PEG had an inhibitory effect on seed germination at the highest levels only ( $\psi < -0.6$  MPa); under these conditions germination percentage anyway exceeded 50% (Tab. 1). However, distinct phenotypic differences were found among cultivars. Line 90-5-2 germinated significantly more than the other two genotypes at all water potentials of solution; at -0.6 MPa germination was still full; the differences with the commercial cultivar ‘Keller’ were clearer at the highest level of water stress (-1.0 MPa). Line CC 101 did not differ statistically from control, although at the greatest level of water stress its seed germination (75.0%) was similar to 90-5-2 and rather higher than control (63.3%). Germination speed was statistically affected by water stress at water potential lower than -0.4 MPa and at -1.0 MPa it lengthened up to approximately 5 days. No differences were observed within cultivars. A  $\psi$  threshold of -1.71, -1.85 and -1.73 MPa, for Keller, 90-5-2 and CC 101, respectively, was estimated.

Tab. 1. Effect of cultivar and water potential ( $\psi$ ) on final percentage germination and  $t_{50}$  in sweet sorghum. Different letters indicate significant differences at  $p \leq 0.05$  by L.S.D test.

$\psi$ (MPa)	Germination (%)				$t_{50}$ (days)			
	Keller	90-5-2	CC 101	Average	Keller	90-5-2	CC 101	Average
0	98.3	100.0	96.7	98.3 ab	2.00	2.00	2.00	2.00 d
-0.2	100.0	100.0	98.3	99.4 a	2.00	2.00	2.00	2.00 d
-0.4	100.0	100.0	95.0	98.3 ab	2.00	2.00	2.00	2.00 d
-0.6	93.3	100.0	93.3	95.6 b	3.00	2.78	2.78	2.56 c
-0.8	88.3	93.3	90.0	90.6 c	3.30	3.00	3.30	3.22 b
-1.0	63.3	76.7	75.0	71.7 d	5.00	4.70	5.00	4.89 a
Average	90.6 b	95.0 a	91.4 b		2.89 a	2.78 a	2.85 a	
Significance	Cultivar	*			Significance	Cultivar	ns	
	$\psi$	***				$\psi$	***	
	Interaction	ns				Interaction	ns	

\*, \*\*\* significant at  $p \leq 0.05$  and 0.001 level, respectively; ns: not significant

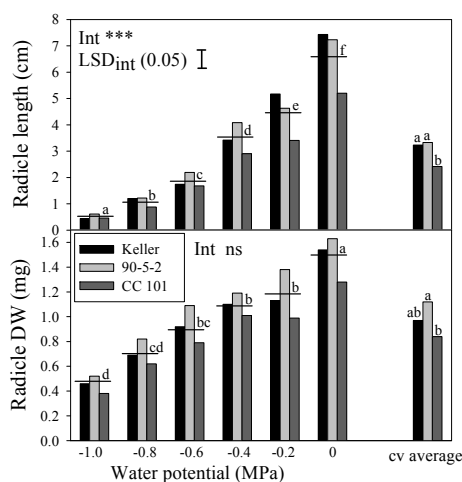


Fig. 1. Radicle length and DW in relation to the experimental factors.

PEG solution had greater inhibitory effects on radicle growth than on final germination since no significant decrease in germination was observed down to -0.6 MPa, whilst radicle growth was significantly reduced already at the lowest level of water stress (Fig. 1). The inhibiting effect of increasing water stress was less evident in cv. 90-5-2 whose radicle elongation and dry weight at the highest water potentials kept greater than those of the other genotypes ( $\psi \times cv$  significant).

## Conclusions

The genetic differences ascertained in germination response to water stress in PEG demonstrates how this procedure may be valuable in evaluating sorghum lines for adaptation to dry sowing in the semi-arid areas, in breeding programs addressed towards the development of drought-tolerant sorghum varieties.

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# May Physiological Traits of Individual Plants Predict the Performance of a Durum Wheat Crop?

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## Introduction

One of the most important tasks most wheat breeders have is further improving yield. The better we understand the physiological bases of a trait the easier it would be to manipulate it. That is why there has been a large effort in the last decades for further understanding yield determination and to provide breeders with practical tools from surrogates of physiological traits that could be used in selection (*e.g.* Slafer *et al.*, 2005). However, most of these efforts were always focused on attributes of the crop canopy. Although this is unquestionably useful in selection of late generations in which breeders work with near-homogeneous populations in a crop stand, the problem remains that the strongest selection pressure is applied in early generations where selection is based on single plant performance.

Individual plant performance is rather independent of crop performance, as lucidly evidenced by Donald half-century ago when creating the concept of ideotype (Donald, 1968). Even though there have been many attempts to offer ideotypes in the literature, it is noticeably small the body of evidences in which morpho-physiological traits of isolated individuals of a particular genotype are actually compared with the performance of the crop of that genotype in dense stands. This sort of comparison might provide breeders with selection criteria to be used in isolated plants of early generations to increase the likelihood of producing a high-yielding cultivar few generations later.

Therefore, the main objective of this study was to identify whether some morpho-physiological traits in individual durum wheat plants could be useful as selection criteria for crop performance.

## Methodology

In order to achieve this objective we grew under field conditions (in rain-fed cropping system of Catalonia, north-eastern Spain), within the same experimental framework, both isolated individuals and dense populations. Treatments consisted of the factorial combination of the four durum wheat cultivars and the two sowing systems. The cultivars were three durum wheat commonly grown in different regions of Spain (Simeto, Claudio, and Vitron) and a durum cultivar known for its adaptation to stress in Syria (Cham-1). Thus, all cultivars possess good adaptation to different Mediterranean conditions providing some expected variability in yield for our experimental site. The sowing systems consisted of isolated plants and dense canopy structure. The isolated plants were sown in a zigzag pattern, in 18 cm apart rows sowing each individual at least 30 cm from the neighbours (*c.* 20 plants m<sup>-2</sup>). The dense canopy structure was a normal sowing system with a planting density of 200 plants m<sup>-2</sup>. All plots were sown simultaneously on 22<sup>nd</sup> February 2007.

At an early tillering stage (DC 2.3) (Zadoks *et al.*, 1974) the crop was fertilised (100 Kg<sub>N</sub> ha<sup>-1</sup>, in a single dose). During the whole season the crop was kept free from diseases, weeds and insects.

At the onset of stem elongation (jointing, DC 3.1), booting (DC 4.1), anthesis (DC 6.5) and physiological maturity (DC 9.5) samples were taken. Each sample was divided into green and senescent laminae, stems, spikes and grains. When the booting and anthesis stages were reached the plant height, leaf green area and SPAD of flag leaves were measured. At maturity, yield and its components were determined.

## Results and Discussion

There was no relationship between the crop yield and the yields of the individual plants (Fig. 1). These results are in line with many previous reports that have shown that selection for yield of isolated plants

in early generations as a mean to develop high yielding cultivars is ineffective since long time ago (*e.g.* Bell, 1963). Thus we can trust that the general picture produced by the cultivars chosen for this study is representative of situations in which breeders do normally work.

Expectedly (due to the lack of relationship between isolated plant yield and crop yield) the yield components and other physiological traits measured in individual plants at maturity did not relate well with crop yield either. The issue would be whether any of the traits measured at early stages in isolated plants exhibited any relationship with crop yield at maturity.

We found some relationships between individual plant traits at anthesis time (the most significant stage of plant development for yield determination; *e.g.* Slafer, 2003) and the yield of the crop at maturity. Plant height and leaf area per plant at anthesis in isolated individuals were related to the performance of the crop at maturity (Fig. 2). Also the spike dry matter per plant at anthesis and plant growth between jointing and anthesis explained well the yield of the crop at maturity, but these two traits may not be useful in selection (destructive and time-consuming) while height and leaf area per plant in isolated individuals could be screened much easily either by eye or with surrogates like refectometry indices adapted to measurements in individual plants (*e.g.* Cabrera-Bosquet *et al.*, 2008). In addition differences in plant growth during stem elongation could be explained by the parallel differences in plant height. This positive association may mainly hold for semiarid conditions as those prevalent in the region of study, in which plants tend to be of short stature due to stressful conditions: for instance, even the highest plants (46 cm) were shorter than those suggested by Donald's ideotype to maximise crop yield.

The positive relationship between plant leaf area and crop yield was reversed when considering only the flag leaf area of the isolated plants or the greenness of the leaves. This emphasises that the collective capacity of capturing resources may prevail over the capacities at lower levels of organisation, and that assessing attributers at levels well below the whole plant may be of little use when attempting to select for complex quantitative traits (see discussion in Slafer, 2003).

## Conclusions

This has been only an initial attempt to find out whether we might be able to uncover physiological traits in isolated plants that might be trustworthily used for selecting in early generations for improved canopy performance. We must still analyse data yet under lab analysis (soluble carbohydrates and isotopic discrimination) and complement the study with larger number of genotypes and contrasting environmental conditions before achieving general conclusions.

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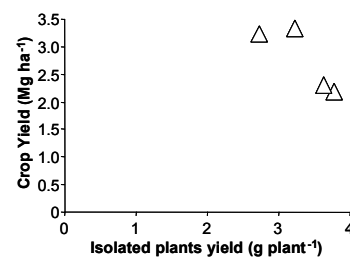


Fig. 1 Relationship between crop and isolated plants yields

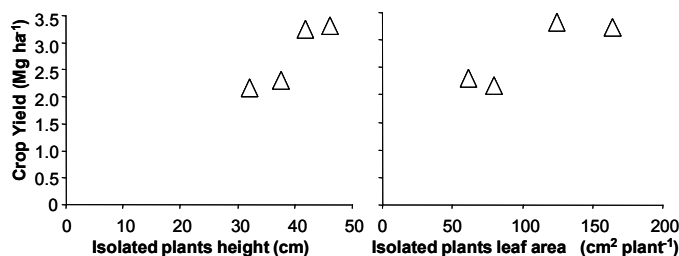


Fig. 2 Relationship between crop yield at maturity and plant height to the base of the Spike (left) and plant leaf area (right) both in isolated plants at anthesis

# Impact of Drought as Climatic Extreme on Agriculture in the Czech Republic

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## Introduction

Drought impact in developing and developed countries, but the features of these effects differ considerably. The capability to manage with drought also differs greatly from country to country and from one region. Nevertheless, it is important for scientists to understand and communicate the probability of drought. According to the strategy suggested of Wilhite (2004) drought impacts on environment would have not sufficed solution only the regional but also it is necessary on global level. Thus, working individually, many nations and regions will be unable to improve drought coping capacity. Collectively, working through global and regional partnerships, the goal of reducing the magnitude of economic, environmental, and social impacts associated with drought in the 21<sup>st</sup> century can be achieved. This study of drought brings important theoretical contributions, since it allows a more detailed and causal knowledge of this event and of its role in the characterization of the climate of the territory of Czech Republic. Also, the study of droughts has a special practical importance since it offers the reference material for the redistribution of crops in the territory, the development of the most suitable agricultural technology and the choice of the species that can resist and produce most under the given conditions. While the drought event has been studied extensively, there is limited discussion of the effects of drought of variation on individual cereal crop yields. The objective of this paper was determining drought impact on fluctuations yield of cereal crops on an example for Czech Republic.

## Methodology

The assessment was conducted meteorological information and historical yield data (1961-2007) of winter and spring cultures of cereal. The agrodatabase available for 47 years are included: spring wheat, winter wheat, spring barley, winter barley, winter rye, oat and maize. In this study, the fluctuations in crop yields over time were calculated on the basis of two components one, determined by the agrotechnical level and/or the climatic conditions and the other – on the agro-meteorological conditions during growing season year to year:

$$y_i^O = y_i^{(T)} + y_i^{(T)} \quad (1)$$

where  $y_i^{(T)}$  - is yield is presented by dynamically mean value (influenced by long-term factors such as cultivation technique and standard management),  $y_i^{(T)}$  - anomaly of yield was represented by the residuals of the detrended yield, because the residual variation reflects the best effects of weather on yield. So, the response of yield is dependent on the meteorological conditions during the growing season as well as during antecedent periods. Technological progress and improvement of societal conditions are responsible for the generally increasing trend of the crop yield. Using the weather-yield model as a measure of the fluctuations in crop yields, it is possible to reflect changes in the favorable and unfavorable agrometeorological conditions and their impacts on crops production every year.

The assumption that in years when real yield  $y_i^O$  was bigger then value mean dynamical, agrometeorological condition has been favorable during the growing season  $y_i^O > y_i^{(T)}$ . A years when real yield  $y_i^O$  was smaller then value mean dynamical  $y_i^O < y_i^{(T)}$ , agrometeorological condition has been considerate unfavorable. Thus, the interannual departures of the regional detrended yield ( $y$ ) of individual cereal plants can be expressed:

$$y_i^{(T)} = y_i^O - y_i^{(T)} / y_i^{(T)}, \quad (2)$$

where  $y_i^O$  - observed crop yield,  $y_i^{(T)}$  - value of the trend in separate year. The significant negative departures were assumed to be primarily an effect of drought event.

## Results

In agreement with developed model the drought risk was associated with cereal residual is smaller then  $y_i^{(T)} \leq -0.5\sigma$ . As defined previously, a landscape is with drought risk if yield residual was  $-0.5\sigma \geq y_i^{(T)} > -\sigma$  a low drought risk;  $-\sigma \geq y_i^{(T)} > -1.5\sigma$  a middle drought risk and  $y_i^{(T)} \leq -1.5\sigma$  a high drought risk. From 1961 to 2007, the negative value years of the anomalies of cereals yield due to drought accounted for 35%. It mean the extreme drought lead to rather significant lowering of yield ( $-\sigma$  to  $-1.5\sigma$ ). In the case of the most serious crop failures in grain production in the CR (1964, 1976, 2000, 2003, 2007), droughts were responsible. It was established drought spells occurs in May caused lower yields of spring barley, spring wheat and oat. If, drought occurs during June, then 70% from number of years with lower yields for maize were found. The effect of drought to cereal crops production depending on duration (as expressed by the consecutive dry days, **CDD** in this study), time of occurrence (during period of higher water requirements) and magnitude (as expressed by the **Si** and **SPI** drought indices). The **Si** combined effects of temperature and precipitation in drought monitoring, while the SPI is based solely precipitation data (Potop V. and Soukup J., 2008). Also the **SPI** used for detecting the early inception and end of drought, although it is a new tool that is being widely used to detect the early emergence of drought in CR and many countries. It was found that the crop-drought function was sensitive to short-period (i.e. days, weeks), because amounts of precipitation distributed at different times during growing season, may have different effect on the crop. In the territory of Czechia recorded the 15 to 20 **CDD** are greatly probability only April, July and September months. In addition to a short episode of drought (18-25 days) occurs on critical crop growth stages, the effects on agriculture were severe (as was the case, during the drought years of 2000 and 2007). Lack of rainfall from April-May 2000 in the Czechia during the critical cereal crop growing period coupled with excessive temperatures caused varying degrees of cereals crop damages. The next severe short drought episode was occurred in 2007, which started in consequence poor winter snow and little spring rain. During April in 2007 year, the drought affected more then 50% agricultural production areas in Czech Republic. Having analyses the features of the drought magnitude in the CR, we can state that approximately every 5<sup>th</sup> year suffers from severe drought during the spring and/or summer. In the meantime, moderate and severe intensity droughts are most frequent in April and July, with a guarantee of 95%, are affecting throughout most of the entire country.

## Conclusions

Taking into consideration the nonhomogeneity of demands cereal crops on hydrothermic condition during the growing season, was observed different the number of low yielding years. Thus, it can be concluded that spring barley and maize yields was much lower than residuals yield in most of the years due to the extreme drought. On the other hand the risk assessment by the model for winter rye and winter barley was not as bad as that spring cereals. Drought impact is variable from crop to crop, making assessment complicated. The degree of damage caused by drought to crop production is mainly decided by such factors as occurrence frequency, duration and severity. This study contributes improve a methodology for assessment of drought impact on cereals production. There is no generally accepted criterion for estimating the degree of damage caused by drought. However, indicators commonly used in CR consider the amount of reduction in crop yields and the economic loss, the percentage of the covered area and the affected area to crop (Chloupek O. et al. 2004.).

**Acknowledgements:** This research was supported by Research Project MSM No. 6046070901.

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# Leaf Senescence during Period of Grain Formation in Wheat Plant: Effect of Environmental Conditions and Nitrogen Supply

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## Introduction

Senescence is a phase of plant development which can be observed in plants by a progressive yellowing of the leaves, and is commonly defined as the sequence of biochemical and physiological events comprising the final stage of development until cell death (Smart, 1994). The earliest and most drastic change in plant cellular structures during senescence is the breakdown of the chloroplasts. A decrease in photosynthetic capacity is caused due to both Rubisco and chlorophyll degradation (Matile et al., 1996, Wingler et al., 1998). In addition to chloroplast disintegration, the decline in photosynthesis, proteins and nucleic acids, senescence also includes mobilization and recycling of nutrients and organics from senescing leaves to young plant organs (Gan and Amasino, 1997, Himelblau and Amasino, 2001). In vegetative organs, including wheat flag leaves, the period of grain filling is characterized by transition from sink to source status (Kichey et al., 2006). Stress conditions, high temperature or water deficiency, during the grain filling period show many negative effects such as inhibition photosynthetic process and the acceleration of leaf senescence. Both heat and water stresses increase the rate of grain filling but they shorten the duration of this period (Tahir, Nakata, 2005). The objective of this study was to estimate the effect of nitrogen starvation and different water supply on the rate of senescence of flag leaf.

## Methodology

Winter wheat (*Triticum aestivum*, L. cv. Nela) was cultivated in the field experiment in Prague-Ruzyně with two levels of N fertilization (0 and 200 kg N per ha) and three different regimes of water supply during grain formation (water shortage, drip irrigation and rain fed as control). Two days before beginning of flowering, fungicide Amistar (Azoxystrobin) was applied to one half of the experimental field. In addition to fungicidal effect, the agent shows a certain stay green effect.

At three terms during the period of grain formation (beginning of flowering, 15 and 25 day after flowering) wheat plants were harvested and flag leaves were analyzed for contents of chlorophyll, soluble protein, total N content and activity of nitrate reductase (NR).

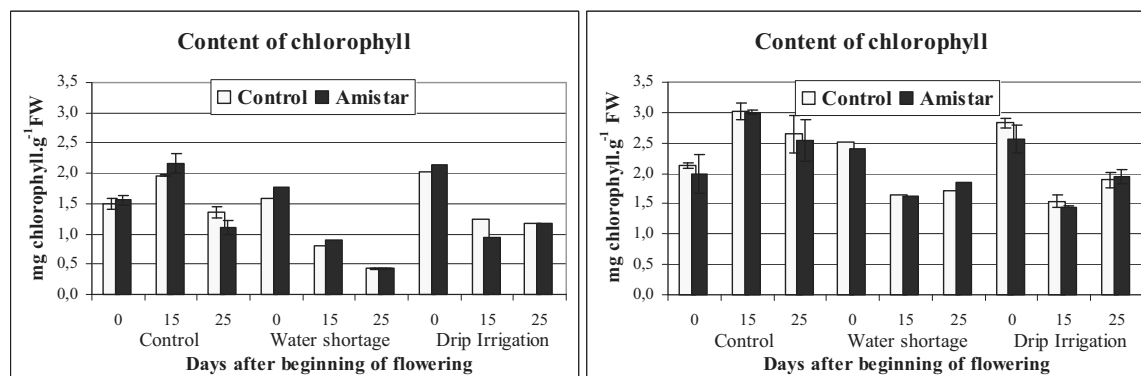
## Results

Unfavourable environmental conditions during period of grain filling induced earlier senescence of flag leaves. This effect was more intensive in plants with insufficient nitrogen supply.

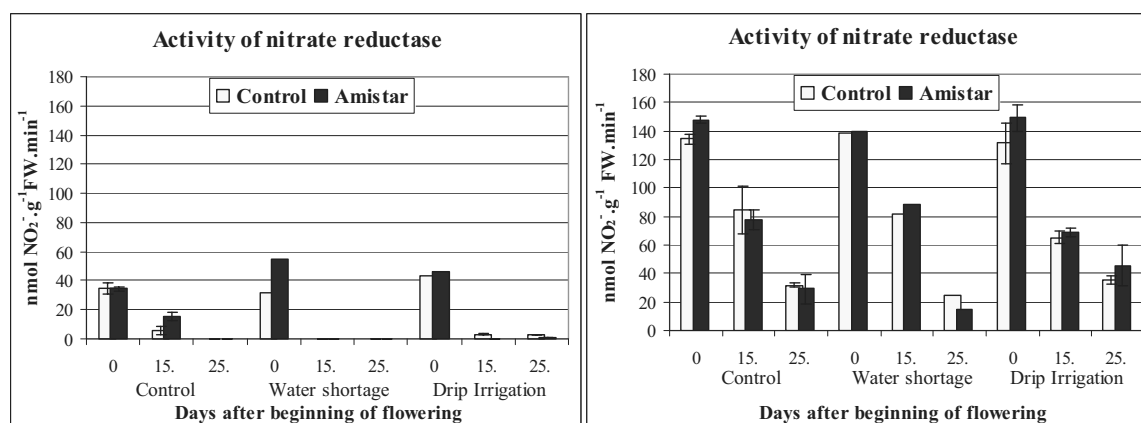
At anthesis, nitrogen starvation reduced chlorophyll content by 30% in all treatments. Similar differences were observed in content of soluble proteins, in control plants it decreased from 37  $\mu\text{g}$  to 26  $\mu\text{g}$   $\text{g}^{-1}$  FW of leaf. Both insufficient water supply and water excess accelerated chlorophyll degradation mainly during early phase of grain filling (Fig.1).

The greatest differences were found in activity of nitrate reductase (Fig.2). At adequate N nutrition NR activity of flag leaf was very high. It reached its maximum at anthesis (130 – 150  $\text{nM NO}_2^-$  per g FW per min.) and it was not affected by water supply.

Application of Amistar delayed the features of senescence. In some cases, the differences were statistically significant.



**Fig. 1** Average chlorophyll content in flag leaf of wheat plants, left part: LN – low rate of nitrogen fertilization, right part: HN – high rate of nitrogen fertilization



**Fig. 2** Activity of nitrate reductase in fresh flag leaf of wheat plants left part: LN – low rate of nitrogen fertilization, right part: HN – high rate of nitrogen fertilization

## Conclusion

Decrease of NR activity, translocation of total N and content of soluble proteins from the senescing flag leaf depends on the dose of N and water supply.

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## Acknowledgements

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# Effects of Deficit Irrigation and Partial Root-Zone Drying on Yield, Fruit Size and Blossom-End Rot of Tomato

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## Introduction

A shortage of water is the most important limiting factor for crop production in the arid and semi-arid regions of Iran. More lands could be cultivated by application water saving irrigation strategies at plant proper time during the growing season, with no benefit loss. Tomato has the highest acreage of any vegetable crop in the world (Ho, 1996), therefore adoption of water-saving irrigation strategies such as deficit irrigation (DI) and partial root zone drying (PRD) could make substantial contribution on water-saving. In the PRD one half of the root zone is irrigated while the other half falls dries out. Irrigated and dry sides are periodically switched (Dry and Loveys, 2000).

In most cases the PRD demonstrated that PRD has the ability to improve the quality of yields in fruits, vegetables and other crops (Dry and Loveys, 2000; Shahnazari et al., 2007).

Soil water stress usually shows a yield depression, together with an increase in blossom-end-rot (BER) (Ho and Adams, 1994). BER is due to calcium deficiency in the distal tissues of tomato (Obreza et al., 1996).

Although several studies have been shown effects water saving irrigation strategies (PRD and DI) on tomatoes (zegbe et al., 2003, Stikic et al., 2003), a thorough assessment of how different irrigation management strategies affect yield, quality and blossom-end-rot is still lacking. The purpose of the present work were to study 1) how PRD, DI, and FI irrigation strategies affected on yield, fruits quality and blossom-end-rot of tomatoes during the growing season, 2) potential water saving by application different level of PRD and DI in semi-arid of Iran.

## Methodology

The experiment was conducted at Shahrekord university, Iran. Six- week-old seedlings were transplanted in to twenty wooden boxes. The boxes were filled with clay loam texture with volumetric soil water content ( $\theta$ ) of 29.1% at full boxes holding capacity and 13.8% at permanent wilting point (PWP).

After 28 days of transplanting, plants were subjected to five irrigation treatments with four replicates: Full irrigation (FI) in which whole root zone (RZ) were watered when 30% soil available water in RZ was uptake by plant and the uptake water were replaced in next irrigation event; deficit irrigation which 50 and 75% of irrigation water in FI divided equally to both sides of the RZ with each irrigation event (DI<sub>50</sub> and DI<sub>75</sub> respectively) and partial root-zone drying which 50 and 75% of irrigation water in FI given only to one side of the RZ with each irrigation event (PRD<sub>50</sub> and PRD<sub>75</sub> respectively). In PRD<sub>50</sub> and PRD<sub>75</sub> treatments the irrigation was alternated between the two sides of the columns every 7–15 days.

Harvests undertaken weekly from 76 to 153 DAT for mature fruits. The fruits were weighted. Fruits were examined for blossom-end-rot (BER) incidence. Tomatoes fruit heavier than 40 gr consider as marketable yield. In each harvest four fruit per replication were randomly chosen at the firm red stage for Calcium (Ca) concentration. The dried fruits were digested with nitric acid and Ca measured by atomic absorption.

## Results

Tomatoes fruit yield are presented in following Table. FI had significantly higher yield compare than PRD<sub>50</sub>, DI<sub>75</sub> and DI<sub>50</sub>. In the marketable fruit yield (heavier (>40gr)), FI and PRD<sub>75</sub> had significantly higher yield compare than PRD<sub>50</sub> and DI<sub>50</sub> treatments. Ca concentration in the tomatoes fruit was significantly higher in PRD<sub>75</sub> and PRD<sub>50</sub> compared to other treatments.

Table Fruit yield(t/ha), marketable fruit yield(t/ha), Ca concentration (meq/l) and blossom-end-rot [BER](%) in tomatoes.

	Treatments				
	FI	PRD <sub>75</sub>	PRD <sub>50</sub>	DI <sub>75</sub>	DI <sub>50</sub>
Fruit yield(t/ha)	57.1 <sup>a</sup>	44.8 <sup>ab</sup>	9.9 <sup>c</sup>	36.9 <sup>b</sup>	9.5 <sup>c</sup>
Marketable fruit yield (t/ha)	27.6 <sup>a</sup>	24.1 <sup>a</sup>	.9 <sup>c</sup>	13.6 <sup>b</sup>	1.4 <sup>b</sup>
Ca concentration(meq/l)	7.5 <sup>b</sup>	11.3 <sup>a</sup>	10.9 <sup>a</sup>	4.8 <sup>c</sup>	8.6 <sup>b</sup>
BER(%)	10.3 <sup>a</sup>	6.7 <sup>b</sup>	3.0 <sup>b</sup>	6.6 <sup>b</sup>	.4 <sup>c</sup>

\* Different letters show significant differences at the 95% level for comparison between irrigation treatments.

## Discussion

In this study similar fruit yield were found in the FI and PRD<sub>75</sub> treatments even the irrigation water application was reduced with 25%. However other water stress treatments (PRD<sub>50</sub>, DI<sub>75</sub> and DI<sub>50</sub>) significantly decreased yield. Similar findings that PRD irrigation causes less yield reduction than DI have been observed in several crops (Zegbe et al., 2003). Beside water saving in PRD technique, higher yield quality has been reported in potatoes (Shahnazari et al., 2007). In our experiment marketable fruit yield was significantly higher in FI and PRD<sub>75</sub>. We can conclude that 75 percent of water saving is optimum for tomatoes in semi-arid such as Iran and more water deficit can reduce yield and quality of tomatoes.

Water stress increases the occurrence of BER (Ho and Adams, 1994) which has been reported by Obreza et al.,(1996) and Pulupol et al.,(1996) in tomato. Our study support Zegbe et al., (2003) report which showed less incidence of BER in PRD in tomatoes. BER has been associated with a calcium concentration in the fruit (Cerde et al., 1979). Based on our result may we can conclude that higher Ca concentration in PRD<sub>75</sub> caused less BER. However it needs to be more studied.

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# Effect of a Transient Thermal Flux on the Dormancy of *Digitaria sanguinalis* Seeds

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*Digitaria sanguinalis* is a summer annual weed in many agro-ecosystems, also in the Mediterranean crops. From the point of view of weed control, as needed to predict seedling emergences, it will be necessary to know the physiological status of the seeds (cycle of dormancy) in the soil bank, and the factors that regulate seed germination. The literature cites water content, bulk density and temperature as the most likely variables to have an effect on the seeds behavior and related with dormancy (Baskin and Baskin, 2001). But in many cases the probability to change the dormancy status is due to where and how the process has developed, as the case of the heat flux. The objective of this study is to relate the thermal properties as a process to rise a determined temperature in which seeds of this species acquire germination capacity.

## Methodology

In this study, we used a soil column device constructed specifically for this experiment. The device was refilled with a calcareous (403 g·kg<sup>-1</sup>) silt loam soil (sand 340 g·kg<sup>-1</sup>, clay 40 g·kg<sup>-1</sup>), with mean bulk density about 1470 kg·m<sup>-3</sup> and mean total organic carbon content about 31 g·kg<sup>-1</sup>, obtained between surface to 30 cm depth, from Llobregat Delta Plain (NE, Spain).

The column device has 33 cm of length. From the bottom, the first 3 cm were refilled with gravels, above 5 cm were refilled with sand, whereas the sample occupied 25 cm to up. Two scenarios were sited inside the device, level **A**=4 and level **B**=12 cm depth. Thermal and hydrodynamic properties were monitored at both levels.

For determining the volumetric water content (**WC**), two EC-5 frequency domain reflectometry probes, one for each level were used. Soil temperature (**T**) was monitored through ECT soil/air sensor. A Decagon Devices EM-50 data-logger was required to collect the data. To determine the thermal properties one small dual-needle sensor (SH-1) was employed (Rubio et al., 2008). This kind of sensor use the heat pulse methodology and yield reliable and accuracy soil thermal diffusivity ( $\alpha$ ) and thermal conductivity ( $\lambda$ ) measurements, meanwhile volumetric specific heat ( $\rho c$ ) according to relation of both properties was estimated. The SH-1 thermal sensor was placed in the middle of two levels, thus obtaining a set of thermal properties data for the soil sample to separate the two scenarios (**A** and **B**). The data were collected using a KD2-Pro reader-logger.

Also, in both levels two split seeds groups (dormant seeds stored at -18°C since their dispersal time **DS**; and non-dormant seeds preserved at room temperature since November of 2006 **NDS**) of *Digitaria sanguinalis* were buried. From the top soil column device a radiation source (50W halogen bulb) was applied during 15 days. The radiation involved a thermal gradient between both levels, that it was monitored using a thermal sensor. To perform the experiment, the soil sample was wetted until to achieve the half field capacity water content. Moreover, support irrigation were carried out.

After 33 days seeds were exhumed. The germination trials were performed at 20°C (12h darkness) and 30°C (12h light). Before incubation, the seeds were rinsed for 10 minutes with 5% diluted sodium hypochlorite for surface sterilization (ISTA, 1985). Five replicates of 40 seeds placed in 9 cm diameter plastic Petri dishes were incubated. Illumination was provided by white fluorescent tubes (3x18 W). Three ml of distilled water was added in each dish at the beginning of the tests, and water was later added as required. Seeds were considered to be germinated with the emergence of the radicle. Germination counts were made for 10 days. The percentages of germination were subjected to analyses of variance followed by Tukey's HSD test in order to identify homogeneous mean groups. Cumulative percentages of germination of the pre-treated seeds were fitted to the three-parameter of the Weibull function (Mas and Verdú, 2002).

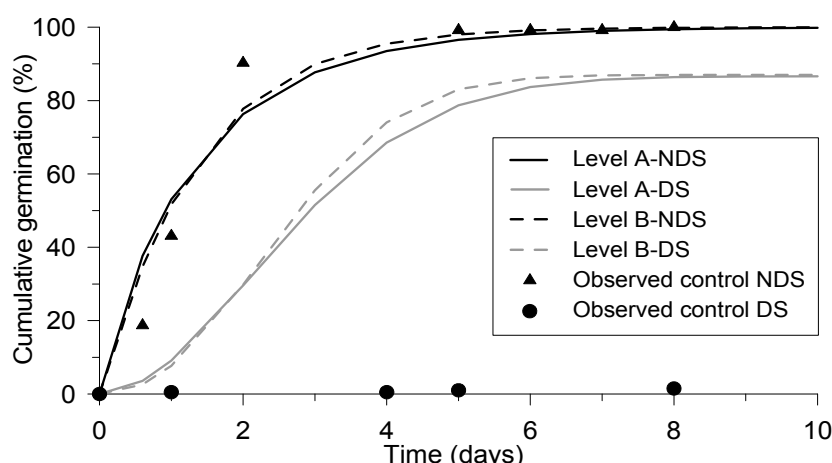
## Results

Table 1 shows the results of the soil variables monitored inside the device. Mean water content at level **A** was higher than level **B**, with differences about 2.5% (vol·vol<sup>-1</sup>). Average temperature values showed that this variable was at steady-state during all experimental process and close to temperature room values (20.4 °C). The thermal integral between both levels presented differences about 15°C, always higher for level **A**, as well in the maximum temperature values (A=27.9 °C; B=22.9°C), hence the radiation source created a slight thermal gradient inside the device. On the other hand, thermal properties showed an homogeneous values, except the volumetric specific heat, with differences about 0.5 MJ·m<sup>-3</sup>·C<sup>-1</sup>, due to the complex moisture scenarios between both levels.

**Table 1.** Statistical variables determined for the soil dataset obtained from the column device. (See text for legend)

	$\lambda$ (W·m <sup>-1</sup> ·C <sup>-1</sup> )	$\rho c$ (MJ·m <sup>-3</sup> ·C <sup>-1</sup> )	$\alpha$ (mm <sup>2</sup> ·s <sup>-1</sup> )	WC-A (m <sup>3</sup> ·m <sup>-3</sup> )	WC-B (m <sup>3</sup> ·m <sup>-3</sup> )	T-A (°C)	T-B (°C)	T-room (°C)
Average	0.546	2.084	0.262	0.130	0.105	21.2	20.5	20.4
Maximum	0.632	2.341	0.289	0.160	0.124	27.9	22.9	22.8
Minimal	0.504	1.875	0.253	0.104	0.091	17.8	18.4	18.1
Integral	----	----	----	----	----	568.3	551.2	507.5

The environment experienced by the **DS** was reflected in their germination capacity (Fig. 1). Both seed splits present a cumulative germination up to 85%, significantly different ( $p < 0.05$ ) from both, **NDS** (all of them) and **DS** (control group). This indicates that an important fraction of the seeds lost the dormancy. Moreover, lots of the two levels significantly differ from the rate of germination during the initial period of the trail. After two days of incubation, **DS** at level **A** attained a 44% of cumulative germination, significantly higher than 31.5% corresponding to **DS** at level **B**.



**Figure 1.** Evolution of the cumulative percentage of germination on *Digitaria sanguinalis* seeds after the burial period. (See text for legend)

## Conclusions

In the monitored microenvironmental conditions of the experimental column device, the loss of dormancy on *D. sanguinalis* seeds was up to 85%. Moreover, the high resolution of the used sensors has allowed to measure the minimal spatial differences for the water content and temperature variables between levels, which would be related to the very small differences observed in the rate of germination of pre-dormant seeds.

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# Development of Snow Mould in Latvia

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Snow mould of winter cereals (especially rye) has been observed sporadically in Latvia. Nevertheless, it was not a serious problem, especially in winter wheat sowings under intensive regime of management. Sharp development of this disease (caused by *Microdochium nivale* (Fries) Samuels & Hallett, previous term *Fusarium nivale*, teleomorph *Monographella nivalis* (Schaffnit) E. Müller) was noticed in 2005-2007. The object of these investigations was development of snow mould depending on different agroecological conditions.

## Methodology

Observations regarding snow mould incidence were done at the Study and Research Farm "Peterlauki" of the Latvia University of Agriculture during 2005-2008, in the central part of Latvia. It is the main region of wheat production. Recording was done on experimental plots designed for investigation of different aspects of winter wheat management. Three varieties of winter wheat ('Tarso', 'Cubus', 'Zentos'), four different dates of sowing ( $30.08 \pm 2$ ;  $10.09 \pm 2$ ;  $19.09 \pm 2$ ;  $29.09 \pm 2$  depending on the year), and three different seed rates (300, 400, and 500 seeds per m<sup>2</sup>) were compared. Incidence of snow mould (% of damaged plants) was determined. The assessment of snow mould was done at the start of wheat vegetation.

## Results

Incidence of snow mould fluctuated from 5-60% in 2005, 14-63% in 2006 and 2-95% in 2007. This disease was not noted in 2008, only some damaged plants were recorded. The winter of 2008 was very mild and snow cover did not develop completely during winter.

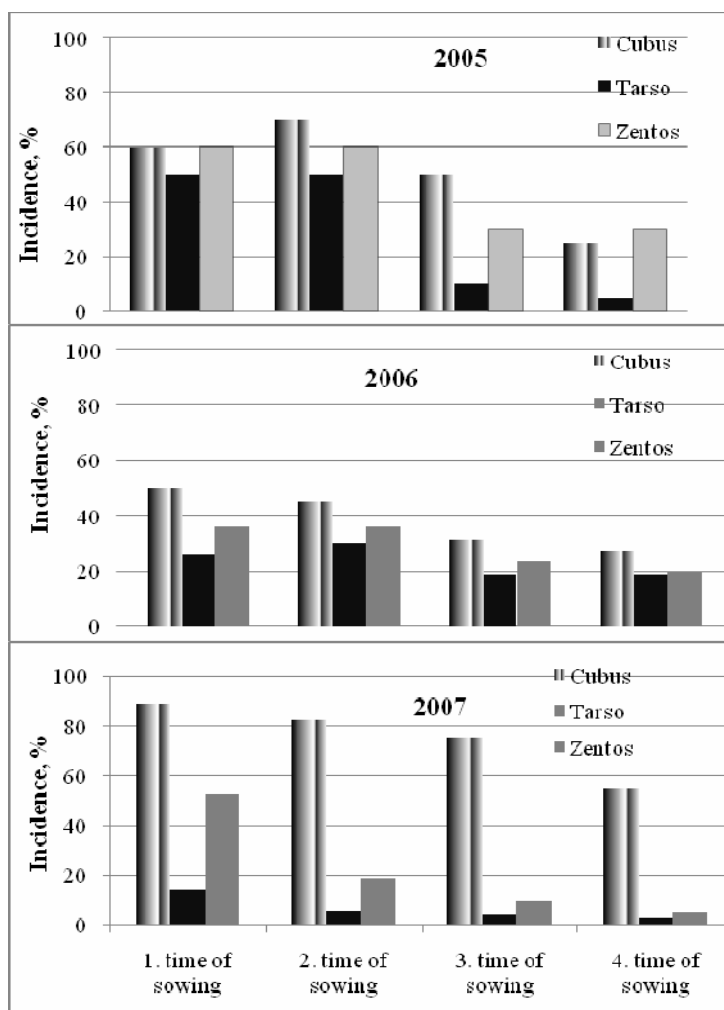
Infection source of snow mould exist in soil permanently, artificial infection of wheat with *M. nivale* demonstrate the role and importance of variety and conditions of overwintering (Ergon et al., 2003). Similar results were obtained in our observations.

Seed rates did not influence the spread of snow mould. It is unexpected result, because sowing density could have influence on overwintering. However, variety and time of sowing were the main factors, which influenced development of snow mould.

Different authors accent the role of varieties (Brennan et al., 2005). The role of varieties was confirmed in our investigations as well. Incidence of the disease was 49% in 2005, 42% in 2006 and 74% in 2007 for 'Cubus', 29%, 23% and 8% for 'Tarso', 42%, 28% and 27% for 'Zentos', respectively. One of the reasons, which influence resistance to snow mould, is different hardiness of varieties (Hommo, 1994).

'Cubus' has low hardiness under conditions of Latvia; this variety was the most susceptible to snow mould.

The main reason of sharp development of the disease is too early sowing time of winter wheat. Incidence of this disease was 55% in 2005, 41% in 2006 and 55% in 2007 at the first time of sowing; 58%, 38% and 43% at the second time of sowing; 28%, 24% and 30% at the third time of sowing and only 19%, 20% and 17% at the late sowing. It is complex of different factors, which influenced plant resistance to snow mould. One of the main reasons for susceptibility to snow mould is high density of wheat stand, which influenced microclimate and amount of oxygen under snow cover. Different chemical composition of plants is another factor. Different planting dates influence accumulation and composition of carbohydrates (Gaudet et al., 2001).



Development of snow mould, depending on varieties and sowing time

The first ten-day period of September is classical time of wheat sowing in the central part of Latvia. Recently, meteorological conditions are atypical to Latvia as autumn has become longer and warmer. Climate change is the reason to overestimate traditional management of wheat production.

### Conclusions

Snow mould has become a serious disease of winter wheat under conditions of Latvia recently. Variety and the time of sowing are the main factors having influence on the development of this disease. Further investigations are necessary for better understanding complex of factors, which influence wheat susceptibility to snow mould.

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# Nitrogen Dynamics under Partial Root-Zone Drying

## Irrigation Strategies in Potatoes

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### Introduction

Potatoes (*Solanum tuberosum* L. cv. Folva) rate fourth in production rate among the world's various agricultural products after wheat, rice and corn (FAO, 1995). As a shallow-rooted crop, irrigation is crucial for growing potato plants even in humid areas. A high level of nitrogen (N) is recommended for potato cropping (Darwish et al., 2003) to ensure acceptable yield. Potatoes are mainly grown on sandy soils with a low water holding capacity and thus with a high risk of leaching of nitrogen under excess irrigation or precipitation events. Introducing efficient irrigation practices may reduce these potential losses (Shahnazari et al., 2008; Shahnazari et al., 2007). During the last decade a novel irrigation strategy, partial root-zone drying (PRD), has been developed (Dry and Loveys, 2000). In PRD approach the root zone is irrigated alternatively so that in each irrigation event half of the root zone is not irrigated. In this study potatoes were grown to investigate how PRD and FI irrigation strategies affected nitrogen content in the soil-plant system during the growing season.

### Materials and methods

Experiments were conducted in 2006 in loamy sand under an automatic rain-out shelter in Aarhus university lysimeters facility to study the effects of full (FI), partial root-zone drying irrigations during the whole season (PRD<sub>1</sub>) and partial root-zone drying imposed after tuber initiation (PRD<sub>2</sub>), on nitrogen (N) dynamics in the soil-plant system of potatoes. FI plants received 100% of evaporative demands, while PRD<sub>1</sub> and PRD<sub>2</sub> plants received 70 % water of FI at each irrigation event. Potatoes were harvested every two weeks after PRD<sub>2</sub> treatment started. Total N content was determined for plant material at the final harvest. Soil samples were taken to determine the top soil layer (0-30 cm) and residual amount of ammonium (NH<sub>4</sub>-N) and nitrate (NO<sub>3</sub>-N) at depths of 0-10, 10-20, 20-30, 30-40 and 40-50 cm from the top of ridge. Physiological N-use efficiency (PNUE, kg tuber yield per kg N uptake), was calculated for various treatments according to Montemurro et al., (2006).

### Results

The PRD<sub>2</sub> treatment resulted in 30% water saving and maintained yield as compared with the FI treatment (Table 1). The PRD<sub>1</sub> treatments resulted in a significant ( $p<0.05$ ) yield reduction. Physiological N-use efficiency (PNUE ) was significantly higher in FI and PRD<sub>2</sub> treatments than PRD<sub>1</sub>.

The residual content of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in the soil were determined in FI,  $\text{PRD}_1$ , and  $\text{PRD}_2$  at the end of season. In the top layer (0-10 cm) the  $\text{NH}_4\text{-N}$  content in the  $\text{PRD}_1$  was significantly higher than other treatments. In the third layer (20-30 cm) the  $\text{NH}_4\text{-N}$  content in the  $\text{PRD}_2$  was significantly lower than  $\text{PRD}_1$ . In the upper soil layers the content of  $\text{NO}_3\text{-N}$  was higher than  $\text{NH}_4\text{-N}$  and in the deeper layers the content of  $\text{NH}_4\text{-N}$  was higher than  $\text{NO}_3\text{-N}$ .

In accordance with the above findings, the total residual mineral N content decreased from the top soil layer to the deeper layers for all irrigation treatments. In the top layer (0-10 cm), the residual mineral N was significantly higher in FI than  $\text{PRD}_2$ . In the third layer (20-30 cm)  $\text{PRD}_2$  had significantly lower residual mineral N than  $\text{PRD}_1$  treatment.

Table 1. N content in the plant-soil system, yield, physiological N-use efficiency during growing season.

	Treatments		
	FI	$\text{PRD}_1$	$\text{PRD}_2$
N uptake by plant ( $\text{kg ha}^{-1}$ )	136.2	133.3	139.1
Residual $\text{N}_{\min}$ after harvest ( $\text{kg ha}^{-1}$ )	34.7 <sup>AB</sup>	38.2 <sup>A</sup>	23.2 <sup>B</sup>
$\text{N}_{\min}$ before planting ( $\text{kg ha}^{-1}$ )	11.6	11.5	11.9
Total N supplied ( $\text{kg ha}^{-1}$ )	150	150	150
Yield, FW (tonnes $\text{ha}^{-1}$ )	36.9 <sup>A</sup>	33.9 <sup>B</sup>	36.6 <sup>A</sup>
PNUE (kg FW tuber per kg N uptake)	271 <sup>A</sup>	254 <sup>B</sup>	263 <sup>A</sup>

\* Different letters show significant differences at the 95% level for comparison between irrigation treatments.

## Conclusions

We conclude that:

- 1) The PRD treatment imposed just after tuber initiation until maturity ( $\text{PRD}_2$ ) is a useful strategy able to maintain yield and save water.
- 2) The PRD treatment increases soil nitrogen depletion during the growing season. The reason for this still has to be explicated

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# The Future of Agriculture within its Competition with Urban Sprawl

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In recent decades, territorial systems and specifically those in European countries, have been significantly transformed, especially as regards the areas that lie outside the compact urban fabric of cities. In these so-called “periurban” settings, the landscape has assumed highly distinctive features typical of both built-up areas and rural ones used primarily for farming.

The study has the general aim of exploring some of the main critical aspects of periurban areas, and specifically those involved by development processes and their interaction with the local agriculture. The specific objective of the paper is to provide elements supporting the interpretation of evolution at the urban fringe.

## Methodology

The analyses refer to four territorial case studies, identified within Italy using a multi-level approach that considered increasing scales of detail: regional, provincial, super-municipal and municipal level. With reference to those study areas, the analytical aspects useful for characterising some of the main processes undergone in periurban areas were considered: land use maps, demography, the built-up system geo-database and town planning zoning were used. Evolutionary analyses of the study areas were performed, and density maps of population and buildings were produced in GIS raster environment by means of areal interpolation.

In the interests of brevity, only the analyses regarding the provincial level are herein reported. This level is represented by the Province of Bologna (3703 km<sup>2</sup>), characterised by significant geomorphologic and landscape diversification (Cavalcoli, 2006), which makes it possible to observe structured dynamics for all the main land-use systems. The analyses involved the comparison between the most recent land-use database (Emilia-Romagna Region, 2006) and a previous one dating back to the Seventies (Emilia-Romagna Region, 1976), in order to investigate the main variations which took place (Tassinari et al., 2007). The databases relating to the two different eras examined were jointly processed, firstly, to define the necessary consolidation of information levels according to suitable criteria to obtain a restricted number of macro-classes of land use common and transverse to both. The reclassification of the geometrical component of both maps according to the macro-classes and the calculation of the total areas for each one, allowed a concise diachronic interpretation of the main evolution in land use.

## Results

In general, the processing performed demonstrated that the integrated analysis of land-use/land-cover, town planning schemes, developed land and population density variables contributes to provide both qualitative and quantitative indications, useful for measuring the level of efficiency of development arrangements and the degree of alteration of the landscape matrix. In particular, the results of the provincial level, briefly summarized in figure 1, show a significant expansion of the inhabited centres in terms of compact urban areas, and the formation and intensification of urban fringes starting from the city of Bologna and extending along the main roads; as well as a fusion between urban territories, with the consequent formation of an enlarged metropolitan area. Moreover farming land has been

reduced due to both urban expansion and sprawl and to land abandonment that, in hill and mountain territories (in the south of the via Emilia, see figure 1), have led to a significant increase in wooded and semi-natural areas. More modest re-naturalisation and recovery initiatives involving areas of environmental value (such as wetlands) also took place in the lowland areas, following the assimilation of European Union regulations.

On the whole, the results underline that the transformation of periurban and rural land caused by urban diffusion gives rise to a situation of territorial and environmental precariousness that presents a complex tangle of critical aspects. In periurban and, more generally, megapolitan settings, the infrastructures, residential lot distribution, commercial and industrial areas and logistic centres overlap with the rural landscape matrix to create an amalgamation of extensive conurbation, where the agricultural landscape is highly fragmented. In these slivers of landscape, the albeit partial permanence of agricultural features and sometimes even traditional landscape elements constitutes a strong distinctive character and potential strong point and, at the same time, a challenge for planning.



Figure 1. Land-use change in the Province of Bologna (Italy) in the 1976-2003 period.

## Conclusions

Due to the gradual dissolution of the monocentric town model and of the polycentric settlement system, which have characterized the European landscapes until the first half of the past century, and whose characters remain today altered and included in the more recent chaotic developments, the discussion of the issues of suburban areas and therefore the identification of their landscape characteristics call for a necessary evolution of the former concept of periurban area itself. Actually, the pervasive processes connected to urban sprawl cause such concept to be no longer appropriate to the interpretation and representation of current situations. The study has therefore developed a contemporary point of view in the study of periurban landscapes, by suggesting their role as an interpretative key for what concerns the urban sprawl processes and related dynamics of agrarian mosaics.

The study thus shed light on the extent of land consumption for development purposes both as part of the current scenario and in terms of an increase compared to recent decades. These phenomena shed light on the common need to adopt suitable sector policies aimed at rationalising land use and restricting the consumption of new resources. In actual fact, despite the progress achieved in recent decades in terms of both knowledge and legislative instruments on town/territorial planning issues, the planned transformations have often been implemented with a high consumption of environmental resources and producing the aforesaid effects of urban sprawl and landscape fragmentation. On the contrary, the urbanocentric concept in which agriculture is attributed residual and ancillary roles should be overcome, without refusing the strong urban 'contaminations' of periurban and fringe spaces.

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# The Analysis of Rural Land Consumption through Areal Sampling Techniques

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Within the theoretical framework of analysing the patterns of change and current configurations of rural land-use, the present study aims to formulate a statistical area sampling methodology which can be applied on an inter-municipal scale to identify sample areas representative of the changes in landscape occurred over a given time span, particularly with respect to human-settlements and farming systems. Such a methodology makes it possible to obtain, from a detailed survey of the selected sample areas, results which can be extrapolated to the entire study area with a known degree of reliability.

## Methodology

For the purposes of the present work, a target study area was chosen, known as the “Nuovo Circondario Imolese” (eastern part of the province of Bologna, Italy) in which the rural space has manifested notable landscape variations, particularly with reference to the post-World War II period. Based on the results of a critical analysis of the theoretical reference framework, a method for the stratified random sampling of the study area was worked out. The steps involved were the definition of an appropriate sampling frame, its stratification according to suitable criteria and variables, the extraction of the sample study areas, the investigation of the sample areas, and the inferential estimation of the parameters under study. The study area was subdivided along physical boundaries into the divisions adopted for the most recent population census (Istat, 2001). The landscape attributes chosen for the stratification were the current *land-use/land-cover* (Tassinari et al, 2007) and *land suitability for agricultural purpose* based on land capability (Klingebiel & Montgomery, 1961). The classes of the first variable are: urban land (class 1); arable crops (class 2); orchards, vineyards, vegetable gardens, plant nurseries, greenhouses (class 3); forest-pasture land, areas with sparse or absent vegetation, wetlands (class 4); water bodies and water courses (class 5). The classes of the second variable are: level land well suited for agricultural use (class a); level land less suited for agricultural use than class a areas (class b); hill-foot and hilly regions with an intermediate suitability for agricultural use (class c); hilly regions moderately suitable for agricultural use (class d); areas with low suitability for agricultural use (class e). The stratification of the study area is shown in figure 1a, where the numbers indicate the classes of land-use/land-cover, and the letters indicate the classes of land suitability. The area sampling methodology was calibrated with reference to the density of buildings (*building number density*,  $N_{bd}$ ) and the proportion of the survey area occupied by buildings (*building cover density*,  $C_{bd}$ ). The analysis of the aforesaid parameters was carried out in a GIS environment by a process of “backward updating” of vector databases (Tassinari and Torreggiani, 2006). The estimation procedure was carried out applying the developed statistical methodology over a significant time span: the period 1975-2005, corresponding to the more recent half of the period of interest for the research.

## Results

The results of the study are represented by the stratification of the sample frame, the definition of the estimators of the parameters under study, the results of the surveys, and the inferential estimation of the parameters.

In particular, the variability of the parameters within each stratum was estimated on the basis of a pilot sample, selected with the method of permanent random numbers (Carfagna, 2007). The surveys

conducted on the pilot sample corresponding to the results for the individual strata are shown in the histograms in figure 1b.

The parameter estimates obtained for the entire study area revealed an increase in the building number density of 16.9 buildings/km<sup>2</sup>, corresponding to a 31% of the amount in 2005, and an increase in the building cover density of 5136 m<sup>2</sup>/km<sup>2</sup>, corresponding to a 44% of the density in 2005. The standard error evaluated on the entire sample was 2.7 buildings/km<sup>2</sup> for the parameter  $N_{bd}$  and 1229 m<sup>2</sup>/km<sup>2</sup> for the parameter  $C_{bd}$ . Consequently, the confidence intervals computed for a significance level  $\alpha$  of 5% were, respectively, 11.5–22.2 buildings/km<sup>2</sup> and 2590–7682 m<sup>2</sup>/km<sup>2</sup>.

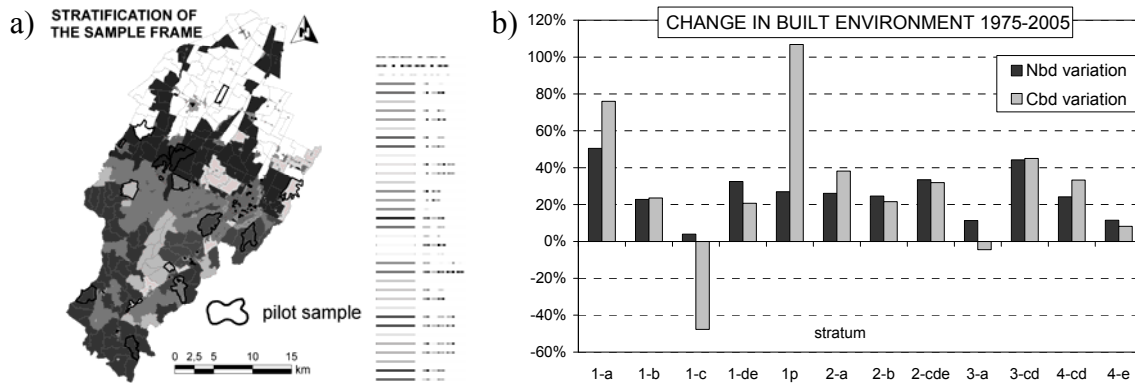


Figure 1. a) Map of the study area stratified, with the indication of the sample areas. b) Histogram of the relative variations of the studied parameters, referred to the values of 2005.

## Conclusions

This work has led to the formulation of an analytical methodology, applied to a study area subdivided along physical boundaries, to extract a random stratified area sample for the purpose of assessing changes in the rural built system. The results showed that it is possible to estimate the changes in specific landscape features descriptive of land-use/land-cover patterns whilst significantly containing survey costs. The methodology, calibrated with reference to buildings, was used to extract and analyse a pilot sample of survey areas on the basis of specific statistical criteria. In future work, it should allow the precision of the estimates to be further refined by augmenting the size of the pilot sample to reach an optimum sample size, computed on the basis of descriptor parameters surveyed on the pilot sample and referenced to periods relevant to the study. The techniques adopted for the extraction of the sample also make it possible to optimise the efficiency of augmenting the sample size. The results confirmed the efficiency of the adopted sampling frame, with respect to a simple random sampling. The general validity of the developed methodology means it can be applied to a variety of landscape features, study areas and time intervals, following verification and, if necessary, redefinition of the stratification variables and the parameters under examination. Moreover, the broader theme of landscape change assessment calls for further consideration of the complex and manifold relations between the trends of the various natural and anthropic features, as well as the indepth study of cultural issues.

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# Evapotranspiration of pistachio trees under deficit irrigation using eddy covariance

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Deficit irrigation strategies have been successfully used in orchards since the eighties (Chalmers et al., 1981); they proved to be effective in reducing the water use of trees, with small or no yield reductions. Most of the work conducted on the subject was based on relationships between the irrigation applied and the obtained yield. Orchard evapotranspiration (ET) was seldom measured; thus, the real water saving attainable with a deficit irrigation strategy remains often unknown. Besides, the few ET measurements performed were never obtained at orchard-scale.

Here we present measurements of ET of a pistachio orchard submitted to deficit irrigation (Goldhamer, 2005), obtained with the Eddy Covariance technique.

## Methodology

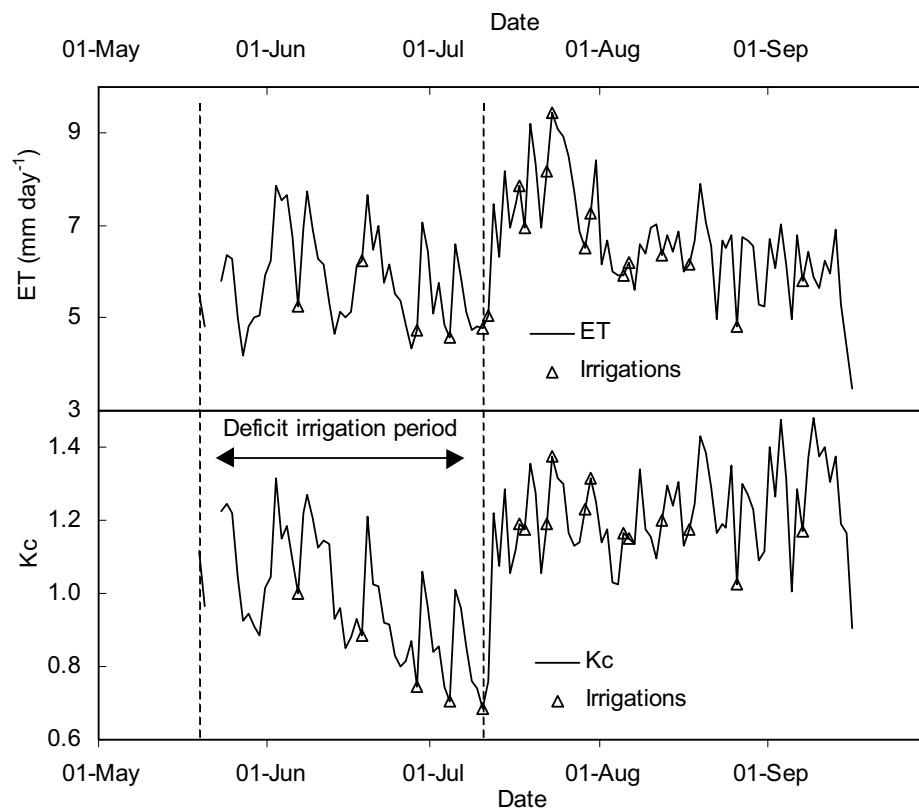
Two eddy covariance (EC) systems were installed in two large plots (32 ha each) of a pistachio orchard located in Madera County, California (USA). The trees were planted at 5.8 x 5.2 m and covered the 57% of the ground; the irrigation system was microsprinkler. The EC systems - placed at 10m height, 6m from the canopy top) were composed of a 3D sonic anemometer and a Krypton hygrometer, sampled at 10Hz. One of the plots (Control) was irrigated with the full water requirements ( $ET_0 \times K_c$ , where  $ET_0$  is the reference evapotranspiration and pistachio  $K_c$  was taken from Goldhamer, 2005). The other one (Regulated Deficit Irrigation - RDI) was irrigated the same as C, except during stage II (Goldhamer and Beede, 2004) - shell hardening, which occurred from 19 May to 11 July - when the orchard was irrigated with the 40% of the net amount of C.

The 10min fluxes of latent and sensible heat were corrected for the effect of frequency response on sensors separation, path-length averaging and signal processing time (Moore, 1986). The latent heat flux was further corrected for air density fluctuations due to heat and vapour transfer and O<sub>2</sub> radiation absorption on the Krypton Hygrometer (Webb et al., 1980, Tanner et al., 1993).

## Results

The upper plot of Figure 1 shows the orchard evapotranspiration; the two vertical dashed lines mark the period of deficit irrigations. When the measurements started, the stage II had just begun. The average ET during the deficit irrigation period was close to 6 mm day<sup>-1</sup>, with strong fluctuations due to the irrigation events. At the end of stage II, when the irrigations were switched to match the potential water requirements, the ET rose to more than 7 mm day until the beginning of August. During the measurement period (from 19 May to 16 September), the total measured ET was 750 mm.

The lower plot of Figure 1 shows the seasonal evolution of the crop coefficient (measured ET/ $ET_0$ ). It clearly shows a decreasing pattern, right from the beginning of the deficit irrigation period. Each irrigation event causes an increase in  $K_c$  of circa 0.3. The minimum (0.74) was reached the last day of the deficit irrigation period. The restart of the full irrigation boosted immediately the  $K_c$  to the high values: during stage III, the  $K_c$  oscillated from 1.05 to more than 1.45 after the irrigation events.



**Figure 1**

### Conclusions

The high time resolution of the eddy covariance technique allows to appreciate the response of the orchard ET and Kc to specific events: the orchard ET responds quickly to irrigations. As no water stress was present after the restart of full irrigation, the higher values of the Kc are an effect of the evaporation from the soil (the irrigation system wetted completely the ground surface).

The Kc of pistachio was very high and, in the unstressed stage II, was in accordance to previous studies (Goldhamer and Beede, 2004). A consistent reduction of the actual evapotranspiration can be achieved by stressing the crop during stage II, with no appreciable reduction in yield (data not presented here).

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# Transpiration of olive trees under deficit irrigation

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The strategy of deficit irrigation (Chalmers et al., 1981; Fereres et al., 2003) has been proved successful for reducing irrigation water supply in fruit trees without a significant decrease in yields. This strategy seems to have great potential in olive (Morianan et al., 2003). The water stress in specific phenological stages, induced by the reduced irrigation water supply, reduces the crop stomatal conductance, thus keeping the transpiration rate below the potential level in periods when the water status is less critical. The actual reduction in the transpiration rate is, nevertheless, rarely measured.

As the natural continuation of the effort that led to the formulation of a model for calculating the unstressed water requirements of olive groves (Testi et al., 2006) we measured the transpiration of a mature, intensive olive orchard submitted to deficit irrigation strategies.

## Methodology

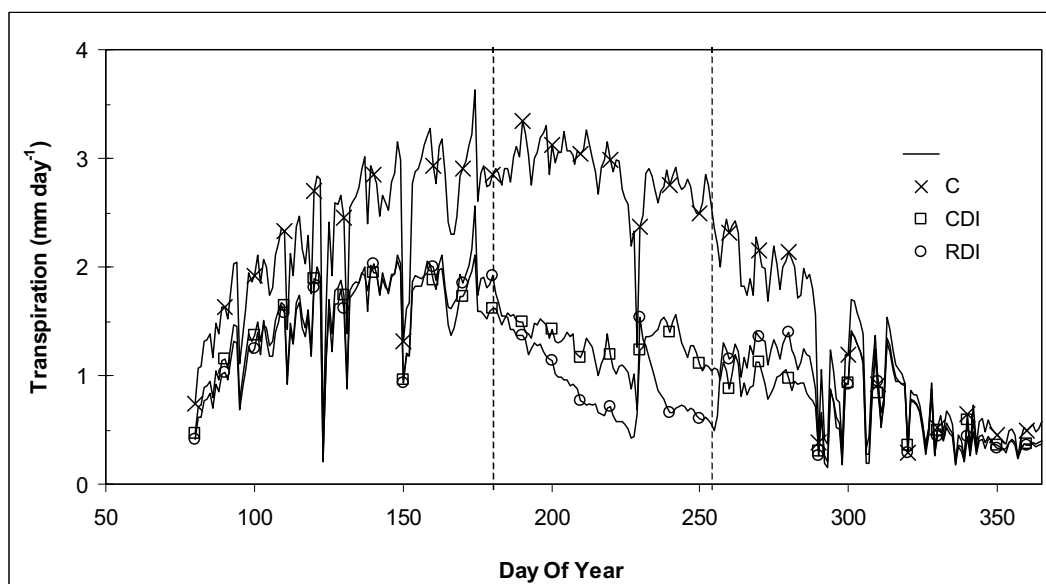
Three treatments with three repetitions in three blocks were set; each elemental plot is composed of 12 trees. The treatments were: Control (C), irrigated with the full crop water requirements (Testi et al., 2006); Continuous Deficit Irrigation (CDI), receiving 25 % of the irrigation amounts of C, during the same irrigation season; Regulated Deficit Irrigation (RDI), which received the same total seasonal amount as CDI, but during July and August the irrigation was completely discontinued.

The two central trees of each elemental plot were instrumented with sap flow probes (two probes each tree), while the others serve as borders. We used the compensated heat pulse method (Swanson, 1962), and the Calibrated Average Gradient method to improve the sensitivity to low sap velocities (Testi and Villalobos, under revision). The probes - designed and manufactured at IAS-CSIC laboratories - consisted of three 2-mm diameter stainless steel tubes: the central one is a 5W high precision heater and the other two contain four thermocouples, spaced 1 cm, that measure the temperature at four depths in the sapwood with a resolution of 0.01 °C. To better deal with the errors induced by the variability in sap velocity and sapwood thickness along the azimuth angle, the calculated flow was calibrated against measurements of soil water balance. The system collected sap flow measurements every 15 minutes from 2004 to 2006, although only data from the year 2006 are presented here.

## Results

The seasonal course of transpiration is presented in Figure 1; the vertical dashed lines mark the period when RDI received no irrigation water. The transpiration in C departs from the deficit treatments very early in the season, while the two deficit treatments - that received similar reduced amounts of water - behave similarly. The ratio between the transpirations of CDI and the deficit treatments was slowly decreasing as the stress increases: around DOY 225 (mid-August) CDI reduced its transpiration to less than 40% of C, while the transpiration rate of RDI (which irrigation was suspended 40 days before) was only 20% of the unstressed control. A 47-mm rainfall on DOY 229 (17 August) mitigated the stress of the deficit treatments, that quickly increased their transpiration rate. This recovering was shorter in the previously more stressed RDI than in CDI (that still received irrigation, albeit reduced). After the irrigation in RDI restarted on DOY 254 (11 September), its transpiration rate quickly increased, slightly exceeding CDI. From the irrigation restart, RDI received more water than CDI (because the total seasonal amount of both treatment had to be the same), which explain this behaviour,

as the soil profile at this moment was very dry (data not presented) thus the only water the deficit treatments were able to access came from irrigation. With the autumn rainfalls that deeply wetted the soil, no significant difference among treatments can be appreciated any more.



**Figure 1**

### Conclusions

The sap-flow measurement system adopted was successful in showing differences in transpiration rate between well-irrigated and deficit treatments. The system proved to be precise enough to discern between very low-transpiring treatments, showing a clear response to different strategies of deficit irrigation.

The deficit irrigation strategies strongly reduced the olive water use, especially during the dry summer. The data presented here will contribute to the development of a model to calculate olive water use that can be applied under water stress conditions. Such a tool will be helpful in evaluating the real water saving that a given deficit irrigation strategy can attain.

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# Time of Sowing Affects Growth, Development, and Seed Yield of Oil Seed Rape

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Oil seed rape (*Brassica napus* L.) is a species that is well adapted to cool and wet climate of north Europe. However, in southern and warmer climates such as in south Europe the seed yield is often limited by heat and water stress (Chen et al., 2005). Heat stress during flowering can cause flower and pod abortion and greatly reduces seed yields (Angadi et al., 2000; Nuttall et al., 1992). The optimum daytime temperature was found to be 20°C for oil seed rape during flowering in the semiarid Canadian prairies (Angadi et al., 1999) and a rise of maximum daily temperature from 21 to 24°C during flowering can result in a substantial seed yield reduction (Nuttall et al., 1992). In addition water stress at flowering and seed filling stages can have a negative impact on seed yield (Nielsen, 1997). Oil seed rape is sensitive to frost injury at the first stages of development and the right time of sowing is important for high seed yield. A change in seeding date can alter the timing of plant growth and development and avoid the negative impact of heat and drought stress at critical growth stages (Chen et al., 2005). Also the importance of oil crops such as oil seed rape has increased in recent years, especially with the interest in the production of biofuels. The objective of the present study was to determine the effect of four different dates of sowing on growth and yield of two hybrids.

## Methodology

A field study was conducted at the Experimental farm of the Aristotle University of Thessaloniki with the objective to find the optimum sowing date of oil seed rape for optimum seed yield and oil content. Four different dates of sowing were used (1) 20<sup>th</sup> of October, (2) 10<sup>th</sup> of November, (3) 30<sup>th</sup> of November, and (4) 21<sup>st</sup> of December and two hybrids. The experimental design was completely randomized block with four replications. Because of the very low temperatures that we had during the winter the forth seeding date was severely damaged by the frost and the plants did not reach the 4-8 real leaves stage which is resistant to frost. The following parameters were determined: seed filling period, plant height at full bloom, seed yield, number of pods per plant, 1000 seed weight, and seed protein content.

## Results

The seed filling period was longer for the first sowing period by 13% and 22 % compared with the second and the third sowing date, respectively leading to higher seed yield (Table 1). Seed filling period is very important and the longer it is the highest the seed yield is (Chen et al., 2005). The plant height was affected by the time of sowing and the plants were taller by 15 % at the first compared with second time of sowing and by 25% at the first compared with the third date of sowing. Titan had the higher seed yield in all treatments compared with Ellan. Seed yield was higher at the first and second date of sowing by 16 % of both cultivars compared with the third sowing date. The number of pods per plant was higher by 20% in both cultivars in the first sowing gate compared with the second and third sowing date. The 1000 seed weight was higher by 10% and 15% at the first sowing date compared with the second and third sowing date respectively. This means that the sowing date affected the development of the seeds as similar response was found by others (Chen et al., 2005; Angadi et al.,

1999). In addition, Titan had the biggest seed (expressed as 1000 seed weight) compared with Ellan. Seed protein content was affected by the seeding date and also by the cultivar. Titan had the highest seed protein content by 23% and 37% at the first seeding date compared with the second and third seeding date respectively. Also Ellan had the highest by 23% and 44% seed protein content at the first compared with the second and third sowing date respectively. Also seed protein content was higher by 15% at the second seeding rate compared with the third seeding date in both cultivars.

Table 1. Effect of different sowing dates on seed filling period, plant height at maturity, yield, 1000 seed weight and seed protein content.

<i>Dates of sowing</i>	<i>Cultivars</i>	<i>Seed filling period</i>	<i>Plant height at full bloom (m)</i>	<i>Yield (Mg ha<sup>-1</sup>)</i>	<i>Pods per plant</i>	<i>1000 seed weight</i>	<i>Seed protein content (%)</i>
1 <sup>st</sup>	Titan	60a <sup>†</sup>	1.26a	3.72a	214.5a	3.34a	27.38a
	Ellan	60a	1.15b	3.11b	195a	3.37a	26.94b
2 <sup>nd</sup>	Titan	52b	1.09b	3.68a	177.2b	3.14ac	22.31c
	Ellan	52b	1.01b	2.91b	165c	2.97cb	21.88d
3 <sup>rd</sup>	Titan	47c	0.99ab	3.16b	177.5b	3.04c	19.94e
	Ellan	48c	0.94b	2.71c	180a	2.81b	18.63e

<sup>†</sup>Numbers followed by the same letter in a column are not significantly different (Duncan's test,  $P=0.05$ ).

## Conclusions

Oil seed rape has the potential to expand to the Mediterranean area and can be grown for the production of oil for biodiesel. Early seeding during fall sowing is the key to achieve a good and stable oil seed rape seed yield. This study indicates that the oil seed rape can be sown between 20 of October and early November can be quite profitable with higher seed yield. Although early winter seeded oil seed rape will encounter warmer soil temperatures and water stress compared with the late seeding date were it will encounter cooler temperatures and frequent frosts. Further studies are needed to test the threshold temperature and duration of oil seed rape genotypes to cold stress and also to test other cultural practises that can affect the growth of oil seed rape under Mediterranean conditions. In addition, there is a need to create cultivars that are better adapted to warmer climates and can tolerate heat and water stress at the reproductive stages.

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# Vulnerability of some herbaceous crops to climate change in Southern Italy

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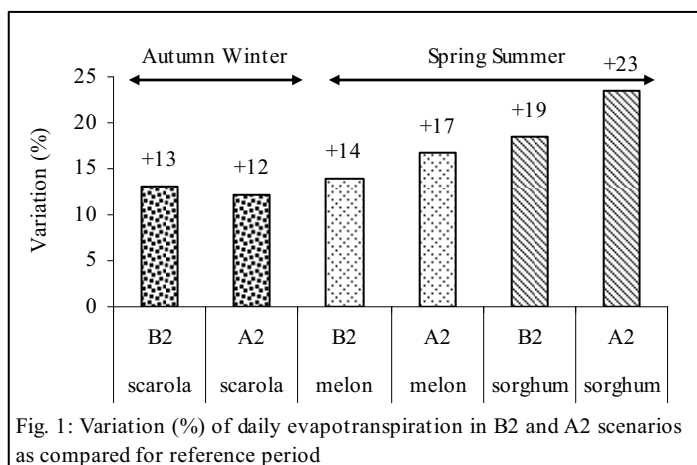
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Continue burning of fossil fuel due to the human activities increases the quantities of greenhouse gases into the atmosphere and it is causing the fastest global warming trend in the history of the Earth. Consequently variations on climatic variables are occurring with a great impact on agriculture activity and water resource availability. The Mediterranean areas seem to be interested by a greater increase of temperature than other regions. However the rainfall predictions are more uncertain because the precipitations depend strongly on local factors like the orographic features and land use.

The climate change (CC) may have a significant impact on plant phenology, crop growth and water requirement. For determinant herbaceous crops a reduction on growing cycle and a consequently decrease of yield is expected. The

contrary could happen for crop with indeterminate cycle, if an increase of water resources will be available. The forecast increase of CO<sub>2</sub> may counterbalance these negative effects, at least for the C3 plants.

From these considerations the complexity to estimate the potential impact of CC on crop productivity appears evident and one of the possible approach is that to use the crop simulation models coupled to the outputs (temperatures, precipitation and global radiation) of General and Regional Circulation Model (GCMs and



RCMs).

In the framework of CLIMESCO (Evolution of cropping systems as affected by climate change) and ADAGIO (Adaptation of Agriculture in European Regions at environmental risk under climate change) projects, the aim of this paper is to present the results of several pilot assessments carried out in order to estimate the vulnerability degree to CC of some important crops of Southern Italy (scarola, water melon, sorghum and tomato), using SWAP and DSSAT models, that adequately describe bio-physical processes of soil, crop and atmosphere.

## Methodology

The models SWAP and DSSAT, previously calibrated, were parameterized using soil and crop data-set collected in studies carried out in Puglia and Basilicata regions in Southern Italy. In this work we used climatic projections generated by the circulation Model HadCM3 (2.5° latitude by 3.75° longitude), developed by Hadley Centre (UK), downscaled at higher resolution by means of a dynamical approach based on HadRM3P for a definitive spatial resolution of 0.44° latitude by 0.44° longitude.

Two emission scenarios (A2 and B2) were selected among those proposed by the Special Report on Emissions Scenarios (IPCC 2000). The climatic daily data (Tmin, Tmax, precipitation and radiation) of

the two scenarios (from 2071 to 2100) and reference period (RIF: from 1961 to 1990) were used as input data for the crop models in order to calculate, at daily scale, the evapotranspiration (Priestley-Taylor equation) and the photosynthetic production and to drive the crop phenology.

The simplified approach of SWAP was used to simulate the water balance of scarola and water melon cultivations analyzing some soil/plant water indicators (transpiration, evaporation, drainage, irrigation requirement). Sorghum simulations were carried out using detailed approach of SWAP. DSSAT was used for tomato simulations evaluating the effects of increasing CO<sub>2</sub>. For these last crops we have analyzed the impact of CC on crop yield.

## Results

The comparison between future scenarios and reference period highlights higher monthly averages of maximum temperatures for A2 and B2 with differences higher than 4-5 °C in summer. Significant reductions of annual rainfall are detected (-17 and -24% under A2 and B2, respectively) particularly in spring and summer. Under A2 the winter precipitations compensate partially the summer drought.

The increase of temperature determines an increase of evaporative demand of the atmosphere (Fig. 1) with significant increases (from 14 to 23%) in spring-summer period for sorghum and water melon. In

winter, the increase for scarola is less than 14%.

In general, a very significant aspect is the shortening of growing season (from -20% to -35%). According with this reduction, precipitation, actual transpiration and evaporation are predicted to decrease at seasonal scale. Compared to these components of water balance, the variation of irrigation requirement are smaller ranging from -9% to +15%.

The yield decrements of sorghum (more than 40%) are correlated essentially to the shortening of

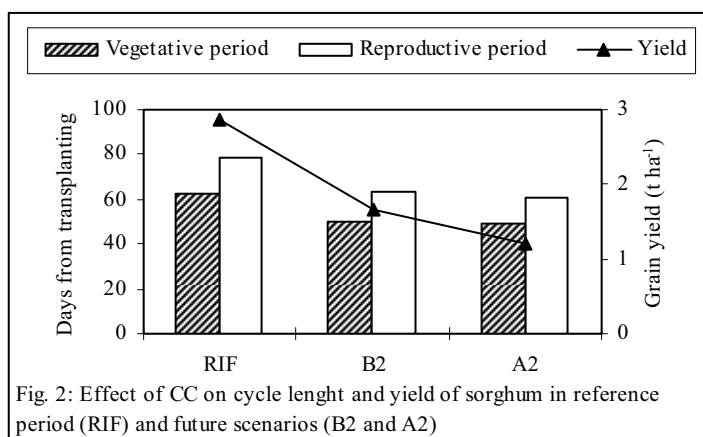


Fig. 2: Effect of CC on cycle length and yield of sorghum in reference period (RIF) and future scenarios (B2 and A2)

reproductive phase when the assimilates accumulate in the grain (Fig.2).

Model DSSAT, applied on tomato, allows to test the contribution of CO<sub>2</sub> increase on the impact of CC on tomato productivity. In particular, not taking into account the CO<sub>2</sub> effect determines significant reductions: -20% and -40% (for B2 and A2 respectively) for yield and harvest index, respectively. On the contrary, when CO<sub>2</sub> is considered, yield decrements are not significant and the irrigation requirements increase under B2 and A2 compared to the past (+9 and +15%, respectively).

## Conclusions

The forecasted warming can be expected to increase the evaporative demand of the atmosphere and consequently the crop water requirement. However at seasonal scale the shortening of the cultivation period counterbalances this effect and smaller variations on irrigation requirements are expected but with significant yield losses as detected on our pilot assessment concerning the sorghum.

Analogous results were obtained in tomato pilot assessment by using the DSSAT model. However for this C3 crop and taking into account the CO<sub>2</sub> effect, the yield decrements are not significant.

Early sowing time and adoption of varieties with longer cycle could be agronomic strategies of adaptation to CC that can be optimized by using this approach based on crop simulation models coupled with climatic models.

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# Production of Quality Croton (*Codiaeum Variegatum*) Plants by Using Different Growing Media

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## Introduction

*Codiaeum variegatum* is one of the most popular grown indoor plants which belongs to family Euphorbiaceae. It is comprised of around 1,300 species which are widespread in tropical regions of the world (Smith, 1986). These tropical plants are native to Malaysia and East Pacific (Mikkelsen, 2003). Croton requires semi shade and well rotten compost for ideal growth. The physical and chemical properties of soil, texture, structure, density, consistency, saturation percentage, color, organic matter as well as nitrogen, phosphorus and potassium concentration of soils are dominant factors affecting the use of soil as a media for plant growth. These properties determine the availability of nutrients to plants, mobility of water into or through soil and penetration of roots in the soil. Soil mixes play an important role in pot plant production. Their chemical and physical properties determine the nutritional status of potting media to sustain better plant growth (Gabriels et al, 1986). A light, rich, well drained soil is considered ideal for croton. These plants are notoriously rank feeders and require abundant quantities of plant food to attain maximum development. Vigorous growth is needed to face the seasonal hazards. Furthermore, croton plants must have tensile strength to sustain low temperature and frost. Keeping in view these points in mind, present study was undertaken to determine the effect of different potting media on the growth and production of the plants so that the best media should be well define. Soil, leaf manure, perlite and mushroom substrate were used for the preparation of potting media in different combinations and to find out the best composition to be used as permanent media. The chemical analysis of these medium are also correlated with growth and production performance of the croton.

## Methodology

In present study, perlite, spent compost of mushroom and leaf manure were used as main source for preparation of media but making different compositions with normal soil, sand, silt and farm yard manure (F.Y.M.). Different Treatment combinations were, T<sub>0</sub>: soil (control), T<sub>1</sub>: sand + perlite (1:1), T<sub>2</sub>: sand + perlite + silt (1:1:1), T<sub>3</sub>: sand + soil + farm yard manure. (1:1:1), T<sub>4</sub>: sand + spent compost (button) (1:1), T<sub>5</sub>: sand + perlite + spent compost (button) (1:1:1), T<sub>6</sub>: sand + silt + leaf manure (1:1:1), T<sub>7</sub>: sand + silt + leaf manure + spent compost (button) (1:1:1:1), T<sub>8</sub>: sand + perlite + spent compost (oyster) (1:1:1), T<sub>9</sub>: sand + silt + leaf manure + spent compost (oyster) (1:1:1:1), T<sub>10</sub>: sand + silt + spent compost (button) + spent compost (oyster) (1:1:1:1), T<sub>11</sub>: silt + spent compost (button) + spent compost (oyster) (1:1:1) on the growth and development of croton. Clay pots (12" size) were filled with different potting media as described and 6" long cuttings of croton cultivar "Gold Sun" were planted in it in the month of August. The experiment was carried out under natural green house conditions and laid out according to Complete Randomized Design having single cutting as experimental unit with three replications. Data were collected fortnightly. Observations on the following parameters were recorded using the standard procedure: Number of sprouted buds, Mortality rate (% age), Number of leaves per plant, Plant height (cm), Leaf area (cm<sup>2</sup>), Number of roots per plant and Root length per plant (cm). Data obtained was analyzed using Fisher's analysis of variance technique. Significant means were compared by using Duncan's Multiple Range Test

at 5% probability level (Steel et al., 1997). Analysis of each medium was procured to get water holding capacity, pH, total nitrogen, available phosphorus and available potassium (United States Salinity Laboratory Staff, 1954).

## Results and Discussion

From results of present study, it was observed that T<sub>7</sub> (sand + silt + leaf manure + spent compost (button) (1:1:1:1) had maximum (1.412) number of sprouted buds followed by T<sub>9</sub> (sand + silt + leaf manure + spent compost (oyster) (1:1:1:1) which gave 1.089 sprouted buds per plant and showed superiority over other treatments. The treatments T<sub>8</sub>, T<sub>3</sub> and T<sub>10</sub> were statistically at par with each other and showed non-significant results while T<sub>0</sub> (normal soil) had the minimum (0.179) number of sprouted buds per plant. An overview indicated that different manures in combination with soil exhibited better results. Observation recorded on height of the plant showed the significant superiority of T<sub>7</sub> (sand + silt + leaf manure + spent compost (button) (1:1:1:1) (16.08) and T<sub>9</sub> (sand + silt + leaf manure + spent compost (oyster) (1:1:1:1) (15.58) over rest of the treatments. This was followed by T<sub>6</sub> (sand + silt + leaf manure (1:1:1) which ranked secured 2<sup>nd</sup> best position, but these were non significant among each other. T<sub>0</sub> (normal soil) (11.09) occupied the lowest height of plants. It was observed that the normal soil alone showed very poor result as compared to other media. Results have indicated the combination of sand, silt, leaf compost and button gave highest plant height as compared to other treatments. It was noted that T<sub>7</sub>, T<sub>6</sub> and T<sub>9</sub> with leaf manure as main source shows more leaf area as compared to rest of the treatments. However T<sub>5</sub> and T<sub>8</sub> with mushroom substrate has attained second position regarding leaf area. While (T<sub>10</sub>, T<sub>2</sub>, T<sub>12</sub>) are non significant with T<sub>8</sub>. T<sub>0</sub> with 100% soil gained lowest position. Data on water holding capacity showed a wide range of overlapping of the treatment means. This gross overlapping is difficult to establish the superiority of a single treatment over the others on the whole, T<sub>10</sub> (sand + silt + spent compost (button) + Spent compost (oyster) (1:1:1:1) got the maximum (71.69) and T<sub>11</sub>: Silt + Spent compost (Button) + Spent compost (oyster) (1:1:1) (62.25) occupied 2<sup>nd</sup> position. T<sub>0</sub> (normal soil) obtained lowest (44.35). The number of buds, number of leaves and plant height have positive correlation with soil nitrogen contents. This may be due to high nitrogen percentage. It was noted that T<sub>7</sub> (sand + silt + leaf manure + spent compost (button) (1:1:1:1) got the maximum (30.46) and T<sub>6</sub> (sand + silt + leaf manure (26.80) occupied 2<sup>nd</sup> position with leaf manure as main source showed more available phosphorus as compare to rest of the treatments. Overall response of croton based on different parameters varied with the pH of the media. Maximum number of buds sprouted / plant, number of leaves and plant height was observed in treatment T<sub>7</sub> (sand + silt + leaf manure + spent compost (button) (1:1:1:1) with pH 7.34. From the all above discussion it is cleared that the T<sub>7</sub> medium (sand + silt + leaf manure + spent compost (button) (1:1:1:1) showed the best result for the production of croton plants. T<sub>7</sub> medium is considered best medium due to its texture, structure, density, consistency, saturation percentage, color, organic matter as well as nitrogen, phosphorus and potassium concentration.

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## **SUB SESSION 3.2**

### **ADAPTATION**

Chairman: Francisco Villalobos



# Adaptation Strategies for Coping with Climate Change

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The greatest challenge of agriculture during the 21<sup>st</sup> century is probably to feed the increasing number of more and more wealthy people on earth while maintaining soil and water resources (Cassmann et al. 2003). Indeed the projections indicate the growth in population and economy over the next 50 years will require a doubling of food production, including a increase from 2 to >4 billion tons of grain per year, and questions on whether this is achievable has been raised (Gilland 2002). Climate change significantly adds to this challenge by reducing the quality of soil and availability of water in many regions and by increasing variability of temperature and rainfall (Tubiello et al. 2007). The increasing confidence in climate change projections (Solomon et al. 2007) and the reluctance and slowness in adopting effective mitigation measures to combat climate change put increasing emphasis effective adaptation measures in all parts of society (Parry et al. 2007).

## **Climate change impacts**

Climate change affect cropping systems through a wide range of direct and indirect pathways (Olesen & Bindi 2002, Tubiello et al. 2007). The effects may be positive or negative depending on current climate and soils, and depending on the direction of change. It should be stressed that the effects of climate change on crops are mediated through the farmer's management of the genotype x environment interactions, which is crucially dependent on available resources, including climate, soil, water, nutrients and genetic diversity. So far, research on climate change impacts in agriculture has given little emphasis on changes in frequency of extreme events. However, the impacts of increased climate variability on plant production are likely to increase yield losses above those estimated from changes in mean climate only (Porter & Semenov 2005). This is primarily linked with changes in the frequency of extreme heat waves and changes in rainfall patterns, including more intensive precipitation events and longer drought periods. Changes in climate variability may be particular difficult for many farmers to adapt to, and adaptation strategies to cope with variability may be different than from those dealing with changes in mean climate. Strategies for adapting to increased variability may include measures to avoid periods of high stress or measures that increase resilience of the system by adding diversity in the crop rotation and improving soil and water resources.

## **Adaptation in cropping systems**

Many management-level adaptation options have been proposed and analysed. Most of these are extensions of current practices to cope with climatic variation or adverse environmental conditions (Howden et al. 2007):

- Altering timing and location of cropping activities
- Altering input inputs, such as crops, varieties, fertiliser rates, irrigation etc.
- Use of technologies that "harvest" and conserve water and soil moisture
- Manage water to prevent flooding, water logging, erosion and nutrient leaching under increased rainfall
- Improve crop protection practices, including changes in crop sequence in time and space
- Diversifying farm activities, e.g. by integrating crop and livestock production
- Use climate forecasting to reduce production risk

There may be several restrictions to effective implementation of adaptation options, including social, institutional and technical ones (Gregory et al 2007). Perhaps equally challenging will be the increasing

scarcity of water for irrigation and also increasing concern in society for reducing environmental impacts of agriculture and maintaining biodiversity, which will also be affected by climate change.

### **Building resilience**

Several of the adaptation measures listed above may be used to increase resilience to climate change in cropping systems. However, when it relates to soil and water resources, building resilient systems may require long-term planning and changes already now in anticipation of climate change. An example of this can be illustrated by the link between climate change and soil degradation, which is one of the greatest threats to global food production (Lal et al. 2007). Most of the processes causing soil degradation are enhanced by climate change, being promoted by higher temperatures, more intense rainfall and longer drought periods, which lead to lower soil carbon stocks, increased soil erosion and salinization (Tubiello et al. 2007). Yet, higher soil carbon contents and better soil structure will be critical for cropping systems to cope with increased climate variability. There is clearly a need within research, advice and policy to focus more on those aspects of agricultural systems that build resilience.

### **Links with mitigation**

Many of the options available for adapting agricultural activities will influence the emissions of greenhouse gases either by enhancing or reducing the fluxes. However, it should be kept in mind that agricultural activities affect several greenhouse gases simultaneously, and it is the net effect on the global warming potential of all gases that should be considered. There may also be differences between short- and long-term responses to introduction of system and management changes, in particular for measures that involve changes in soil management and input of carbon and nitrogen to the soil. There are very few studies linking adaptation and mitigation in agriculture, and further studies are warranted.

### **Conclusions**

The challenge of adapting agriculture to climate change is not only one of ensuring crop production to maintain food security. It is also a task of maintaining our soil and water resources while at the same time protecting environment and biodiversity. This puts new challenges to agronomic research (Ingram et al. 2008), which in future must consider larger spatial and temporal scales and also work with a range of other scientists to ensure that adaptation strategies are effective not only in terms of crop production, but also environmentally and economically robust, at landscape and regional scales.

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# From Detailed to Summary Models of the Crop-Soil System for Larger Scale Applications

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Crop growth models developed at field level are increasingly used in larger scale studies often in combination with other models to explore management options at the whole-farm scale or for integrated assessment at regional level (e.g. Giller et al., 2006; van Ittersum et al., 2008). Unclear is whether the mechanistic detail of dynamic simulation crop models is required for such applications or whether summary models may be sufficient. To address this issue and to identify relationships that need specific attention in future research, we compared simulations of crop production by two models with a different degree of mechanistic detail.

## Methodology

The models considered are APES and FIELD. The crop module of APES (Agricultural Productivity and Externalities Simulator - Donatelli et al., 2007) was developed to simulate crop growth and production for the European agriculture. The crop module of FIELD (Field-scale resource Interactions, use Efficiencies and Long-term soil fertility Development - Tittonell et al., 2007) calculates crop production based on resource (light, water, nutrients and labour) availability on the farm, aggregated over a season. The crop module of FIELD summarises processes regulating resource utilisation in the form of functional relationships. For this study APES was calibrated for maize using leaf area index, date of flowering and physiological maturity collected at Bouillac, Midi Pyrenees, France during the years 1999 and 2000. APES was used to derive functional relationships for maize to be used in FIELD. Crop production was calculated with FIELD using these functional relationships. We compared yields predicted on the basis of radiation and water availability by these two models against measured data for the years 1999 and 2000 and compared yield predicted from both models for a time series from 1982 to 2006.

## Results

The two years chosen for the calibration/testing of APES were ‘average’ years for the region, in terms of weather, with similar amounts of rainfall and radiation captured by the crop during the growing season (Table 1). Using APES we derived two relationships for the model FIELD, specific to Bouillac in Midi Pyrenees:

$$Y_l = I_{PAR} * F_{int} * \varepsilon_l \quad (1)$$

$$Y_{wl} = R_{cum} * F_{cap} * e_w \quad (2)$$

Where,  $Y_l$  is the light determined yield,  $I_{PAR}$  the incident photo-synthetically active radiation (PAR) received by a crop canopy over the growing season,  $F_{int} = 0.5$  is the fraction of PAR intercepted by the crop over the season, derived for maize in Midi Pyrenees using APES, and  $\varepsilon_l = 3.3$  g DM (dry matter) MJ<sup>-1</sup>, the average (intercepted) radiation use efficiency over the season.

$Y_{wl}$  is the water-limited yield,  $R_{cum}$  the amount of water (rainfall and irrigation) given to the plant during the growing season,  $F_{cap} = 0.37$ , is the water capture efficiency, or the fraction of the total water available ( $R_{cum}$ ) that is transpired by the crop as derived with APES for maize in Midi Pyrenees. The coefficient  $e_w = 10.24$  g DM m<sup>-2</sup> mm<sup>-1</sup> represents the transpiration conversion efficiency, i.e. the amount of biomass produced per mm of water transpired by the crop canopy over the growing season.

Figure 1 shows the yield predicted by both models compared with the observed yields at Bouillac for the years 1999 and 2000. Maize productivity was 8.4% lower in 2000 than in 1999, although average radiation and cumulative water available to the crop was almost similar in both years (Table 1). This lower productivity is due to the higher water stress during the grain filling period (less rain during this period) in the year 2000. A mechanistic crop growth model such as APES, simulating the dynamics of crop growth during the growing season is able to account for the effects of water stress during critical phases. APES simulated maize yields that were 26% lower in 2000 than in 1999, with yield differences with respect to measurements of +15% for the year 1999 and -7.2% for 2000. The summary model FIELD, which does not consider intra-season rainfall variability, simulated maize yields that were 13% larger for 2000 than for 1999, overestimated water-limited grain yields in the year 2000 by 16% with respect to measured yields.

Table 1: Radiation and water available for the crop during the growing season and water stress indexes experienced by a maize (calculated by APES) at Bouillac in 1999 and 2000.

	1999	2000
Incoming PAR ( $\text{MJ m}^{-2}$ )	1602	1644
Available water for the crop (mm)	502	569
Average water stress index	0.15	0.19
Water stress index during grain filling	0.08	<b>0.40</b>

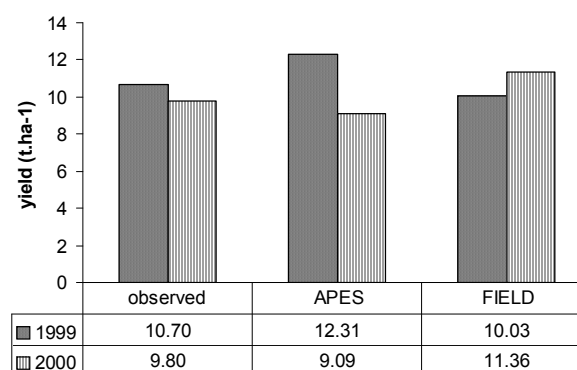


Figure 1: Comparison of models prediction with observed yield : Bouillac, Midi Pyrenees, France

## Discussion and conclusions

Results from the simulation of water-limited maize grain yields in Midi Pyrenees over a 25 years period (not shown) indicate a difference of 20% between yields predicted by FIELD and APES. The advantages of summary models for farm and regional applications reside in that they require less data for parameterisation, once generic functional relationships have been derived for a certain region (cf. Equations 1 and 2). For exploration of medium- to long-term changes in crop productivity and soil quality such summary models may suffice (Bouman et al., 1996). However, the preliminary results of this study suggest that the intra-seasonal variability of resources such as water may have a noticeable effect on maize productivity (overestimation of 16% for water limited grain yield). Such an effect may be larger in situations without irrigation, when droughts coincide with pollination, or for maize genotypes of shorter cycles. On the other hand, this effect may be smaller for other crop types with a more spread reproductive period. Further analysis with longer time series are required (and are ongoing) to derive relationships that consider the effect of intra-seasonal rainfall variability in summary models to enable yield estimations at large scales.

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# Using Long-Term Data and Crop Modelling to Assess Climate Change Impacts on Durum Wheat Production in the Mediterranean

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Future projections of climate for the Mediterranean area indicate an increasing trend for temperature and a reduction in annual rainfall amounts during the next decades (IPCC, 2007). Different crop models were recently used to predict responses of crops to climate changes, to analyse the most appropriate actions to mitigate potential effects, and to propose guidelines for adaptation (Ludwig and Asseng, 2006; Tubiello 2000). In this study, 30-year climate and durum wheat yield datasets from two experimental sites located in southern Sardinia, Italy, were used for CERES-Wheat model calibration and validation, with the purpose of determining the impact of climate change on production.

## Methodology

CERES-Wheat model calibration was performed using an iterative procedure until differences between measured and simulated data of yield, anthesis dates, and seed weight, were below defined error thresholds. Errors in predicting yield, anthesis date and seed weight for *Creso*, *Duilio* and *Simeto* varieties in two experimental sites located in Southern Sardinia (Italy) were evaluated using several statistics. In addition, a total of 48 simulated climate change scenarios were computed as incremental differences from the observed time series (1973-2004) of air temperature (1°C to 6°C) and/or decremental percentage of rainfall (5% to 30%). The simulated effects of climate change scenarios on crop yield, anthesis and seed weight of durum wheat varieties were determined for each site and variety.

## Results

Crop modelling results were consistent with yield and anthesis field data both in the calibration and validation phases and over all varieties (table 1). High significant values of correlation coefficients ( $r$ ) were found both for anthesis date and yield. Coefficient of residual mass (CRM) negative values indicate a slight tendency of the model to overestimate the observed values. The positive value of modeling efficiency index (EF) indicates that the model is a better predictor than the mean of measured values, particularly for anthesis. In addition, the low values of root mean square error (RMSE) values indicate a low level of bias and a good performance of the model (table1).

The effect of future increasing temperature was a gradual reduction in yield for all varieties and sites. *Simeto* was the most sensitive variety, and it showed the highest yield loss when temperature increases were combined with rainfall decreases (figure 1, left). For the other varieties yield reduction due to combined climatic changes scenarios ranged from -2.2% to a maximum of -38.3% from mean observed yield (table 2). In general, increasing temperature led to shortened cycle. As expected, decreased rainfall had no significant effect on flowering occurrence dates as in the CERES-Wheat model rate of development is function of temperature (figure 1, right). An advancement ranging between 2.5 to more than 12 days from the mean observed flowering dates was simulated (table 2). The model showed low accuracy for predicting seed weight with a slight trend of increasing weight for the combined-scenarios. This tendency was observed for all varieties and sites (data not shown).

	Anthesis date (dap)	Yield (kg ha <sup>-1</sup> )
CALIBRATION		
R <sup>2</sup>	0.84	0.62
RMSE	5	802
VALIDATION		
r	0.80 (p<0.001)	0.80 (p<0.001)
RMSE	9	894
EF	0.98	0.63
CRM	-0.03	-0.05

Table 1. Calibration and validation results for anthesis date (dap, days after planting) and yield (kg ha<sup>-1</sup>) simulations performed using wheat data (*Creso*, *Simeto* and *Duilio* varieties) from Benatzu and Ussana sites (Southern Sardinia, Italy). Values of the coefficient of determination (R<sup>2</sup>), correlation coefficient (r), root mean square error (RMSE), modeling efficiency index (EF), and coefficient of residual mass (CRM) are reported.

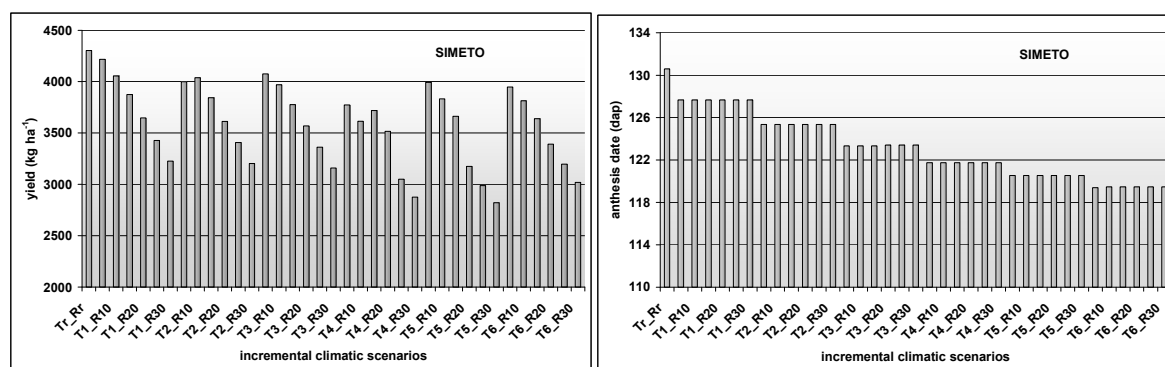


Figure 1. Effect of incremental climatic scenarios on yield (left) and anthesis date (right) for *Simeto* variety at Benatzu site.

	YIELD (%)		ANTHESIS (days)	
	Benatzu	Ussana	Benatzu	Ussana
CRESO	-2.2% / -27.2%	-2.6% / -29.3%	-2.5 / -7.6	-2.9/ -9.0
DUILIO	-2.6% / -26.0%	-3.3% / -25.0%	-2.9 / -11.7	-3.0 / -12.4
SIMETO	-2.0% / -34.4%	-6.7% / -38.3%	-2.9 / -11.2	-3.1 / -12.5

Table 2. Effects of the simulated climate change scenarios on crop yield and anthesis of *Creso*, *Duilio* and *Simeto* varieties. Maximum and minimum values of changes relative to the mean observed yield values (percentage) and anthesis dates (number of days) were reported.

## Conclusions

The analysis reported in this study showed that crop modelling was a useful and efficient tool for predicting the yield and phenological responses of durum wheat to climate changes. Their application in the Mediterranean area can be a valuable support for developing adaptation strategies.

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# ALOMYSYS: a Model for Evaluating and Developing Cropping Systems for Integrated Weed Management

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Weed management is usually based upon systematic and frequent herbicide applications, which results in the appearance of herbicide-resistant weeds and in the presence of herbicides in the environment (e.g. groundwater). It is therefore necessary to reduce herbicide use and thus to reason the whole cropping system to manage weeds, while taking account of farmers' constraints and objectives. The objective of the present work was to use a weed dynamics model to test a wide range of cultivation techniques and cropping systems, to evaluate existing cropping systems for their weed management and effect on environment and to propose integrated weed management solutions adapted to contrasted cropping systems.

## Material and Methods

A frequent and harmful winter weed, i.e. *Alopecurus myosuroides* Huds., was chosen as a model species. Weed dynamics were simulated with the ALOMYSYS model (Colbach et al., 2006, 2007), using the crop succession (including catch crops, associated crops, perennials), cultivation techniques (tillage tools, depths and dates; sowing date and density; herbicide rates, active ingredients and dates; nitrogen rates and dates; organic fertilizer rates, concentrations and dates; dates, tools, working depths and speed of mechanical weeding; harvest dates) and climate as input variables. The model simulates for each day the density of dormant seeds, germinated seeds, emerged seedlings, adult plants, mature ears, seed production and viable seeds in soil. It was evaluated by comparing its simulations to independent field observations assessing weekly emergence over two years and crop infestations over 10 years. This evaluation showed the model to predict weed emergence and infestation correctly, except if fields were left untilled; then seed survival close to soil surface was overestimated.

In a first step, ALOMYSYS was used to simulate the effect of individual cultivation techniques (e.g. sowing date, choice of tillage tools) on weed dynamics, repeating each scenario with 14 different climate years. In a second step, farms were surveyed in Burgundy to identify contrasted cropping systems which were evaluated for long-term weed dynamics with ALOMYSYS and for their environmental impact with agro-environmental indicators (i.e. IPEST and SALCA). The last step consisted in simulating changes in these cropping systems to identify solutions for managing weeds while reducing the impact on environment.

## Results

Table 1 shows that delaying the sowing of winter crops reduces infestation by *A. myosuroides* but that infestation is only consistently reduced for sowings later than 31 Oct. In early October, a delay in sowing dates can even lead to an increase in emergence when the associated tillage is carried out soon after a rain on dry soil, thus triggering a germination flush. Delayed sowing systematically reduces weed emergence only in moist autumns.

The farm surveys were carried out in autumn 2007 in Burgundy and led to the choice of four contrasted rotations. The levels of weed infestations of these rotations were simulated with ALOMYSYS (Fig. 1). The infestation risk increased with the proportion of winter crops in the rotation as these crops are most favourable to weed reproduction. Though *A. myosuroides* produces little or no seeds in perennial crops, the latter are not efficient in reducing infestations as the field is not tilled, thus reducing seed bank decrease through fatal germination.

Table 1. Example of the evaluation of a cultivation technique. Effect of a one-week delay in winter wheat sowing on *Alopecurus myosuroides* emergence in winter wheat. Means of 14 climatic repetitions carried out with ALOMYSYS. Numbers of a column followed by the same letter are not significantly different at  $\alpha = 0.05$  (least significant difference test)

Delay sowing		Relative variation (%)	
from	to	North	Burgundy
3 Oct.	10 Oct.	-0.9 a	22.5 a
10 Oct.	17 Oct.	-1.0 a	7.2 ab
17 Oct.	24 Oct.	2.0 a	4.1 ab
24 Oct.	31 Oct.	-0.8 a	-7.9 abc
31 Oct.	7 Nov.	-8.8 b	-15.2 bc
7 Nov.	14 Nov.	-13.3 b	-27.2 c

Figure 1. Evaluation of four contrasted crop rotations for their *Alopecurus myosuroides* infestation simulated with ALOMYSYS. Means of 10 repetitions with randomly chosen climate sets. (●: OSR = oilseed rape, ■: WW = winter wheat, ◆: WB = winter barley, Δ: sb = sugar beet, \*: 3L = 3-year lucerne, □: m = maize)

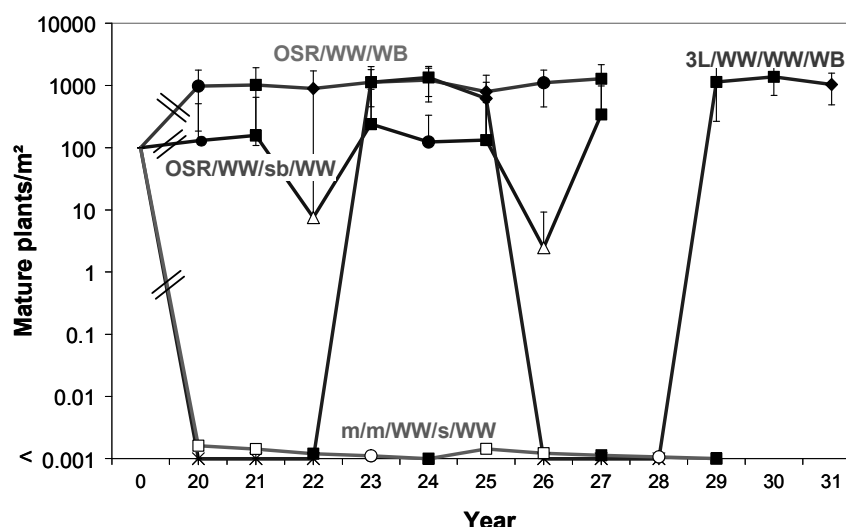


Table 1. Environmental and operational evaluation of the analyzed crop rotations (means per crop over rotation)

Rotation	Herbicide toxicity (IPEST)	Number of operations				
		Total	Herbicides	Mouldboard ploughing	Other tillage	Mechanical weeding
OSR/WW/sb/WW	0.55	6.25	2.25	0.25	3.75	0.0
m/m/WW/s/WW	0.15	6.40	2.20	1.00	2.80	0.4
OSR/WW/WB	0.27	5.00	1.33	0	3.67	0.0
3L/WW/WW/WB	0.25	3.50	2.00	0	1.50	0.0

The rotation with the lowest weed infestation was also the one with the least toxic herbicides (i.e. maize/maize/wheat/soybean/wheat rotation). However, it used more operations than the other rotations, especially mouldboard ploughing and mechanical weeding.

## Conclusion

Managing weed infestation with crop rotations and cultivation techniques other than herbicides is possible. Using less toxic herbicides does not necessarily lead to an increase in weed infestation. The present results must now be completed by simulations covering all aspects of cropping systems to identify solutions for managing weeds while reducing the impact on environment.

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# Simulation of Growth and Production of Open-field Bell Pepper under Mediterranean Conditions

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Despite the importance of open-field grown bell pepper (*Capsicum Annuum* L.), little information is available on the physiological determinants of growth and yield of this open-field crop.

The aim of this study was to construct a growth model to simulate potential and water-limited growth and production of a pepper crop under Mediterranean conditions.

## Methodology

Field trials with bell pepper (*Capsicum annuum* L. cv. Capistrano) were conducted in South-western Portugal (latitude: 37° 30' N, longitude: 8° 45' W, altitude: 106 m above M.S.L.), from 2000 to 2002, consisting of three planting dates per year (P1, P2 and P3 respectively second fortnight of April and first and second fortnight of May). In 2001 and 2002 there were two water treatments in the second sowing date. In the R1 treatments soil moisture was kept above 60% of the available water capacity; in the R2 treatments, only half of the amount of water was applied. In 2003, two grower-fields, located in Central Portugal, were monitored and essential data were collected and added to the validation dataset. Throughout the crop cycle, measurements were done in the soil (soil water profiles and rooting depth), on the plants (detailed dry-matter and area partitioning and phenological development stages), and in the atmosphere (temperature, humidity, incident, reflected, and transmitted radiation, both in the total and PAR wave band, and precipitation) (Vieira, 2006).

The model, coded in Visual Basic 6.0, contains three objects: Astromet, Crop and Soil. The object Astromet generates the astronomical and weather variables needed by the model using the available weather data. The object Crop simulates absorption of radiation of this discontinuous canopy, photosynthesis, respiration, potential and water-limited crop growth, and dry matter (DM) partitioning. This object also incorporates high temperature reduction of growth and development, potential and actual fruit growth, fruit size grades, fruit colour and fruit placenta-pulp ratios. The object Soil computes the soil water balance and its components. Harvest criteria are user-defined and are based on minimum and maximum fruit size, colour and/or date (Vieira, 2006).

Most sub-models and the complete model were calibrated using the data yielded by the 2000 and 2001 trials. The validation dataset consisted in the data collected in 2002 and 2003, totalling six crops.

## Results and Discussion

Development was simulated using normalized thermal time (NTT), where high-temperature reduction of thermal time accumulation was accounted for. Empirical functions were fitted to the fractions of the various plant parts versus NTT and other functions were fitted to the course of specific areas of the same plant parts in relation to NTT. These functions were validated in an independent dataset. Since their performance was very good, we used them to simulate dry-matter allocation to the different plant compartments and crop area index. (Vieira et al., 2004).

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The computed  $X$ -parameter of the ellipsoidal leaf-angle distribution model (Campbell, 1986) was 2.48 and 2.89 and radiation-use efficiency was 1.6 g per MJ of intercepted PAR. The area covered by a single plant is:

$$A_c = \pi \left( 1.33 \sqrt[3]{A_L} + 0.64 \right)^2, \quad (1)$$

where  $A_L$  is the area of the leaves in  $\text{m}^2$  ( $r^2 = 0.98$  and  $\text{SE} = 0.03 \text{ m}^2$ ) (Vieira, 2006).

The daily intercepted total solar radiation was closely simulated by the model. The regression analysis of predicted versus observed yielded a regression line with a slope of 0.98, an intercept of 0.94 and a  $r^2 = 0.94$ .

Using the validation dataset, the linear regression line of predicted versus observed values of shoot DM (present plus harvested, in  $\text{kg m}^{-2}$ ) was  $y = 0.97x$ , with a  $r^2 = 0.98$ , while modelling efficiency was 0.98. The linear regression line of predicted versus observed values of fruit DM (present plus harvested, in  $\text{kg m}^{-2}$ ) was  $y = -0.02 + 1.07x$ , with a  $r^2 = 0.96$ , while modelling efficiency was 0.95.

The model (Fig. 1) accurately described the time-course of fruit fresh-weight present on the plants. The linear regression line of predicted versus observed values of fruit fresh-weight present on the plants (in  $\text{kg m}^{-2}$ ) was  $y = -0.19 + 0.99x$ , with a  $r^2 = 0.64$ , while modelling efficiency was 0.85. The performance of the model in this case was lower due to discrepancies between simulated and observed dates of harvest and fruit water content.

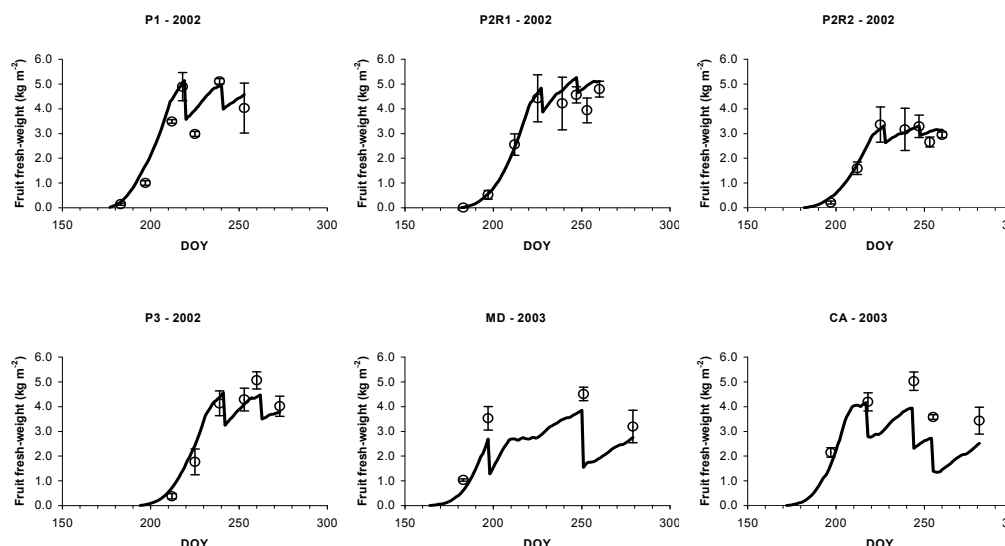


Fig. 1. Fruit fresh-weight: simulated (—) and measured (O) values ( $\pm$  standard errors)

## Conclusions

The model was able to describe very well the courses of total shoot and total fruit DM (present plus harvested). Its performance was somewhat lower when describing the fresh-weight of the fruits present on the plants. This model may be used to assist the food industry in planning the date of planting and areas needed, in order to maximize the overall profit and optimize plant operation.

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# Functional-Structural Plant Modelling, Applied to Tillering in Wheat

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Functional-structural plant models (FSPMs) are a new tool to analyse problems in which the spatial structure of a plant is an essential factor contributing to the explanation of the behaviour of the system of study (Vos *et al.*, 2007). FSPMs treat plants as multiples of basal units (phytomers, consisting of a leaf, a node, an internode, and an axillary bud). FSPMs simulate spatiotemporal development of these basal units in 3D, as influenced by environment and plant functioning.

This paper presents the philosophy of FSPM, the construction of an FSPM for wheat (ADEL-wheat) and barley and a first application to study effects of the light environment, represented by the red / far-red ratio (R:FR) in the canopy, as varied by plant population density on tillering.

A brief account is given of other extensions and applications of FSPM studied by the author group and their associates. The longer-term aim of the work is to accomplish the integration of physiological, morphological, and genetic information in a single FSPM in order to analyze phenotypic plasticity in wheat and other cereals.

## Methodology

ADEL-wheat is an architectural model of wheat (Fournier *et al.*, 2003), based on the open L-system principles and the cpfg language and simulation program (Prusinkiewicz *et al.*, 2000). Though originally designed for winter wheat, the model was adapted to spring wheat (Evers *et al.*, 2005) growing in well-watered and non-limiting nutritional conditions. Dedicated experiments delivered parameter values for rates and duration of appearance and expansion of leaves and tillers. Digitization using a Polhemus magnetic digitizer, provided the basis of analysis of geometric properties, *i.e.* shape and spatial orientation of organs. Stochastic variables in the model include seed orientation (*i.e.* orientation of the first leaf), basal angle and curvature of leaf blades, and leaf and tiller azimuth angles and were based on distributions derived from experimental data.

Interception and scattering of the light that was falling on each element of the structure was simulated with the nested radiosity method (Chelle *et al.*, 2007). The differences in leaf optical properties for red and far-red light were taken into account, thus enabling simulation of R:FR of the light scattered within the canopy. Bud break, which results in tiller growth, was made dependent of R:FR perceived by the plants by means of a R:FR threshold below which bud break was arrested.

## Results

FSPMs aim at an adequate representation of each element of the 3D structure. Conservative patterns of coordination and recurrence of properties across the structure help to achieve that goal. For instance, there is a very conservative schedule in the development of successive phytomers along a shoot, with all leaves having the same duration of linear extension, whereas a close coordination was observed between the emergence of leaf tips and collars and the phases of extension of blade and internodes of adjacent phytomers (Fournier *et al.*, 2005). The time of appearance of a particular tiller always coincides with a particular stage of development of the parent shoot. The concept of ‘relative phytomer number’ provides an example of recurrence of properties, applicable to a wider range of gramineous cereals. Phytomer components (*e.g.* leaves, internodes) with equal RPN show similar values of properties (*e.g.* leaf area, internode length). The RPN of main stem phytomers is equal to their rank

number (counted acropetally); for phytomers on tillers RPN is their rank number plus a shift value. The latter is unique for each tiller type.

The established parameterization of various parts of the model proved suitable for the simulation of canopy development of spring wheat for contrasting plant population densities and light conditions (Evers *et al.*, 2007a). This justified to go a step further and test whether simple hypotheses on the effects of R:FR could explain tiller number for different plant population densities. Several population densities and R:FR threshold values were tested, and the study confirmed that the virtual plant approach based on L-systems is suitable to model the environmental influence at the plant organ level on the development of a canopy of plants (Evers *et al.*, 2007b).

The model and its application described so far were essentially descriptive models, that is growth is not coupled to light interception. Recently we have implemented routines to simulate photosynthesis at the level of the individual plant organs using a detailed Farquhar-based approach (Yin *et al.*, *in prep*), and to simulate carbon distribution through the plant structure based on the widely accepted relative sink strength concept (Marcelis, 1996). With these extensions the model truly became a FSPM, combining the simulation of structure and basic plant functions, enabling the study of plant plasticity.

The author groups have addressed other extensions and applications of their FSPM, including modelling of the (i) action of known Mendelian genes associated with gibberellic acid metabolism in barley (Buck-Sorlin *et al.*, 2008), (ii) nitrogen and light distribution in wheat (Bertheloot *et al.*, 2007) and (iii) analyses of spore dispersion of fungal diseases in relation to rain splash and canopy architecture (Robert *et al.*, 2007).

## Conclusions

Though the methodology needs further improvement, FSPM can currently already address the same issues as classical crop growth models, but offer particularly advantage if the third dimension needs to be taken into account for a proper explanation of the phenomena under study.

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# Yield Modelling of Mediterranean Crops: a Probabilistic Approach

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The probabilistic approach to evaluate crop response to climate change is a time-consuming process due to the large number of model runs needed for such a procedure. An alternative method relies on the set up of impact response surfaces (RS) with respect to key climatic variables on which a probabilistic representation of projected changes in the same climatic variables may be overlaid (Fronzek et al. 2008).

This approach was exploited within the ENSEMBLES EU Project aiming at assessing climate change impact on durum wheat. This work presents the preliminary results of the project with a particular concerning about i) developing a simple statistical model that emulates mechanistic durum wheat yield model; ii) creating yield response surfaces for a pilot study area, and iii) obtaining preliminary estimates of the likelihood of exceeding fixed thresholds using probabilistic information about future climate.

## Methodology

The crop growth simulation model SIRIUS Quality (Jamieson et al., 1998) was selected to simulate durum wheat yield in a scenario sensitivity analyses conducted for 9 different grid points (50 Km x 50 Km), representatives of the climatic variability over the Mediterranean Basin. The baseline climate consisted of 30 years (1975-2005) of daily temperatures, rainfall and global radiation extracted from the MARS JRC Archive. The sensitivity analysis was performed for precipitation changes (from -40% to 20%) and temperature changes (from 0°C to 8°C), uniformly applied across all the year. For each of the resulting scenarios, SIRIUS model was run for 4 CO<sub>2</sub> concentration levels, 3 different soil types and 3 N-rates. For each scenario combination the average grain yield over the 30-years period was calculated.

In order to further reduce the computing-time needed to construct the RSs with the mechanistic model, an Artificial Neural Network (ANN) was used to emulate SIRIUS outputs in response to meteorological and crop management parameters. Following the approach proposed by Olesen et al. (2007), 5 input variables were used to train the ANN over all the 9 grid points: soil water content, N level, CO<sub>2</sub> concentration, mean temperature and cumulated rainfall over the period April-June. The trained ANN was applied to create RSs by altering the 30-years baseline climate.

Future yields were estimated by applying a bilinear interpolative method to overlap, onto the RSs, the data from perturbed physics experiment of Hadley Centre for future scenarios (joint distribution of annual temperature and rainfall changes).

Critical thresholds of impacts were determined by calculating, for each grid cell, the distribution of the 30-years average yield according to the joint distribution data for present period (1990-2010) and selecting the values that correspond to the 20<sup>th</sup> percentile of cumulative probability.

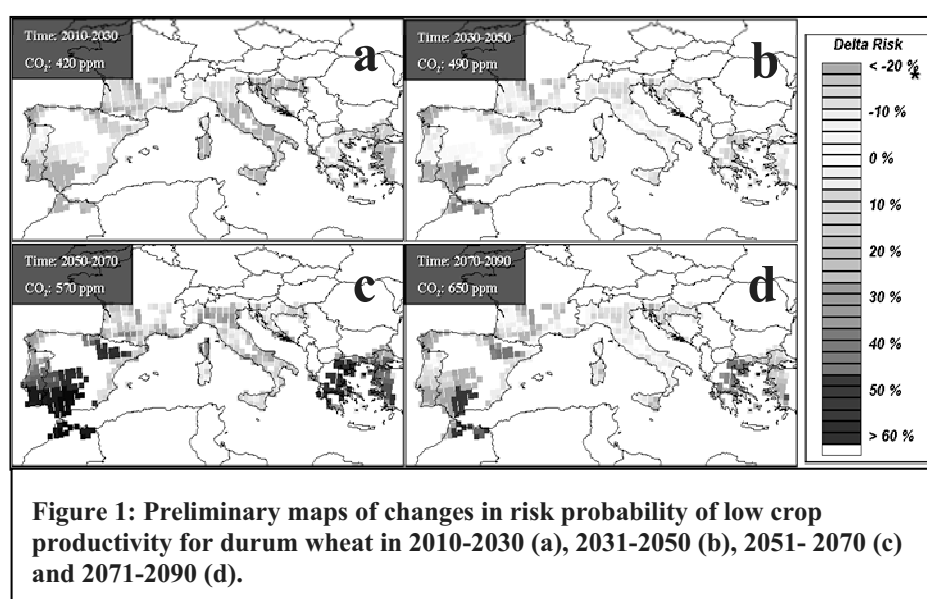
Future yields were compared with yield threshold to assess risk probability that, in each time period, was defined as the percentage of yields that not overcome the selected threshold.

The above procedure was applied to define preliminary risk probabilities over the Mediterranean Basin. The study area consisted of 445 grid cells in 50 Km x 50 Km spatial resolution. Response surfaces for each grid point and at different time periods were obtained by altering the baseline climate from MARS

JRC and considering a CO<sub>2</sub> concentration according to the A1B IPCC scenario. Soil properties were from the Eusoils database and 170 Kg N ha<sup>-1</sup> was uniformly adopted as nitrogen availability.

## Results

In figure 1 is depicted the change in risk probability with respect to present period. Delta risk was calculated as differences between the percentage of yields that not overcome yield threshold in present and A1b scenarios. With the exception of Portugal and Southern Spain, in the next 30 years risk probability shows an overall reduction (Fig 1a). Thereafter, the risk progressively increase starting from lower latitudes (Fig. 1b). Maximum risk was estimated in 2060 when strong reductions in yield were accounted all over the Mediterranean Basin (Fig. 1c). Slightly reductions in risk were accounted for the end of next century (Fig. 1d), probably due to the greater uncertainty of future data. South Portugal, South Spain and Peloponnesus resulted the most vulnerable areas showing increase in risk probability



up to 50%, while risk probability in Galicia, Slovenia, Croatia and central-southern France always resulted lower than present time

## Conclusions

These dynamics could be explained considering the fertilizing effect of the CO<sub>2</sub> increase that will compensate, at least in the first period, for negative effects due to rising temperatures and rainfall reductions. Furthermore, water use efficiency will enhance as a result of the CO<sub>2</sub>-induced stomata closure and consequent reduced transpiration. In these conditions, it is likely that the reduced yield, as expected as a consequence of reduced growing season due to high temperatures, could be compensated by the increased radiation use efficiency supported by adequate water availability.

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# Land, Climate and Resources (LandCaRe) 2020 – Foresight and Potentials in Rural Areas under Regional Climate Change

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## Introduction

Climate change may have positive or negative impacts on agriculture, depending on actual site potentials and boundary conditions. Besides the reduction of greenhouse-gas emissions, adaptation of agriculture and landscape management to climate change and extreme weather events is needed. The project LandCaRe (Land, Climate and Resources) 2020 ([www.landcare2020.de](http://www.landcare2020.de)) investigates effects of regional climate change on agricultural production as well as water and matter fluxes to provide a knowledge-based framework for adaptation. The project consists of ten sub-projects co-ordinated by the Department of Meteorology at the Technische Universität Dresden and conducted at six research institutes in Germany. Funding is provided by the German Ministry for Education and Research within the funding priority "Research for climate protection and protection from climate impacts" ([www.klimazwei.de](http://www.klimazwei.de)) focusing on the development of strategies for action and practical solutions.

## Conceptual Approach and Methodology

Central objective of the project LandCaRe 2020 is a web-based, dynamic decision support system (DSS) exemplarily developed for two contrasting regions of eastern Germany in the dry lowlands of Brandenburg and in the humid mountain area of Saxony. Potential future users of the DSS like agricultural administrations, farmers, water agencies and agro-business participate in the project. As far as possible their specific demands of knowledge and decision support will be considered. The spatial DSS is dynamic because it allows new model runs with various sets of scenarios and parameters by the user. It includes regional climate trends and weather statistics of the past recorded by climate stations of the German Weather Service (DWD) since 1950 as well as different future climate scenarios based on the ECHAM5 Global Circulation Model with dynamic downscaling steps (20 to 1 km grid length) using the Europe-wide regional climate model CLM (Böhm et al. 2006) and results of the statistical-dynamic model WETTREG (Enke et al. 2005) (Figure 1). Besides climate scenarios, socio-economic

scenarios are provided by the agricultural information system RAUMIS to derive the actual and potential future land use and to provide data for the transfer of the system to other regions in Germany.

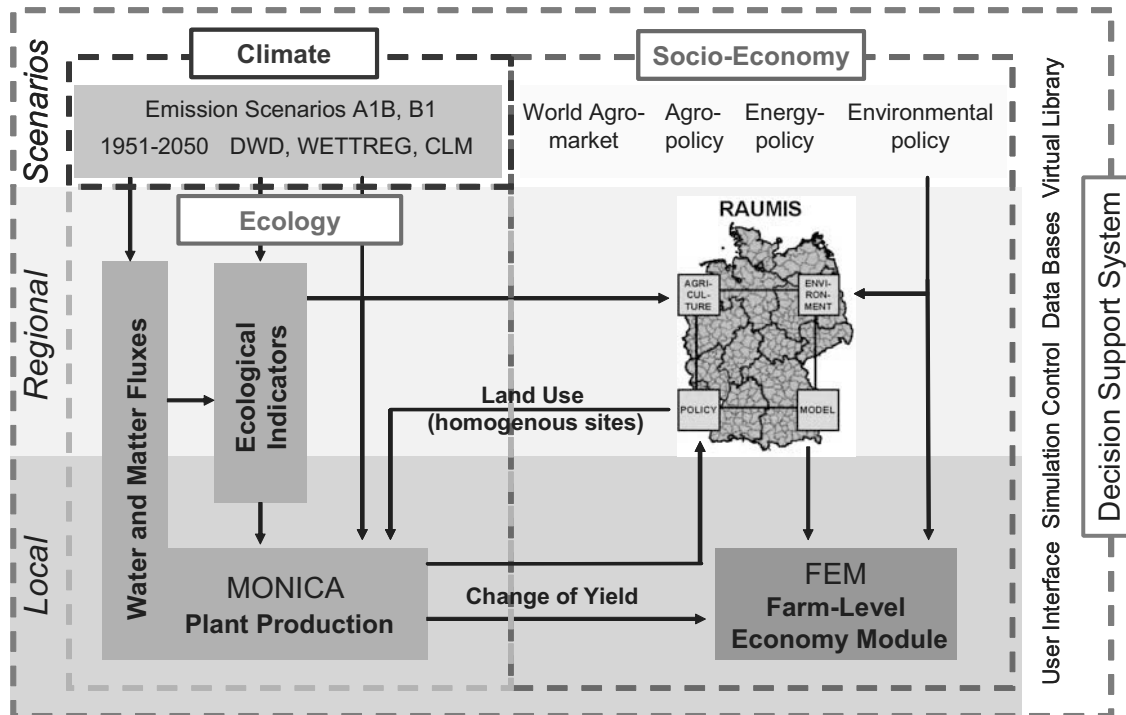


Figure 1: Conceptual framework and levels of integration of the different modules in the LandCaRe-DSS

The ecology module comprises simulations of water, carbon and nitrogen fluxes as well as yield predictions of crops and grassland. Simulations are performed by the central, CO<sub>2</sub>-sensitive Model MONICA developed from components of previous models like THESEUS, HERMES and AGROSIM. Further models and algorithms are used to derive ecological indicators related to nitrogen and water fluxes, water-use efficiency, primary production, greenhouse-gas emission, soil erosion, phenology, and site potential. Modelling is supported by data from FACE (free-air carbon dioxide enrichment) experiments with agricultural crop rotations at vTI Braunschweig. Besides completed experiments with the C<sub>3</sub> species sugar beet, wheat and barley, the C<sub>4</sub> species maize is currently investigated under high CO<sub>2</sub> (550 ppm). The experiments allow to include the CO<sub>2</sub> fertilizer effect in modelling which is crucially important for the prediction of crop yield with respect to quality, quantity and implications on the soil water and energy budget.

### Perspectives

Transfers of the LandCaRe-DSS to other regions within Germany are currently prepared and may be extended to further European countries. The regional to local focus of the DSS will allow to develop more specific action of adaptation to climate change. It will further provide a better understanding of the potential range of climate change impacts and specify required research to improve model parameterisation and validation by future experimental work.

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# IXIM: A New Maize Simulation Model For DSSAT v4.5

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The Decision Support System for Agrotechnology Transfer (DSSAT) is a suite of crop simulation models and associated tools for simulating growth, development, and yield of 25 crops. The maize simulation model in DSSAT is CSM-CERES, the modular version of CERES-Maize, which was first published in 1986. The newest release of DSSAT, version 4.5, provides users with the opportunity to run an alternative maize simulation model. IXIM (eeh-sheem), the Mayan language for maize, is a new, more mechanistic, maize simulation model fully compatible with DSSAT. The purpose of this work is to compare seasonal simulations of maize growth and N uptake using CSM-CERES and IXIM.

## Methodology

IXIM was modified from CSM-CERES and includes a number of improvements and new modules. Leaf area expansion and senescence are simulated using sigmoidal functions to describe expansion, longevity, and senescence of individual leaves (Lizaso et al., 2003). Leaf rolling per-leaf is calculated as a function of time of the day, atmospheric transmission of radiation, and water stress intensity. Instantaneous assimilation of each green leaf is calculated as a function of hourly-absorbed photosynthetic photon flux density, leaf age, and air temperature (Lizaso et al., 2005). Canopy gross assimilation is calculated by integrating the contributions of successive vertical leaf classes. Canopy respiration accounts for maintenance respiration and growth respiration for tissue synthesis (Lizaso et al., 2005). Specific leaf area determines potential allocation of biomass to individual leaves. Specific leaf area of each leaf is a function of light intensity, temperature, and leaf position within the canopy. Partitioning of assimilates among organs was completely modified. Ear growth occurs within a thermal time window of 250 degree-days before silking, until 100 degree-days after silking. Kernel number per plant is calculated as a curvilinear function of the daily shoot growth rate averaged over the same thermal time window around flowering used for ear growth. Maximum daily nitrogen uptake is limited by a curvilinear function of daily growth rate. This constraint recognizes the energy cost for N uptake, reduction, and assimilation. IXIM requires two additional genetic inputs relative to CSM-CERES: 1) Surface area of the largest leaf (cm<sup>2</sup>), and 2) Longevity of the most long-lived leaf (degree-day). Model parameters are included in a new maize species file and are accessible to the user. Data distributed with DSSAT (UFGA8201, Irrigated and non-irrigated during the vegetative phase treatments) were simulated and compared. Willmott's d index (Willmott, 1982) was used to evaluate model accuracy. Index d has values within the range 0-1 with higher values indicating more accurate simulations.

## Results

Both models produced better results when simulating non-stress conditions as compared to the water stress treatment (Figure 1). CSM-CERES consistently overestimated leaf area in the irrigated treatment. However, it was able to accurately simulate biomass accumulation and grain yield (d values of 0.991 and

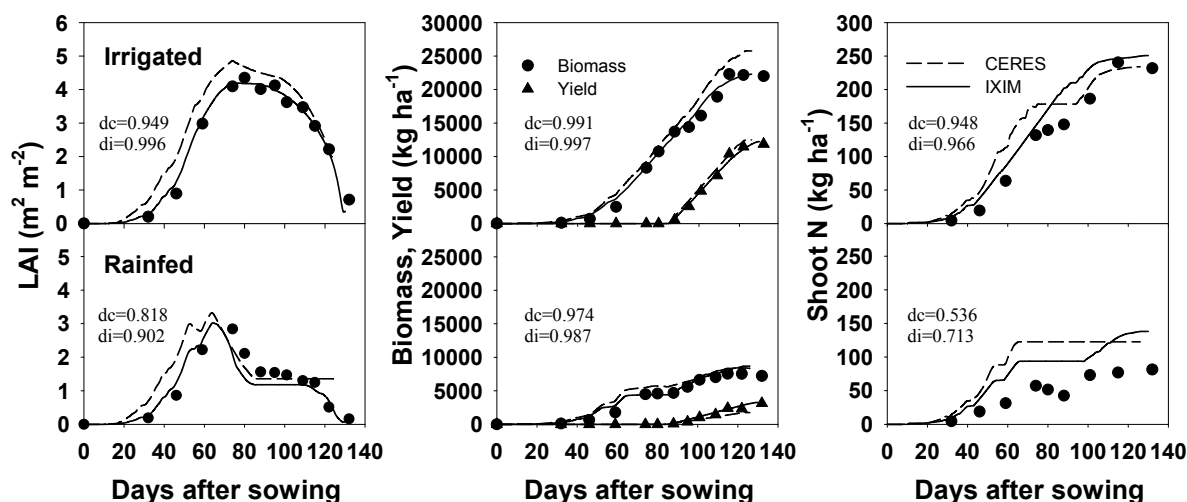


Figure 1: Leaf area index, biomass accumulation, grain yield, and shoot N uptake in maize irrigated and water stressed during the vegetative phase (Rainfed), simulated with CSM-CERES (dashed lines) and with IXIM (continuous lines) compared to observed (symbols). Index of agreement (Willmott, 1982) calculated with CERES simulations (dc) and with IXIM simulations (di).

0.973). It also calculated faster than observed shoot N uptake especially immediately after applying fertilizer.

CSM-CERES overestimated leaf senescence in response to soil water shortages during the vegetative phase. However, simulations of biomass and grain yield were adequate with d values of 0.974 and 0.977 respectively. The model simulated, at flowering, more than two-fold greater than measured N uptake.

On the other hand, IXIM improved consistently the estimations of growth and N uptake. Willmott's d index (Willmott, 1982) improved 5 and 10% when simulating leaf area of irrigated and vegetative water stress treatments. Simulations of biomass and yield showed minor differences between models, but N uptake simulations improved 2 and 33% for irrigated and rainfed treatments. Partition of dry mass between leaves and stems was also simulated closer to measured by IXIM as compared to CSM-CERES (not shown).

In summary, IXIM outperformed CSM-CERES when simulating growth and N uptake of non-stressed and water stress treatments. Values of d index fluctuated between 0.966 and 0.997 under irrigation, and between 0.713 and 0.987 for water stress conditions. Corresponding ranges for CSM-CERES were 0.948 and 0.991 for irrigated and 0.536 and 0.977 for rainfed conditions. Simulation of N uptake, especially under stress conditions needs further attention.

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# From site-specific modelling supported by monitoring data to regional application

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A fundamental task in agro-ecological research is the quantification and prediction of the potential effects of agricultural management practices on crop growth, soil water dynamics and nitrogen leaching which can affect the quality of groundwater. Continuous maize cropping systems traditionally have an high potential risk of nitrate leaching because of a large supply of nitrogen and water (Acutis et al, 2000). Efforts have to focus especially on this issue in order to guarantee European Union Directive 91/676 compliance. Here objectives, methodologies and some results of the project ARMOSA (AA. VV., Regione Lombardia, 2007) are presented. The project is developed by the Region Lombardy in order to set up a soil monitoring network to measure the nitrate leaching, supported also by a crop model development.

## Methodology

In order to assess the actual nitrogen losses due to leaching phenomena in Po Valley a project called ARMOSA and carried out by ERSAF with the partnership of the Universities of Milan and Naples and the National Research Council has been formulated.

Related to the nitrate leaching risk two main tasks are provided by the programme: (i) setting up a soil monitoring network to measure the nitrate leaching from different cropping systems in different pedoclimatic conditions; (ii) identifying alternative and sustainable nitrogen management strategies, at farming level, both aware from the environmental point of view and economically acceptable by farmers. Five monitoring sites, representative of the region pedoclimatic conditions and cropping systems, have been set up. Soil profile description and both hydrological and micromorphological characterization and analysis of soil horizons have been measured. Each site is equipped with a meteorological station, TDR probes, tensiometers, suction cups and soil-temperature probes at various depths to 150 cm. Data collected allowed for the calibration and validation of mathematical environmental models simulating the N-cycle for the Lombardy environment, hydrological dynamics and cropping conditions. Once calibrated, these models will be used to develop a new one, able to implement all the mathematic approach coming from the previous ones, as SWAP, MACRO, SOIL-N and CropSyst. ARMOSA model system simulates agro-meteorological variables, soil water balance, crop growth and development. Three components integrate (1) a micrometeorological model (MM) simulating the energy balance; (2) a crop growth model which uses the outputs of the MM model (radiation and temperature), and (3) a soil water balance model. The model outputs are aggregated as risk indicators, which are related to crop performance and management. The model system is developed using Object Oriented Programming (OOP) to ensure modularity and re-usability of components. The programming language adopted for all model components is Visual Basic 6.0. Such model, ARMOSA, has run to compare different scenarios at municipal scale, extending the previous results of the monitored sites, in order to estimate leaching events in five municipalities. Five municipalities in Lodi area have been chosen as simulating scenarios, whom N-load, land use and arable crop area values were available. We hypothesized three common cropping systems for each municipal areas (Continuous silage maize, grain maize-winter wheat, meadow) under four years (2003-2006). Here some simulation results, such as crop yield and N-NO<sub>3</sub> soil content, are presented. Other

data and parameters of tillage and irrigation required to run the model are derived from typical management of dairy and arable farms.

## Results

The first applications of ARMOSA gave interesting results of fitting values, as shown in Tab.1 for two of the monitoring sites in Lodi and Mantova. With respect to soil N-NO<sub>3</sub> content, ARMOSA well estimated it especially in the shallow layers.

Tab.1. Fitting values. Biomass and N-uptake parameters values are the average of Lodi and Mantova.

<i>PARAMETERS (kg ha<sup>-1</sup>)</i>	RRMSE	EF	CRM	R <sup>2</sup>
AGB	11.62	0.67	0.00	0.67
N-NO <sub>3</sub> <sup>-</sup> plant uptake	16.83	0.57	-0.04	0.6
LODI: N-NO <sub>3</sub> soil content	48.28	0.11	0.09	0.31
MANTOVA: N-NO <sub>3</sub> soil content	70.72	0.39	0.3	0.51

After a good evaluation of the model in simulating crop yields, we ran the model in order to estimate N loss for leaching. Annual mean values of N-NO<sub>3</sub> leached for each municipality

application are shown in Tab. 2. They are average of the simulated annual values for each cropping system adopted. In the case of meadow system, results (Tab.3) show values noticeably lower, especially for the municipality in which clay loam soils prevail as Brembio, Massalengo e Ossago.

Tab. 2. N-NO<sub>3</sub> leached: annual mean values of the three cropping systems.

N-NO <sub>3</sub> LAECHED					
Year	BREMBIO	CORNEGLIANO LAUDENSE	MASSALENGO	OSSAGO LODIGIANO	SAN MARTINO IN STRADA
2003	60	65	45	53	68
2004	94	114	73	89	109
2005	62	118	44	68	93
2006	69	87	49	51	74
<i>Average</i>	<i>71.3</i>	<i>96</i>	<i>52.8</i>	<i>65.3</i>	<i>86</i>

Tab. 3. N-NO<sub>3</sub> leached: annual mean values for meadow system.

N-NO <sub>3</sub> LAECHED: ANNUAL MEAN VALUES for meadow SYSTEM					
Year	BREMBIO	CORNEGLIANO LAUDENSE	MASSALENGO	OSSAGO LODIGIANO	SAN MARTINO IN STRADA
2003	14.57	22.60	13.09	13.74	21.10
2004	19.59	28.66	17.49	18.86	27.39
2005	22.12	31.50	19.57	20.78	30.74
2006	17.35	25.00	15.49	15.12	23.77
<i>Average</i>	<i>18.8</i>	<i>27.6</i>	<i>16.7</i>	<i>17.8</i>	<i>26.4</i>

## Conclusions

ARMOSA model applied in the project monitoring sites gave good results that have encouraged an application at regional scale, as a decision support. The model has been used in order to provide an estimation of N-NO<sub>3</sub> leached under three different cropping systems in a regional application. Results show that a continuous silage maize involves higher values of leaching; they suggest a reduced application of inorganic N-fertilizers in the case of maize and wheat with respect to economic and environmental benefits.

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# Using Agro-Meteorological Metrics to Communicate Climate Change Impacts to Land Managers

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## Introduction

Climate change has been identified by UK and Scottish sustainable development strategies as a key threat to rural communities. Yet for many land management stakeholders the issues of climate change and sustainable development are swamped by other policy and market changes with more immediate consequences. There is, however, the need for effective engagement between researchers and stakeholders if adequate adaptation and mitigation measures are to be enacted through informed decisions. Agro-meteorological metrics are useful indicators of conditions that aid farm scale decision making. Metrics derived from estimated future climate scenarios provide an opportunity to characterise the potential impacts of climate change on agriculture. Such indications are vital to determine how adaptations to farming systems can be achieved. However, Regional Climate Model (RCM) estimates are determined for spatial scales considerably larger (typically 50×50 km) than those at which agro-metrics are applied. Therefore there is a need to evaluate the quality of RCM estimates at specific locations, and where necessary, conduct downscaling to allow for differences in scale. It is then important to engage with stakeholders to assess the utility of the agro-metrics and use them as the basis for dialogue in order to communicate the potential impacts of climate change and develop appropriate adaptation strategies.

## Methodology

A process of RCM evaluation (Rivington et al 2008a) was used to better understand the quality of the projection data and potential for introduced uncertainty. RCM data were then downscaled (Rivington et al 2008b) to increase utility for the generation of site-specific agro-metrics. Agro-metrics were derived from observed and future downscaled data (A2 medium-high emissions scenario) for six sites in Scotland. The agro-metrics were presented to stakeholders for evaluation during a series of workshops, during which stakeholders also deliberated on the potential CC impacts and what adaptation steps they could take to cope with the projected changes. This enabled the best form and combination of metrics to be identified ([http://www.macaulay.ac.uk/LADSS/agromet\\_cc\\_indicators.html](http://www.macaulay.ac.uk/LADSS/agromet_cc_indicators.html)).

## Results

Results show the RCM made both good and poor estimates of weather variables for the past climate. Errors were greatly reduced by downscaling, increasing confidence in the utility of the future projections and derived agro-metrics. Future agro-metrics indicate that whilst the growing season will start earlier, access may be no earlier than at present, as the date of the end of field capacity did not change. The period when crop growth is water-limited, and the maximum soil water deficit are both estimated to increase, hence in increased risk in plant water stress. Whilst stakeholders could discern possible effects of climate change directly from meteorological summaries (i.e. mean monthly temperature), the use of agro-metrics enhanced their ability to generate specific adaptation strategies. Metrics of greatest interest were the distribution of growing season and access days, potential for increased soil moisture deficits, reductions in the period of frost and the potential for plant heat stress.

Agro-metric	Aberdeen	Mylnfield	Galashiels	Dumfries	Auchincruive	Prabost
Average Daily Temp. (°C)	➤ 2.8	➤ 3.1	➤ 3.0	➤ 3.3	➤ 2.8	➤ 1.2
Average Annual Rainfall (mm)	➤ 36	➤ 26	⬅ 16	➤ 100	➤ 70	➤ 310
Start of Growing Season (day)	⬅ 48	⬅ 35	⬅ 36	⬅ 27	⬅ 14	⬅ 30
Tsum200 (day)	⬅ 22	⬅ 22	⬅ 25	⬅ 22	⬅ 16	⬅ 16
End of Field Capacity (day)	⬅ 3	⬅ 2	⬅ 4	➤ 2	⬅ 3	⬅ 6
Last Air Frost in Spring (day)	⬅ 42	⬅ 41	⬅ 32	⬅ 36	⬅ 37	⬅ 63
Return to Field Capacity (day)	➤ 14	➤ 18	➤ 26	➤ 23	➤ 18	➤ 10
End of Growing Season (day)	➤ 17	➤ 17	➤ 20	➤ 19	➤ 20	➤ 16
Dry Soil (days)	➤ 3	➤ 11	➤ 12	➤ 13	⬅ 0 ➤	⬅ 0 ➤
Growing Season Length (days)	➤ 64	➤ 63	➤ 62	➤ 60	➤ 55	➤ 58
Access Period Length (days)	➤ 11	➤ 19	➤ 36	➤ 47	➤ 30	➤ 3
Access during Growing Season (days)	➤ 20	➤ 26	➤ 42	➤ 51	➤ 33	➤ 3

Table 1. Summary of key agro-metrics. Arrows to left indicate less or earlier, arrows to right indicate more or later.

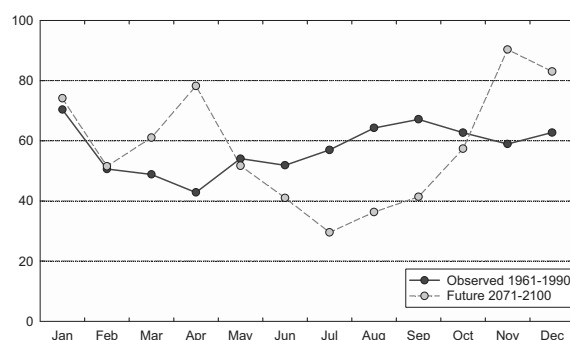


Figure 1. Mean monthly precipitation (mm) at Mylnfield.

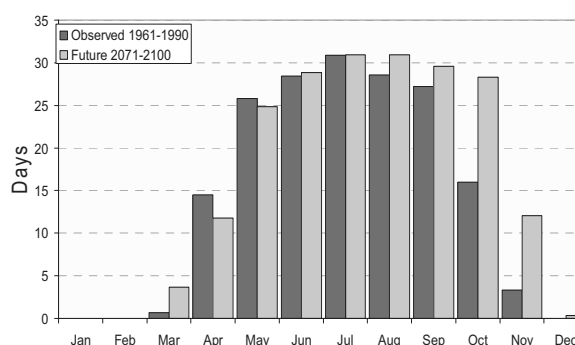


Figure 2. Combined growing season and field access days at Mylnfield

## Conclusions

This work has demonstrated the value in conducting detailed evaluation, and where necessary, a form of downscaling by bias correction, in order to increase the utility in RCM future projections. A simple downscaling method greatly improves the utility of the projection data, helping to build confidence in the use of the agro-metrics when presented to stakeholders. Evaluation of the form of presentation of the metrics by stakeholders was highly valuable, enabling improvement in both the type of metric and form of presentation. Using the agro-metrics framework with stakeholders was more effective in communicating the implications of the climate change scenarios than meteorological summaries alone. The agro-metrics were particularly effective in encouraging stakeholders to consider both impacts and adaptation. The stakeholders were willing and able to engage with the more complex agro-metrics where they could see their potential benefits as decision making indicators. By presenting time series of the soil water balance metrics for example it was possible to identify particular iconic events in the historical dataset and to make comparisons with the future scenarios both in quantitative and qualitative terms. The outputs from these more complex analyses that integrate several weather variables also stimulated the stakeholders to question further the nature of the changes in patterns of weather and thus close the circle from impacts and adaptations to the climate drivers and their causes.

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# A Model Framework For Simulating Plant Disease Epidemics

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The Agricultural Production and Externalities Simulator (APES) is a modular simulation model system for estimating the biophysical behaviour of agricultural production systems in response to the interaction of weather, soil and agro-technical management options (APES, 2008). APES has been developed to allow the incorporation of other components which might be needed in order to simulate processes not yet included in the current version. Among these processes, plant diseases are interesting candidates because of their effects on crop yield and because of the implications as agro-chemicals use. A Diseases software component is currently under development adopting the design summarized by Donatelli and Rizzoli (2008). It aims at estimating the impacts of plant disease epidemics on plant growth and yield. It consists of four modules providing a generic frame to simulate disease development: 1) Disease progress, 2) Inoculum pressure (initial conditions), 3) Impacts on plants, and 4) Agricultural management impact on pathogen populations. The Disease progress module, developed as a generic model framework to simulate the epidemics caused by fungal pathogens, is briefly described herein.

## Methodology

According to the Systems Analysis (Leffelaar, 1993), the interrelated elements of a generic pathosystem were defined as state, rate, auxiliary, and exogenous variables. Approaches and terminology used in the model development follow Van der Plank (1963), Campbell and Madden (1990) and Rossi et al. (1997).

Mathematical equations operating within the model make it possible to calculate the state variables, the rates at which they change over time (with a step of one day) in response to auxiliary and exogenous variables, and parameters. These equations were taken from literature or elaborated *ad hoc*.

## Results

The Disease progress module simulates the epidemics of a generic air-borne fungal pathogen, considering the following components of the infection process (auxiliary variables): infection, incubation, latency, infectiousness, sporulation, spore dispersal and landing. These processes, which are driven by weather conditions and interactions with the host plant, are modelled as a function of meteorological variables and parameters specific for each host-pathogen couple.

Proportions of the host tissue affected by the disease are classified in different states on the basis of the following disease stages: incubation, latency, infectiousness, and lesion senescence. The states of the host tissue are, therefore: i) healthy, ii) latent (with latent infections not yet visible), iii) visible (with visible but no sporulating lesions), iv) infectious (with sporulating lesions), v) old (with old and sterile lesions, i.e. no longer sporulating). States ii to v represent the total proportion of host tissue affected by the disease (Figure 1).

Healthy tissue enters the latent state when infection occurs. Infected host tissue in the latent state evolves to the state of tissue with visible lesions once the incubation period is over. The subsequent two states of host tissue with sporulating lesions and with old lesions occur when the latent and infectious periods are finished, respectively. The incubation, latent, and infectious periods are estimated as a function of temperature using parameters specific for the pathosystem under simulation. The portions

of health host tissue, which become infected and therefore evolve to the state of host tissue latent, are estimated based on portion of host tissue vulnerable to infections (susceptible and not affected yet) and rate of infection. The infection rate depends on two factors: sporulation which is estimated as a function of temperature and vapour pressure deficit, and dispersal (of spores produced on sporulating lesions which are transported and deposited on vulnerable host tissue) which is simulated as a function of either rainfall or wind speed (according to the pathogen). Once deposited on the surface of a vulnerable host tissue, new infections take place under favourable conditions of temperature and humidity.

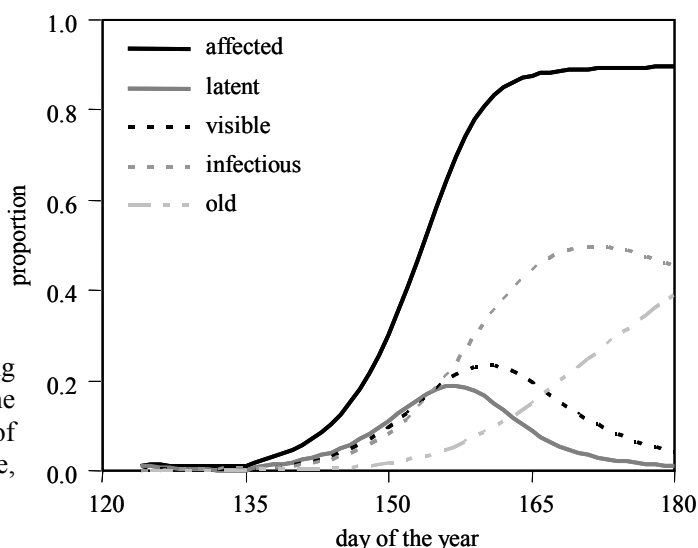


Figure 1. Outputs of the model simulating the progress of plant disease epidemics. The host tissue affected results from the sum of different tissue's categories: latent, visible, infectious, and old proportions.

## Conclusions

The Disease progress module estimates the proportion of host tissue affected compared to the total host tissue. It uses a set of functions which can be used to simulate the progress of the epidemics caused by several pathogenic fungi on several crops, by simply changing specific model parameters. The same approach can be used to simulate the effects of agriculture management options on disease progress (fourth module of the Diseases component). The model framework is implemented as a software component and it will be publicly available within 2008.

## Acknowledgements

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# Intercropping Winter Wheat with a Cover Crop in No-Till: a Modelling Approach

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## Introduction

Crop management systems based on little or no soil disturbance along with permanent soil coverage have been proposed to solve economical and environmental issues in agriculture (Lahmar *et al.*, 2006). Nevertheless, these management systems may present short-term drawbacks, such as an increase in herbicide use or a decrease in the physical quality of the soil. Intercropping winter wheat with a cover crop may partially overcome drawbacks of no-till thanks to facilitation relationships: weed control, nitrate recycling and soil conservation. Cover crop can however also compete with wheat for resources and hence decrease yield (Carof *et al.*, 2007a, b).

The aim of this work is to study facilitation and competition for water, nitrogen and light between wheat and red fescue intercrops through a modelling approach that evaluates the agronomic and environmental performance of intercropping in no-till. We investigate the suitability of the STICS model to accurately reflect the facilitation relationships between the two crops. More specifically, we ask if the model can accurately evaluate the facilitation and competition for water, nitrogen and light between wheat and red-fescue under no-till conditions.

## Methodology

An intercropping extension of the sole crop model STICS has been used (Brisson *et al.*, 2004). It integrates input variables related to climate, soil and the crop system to calculate agricultural and environmental variables (yield, water and nitrogen fluxes). The model was parameterized and evaluated using data from 1999 and 2000 field experiments at the INRA Grignon experimental station (Ghiloufi *et al.* 2001) involving winter wheat and red-fescue grown alone and winter wheat intercropped with red fescue under no-till conditions. The varietal parameters were calibrated for wheat and fescue using data from the sole treatment. After calibration, the model was evaluated with data from intercropping treatments for the two years. The root mean square of error (RMSE) was the statistic criteria used to compare observed and simulated results.

## Results

### 1) Evaluation of STICS intercropping model

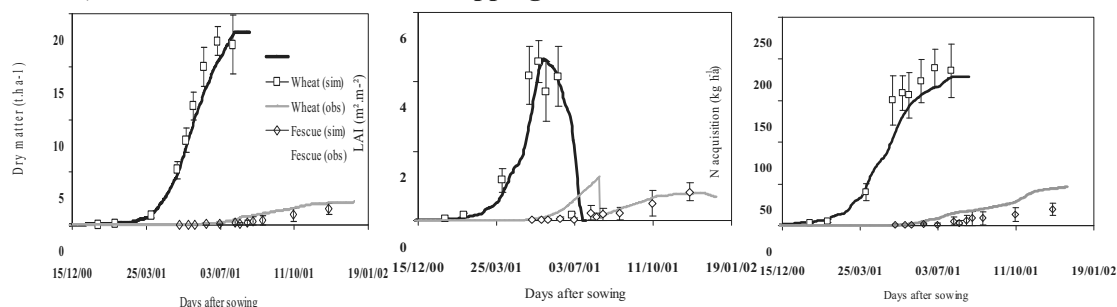


Fig.1. Simulated and observed above-ground dry matter (a), Leaf area Index (b) and N acquisition (c) in wheat and fescue intercropped (2000).

There is a good agreement between simulations and measurements for dry matter, leaf area index (LAI) and N uptake evolution in intercrops (figure 1) as indicated by the RMSE values: 0.38 t.ha<sup>-1</sup> (fescue) and 1.00 t.ha<sup>-1</sup> (wheat) for dry matter, 0.44 m<sup>2</sup>.m<sup>-2</sup> (fescue) and 0.86 m<sup>2</sup>.m<sup>-2</sup> (wheat) for LAI and 11.04 kg N.ha<sup>-1</sup> (fescue) and 18.49 kg N.ha<sup>-1</sup> (wheat) for N acquisition. However, the model over-estimates the LAI of fescue in the intercropped period probably due to difficulties in the experimental measurement, but this has had no effect on the simulation of the other variables.

The results of the simulation of nitrogen and water stocks in the soil were less satisfactory (RMSE: 243 mm for water and 16.35 Kg N.ha<sup>-1</sup> for nitrogen). By contrast, the model well reproduced the dynamics of the water and the nitrogen in the soil profile compared to the measurements: this lets suppose that water and nitrogen fluxes (evaporation, drainage and mineralization) were correctly simulated by the model.

## 2) Competition and facilitation for water, nitrogen and light

The simulation results confirmed that the wheat yield was not overly affected by fescue development in the intercropping system: yield differences between sole and intercropped wheat were about 4%. Neither water nor nitrogen appear to explain the competitive edge of wheat. As shown in figure 2, the interception of light radiation during the intercropping period may well be a partial explanation: fescue intercepted 4.8 % of the total incident radiation during intercropping.

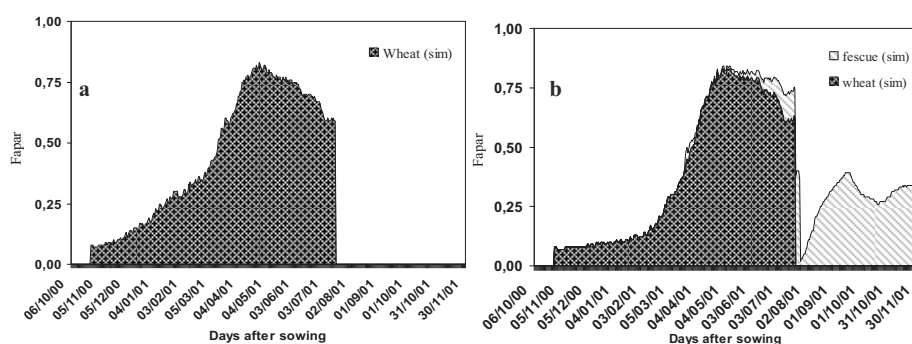


Fig.2. Simulated daily PAR interception efficiency of sole wheat (a) and wheat-fescue intercrops (b)

## Conclusion

The STICS model is a suitable tool for simulating the growth of the two species and for reproducing the environmental conditions in wheat-fescue intercrops. The model reveals that the capacity of wheat to intercept light gives a competitive edge. The model will be helpful in calculating water and nitrogen fluxes as well as daily PAR interception efficiency of fescue after wheat harvest, in turn to assess the facilitation effects of the cover crop.

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# The Vamos Project: Regional Scale Validation of Two Models Predicting Soil N Availability for a More Sustainable Fertilization of Wheat and Peach Crops

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Models are potentially useful tools for both growers and policy makers, to guide the nitrogen fertilization in order to reconcile environmental and productive issues. This research, funded by the regional government of Emilia-Romagna and Crpv in view of testing the regional nitrogen application policies for a more sustainable crop production, aims at the extensive testing of water balance and nitrogen transport models in actual cropping conditions in a rather large area of the eastern Po river plain.

## Methodology

We performed a large scale monitoring of soil water content at four depths (0-0.25, 0.25-0.50, 0.25-0.75 and 0.75-1.00 m) and soil nitrogen content (N-NO<sub>3</sub> and N-NH<sub>4</sub>) at two depths (0-0.50 and 0.50-1.00 m). Measurements took place every year from 2005 to 2008 in 130 winter wheat fields and 10 peach orchards located in more than forty farms in three Central and Eastern provinces of Emilia-Romagna, an agricultural region of Northern Italy (Figure 1). Together with measurements in the field a large amount of information was collected from farmers about preceding crop and crop husbandry practices and dates, soil fertilization type, amounts and dates, irrigation amounts and dates (for peach orchards). All the data collected were stored in the Vamos project relational database. Weather data (daily precipitation and extreme temperatures) and watertable depth data from stations located in the



Figure 1 - Study area with experimental fields located in Emilia-Romagna, Italy.

area were available. Weather data were interpolated on a 2.5 km regular grid. Soils were characterized in terms of the regional soil survey standards. The collected data were used for the validation, in different soil and weather conditions, of two soil water and nitrogen balance models: the MACRO/SOILN (Larsson and Jarvis, 1999) model and the CRITERIA model (Marletto and Zinoni 1996; Marletto et al., 2001) integrated with nitrogen routines (Hutson and Wagenet, 1992), to be used for prediction of water content and nitrogen availability in soils.

## Results

The Vamos project ends in 2008 and final results will be available next year. With the CRITERIA model (Figure 2) general nitrogen dynamics are quite well represented but timing of the main processes and quantitative values can be improved. In spring and summer, until harvest, nitrogen uptake by the crop exceeds nitrate release by nitrification so nitrate content gradually decrease. Abundant precipitations in April lead to nitrate leaching and to a sudden decrease in nitrate content. The coupled MACRO-SOILN model performed well in estimating soil water content but the model tended to underestimate the N content in the upper 0-0.5 m soil layer while overestimating the deeper one, from 0.5 m up to 1.0 m depth. Nevertheless, the measured soil N content pattern was partially reproduced (Figure 3).

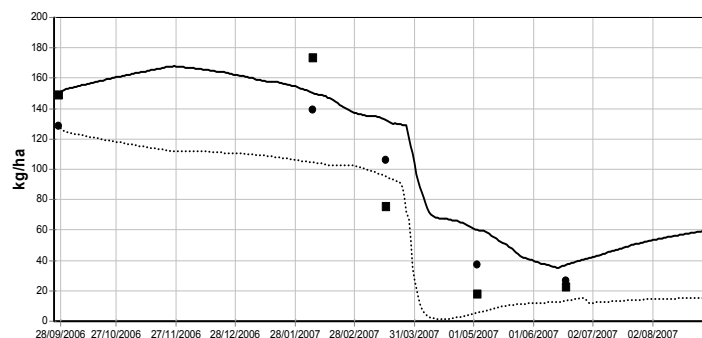


Figure 2 – N-NO<sub>3</sub>, example of comparison between measured data (squares: 0-0.5 m, circles: 0.5-1 m) and the CRITERIA model simulation (solid line: 0-0.5 m, dashed line: 0.5-1 m) in a wheat field.

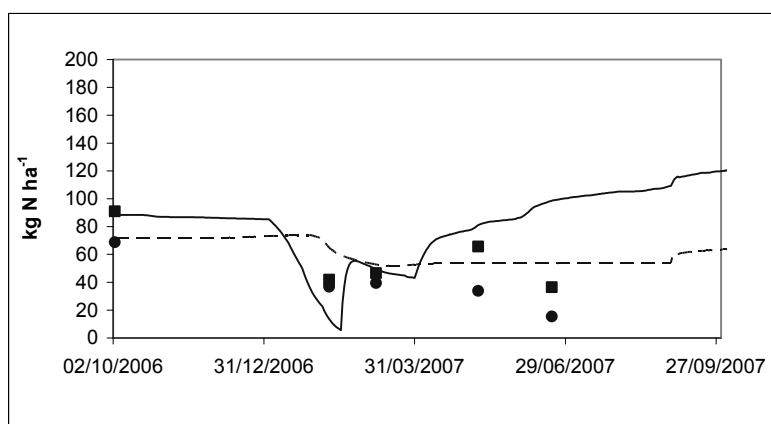


Figure 3. N-NO<sub>3</sub>, example of comparison between measured data (squares: 0-0.5 m, circles 0.5-1.0 m) and the SOILN model simulation (solid line: 0-0.5 m, dashed line: 0.5-1.0 m) in a wheat field.

## Conclusions

The results of the Vamos project are still preliminary. Efforts on calibration of the CRITERIA nitrogen routines are still to be made in order to improve the simulation of the main nitrogen processes, particularly water table dilution of nitrates, which is of great environmental relevance. A better simulation of soil N depletion with the MACRO-SOILN model during the growing season could also be obtained by improving the N transformation processes parameters.

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# Crop sequences modelling faced to landscape dynamics

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## Introduction:

How do farms contribute in building and changing features of the landscape? This issue has drawn increasingly more attention since the pioneer work "Pays, Paysans, Paysages" (Countries, peasants and landscapes) (INRA-ENSSAA, 1977) has been launched.

A new challenge in crop sequences designing is drawing face to environmental consequences of cropping systems (Benoît et al, 2007). Preventing runoff erosion and the associated pollution of surface water (especially by pesticides) needs grassland strips, ditches, or other structures placed in suitable strategic locations in a catchment. Again, similar conclusions could be drawn about many other environmental targets, such as biodiversity, or landscape quality and accessibility (Benoît *et al.*, 2006). Now, a lot of works focused on crop rotations, as central object for agronomists. Recent researches have proposed to use stochastic modelling (Mari & Schott 2001) or symbolic computer models (Stefik, 1995) in order to describe the crop rotations built by farmers practices (Largouet & Cordier, 2001; Le Ber et al, 2006; Castellazzi et al., 2007). So, we have to progress on a new way : how to model crop sequences built BY farmers to be able to design new landscapes WITH farmers?

Our aim is to propose a methodological approach of crop rotations designing by farmers. This proposal is built on three interactive levels of crop rotations designing: field, farm and landscape. To develop our proposal we define our concepts, propose an european nomenclature, and describe the time and space dimensions of crop sequences through examples in Europe.

## 1- Methodology:

Agricultural landscapes can be modelled as a complex and dynamic pattern of fields. We propose two points of view of this complexity:

-The crop pattern as an instantaneous aspect (Fresco *et al.*, 1993), can be described in their dynamic (i.e. crop sequences) by Hidden Markov Models (Mari & Le Ber, 2006). So, we are able to identify **regularities in landscape** through sequences of crops and permanent grassland use types at divers time and space scales with HMM. These descriptions are a basis of a european crop sequences corpus.

- The farmer practices involved in this building, with a designing point of view, can be described as ergonomical rules through agronomy (Benoît *et al.*, 2006). Here, the description of the crop sequences is a more qualitative one, through **farmer rules** as they are described, managed and decided by the actors: rules used by farmers to explain their choices are modelled with AI modelling. Three steps are necessary for this modelling: (i) the identification of each crop or grassland rotation made BY the farmers, (ii) the identification of land patterns WITH the farmers definitions, (iii) the building of logics rules WITH the farmers decision rules as background.

## 2- Results:

### - Farmer decisions are the motor of crop sequences in Europe:

With comprehensive surveys, we can reach the decisions taken by farmers concerning their land. A lot of factors are described by european farmers: land tenure, accessibility (roads, ways), field soil, slope, boundaries (forests, roads, rivers), distance from the farmstead, size of this field, proximity of other fields, irrigation suitability, ... All of them have a specific local weight. We propose to use the Artificial Intelligence capabilities to modelize these building rules of landscapes (Morlon, Benoît, 1990; Le Ber, Benoît, 1998).

### - Landscape as a result of sequences sequences pattern

We develop some examples to explain the major effect of crop rotations choices in land designing in Europe. For us, as agronomists, land pattern and long term soil fertility are results of farmer decisions mainly on

field size and crop rotations choices. To model this design process we use Hidden Markov Chains to identify time and spatial regularities in European landscapes.

## Conclusions

All over Europe, the farmers have each year to allocate their crops and grassland uses in their own territory. This annual adjustment between chosen crops and field plots results in different perennial sequences of crops and grassland use types. For example, in Denmark : maize-maize- winter Wheat-Barley, in south west of France without irrigation : sunflower- winter Wheat- barley, in East of France : oil rapeseed- winter wheat, in the plain of Rhine in Vorarlberg (Austria) : maize-maize-temporary grassland for mowing (3 years).

These crop sequences have two interesting properties for agronomists: (i) They are one of the cropping system components in farm management (Benoît *et al*, 2006), (ii) They increase the stability of our modelling with time resilience. On this second aspect, we insist on one central point: if we can see a wheat field in a landscape, it is not the same logic rules if this wheat is in the first or the third rotation we described above. **Our main hypothesis is : in Europe, the farmer decisions are not built “crop per crop” but on a crop/grassland sequences managed with their spatial opportunities/constraints.**

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# Parameterisation of two Wheat Phenology Models for an Extensive Data Set from Germany

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Accurate predictions of crop phenology are a prerequisite in the application of crop models. The aim of this study was to compare the accuracy of a phenology model based on CERES Wheat (Ritchie 1991; "CW") and the phenology model of Wang and Engel (1998; "WE"). For the parameterisation and validation of the models an extensive data set originating from surveys carried out routinely at several locations and years throughout Germany has been used to get a model parameterization valid for a larger subset of the whole environmental conditions within the wheat growing area of Germany.

## Material and Methods

Both models were implemented in the modular object oriented modelling environment HUME which allows to integrate simulation runs and data for several sites and years into the parameter estimation process. The CW model had to be modified to calculate development stages as BBCH values. Furthermore the model structure was changed for the stage of leaf growth from a fixed temperature sum to a calculation of this phase based on the number of initiated leafs and the rate of leaf expression. The length phase during which leafs are initiated depends (as in the original CW) on a temperature sum modified by the influence of vernalisation and photoperiod while the rate of leaf initiation is according to the plastochron which is assumed to be a fixed temperature sum. Therefore the number of initiated leafs and consequently the duration of leaf expression is variable.

Both models were parameterized by a step by step approach; starting with the early development stages and stepping further on to the later stages. Some parameters like the phyllocron were estimated from linear relationships between measured BBCH stages and the temperature sum. For other parameters the Levenberg-Marquardt algorithm was used including data points of the developmental stages that are directly affected by the respective parameter. As the BBCH scale is not continuous but has gaps in certain parts this had to be accounted for in using the data.

The data set used to calibrate the models was obtained from phytopathological surveys carried out yearly and routinely on different locations situated throughout Germany. The data set was divided into a calibration data set and a validation data set which was enlarged with additional data.

## Results

The change in the model structure of CW for the leaf growth results in differences in the number of initiated and expressed leafs and in the duration of this phase depending on year and location. This corresponds to differences in the observed BBCH stages (fig. 1). The prediction accuracy for the BBCH stages 30 to 39 was substantially increased by this modification.

The overall prediction accuracy of the modified CW is better than the WE model (Tab 1). Root mean squared errors (RMSE) for the total validation data set are 4.7 and 5.5 BBCH units or 11.6 and 13.0 days for CW and WE, respectively. The parameterization proved to be very robust for different years and valid for many different locations spread throughout Germany.

Table 1: Slope and intercept of the linear regression between measured and predicted BBCH values for the calibration and validation data set of the CW and WE wheat phenology models as well as number of observations (n), correlation coefficient  $r^2$  of the linear regression and RMSE of the prediction in BBCH values and in days of the occurrence of a certain growth stage.

	Slope	Intercept	n	$r^2$	RMSE	
					BBCH	days
CW calibration	1.03	-1.31	593	0.97	4.3	13.8
CW validation	0.98	-0.41	5764	0.93	4.7	11.6
WE calibration	1.01	0.85	593	0.95	5.4	15.9
WE validation	1.02	0.57	5764	0.91	5.5	13.0

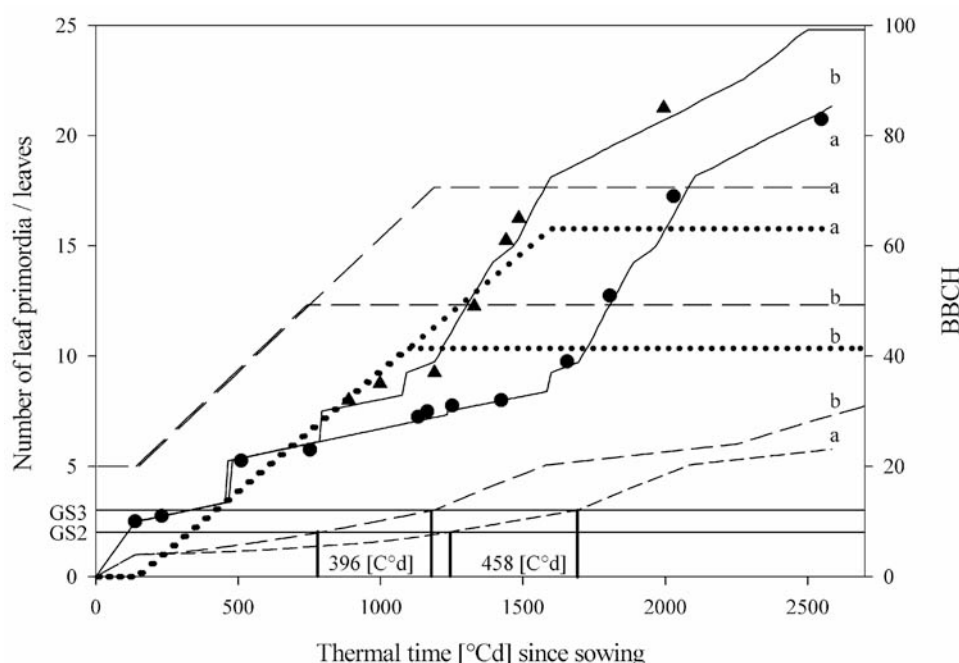


Figure 1: Number of leaf primordia, leaves, growth stages (GS) and BBCH over thermal time since sowing for two locations (a, b). The thermal time interval from GS 2 to GS 3 is given for the two locations ( — predicted BBCH, — — predicted number of leaf primordia, ..... predicted leaf number, --- GS, • observed BBCH site a, ▲ observed BBCH site b)

## Conclusions

Our study shows that a robust parameterization of wheat phenology models can be achieved using an extensive data set which has not been collected from trials especially designed for model calibration. Thereby we could show that despite the long history of phenology models it is still possible to increase the accuracy by small changes to the structure of a commonly used model approach.

The higher number of parameters within the modified CW model seems to be not a serious drawback as we could show that the available data were sufficient to obtain stable parameter values and the simpler WE model in contrary may not be able to depict all available information through corresponding parameter values.

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# Modelling Agricultural Diffuse Pollution and Water Quality in the Venice Lagoon Watershed (Italy): I - A Method for Building Spatially Distributed Scenarios of Agricultural Systems

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## Introduction

During the 1980's and the early 1990's, high nutrient loads in the Venice Lagoon Watershed (VLW), in Northern Italy, have been responsible of a severe eutrophication of the Venice Lagoon (VL). While municipal and industrial loads have been reduced during the past decade by waste water treatment plants activity, nutrient loads from agriculture and animal rearing activities still remain a significant pollution source. In the Master Plan 2000 the Regional Government has planned interventions and actions to reduce the pollutants discharged into the VL. Therefore modelling water cycle at watershed scale is an important task also for scenario analysis. Therefore simulation models for diffuse pollution of agricultural origin provide a great potential within this context. In this study the development of a territorial model to support SWAT (Soil and Water Assessment Tool) simulations is presented.

## Methodology

The present work is focused on improving space-time phenomena descriptions with reduction of work required to implement a scenario analysis. This is a part of a broader project with the main objective of building a Decision Support System (DSS) based on chemical and hydrological simulations obtained applying QUAL2E (US-EPA, Brown and Barnwell, 1987) and SWAT (Di Luzio et al., 2002) models and on the mDSS software (Giupponi, 2007). This work presents the statistical and geographical elaborations carried out to produce the *agricultural systems map*, describing agricultural productions of the VLW subsequently utilised as an input for the SWAT hydrological model. The proposed approach is an original one that permits to identify agricultural systems, i.e. territorial homogeneous units, based on geographical distributions of crop productions and livestock rearing activities. Agricultural systems are characterised by: mix of crops (organised in multiannual rotations), livestock live weights and manure productions, agro-techniques (fertilisations, irrigations, mechanisation, etc.). The methodology developed allows to integrate data from agricultural census (ISTAT, 2000), land cover map (ARPAV, 2003) and data obtained from an extensive farm survey (approx. 550 farms; Giupponi et al., 2004). When the unique objective of the study is the simple analysis of the existing, land cover map integrated with data related to agro-techniques could be sufficient. Nevertheless, considering a multi-temporal perspective and furthermore the necessity of creating alternative scenarios for the subsequent multi-criteria analysis with mDSS, a more efficient approach is needed, in order to create representative territorial units of plausible agricultural systems suitable for supporting scenario simulations, representing the action of varying *driving forces* and constraints. The SWAT model provides the *Hydrologic Response Units* (HRU) through an *overlay mapping* procedure among informative layers related to land cover, soil and climate. It is important to highlight that the land use identified in the SWAT model is used for multiannual simulations, so the different land uses should be described with a robust representation of agronomic managements. Consequently it is necessary to identify land uses through crop rotation instead of single crop to avoid technical non sense such as sugar beat cultivated on the same field for more than one year, but also to avoid problems of hydrological or erosion unbalances as consequence of cumulative effects due to the unrealistic simulation of monocultures. As stated before with the *agricultural systems map* is possible to represent alternative scenarios, which in our case study represented the effects the introduction of measures to reduce nitrate pollution (Veneto Region, 2004). Furthermore, these changes could be effectively propagated in a manageable number of agronomic territorial system units. The algorithm for the “agricultural systems map” construction is presented through 9 steps:

- 1) re-elaboration of ISTAT data at municipal level considering most representative crops;
- 2) elaboration of data related to livestock live weights, converted in units of nitrogen excreta;
- 3) *cluster analysis* with *k-means* methods, on statistical data obtained from the elaboration phases 1 and 2, adding the coordinate of municipal centroids normalised, obtaining: 5 crop mixes and 2 livestock intensities;
- 4) *overlay* of the two thematic layers (crops & livestock), thus identifying 8 typologies (e.g. maize with high livestock loads) and creation of the map “Agricultural productions” of the VLW;
- 5) identification of 9 rotation typologies, according to information collected in farm survey data;
- 6) allocation of the 9 crop rotations to the 8 *agricultural production typologies* to obtain multiannual land uses compatible with VLW distribution of cultivated areas per crop and livestock loads;
- 7) creation of a cartographic model through random assignment of rotations to pixels identified as agricultural area by the Land Cover, respecting the constraints of crop and livestock distributions;
- 8) construction of the map of “Agricultural systems” (considering both crop rotations and livestock rearing activities) distribution of the VLW (Figure 1);
- 9) definition of cropping system itineraries (typical sequence of agro-techniques) for the main crops, assigned to the different agricultural system types.

## Results

The “agricultural systems map” (Figure 1) permitted to run SWAT model for calibration and validation of the hydrological model. This model offers an agronomic and geographically faithful representation of the agricultural land use of the watershed. This map was the bases of an effective simulation of alternative agricultural scenarios. It is important to highlight that agricultural and breeding activities are located in pixels that are classified as agricultural pixels in Land Cover 2000 map, moreover pixels represent in the map minimal territorial units are very close to the spatial-temporal situation of the real crop units of the farms in the VLW.

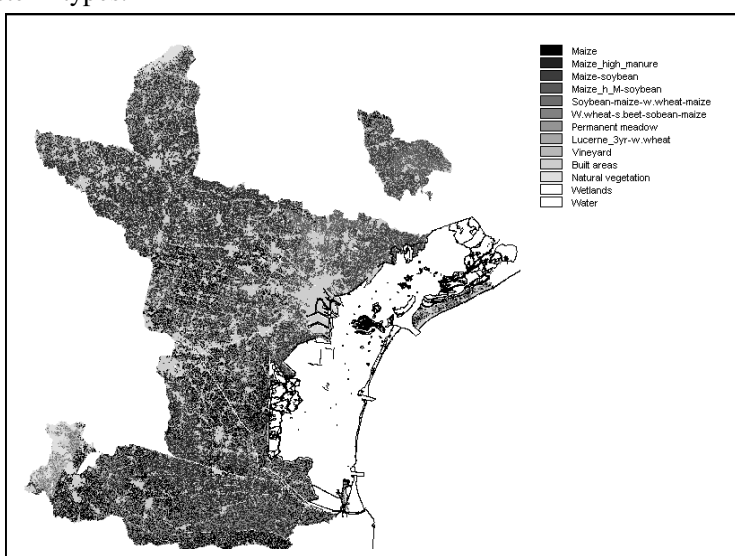


Figure 1 – Agricultural systems map

## Conclusions

The *agricultural systems map* allowed to provide information for the model calibration and scenario analyses utilizing the SWAT model. Moreover the map offered an effective system to analyse the present situation and to assess alternative scenarios of agricultural non-point pollution and its effects on surface water quality. The map regroups different macro-areas of the territory considering homogeneous characteristics allowing to preserve the model spatial representativeness without losing information also at different spatial scale level: VLW, sub-basins and municipalities. This method could manage in an effective, transparent and repeatable manner the agricultural land use description of the current situation, but it is also flexible enough to represent, through convenient modification, possible scenarios that could be imposed by external driving forces (e.g. changes in European agricultural policy) or internal one, like measures or political intervention for environmental or agro-environmental purposes.

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# New Cropping Systems under Constraints

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According to the evolution of economic and agronomic context, new cropping systems have to be proposed, accounting for actual constraints to farms. On the one hand, many studies have been carried out on the design and assessment of crop management techniques used to define new systems (Vereijken P. 1997; Loyce et Wery, 2006; Reau et Doré, 2008) or new cropping systems (Debaeke P. et al. 1996). On the other hand, the cropping system performances could be assessed by models. Meinke et al. (2001), Dogliotti et al. (2004) pointed out the limits of such assessments. Particularly, all the components of the agro-eco-system are not taken into account in the models. These authors concluded that long-term field trials are still necessary, for which methodological frameworks already exist (Debaeke et al., 2008).

The objective of this project has been broken down into three major steps: designing new cropping systems from expert knowledge according to specific constraints, assessing them using models, and implementing the most promising ones in a long-term field trial. The far-reaching objective is to improve cropping systems for arable crops in northern Europe.

## Methodology

First step: new cropping systems were designed according to four different objectives. The first objective was to fulfil several environmental friendliness criteria (bio diversity, pollution, energy use) while maintaining a high yield, thereby meet the farmer's economical requirements. The second objective encompassed the same basic requirement and an added constraint: no pesticides were allowed. In the third objective, the added constraint was that the use of fossil fuel products had to be reduced by half compared to the current cropping systems. The added constraint in objective 4 was that the production of greenhouse gases had to be reduced by half. Expert knowledge was used to design alternative candidates for each of these goals.

Second step: the candidates were assessed by different models. The INDIGO model (Bockstaller et al., 2000) estimates environmental friendliness of the systems, via indicators of bio diversity,  $\text{NO}_3^-$  leaching, fossil fuel consumption, water and air pollution by pesticides... The AMG model (Andriulo et al. 1999) estimates the sequestration of organic C in soil. The CERES-EGC model (Gabrielle et al., 2006) estimates the emissions of  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and  $\text{CO}_2$ .

Third step: for each cropping system, the most relevant candidate will be tested in a long-term field experiment at Grignon (78, France). The agronomical, environmental and economical performances of the cropping systems will be recorded over a long period, and the discrepancies between real and intended performances will be analysed. A monitoring of soil physical, chemical and biological characteristics will be performed.

## Results

The following specifications were identified for each objective.

In order to reach objective 1, cropping systems should include: a long rotation (5 years instead of 3, as is currently observed in Ile-de-France in France) to increase biodiversity and decrease pesticides use,

legumes at least once in the rotation to reduce the nitrogen leaching and fossil fuel consumption, reduced tillage to increase soil organic matter and reduced fuel consumption. The specifications for objective 2 (no pesticides) are: very long rotation (6-8 years), mechanical weeding, alternation of winter and spring crops, use of high resistance varieties, association of species or mixture of varieties, associated with optimal sowing dates and densities to reduce sensitivity to insects and diseases.

Objective 3 (fossil fuel consumption) can be attained by reducing tillage (only 1 year out of 5), sowing as many legumes as possible (main crop, catch crop, association with cereal) and using low N requirement species in order to decrease mineral N fertilisation.

In order to reach objective 4 (greenhouse gases), crops with a high residues production are favoured (maize, wheat...), and their residues are buried. In order to reduce the emissions of NO<sub>2</sub>, mineral N fertilisation is optimized.

Results from the long term field trials are not available yet, as the first crops will be sown in September 2008.

### Conclusions

Expert knowledge allowed us to identify the key specifications that a cropping system has to obey in order to meet the requirements of the 4 objectives and to select, among the remaining elements of the system, those that were compatible with the specified characteristics. This led us to propose up to 100 candidates cropping systems for each objective. The model-based evaluation of these candidates, based on environmental indicators, is currently under progress and should provide us with a reduced number of cropping systems to be tested in the field.

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# Predicting Phenology of *Vicia faba*: Parameter Estimation with CROPGRO-fababean Model Using Multiple Sowing Date Experiments

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Crop models have become valuable tools for designing efficient cropping systems, particularly once model reliability is documented for a given environment. For this use, the timing of crop phenology has to be accurately simulated to predict life cycle and the correct allocation of assimilates to yield components. The CROPGRO-Fababean model was developed based on adaptation of the generic CROPGRO legume model to simulate faba bean grown in Cordoba, Spain (Boote et al., 2002) but the model has not been tested extensively in other environments. Therefore, the model needs to be tested for additional environments, and may need to be modified to improve its reliability under a wide range of field conditions. For the initial model version, phase durations were calibrated against field data collected at Córdoba; however, the cardinal temperatures that affect phenology were derived from the literature. Because our goal was to use these parameters to make reliable predictions in new field environments, we propose that the best way to solve the coefficients is through a calibration process based on field data obtained under varying daily and seasonal temperature and daylength, similar to the method used successfully to calibrate the SOYGRO model phenology.

The objective of this work was to determine quantitatively the effects of temperature and daylength on rate of vegetative node expression, time to flowering, time to beginning pod, time to beginning seed, and time to physiological maturity with the ultimate goal of making the CROPGRO-Faba bean model more reliable over a wide range of sowing date environments.

## Methodology

In order to obtain a data base of faba bean phenology progression under a wide range of temperatures and daylength during the crop growth cycle, three years of experiments with cv. Alameda and multiple sowing dates under non limiting conditions were implemented. The first two years of sowing date experiments were used for parameter calibration, while the third year was used to validate the calibration process. The study was carried out over a period of three agricultural seasons (October 2004 to August 2007) in an experimental field of the Escola Politécnica Superior (EPS) of the University of Santiago de Compostela (USC), located in Lugo (43°04' N; 7°30' W; altitude 480 m).

Because we wanted to develop coefficients that would be useful for improving the CROPGRO-Faba bean model, we used the model itself as a tool for optimizing cardinal temperature and daylength coefficients affecting vegetative and reproductive processes. The optimization algorithm used a simulated annealing method (Goffe et al., 1994), which program was linked with the CROPGRO program, where the two executables alternately share files. The CROPGRO program outputs simulated node number on given day of year or outputs the simulated flowering date or other reproductive phenological event, and these values are then used by the optimization program to calculate sum of squares from simulated versus observed values.

## Results

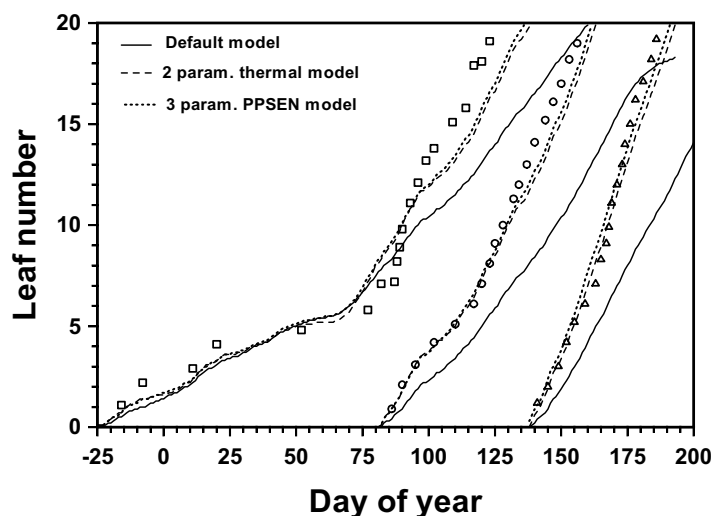


Fig. 1. Predicted and observed values of leaf appearance for three sowing dates of the first experiment (November 5 2004, February 17 2005 and May 5 2005). Default model:  $T_b = 0^{\circ}\text{C}$ ;  $\text{Topt1} = 27^{\circ}\text{C}$ ;  $\text{Topt2} = 30^{\circ}\text{C}$ ;  $T_x = 45^{\circ}\text{C}$ ;  $\text{TRIFOL} = 0.33 \text{ node/PTD}$ . 2 param. Thermal model (calibrated  $\text{TRIFOL}$  and  $T_b$ ):  $T_b = 3.9^{\circ}\text{C}$ ;  $\text{Topt1} = 23^{\circ}\text{C}$  (fixed);  $\text{TRIFOL} = 0.516 \text{ node/PTD}$ . 3 param. PPSEN model (calibrated with daylength effect):  $T_b = 2.2^{\circ}\text{C}$ ;  $\text{Topt1} = 23^{\circ}\text{C}$  (fixed);  $\text{TRIFOL} = 0.516 \text{ node/PTD}$ ;  $\text{CLDL} = 24 \text{ h}$  (fixed);  $\text{PPSEN} = -0.0426 \text{ (1/h)}$

The original model parameters need some adjustments to allow good predictions of leaf appearance rate and main phenological events under wide environmental conditions. This was confirmed by the validation process. Leaf appearance rate predictions improved considerably with higher  $T_b$ , but there was minor effect of daylength. Daylength was very clearly needed for flowering date prediction but later phase durations after flowering did not require daylength effect.

## Conclusions

The simulated annealing program coupled with COGRO-Faba bean and field collected data under a widely variable environment has proved to be an appropriate method to establish the CROPGRO-Faba bean coefficients needed to predict correctly crop phenology.

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# A Simulation Model for the Grain Durum Wheat Quality Traits

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The improvement of quality characteristics of durum wheat grain is one of the objectives of the modern Sicilian durum wheat cultivation. The possibility to estimate these characteristics can represent a valuable aid for the choice of the farmer. The factors influencing the process of nitrogen intake during the filling of caryopsis are due, as well as the characteristics of the variety used, temperature and the water availability and the uptaken N in the soil. The protein content is the main parameter for estimating the grain quality in the countries mainly interested to the durum wheat production.

The grain protein content does not explain all the differences in durum wheat quality; are also important some low molecular weight glutenin subunits denominated LMW1 and LMW2. The production of high quality grain must therefore take into account both its protein content, its protein types, dependent on interactions between the genotypic, environmental and agronomical factors. Very important are the availability of nitrogen in the soil, the air temperature and the soil moisture content during the formation of grain.

On this basis a mathematical model for the estimation of some quality characteristics of durum wheat grain and semolina was developed and calibrated on independent data.

In particular were taken into consideration the following characteristics of quality:

- for grain: protein content, shrunken kernels, starchy kernels, hectolitre weight;
- for the semolina: protein content, gluten index, dry gluten content, W alveographic index, P alveographic index, L alveographic index, G alveographic index, P/L alveographic index.

## Methodology

A previously developed model (Cosentino *et al.*, 2001) was further improved in order to simulate more quality characteristics. In particular the following traits were taken into consideration:

The main case of the model simulate the protein content of the durum wheat seed during its ripening. This character is obtained using as driving factors: maximum air temperature, soil water content, soil nitrogen availability and seed yield.

The protein concentration of semolina is directly linked to the seed protein content and it is determined as the product of multiplying the seed protein content by a genotypic controlled reduction factor ( $S.P. = GP \times Fred$ ).

Taking into consideration the relationship between the protein concentration of semolina and the dry gluten concentration, which is highly significant in many varieties (in “Mongibello” cv.  $R=0,99^{**}$ ), the dry gluten (DG) concentration was simulated according to the following formula

$DG = (DG_a + DG_d + SP) \times K_{gluten}$  where DG is only gluten concentration,  $DG_a$  and  $DG_b$  are coefficients SP is semolina protein concentration and  $K_{gluten}$  is (nitrogen in the soil  $\times K_1$  + (nitrogen fertiliser at sowing + nitrogen fertiliser at top dressing  $\times K_2$ ), where the nitrogen in the soil before sowing is in  $kg\ ha^{-1}$  and  $K_1$  and  $K_2$  are coefficients which takes into account the effect of the preceding crop ( $K_1$ ) and the effect of top dressing ( $K_2$ ).

The values of alveographic indexes was obtained from the observation of the relation between dry gluten concentration and the above said indexes:

$$y = Wa + (Wb + Gs) \text{ for the alveographic index W (strength of dough)}$$

$y = Pa + (Pb + Gs)$  for the alveographic index P (tenacity of dough)  
 $y = La + (Lb + Gs)$  for the alveographic index L (extensibility of dough)  
 $y = Ga + (Gb + Gs)$  for the alveographic index G (baking of dough)

The alveographic index P/L was obtained from the relation between the alveographic index P and L and it shows the incidence of the tenacity compared to the extensibility.

The reliability of simulated results was assessed according to the following formula:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n \left( \frac{x_i - \bar{x}_i}{x_i} \right)^2}{n}}$$

where  $x_i$  and  $\bar{x}_i$  are the observed and the simulated value.

## Results

The calibration of the simulation model was carry out using data of a sample survey conducted in Sicily on 2004 in organic farms cultivating the durum wheat cv. Mongibello. The procedure provides the definition of the coefficients of the different functions in each algorithm in the model. The coefficients derived from the data of experimental trials were used and subsequent adjustments concerned some alveographics indexes have been made.

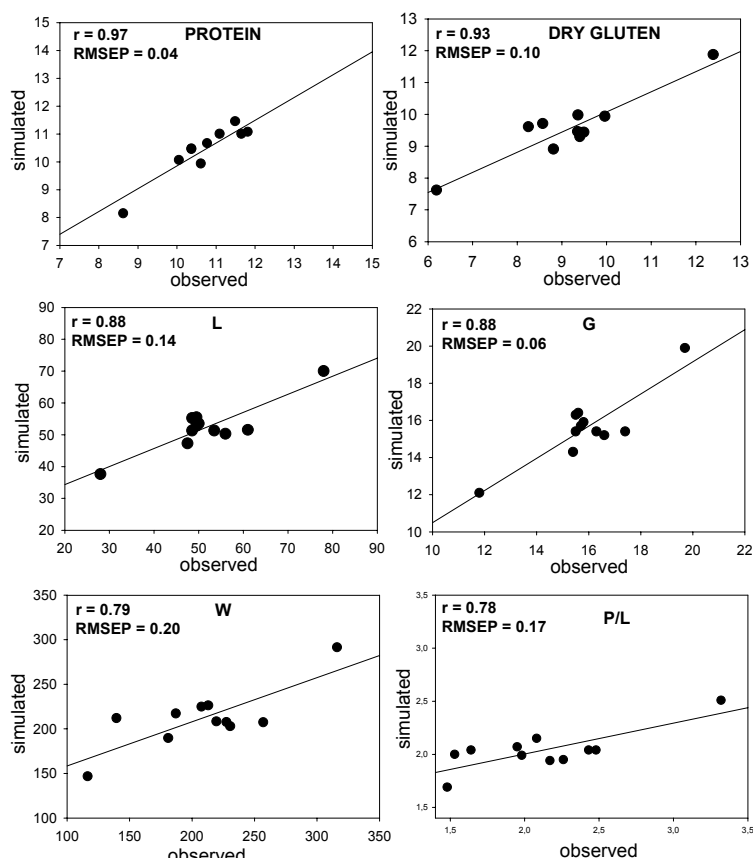
The model was able to simulate reliably the seed protein concentration ( $r = 0.97$  RMSE= 0.04) showing a sound validity of the basic hypothesis. The dry gluten concentration was reliably simulated ( $r = 0.93$ ) even if a certain over estimation of low values and under estimation of high values was observed (RMSE= 0.10). A quite good simulation of G alveographic index was also observed ( $r = 0.88$  RMSE= 0.06), whereas W, L and P/L alveographic indexes showed the same behavior of dry gluten concentration (RMSE 0.14, 0.17, 0.20 respectively for L, P/L and alveographics indexes).

## Conclusions

The simulation of the quality traits of durum wheat semolina of the crop cultivated in organic farming seems to be promising. However a deep analysis of this preliminary results may lead to an improvement of some algorithms.

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# Long Term Effects of Nitrogen Fertilization on Soil Organic Matter: Applications of the DSSAT Model

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## Introduction

The conversion from natural to agricultural ecosystems and unsustainable land management and agricultural practices have often lowered the soil organic matter (SOM) content (Doran, 2002). An increase of SOM is required almost everywhere (Triberti et. al 2008), since it plays a key role to maintain sustainable cropping systems. It prevents soil degradation by limiting soil cracking and erosion, reduces pollution risks by adsorbing toxic substances, improves soil structure, plant nutrients availability and soil microbial biodiversity. Part of SOM which has been lost can be re-sequestered through adoption of recommended soil and crop management practices. DSSAT 4.02 (Jones et al., 2003) has been recently integrated with the CENTURY SOM module (Gijssman et al., 2002) and the module for tillage effects on soil processes, to simulate the long term dynamics of SOM.

The objective of this study was to analyze the long term impact of tillage and fertility management on soil organic matter fractions in a durum wheat-corn rotation in a hilly rainfed area using field experiments and model simulations. In this paper we report the results of the effect of the nitrogen fertilization on SOM.

## Methodology

This study is based on a long term field experiment established at the farm of the Faculty of Agriculture of the Polytechnic University of Marche, in Agugliano (100 m a.s.l., 700 mm mean annual rainfall), in a hilly area (slope: 10-15%) with a silt-clay soil type. The experiment has been designed to compare the effects on SOM of three different soil tillage practices (no till vs 25 cm deep scarification and 40 cm deep plowing) and three levels of nitrogen fertilization (0-90-180 kg ha<sup>-1</sup> N) using a split-plot randomised block design with two replicates (2 for each crop). Results reported in this paper refer to fertilization treatments under conventional tillage (ploughing). The sub-plot size was 500 m<sup>2</sup>. Wheat and corn were alternatively sown on two adjacent groups of 6 sub-plots (3N x 2rep), so that both crops were sown every year.

The long term effect of nitrogen fertilization on SOM was simulated by DSSAT. Observed daily meteorological data (Tmax, Tmin, precipitation) from 1998 to 2006 and daily radiation estimated by Radest 3.00 (Donatelli et al., 2003) were used as meteorological inputs. Soil texture, bulk density, organic carbon, cation exchange capacity, pH, total nitrogen were measured from sixteen different soil profiles within the experimental field, while wilting point, field capacity, saturation hydraulic conductivity were estimated by pedo-transfer functions (Saxton and Rawls, 2006). Grain yield and main yield components were measured in the field for both crops. According to local farm surveys, a 50-year time interval and a durum wheat-corn rotation regularly ploughed and fertilized with 140 kg ha<sup>-1</sup> of N were considered to initialize soil organic matter fractions starting from default values of model (tab. 1).

## Results

Simulation outputs were consistent with field data collected from the long term trial. The long term (i.e. 12 years) dynamics of three different soil organic pools was analysed in relation to contrasting nitrogen

fertilization rates (0-90-180 Kg ha<sup>-1</sup>) and a conventional tillage technique. DSSAT simulations were carried out for the same time interval of the field trial (1994-2007) and results are reported as total soil organic carbon (SOC) in the upper 30 cm of soil (figure 1).

Table 1 – Soil organic matter fractions obtained after initialization, default values in brackets.

Soil texture	Depth	SOM1	SOM2	SOM3
Silty clay	0-30 cm	0.03 (0.03)	0.63 (0.38)	0.34 (0.59)
	>30 cm	0.01 (0.01)	0.09 (0.22)	0.90 (0.77)
clay	0-30 cm	0.02 (0.02)	0.63 (0.34)	0.35 (0.64)
	>30 cm	0.01 (0.01)	0.09 (0.17)	0.90 (0.82)

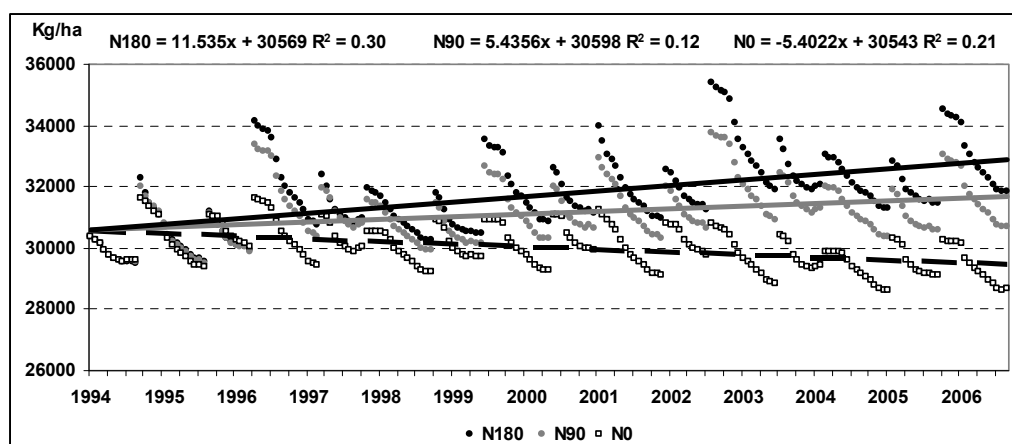


Figure 1 – Soil Organic Carbon content simulated in relation to three nitrogen fertilization levels

SOC showed a slight negative trend in the unfertilized treatment ( $-0.08 \text{ t ha}^{-1} \text{ year}^{-1}$ ), while a slight positive trend was observed with  $90 \text{ kg ha}^{-1}$  of N ( $0.08 \text{ t ha}^{-1} \text{ year}^{-1}$ ). The highest fertilization level ( $180 \text{ kg ha}^{-1}$  of N) resulted in increased SOC sink rate ( $0.17 \text{ t ha}^{-1} \text{ year}^{-1}$ ), mainly as a consequence of the increased SOM2, the intermediate soil organic matter pool.

However, N180 treatment leached  $37.9 \text{ kg [N] ha}^{-1} \text{ year}^{-1}$ ; significantly more than  $23.0$  and  $13.6 \text{ kg ha}^{-1} \text{ year}^{-1}$  leached by N90 and N0 treatments respectively.

## Conclusions

The model estimated a positive trend of SOC under N180 fertilization scheme, but, at the same time, this resulted in higher N leaching. However, leaching was mostly attributed to the long bare soil period between wheat harvest and corn seeding under conventional tillage, in a period in which soil water surplus is very likely to occur. The long term effects on SOM dynamics of different tillage techniques on soil organic carbon dynamics is being considered for further simulations with DSSAT.

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# EPIC Model Simulation of a Central-West Sardinia Traditional Cropping System

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In the '80, EPIC model was developed in order to evaluate soil erosion and soil productivity relationship (Williams *et al.*, 1984). Since its inception, EPIC has evolved into a comprehensive agro-ecosystem model for analysis of cropping systems and cultural practices on production, soil and water quality, water and wind erosion (Williams, 1995). Here we describe the calibration and the utilize of the EPIC model in Arborea (39° 47' 44.2"N latitude and 8° 33' 42.0"E longitude), a Central-West of Sardinia (Italy) area characterized by dairy cow breeding farms in a double cycle maize – annual ryegrass.

## Methodology

Several data sets were developed as EPIC input files. Data sets includes climatic, soil, crop and management information. Data on daily net radiation (MJ/m<sup>2</sup>), maximum and minimum temperatures (°C), precipitations (mm), relative humidity (%) and wind speed (m/s) from 1995 to 2007 were collected from Regional Agrometeorology Service of Sardinia, SAR. The study area soil was classified as Haplic Lixisols (Epidystric, Epicarenic) within the World Reference Base of Soil Resources (WRB), (FAO-IUSS 2006). Soil sampling were carried out in order to determine bulk density, sand, silt and rock content and to perform the chemical analysis to determinate pH, sum of base, calcium carbonate and cation exchange capacity. Soil layers thickness was also measured. Wilting point, field capacity and saturated hydraulic conductivity was estimated using the SPAW model (Saxton and Willey, 2006). For the model calibration, the descriptions and timing of management practices are required. EPIC allows the user to simulate complex crop rotations with several irrigation, fertilizing, pesticide and tillage control options. Historical management data were obtained by farmers interview.

In EPIC model, default crop data have been established by the USDA and do not need to be modified if there is no specific knowledge or specific application (Yang P. et al, 2003). In our case, default data on maximum crop height, maximum leaf area index and plant population both for maize and annual ryegrass were modified by direct sampling. For the calibration of the EPIC model, average production of maize and annual ryegrass from 1995 to 2007 were considered and compared to the simulated production.

## Results

### Model validation

For model validation, the real productions of the last three years (2005, 2006, 2007) of the two crops were compared to the simulated ones and statistical differences were not evidenced between observed and simulated values (Table 1).

### Alternative irrigation and fertilization simulation

Other simulations were carried out in order to evaluate the forage production and number of stress days by a modified irrigation and fertilization adoption. Model calibration was carried out considering the real irrigation timing of 7 days. A new simulation on maize was carried out, considering a water supply to the crop every 4 days. In this simulation, the seasonal water supply was increased 20% in order to

compensate the efficiency reduction caused by the increased number of irrigation operation. Production and number of water stress days estimated by the new simulation was compared with the calibration simulation ones.

Table 1 – Observed and simulated values of crops dry matter yield

Year	Maize yield (t ha <sup>-1</sup> dry matter)		Annual ryegrass yield (t ha <sup>-1</sup> dry matter)	
	Observed	Simulated	Observed	Simulated
2005	17.00	17.50	6.90	7.87
2006	15.50	15.02	6.90	7.19
2007	18.60	16.93	7.70	9.86
<b>Mean</b>	<b>17.03 a</b>	<b>16.48 a</b>	<b>7.17 a</b>	<b>8.30 a</b>

Means values followed by different letters in each row are significant different at P ≤ 0.05 probability level according to LSD test

Table 2 – Maize: dry matter yield and number of water stress days during the crop cycle

	Yield (t ha <sup>-1</sup> dry matter)		Day of water stress	
	Irrigation interval (days)			
	7	4	7	4
Mean (1995-2007)	16.557 a	16.883 a	2.738 a	0.452 b

Means values followed by different letters in each row are significant different at P ≤ 0.05 probability level according to LSD test

Between simulations, statistical differences were evidenced only on number of water stress days during the cycle (Table 2). Another simulation, both for maize and annual ryegrass, was carried out in order to evaluate the effects of manure distribution on production and days of nitrogen stress. Only results on ryegrass are reported below due to the maize ones weren't statistically different.

Table 3 – Annual ryegrass: dry matter yield and number of nitrogen stress days

	Yield (t ha <sup>-1</sup> dry matter)		Day of nitrogen stress	
	Manure application	No manure application	Manure application	No manure application
<b>Mean (1995-2007)</b>	<b>6.923 a</b>	<b>5.406 b</b>	<b>0.007 a</b>	<b>30.680 b</b>

Means values followed by different letters in each row are significant different at P ≤ 0.05 probability level according to LSD test

## Conclusions

After calibration the model was able to simulate the yearly forage production variation without statistical difference. About the different irrigation technique, the simulation by EPIC model evidenced the reduction of the number of water stress days, according to the irrigation technique for sandy soils. Moreover, the simulation on manure application demonstrate that only the ryegrass production was influenced, due to the high quantity of chemical fertilizer usually applied on maize. EPIC model can be considered an useful instrument to study many cropping system, thanks to its simulation capacity of the whole system. In the future, we will utilize the EPIC for the study of soil carbon sink dynamic and nitrogen leaching in the Arborea area.

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# Real Time Production Of Phenological Maps For Italy The Experience Of The Iphen Network

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IPHEN (Italian PHEnological Network) is a co-operative effort for the real time production of phenological maps at Italian national scale, proposed and developed starting from the 2006 growing season. Iphen project was promoted by a group of phenologists and crop modelers, belonging to some universities, agrometeorological services, local extension services, Cnr (National Research Council) and Cra (Agriculture research council). The whole activity in this prototypal phase is referred to vine (*Vitis vinifera* L.) and black elder (*Sambucus nigra* L.) but only the case of vine is hereafter discussed. Data carried out by observers in different areas of Italy are processed at Department of Crop Science of University of Milano and internet broadcasting is carried out by Cma ([www.ucea.it](http://www.ucea.it)).

## Methodology

Maps for vine, referred to the BBCH scale, are produced for two varieties (Cabernet Sauvignon – CS - and Chardonnay - CH). The whole work was referred to a Digital Terrain Model (DTM) of Italy with pixel of 2x2 km and geo-referenced with UTM 32T system.

Coherently with the objective, the extended BBCH phenological scale (Meier 2001) has been adopted. The grapevine BBCH phenological scale represents the conversion of the Eichhorn and Lorenz (1977) scheme into the general one of the BBCH phenological coding system. Differently from the still now frequently used Baggiolini (1952) scale, this methodology allows to constantly assign a phenological growth stage to the grapevines, all through the annual cycle, without any gaps of data.

To produce maps which could include most of the Italian variety assortment and, at the same time, could be comparable with other geographical proveniences, two widespread international cultivar have been selected: Chardonnay, a variety early both in bud break and grape ripening, and Cabernet sauvignon, late in bud break and medium-late in grape ripening.

Phenological phases were estimated on the base of NHH, an analogous of the chill units adopted to evaluate thermal resources. The procedure of normalization was based on a generalized response function which varies from 0 to 1 (each hour at a given temperature receives a weight in the 0-1 range in function of the level of optimality). The function adopted is the beta function discussed in Wang and Engel (1998) which gives 0 for temperatures outside minimum and maximum cardinals (respectively  $T_{min} = 7\text{ }^{\circ}\text{C}$  and  $T_{max} = 35\text{ }^{\circ}\text{C}$ ) and 1 for temperatures at optimum ( $T_{opt} = 26\text{ }^{\circ}\text{C}$ ).

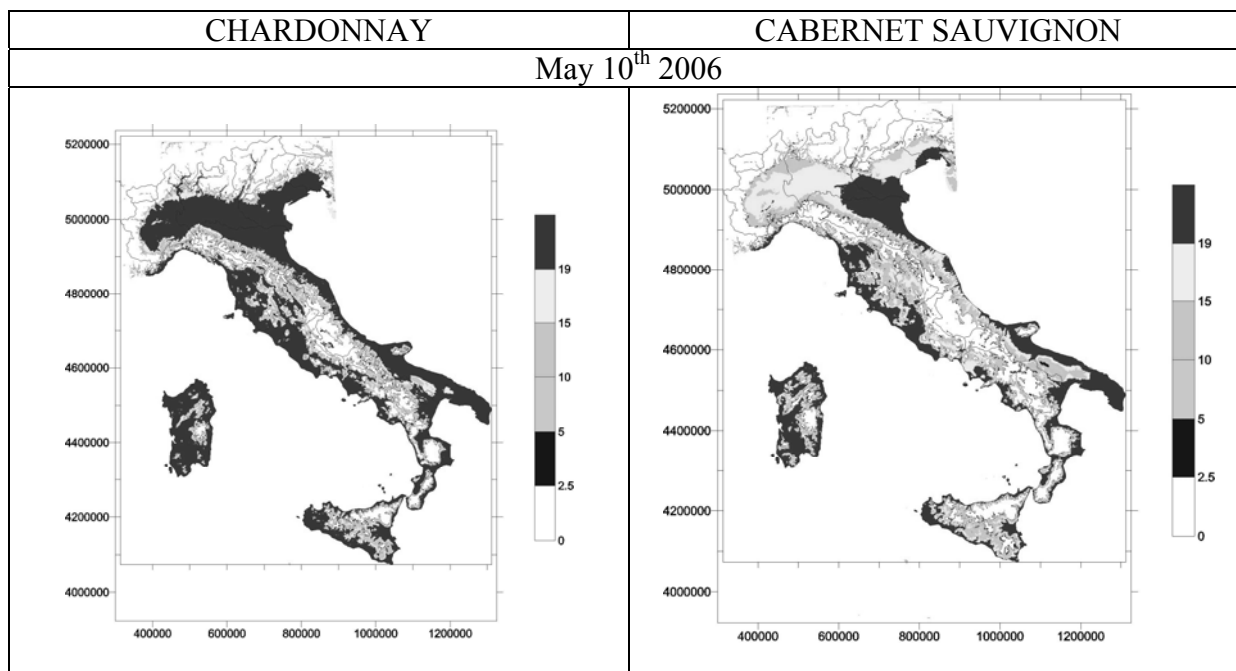
The averages of daily maximum and minimum temperatures ( $T_x$ ,  $T_n$ ) were calculated for 92 meteorological stations of the Italian area belonging to the networks of UCEA – CRA and Air Force Meteorological service

Thermal fields ( $T_n$  and  $T_x$ ) for Italian area were produced by means of the method of inverse square of distance weighted interpolation, applied to the whole set of known data points previously homogenized to the unknown point for height and aspect. Once obtained the mean fields of  $T_x$ ,  $T_n$  for the whole Italian area, the mean hourly data for each grid point were produced applying the Parton & Logan algorithm (1981).

NHH method applied to these hourly data produced a field of NHH for the given day. From the map of NHH cumulated from the beginning of the year a First Guess Field (FGF) of BBCH stages is produced by means of empirical equations calibrated on 2006 and 2007 observations. A match of simulated data with data coming from observers was used to produce an error field (EF) that was adopted for the correction of FGF. In this way a Final Field (FF) was obtained; FF was used to produce maps with a standard Gis program.

## Results

Maps of BBCH phenological phases for Cabernet Sauvignon and Chardonnay were produced in 2006, 2007 and 2008 for the vegetative period (March – October) with a time step of 15 days and are available on the web site [www.ucea.it](http://www.ucea.it). Examples referred to May 10th 2006 are hereafter presented.



## Conclusions

The knowledge of crop phenological course at national scale is an important objective for a number of practical and scientific reasons and can be approached adopting modelling tools which are essential also to get out of the main actual problem of agrophenological networks: the high running costs of a network based on professional observers. Models aren't alternative to human observers due to the fact that phenological behaviour is a result of a quite complex mix of physical, biological and agronomical factors but, on the other hand, models can significantly reduce the need of human observers.

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# Spatially-Explicit Modelling of Mixed Cropping Systems

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Management of the ecological interactions among components of the agroecosystems is a fundamental principle of sustainable crop production. A typical application of this principle is intercropping, where two or more plant species are grown together to benefit from complementary features to optimise use of resources and increase weed suppression ability. Another example is integrated weed management, where containment of weed populations is obtained implementing long-term strategies which enhance the natural competitiveness of crops against weeds.

Spatially-explicit modelling could be an effective tool for supporting decision making in such situations, since the variability in both spatial disposition and emergence time of the coexisting species (weeds and/or intercrops) can strongly affect the system performance.

This idea stimulated the development of MICROS (*Mixed Crop Simulator*), a computer program designed to simulate the growth of multiple coexisting species at any spatial arrangement.

The program allows to quantitatively assess resource partitioning among competing species, with emphasis on light interception, in relation to site-specific combinations of environmental factors. With this approach, crucial determinants of intra- and inter-specific competition can be identified and used to manipulate the competitive relationships.

## Methodology

The modelling approach analysed the soil-plant-atmosphere system following a hierarchical distinction between “low-“ and “high-level” subsystems. The former are subsystems which can be described with no or minimal dependency from other subsystems, while the latter involve interactions among multiple subsystems.

It was therefore defined a higher-level “Environment” subsystem, where bio-physical processes at stand level (e.g. light interception and water relations) take place, and a set of lower-level subsystems, (soil, climate, plant, management).

The system general architecture (Fig. 1), which was developed with a component-oriented approach, maps the conceptual model to a framework of *class libraries*. (Microsoft .NET terminology).

An *Environment* class library simulates the stand-level processes, and interacts with all the other components (soil, plant, climate and management).

The plant growth processes are simulated by a *Plant* class library, which implements a three-dimensional description of individual plant architecture, based on an *IPlant* interface. The individual crown architecture is described as an array of discrete size cubic volumes, termed *Elementary Leaf Units*, which are data structures containing information about area density, orientation and optical properties of leaves. The same approach is adopted for modelling the three-dimensional structure of the root system.

Multiple instances of *IPlant*-derived “plant-objects” can be run simultaneously under the control of *Environment* to simulate a multi-specific plant stand, with each species having its own model to drive growth in responses to environmental stimuli experienced locally.

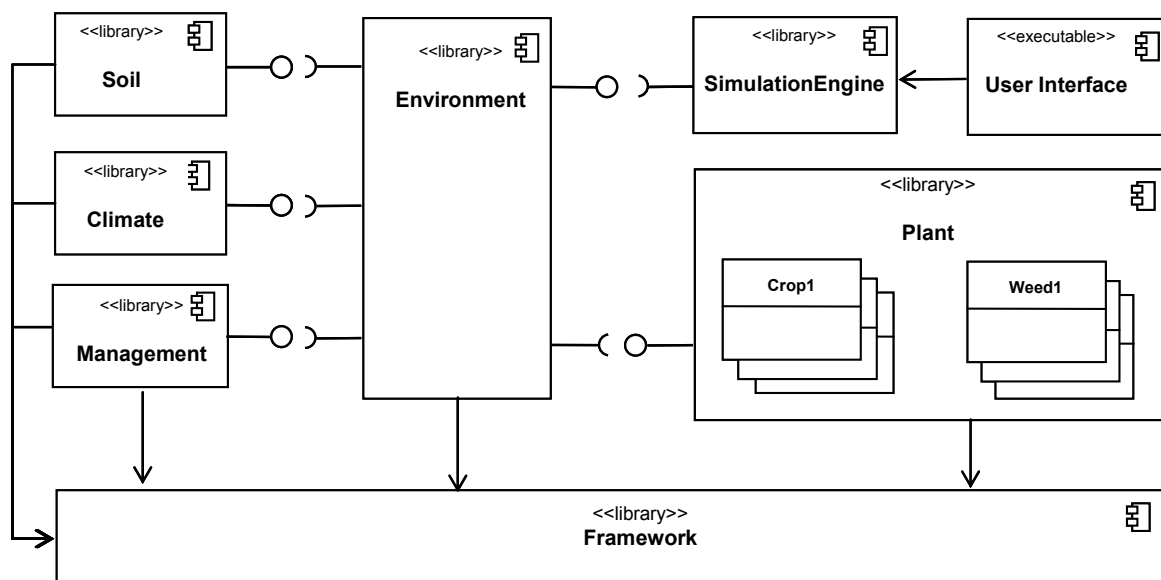
Since plant-objects may incorporate very different growth models, the *IPlant* interface does not expose model parameters. Access to parameters is therefore allowed by means of a metadata exchange protocol mediated by a “*ParameterSet*” class.

The program calculates with a hourly step the distribution of intercepted light within the canopy and the partitioning among the individual plants, then each plant updates its status according to the daily cumulated intercepted light received, and its specific internal growth model.

Different patterns of intra-crown light distribution trigger appropriate plastic responses, e.g.: a strong vertical light gradient can stimulate stem elongation, while a uniform distribution can be associated with higher tillering or branching.

Various crops and weeds growth models are available from the release package or can be loaded from external libraries with a “plug and play” approach.

The other components of the system are Framework (type specifications and basic system functionality), Climate (weather data input), Management (sowing dates and irrigations) and Soil (water and nitrogen dynamics; still under development). SimulationEngine administers the simulation, and User\_interface is the executable providing a graphic interface to allow user’s interaction with the system.



**Figure 1** - UML –component diagram of MICROS

## Conclusions

MICROS was conceived as a tool for designing cropping systems with complex spatial arrangement. In the planning phase of sustainable weed management strategies, the program could be employed to test various combinations of agronomical factors, such as planting scheme, sowing date or choice of cultivars, to maximise crop competitiveness against weeds. Another possible application is the design of intercropping systems, where the objective is to minimise competition effects between the associated crop in order to optimise yield per unit area. A last important application area is crop breeding, where the system could support early testing of architectural traits during the selection process.

# Prediction of Pea Yield (*Pisum sativum*) for North-Central Poland Using Weather-Crop Model

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Pea is one of the most popular leguminous plants in Western Europe; also in Poland it is of particular importance and because of the short vegetation period it is cultivated all over the country. Because of its values an increasing number of publications on modeling the yield of that crop is found.

## Methodology

The paper presents the results of studies on determining the influence of meteorological factors (global radiation, air temperature, precipitation) on growth, development and yields of pea, Karat and Agra cultivars in 3 experimental stations in North-Central Poland. The yield was described using the linear and square multiple regression functions for consecutive phonological phases (tab. 1). The models were verified by cross-validation test. The selected weather-crop model was applied to determine the yield of pea in 2030. The prediction of yield was done using the generated daily data on global radiation, maximum temperature, minimum temperature and precipitation (Kuchar 2004, Rosenzweig 1989) according to the GISS climate change scenario for 2030 and achieved using the WGENK model.

## Results

Table 1 presents the regression equations designed for individual pea development periods, which indicate that the influence of studied variables on the yield was diversified; the coefficients of determination ranged from 55 to 90%, and after passing through the cross-validation test 49-78%.

Table 1. Coefficient of correlation ( $R^2$ ,  $R^2_{adj}$ ,  $R^2_{pred}$ ) and mean square error (RMSE) for selected variables in regression equation.

Station	Period	Variables (in regression equation)				n	R <sup>2</sup>	R <sup>2</sup> <sub>adj</sub>	R <sup>2</sup> <sub>pred</sub>	RMSE
Karat cultivar										
Radostowo	5	TSR4	P3	P5	KP5	15	0.73***	0.62**	0.54**	0.7
Chrzastowo	4	KP2	P2	P4		12	0.72***	0.62**	0.49**	0.8
Karżniczka	5	SR5	KSR5			11	0.55**	0.43*	0.18	0.7
Agra cultivar										
Radostowo	3	TSR3	KTSR3			10	0.62**	0.51*	0.29	0,8
Chrzastowo	3	KSR1	P3			11	0.90****	0.88****	0.78***	0,6
Karżniczka	3	SR3	KSR3			11	0.73***	0.66**	0.56**	0.6

\*, \*\*, \*\*\*, \*\*\*\* - represent significance level at  $\alpha = 0.1, 0.05, 0.01, 0.001$

n - number of observations, K – squared variable

Variable: SR – global radiation (MJ/m<sup>2</sup>)

TSR – average temperature of air (°C)

P – precipitation (mm)

Period: 1 sowing – germination

2 germination – beginning of flowering

3 beginning of flowering – end of flowering

4 20 days before flowers to 10 days after the flowers

5 end of flowering – complete maturity

One of the models obtained was applied to determine the yield of Karat cultivar at the experimental station in Radostowo in 2030. The computed and observed yield values were compared as concerns the average, maximum and minimum values, standard deviations and coefficients of variation (tab.2).

Table 2. Descriptive statistics of observed and simulated yields

Parameter	Simulated	Observed
n – no. of observations	60	15
y – average	4.79	4.93
s <sup>2</sup> – variance	1.08	1.49
s - standard deviation	1.04	1.22
ymin - minimum	2.22	3.41
ymax- maximum	7.68	7.90
ymax-ymin - range	5.46	4.49
v- coeff. of variation	2.17	2.48

The average actual yields were slightly higher than the estimated ones (0,14 t), while the variation of estimated yields was lower, which is also visible in the graph (fig. 1) of the yields distribution probability (Weibull).

Also the maximum yields are lower for the simulated yields. This happens so because the variation of meteorological factors, in particular precipitation, also changed.

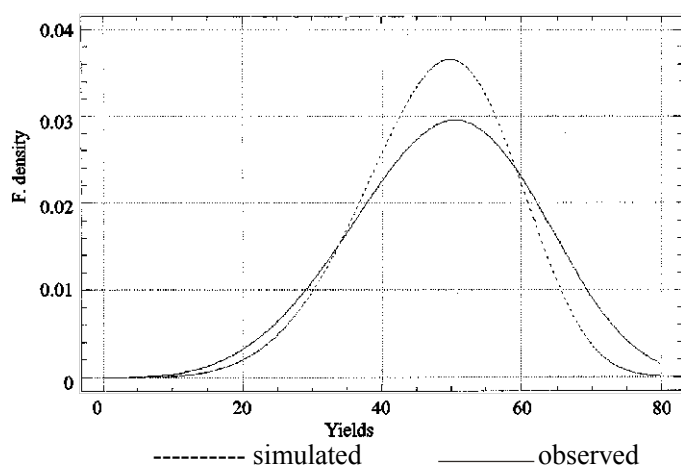


Fig. 1. Probability distribution of observed and simulated yield

## Conclusions

1. The influence of global radiation, average air temperature and precipitation on the yields of pea was diversified depending on the station location, cultivar and vegetation stage.
2. The investigated relations between meteorological factors and yields of seeds of different pea cultivars allow application of selected models for current forecasting of yields at any stage of plants development and at the moment of its ripeness.
3. The comparison of the distributions for actual and simulated yields (based on the synthetic meteorological data and GISS climate change scenario) indicates that the actual yields are slightly higher than those generated for 2030.

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# Sustainable Water Use Securing Food Production in Dry Areas of the Mediterranean Region – An Introduction to a New EU FP7 Project

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## Abstract

The strategic objective of the project “Sustainable water use securing food production in dry areas of the Mediterranean region (SWUP-MED)” is to improve food crop production in the Mediterranean region, influenced by multiple abiotic stresses. These stresses are becoming even more pronounced under changing climate, predicted to result in drier conditions, increasing temperatures, and greater variability, causing desertification. The project will work mainly in farmers’ communities to improve farming systems, by strengthening a diversified crop rotation and using marginal-quality water for supplemental irrigation, aiming at:

- Introduce and test new climate-proof crops and cultivars with improved stress tolerance, selecting promising varieties of cereals, grain legumes and new crops. Climate-proof traits will be identified for breeding programmes using advanced physiological and biochemical screening tools. Supplemental irrigation will be performed as deficit irrigation by different sources of water.
- Investigate the sustainable field applicability of the farming systems, such as environmental effects related to irrigation water quality assessed by monitoring groundwater and soil quality. Financial implications for the farmer and economic costs and benefits in the food sector will be analysed.
- Develop a research synthesis in dialogue with food sector, based on experimental results and advanced simulation modelling to improve farming systems management, utilizing dynamic tools that ease adaptation to the effects of a variable and changing climate. The approach is participatory, involving the farmer’s community and the market and political level.

The expected outcome is improved productivity and sustainable use of agricultural lands by developing a more diverse farming system, supporting economic development in non-European Mediterranean countries while ensuring mutual interest and benefit with the EU. It will accelerate adoption of improved agricultural practices and technologies to meet future constraints imposed by climate changes.

## Strategic objective

Food crop production is restricted in the Mediterranean region. Typical crop cultivation under semi-arid and arid conditions in Mediterranean countries, affected by multiple abiotic stress factors further influenced by climate change, are cereals in low yielding monoculture or eventually combined with fallow. The strategic objective of the project therefore is:

*Improve food production by introducing climate-proof varieties in crop rotations of wheat, grain legumes and new crops (potentially high value food cash crops), in a rainfed system with supplemental deficit irrigation using marginal-quality water and harvested rainwater. This will accelerate adoption of improved agricultural practices supporting small farmers’ livelihood and income levels.*

## Expected results of SWUP-MED

### *New climate-proof crops and farming systems’ management*

Based on an assessment of the farming systems, new varieties and species with improved tolerance to drought and salinity will be introduced in the crop rotations of rainfed and irrigated farming systems. This is expected to interact synergistically with improved agronomic practices. Tolerance to multiple abiotic stresses will be assessed by physiological, biochemical and molecular methods, and new crops with outstanding properties (food quality and multiple stress tolerance) will be selected from the test-material. New irrigation techniques will be tested, such as deficit irrigation, applied as supplemental

irrigation. Three water sources will be mobilized, from rainwater harvesting, saline and treated wastewater, which all contribute to saving of fresh water, and boosting yield of the farming systems of the small-scale farmers of the Mediterranean countries.

#### *Sustainable field applicability*

The environmental effects will be assessed by monitoring groundwater quality and level. Soil related parameters will be evaluated such as salinity, sodicity, hydraulic properties, and organic matter content. The impact of treated wastewater will be evaluated monitoring the levels of potential contaminants (nitrate, metals) in surface and groundwater as well as indicator pathogens. Barriers such as legal issues for the use of the combination of marginal-quality water and improved irrigation systems, and other agronomic strategies, as well as crop species and varieties with improved tolerance to multiple abiotic stresses, will be identified and discussed with key stakeholders. The environmental impact assessment of the introduced interventions will lead to the development of guidelines for best practices to avoid adverse impacts. The financial implications for the farmer, and the economic costs and benefits for the food sector, will be analysed.

#### *Research synthesis in dialogue with food sector*

Important deliverables of the project are crop, field and water management tools for end-users in the agricultural and irrigation sector, and for the environmental sector and water policy planners. SWUP-MED comprises a range of subjects that are of general interest to the public, such as food and water resources. Most of the research will be performed in a participatory manner, involving farmers and their communities directly in the work, and in addition a strong list of stakeholders has been created, to secure constant interaction and mutual benefit with private companies and governmental bodies.

Market is increasing for a range of new crops, which have great potential for increased production to satisfy the increasing demand. New crops introduced by the project will be analysed regarding market and social acceptance. This will be achieved by conducting market surveys for the new crops.

Guidelines and recommendations for the food sector based on field results, surveys and modelling, will be developed as a farmers' application tool for optimising irrigation management schemes, and other agronomic practices optimizing yield, quality and water use efficiency, as well as selection of adequate crops for the farming system. The model will be used to analyse results of the project, and designed to assist farmers and decision makers in the future to better be able to understand the effects of a changing climate, of multiple abiotic stresses, and tools to combat this, applying both agronomic measures and genetic improvement. The project website will be ready at an early stage both for beneficiaries to present results and keep up to date with other parts of the programme, and to provide a focus and point of contact for interested outside parties. Either the tool is for farmers or for the food sector.

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# Combining Methods for Water Saving in Western Balkan

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## Introduction

Drought is one of the most common environmental stresses that may limit agricultural production worldwide. Climate model projections suggest a general increase in temperature together with drier conditions in the south and centre of Europe (IPCC, 1998). The Western Balkan region in general is already suffering from drought, especially during the growing season, and the climate predictions are for the intensity, frequency and duration of these droughts to increase. Serbia is a region rich in natural resources including water for irrigation, but because of financial difficulties in the last decades much of the irrigation system has fallen into disrepair, particularly around the Danube flood plain (at least 75% out of use). 188,000 ha in Serbia is covered with irrigation systems, though irrigation is regularly practiced on only 30,000 ha, that is 3% of total agricultural land. In addition, many surface and underground water supplies in Serbia are seriously contaminated with chemical and microbiological pollutants. In Macedonia there was a trend for decreased irrigation efficiency which is expected to continue because of unfavourable climatic conditions during the main growth season. A reduction of the area under irrigation has also occurred because of administrative and operational difficulties, as well as farmer reluctance to pay water charges. The strategic objectives of the project Water Resource Strategies and Drought Alleviation in Western Balkan Agriculture (WATERWEB), EU FP6, were:

- to contribute to development in the Western Balkans (WB) by introducing strategic water management for drought alleviation and sustainable agricultural practices in the WB,
- to establish and reinforce research expertise in the WB in a range of technologies for water and crop management.

## Main results

The investment, installation and data collection from hydrological equipment and the creation of GIS databases for the Land-Water-Economy Information System (LWEIS) is an important tool to introduce appropriate irrigation systems in the WB region, and to analyse quantity and quality of groundwater and surface water with respect to the quality of the water to be used for irrigation purposes. There was no permanent N contamination of irrigation water, however, periodic increases of N in Serbia originating from sewage leakage was alarming. Nitrate ( $\text{NO}_3^-$ ) represents the most common contaminant introduced to groundwater systems from growing anthropogenic sources. Agricultural practices such as N fertilizer applications, mismanagement of irrigated crops, livestock waste disposal and virgin land cultivation stand as the most extensive sources of  $\text{NO}_3^-$ . The easy transport of nitrate in groundwater systems might promote surface water eutrophication or threaten human and livestock as well as the environment. 57% of water samples had nitrate levels above WHO water quality standards (50 mg  $\text{NO}_3^-/\text{l}$ ). Water quality analyses of the groundwater pointed at anthropogenic source of pollution. High concentrations of  $\text{Ca}_2^+$ ,  $\text{Mg}_2^+$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  reveal the polluted nature of the groundwater due to the application of chemical fertilizers  $(\text{NH}_4)_2\text{SO}_4$  and  $(\text{Ca}, \text{Mg})\text{CO}_3$ . Analysis of data that include chemical composition of groundwater at farm PKB "7. Juli", showed that water quality at the experimental field was not recommended for irrigation, because of  $\text{HCO}_3^-$  toxic effect, and low toxic effect of  $\text{Na}^+$  and  $\text{Cl}^-$ .

Also contamination of vegetables was detected, induced by washing with channel water containing *E. coli* and *Salmonella*. Different decontamination procedures of the crops included disinfection by UV or organic solutions, however, prevention of contamination is preferred. In Macedonia all water samples were seriously contaminated with *E. coli*, and therefore they could not be used for any purpose. Modern methods for detection of pathogens and other contaminants in water, and technologies for reducing contamination impact, will be of importance in the WB region, where legislation and implementation of EU standards targeting water for irrigation is low. New methods and more focus on the subject of water, as gained through Waterweb, will help to improve existing quality criteria for water for irrigation in Serbia and Macedonia.

Climate predictions for Serbia and Macedonia are an increase in drought events, which will increase their dependence on irrigation and especially on techniques for more efficient water use, such as deficit irrigation (DI). Further investigations of DI techniques will favour agricultural production in Western Balkan. Implementation of DI, for instance alternate irrigation has the potential to save water, increase water use efficiency, improve end product quality, and reduce the risk of leaching of nutrients. Alternate irrigation is a novel irrigation technique for Western Balkan area, where it was tested in grapevine, tomato and maize with promising results, with an improvement of fruit quality, and an increased water-use efficiency. Field studies under various climatic and soil conditions, and knowledge of drought sensitive stages of each crop are needed in order to optimize the strategies.

Farms supplying foreign-owned buyers are noticeably larger (mean of 16.45 ha) than those supplying domestic buyers (6 ha). It was demonstrated that these small-scale producers in Serbia risk being marginalised into low-value markets. One option as a new crop for Macedonia and probably Serbia could be quinoa, which has a high nutritional value, with a high content of protein, a perfect amino acid balance, and a high level of a range of vitamins and minerals. It makes it interesting as a new crop for high quality food in Macedonia and in Europe in general. The water reform has stimulated a significant increase in cost recovery rates, and now a majority of farmers in Macedonia have joined a water community.

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# Synthetic Assessment of Sustainable Agricultural Development for Poland's Regions

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## Introduction

Agricultural areas in Poland are strongly differentiated with regard to the environmental qualities, agricultural production capacity and the level of productivity (Harasim, 2001). In an assessment of agricultural performance of Polish regions, the analysis of productive efficiency and economic efficiency of production factors (land, labour and capital) has prevailed by now. In this approach there are not considered environmental effects of agricultural activities which for the reason of their unproductive character are difficult to be linked directly with the effects of production. At present, there is a growing need to apply a holistic approach for the assessment of agricultural production, both at the farm scale and the regional scale. It is indicated by the EU strategy calling for the harmonious, balanced treatment of main pillars (environmental, economic and social) of sustainable development (EU-COM, 2001). Since the recognition of sustainable development as a strategic goal for the EU policy many criteria and parameters have been created for the assessment of this process in agriculture. However, they allow only for the partial, fragmentary appraisal of different aspects of farming. The undertaken attempts of aggregating various indicators by the different methods of grouping are not satisfactory because of subjective nature of weighting process by which different indicators are combined. The most important task in holistic description of sustainability is integrating all indicators, both of quantitative and qualitative nature, into one single synthetic index capturing multi-dimensional character of sustainability (Zhou et al., 2006). The aim of this research was to obtain the synthetic assessment of sustainable agricultural development for Poland in relation to regions.

## Methodology

Regional data on agricultural production and economic performance were collected from Poland Statistical Yearbook (GUS, 2006). Nitrogen and phosphorus balances as well as nitrogen emission into atmosphere at the regional level were determined with use of model of nutrient flow in farms (Bieńkowski et Jankowiak, 2006). For measurement of sustainability development level, the non-parametric DEA method of optimization was applied (Despotis, 2005). Assessment of the synthetic sustainability index was achieved in a three-step process. In the first step allocation of indicators (variables) into four sub-groups was carried out based on their similarity. In the second stage, indicators within each of sub-groups were subjected into aggregation in the DEA model in order to generate sub-indexes. Principle behind the choice of sub-indexes was that they had to consider different types of basic functions performed by agriculture in the regions. The following sub-indexes were distinguished: environmental efficiency, spatial efficiency, economic efficiency and productive efficiency. In the last stage, these sub-indexes were grouped on the "output side" in another DEA model to create synthetic index of sustainable agricultural development (IS). By integration of component sub-indexes describing different activities into one, composite index, by the DEA method, it was possible to exclude bias preferences for the analyzed sustainability dimensions.

## Results

It has been shown that synthetic index is spatially differentiated (Fig. 1). The group of six voivodeships (zachodniopomorskie, lubuskie, dolnośląskie, opolskie, mazowieckie and podkarpackie) has scored

values of synthetic index above 0,95, indicating a high level of realizing the sustainability principles by them. However, only zachodniopomorskie voivodship has proved to be fully efficient in terms of the synthetic index.

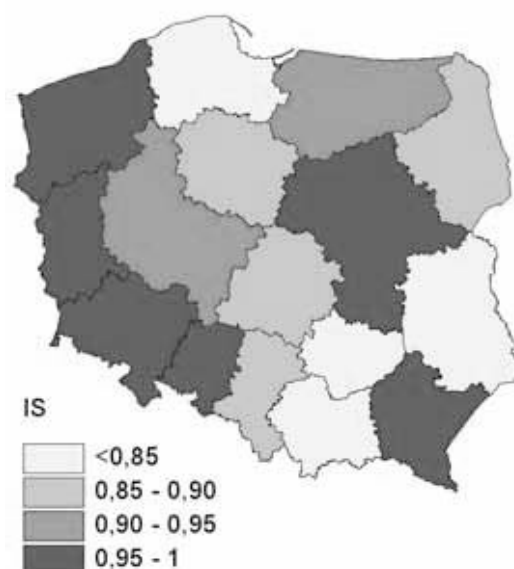


Figure 1. Spatial differentiation of synthetic index (IS) according to regions

## Conclusions

1. Presented method of synthetic assessment of sustainable development for Polish regions, due to additional inclusion of environmental and spatial aspects can be used as a basis for the comparative, extended evaluation of agriculture performance among the regions.
2. Synthetic index of sustainability performance of agriculture can be an effective tool of informing about the threats for the functioning of economical and environmental processes at the regional level.
3. The research indicates that the development of agriculture in the majority of regions does not proceed in a sustainable way, taking into account environmental and economic issues. The reasons for lower sustainability level were different depending on the region. In the south-east regions lower values of synthetic index were caused by the decreased economic efficiency, while in the Wielkopolska and Kujawsko-pomorskie regions – by the reduced environmental efficiency. Thus, realizing the strategy of agriculture development specific solutions should be directed either, depending on the regions, to the improvement of the state of the environment or to creating better conditions for agricultural economic processes in regions.

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# The Advantages of Alternating Over Common Deficit Irrigation in Tomato Plants

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Irrigation water resources are limited worldwide and there is an urgent need to increase water use efficiency (WUE) of crops. Deficit irrigation (DI) and alternating deficit irrigation causing partial root-zone drying (PRD) are water-saving irrigation strategies presently being investigated in many countries. DI is a method of irrigating the entire root zone with an amount of water less than the water losses by evapotranspiration while PRD involves irrigating only one part of the root zone leaving the other part to dry to a predetermined level before the next irrigation. In the present experiment we studied physiological reactions and fruit quality of tomato plants under different deficit irrigation treatments.

## Methodology

A long-term PRD pot experiment on fresh tomato plants (cv. Cedrico) was performed in a glasshouse in 2007. The plants were grown in split-root pots filled with a mixture of peat, clay soil and sand (4:1:1 v/v/v) in a glasshouse at 26/20 °C day / night. From flowering (March) to the end of fruit harvesting (July) plants were subjected to three watering regimes: (1) full irrigation (FI), in which the whole root system was irrigated close to field capacity; (2) deficit irrigation (DI), in which only 62 % of water for FI was irrigated to the whole root system; (3) PRD, in which 62 % of water for FI was irrigated to only one half of the root system and the irrigation was shifted between sides every 6 days. For each treatment 16 plants in four blocks were used. The blocks differed in air humidity and temperature also in the vapour pressure deficit (VPD) because of heterogeneous forced airflow and insolation in the glasshouse. The stem growth apex of the plants was removed after the appearance of one leaf above the 5<sup>th</sup> cluster. Before onset of the treatment, fresh weight (FW) and dry weight (DW) of roots, leaves and stems were recorded and samples of roots and leaves were collected for abscisic acid (ABA) analysis. During the drying cycles the following measurements and samplings were performed: (1) Soil water content manually with TDR system CS620 HydroSense® System daily in all pots; (2) Chlorophyll fluorescence analyses by fluorometer PAM 2000, every 2 days, twelve replicates per treatment; (3) Stomatal conductance by porometer Delta A4, every 2 days, twelve replicates; (4) Leaflet elongation, every 2 days, twelve replicates per treatment; (5) Water potential (WP) by pressure chamber, once a week. The leaflets cut for WP measurements were collected for osmotic potential (OP) and ABA content measurements. During the 55 days of the experiment FW and DW (after 7 days at 60 °C drying) of rape fruits of the first five clusters were determined. In the fruits the content of reducing sugar as well as pH, osmolarity and content of antioxidants of fruit sap were measured by DPPH method. After 16 drying cycles the following measurements and sampling were done: (1) leaf area, FW and DW of leaves and stems were determined; (2) sampling and freezing of leaves and roots for ABA measurements were done; (3) extracting roots from six pots for each treatment and determining their DW were performed; (4) collecting samples of soil from six pots from each treatment for soil analyses (N content analyses in the form of nitrate and ammonium, pH value) was done; (5) sampling (ca. 1 g DW) from DW of leaves, stems and roots was done for total N content analyses by N/protein analyzer.

## Results and Discussion

Long-term (100 days) deficit (DI) and alternating deficit (PRD) irrigations show a significant interaction between the irrigation method and the water vapour pressure deficit (VPD) as well as the evapotranspirative demand around plants in blocks (rows of plants). The advantages of PRD irrigation were most evident in the peripheral row, in which the evapotranspirative demand was highest. During long lasting deficit irrigation an irreversible adaptation of tomato plants to water shortage conditions was observed. As consequences of the general adjustment we observed: (1) reduction of the yield of fruits, which was smaller in PRD than DI; (2) increase in water use efficiency (WUE), which was significantly bigger in PRD than DI (Fig. 1). The reason for this was probably the partial closed stomata in PRD plants during drying cycles. The stomatal closure was more pronounced in PRD than in DI. The partially closed stomata did not, however, limit the photochemistry efficiency in PRD plants as measured by chlorophyll fluorescence parameter effective quantum yield of PSII (3) increase in sugar and antioxidants content of fruits, which was significantly higher in PRD than in DI (Fig. 2).

## Conclusion

When comparing PRD with DI, PRD caused smaller fruit yield reduction, higher WUE and higher quality of fruits than DI. The advantages of PRD compared to DI were greatest at high VPD.

## Acknowledgements

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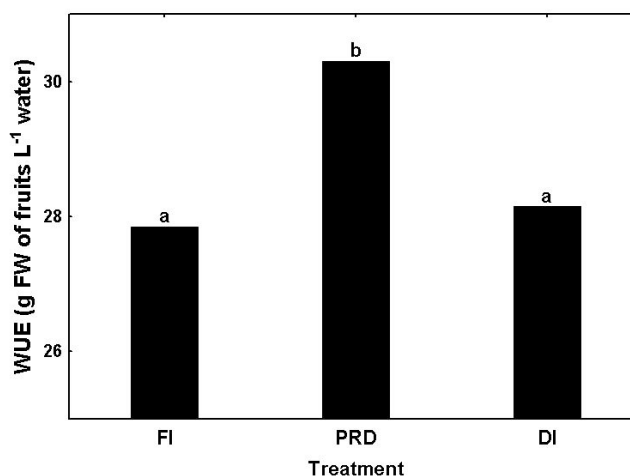


Fig. 1. Water use efficiency (WUE) in the production of fruits by tomato plants under different irrigation types: FI - full irrigation, DI - deficit irrigation, PRD – alternating deficit irrigation.

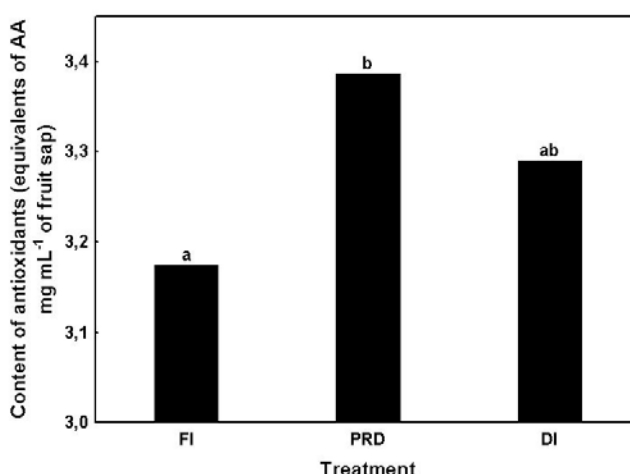


Fig. 2. Content of antioxidants as an equivalent of ascorbic acid (AA) in the fruit sap of tomato grown under different irrigation types: FI - full irrigation, DI - deficit irrigation, PRD - alternating deficit irrigation. Values denoted with the same letter do not differ significantly at  $P < 0.05$  according to Duncan's multiple range test.



# SIPPOM-WOSR: a Simulator for Integrated Pathogen Population Management to Design Control Strategies against Phoma Stem Canker on Winter Oilseed Rape, Maintaining the Efficiency of Specific Resistances

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Combining pest control methods in space and over time allows satisfying economical, ecological and toxicological requirements of Integrated Crop Management (ICM), and to preserve the efficiency of control methods. The use of resistant varieties is one of these pest control methods. However, the wide use in a region of cultivars with the same specific resistance gene to control a disease often leads to the total loss of efficiency of this resistance in a couple of years. To enhance the durability of specific resistances, Integrated Avirulence Management (IAM, Aubertot et al., 2006) consists in limiting the selection pressure exerted on pathogen populations by reasoning the cultivar deployment, and reducing the size of pathogen populations by combining control methods. When dispersion of spores exceeds the field scale, it is necessary to design combinations of control methods at the regional scale. And because of polyetical processes in epidemic cycles of diseases, it is necessary to manage the disease at the pluriannual scale. At these scales, field experiments are difficult to set up, and modeling is a suitable approach.

## Methodology

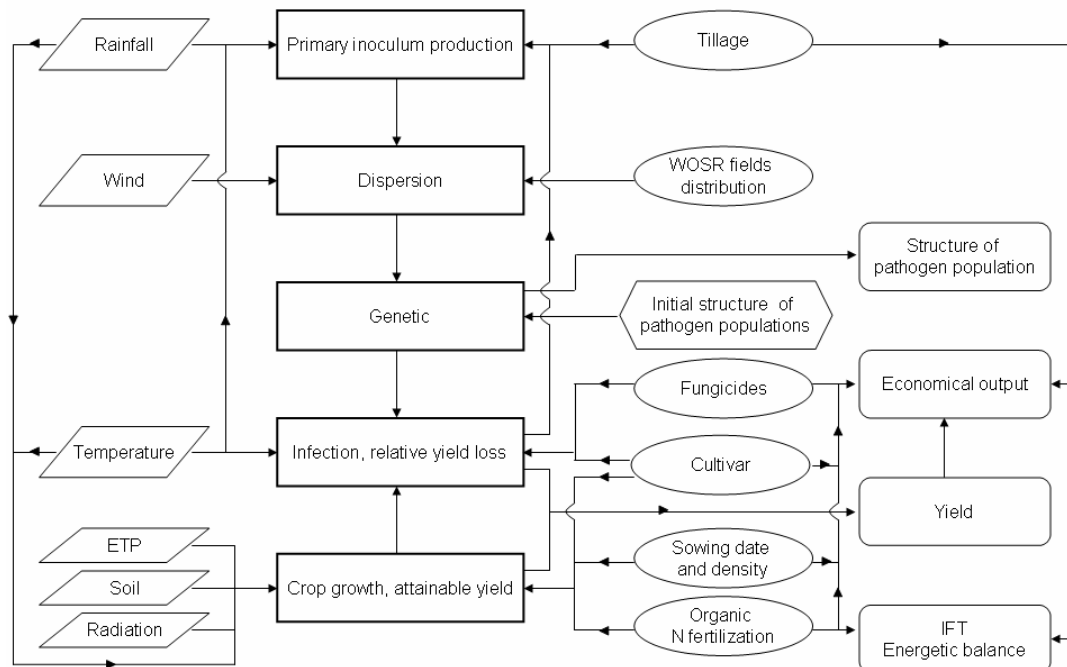
A model was developed for the control of phoma stem canker, one of the most economically damaging diseases of winter oilseed rape, caused by the species complex *Leptosphaeria maculans*/*L. biglobosa*. The main efficient control method against phoma stem canker is genetic through the use of cultivars with specific or quantitative resistances. Infection of a specific resistant cultivar occurs only if ascospores (the primary inoculum) don't have or have lost the corresponding avirulence gene, but specific resistance lacks durability. Quantitative resistances limit the systemic progression of the fungus within the plant and therefore the canker severity (responsible for yield loss). Fungicide treatments can limit the primary infection. Cultural practices, such as sowing rate or organic nitrogen application before sowing, have an impact on the leaf area receiving ascospores, and thus on the risk and intensity of infection. Shifting the sowing date can prevent the coincidence between ascospores release and the most sensitive stage of oilseed rape to infection. After harvest, soil tillage can reduce the quantity of primary inoculum by burying infected stubble and preventing pseudothecial maturation, and thus release of ascospores. Disease control can be improved by reducing spore flow between fields, which requires considering the spatial distribution of oilseed rape fields within the landscape.

A spatially explicit model has been implemented, SIPPOM-WOSR, a Simulator for Integrated Pathogen POpulation Management, to simulate the effects of cropping systems and their spatial distribution on phoma stem canker epidemics over years, at a regional scale, along with the adaptation of *L. maculans* populations to oilseed rape specific resistances. Most sub-models have been evaluated independently, and a sensitivity analysis to parameters variation has been carried out to test the stability of the ranking of control strategies. Simulations with contrasted crop managements and cultivar deployments have then been performed to test the behavior of SIPPOM.

## Results

SIPPOM is composed of 5 sub-models simulating primary inoculum production, dispersion of ascospores, changes of the genetic structure of pathogen populations over time, infection and yield loss, and crop growth dynamic (Figure 1).

Figure 1. Flow chart of SIPPOM-WOSR. Sub-models are represented in squares, climatic and soil inputs data in diamonds, technical inputs data in ovals, and outputs data in rounded squares. The structure of pathogen populations is an input variable (initial structure), a state variable (simulated each year) as well as an output variable. The quantity of inoculum also depends on the severity of the disease in the previous year (Lô-Pelzer et al., 2008)



The user of SIPPOM determines the spatial distribution of fields in the landscape, the crop sequence associated to each field and all the cultivation techniques: tillage, organic nitrogen, sowing date and rate, and oilseed rape cultivar (specific and quantitative resistances, potential yield), chemical treatments, fertilizations. Soil characteristics have to be described for each field, along with daily climatic data. Output variables of the model are epidemiologic (disease severity, yield loss), agronomic (yield), economic (simplified gross margin), environmental (Treatment Frequency Indexes and energy cost of cultural practices), and genetic (pathogen population structure), in order to consider several criteria of assessment of ICM strategies.

Preliminary simulations have shown the capacity of SIPPOM to represent the effect of cropping systems and their spatial distribution on phoma stem canker and on the preservation of efficiency of specific resistance. In particular, they demonstrated that it is possible to enhance the durability of the efficiency of a specific resistance gene if a cultivar management limiting the pathogen population size is applied on fields with the specific resistant cultivar.

### Conclusions

SIPPOM is the first spatially explicit model that takes into account genetic, cultural and chemical control methods together, and the spatial distribution of cropping systems to evaluate management strategies of specific resistances. It can be used to rank integrated management strategies of a disease according to how well they maintain efficiency of specific resistances against phoma stem canker, and how well they limit environmental costs and enhance economical profits. SIPPOM-WOSR could also be adapted to other pathosystems.

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# Effect of Climate Change on Water Use and Irrigation Requirements of Muskmelon and Broccoli in Southern Europe

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## Introduction

CO<sub>2</sub> atmospheric concentration is increasing and model forecasts suggest there will be a consequent global warming and changes in precipitation patterns (Kimball, 2004). The expected temperature increase and the change of rainfall patterns require an in-depth analysis of the assessment of crop evapotranspirative needs for optimizing irrigation practice. In this study, the change in water use and irrigation requirements of two crops (broccoli and muskmelon), typical of Mediterranean agriculture areas of Basilicata region (southern Italy), is reported.

## Methodology

Water and irrigation need assessment was carried out on broccoli and muskmelon crops. Agro-meteorological data from the period 1961-2006 measured at the Metaponto station (MT, 40°00' N, 16°48' E), statistically processed in order to point out the mean time trends, were taken into account. Measured data were temperature, rainfalls, wind speed, solar radiation and relative humidity. The

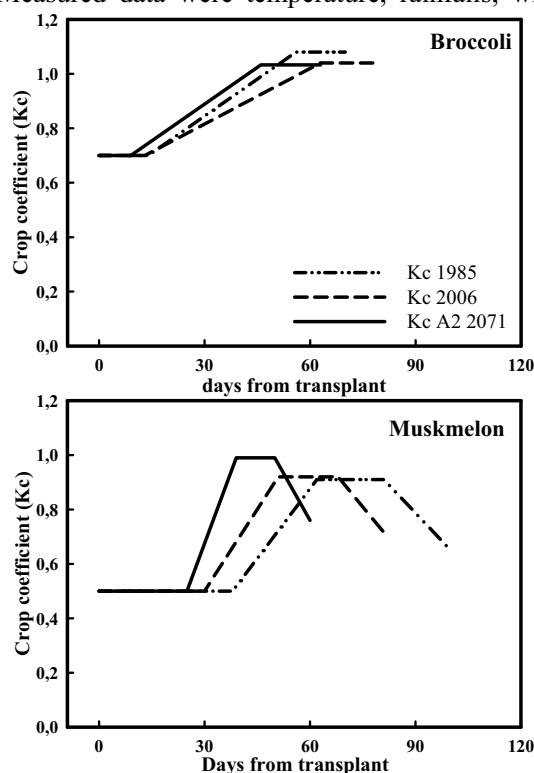


Fig.1 Daily pattern of crop coefficients (Kc) during broccoli and muskmelon cycle in 1985, 2006 and A2 2071.

measured climatic trend was compared with a future climatic scenario for the 2042-2071 period obtained by a regional circulation simulation model (HadRM3P), having a spatial resolution of 0,44° latitude and 0,44° longitude and representing the result of a dynamic downscaling. Simulation model was validated and calibrated using data from the 1961-2006 period measured in the investigated geographic area. In order to simulate the climate change an emission scenario was taken into account (A2, 900 ppm CO<sub>2</sub>) among those proposed by the Special Report on the emission scenarios of IPCC (2000). The potential water deficit was obtained assessing reference evapotranspiration (ET<sub>o</sub>) by the Penman-Monteith equation, on daily basis, properly recalibrated in the resistive terms for the future scenario (Kimball, 2004). Effective rainfalls were calculated using the USDA method. The two step approach (ET<sub>c</sub>= ET<sub>o</sub> x K<sub>c</sub>) was followed to assess water needs, where ET<sub>o</sub> was assessed with the Penman-Monteith equation, while the K<sub>c</sub> for both crops (broccoli and muskmelon) were corrected taking into account the data of relative humidity and wind speed actually measured in 1985, and simulated for 2071 according to the suggestions given in the FAO Paper n.56 (Allen et al., 1999).

The impact of climate changes on phenological phase length was evaluated using the thermal sum method. The temperature of 5°C was considered as the zero vegetation value for broccoli, while 12°C was the zero vegetation value in muskmelon.

## Results

About the historical measured period, from data analysis it came out that starting from 1985, mean annual temperatures showed a trend to increase. Moreover the pluviometric regime changed compared to the trends measured in the previous years. In 1985, a mean annual temperature of 15,5°C and a total

Table 1 – Changes of temperature, rainfall and water deficit mean values in 2006 and A2 2071, compared to 1985.

	2006	2071
Mean annual T (°C)	+ 1.3	+ 4.8
Rainfalls (mm)	+ 83	- 77
Water Deficit (mm)	- 90	+ 327

year rainfall of 486 mm were recorded. Table 1 shows that, compared to 1985, mean annual temperature increased by 1,3°C in 2006 and, on the basis of data obtained from the A2 simulation, a further increase of 4,8°C is expected in 2071. Despite a strong annual variability, rainfall statistical analysis pointed out a simple tendency to a moderate decrease. In such a defined climate situation the potential water deficit in 2006 was roughly similar to that measured in 1985, while in 2071 its increase is expected (+327 mm).

Figure 1 shows broccoli's and muskmelon's crop coefficients change in the three compared climatic scenarios (1985, 2006, A2 2071). As it is clearly shown in figure 1, numeric values of crop coefficients of both crops don't decisively change, but the length of the single phases do. Especially in muskmelon, the length of the whole crop cycle decreases by 40 days going from the 1985 to the 2071 scenarios. In broccoli, a reduction in water need from 1985 to 2006, due to abundant rainfalls (186 mm useful rain), while as regards to 2071, water need is basically similar to that measured in 1985 (Figure 2). As regards to muskmelon, both ETo and ETc decrease going from 1985 to 2006 and 2071, due to the significant crop cycle reduction, especially in 2071 (Fig. 2). Rainfalls, almost unchanged between 1985 and 2006, drop dramatically (around 90%) in 2071. However, in melon, the significant shrinkage of crop cycle compensate for the great rainfall reduction. For this reason, water need in 2071 doesn't change basically compared to 1985.

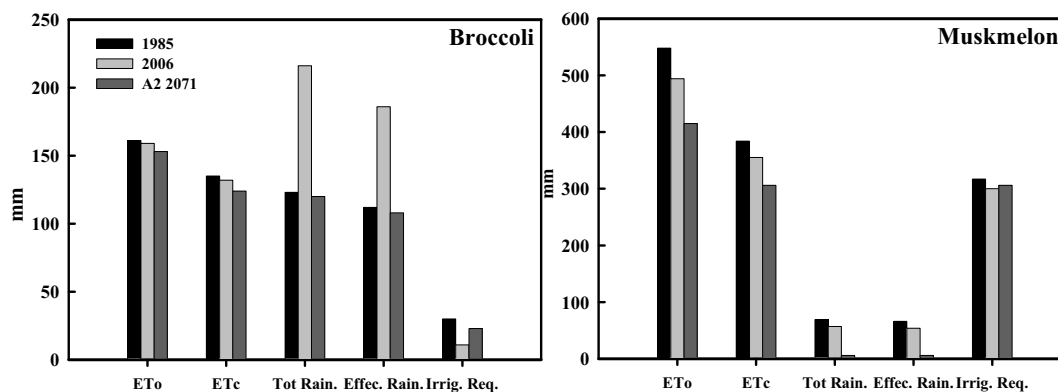


Fig.2 ETo, ETc, total rainfall, effective rainfall and irrigation requirements of broccoli and muskmelon in three climatic situation considered (1985, 2006, A2 2071).

## Conclusions

In the future climate scenario we took into account, despite the temperature increase and the contemporary expected rainfall reduction bringing to a rise of the potential annual water deficit, from the analysis of the results came out that water use and irrigation requirements could stay unaltered due to the significant reduction of crop cycle length determined by the increase of thermal regime, especially for spring-summer crops as muskmelon.

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# Stream Flow Modeling Using WEPP (Water Erosion Prediction Project) in a Northern Italian Watershed

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## Abstract

This paper illustrates the Water Erosion Prediction Project (WEPP) model's performance, to predict stream flow in a study conducted in a watershed in the Apennines Mountain Range, northern Italy. Stream flow and weather information were measured on a daily basis for 9 years. Topography, soil and land use data were also collected. A sensitivity analysis showed that the most sensitive parameters were the soil hydraulic conductivity and soil albedo. A comparison between observed and modeled stream fluxes indicated that the model provided reasonable estimates, although the model tended to overestimated the large events and under estimate the low events. Since low flux events were quantitatively dominant, overall the cumulative annual fluxes were underestimated.

## Methodology

The model application was performed at the experimental watershed of the University of Bologna (area 192 ha and elevation a.s.l. 200 m), which is located in the Centonara watershed (44°24'N, 11°28'E), southeast of Bologna, Italy. Observed fluxes from the Centonara stream were collected on a daily basis. Temperature, relative humidity, wind velocity and direction as well as net solar radiation were measured and provided as input to the model, as well as topography, soil properties and land use. The WEPP (Flanagan and Nearing, 1995) model was employed to simulate the water balance and stream flow for a nine years period (1999-2007). Model's sensitivity was tested applying a sensitivity test, while model performance was evaluated by comparing observed and modeled annual and daily stream fluxes, using the Root Mean Square Error (RMSE) and the Nash-Sutcliffe (NS) statistical indexes.

## Results

The results showed low model's sensitivity to anisotropy of hydraulic properties and soil depth, and high sensitivity to soil hydraulic conductivity and soil albedo. Figure 1 showed daily precipitation and a representative observed and simulated hydrograph, for the year 2002. Modeled and observed fluxes were in good agreement for the years 1999, 2000, 2001, 2002, 2003, 2006 and 2007 with low NS indexes, but with inter-annual variability. For 2004 and 2005, larger errors in model performance were found and due to lack of experimental data, as indicated by the high percentage of missing data. A detailed event-based analysis showed that when the stream fluxes were of high intensity, the modeled stream fluxes were over predicted, in particular the model error increased when intense stream fluxes occurred after a dry period (Figure 1). The variability in model performance may also be due to the need of providing more detailed inputs to the model (such as soil tillage), and spatial variability in soil depth across the watershed, that were not provided as input to the model (the soil depth was assumed to be uniform across the watershed).

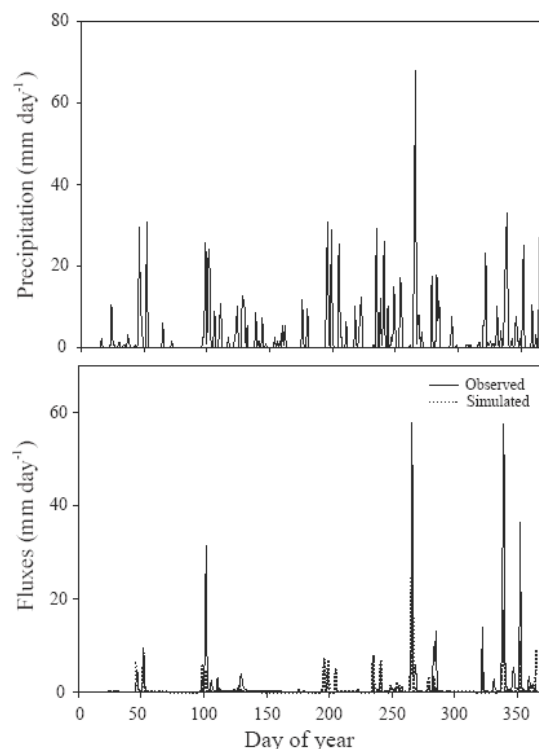


Figure 1. Daily precipitation (above), and observed and WEPP simulated stream fluxes (below) for the year 2002.

## Conclusions

Overall, the model provided good estimates of stream fluxes for seven of the nine years (1999, 2000, 2001, 2002, 2003, 2006 and 2007). For 2004 and 2005, the poor performance was due to missing experimental data preventing an effective model evaluation. On an event-based, the model overestimated the large events and underestimated the low events. Since low flux events were quantitatively dominant, the cumulative annual fluxes were underestimated. The reasons for these differences may be the lack of input data for annual variations in land use and tillage, as well as missing spatial variability of soil depths. More efforts should be done to provide WEPP with more detailed management input, to improve the model's performance. However, considering the complexity of the topography, land use and management of the watershed, the model showed promising potential for a correct quantification of stream fluxes.

## Acknowledgments

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# Advanced-Canopy-Atmosphere-Soil Algorithm (ACASA Model) for Estimating Mass and Energy Fluxes

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## Introduction

There is a recognized need to improve land surface models that simulate mass and energy fluxes between terrestrial ecosystems and atmosphere. In particular, long-term land planning strategies at local and regional scales require better understanding of agricultural ecosystem capacity to exchange CO<sub>2</sub> and water. One of the more elaborate models for flux modelling is the Advanced Canopy-Atmosphere-Soil Algorithm (ACASA) model (Pyles et al., 2000), which provides micro-scale and regional-scale fluxes. The ACASA model allows for characterization of energy and carbon fluxes. It is a higher-order closure model used to estimate fluxes and profiles of heat, water vapor, carbon and momentum within and above canopy using third-order closure equations. It also estimates turbulent profiles of velocity, temperature, humidity within and above canopy. The ACASA model estimates CO<sub>2</sub> fluxes using a combination of Ball-Berry and Farquhar equations. In addition, the effects of water stress on stomata, transpiration and CO<sub>2</sub> assimilation are considered. The model was mainly used over dense canopies (Pyles et al. 2000, 2003) in the past, so the aim of this work was to test the ACASA model over a sparse canopy for estimating mass and energy fluxes, comparing model output with field measurements taken over a vineyard located in Montalcino, Tuscany, Italy.

## Methodology

The experiment was conducted at Col d'Orcia vineyard near Montalcino, Tuscany, Italy (43° 05' N, 11° 48' E, 220 m elevation above sea level). The canopy was estimated to have about 50% ground cover. ACASA model results were compared with eddy covariance (EC) measurements taken during the fruit set (June-July) and veraison (August) phenological stages in 2005 and 2006. During 2006, measurements covered the entire period from the end of June to August. EC system consisted of a sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA), and an open path gas analyzer (LiCOR 7500, Lincoln, NE, USA). Net radiation, soil heat flux, air temperature, humidity, and precipitation were also measured.

The model was set to use ten air layers within the canopy and ten above it. The number of soil layers was set to 15 with adjustable thickness per layer. Input files required to run the model, include: (1) plant and soil data, leaf optical and physiological characteristics, (2) meteorological (30 minute) data, and (3) initial soil data. ACASA was run initially to select morphological input that gave better results. The August 2005 measurement period was used for model parameterization, and the other measurement periods were used for model validation. ACASA model accuracy was evaluated using linear regression, the root mean squared error (RMSE), the mean absolute error (RA), and the mean bias error (MBE). Regression significance between simulated and measured fluxes was evaluated by the F test. Significance was tested at 0.95 and 0.99 levels.

## Results

Simulated energy and mass fluxes during 2005 and 2006 were compared with measurements at half-hourly time steps. Modeled data showed a good energy balance closure. ACASA estimates of net radiation were excellent. Sensible (H) and latent heat (LE) flux predictions exhibited only small differences between modeled and observed data (Figs. 1, 2). The ACASA soil heat flux was slightly lower than observations. The model most likely did not properly account for more exposed, bare soil in

the sparse canopy. Regarding CO<sub>2</sub> flux, model estimates were good with both positive and negative fluxes well predicted (Fig. 3). In addition, ACASA was able to capture the increase in respiration (positive net ecosystem exchange), which occurred after rainfall events. Statistical analysis showed that errors of ACASA predictions were low.

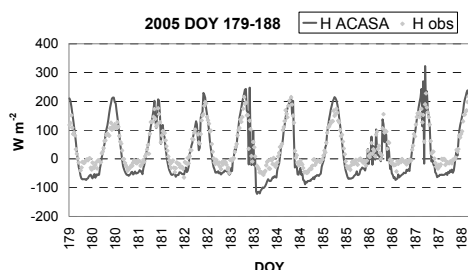


Figure 1. Comparison between simulated and observed sensible heat flux (H) during June-July 2005.

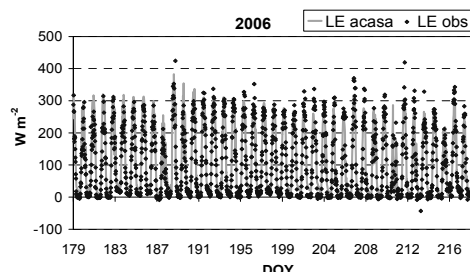


Figure 2. Comparison between simulated and observed latent heat flux (LE) during June-August 2006.

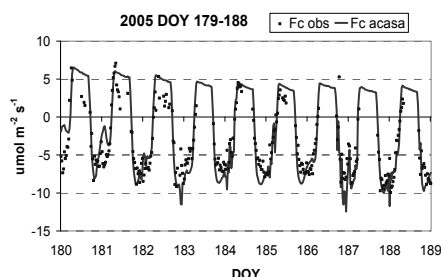


Figure 3. Comparison between simulated and observed CO<sub>2</sub> flux (Fc) during June-July 2005.

## Conclusion

The use of ACASA to predict energy and mass fluxes between the vegetation and atmosphere is promising. After some refinements to the input parameters and model codes, the ACASA model could greatly improve our ability to estimate fluxes over agricultural ecosystems at both local and regional scales.

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# Evaluation of the CROPGRO/DSSAT Model Performance for Simulating Plant Growth and Grain Yield of Soybeans, Subjected to No-Tillage and Conventional Systems in the Subtropical Southern Brazil

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## Introduction

During crop cycle, growth and phenological development are influenced by different factors such as weather conditions, genetic potential, soil fertility, and system management. Management practices influences soil water retention properties and consequently crop growth and yield. In Brazil, a paradigm change in soil management has lead conventional tillage system to give room to no-tillage cropping system, due to impacts such as erosion processes and soil organic carbon losses. No-tillage is considered a conservationist system because it includes crop rotation, mulching and only minimum soil mobilization on the seeding line. Those practices promote higher soil water storage, which are attributed to improvement of mesoporosity and non-saturated soil hydraulic conductivity. Knowledge on soil-water-atmosphere system dynamics is required on modeling crop growth and yield for supporting decision systems. Among several options, the Decision Support System for Agrotechnology Transfer (DSSAT) seem to be the most suitable for practical application since it includes more than 18 different crops models tested worldwide. After model calibration against field data, it is possible to simulate realistic scenarios for decision-makers (farmers, managers, agricultural technicians and government), as well as to identify crop constraint for scientists defining research priorities. This study aimed to evaluate CROPGRO/DSSAT model performance to simulate soybean growth, development and crop yield under no-tillage and conventional tillage systems in subtropical climate conditions, in order to support decision making in soybean cropping of Brazil.

## Methodology

CROPGRO model integrated to DSSAT Version 4.0 was used to simulate a soybean crop during 2003/04 cropping season, in Eldorado do Sul (30°05'S and 51°40'W and 46 m altitude), RS, Brazil. Simulations were compared with data from an experiment conducted in a area of 0,5 ha of a typical Argissolo Vermelho Distrófico. The area had been conducted under two soil management systems (no-tillage and conventional tillage) since 1995, with maize grown in summer and oat and vetches cultivated in winter. The experiment was conducted in a stripe design, with cultivar Fepagro RS-10 sown in November 20th, 2003, in 0,40 m row spacing and 300,000 plants per hectare. The treatments were conventional tillage and no-tillage, with irrigation and no-irrigation. Soil water potential was measured with tensiometers to be used on the soil retention curves, determined experimentally, to obtain observed soil moisture. Evapotranspiration rates were measured in a weighing lysimeter and meteorological data were determined in an automatic weather station. Leaf area index (LAI) and dry biomass (leaves, stems, pods and seeds) were determined weekly. Model inputs included minimum and maximum air temperature (°C), precipitation (mm) and solar radiation (MJ m<sup>-2</sup>). Soil inputs included soil classes, soil physical-hydraulic and chemical properties, in addition of crop management information (weed control, variety, planting date and irrigation). Calibration was performed using genetic coefficients of cultivar Fepagro RS-10, as described by Martorano (2007). Willmott et al. (1985) concordance index “d” was used to compare CROPGRO-Soybean estimates with observed data.

## Results

Figure 1 shows high accuracy of CROPGRO-Soybean model to simulate crop phenological stages, as demonstrated by the low scattering of points around the 1:1 line, mostly for treatments with irrigation.

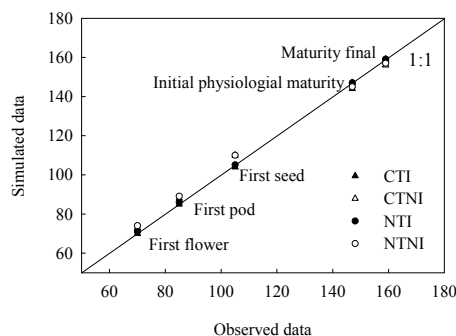


Figure 1. CROPGRO-Soybean model simulated and observed phenological stages (in days after sowing) for different treatments in Eldorado do Sul, RS, Brazil, 2003/04. CTI is conventional tillage irrigated, CTNI is conventional tillage non-irrigated, NTI is no-tillage irrigated and NTNI is no-tillage non-irrigated

The model was efficient to simulate the crop phenology ( $d \approx 1$ ) and to estimate the canopy biomass. Crop emergence occurred 8 days after sowing (DAS) and first flower appearance was about 71 DAS, with a difference of three days between irrigated and non-irrigated treatments. In

conventional tillage with irrigation, comparison among simulated and observed data showed  $d$  values of 0.83 for LAI, 0.96 for plant height, 0.93 for leaf weight, 0.97 for total dry biomass, 0.90 for pod weight and 0.98 for seed weight. In no-tillage system with irrigation,  $d$  were 0.82, 0.87, 0.89, 0.94, 0.88 and 0.91, respectively. The model had lower accuracy under water deficit (non-irrigated treatments), especially in no-tillage system. Observed crop grain yields were 3,597 kg ha<sup>-1</sup> (CTI), 3,816 kg ha<sup>-1</sup> (NTI), 1,559 kg ha<sup>-1</sup> (CTNI) and 1,894 kg ha<sup>-1</sup> (NTNI), while simulated values were 3,108 kg ha<sup>-1</sup> (CTI), 2,788 kg ha<sup>-1</sup> (NTI), 824 kg ha<sup>-1</sup> (CTNI) and 818 kg ha<sup>-1</sup> (NTNI). Differences in crop grain yield between observed and simulated data of non-irrigated treatments suggest the necessity in adjusting soil water parameters in order to improve the performance of the model in simulating the crop growth and yield. Faria & Bowen (2003) after introduction of Darcy's equation to calculate soil water flux significantly improved soil moisture estimates.

## Conclusions

CROPGRO-Soybean model presented higher performance to simulate phenological stages, growth variables and yield components under irrigated conditions than in non-irrigated treatments, especially in conventional tillage. Crop yield results for no-tillage system presented low accuracy, mostly for water deficit conditions (non-irrigated). Lower values of Willmot's test for agreement index " $d$ " in no-tillage system than in conventional system suggest that adjusting model parameters is essential for applying CROPGRO/DSSAT model in Brazil, where no-tillage system area is increasing significantly.

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# Land Suitability and Potential Yield Variations of Wheat and Olive Crops Determined by Climate Change in Italy

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There is significant concern about the potential impacts of climate change and its variability on agricultural production worldwide. A good case study for analyzing vulnerability to climate change is represented by the Mediterranean area, which accounts for most of the global olive oil and more than 10% of the total cereal production. Considerable efforts have been recently expended on analyzing the effects of climate variability on agricultural crops using the scenario approach.

The general goal of this work is to conduct a land suitability analysis for selected Mediterranean crops (wheat and olive) in Italy and to assess the impact of climate change on yield.

## Methodology

The analysis is based on the Agro-Ecological Zoning (AEZ) methodology developed by FAO/IIASA (Fisher et al. 2005) that provides a standardized framework for the characterization of climate, soil and terrain conditions relevant to agricultural production. Crop modelling and environmental matching procedures are used to identify crop-specific limitations of prevailing climate, soil and terrain resources, under assumed levels of inputs and management conditions. This AEZ methodology provides maximum potential, agronomically attainable crop yields for basic land resources units. The AEZ results quantify impacts on land productivity using historical climate variability as well as potential future climate change.

In this work, the AEZ model was applied to the baseline climate period of 1961–1990 and to climate change scenarios based on two General Circulation Models (HadCM3 and CSIRO) SRES scenarios A2 and B2 (IPCC 2000, 2001a, Easterling et al. 2007). Specifically, AEZ employs the FAO/UNESCO digital soil map of the world (DSMW) at 5'x5' latitude/longitude global grid, a global digital elevation map (DEM), a current climate database based on the climate research unit (CRU) of the University of East Anglia and a land cover/ land use layer specifying distributions of aggregate land cover classes. Using the 1961–1990 data, the AEZ model output was compared with existing production data to gain confidence for future scenario output.

## Results

The results include the assessments of land suitability and crop productivity for olive and wheat in Italy under rain-fed conditions, with a high level of input for the baseline period and the future periods (2020s, 2050s, 2080s). Under climate change, the Italian regional analyses of AEZ results indicate expansions of suitable land area for both crops and a decrease of area with severe constraints by the 2080s. In particular, lands suitable for wheat (figure 1, classes 1, 2 and 3) increased from 36.25% to 37.82% (with a decrease of no suitable area from 7.9% to 1.6%) in Northern Italy, from 13% to 15% in Central Italy and from 19.85% to 22.5% in the south. For olive (figure 2) the major increase of suitable area was noticed in Northern Italy where lands suitable increase from 0.2 % to 24.4%, (in Centre Italy from 1.4% to 16.5% and in the south from 25.5% to 37.4%).

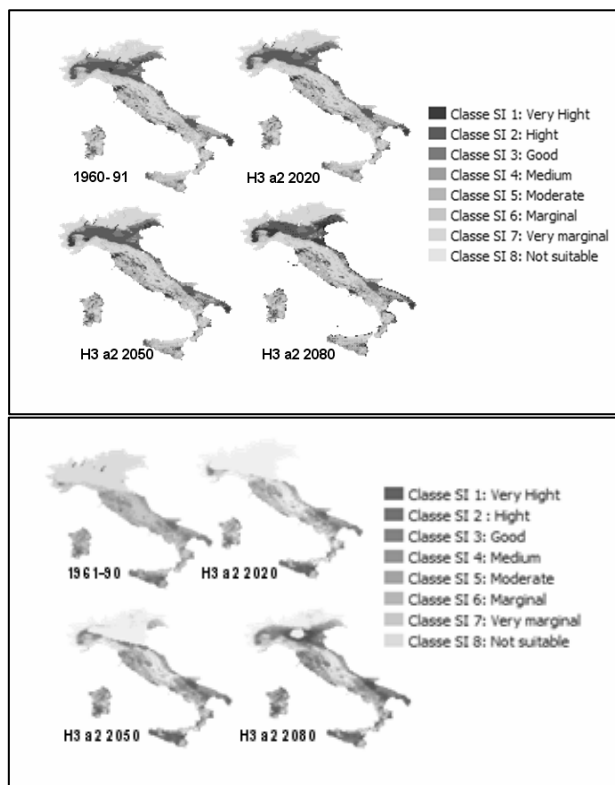


Figure 1. Wheat Land Suitability for the baseline period and the future periods (2020s, 2050s, 2080s) with HadCM3 A2.

Figure 2. Olive Land Suitability for the baseline period and the future periods (2020s, 2050s, 2080s) with HadCM3 A2.

Consequently, both SRES scenarios showed an increase of potential agricultural land for olive and wheat production as presented in table 1.

	OLIVE		WHEAT	
	1961-1990 (q ha <sup>-1</sup> )	HadCM3 A2 2080 (q ha <sup>-1</sup> )	1961-1990 (q ha <sup>-1</sup> )	HadCM3 A2 2080 (q ha <sup>-1</sup> )
NORD	0.2	7.1	20.9	24.9
CENTRO	4.9	8.3	17.8	19.3
SUD	8.9	12.7	21.2	24.2

Table 1. Mean potential crop yield for the baseline period and future period (2080s) with HadCM3 A2.

## Conclusions

The agro-climatic assessment with climate projections of two GCMs for Italy up to 2080 showed significant impacts on the extension and location of cultivation areas of wheat and olive. The study also showed a potential for increased yield.

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# Modelling Yield and Yield Loss Distribution on European Scale

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Climate change may have in fact significant consequences for crop growth and yield, since these are largely determined by the weather conditions during the growing season. Accordingly, also the expected increase of extreme events such as prolonged drought periods or heat waves should be carefully taken into account due to their effect on yield quality and quantity. Generally the climate-risk in agriculture has been evaluated, at different scale, characterizing the shape of crop yield distribution. Yield distribution has in fact important implications for modelling crop insurance programs, production decisions under uncertainty, and risk-efficient farm planning and climate change is expected to alter the present trend. Additionally, yield loss distribution, due to extreme events, has not yet been characterised. On these bases, a framework is presented to assess climate change impact on yield and yield loss distributions on European scale, which represents a basis to test adaptation options in agriculture. As a first step of this framework, the yield probability density function (PDF) of different crops was calculated for the present period at European scale.

## Methodology

Cropsyst simulation model (Stokle et al., 2003) was used to simulate crop yields (wheat, barley, sunflower, maize, soybean, bean and sugar beet, potatoes) across Europe and these data were fitted by different PDFs. CropSyst is a multi-year, multi-crop, daily time step crop growth simulation model (Stokle *et al.*, 2003). It simulates soil-water budget, soil-plant nitrogen budget, crop canopy and root growth, phenology, dry matter production, yield, yield loss due water and heat stress, residual production and decomposition. Daily meteorological data driving the simulations (Tmin, Tmax, rainfall and global radiation) on a grid scale of 50Kmx50Km were provided by MARS project,

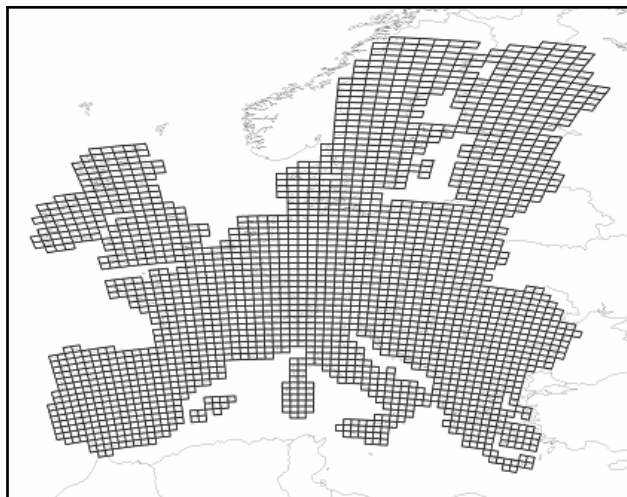


Figure1. Grid points (resolution=50Kmx50 Km) provided by MARS project

(<http://agrifish.jrc.it/marsstat/default.htm>) for the time-slice 1975-2005 (Fig. 1).

Since crop yield distribution is better fitted by a PDF if a large series of yield data are available, MARS time series for each grid point (31 years) was enlarged by means LARS-WG stochastic weather generator (Semenov and Brooks, 1999). Accordingly, after a proper validation, LARS-WG reproduced stochastically 100 years of daily meteorological data for each grid point were used as input variables of Cropsyst. The yield data were then simulated for each crop and their statistical distribution was analysed by means of R software. Different kinds of PDFs were fitted to yield simulated data and the

relevant performances were compared by goodness of fit test. Skewness (a measure of distribution symmetry) and kurtosis (a measure of distribution variability) of each distribution were also calculated. Additionally, yield losses due to both heat and water stresses were calculated and their statistical distribution was analysed as well.

## Results

The results indicated that generally crop yields didn't have normal distributions being negatively or positively skewed (Fig. 2) according to the crop growth conditions. Areas where the general climate conditions were suitable for a crop, crop yields showed a negative skewness (elongated tail to the left, where yields are close to their maximum potential); whereas where the conditions were less favourable,

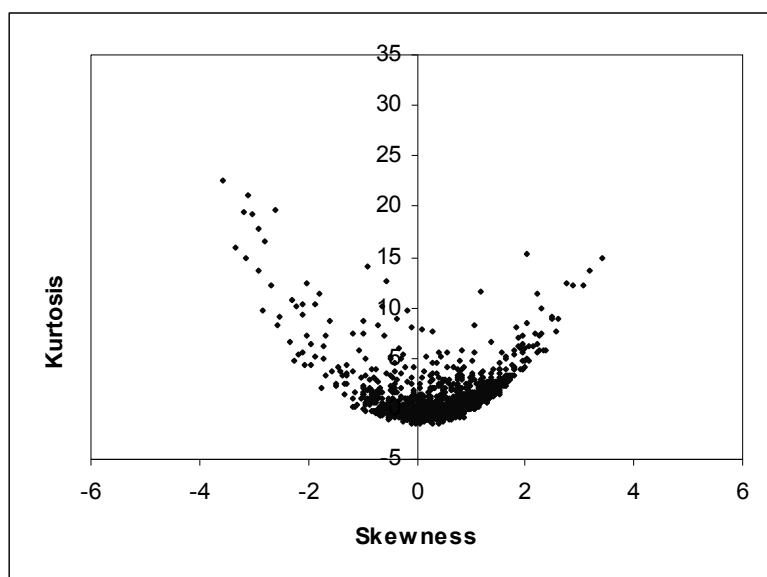


Figure 2. Skewness-kurtosis graph showing the deviations of durum wheat yield from normal distribution (where skewness=0; kurtosis =0)

the skewness was positive (elongated tail to the right, where yields are strongly reduced closer to 0). Gumbel and Lognormal function generally fitted better when skewness was positive; whereas Logistic function performed better when skewness was negative.

Yield losses due to both heat and water stresses were generally positively skewed resulting well approximated by a Gumbel function.

Summer crops performed generally better (PDF negatively skewed) in Northern Europe; whereas winter crops performed better over the Mediterranean basin.

## Conclusion

Yield probability distribution for several crops was characterized at European scale using a gridded meteorological dataset spaced 50Kmx50Km. Different kind of information can be consequently derived from this analysis such as yield or yield loss return period. Accordingly, the same approach may be applied in future scenarios for assessing climate change impact in agriculture and to introduce reliable adaptation options.

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# Impact of Climate Change on Grapevine (*Vitis Vinifera* L.) at regional Scale Phenology, Yield and Biotic Stress Responses

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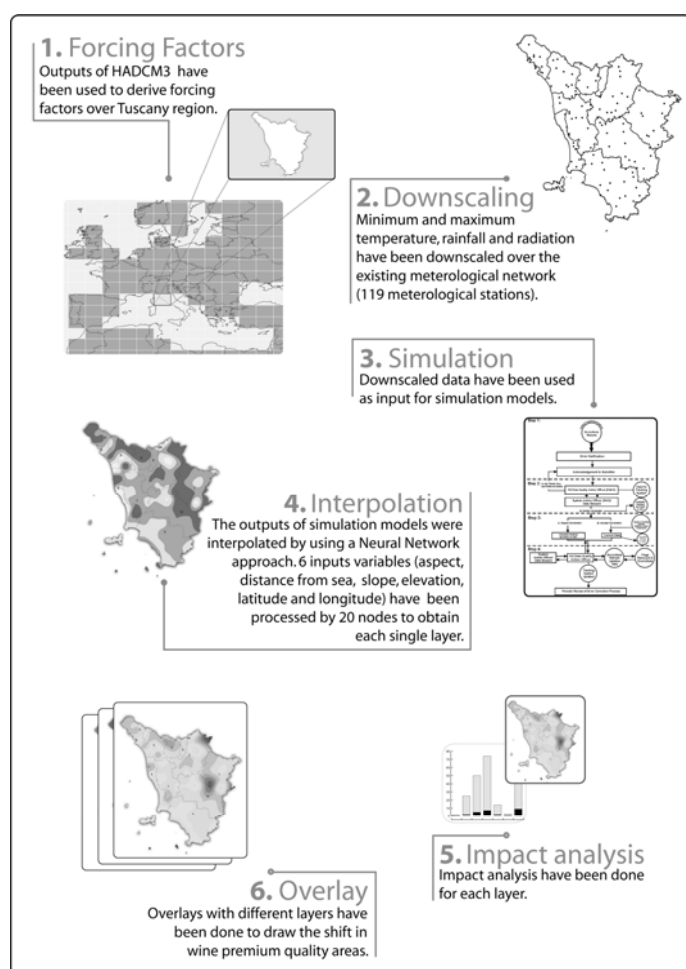


Figure 1. Methodological framework for climate change impact assessment on grapevine in Tuscany region.

Climate change is expected to have important impacts on different economic sectors (e.g. agriculture, forestry, energy consumptions, tourism, etc.). In particular, agricultural sector will be extremely sensitive to climate change, since animal and crop growth are largely determined by the weather conditions during their life cycles. However, these impacts will be not consistent across Europe. In northern areas, climate change may produce positive effects through increases in productivity and in the range of species grown; whereas in southern areas the disadvantages will be prevalent with lower harvestable yields, higher yield variability and a reduction in suitable areas for traditional crops. Thus, the assessment of climate change impacts for crops like grapevine, that has a very important social, economic, cultural and tourist role in many Mediterranean areas (e.g. Tuscany Region, Italy), will be very important for future development planning of these rural areas.

In this work, climate change impact on grapevine was assessed in Tuscany Region (Central Italy) by coupling crop growth and disease simulation

models with statistically downscaled climate data obtained from a regional meteorological network (Fig. 1). Forcing factors, provided by General Circulation Model (GCM) HadCM3 for the period 1990-2100, was used to drive minimum and maximum temperature, rainfall and radiation (Tmin, Tmax, R and RAD respectively) downscaling procedure for A2 and B2 greenhouse gases emission scenarios.

## Methodology

A methodological framework was set up to assess climate change impact on the viticulture in Tuscany region (Central Italy; 9°-12° East longitude and 42°-44° North latitude) (Fig. 1). At first, the outputs of

GCM HADCM3 were used, as forcing factors of LARS WG (Semenov et al., 1997) for the statistical downscaling of Tmin, Tmax, R and RAD over the existing meteorological network (119 meteorological stations) for the period 1975-2100 (Fig. 1, stages 1-2). Both A2 and B2 greenhouse gases emission scenarios (A2: [CO<sub>2</sub>]= 700 ppm in 2100; and B2: [CO<sub>2</sub>]= 550 ppm in 2100) were considered in this analysis. Each time series was split in 4 sub-set data called respectively present period (PP, 1975-2005), future period 1 (FP1, 2006-2037), future period 2 (FP2, 2038-2069), future period 3 (FP3, 2069-2099). The downscaled climate data were used as input variables to drive simulation models, including epidemiological (Sall, 1979) and growth models (Bindi et al., 1997) and a bio-meteorological index (e.g. Winkler index), for assessing climate change impacts (Fig. 1, stage 3). Additionally extreme event frequency at sensible growth stages (frost events at bud-break and heat waves at berry ripening) was calculated. Next, site simulation models outputs and extreme events data were interpolated at regional level using a Neural Network approach (Fig. 1, stage 4) and spatial maps were analysed (Fig. 1, stage 4). Finally, the variables affecting wine quality (i.e. Winkler index, frost and heat waves frequency) were combined in a Multi Criteria Evaluation Approach to derive regional maps showing possible shift or contraction of best premium wine production areas (Fig. 1, stage 6).

## Results

The results indicated a general and progressive increase in temperature and a decrease of rainfall during the growing season and these changes were more evident in A2 scenario than in B2. Consistent with the increasing temperatures, grapevine development was shown to be faster, thus phenological stages were reached earlier and growth period lengths were shorter. As a consequence of a shorter time for biomass accumulation, lower rainfall and longer dry spell during the growth season, final yield gradually decreased. Additionally, expected increases in heat stress frequency during berry ripening from PP to FP3 may strongly affect also yield quality. The risk of powdery mildew infection will be less effective. High quality grapevine varieties will be likely replaced by low quality varieties in flat areas; whereas in FP2 and FP3 high elevation areas (>600m asl) will become viable for premium quality wine production.

## Conclusion

The results showed that climate change will affect the quantity and quality of grapevine yields in Tuscany. Thus, to cope with these negative impacts adaptation options should be adopted. These, however, will be not easy to define since many production and environmental aspects need to be considered. The modification of canopy structure, through reduced plant density or reduced foliage heights may be very useful to respond to water limitation during spring and summer period, but at the same time these may alter grapevine quantity and quality (e.g. acids/sugar ratios). The same was for the reduction of grass cover within the vineyard rows that would increase the plant water availability, but at the same time may increase soil erosion in hilly areas due to expected more intense precipitations. The choice of the plant material, in terms of cultivar precocity and shorter growing cycle length, may be another adaptation option to a drier climate.

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# Climate Change Impacts on the Guadiana River Basin: a Preliminary Study to the Adaptation Strategies

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The Guadiana River Basin lies between Spain and Portugal and constitutes one of the three main drainage units of the Iberian Peninsula, with a total drainage area of 66,800 km<sup>2</sup>. The main environmental problems within the river basin are the overexploitation of the aquifers through large extractions for agricultural use, agricultural contamination and river fragmentation by dams (Cosme, Sousa *et al.* 2003). These problems make the Guadiana River Basin extremely vulnerable, and at the same time very interesting to evaluate the role that both climatic change impacts and adaptation strategies may have on it.

This work presents the preliminary results concerning the assessment of the impact of climate change on this region. Growth simulation models, driven by meteorological data, were used to simulate the impact of a warmer climate on the most representative agricultural and natural ecosystems over this 'hotspot' region.

## Methodology

Main crop yields, olive tree suitable area and forest fire weather risk were evaluated for the present (from 1961 to 1990) and the future (from 2071 to 2100) according to the IPCC emission scenario A2. Daily weather data of minimum and maximum temperature, rainfall and global radiation for both time slices were from the climate high resolution regional model HIRHAM (12.5 Km x 12.5 Km).

Yield of maize, wheat, soybean and barley were simulated using the crop growth simulation model CropSyst (Stockle *et al.* 2003). This model is able to simulate, on a daily basis, growth and production of crops depending on cultivar specific, management and soil parameters. Similar inputs were requested for the model elaborated by Bindi *et al.*, (1997) that was used to estimate present and future yields of grapevine. Furthermore, the Winkler Index (Fregoni, 1985), was also applied because it is able to create a useful link between temperature and the potential area for grapevine cultivation (Bindi & Moriondo, 2006).

Olive trees (*Olea europea* L.) suitable area was forecasted for the future by using the methodology developed by Moriondo *et al.* (2007). Finally, the Fire Weather Index (FWI) was used to estimate the effect of climate change on fire risk (Moriondo *et al.*, 2006). This index is a daily meteorological-based index used worldwide to estimate fire danger.

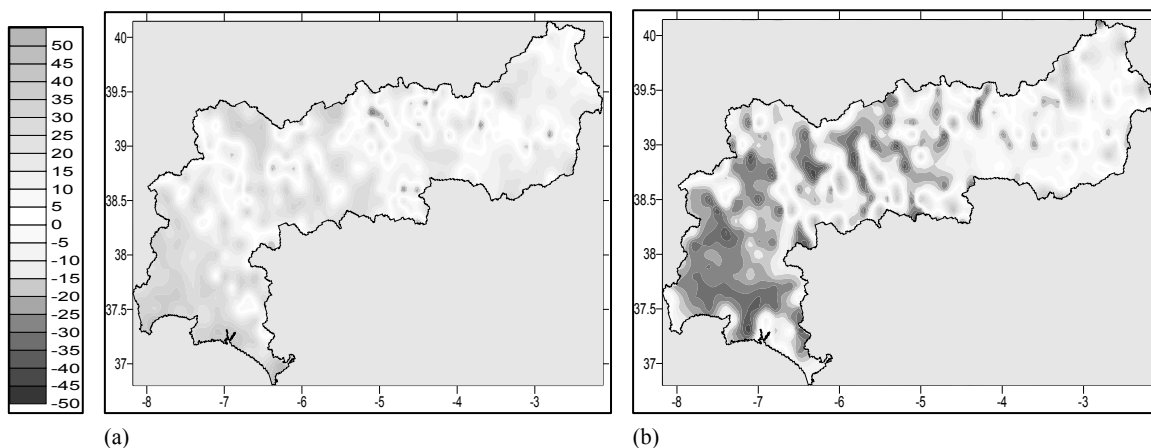
## Results

Preliminary results indicated that, in general, winter cereals will take advantage in changed climatic conditions as simulated in the IPCC emission scenario A2. In particular, on the most part of Guadiana basin and between the two time slices (1961-1990 and 2071-2100) wheat mean yield will increase by 14 % (Fig. 1a). On the other hand, the warmer conditions of the future period will cause summer crops to undergo a reduction in yield. For soybean, our results indicated an overall negative variation of yield with respect to present time (Fig 1b). In particular, the greater reductions were recorded on the southern side of the Guadiana basin (-20.2%), where greater reductions in rainfall (-40 %), during spring and summer seasons, were forecasted (data not shown).

Also, grapevine average yields will generally decrease (-4.4%). Furthermore, from the analyses of the Winkler index resulted that the cultivation of high quality grapevine varieties will shift on to the high elevation areas. Increased temperatures and reduced rainfalls as simulated in southern Spain, will affect olive tree suitable area that will be gradually shifted northwards.

Forest fire risk areas will increase and will tend to spread eastwards with considerable impact on natural vegetation distribution.

Figure 1 - Wheat (a) and soybean (b) yield variations expressed as percentage between two time slices: 1961-90 and 2071-2100. Image elaborated by DISAT (2007).



## Conclusions

The main climatic trends forecasted for the IPCC emission scenario A2 in the Guadiana basin, will be characterized by an overall rainfall reduction and a seasonal temperature increase. These variations will affect future crop yields in different way: some crops will have a yield increase, while others will undergo a yield decrease. Furthermore, there will be also some cultivated area shiftings.

This work took in account climate and agro-environmental aspects to preliminarily describe the future territorial conditions in the Guadiana basin. Nevertheless, a complete vision on this issues was not reached. Consequently, the implementation of future agricultural adaptation strategies will require a more exhaustive survey of the climatic change in the rural area including meteorological, agro-economic and sociological on-field data.

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# Simulating Changes of Organic Carbon Content in Soil Following Tillage Intensity and Fertilizer N Rate Reduction

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Economic, environmental, and societal benefits are expected from good practices of soil carbon (C) management (Kimble et al., 2007). However, new tools are required for national and local policymakers to accurately estimate the effects of different agricultural management practices on greenhouse gas emissions and removals. The *2006 IPCC Guidelines for National Greenhouse Gas Inventories* (IPCC, 2006), which provide methodologies for estimating national inventories of anthropogenic emissions and removals of greenhouse gases, together with the *Good Practice Guidance for Land Use, Land-Use Change and Forestry* (IPCC, 2003), provide internationally agreed methodologies for this purpose, and include the possibility of using simulation models as *higher order* methods for gas inventories in the Agriculture, Forestry and Other Land Use (AFOLU) sector. According to the *IPCC Guidelines*, models should undergo quality-checks, audits, and validations and be thoroughly documented.

Tillage intensity reduction is known to increase the soil organic C sink potential (Paustian et al., 1995; Al-Kaisi and Yin, 2005), whereas the effect on C sink of supplying soil with N fertilizer is less understood (Khan et al., 2007). The main objective of this study was to estimate the change of organic C content in the cultivated soil layer as a function of change in tillage intensity, in interaction with different fertilizer N rates. The Daisy model was used for this purpose, in view of its possible future application at regional scale for the estimation of AFOLU-related greenhouse gas emissions.

## Methodology

The simulated system refers to an experiment carried out on a silty clay soil at Agugliano (Ancona, Italy), in the 1994-2006 period. The non-irrigated cropping systems were: durum wheat-sunflower, from 1994 to 2001; durum wheat-maize, from 2002 to 2006. Compared treatments were: tillage (conventional vs. no-till) × N fertilization (fertilized vs. unfertilized), in a split-plot experimental design with 2 replications. Crop residues were always left on the field. Climatic dataset was obtained from multi-year time series measurements at Agugliano (43° 54' N, 13° 39' E; altitude 255 m) and Jesi (43° 32' N, 13° 17' E; altitude 96 m). The following information was used for the model parameter setting: soil measurements (texture, organic C and Kjeldahl N); crop height at harvest (measurement-based) and maximum rooting depth (from expert-estimate). The other parameters were kept at the value suggested by the model library. The hydrological parameters were estimated by means of pedotransfer functions (Saxton and Rawls, 2006). Starting soil conditions were referred to tilled, well fertilized crops (conventional management). The soil profile was simulated up to 1.60-m depth. The simulations started on 1 Aug 1994 and ended on 31 Dec 2006.

## Results

According to the model simulations, when no N fertilizer was supplied, the total organic C stock, that is surface C plus soil C in the top 0.45 m soil layer (corresponding to the mean depth of the Ap horizon, for the soil of the experiment) decreased, both in tilled and in non tilled plots (Table 1). In plots

receiving N fertilizer, a net increase of total soil organic C content (surface + soil C) was simulated, higher for the non tilled ( $+0.90 \text{ t C ha}^{-1} \text{ y}^{-1}$ ) than for the tilled plot ( $+0.15 \text{ t C ha}^{-1} \text{ y}^{-1}$ ). According to the model, in the non tilled plots the crop-residue organic matter partly accumulated onto the soil surface as litter, was partly bioincorporated to soil. Carbon bioincorporation was higher, and soil biomass respiration lower in non tilled than in tilled plots.

Table 1. Simulated surface and soil organic C ( $C_{\text{org}}$ ) balance components, in no-till (NT) and conventional tillage (CT) plots. Accumulated values for the 1994-2006 period, top 0.45-m soil layer, Agugliano (AN, Italy).

C balance components	NT	NT	CT	CT
	unfertilized	+ N fertilizer	unfertilized	+ N fertilizer
	-----Kg C ha <sup>-1</sup> -----			
<b>Surface</b>				
Residual top C	13,939	38,509	13,287	38,589
Bioincorporated from surface	-13,296	-25,578	-9,654	-19,303
Removed from surface by tillage	0	0	-3,633	-19,286
<b>Change in surface <math>C_{\text{org}}</math> (final - initial)</b>	<b>643</b>	<b>12,931</b>	<b>0</b>	<b>0</b>
<b>Soil</b>				
Bioincorporated to soil	5,310	10,214	3,855	7,708
Dead roots added to soil	3,769	9,611	3,584	9,461
Added to soil by tillage	0	0	3,633	19,286
Soil biomass respiration	-12,877	-21,522	-14,541	-34,552
<b>Change in soil <math>C_{\text{org}}</math> (final - initial)</b>	<b>-3,798</b>	<b>-1,697</b>	<b>-3,469</b>	<b>1,903</b>
<b>Total change in the <math>C_{\text{org}}</math> reserve</b>	<b>-3,155</b>	<b>11,234</b>	<b>-3,469</b>	<b>1,903</b>

The observed trends in soil total organic C content following tillage intensity reduction are in agreement with the majority of the experimental results reported by relevant scientific literature. Nitrogen fertilizer seems to positively affect the soil C sink by increasing the amount of crop-residue C that may be incorporated into the soil.

## Conclusions

The results of our model simulations confirm the positive role played by the no-till management practice on soil organic C content, already observed in several, literature-reported, field experiments, and suggest a positive effect of N fertilization as well. The available soil samples from the long term field experiment will provide a benchmark for the assessment of the model performances.

## Acknowledgements

This study is part of the research project *SOILSINK (Climate change and agro-forestry systems. Impact on carbon sink and soil microbial diversity)*, supported by the national government grant *FISR (Research Integrative Special Fund)*.

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# Best Practices in Agriculture: The Code of Attitudes to Prevent Mutual Impact Between Agriculture and Climate Change

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Climate change will have dramatic affects many economy sectors in Europe. Climate affects agriculture and agriculture affects climate. This mutual impact becomes even more evident now, when climate change and variability are widely recognized. Agriculture is the sector of the economy which will be the first and most severely hit by the effects of climate change, even if it potentially could have a positive and massive contribution, like ensuring greenhouse gas reduction for example by the trees carbon absorption from the atmosphere, or also, organic biomasses perfectly could replace fossil fuels. It has to considered on the contrary that agriculture has an effect on global climate change, for example, a huge part of the greenhouse gases like methane and nitrous oxide originate from agriculture sector (Brumbelow K. and Georgakakos A. 2007; Seguin B. et al. 2007). The awareness of the mutual interdependencies between agriculture and climate change is the starting point of the ACCRETe project "Agricultural and Climate Changes: How to Reduce Human Effects and Threats", EU co-funding within the program INTERREG IIIB CadSES. The target of the project is to make private and public actors of the agriculture sector, farmers and the scientific sector sensible to the consequences of climate change on agriculture and environment. Among projects outputs and deliverables, a Code of Attitudes for farmers was produced, which summarizes mutual risks for agriculture and climate, promoting good attitudes which should help concretely address the needs for farmers to adapt to climate change and to contribute to the mitigation of agricultural impacts on climate. The main agricultural activities impacting climate are outlined, together with the list of good practices farmers can adopt to reduce impacts and vulnerability.

## Methodology

The Code has been produced as a collective effort from the staff belonging to the Project Accret-e. Ten partners worked together to develop tools to raise awareness and to inform about the link between agriculture and climate change. The project partners that contributed to the realization of the document were PROVINCE OF PARMA - ITALY (lead partner); BASILICATA REGION – ITALY; IASMA - AGRO INSTITUTE SAN MICHELE ALL'ADIGE – ITALY; MUNICIPALITY OF CHRISOUPOOLIS – GREECE; UNIVERSITY OF ROSTOCK – GERMANY; MUNICIPALITY OF AEHGIO – GREECE; CZECH HYDRO METEOROLOGICAL INSTITUTE - CZECH REPUBLIC; AGRICULTURAL INSTITUTE OF SLOVENIA – SLOVENIA; NATIONAL METEOROLOGICAL ADMINISTRATION – ROMANIA; UNIVERSITY OF THESSALY – GREECE. The scientific and coordination committee was: CNR – IBIMET BOLOGNA – ITALY; CRA – UCEA ROME – ITALY; COME.S VIAREGGIO – ITALY. The key aspects considered was adaptation, as decisions for reducing climate risk exposure and mitigation, as strategies mainly suggest to reduce GHG emissions, including methane and nitrous oxide, and eventually increase absorption tools, mainly farmer oriented. Effort to offer suggestions in taking appropriate adaptation measurements, as increasingly important elements in the management "toolkit" for farmers, has been done to produce this Code.

## Results

A Code of Attitudes has been produced at the beginning of 2008 as a result of the Inter-reg project ACCRETe.

The main agricultural activities impacting climate are outlined, together with the list of good practices that farmers can adopt to reduce impacts and vulnerability of the agricultural sector.

The code starts with an introduction on climate change and the mutual relationships with agriculture, then focalizing on the agricultural activities impacting the climate and the good practices to reduce the mutual impacts. For each of the main agricultural activities, specific aspects are treated and organized in a single sheet to facilitate the consultation. A basis explanation of its mutual interactions with climate is reported together with suggestions (good practices) and benefits for farmers and environment (Fig. 1).

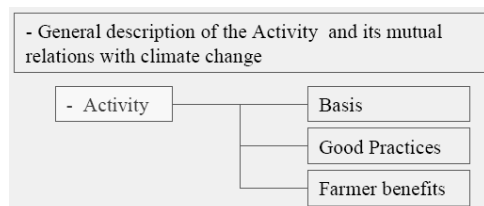


Figure 1. Procedural scheme for the suggestion of the good practices for each treated specific aspect.

Treated topics are involved in Crop and Land Use (varietal selection, cropping system and cropping design, pest and weed control, practices favouring C sequestration), Soil management and fertilization (water erosion and tillage erosion, tillage methods, mineral fertilization, organic matter and fertilization), Livestock managements (genetic improvement, formulation of the diet, animal housing and grazing, manure storage techniques), Water management (irrigation best management practises, choosing an irrigation method, save water techniques, tools in irrigation: the irrigation systems, tools in irrigation as the water balance), Renewable energies and energy efficiency (solar energy for the farm: thermal and photovoltaic, wind energy for the farm, hydroelectric energy for the farm, energy from BIOGAS for the farm, energy from biomass as wood and raw material). The Code provides also a list of definitions of many terms related to the treated topics, based also on the European Environmental Agency multilingual glossary web site (<http://glossary.eea.europa.eu/EEAGlossary/>). The code has been translated and distributed, also on digital support in the involved Countries and published on the web site [www.accrete.eu](http://www.accrete.eu).

## Conclusions

The produced code of attitudes wants to be a summary of Best Practices for farmer, to prevent mutual impact between agriculture and climate change, that will bring to a balanced use between of the resources and their protection, reaching a sustainable agriculture with a socio-economic and climate equilibrium.

## Acknowledgements

This work has been conducted in the framework of the ACCRETe project "Agricultural and Climate Changes: How to Reduce Human Effects and Threats", EU co-funded within the program INTERREG IIIB Cadres.

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The complete list of the used references is reported on the produced Code that can be consulted at the website address [www.accrete.eu](http://www.accrete.eu).

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# Agro-Environmental Approach and Management of Mediterranean Archaeological Areas

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## Introduction

Traditionally, archaeological sites have long been considered only for their historical and cultural aspects, without attributing any particular importance to the environmental context or to the landscape in which they are included. More recently, following a more integrated approach among different components, archaeological sites have started to be considered also for the physical and biological environment around them (Catizone, 1989; Celesti-Grapow & Blasi, 2004) and for the landscape they are part of (Speranza et al., 1993; Sanchez-Palencia & Hoejring, 2002). Conservation and management of an archaeological site is thus becoming a multidisciplinary topic, that requires skills and knowledge of the territory around the site, as well as the ability to use instruments and methods of territorial analysis.

We present here a case study at the archaeological excavations of Tilmen Höyük (South-East Anatolia, Turkey) where a multidisciplinary approach, involving topography, agronomy and plant ecology expertises were adopted to define an integrated sustainable management of the site, enhancing the existing environmental features.

## Methodology

In order to obtain a rigorous topographical and cartographical characterization and a correct geo-referentiation of the Tilmen Höyük site, the core technique of the Global Positioning System (GPS) was used. GPS measurements were realized by geodetic instruments (single and double frequency) using two different methods: 1) absolute positioning by static measurements of long duration and processing of the collected data together with those coming from independent GPS permanent stations (baselines up to hundreds of kilometers) in the ITRF2000 system; 2) local surveys performed by rapid-static method for establishment of other stations with a precision at the centimeter level or in kinematic way. Regarding the climate parameters, precipitation, air temperature, wind velocity and direction, atmospheric pressure, global solar radiation and relative humidity were measured. Data were collected by data loggers and post-processed to obtain hourly and daily values of the measured variables. Investigation of soil properties (sand, silt and clay content, calcium carbonate, potassium, nitrate, cation exchange capacity, salinity, pH and organic matter) was performed through drilling, core sampling and collection of disturbed and undisturbed soil samples at different georeferenced points. The site was investigated hydrologically to identify the position of the groundwater level and the dynamics of soil water. A ground-penetrating radar (GPR) was used to identify groundwater depth in the south-east part of the city. The plant cover was subjected to a detailed analysis by means of a series of field surveys (thirty-five plant community relevés) distributed across the whole area of the site that allowed us to establish the type and the number of plant species present in the Tilmen Höyük site (flora) and how they combine to form communities of species, in relation to the different environmental conditions (vegetation). All the information described above was georeferenced to the WGS 84 Datum UTM 37

Coordinate System and incorporated into a GIS managed by the software Arcview 3.2 (ESRI Inc.) and its extension, 3D Analyst. Topography, soil data, groundwater level, archaeological buildings, plant cover types represent as many layers within the GIS. All these layers are linked with their own attribute table, so that a continuous update of the stored information will be possible.

## Results

The topographic survey provided a good metric knowledge of the site of Tilmen Höyük, including 3D relationships between single structures or parts of the city, but also good contextual, cartographic information, useful for studies at a larger level (e.g. at regional level).

The analysis of meteorological and climatic data showed that the climate is continental, with hot dry summers (average temperatures often above 30 °C, dry period, typically from May to September) and cold winters (average temperature is below zero for a few days during December and January). Annual rainfall is 900 mm and mean annual air temperature is 16 °C. Overall wind speed at the site (during 2007) was relatively low with a maximum speed of 6.18 m s<sup>-1</sup> and an average annual speed of 2.34 m s<sup>-1</sup>. The dominant direction for the average wind speed was 360°; however, when wind speed increased, the dominant direction changed to north-easterly.

Soil analyses showed a relatively high content of nutrients and organic matter, due to grazing, but also a general vulnerability to erosion because of the shallowness of the soil itself. The soil is permeable and well drained, and therefore the groundwater level depends on the proximity to the water system surrounding the Höyük.

The study of the flora highlighted a remarkable taxonomic diversity (221 different plant *taxa* in an area of 25 ha) and emphasised the great interest of the site from the point of view of the presence of rare *taxa* endemic to the nearby Amanus mountains, as well as of species considered to be the wild relatives of important crops. The analysis of the plant communities revealed that the recently excavated areas are the most critical ones from the point of view of the development of management protocols in order to eliminate all the vegetation on the archaeological buildings. Weed control was performed both on annual and perennial plants, using full spectrum herbicides such as glyphosate and glufosinate. The vegetation analysis also identified the most interesting naturalistic-environmental areas, in which site management protocols should aim for the maintenance and preservation of the corresponding plant communities.

## Conclusions

The design and management of an archaeological park with modern criteria should consider the archaeological component set within an environmental context that is the result of the interaction of a wide variety of physical and biotic factors. Concerning the site of Tilmen Höyük, the study of physical factors (rainfall, temperature, soil, hydrology) help identifying specific risks concerning the possible soil erosion or degradation of the walls and ancient structures built of mud bricks. In designing the covers for the more exposed excavated areas, the climatic conditions of the site were taken into consideration, an important factor also in the planning of vegetation control interventions. The study of biotic factors such as the plant cover and floristic biodiversity of the site, highlighted the remarkable naturalistic patrimony present in the archaeological area, which deserves to be protected and also promoted, thus providing an integration to the historical-cultural wealth of the site.

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# Sources of Uncertainties in Projections of Climate Change Impacts in Cropping Systems

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This work seeks to identify sources of uncertainty related to impact assessments when crop models are linked to various Regional Climate Models (RCMs). This research will build on our experience in the EU project PRUDENCE (Mínguez et al. 2007). In the scientific problem, the current hypothesis is that use of various high resolution climate models-RCMs ‘linked’ to various crop/impact models allows for evaluation of projection uncertainties, and improves capture of extreme events. High resolution models for regional climate simulations are needed for establishing regional impacts in the particular case of the Iberian Peninsula because of its small size and complex orography (Castro et al. 1995). Nevertheless, high-resolution projections also introduce uncertainties of their own to the process of climate simulation (Giorgi et al., 2001). Uncertainties are introduced all along the simulation chain beginning with emissions scenarios and continuing through climate models, crop/impact models and up-scaling of results (Ludwig and Asseng, 2006). In crop simulations, uncertainties are specially related to crop models *per se*, soil information, water balance and water stress impact, and simulation of impact of CO<sub>2</sub> and temperature rise. Quantification of uncertainties is one main step to improve interpretation of impact/adaptation projections.

## Methodology

The EU project PRUDENCE (<http://prudence.dmi.dk/>) provided climate scenarios for impact simulation. CERES wheat and maize models previously calibrated for certain locations were used to perform simulations for 30 years of baseline (1969-1990) and future (2070-2100) climate (SRES A2 IPCC scenarios) generated by 10 RCMs participating in PRUDENCE, and nested in the GCM HadAM3H. Simulations were run for the main Spanish agricultural areas. Wheat and maize were used in rainfed and irrigated conditions, respectively. This choice allowed comparison between winter and summer growth seasons, and in addition, two wheat cultivars with different vernalisation requirements were included. Uncertainties were measured as degree of variation among impact projections obtained. Thirteen locations were selected to represent different conditions across Spain, so simulations for 3 crops and 20 climate data sets for each location provided a total of 780 simulations. Crop yield was selected as the impact indicator. For each combination of crop and location, 30 years time series of simulated yield from baseline and A2 scenarios from the 10 RCMs data were compared, ie 20 time series. Two-by-two comparisons of means from time series were performed, together with ANOVA analyses, to investigate the contribution to uncertainties of RCMs, seasons, crops, management and location. From these tests, ‘number of pairs’ (**Np**) showing non-statistically significant differences, was selected as indicator of degree of coincidence of the projections. The larger the value of **Np**, the smaller the uncertainty associated. The percentage of statistical differences (**Pd**) found between A2 and baseline simulations was used to measure discrimination between scenarios. Locations and RCM-driven simulations showing the largest and the smallest **Np** were also computed. Spatial analysis of yield variations was performed. Maps showing regional trends were built, showing degree of agreement of 10 RCM-driven simulations on impact sign – ie increase or decrease.

## Results

**Np** values were always greater for future climate scenarios than for baseline simulations, showing that A2 impact projection range and uncertainties were smaller than for baseline simulations. **Np** values were larger for both scenarios of maize, followed by rainfed spring wheat (RSW) and finally rainfed winter wheat (RWW). Smaller uncertainty for maize may be explained by high summer temperatures projected by all RCMs combined with management (irrigation), which excludes precipitation and soil properties as uncertainty sources. Locations where maximum and minimum **Np** values appeared are identified in Figure 1 for RSW and RWW, and in general these areas coincided also for maize. Projections for the Central Plateau presented smallest uncertainties for all crops simulated, while Galicia (NO) and Navarra (NE) are regions of maximum uncertainty. Comparing **Pd** for each crop in baseline and future scenarios, RSW and RWW presented comparable **Pd** values (36 and 40%), while **Pd** for maize simulations was double that of wheat (80%). For RSW and maize, locations of maximum uncertainties also presented greater values of **Pd**, while minimum **Pd** values were obtained for locations of small uncertainties. However, the opposite combination was found for RWW.

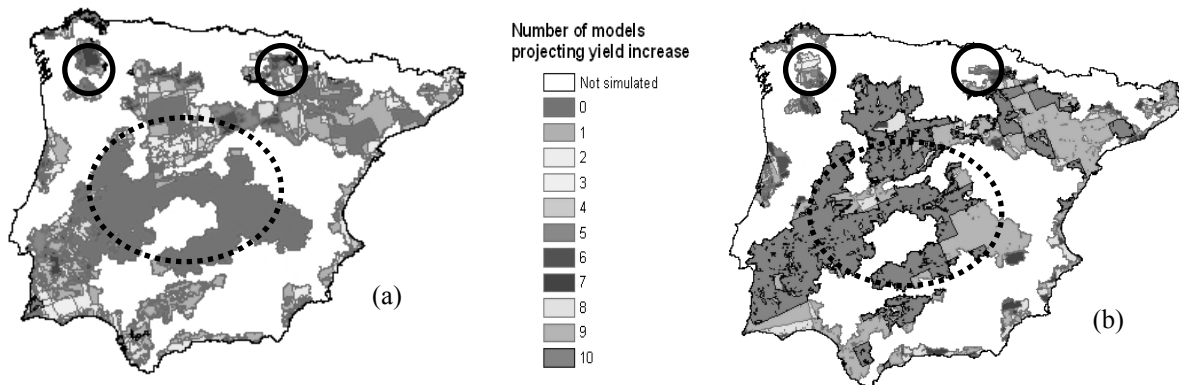


Figure 1. Coincidence maps of yield impact trends for projections generated with 10 RCMs data for RWW (a), and RSW (b). Red colour indicates 0 models projecting yield increase and green colours 8, 9 or 10 models projecting yield increase. Red and green are also areas of small uncertainty. Blue colour represents inconclusive areas with large uncertainty, with *ca.* half of the models projecting yield increase and the rest a decrease. Solid and broken lines specify areas of maximum and minimum uncertainties respectively for RWW, RSW and also for maize.

## Conclusions

The results have enabled establishment of regional trends, identifying regions with maximum and minimum uncertainty in impact projections, depending of crops, seasons, management and climate scenarios. Uncertainties were smallest in summer projections, while in Northern Spain presented projections for all crops were less certain than for Central areas of the Iberian Peninsula. Projections for irrigated maize presented least uncertainty, and greater differences between A2 and baseline scenarios. Irrigation in winter crops will allow further testing of these conclusions.

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# Sources of Uncertainties in a Range of ETo Projections under Climate Change

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Various climatic variables, for instance wind speed or precipitation, generated through Regional Climate Models (RCMs), nested in the same General Circulation Model (GCM), have been shown to have different levels of uncertainties. With this experience, the possibility to choose optional ETo estimation methods may provide analyses of IPCC scenarios with more accurate estimations of uncertainties. In addition, a question arises about the relative contribution to projection uncertainties of any ETo calculation method within the context of the entire input data for RCM and IPCC scenarios. This work quantifies the effect on projection uncertainties of three methods currently used to estimate ETo in areas with Mediterranean-type climate.

## Methodology

The EU project PRUDENCE (<http://prudence.dmi.dk/>) provided the climate scenarios for ETo projections used here. They comprised baseline (1969-1990) and future (2070-2100) climate (SRES A2 IPCC scenarios), generated by 10 RCMs included in PRUDENCE, and nested in the GCM HadAM3H. For these projections, thirteen sites with contrasting climate conditions across main Spanish agricultural areas were selected. Monthly ETo projections from climate scenarios and observed data were computed using three calculation methods differing in data requirements: 1) Penman-Monteith (PM; FAO, 1998); 2) Hargreaves (H; FAO, 1998), and 3) Priestley-Taylor (PT; Priestley and Taylor, 1972). In total, projections were calculated for 20 climate scenarios and observed data, using the three methods to provide a total of 63 different projections for each location comprising monthly data for 30 years (scenarios) or 15 years (observed). Previous work on quantifying uncertainties of agricultural impacts of climate change (Mínguez et al, 2007; Ruiz-Ramos and Mínguez, 2008) sought to identify regions with maximum uncertainties and one site among them was selected, Lugo in northern Spain, to cover the widest range of ETo projections.

Estimations based on observed weather (OETo), baseline (BETo) and A2-scenarios (AETo) were compared for Lugo using the three ETo calculation methods. The results are coded as in the following example. OETo-PM represents the time series of monthly ETo projections calculated from observed data by the PM method. Uncertainties of ETo projections for Lugo were measured as the degree of variation among them. Two-by-two comparisons of means from time series were performed, together with ANOVA analyses, to investigate the contribution to uncertainties of RCMs, scenarios and ETo calculation method. From these tests, 'number of pairs' (**Np**) showing non-statistically significant differences, was selected as indicator of degree of coincidence of the projections. The larger the value of **Np**, the smaller the uncertainty associated.

## Results

OETo-PM was never different to either OETo-H or OETo-PT, but the results of those two methods differed between themselves. In Table 1, **Np** (expressed as a percentage of coincident pairs) measures the degree of coincidence among the 10 RCM projections from the selected time series. As expected, OETo provided a better match to BETo than to AETo (Table 1, analyses 1 and 2). Uncertainty was

smaller for BETo (greater Np, analysis 3) than for AETo (analysis 7) when all ETo estimation methods were considered. When specifying Np for each method for BETo (analyses 4 to 6) and for AETo (analyses 8 to 10), ETo-PT projections presented the smallest variation range and largest coincidence, and therefore smallest uncertainty.

Analysis	Time series	Np(%)
1	OETo/BETo	47
2	OETo/AETo	7
3	BETo	42
4	BETo-PM	38
5	BETo-H	32
6	BETo-PT	55
7	AETo	39
8	AETo-PM	35
9	AETo-H	36
10	AETo-PT	47
11	OETo-PM/ BETo-PM	40
12	OETo-PM/ BETo-H	20
13	OETo-PM/ BETo-PT	60
14	OETo-H / BETo-PM	80
15	OETo-H / BETo-H	50
16	OETo-H / BETo-PT	90
17	OETo-PT / BETo-PM	20
18	OETo-PT / BETo-H	20
19	OETo-PT / BETo-PT	40
20	OETo/BETo-PM	47
21	OETo/BETo-H	30
22	OETo/BETo-PT	63
23	BETo/OETo-PM	40
24	BETo/OETo-H	73
25	BETo/OETo-PT	27

Table 1. Np, expressed as a percentage of coincident pairs, from comparisons of means for different combinations of time series ETo projections. When not specified, analysis included three methods of ETo calculation.

Analyses 11 to 25 in Table 1 dissect the Np obtained in analysis 1, according to the computation method of ETo. When each method for OETo estimation was compared to the three methods for BETo (analyses 11 to 19), only OETo-PT showed greater degree of coincidence with the same method of BETo. BETo-PT presented the greatest coincidence to OETo (analyses 20 to 22), while OETo-H presented the greatest coincidence when considering all BETo (analyses 23 to 25). If temperature is the main factor of scenario differences, the H method, that only uses radiation and temperature, provided observed-based data closer to mean baseline projections. PT considers heat fluxes and more variables than H, but does not include wind speed data of high uncertainty as does PM. This could explain why BETo-PT predictions were the most similar to the mean of observed-based series.

These results indicate that ETo method was not the driving factor in the statistical differences. Rather, analyses 1-2 suggest the main factor to be scenario, followed by RCMs, and lastly the ETo estimation method. Other Np results (not shown) confirmed these hypotheses and allowed for testing the ability of methods and RCMs to discriminate between series. H discriminated better between A2 and baseline scenarios, followed by PM and finally PT. For RCMs, the climate model that produced maximum differences between ETo projections between scenarios was the one that also showed smaller sensitivity to ETo calculation method and vice versa. The RCM showing poorest discrimination between A2 and baseline, showed more differences among its ETo projections calculated by different methods. This could point to a relationship between both responses: in RCMs projecting smaller changes in temperature, other variables have greater relative importance and can produce larger differences in ETo estimations when using methods that consider more variables.

## Conclusions

The results for one location of Northwestern Spain, indicate that even detecting significant differences between two ETo calculation methods for observed-based estimations, climate scenario (first) and RCMs (second) have a greater influence on creating heterogeneity on ETo estimations than the calculation method of ETo. This exercise needs to be extended to other sites with smaller uncertainty of climate projections to test the conclusions further.

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# The Effect of Weather Conditions During the Growing Period on Potato Plant Development and Chlorophyll *a* Fluorescence Activity

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The potato is a crop plant typical of temperate climate and, among other factors, it is for this reason that it is commonly cultivated in Poland. It is characterized by specific temperature requirements and develops best at a temperature of about 20°C. However, the limits and optimal values for the growth of the above-ground part of the potato plant and for the growth of the tubers are different. From experiments conducted in growth chambers it is known that haulm growth is fastest in the temperature range of 20-25°C whereas the optimal range for tuberization and tuber growth is 15-20°C. At a temperature lower than 9°C total inhibition of potato growth takes place.

For a number of years now, increasingly higher temperatures have been recorded in Poland (Table 1). In the decade 1977-1986, the average air temperature in Jadwisin (central Poland) in July was 17.3°C, and in the decade 1997-2006 it was higher by 1.79°C. According to the National Meteorological Service, in many regions of Poland the maximum air temperature has exceeded 30°C and has been as high as 36.5°C. Such high temperatures have been recorded for up to 23 days.

Table 1. July and August air temperatures in Jadwisin since 1967 (°C)

Month	Years			
	1967 - 1976	1977 - 1986	1987 - 1996	1997 - 2006
July	17,89	17,39	18,66	19,18
August	17,68	17,32	17,91	18,04

In connection with global warming projections, the problem of tolerance of potato cultivars to environmental stress, including high-temperature stress during the growing season, has increased in recent times. A method particularly useful for determining the physiological state of a plant under the influence of various environmental factors is the method of chlorophyll *a* fluorescence. Conducting research by means of this non-invasive technique allows the photosynthetic activity to be studied *in vivo*. The method is increasingly widely used in studies of different plant species, but so far there has been little information with respect to the potato plant.

The aim of the work was to determine the level of tolerance of potato cultivars to unfavourable temperature conditions during the growing period using the technique of chlorophyll *a* fluorescence.

## Methodology

Fourteen cultivars of potato were investigated: Irys (very early), Cekin, Ibis, Irga, Kolia, Oda, Tajfun, Tara (medium early), Ikar, Pasja, Syrena, Zeus (medium late), Jasia and Rudawa (late).

The experiment was carried out in 2007 at the Plant Breeding and Acclimatization Institute in Jadwisin, on a sandy-clay soil, in a split-block design with four replicates. Daily weather conditions in the experimental field were monitored with the use of a Campbell Meteorological Station. The physiological state of the plants and photosynthetic activity during the growing period were determined by approximately weekly measurements of chlorophyll *a* fluorescence parameters (Fv/Fm – maximum quantum efficiency of photosystem II, and PI – vitality index of PS II) with a Pocket PEA Chlorophyll Fluorimeter, and of the relative chlorophyll content in the leaves with a Chlorophyll Content Meter CL-01 (both instruments made by Hansatech Instruments Ltd., UK).

## Results

The weather conditions during the growing period in 2007 were favourable to the development of the potato plants. In contrast to some previous years, the expected high temperatures did not occur (Table 2)

Table 2. Air temperature in Jadwisin during the growing period in 2007.

Air temperature in °C	Month				
	April	May	June	July	August
Average air temperature	7,8	13,1	15,7	17,6	17,8
Deviation from many year average	+ 0,1	- 0,5	- 0,8	0,8	0,0

However, at the beginning of July, the air temperature dropped below 9°C, and on 9 July it fell to 6.5°C at a level of 5 cm above the ground. It caused plant growth inhibition, at first invisible to human eye, but the over-cooling of the photosynthetic apparatus was recorded by the Pocket PEA meter. A highly significant decrease in the vitality index of PS II (PI) on 9 July was confirmed (Fig. 1). All the tested cultivars responded similarly, irrespective of the length of their growing period.

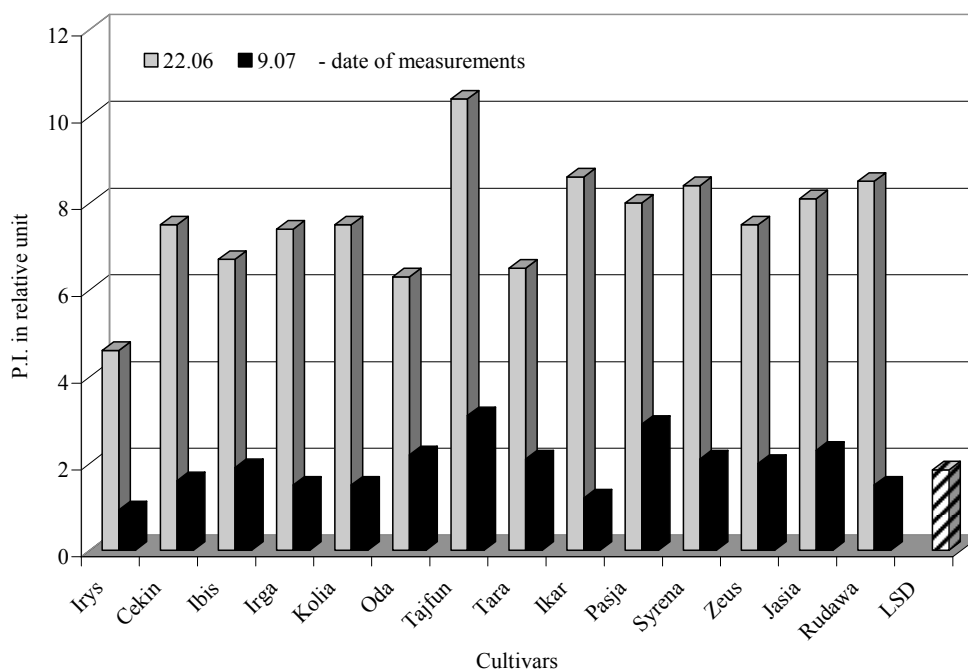


Fig. 1. Vitality index of PS II (PI) depending on cultivar and date of measurement.

## Conclusions

The study carried out with 14 potato cultivars revealed a high sensibility of potato plants to low temperature during the growing season. The parameters of chlorophyll *a* fluorescence allowed early detection of their response to low-temperature stress.

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# Simplified Phenological Models for Open Field Tomato in Sardinia

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Nowadays regional, national and international markets of horticultural products demand a careful planning regarding the cultivation cycle in relation to the different harvest period. Aim of the research is to calibrate simplified model based on heat units summation in order to determine the phenological stages and harvest time in open field tomato (*Solanum lycopersicum* L.).

## Methodology

The trial has been carried out during spring-summer 2005 in 3 sites (North, Centre and South of Sardinia, Italy), chosen on the basis of different thermopluviometric trend: 1) Nuraxinieddu (OR) - (39°55'40"N, 8°36'18"E); 2) Samassi (CA) - (39°27'10"N, 8°56'48"E); 3) Valledoria (SS) – (40°55'14"N, 8°52'10"E). In each experimental site, "Oxford" hybrid plants were transplanted (0.2 x 1.0 m spacing) in two transplanting time (first week of May – last week of May). According to traditional practice, crop management was carried out by the farmers involved in the experiment.

Phenological stages have been weekly monitored from the transplanting date to the harvest. Per each combination (site x transplanting time), 20 randomly chosen plants have been monitored, using the extended BBCH-scale (Hack et al., 1992). Maximum and minimum air temperature in the experimental sites have been daily collected. The heat units were calculated by daily average temperature minus base temperature summation ( $GDD = \sum (\text{Daily average } T^{\circ} - \text{Base } T^{\circ})$ ). The base temperature is not the stop growing temperature but it's a statistic parameter chosen to minimize the total heat units variation coefficient among plants. Base temperature was determined through two methodologies: 1) x-intercepts (Arnold, 1959); 2) coefficient of variability methods (CV). For each phenological interval, base temperature has been calculated and applied for a specific model setting, then the base temperature of the complete cycle has been applied in the heat units calculation for all phenological intervals of the crop. Prediction efficiency of the two approaches (specific or general model) were evaluated and compared to the duration in days of the phenological phases.

## Results

The two methodologies of base temperature determination have not shown significant differences (Table 1). For all phenological stages, heat units showed lower coefficients of variation than the standard method of counting days. Although not always sustained by elevated values, for all the calculated linear regressions, the coefficients of determination have been resulted highly significant. The complete cycle general model showed lower values of variability coefficients and a good prediction capacity of the cycle duration

(Table 2). All the  $R^2$  related to the regressions between counting days and heat units have been resulted elevated and highly significant.

Table 1 - CV and x-intercepts application for each phenological interval

Phenological intervals from transplanting (BBCH Cod.)	CV method		x-intercepts method	
	Base T°	CV	Base T°	R <sup>2</sup>
6 <sup>th</sup> leaf (16)	11.8	21.6	11.8	R <sup>2</sup> =0.64**
7 <sup>th</sup> leaf (17)	10.4	16.9	11.6	R <sup>2</sup> =0.77**
8 <sup>th</sup> leaf (18)	9.6	16.3	11.4	R <sup>2</sup> =0.76**
9 <sup>th</sup> leaf (19)	8.6	17.5	11.0	R <sup>2</sup> =0.64**
Flowering on main steam (61)	1.6	20.8	1.9	R <sup>2</sup> =0.20**
Flowering on secondary steam (601)	0.4	12.2	-2.1	R <sup>2</sup> =0.32**
Maturation on main steam (81)	-13.3	7.5	-13.5	R <sup>2</sup> =0.22**
Maturation on secondary steam (801)	-13.3	5.7	-14.5	R <sup>2</sup> =0.62**
Harvested product (92)	-33.8	4.3	-44.7	R <sup>2</sup> =0.09**

\*\*=P<0.01 D.F.=120

Table 2 - Means and coefficients of variability of counting day and GDD calculated using base T° of transplanting-harvesting interval

Phenological intervals from transplanting (BBCH Cod.)	Day		GDD CV method			GDD x-intercepts method		
	Mean	CV	Mean	CV	Base T°	Mean	CV	Base T°
6 <sup>th</sup> leaf (16)	10.9	33.2	556.8	31.0	-33.8	686.5	31.3	-44.7
7 <sup>th</sup> leaf (17)	13.6	27.0	702.6	24.7	-33.8	851.0	25.1	-44.7
8 <sup>th</sup> leaf (18)	15.8	25.2	817.1	22.9	-33.8	989.6	23.3	-44.7
9 <sup>th</sup> leaf (19)	18.2	23.9	945.5	22.0	-33.8	1144.7	22.3	-44.7
Flowering on m. steam (61)	23.1	22.8	1208.6	21.8	-33.8	1461.7	21.9	-44.7
Flowering on s. steam (601)	33.3	14.9	1770.3	13.4	-33.8	2134.5	13.7	-44.7
Maturation on m. steam (81)	76.8	8.4	4269.7	7.6	-33.8	5109.7	7.7	-44.7
Maturation on s. steam (801)	86.7	6.1	4865.9	5.7	-33.8	5814.8	5.8	-44.7
Harvested product (92)	101.6	4.6	5708.7	4.3	-33.8	6820.4	4.3	-44.7

## Conclusions

Considering crop cycle duration in term of days, heat units can be remarked as more efficient instrument to predict the principal phenological intervals duration. X-intercepts method for base temperature determination should be preferred due to its simplified calculation. As highly significant results, it's promising to use, for all phenological stages in tomato, a unique predictive model calibrated on the complete cycle of the crop. In the future, the available data base will be utilized to improve the predictive model by considering the influence of the photoperiod on tomato phenology.

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# Assessing Farmers' Adaptation Capacities and Land Use Changes in Response to Forest Conservation in Madagascar

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In our opinion, the key failure of integrated conservation–development projects at local scale lies in their founding on a limited understanding of a primary process: farmers' adaptation capacities to cope with changing environmental, economical and political conditions. The determinants of adaptation strategies could be used as target variable to enhance farm's possible evolution in the current perspective of agriculture role in land management and forest conservation. However few theoretical works allow to explore internal and external determinants at farm level that cause convergence or divergence between the needs of rural population and appropriate environmental policy. The objective of this paper is to provide an analytical framework for assessing farmers' adaptation capacities and subsequent land use changes in response to conservation. We assume that constraints from conservation measures impacting households vary considerably depending on i) the degree and manners that households incorporate forest resources into their livelihoods and ii) the correspondence between ecological zoning plans and territorial agricultural dynamics. From case studies in the eastern rainforest of Madagascar under community-based forest management contracts since 2002, we adopted a spatial approach at farm and village territory levels, using a combination of concepts and tools from both agronomy and geography. We built the framework from empirical research focusing on livelihood strategies and land use pre- and post-conservation, based on data collected in 2004-2007.

## Methodology

In the tropics, numerous stresses, such as cyclic fluctuations in resource abundance, followed by annual lean periods, regular climatic disasters and economic hardship, are continuously impinging on livelihoods. A new environmental policy with limitations on agricultural practices and forest use in delimited protected areas is considered as a shock. It creates a major peak in pressure beyond the normal range of variability in which households operate (Turner *et al.*, 2003). The degree of pressure arising from conservation measures depends on the degree and manners that households incorporate forest resources into their livelihoods. The more the constraints affect the satisfaction of daily needs, the more the sensitivity of household is high. If constraints are postponed, according to the future evolution of household's needs, sensitivity is "weak" under the assumption that it is easier to find adaptation strategy in the long term. The needs of households evolve along with the family cycle (beginning, growth, maturity or retirement) which is decisive in household assets, labor force, access to land and individual and collective objectives, in other words in the farm-household system functions (Lericollais and Milleville, 1997). In response to those perturbations, adaptation strategies can be further classified as reactive or anticipatory, depending upon when they are initiated. A reactive strategy is an immediate change in the farm-household system in response to new constraints. An anticipatory strategy enables to react to possible constraints arising in the middle-term or in the long term. Anticipatory and reactive adaptation strategies aim at reducing shock and maintaining or enhancing farmers' livelihood options by adjusting their lives. In this way, we define adaptation capacity as the aptitude to maintain or develop the greatest part of the farm-household system functions existing before the implementation of conservation measures (subsistence or accumulation).

In order to inform adaptation strategies in relation to a degree of sensitivity, we adopted a spatial and territorial approach. Agricultural changes have spatial footprint and produce new territorial organization; *vice versa* the characteristics of the territory (spatial arrangement of landscape units, stakeholders, their relationships, activities and land uses) influence agricultural changes. In a land planning perspective, the territory must be taken into account as one of the determinants and also a support for adaptation strategies (Lardon *et al.*, 2001). At the territory level we explored socio-demographic, geographical, and agro-ecological factors so as to represent landscape units where land use options for farmers were similar. This first mapping process helps to generate a representative sample of households' diversity. Farm-level survey was divided into three parts. The first part aims to characterize the household structure, its history of settlement, its farming system, and main sources of income. The second section focuses on land-use changes, combining field observations and semi-structured questions about the history of the farmland development, its past and present uses, and the reasons for each change in land-use strategies. The third part deals with farmers' perceptions of benefits and constraints resulting from conservation measures, and the impacts of these measures on their livelihood strategy. Farm-households are then categorized in function of their structural characteristics, livelihood strategy pre-conservation and sensitivity. Adaptation strategies and capacities are analyzed for each group. Graphic models of each type of household connect land use changes at farm and territory levels (Lardon *et al.*, 2005) and allow to represent the consequences of adaptation strategies in term of forest conservation and land management. We developed and tested the approach and the typology in two village territories with contrasted farming systems. They are located in the agricultural frontier on each side of the Fianarantsoa forest corridor separating the Indian Ocean lowlands (400 m a.s.l.) and the island's central highlands (1,200 m a.s.l.).

## Results

Results obtained make it possible to identify the particular determinants both at household and territory scales that are important in facilitating or inhibiting adaptation capacities. We showed that: i) the distribution of the five farm-household types identified varied from a study site to another according to the spatial distribution of landscape units within village territories; ii) Farm-household types are more or less capable of adaptation; each type combined specific reactive and anticipatory strategies, spread out over time, based on specific internal (household holdings, land access or labor access) and external (local rules, infrastructure, market access or off-farm employment) determinants (in each case, adaptation capacity is related to the familial life cycle and farmland characteristics); iii) Adaptation strategies are similar from a study site to another but households use specific resources of their territory iv) The processes of land use changes (intensification, extensification, diversification or abandonment) are similar among each landscape units.

## Conclusions

Our framework based on the three concepts "household strategy - territory - adaptation capacity" gives insight into relationships between agricultural development and forest conservation at local scale. We identified determinants that can facilitate processes at farm level allowing farmers to switch over to activities directed toward supporting conservation outcomes without threatening their survival. Results also suggest that the zoning-based management scheme for conservation would benefit from incorporating a more detailed farm-level land-use approach.

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# Management of Herbicide-Resistant Weed Beet: a Simulation Study

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In regions of sugar beet cultivation, weed beet infestations are responsible for economic losses. Weed beet belongs to the same species as the cropped plant, thus rendering herbicide control impossible in conventional sugar beet where costly practices such as manual weeding of bolters must be carried out instead. Genetically-modified herbicide-tolerant (GMHT) sugar beet varieties might provide an alternative in fields heavily infested with weed beet. However, accidental bolting of GMHT plants would result in pollen-mediated transgene flow towards weed beets. The objective of the present paper was to use a spatio-temporal simulation model for comparing 3 production systems with different cultural practices to evaluate the risk of herbicide-tolerant weed beet populations in a small agricultural region where GMHT and conventional sugar beet coexist.

## Methodology

The GENESYS-Beet model was specifically designed to evaluate different cropping systems for their potential impact on weed beet infestation and transgene dispersal through time and space (Sester et al., 2007; 2008). Model inputs are (1) the regional field pattern, (2) the crop succession in each field, (3) the cultivation techniques used to manage each crop; (4) the genotype of the sugar beet varieties (e.g. GM vs. non-GM, male sterility genes); (5) daily climate and soil conditions. The model simulates the life-cycle of weed beet in every field as a function of abiotic constraints and cropping system variables (i.e. crop, tillage operations, herbicide treatment etc.). The model operates on a daily time step with life-stage densities and genotype proportions as state variables. Genotype proportions change when the herbicide associated to the transgene is applied (i.e. glyphosate in case of GM glyphosate-tolerant sugar beet) and during pollination. Gene flow is calculated from the pollen production of flowering plants in each field and the individual pollen dispersal function integrated over the whole region for every recipient field.

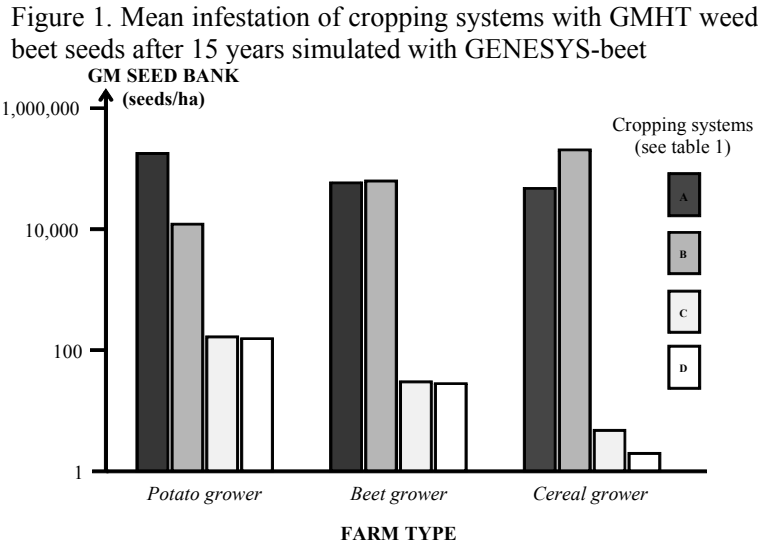
Table 1. Coexisting cropping systems in farm-type simulations	Cropping system	Sugar beet variety	% regional area	Ploughing	Manual bolter- weeding	Initial weed infestation (seed/m <sup>2</sup> )
	A	GM	11	Yes	none	300
	B	non GM	45	Yes	none	0
	C	non GM	22	Yes	2	0
	D	non GM	22	No	none	0

The GENESYS-Beet simulation model was used in a case study of a French sugar beet production region where 3 types of intensive production systems had been identified (Messéan et al., 2006): (1) "potato grower", with a sugar beet/potato/winter wheat/legume/winter wheat/potato/winter wheat rotation; (2) "beet grower", with a sugar beet/winter wheat/set aside/pea/winter wheat rotation; and (3) "cereal grower", with a sugar beet/winter wheat/pea/winter wheat rotation. The three production systems were successively simulated on a real 149 field map extracted from the studied region. At the onset of each simulation, the crops of the rotation were randomly distributed in the region. In addition, four cropping systems were randomly allocated to these fields (table 1). During the subsequent years,

crops depended on crop rotation. A field once grown with a GM variety was never cultivated with a non-GM variety and vice versa. Initial seed banks were empty except for GMHT fields where infestations of weed beets justify the adoption of herbicide-tolerant varieties. Simulations lasted 15 years with the GMHT weed seed bank as the output variable.

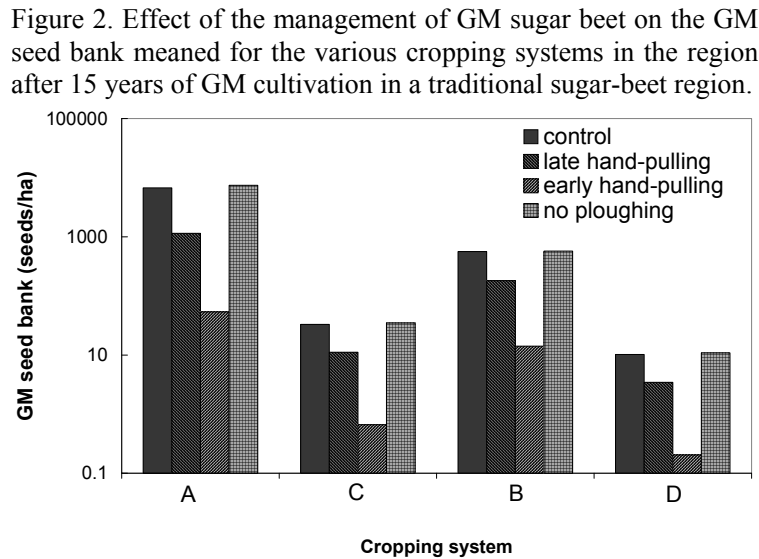
### Results

Whatever the production system, all the fields were infested with GM seeds after 15 years. GM seed density was largest in GMHT sugar beet fields and ploughed conventional sugar beet fields without any manual weeding (Fig. 1). In unploughed and twice-weeded fields, GM seed density decreased considerably. No ploughing was even more efficient in the “cereal grower” system because the seeds were left to germinate on soil surface after sugar beet harvest, thus reducing seed survival in soil. This option is though only feasible if the following crop is a wheat where weed beet produces few seeds. Changing the management of the GM field also contributes to reducing GM seed bank in both GM and non-GM fields (Fig. 2).



### Conclusions

Our simulation results show that GM gene flow is inevitable, both in time and in space, but that it varies considerably with the cropping system. Similar results were obtained for oilseed rape. Simulation models proved to be powerful tools for predicting the effect of alternative methods on gene flow to and from weed populations. But proposals still require economic feasibility studies.



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# Evaluating the capability of a current irrigation design to cope with the estimated Climate Change conditions for northern Spain

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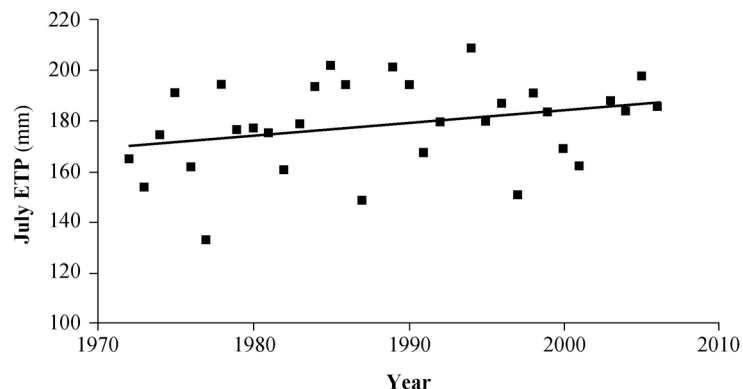
## Introduction

A large effort to modernise irrigation systems is being conducted in Spain (MAPA, 2005). The goal is to reduce water and energy losses in an environmentally sustainable way. The investments are covered by public funds and by farmers. The new irrigation systems have been designed taking in account the historical maximum evapotranspirations for the typical crops of the zone, considering the published crop Kc coefficients.

However, the increment in evapotranspiration rates, due to Climate Change, could yield that these new and expensive irrigation systems become not able to fulfil the crop water requirements during this century. Furthermore, the expected increment in CO<sub>2</sub> concentration would reduce crop transpirations and hence the Kc should be recalculated accordingly. Due to the importance of this subject, calculating future irrigation requirements is one of the main actions in the Spanish Strategy for Climate-Change Adaptation (OECC, 2006). Present paper shows a methodology to estimate water requirements of the main irrigated crops in the Spanish Northern Plateau for the XXI century, using the available Climate-Change scenarios. The reliability of an existing system is evaluated according to the estimations.

## Methodology

MAPA (2005) provided the general guideline for designing irrigation systems under the Spanish Irrigation Plan. The crop water requirements for the design are calculated from the historical average of July, the month of maximum evapotranspiration (ETP) rates.



No significant future changes are considered, although July ETP has raised about 0.45 mm yearly in Valladolid since 1970, as can be seen in Fig. 1.

According to the design approach, the relative water requirements of the main crops in the zone are calculated from their relative areas, assuming that there will be not significant changes in crop relative distributions in the future.

Fig. 1. July-recorded Priestley and Taylor evapotranspirations in Valladolid from 1970 to 2006. The dashed line shows the regression.

The conducted Climate-Change impact assessment used the regionalized climate-change scenarios for Valladolid, northern Spain, provided by the National Institute of Meteorology (Brunet et al., 2007) for the periods 2011-2040, 2041-2070 and 2071-2100; considering the ECHAM and CGCM global circulation models and the A2 and B2 emission scenarios.

A historical series of Valladolid, comprising more than 50 years of daily data was used to generate Priestly and Taylor maximum evapotranspiration rates for each of the above periods, GCM models and emission scenarios, using the LARS-WG weather generator.

The average crop water demand calculation was made considering 40% of maize, 35% of sugarbeet and 25% of potato; as well as the standard Kc coefficients usually employed. Those are typical crops in the zone where the irrigation design was made.

Furthermore, each Kc was reduced for each period and emission scenario; according to the relationships between CO<sub>2</sub> concentrations and transpiration obtained by Kruijt et al. (2007).

## Results

Table 1 shows the estimated July maximum evapotranspirations and each of the crop water requirements considering the reduction in Kc coefficients according to CO<sub>2</sub> concentration rising. The results with the unmodified Kc are shown as well.

The ETP results agree with previous assessments on Climate-Change impacts in the region. The maximum water requirements correspond to the A2 emission scenario. Both GCM models provide similar results.

Besides, CO<sub>2</sub> concentration rising would notably reduce the crop evapotranspirations despite of temperature increments.

This result must be verified, since some feedback effects were not taken in account and the CO<sub>2</sub> effect data used for some regressions was not adequate (Kruijt *et al.*, 2008).

Table 1.

		ETP	Maize		Potato		Sugarbeet	
		Mean	Kc	CO <sub>2</sub>	Kc	CO <sub>2</sub>	Kc	CO <sub>2</sub>
1970-2006		162	165	167	122	123	170	172
2001-2040	CGCM	B2	178	182	163	134	123	187
		A2	182	186	162	137	123	191
	ECHAM	B2	177	181	162	133	122	186
		A2	182	186	162	137	123	191
2041-2070	CGCM	B2	173	176	145	130	112	182
		A2	193	197	147	145	116	202
	ECHAM	B2	178	182	149	133	115	187
		A2	196	200	149	147	117	206
2070-2100	CGCM	B2	195	199	144	146	114	205
		A2	212	217	111	159	98	223
	ECHAM	B2	192	195	141	144	112	201
		A2	206	210	108	155	95	217

Table 2 shows the estimated water supply for the new Valladolid irrigation system, following the same applied methodology (MAPA, 2005). The Table comprises results considering the mean values, as well as the 95<sup>th</sup> percentiles of the ETP distributions.

Table 2

Water supply			Mean		95 <sup>th</sup> percentile	
			Kc	CO <sub>2</sub>	Kc	CO <sub>2</sub>
1970-2006			0.820	0.828	1.019	0.928
2001-2040	CGCM	B2	0.901	0.821	1.037	0.944
		A2	0.920	0.817	1.041	0.925
	ECHAM	B2	0.896	0.816	1.075	0.980
		A2	0.922	0.819	1.082	0.961
2041-2070	CGCM	B2	0.875	0.749	0.973	0.833
		A2	0.975	0.757	1.191	0.925
	ECHAM	B2	0.900	0.760	1.038	0.876
		A2	0.990	0.769	1.155	0.896
2070-2100	CGCM	B2	0.986	0.746	1.119	0.847
		A2	1.074	0.614	1.395	0.798
	ECHAM	B2	0.969	0.733	1.134	0.858
		A2	1.043	0.597	1.245	0.712

The selected water supply in the reference irrigation design was 1 l/s.ha, which is adequate to satisfy the average crop water requirements in the zone. However, this design might be not enough at the end of the century if CO<sub>2</sub> effects CGCM are lower than expected, particularly for the A2 scenario. Furthermore, the system might hardly work under the case of extreme events, even considering the CO<sub>2</sub> effects.

### Conclusions

The methodology for designing irrigation systems in Spain seem to be valid still under Climate-Change conditions. The rising of CO<sub>2</sub> concentrations yields to

important reductions of crop water requirements in the future, despite of the increments on potential evapotranspirations.

However, this issue needs to be verified with additional experiments. Besides, the increment of ETP variability associated with Climate Change seems to posses the main challenge to the irrigation systems reliability in the near future.

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# The Effect of Temporal Aggregation of Weather Input Date on two Important Processes in Crop Growth Models

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In recent years, application of crop growth modelling has been extended from the plot and field scale to regional or even global scale, which also includes applications for longer time horizons (100 years or more), such as for climate change impact studies (Ewert 2004). Daily weather data are important inputs for crop growth models that originally were developed for field level applications. However, daily weather data are often not available, especially when models are to be applied at regional scale and/or for long time periods.

To overcome the problem of missing daily weather data, it is possible to derive these data, through linear interpolation, from average monthly weather data, as typically available at this scale. As many weather-crop relationships are non-linear (Semenov et al. 1995), simulation results, using average monthly weather data are likely to deviate from those with daily weather data. Using temporally aggregated weather data will have differential effects on different processes, the consequences being dependent on the level of modelling detail.

In this research, the effects of aggregating weather data on two processes, leaf area development and biomass production, will be analysed, using spring wheat as an example. The aim is to examine relationships between the modelling detail and the (temporal) resolution of input data to support upscaling of important crop growth processes from the field to the regional level.

## Methodology

Growth of biomass can be calculated based on either the summarized radiation use efficiency approach (Sinclair et al. 1999) or on the detailed photosynthesis model according to Farquhar et al. (1980). For leaf area growth, a rather summarized approach can be applied, with only temperature as input (Bondeau et al. 2007), and a more detailed approach that also considers the supply of assimilates (Spitters et al. 1990). To be consistent with the allocation of biomass to the different crop organs, the original allocation scheme belonging to each leaf area development approach has been used. Three combinations of the processes have been tested (Fig. 1). Two different climates [De Bilt, The Netherlands (52°6',5°10') and Sevilla, Spain (37°25',-5°52')] have been used, for the year 1982. Biomass production under potential production conditions has been considered for the comparisons.

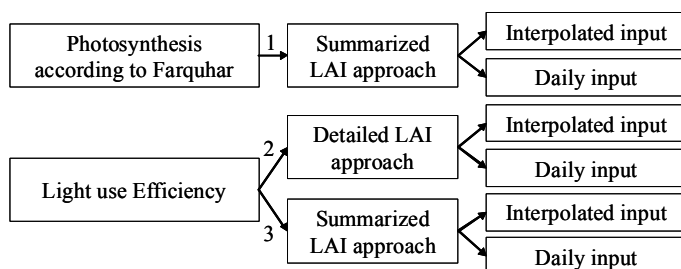


Figure 1. Schematic overview of model exercises with the three combinations of approaches.

To derive interpolated weather data over time from non-interpolated data, a linear interpolation method has been applied, using monthly averages for temperature and radiation, which are set to the middle day of each month.

## Results

In Figure 2 an example of the results of the interpolation method is given for radiation data from the Netherlands. Weather for Spain (not shown here) showed a similar temporal pattern; however with much smaller day-to-day variability for radiation and temperature.

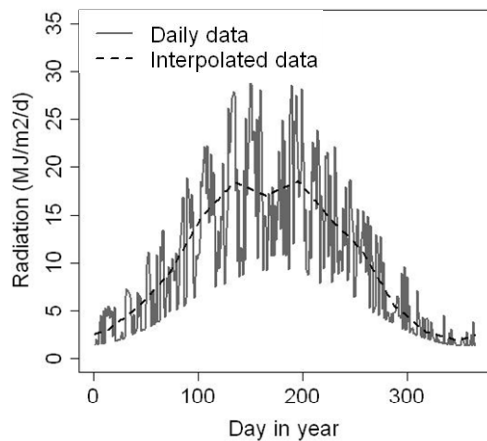


Figure 2. Daily observed and interpolated radiation data in De Bilt, the Netherlands, 1982.

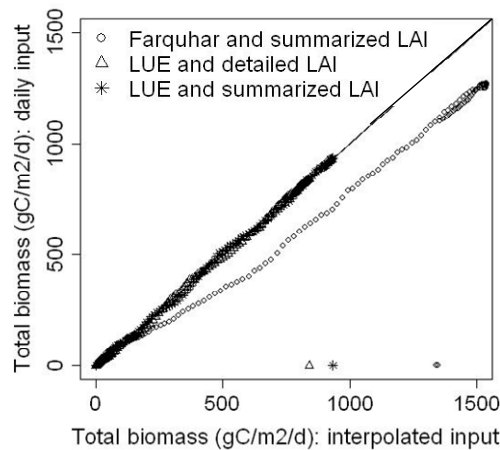


Figure 3. The effects of using interpolated data vs. daily input data for the Netherlands, on weight of total biomass.

Figure 3 shows the effects on total biomass of using daily vs. interpolated input data, with input data from the Netherlands. Using interpolated data in approach 1, results in overestimation of 200 g C/m<sup>2</sup> at the end of the growing season for total aboveground biomass, but in approaches 2 and 3 the difference is negligible. For the Spanish weather data, the results are less sensitive to interpolation of input data in all three approaches.

## Conclusions

Many weather-crop relations are non-linear, and use of interpolated weather data instead of daily data may lead to unrealistic results. The results of this study indicate that especially the detailed photosynthesis approach is sensitive to the use of interpolated data, particularly in climates with a high day-to-day variability in weather conditions. As future climates are projected to be more extreme (Salinger et al. 2005), the use of interpolated input data in combination with detailed models is likely to increase simulation errors.

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# Modelling agricultural diffuse pollution and water quality in the Venice lagoon watershed (Italy): II - a spatial decision support system for the evaluation of alternative scenarios

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The Venice Lagoon Watershed (VLW) is considered by the national legislation (D.Lgs.n°152/99) a sensitive area for the water protection against pollution. Nutrient loads from agriculture and animal rearing activities represent one of the main pressures on the quality of surface and ground-water. The study reported herein refers the development of a decision support system (DSS) aimed at providing the Regional Government with insights in the effectiveness of alternative agro-environmental policies, mainly consisting in the reduction of fertilizer application.

## Methodology

In this study the SWAT model (Arnold et al., 1996), is used to describe water and nutrient cycles, to quantify source apportionment and to perform a scenario analysis (Salveti et al., 2007). Two alternative scenarios are simulated: “business as usual” and “low impact agriculture”. The two scenarios are characterized by different agro-techniques, the second representing the effects of the introduction of reduced mineral fertilizers and livestock manure inputs, in such a way that the total amount of Nitrogen ( $\text{kg ha}^{-1}$ ) is reduced of 30% and the total amount of Phosphorous of 14%.

The DSS is given the task of facilitating the communication and uptake of SWAT modelling outputs in the decision making process. The mDSS tool (Giupponi 2007) was selected and interfaced with SWAT, for integrating spatial and multi-criteria analysis (MCA) functionalities.

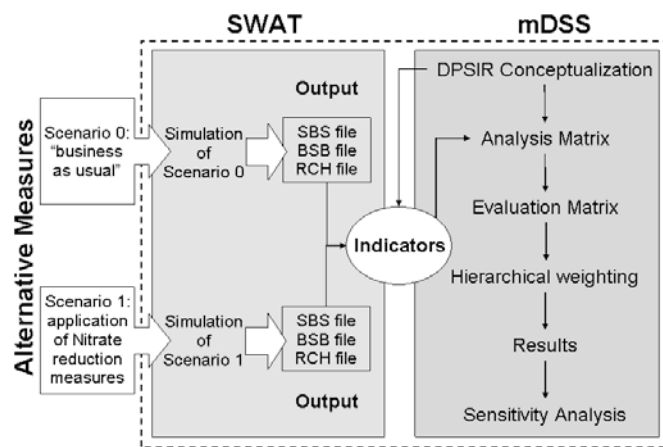


Figure 1: SWAT-mDSS integration for scenario analysis.

In the mDSS software the classic Simon's model for decision problems (Simon, 1960), is combined with the DPSIR (Driving forces-Pressures-State-Impact-Responses; EEA, 1999).

In the initial Conceptual (or Intelligence) Phase, the spatial view functionality of the mDSS software is used to visualize the spatial distribution of the values of DPSIR indicators built upon model output variables. This allows to better understand the decision problem and to select the most significant indicators for the scenario analysis and evaluate the Responses in terms of agro-environmental measures. Among the selected indicators, a first set describes the general conditions within the watershed, while a second set is related to water quality at the sub-basin outlets, in terms of water, sediments and nutrients; finally, an indicator quantifies the variations in the average crop yields.

In the subsequent Design phase, the indicator values are sent to the analysis matrix, which is filled in with the results of the SWAT simulations.

In the final Decision phase, a multi-criteria decision functionality allows the system to elicit users' preferences and to aggregate the performances of the previously defined options with regard to the selected decision criteria. MCA is performed both in a scalar way, by considering aggregated indicator values, and in a spatial way, by analysing the distribution of the selected indicators across micro-basins.

## Results

The effects of low-impact techniques simulated by the SWAT model show in general a reduction of total N discharge in the lagoon, a decreasing value of the parameters related to water balance and an increasing sediment transport, both at the watershed level and in terms of sediment transport. These general trends are verified for all the examined sub-basins. The comparison of scalar and spatial analyses show that while at the aggregated level the "low input" scenario performs better in most of the examined sub-basin, at the disaggregated micro-basin level the positive effects of the "low input" scenario is much less evident. In several micro-basins the performance of the "business as usual" scenario overcomes the performance of the agricultural scenario.

Such results are still to be explored in further project developments, but it is already evident that the weighting of the various criteria, expressing the preferences of different actors have a crucial role. For example, when putting emphasis on those indicators describing discharges at the sub-basin outlets, trade offs are to be expected with the quality of water across the basin.

## Conclusions

This study demonstrates how the outputs of a complex model, such as the SWAT model, can be interpreted through the mDSS decision support in order to help the decision maker both to better understand the system's behaviour and to take better decisions.

Moreover, the possibility provided by the developed system of performing the MCA at a spatially disaggregated level allowed a distributed representation of the performance of each alternative policy option, which allows the decision maker to know not only which alternative performs better, but also in which sector of the watershed. Such information is important in order to improve the efficiency and the effectiveness of the application of agro-environmental policies and measures.

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### **SUB SESSION 3.3**

#### **GREENHOUSE GAS EMISSIONS FROM AGRICULTURE**

Chairman: John R. Porter



# Agriculture, Climate Change and Mitigation - What Can Agriculture Do?

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Climate change is a major concern of our modern civilisation. Agriculture, like every human activity, has an impact on climate. Agricultural activities greatly contribute to the global net flux of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> from the terrestrial biosphere into the atmosphere. Globally, agriculture is the main source of nitrous oxide and methane emissions to the atmosphere and held responsible for at least 15% of total greenhouse gas emissions (Soussana et al. 2004; Smith et al., 2004). CO<sub>2</sub> is emitted mainly from land use change (i.e. deforestation or ploughing up of permanent grassland); this contribution of CO<sub>2</sub> is still relatively uncertain and is generally poorly represented in the emission calculations. On the other hand, agriculture has a potential to contribute to the removal of CO<sub>2</sub> through sequestration of carbon in soil organic matter. Agriculture as such is a minor contributor to CO<sub>2</sub> from the use fossil fuels.

## Emissions of greenhouse gases

The paper will identify the major sources of emissions of greenhouse gases and of sequestration of CO<sub>2</sub> in agriculture as well as the driving forces for these fluxes. These driving forces include several hierarchical levels (Oenema et al., 2001), i.e. societal and farm level. There is ample evidence that agricultural activities over the last decades have increased the emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. The increased fluxes follow from the changes and intensification of land-use in order to feed the needs of the rapidly growing human population with changing preferences in their diet. This requires more soil cultivation, increased number of livestock (especially ruminants) increased acreage of paddy rice fields and increased use of nitrogen fertilizer (Oenema et al., 2005). Though the global budgets of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O seem relatively well constraint and accurate, there is large uncertainty in the source strength of sources at local and national scales, especially in agriculture. The uncertainty in the estimates mainly follows from the complex nature of agricultural practices and management and character of the agricultural sources.

## Policies and measures

Agriculture, as a consequence of its (growing) emissions, must seek solutions to mitigate its impact on climate. In the Kyoto and post-Kyoto Protocol contexts, the European Commission wants the agricultural sector to contribute to the cutting of greenhouse gases emissions. One of the priorities of the EC Rural Development Strategy 2007-2013 is to support actions to mitigate climate change. The major challenge of policy makers is to formulate effective and efficient policies and measures, using the potentials of the mitigation measures proposed so far, and in an international setting with still highly uncertain cause-effect relationships. Major constraints for policymakers follow from the complexities and possible feed back and side effects of mitigation measures, from the many stakeholders involved, often with contrasting views, and from the unfamiliarity of farmers with the problem of climate change. Because of the many complexities and interactions involved, policy makers can follow two tracks. chain-oriented measures, i.e. measures that aim at an increased carbon, nitrogen and water use efficiencies in the whole food chain, and source-oriented measures, i.e. measures that aim at decreased emission from specific sources. Chain-oriented measures should fit in with other environmental policies that aim at increasing resource use efficiency, to be effective and efficient.

## Results

Several papers address the options that are available to decrease emissions of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> from agriculture (Oenema et al., 2005; Smith et al., 2007; Velthof et al., 2008). Generally these include options for: reducing emissions, enhancing removals and avoiding or displacing emissions. They include actions favouring major changes in land use, such as a switch to growing biomass crops, afforestation or significant changes in livestock production approaches. However, less substantial changes to existing agricultural practices towards optimised land management can also have a positive climate change mitigating effect, while also being beneficial to soil condition (Smith et al., 2008). This includes for example zero- or reduced-tillage techniques, use of deep-rooting crops, different types of set-aside, conversion of arable to grassland (including field strips), improved rotations, winter cover or maintenance of terraces.

These options depend on climate and local soil conditions and require regional approaches in order to be successful and acceptable for the farmers. Common to most of these mitigation measures is that the suggested potentials to decrease the emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O from agriculture are large. Common to most of the measures is also the 'single gas' and 'source-oriented' approach. In most papers it has been implicitly assumed that farmers are able and willing to implement the proposed measures. So far, none of the measures has been consciously implemented and tested at farm scale.

I will present results from the EU project on Policy Incentives for Climate Change Mitigation Agricultural Techniques (PICCMAT). This project, launched in January 2007, aims to identify farming practices that reduce greenhouse gas emissions, and to suggest policy instruments to support the necessary changes in land management to stakeholders and policy makers. Our studies further suggest that 'balanced N fertilisation' is among the most promising mitigation measures, simultaneously reducing fluxes of NH<sub>3</sub> volatilisation, N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> leaching, while avoiding pollution swapping to CH<sub>4</sub> (Velthof et al, 2008).

## Outlook

There is a strong need for and significant benefit from integral mitigation measures that enable to decrease not only emissions of N<sub>2</sub>O but of NH<sub>3</sub> and NO<sub>3</sub>, losses of C and emissions CO<sub>2</sub> to the environment without pollution swapping to i.e. CH<sub>4</sub> emission. Such integral measures can only be based on sound mechanistic understanding of the controls C and N pathways in the soil. We will review state of the art understanding of the controls of the relevant greenhouse gas emissions at the field scale. Furthermore, measures need to be cost-effective and feasible for individual farmers and the results of actions by farmers need to be shown in national calculations and reporting of emissions of greenhouse gases. And finally, their implementation may be constrained by political priorities and diverging interests and markets for specific products, i.e emerging need for bioenergy en biofuels.

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# Estimates of regional ammonia emissions from agriculture for Poland

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## Introduction

Intensively managed agricultural areas are the most significant contributors of the ammonia emission to the environment. There is a wide international concern over its direct environmental impact due to the ammonia toxicity to aquatic organisms and its role in the aerial transport of acidic pollutants. Total ammonia emission for Poland in Gothenburg Protocol is not to exceed 486 ktons in 2010 (Economic Commission for Europe, 2004). Between 1990 and 2000, ammonia emissions from agricultural sources in Poland were reduced by 37%, mainly due to a marked reduction in livestock numbers and fertilizer use. Since then there has been a recorded improvement in economic conditions of farming and slowly growing livestock densities in some areas, which might have reversed the downward trend of ammonia emission. The aim of this research was to generate estimates of the current, overall ammonia emissions from agriculture for the whole country and across the regions using region-specific activity data for ammonia sources.

## Methodology

The ammonia inventory was produced for 16 regions using agricultural statistical data for 2005 (GUS, 2006). There were considered three important sources of ammonia emission: 1) animal wastes during housing and storage, 2) faeces and urine deposited on the soil, and 3) mineral fertilizers containing nitrogen. Emission rates for livestock were calculated according to emission factors for different animal categories and types of manure management using EMEP/CORINAIR methodology (2007). To calculate ammonia emissions from fertilized cultures, the detailed methodology estimates of ammonia emission for the 3<sup>rd</sup> group of European countries was chosen.

## Results

For 2005, the countrywide ammonia emission was 388 ktons. Approximately, 89% of this total was attributed to livestock production. Other contribution to the emission (11%) originated from the application of synthetic nitrogen fertilizers. The major part of the emission associated with livestock activities came from dairy cattle production (44.9%) and from pig production (38.5%). The remaining part of the ammonia emission was linked to the poultry operation and other types of animals. Seven regions out of the total of sixteen had relative contribution of around 72% to the total ammonia emission (Fig. 1). The cluster with a higher relative share of ammonia emission (above 5%) contained regions placed in the central and mideast parts of Poland. Two regions (Wielkopolska and Mazowieckie) accounted for the high proportion (34%) of the national total annual emission of ammonia. There was a clear pattern of spatial differentiation of ammonia emission (Fig. 2). Estimated annual rates of emissions were higher in the regions where more intensive livestock operations concentrated. Regionally differentiated inventory showed that the highest values of average annual ammonia emissions exceeding  $2.3 \text{ t km}^{-2}$  were in the Wielkopolska region, while other ones had emissions estimates below  $2.0 \text{ t km}^{-2}$ .

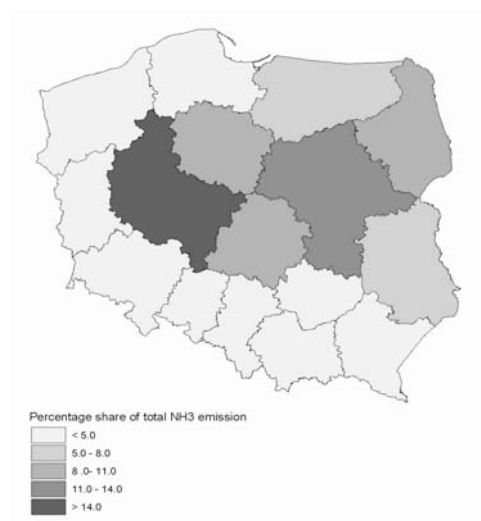


Figure 1. Relative contribution of regions to the countrywide ammonia emission from agriculture

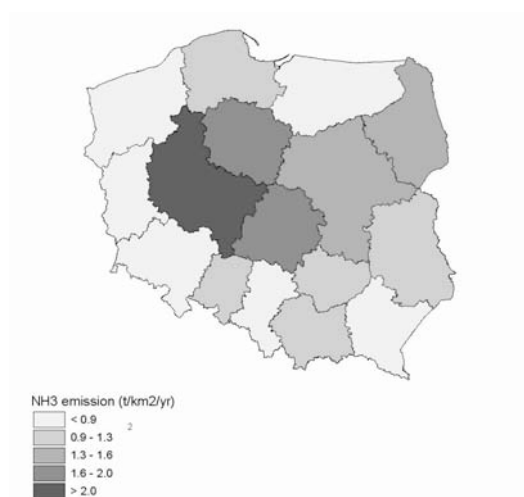


Figure 2. Regionally differentiated ammonia emission from agriculture in Poland, expressed per 1 km<sup>2</sup>

## Conclusions

1. Present estimates of the total ammonia emission from agriculture sources are markedly below the emission limit in 2010. However, if the increasing livestock production continues then it will be difficult to keep the ammonia emission in future below its current level.
2. The leading regions for annual emission of ammonia are shown to be localized in the central and mid-east part of Poland.
3. To control ammonia emission in regions with high intensity of livestock production, it will be necessary to improve manure management practices and adjust feeding management.

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# Should We Store Straw Carbon For Reducing Net Carbon Dioxide Emissions?

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The rise of carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere, and the related threat to global climate change, makes carbon sequestration one of the most important ecosystem services that land-use strategies can provide to human society. Several land-use alternatives have been proposed to curb the rise of CO<sub>2</sub> in coming decades: i) massive use of biomass for bio-fuels; ii) grassland conversion of agriculturally degraded soils, iii) saving and restoring forests (Fargione et al., 2008). It is unfortunate that these “carbon-oriented” options are almost always in competition for available land with the overwhelming objective of agriculture, which is to provide food, in good quantity and quality, to human populations. In fact, growing forests maximize carbon storage but provide little, if any, source of food for mankind. On the other hand, land conversion from forests or grasslands into arable crops notoriously determine a lowering of carbon storage compared to original ecosystems. Therefore, it is apparent that “win-win” land-use solutions for food production and carbon sequestration can hardly be devised. In this contest, the ongoing debate concerning the effectiveness of land use options is becoming a primary criteria for evaluating land use strategies at local and global level (Searchinger et al., 2008). The objective of this study was to explore the potential contribution of a new idea: setting aside straw bales within farming systems might provide transparent and verifiable carbon sequestration coupled with food production.

## Italy's greenhouse gas emission trend

Within the Kyoto protocol agreements, Italy is committed to a 6.5 % reduction of its greenhouse gas emission of base year 1990, amounting to 517 Mt carbon dioxide equivalents (CO<sub>2</sub>eq). Thus, in order to accomplish the Kyoto target, annual emissions should drop to 483 Mt CO<sub>2</sub>eq within 2012 (Fig. 1). Unfortunately, the national trend goes in opposite direction: the emissions of year 2006, about 568 Mt CO<sub>2</sub>eq, far outweigh the target of about 85 million tonnes (Romano et al., 2008). Thus, urgent initiatives should be undertaken to reduce the net emissions, at least in the post Kyoto period after 2012.

## The potential role of wheat straw storage

In Italy, during the period 2000-2007 the average area cultivated annually with wheat (including soft and durum wheat) was 2,223,231 hectares ( $\pm 163,076$  sd), with an average grain yield of 3.36 t ha<sup>-1</sup> ( $\pm 0.39$  sd) (ISTAT, 2007). Assuming a dry matter content of 87%, an harvest index of 50%, and a 44% carbon (C) fraction on dry matter, the C embedded in straw is equivalent to about 10.5 Mt of CO<sub>2</sub> equivalent, which is equivalent to 12 % of the gap between 2006 net emission and the Kyoto target. Gabrielle and Gagnaire (2008) pointed out that if straw is incorporated into the soil, only 5-10% of its C results in sequestration within 30 years. On the contrary, with proper attention to moisture control, straw bales can assure a long lasting conservation. Obviously, in order to maintain soil fertility, wheat should be rotated with other crops (e.g. maize or alfalfa) providing abundant residues to the soil. Unproductive areas could serve as straw storage: non-cultivated portions of the farms and degraded areas (e.g. exhausted gravel pits) are potentially suited. Proper measures for preventing fire accidents

should be certainly adopted, but this is also needed for growing forests. Straw storage could mimic a “virtual forest” in which carbon is accumulated for a long time span in the aboveground biomass of growing three. Considering an annual straw production ranging from 2,5 t ha<sup>-1</sup> (Southern Italy) to 5 t ha<sup>-1</sup> (Northern Italy), an amount of 0,96 to 1,91 t C ha<sup>-1</sup> of cultivated wheat could be set aside. Yet, assuming that wheat could return on the same field every 4 years, the annual rate of storage per hectare of arable land would range from 0.96/4=0.24 to 1.91/4 = 0.64 t C ha<sup>-1</sup>. Interestingly, these values are within the range of average C sequestration, from 0.1 to 3.6 t C ha<sup>-1</sup>, reported for forests growing between 42° and 45° latitude (Magnani et al., 2007).

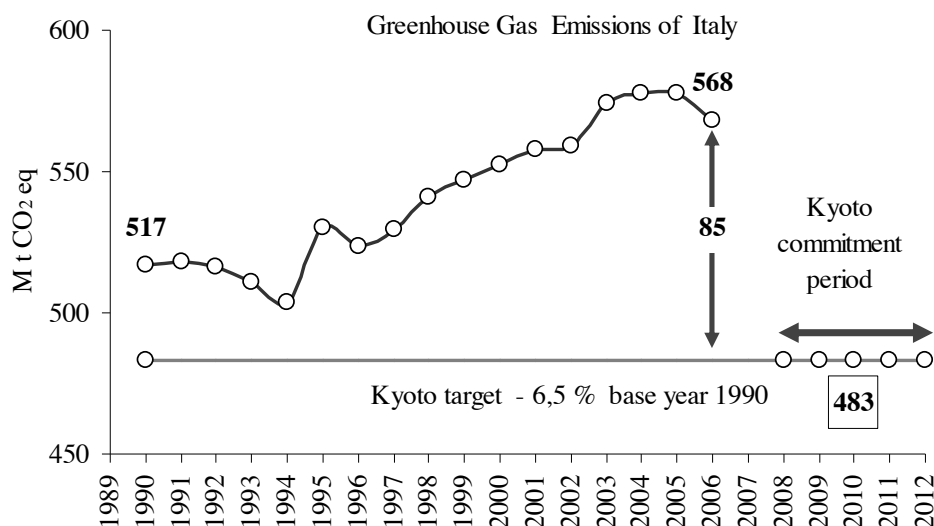


Figure 1. Time course of national GHG emissions and Kyoto commitment for Italy.

The idea of hoarding CO<sub>2</sub> in standing biomass, originally proposed by Dyson (1977) is now being implemented under the Kyoto Protocol, but solely with regard to afforestation and reforestation activities (Shoene and Netto, 2005). We suggest that straw storage should be made eligible to receive carbon credits, because this would imply several advantages: i) all the carbon captured by the wheat crop in its inedible aboveground biomass (about 50%) could be exploited for carbon storage whilst food production is not displaced; ii) carbon could be stored in transparent and cost-effective manner, because straw bales allows reasonably accurate accounting at farm level (i.e. the verifiability issue is satisfied); iii) the carbon embedded in straw is associated with low nitrogen (N) content, because most of the nitrogen taken up by the crop is accumulated in storage organs (C/N ratio for straw is about 90).

## Conclusions

We advocate that straw storage should be made eligible to receive carbon credits. In this way the traditional wheat role of providing food could be complemented by a verifiable carbon sequestration.

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# Does Conservation Agriculture Mitigate the Negative Effects of Climatic Change on Crop Production: a Modelling Analysis for a Case Study in Zimbabwe

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## Introduction

Conservation agriculture (CA) is seen as a new paradigm to conventional agriculture that uses soil tillage. Three practices underpin CA: (1) minimizing soil disturbance by reduced or zero-tillage; (2) retaining residues on the soil surface and (3) using crop rotations. There is a consensus among climate specialists that Southern African regions will become dryer with more irregular rainfall over by the end of the 21st century. In the global context, maize in Southern Africa is seen as one of the most important crops in need of adaptation investment. Can CA mitigate these negative effects of climate change on crop production? It is known that the water conserving effect of CA practices can stabilise crop yields under drought conditions, but the same effect exacerbates poor drainage. We developed a simulation modelling approach to better understand the potential role of CA under changing rainfall patterns. We present in this paper the results for a case study in Zimbabwe.

## Material and Methods

The crop growth model DSSAT-CSM (Jones et al., 2003) was adapted to simulate CA practices, and then calibrated and tested using data from a soil tillage experiment at the Henderson Research Station (17°35' S, 30°38'E, 1136 m.a.s.l.) near Harare in Zimbabwe. The region is characterized by a subhumid subtropical climate with an average annual rainfall of about 880 mm. Rain falls during summer from November until early April. Average annual temperature is about 22°C. The site has a slope of about 5 to 7 % and the soil was classified as a dystic Arenosols. For this study, 2 tillage treatments were considered: (1) the conventional farmer's practice of ploughing the soil to a shallow depth (10 to 15 cm) without retention of crop residues (CT); (2) the no-tillage practice with retention of crop residues (about 2 ton DM/ha) using a direct seeder (CA).

DSSAT-CSM uses daily weather, crop and soil parameters as input to predict growth and yield of a range of crops. Model adaptations included the influence of crop residue cover and tillage on soil surface properties and the soil water balance. With the model we assumed that the following four soil properties vary with tillage: 1) bulk density, 2) saturated hydraulic conductivity, 3) the 'Soil Conservation Service' runoff curve number and 4) soil water content at saturation. The soil properties after a tillage event are input and they change back to a settled value, following an exponential curve that is a function of cumulative kinetic energy since the last tillage operation (Andales et al, 2000). A mulch of crop residues affects three soil water-related processes in the model: 1) rainfall interception by the mulch, 2) reduction of soil evaporation rates, and 3) reduction of surface water runoff.

We ran the model to simulate maize production for water-limited conditions under the present climate using 45 years of daily climatic data (baseline scenario, BS) from Harare and under three plausible future rainfall scenarios for the region (Lobell et al., 2008). These were: (1) a 15% decrease in annual rainfall, RS; (2) a 15% increase in the duration of dry spells, DS; and (3) the combination of scenarios 1 and 2, RDS. Each scenario also comprised a temperature increase of 1.1°C. The scenarios were constructed using the stochastic weather generator LARS-WG (Semenov and Barrow, 1997)

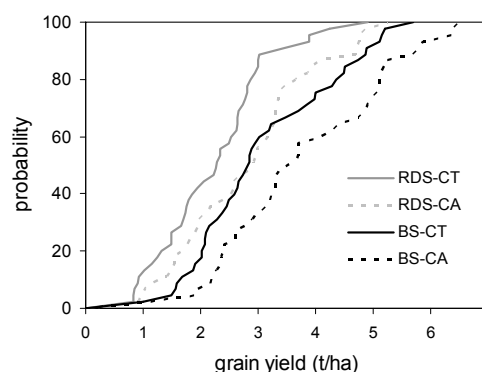
## Results

Using DSSAT-CSM we predicted water-limited maize grain yield for the Henderson site under the 4 weather scenarios (including the baseline climate) and for the 2 tillage treatments (CT and CA). Planting date was during the last week of October. For the baseline scenario (BS) simulated maize grain yield was on average about 720 kg/ha higher under CA than under CT (Table 1). This was mainly due to increased water availability as a result of decreased runoff under CA compared to CT. Predicted yields varied broadly, from a minimum of 1003 kg/ha to a maximum of 6483 kg/ha depending on seasonal rainfall amount and distribution. As expected average grain yields for both tillage practices were lower for future climate scenarios (Table 1). The simulation results indicate that the impact of a 15% increase in the duration of seasonal dry spells (DS scenario) is at least as large as that of a 15% decrease in annual rainfall (RS scenario). Under the RDS scenario of decreased rainfall with longer dry spells model predictions suggest a decrease in maize grain yields of about 25 to 30%, which is in agreement with the value (30%) projected for Southern Africa in a broad-scale analysis by Lobell et al (2008).

The cumulative distribution functions of simulated maize grain yield for the BS and RDS climate scenarios under CT and CA are presented in Fig. 1. Under the current climate the probability of producing at least 3000 kg/ha grains is 41 and 67 % for respectively CT and CA. Under future climate, due to water stress the probability drops to respectively 15 and 43%. The results indicate that the negative impact of climate change can be mitigated by adopting CA in the 'normal' years, but with a higher risk of lower yields in the 'good' and 'bad' years.

**Table 1:** Effect of climate change on maize yield (kg/ha) as simulated by DSSAT-CSM under conventional tillage and CA for the Henderson site nearby Harare, Zimbabwe. Variation coefficient in parenthesis

	BS	RS	DS	RDS
CT	3107 (0.39)	2607 (0.35)	2577 (0.41)	2254 (0.43) <sup>o</sup>
CA	3830 (0.35)	3166 (0.34)	3328 (0.37)	2832 (0.40)



**Figure 1.** Cumulative probability functions of maize grain yield as simulated by DSSAT-CSM for the BS and RDS climate scenarios under CT and CA practices.

## Conclusions

The simulation results show that climate change will have a major impact on maize productivity in the study region. CA practices have a potential to reduce climatic risk for farmers in southern Africa. However, the question remains how these practices fit in their farming systems. Crop residue mulching profoundly alters the flow of resources at the farm, and there are trade-offs in the use of crop residues at farm level. Crop residues, and in particular cereal stover, is a highly-valued fodder for livestock in smallholder farming systems in Africa.

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# Micrometeorological Techniques For Field Measurements Of Ammonia Volatilisation From Arable Lands

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In last decades, many studies have put an increasing attention on the contribution of the agriculture on greenhouse gases emissions. Even if atmospheric ammonia (NH<sub>3</sub>), which mainly originates from agriculture, is not a greenhouse gas, NH<sub>3</sub> has an impact on climate change and air quality through formation of secondary aerosols. Moreover, NH<sub>3</sub> dry and wet deposition leads to ecosystem eutrophication and soil acidification (Erisman et al., 1998).

Ammonia losses by volatilisation following application of both organic and mineral fertilisers have long been studied, even if these measurements are particularly difficult. New technologies are in development for both advance and low-cost N-flux measuring systems to be used on a wide scale for monitoring the processes and mechanisms involved in the NH<sub>3</sub> emissions. A variety of techniques have been developed to measure surface-atmosphere trace gas exchanges, including enclosure and micrometeorological approaches (Denmead and Raupach, 1993). These latter do not disturb the environmental and soil processes and allow continuous flux measurements over area of few hectares. In any cases measurements of gas concentration are needed: considering ammonia, the analytical methods are rapidly evolving (Harper, 2005). In this context, a new apparatus, called ROSAA (RObust and Sensitive Ammonia Analyser) has been developed for measuring NH<sub>3</sub> fluxes by means of the aerodynamic gradient method (Loubet et al., 2008). First observations and considerations regarding the use of this innovative system are reported.

## Methodology

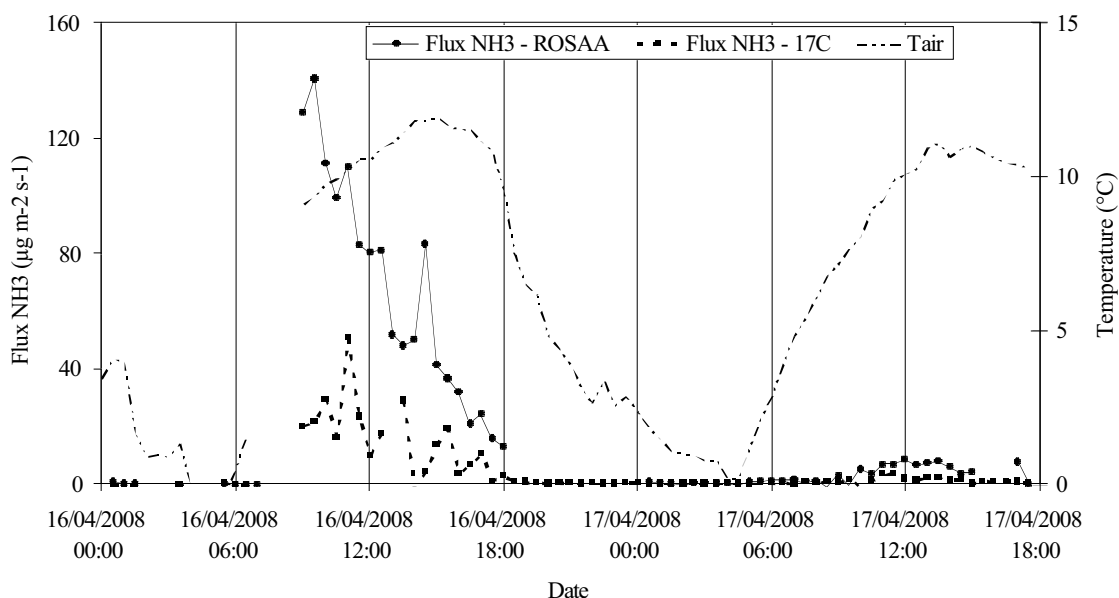
The ROSAA system is aimed for field measurement in conditions of high emission, greater than 1 µg m<sup>-2</sup> s<sup>-1</sup>, and low deposit, less than 50 ng m<sup>-2</sup> s<sup>-1</sup> (Loubet et al., 2008). This system is based on mini-continuously wetted diffusion denuders and it relies on the coupling of ammonia capturing by means of an acid stripping solution, which converts ammonia in ammonium, and the analysis of the ammonium concentration by means of a detector based on a temperature controlled conductivity cell, continuously calibrated against NH<sub>4</sub><sup>+</sup> standard solutions. The coupling is obtained with use of an injection valve.

A first field test of the ROSAA system has been done in comparison with a chemiluminescence analyser 17C (Thermo Environmental Instruments, USA). NH<sub>3</sub> concentrations were measured at the same three levels above ground (0.3; 0.7; 1.5 m) for both the systems. The experimental campaign was carried out during cattle slurry spreading at Grignon site (48°51' N, 1°58' E), located 30 km at west-south of Paris (France) on a field of about 20 ha, immediately after cutting of mustard. The mean annual temperature is 11.5 °C with a mean annual precipitation of 700 mm. The surface spreading was carried out by means of a tanker fitted with a splash-plate and the spread started at 7:00 a.m. (GMT) of 16<sup>th</sup> April 2008 with the slurry incorporation two days after. The field received approximately 120 kg ha<sup>-1</sup> N-NH<sub>3</sub>. During the spread the weather was mostly fine. The experimental field is monitored since 2004 performing soil, plant and atmosphere measurements as part of the NitroEurope IP and CarboEurope IP projects.

## Results

Figure 1 shows the pattern of ammonia volatilization measured by the ROSAA and chemiluminescence gradient systems. The fluxes are shown for the first two days of experimental campaign. The ROSAA fluxes are larger than the 17C fluxes, which in a first analysis can be explained by a lower gradient

measured by the 17C, due to line averaging. Although the amplitude of the fluxes is different, they clearly show the typical pattern of ammonia volatilization after slurry spreading, with the  $\text{NH}_3$  volatilization rate rapidly decreasing within a few hours. The peak of ammonia flux is recorded the day of slurry spreading by both systems. In both cases it is evident that the dependence of  $\text{NH}_3$  volatilization by weather conditions with a diurnal cycle of  $\text{NH}_3$  flux which is indicative of the positive relationship between ammonia volatilization and global radiation (air temperature).



**Figure 1.**  $\text{NH}_3$  fluxes measured by ROSAA and chemiluminescence (17C) systems. Air temperature is also shown.

### Conclusions

The improvement of ammonia concentration and fluxes measurements are needed in order to widely monitor the emissions of this nitrogen compound that indirectly is involved in climate change. A new device, ROSAA, has been developed and its first experimental campaign has given pretty good results, even if a further analysis on the data is needed to explain the difference of ammonia emission recorded by the ROSAA system and the chemiluminescence one.

### Acknowledgements

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# Soil Carbon Stocks, Carbon Dioxide Sequestration and Tillage Techniques

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C sequestration via agricultural soils can be accounted for, under Article 3.4 of the Kyoto Protocol, provided that specific measures are implemented. Sound cropland management can play a positive role in mitigating GHGs emissions from other sources, and carbon dioxide in particular, through a decrease of soil organic carbon (SOC) losses, an increase of organic material inputs or combining these two options. Literature data estimate about 1,500 Gg SOC to 1 m depth (Eswaran et al., 2000), in comparison with 4,000 Gg C of fossil fuels, indicating SOC as the largest sink of C after fossil fuels. Vegetation (600 Gg) and atmosphere (760 Gg) store considerably less C than soils.

Carbon sequestration can occur through a reduction in soil disturbance, since more carbon is lost as CO<sub>2</sub> from tilled soils in comparison with reduced or sod-seeding systems: no tillage systems may absorb on average 0.7-1.4 t CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> (INRA 2002, ECCP 2003).

This paper compares the effects of conventional tillage and sod-seeding on SOC, in a two-years rotation long term field experiment (durum wheat-sunflower 1994-01; durum wheat-corn 2002-06).

## Methodology

A long term field experiment was established in 1994 at the farm “P. Rosati” of the Politechnic University of Marche, in Agugliano (43° 32' 17"N, 13° 22'03"E, 88 m a.s.l.), a hilly area (slope 12%) with a silty-clay soil classified as a Calcaric Gleyic Cambisol (FAO, 2006). We report in this paper results on the effects of SOC of two tillage techniques (C: conventional, i.e. ploughing at 40 cm depth and double harrowing, vs. S: sod-seeding) and two nitrogen fertilization rates (0 vs. 90 kg N ha<sup>-1</sup>), according to a 2x2 factorial experimental design. SOC was determined in 2006 after 12 years of continuous ploughing vs. sod-seeding on 16 soil profiles, with four replications for each set of plots, i.e. CN90, CN0, SN90, SN0. The amount of SOC was determined according to Batjes (1996), considering the bulk density, the proportion of SOC, and the thickness of the layers for individual soil profiles at three depth intervals: 0-30, 30-50 and 50-100 cm; the equivalent CO<sub>2</sub> was derived from SOC. The availability of previous SOC determinations on four of the sixteen profiles allowed comparing SN90 and CN90 plots (depth 0-30 cm) after a period of ten years (1996-2006).

## Results

Differences of carbon stocks among treatments in 2006 in the 0-100 cm soil layer were not statistically significant (table 1). This can be attributed to the relatively high coefficient of variation (19%). Differences between tillage techniques in the topsoil (0-30 cm) were significant (P<0.05) but not so between fertilizations. In fact, in this layer, S treatments stored +5.3 t ha<sup>-1</sup> SOC more (+19%) than C treatments (i.e. +19.4 t ha<sup>-1</sup> CO<sub>2</sub>). Considering the 0-100 cm layer, 69% of the SOC was stored in the 0-50 cm layer and 44% in the 0-30 cm layer. Results indicate that, in the analysed context, tillage effects on SOC after 12 years were limited to top layers, while deeper layers were not affected. These results

are in agreement with Blanco-Canqui et al (2008), which found in some cases SOC may be higher in plowed than in no-tilled soils.

Table 1. SOC stocks and CO<sub>2</sub> sequestration (t ha<sup>-1</sup>) as affected by 12 years of contrasting tillage and fertiliser treatments. See text for abbreviations (means ± standard error; CV = coefficient of variation)

Layer:	0-30 cm		30-50 cm		50-100 cm		0-100 cm	
Treatment	SOC	CO <sub>2</sub>	SOC	CO <sub>2</sub>	SOC	CO <sub>2</sub>	SOC	CO <sub>2</sub>
SN90	34.3±1.7a	126±6	16.8±1.0	62±4	23.6±3.2	87±13	74.7±5.3	274±19
SN0	33.1±1.7a	121±6	18.1±1.0	66±4	20.9±3.2	77±13	72.1±5.3	264±19
CN90	28.6±1.7b	105±6	19.0±1.0	70±4	25.5±3.2	94±13	73.1±5.3	268±19
CN0	28.1±1.7b	103±6	16.2±1.0	59±4	18.8±3.2	69±13	63.1±5.3	231±19
CV	16%		17%		26%		21%	

When comparing 1996 and 2006 data (Table 2), results showed a tendentially increase of soil carbon stocks and CO<sub>2</sub> sequestration in the no tilled plots (SN90), and a decrease in the conventional system (CN90), with differences of the same order of magnitude, i.e. ± 0.3 and ± 1.1 t ha<sup>-1</sup> yr<sup>-1</sup> of SOC stocks and CO<sub>2</sub> sequestration respectively, the treatment x year interaction being nearly significant.

Table 2. Differences in SOC and CO<sub>2</sub> sequestration (t ha<sup>-1</sup>) measured in the 1996-2006 interval.

Layer	0-30 cm (t ha <sup>-1</sup> )		0-30 cm (t ha <sup>-1</sup> yr <sup>-1</sup> )	
Treatment	SOC	CO <sub>2</sub>	SOC	CO <sub>2</sub>
SN90	3.1	11	0.3	1.1
CN90	-3.0	-11	-0.3	-1.1

## Conclusions

The long term field experiment provided some evidences that no till can effectively contribute to increase soil C-sink of the rainfed cereal cropping systems of central Italy. However, 12 years were just sufficient to evidence some trends and further efforts may be necessary to achieve clearer figures.

No tillage techniques are not so widespread in Italy. Recent available data from the European Conservation Agriculture Federation report 560,000 ha under conservation agriculture and only 80,000 ha under no-tillage, i.e. less than 10% of the arable land, while in central Europe figures are 30% for United Kingdom, 20% for Germany, 17 % for France.

Considering that in 2005 arable crop area in Italy was 7 million ha, assuming that our findings are applicable to such area, we could estimate a potential additional SOC stock of 2.1 million tons per year in the top soil, and a CO<sub>2</sub> sequestration of 7.7 million tons per year in case no-till systems are extensively used.

## Acknowledgements

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# New Insight into Plant Gas Exchange Models including Chemical and Hydraulic Signaling

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## Introduction

One of the oldest and most widely used models for simulation of gas exchange in plants is the empirical equation proposed by Ball et al (1987) known as the Ball-Berry (BB) model. Furthermore, it has been shown that stomata tend to close when the plant is exposed to drought and root senses dry soil. In these conditions, the stomata react to the xylem [ABA], which is mainly a root-sourced chemical signal. Tardieu (1993) and Gutschick and Simonneau (2002) modified the BB model with a decreasing exponential factor to consider the effect of xylem [ABA]. It is shown that at moderate soil water deficit chemical signaling mainly controls the stomatal behavior, but at severe soil water drought, both chemical and hydraulic signals are involved in stomatal control. Leaf water potential ( $\psi_l$ ) is known and referred to as the key hydraulic signal (Tardieu 1993). Tardieu and Davis (1992) showed that stomatal response to [ABA] is a function of the plant water status and suggested that studies concerning modeling of plant and soil water relations should take into account  $\psi_l$  as well as ABA supplied by root system. They showed lower stomatal sensitivity to [ABA] at high  $\psi_l$ . Tardieu (1993) proposed a model to account for the interaction of leaf water potential and xylem [ABA]. Below is a summary of the models discussed.

Ball et al. (1987) 
$$gs = mA \frac{hs}{cs}$$

Modified Ball et al. (1987) (Gutschick and Simonneau 2002) 
$$gs = mA^\lambda \frac{hs^\alpha}{cs}$$

Tardieu (1993) 
$$gs = gs_{\min} + a \exp\{-\beta[ABA] \exp(-\delta[\psi_l])\}$$

where:

gs: stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ); A: photosynthesis rate ( $\mu \text{mol m}^{-2} \text{s}^{-1}$ ); hs: relative humidity at leaf surface; cs: intercellular  $\text{CO}_2$  concentration ( $\mu \text{mol mol}^{-1}$ ); [ABA]: xylem ABA concentration ( $\mu \text{mol m}^{-3}$ );  $\psi_l$ : leaf water potential (MPa);  $m, a, \beta, \delta, \lambda, \alpha$ : fitted parameters.

## Methodology

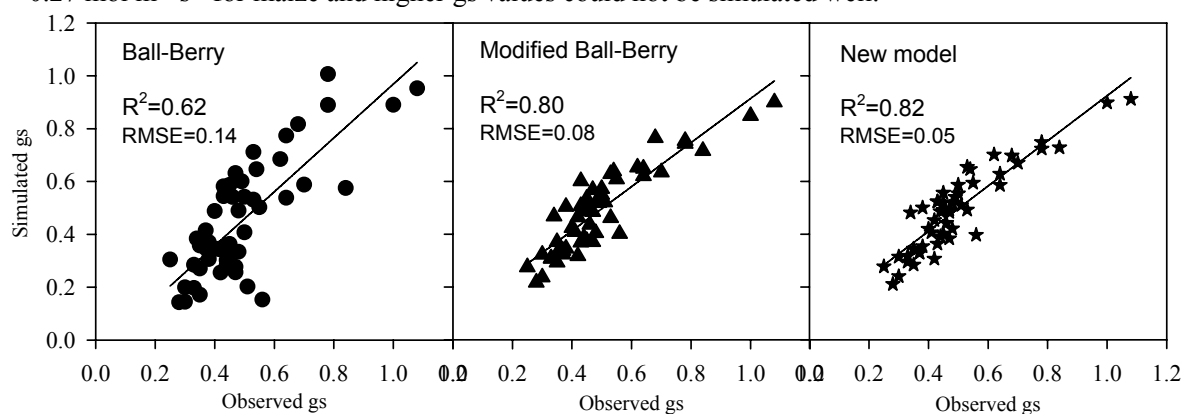
A semi-field study was run in summer 2007 at Research Center Foulum, Aarhus University, Denmark to check the accuracy of a newly developed gas exchange model on potato (cv. Folva). Three subsurface irrigation methods were applied on three soils sandy loam, loamy sand, and coarse sand. The irrigation treatments were full, deficit (DI), and partial root-zone drying (PRD). The plants were fully irrigated until tuber bulking and then irrigation treatments started as DI and PRD plant received 65% of fully irrigated plants. Three diurnal measurements of gas exchange, leaf and stem water potential were made on 10, 15 and 20 July 2007. ABA samples were collected as many as possible. 20 July had the most complete dataset. In our study, we adopted a new and modified gas exchange model based on the previous studies as follows:

$$gs = mA^\lambda \frac{hs^\alpha}{cs} \exp(-\beta[ABA]) \exp(-\delta[\psi_l])$$

Hydraulic and chemical signals of the Tardieu (1993) model are separated into two different terms, and  $\psi$  could be either leaf or stem water potential (Tardieu and Davis, 1992). The data belonging to the sandy loam and coarse sand on 20 July were used for model parameterization using a nonlinear regression procedure. The remaining data was kept for model validation.

## Results

The following figure shows the comparisons between the BB, the modified BB (MBB) and the new model. It is seen that BB model could not simulate  $g_s$  as well as the other models and has lower  $R^2$ . However, MBB model considerably improved the  $g_s$  simulations ( $R^2=0.80$ ) and showed that humidity and photosynthesis rates are not linearly related to  $g_s$ . The BB and MBB models can both be considered as special cases of the new model (i.e. with parameter restrictions). Partial  $F$ -test revealed that the new model significantly ( $p=0.05$ ) improved  $g_s$  simulations over the MBB model, and the MBB model over the BB model ( $p=0.001$ ) implying that adding the water stress terms improve the  $g_s$  simulations and the modified BB model is not reliable for  $g_s$  simulation under limited water conditions (Tuzet et al., 2003). Moreover, results showed that separation of the water stress terms in Tardieu model gave more robust results than the original model of Tardieu (1993). While our model could simulate both low and high  $g_s$  values on different soil types and soil water conditions, Tardieu et al. (1993) claimed that their model was valid for  $g_s$  up to  $0.27 \text{ mol m}^{-2} \text{ s}^{-1}$  for maize and higher  $g_s$  values could not be simulated well.



## Conclusion

We proposed a new model for simulating  $g_s$  coupled with both chemical and hydraulic signals. It is valid for a wide range of environmental conditions and may improve current dynamic crop growth models.

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# Soil CO<sub>2</sub> Emissions under different Management Systems

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Soil CO<sub>2</sub> efflux patterns during the growing season may predict soil organic carbon (SOC) storage patterns in soil under different tillage practices (Gayoung and Wander, 2006).

Conventional tillage (CT) usually increases SOC mineralization and thus CO<sub>2</sub> evolution rates because tillage aerates soil, breaks up the aggregates and incorporates residues into soil profile. On the other hand, no tillage (NT) may increase physical protection of SOC within the aggregates resulting in less CO<sub>2</sub> evolving from soil.

The objective of this study was to evaluate the influence of tillage, crop rotation and nitrogen fertilization on soil CO<sub>2</sub> emissions under the semiarid conditions from the central region of Spain.

## Methodology

Experimental design consists of 60 plots (5x3 with 4 replicates) where tillage is the main treatment (conventional, minimum and no tillage) and a 4 year crop rotation (fallow-wheat-pea-barley) is compared to wheat monoculture. A fertilization experiment was also carried: conventional fertilization (200 kg ha<sup>-1</sup>, 8/24/8) was compared to rational fertilization calculated from N soil content, cereal expected productivity and cereal nitrogen needs (from 0 to 144 kg ha<sup>-1</sup>).

CO<sub>2</sub> -C evolving from soil surface (kg CO<sub>2</sub>-C ha<sup>-1</sup> d<sup>-1</sup>) was measured in all the plots three times during the cereal growing season: tillering, earing and heading. PVC rings were inserted into the soil one week before the readings. Measurements were taken using a soil respiration chamber and a soil temperature probe connected to an infrared gas analyzer (Model EGM-4, PP Systems). Soil water content was measured with a TDR probe in the first 7.5 cm depth. Rainfall was collected from the meteorological station near the experimental site.

Statistical analyses of data were performed using SAS software. GLM process was used to compare tillage treatments, crops and fertilization. Means were tested using Tukey's test (p<0.05).

## Results

Soil CO<sub>2</sub> emissions under different tillage and cropping systems are shown in Figs. 1 and 2.

CO<sub>2</sub> fluxes measured in tillering (end of February) did not show any significant differences among tillage and crops and varied between 13.5 and 24.3 kg CO<sub>2</sub>-C ha<sup>-1</sup> d<sup>-1</sup>.

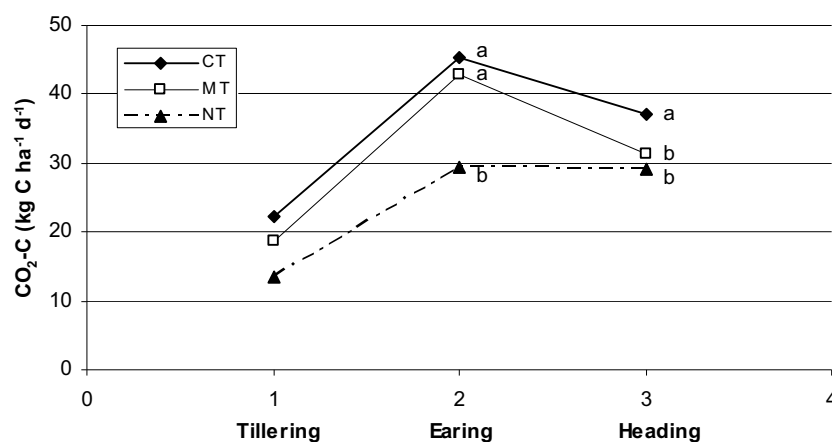
During earing and heading CO<sub>2</sub> fluxes were higher (from 29.3 in NT to 45.4 in CT, and from 29.3 in NT to 37.2 kg CO<sub>2</sub>-C ha<sup>-1</sup> d<sup>-1</sup> in CT respectively earing and heading) probably due to the precipitation fallen during April and May (83 mm till earing and 67.5 mm till heading). However a small relationship was obtained between surface soil water content and soil CO<sub>2</sub> flux. Under semiarid conditions, Álvaro-Fuentes J., *et al.* (2008) did not obtained any significant relationship between soil water content and CO<sub>2</sub> flux. They attributed it to the limitations of the technique applied, that does not differentiate between heterotrophic-derived CO<sub>2</sub> and root respiration.

The lower values of CO<sub>2</sub> emission were recorded under NT practices and the higher values were under CT (Fig.1). Fallow low CO<sub>2</sub> emissions (Fig. 2) could be attributed to the absence of root respiration. Among cereal and pea no significant difference were found.

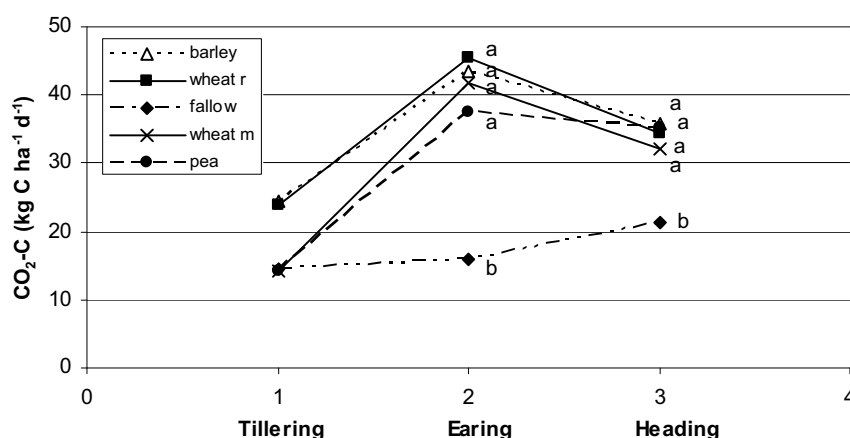
Nitrogen fertilization had little effect on soil respiration. Traditional fertilization showed lower CO<sub>2</sub> flux values than the rational but without significant differences (data not shown). This agrees with the results obtained by Wilson, and Al-Kaisi (2008).

No relationship was found between soil temperature and CO<sub>2</sub> flux.

Since the start of the experiment 14 years ago, a significant increase of SOC was observed under NT compared with CT. Soil respiration data are consistent with this results.



**Fig. 1. Soil CO<sub>2</sub> fluxes as influenced by tillage (CT, conventional tillage; MT, minimum tillage; NT, no tillage) during the growing season. Different letters indicate significant differences among tillages treatments within the same cereal phenological state.**



**Fig. 2. Soil CO<sub>2</sub> fluxes as influenced by crop during the cereal growing season. Different letters indicate significant differences among crops within the same cereal phenological state.**

## Conclusions

NT practices may increase SOC content due to slowly organic matter mineralization and this leads to lower soil CO<sub>2</sub> emission values when comparing to CT.

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# Carbon Sequestration In Tilled Soils As Influenced By Agronomic Practices

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The recent increase of carbon dioxide emission is raising global temperature by blocking solar radiation reflected from the Earth surface (Lal 2004, Ramaswamy 2001). Kyoto Protocol (3.4 article) emphasizes that agriculture can diminish air CO<sub>2</sub> by increasing soil organic carbon (SOC). Moreover a high organic matter (OM) content in soil is always beneficial: to prevent soil degradation, enhance its fertility and reduce pollution risks (Swift, 2001). This aim can be pursued by sustainable agricultural practices, but long-term experiments are required for the slow SOC dynamic. This paper reports results on SOC obtained in long lasting trials that DiSTA is carrying out in the Po Valley, near Bologna.

## Methodology

In Cadriano (Bologna) there are two field trials started in 1966. The first one compares crop rotations (continuous wheat WW, continuous corn CC, corn-wheat CW, sugarbeet-wheat SW, and 9-year rotation: alfalfa 3-year's meadow followed by CWCWCW), organic supply (with M0 or without M1, manure), and inorganic NP rates (N0P0, N1P1, N2P2) in a split-plot replicated twice (56 m<sup>2</sup> sub-sub-plots). In the second trial a same supply of organic materials (none, manure, slurry and crop residues) on a dry matter basis is factorially tested with mineral N rates (N0, N1, N2 and N3) on a corn-wheat rotation in a split plot with 4 replicates (33 m<sup>2</sup> sub-plots). Two other experiments, begun in 1976 and interrupted in 2000, tested tillage treatments and N rates (N0, N1, N2) in split-plots with three replicates (42 m<sup>2</sup> sub-plots). Basically, ploughings to increasing depth (25, 50, 75 cm) were compared to chisel or disk harrow (MT). In all trials crop residues have were always removed from the field and soil was sampled every year for SOC content determination along the tilled soil profile.

## Results

SOC content declined in the first 20 years of all trials, likely because compared managements were more intensive than previously. Then it increased at different paces, depending on treatments.

Period	9-yr	CW	SW	WW	CC
1966 (Start)	35.7	35.7	35.7	35.7	35.7
1972-1975	31.4	31.8	27.5	32.4	30.3
1981-1984	28.7	27.1	23.6	30.3	25.5
1990-1993	32.3	30.0	25.0	34.1	27.2
1999-2002	37.3	33.5	26.6	38.7	29.2

*Table 1. Crop rotation effects on the SOC content in the 0-0.40 m soil layer (t ha<sup>-1</sup>). Each value, except 1966, is a mean of the last 4 years of the 9-year cycles.*

The 9-year rotation and continuous wheat smoothed the initial drop (-11% year<sup>-1</sup>); then they both increased SOC at a rate of 467 kg ha<sup>-1</sup> year<sup>-1</sup>, with a mean C stock of 32.0 t ha<sup>-1</sup> over the 36 years (Table 1). Sugarbeet-wheat mostly reduced SOC (-23% year<sup>-1</sup>) and caused the slowest subsequent augment (130 kg ha<sup>-1</sup> year<sup>-1</sup>) with a mean SOC stock of 26.7 t ha<sup>-1</sup> (Table 1). The other rotations gave intermediate results. Thus both the amount and quality of crop debris played an important role in SOC dynamic. Manure reduced the initial SOC decrease (-9% year<sup>-1</sup>) compared to mineral fertilizers alone (-15% year<sup>-1</sup>, on average). Subsequently it promoted a faster SOC build up (+381 kg ha<sup>-1</sup> year<sup>-1</sup>), than mineral fertilization (+272 kg ha<sup>-1</sup> year<sup>-1</sup>). The influence of NP rates on SOC was slight (Table 2).

In the second trial (Table 3) CO<sub>2</sub> sequestration capacity of manure (0.26 t ha<sup>-1</sup> year<sup>-1</sup> of SOC increase) was 1.4 and 1.6 times higher than that of slurry (0.16 t ha<sup>-1</sup> year<sup>-1</sup>) and crop residues (0.26 t ha<sup>-1</sup> year<sup>-1</sup>). This result can be ascribed to polycondensed humic compounds in manure that are resistant to microbial attack.

Period	N0P0M0	N1P1M0	N2P2M0	N0P0M1	N1P1M1	N2P2M1
1966 Start	35.7	35.7	35.7	35.7	35.7	35.7
1972-1975	30.8	31.5	32.1	32.8	33.8	34.1
1981-1984	26.3	27.1	26.9	29.9	29.3	29.1
1990-1993	27.7	28.7	29.7	32.2	33.0	33.5
1999-2002	29.8	31.5	32.3	36.8	36.7	37.3

Table 2. Fertilisation effects on SOC in the 0-0.40 m soil layer (t ha<sup>-1</sup>). Each value, except 1966, is a mean of the last 4 years of 9-year cycles.

	Equation	r <sup>2</sup>	Probability
Manure	y = 0.26 x - 487	0.55	***
Slurry	y = 0.18 x - 324	0.38	***
Residue	y = 0.16 x - 285	0.39	***

Table 3. Regression over time of the SOC stock (t ha<sup>-1</sup> year<sup>-1</sup>) in the 0-0.40 m soil layer, with the supply of various organic materials.

	1976	1980	1984	1988	1990	1992	1994	1996	1998
MT	120.1	117.1	117.1	118.2	109.4	114.7	120.7	110.8	114.3
T25	120.1	108.9	105.5	103.9	114.4	117.1	116.8	116.7	113.3
T50	120.1	104.3	93.8	96.5	108.2	111.6	112.7	108.5	109.3
T75	120.1	107.2	95.1	97.3	105.0	104.9	108.1	103.9	102.0

Table 4. Tillage effects on SOC stock (t ha<sup>-1</sup> in 0-0.75 m soil layer). Means of the two trials.

In the other trials tillage depth significantly influenced SOC distribution along the soil profile, whilst tillage equipment had a slighter effect (Table 4). Deeper ploughings reduced SOC content, both for OM dilution along the soil profile and its faster degradation in well-aired soil. During the 20 years following the initial drop, SOC steadily increased with 25-cm ploughing. Instead, minimum tillage caused no evident build up, probably because of less residue produced (smaller OM yearly input).

## Conclusions

A suitable agronomic management can favour CO<sub>2</sub> sequestration in tilled soils. However SOC dynamics revealed slow (likely due to microbial soil buffering capacity). The amount and kind of buried OM proved important. Manure resulted the best material, but, considering C wasted during its formation, C balance at farm scale should not be so favourable. Cereal straw was found a good soil OM input mean, probably for its high lignin content, together with the 9-year succession, likely thanks to the great quantity of residues left by alfalfa. A shallow ploughing appeared a valid mean to maintain a high SOC level. Instead, in our environment, minimum tillage revealed inadequate, likely because it lowers too much crop yield and consequent residue production.

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# Can a Unique Model Simulate C and N Dynamics of Different Liquid Dairy Manures?

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Efficient management of animal manures reduces water pollution and economic costs. Nitrogen (N) dynamics in the soil after manure application are extremely complex, due to manure heterogeneity and to interactions with soil matrix and microbial biomass. Simulation models of the dynamics of soil organic matter (SOM) are useful as research and management tools, and can be effectively used also in manure studies. A particularly complete and promising SOM model is CN-SIM (Petersen et al., 2005). In a previous experiment (Marino and Bechini, 2007), we found out an extremely high variability of C and N dynamics for five different liquid dairy manures incorporated into three soils of increasing clay content (102, 209 and 337 g kg<sup>-1</sup> for soils 1, 2 and 3). In this research, we wanted to verify whether a single SOM model (CN-SIM) could simulate that variability.

## Methodology

The experimental data consisted of 12 measurements of respired CO<sub>2</sub> and concentrations of NH<sub>4</sub>-N and NO<sub>3</sub>-N during 180 days under standard laboratory conditions (25°C and optimal soil water content). The CN-SIM model uses seven organic pools, two representing the added organic materials (AOM1 and AOM2), two for the microbial biomass, and three for soil organic matter. As this model structure is very complex, a preliminary sensitivity analysis was carried out to identify the most important parameters for the simulation of the net effect of manure addition on C and N dynamics. Model calibration was carried out with an automatic procedure, aimed at minimising the relative root mean square error (RRMSE) between measured and simulated net CO<sub>2</sub> and soil mineral nitrogen (SMN = NH<sub>4</sub>-N + NO<sub>3</sub>-N), separately for each treatment. Nine parameters were calibrated: five describe C and N pools of the manure and their decomposition rates, two describe the C/N ratio (CN<sub>SMR</sub>) and the decomposition rate (k<sub>SMR</sub>) of soil microbial residues (SMR), one is the ratio 'residues produced / (maintenance + death rate)' of zymogeneous microbial biomass, and one is the nitrification rate. We chose these parameters because they can influence the simulation of the complex dynamics observed in manure mineralisation studies, where part of manure N is quickly immobilised by microbial biomass and subsequently remineralised.

## Results

Eleven out of 15 treatments were simulated with a RRMSE lower than 5% (Table 1), indicating a good fit of the model to the experimental data. As shown in Fig. 1, the model simulated rather well C and N dynamics of liquid dairy manures that differ in net N mineralisation. Calibrated parameters discriminate manures according to size (f<sub>AOM1</sub> and f<sub>AOM2</sub>) and relative decomposition rate (k<sub>AOM1</sub> and k<sub>AOM2</sub>) of the two organic manure pools. Manure 1 has a relatively fast and N-poor labile pool (AOM2) that forces N immobilisation in the first weeks; its resistant pool (AOM1) continues to immobilise N later, due to high C/N ratio. Manure 2 has the lowest k<sub>AOM1</sub> and k<sub>AOM2</sub>, as confirmed by the low respiration (Fig. 1a; 52, 40 and 49% of C applied after 180 days on soils 1, 2 and 3); the relatively low C/N ratio of the labile pool does not generate immobilisation in the first stage of decomposition (Fig. 1b). Manure 4 has a large and fast labile pool that generates a high flux of CO<sub>2</sub> in the first days of incubation (Fig. 1a) and the largest total respiration (67, 55 and 59% after 180 days on the three soils), associated to net N mineralisation due to its low C/N ratio. Notwithstanding these good performances, the upper or lower boundaries of model parameters were reached in 11 out of 15 cases, indicating that the optimisation tried to obtain a good fit of the model by exaggerating the importance of some

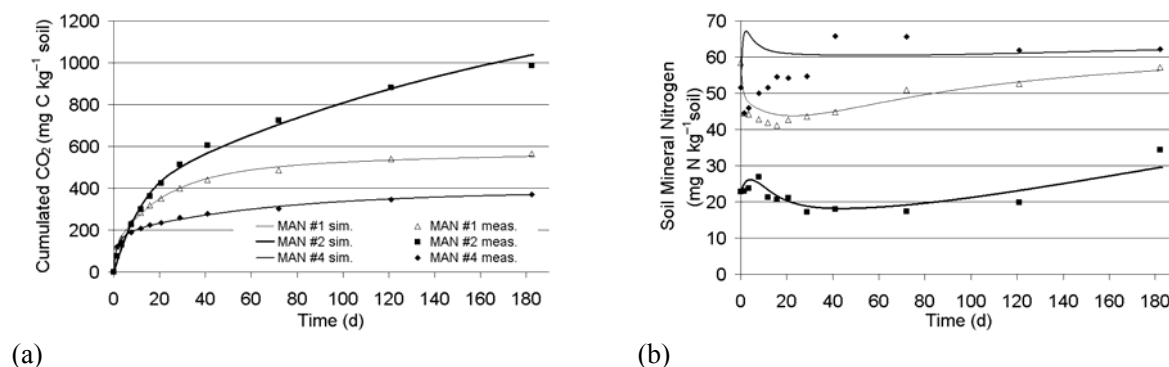
Table 1. Calibrated parameters of the CN-SIM model for three liquid dairy manures incubated in three soils of increasing clay content (102, 209 and 337 g kg<sup>-1</sup> for soils 1, 2 and 3).

Manure	Soil	k <sub>AOM1</sub> (d <sup>-1</sup> )	k <sub>AOM2</sub> (d <sup>-1</sup> )	f <sub>AOM1</sub> (-)	f <sub>AOM2</sub> (-)	CN <sub>AOM1</sub> (-)	CN <sub>AOM2</sub> (-)	RRMSE (%)
1	1	0.0097	0.121	0.54	0.46	24	13	4
	2	0.0279	0.450	0.56	0.22	43	10	4
	3	0.0146	0.310	0.66	0.28	17	25	3
2	1	0.0012	0.022	0.55	0.37	546	12	8
	2	0.0015	0.104	0.72	0.22	175	8	15
	3	0.0017	0.034	0.52	0.42	127	10	7
4	1	0.0170	0.277	0.11	0.56	23	7	2
	2	0.0120	0.333	0.17	0.53	15	8	4
	3	0.0045	0.294	0.19	0.50	15	7	5

~ 10<sup>-4</sup> d<sup>-1</sup>, compared to ~ 10<sup>-3</sup> d<sup>-1</sup> in the original) reduces its subsequent remineralisation.

Some of model failures and the extreme values reached by model parameters during optimisation can be explained by recalling that more sinks of N (other than those simulated by the model through organic matter flows) could have contributed to decrease SMN, namely denitrification and clay fixation. In addition, manure organic matter (and eventually microbial biomass) could be partitioned in more than two model pools, to match the observed fluctuations of CO<sub>2</sub> and SMN rates.

Fig. 1. Simulated (sim.) and measured (meas.) dynamics of (a) cumulated CO<sub>2</sub> respiration and (b) SMN for three liquid dairy manures (MAN) incubated in soil #3 (clay content of 337 g kg<sup>-1</sup>).



## Conclusions

A unique model structure can be used to simulate manures that differ in composition and decomposition dynamics. The problems encountered in this study might be amplified when the model will be calibrated, for each manure, on the three soils simultaneously.

## Acknowledgements

We are grateful to Marco Acutis for providing the optimisation code, and to Bjørn M. Petersen for support with model implementation. Sensitivity analysis was carried out with SimLab (<http://simlab.jrc.ec.europa.eu/>).

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# CO<sub>2</sub> Emissions Following Soil Application of Different Animal Manures in Mato Grosso (Brazil)

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Soil microbial activity includes all the metabolic processes and their interactions performed or mediated by soil microbial communities for obtaining energy and nutrients (Moreira and Siqueira, 2002).

When an organic material is applied to the soil, microorganisms decompose it, following slow or rapid dynamics according to several factors, including soil moisture, pH, temperature, carbon (C) and nutrients availability. The emission of carbon dioxide (CO<sub>2</sub>) from soil is related to both the freshly added and stable soil organic matter decomposition.

The objective of this work was to evaluate the microbial decomposition dynamics following the application to the soil of different animal manures. As an indicator of microbial decomposition, we measured CO<sub>2</sub> emissions.

## Methodology

The experiment was carried out in Cáceres, Mato Grosso (Brazil). The site is characterized by a tropical climate (mean annual precipitation of 1,348 mm and mean annual temperature of 25.2 °C) and a loamy sand Hapludalf soil (8% clay, 84% sand).

The treatments included three different types of animal manures: bovine excreta (faeces and urine from dairy cows), poultry waste (faeces, urine and waste feed from a “Frango Caipira” livestock), and a mixture of bovine excreta and poultry waste. A not-fertilised treatment was also present. The animal manures were applied to the soil at doses normally adopted in the region (50 Mg ha<sup>-2</sup> of bovine excreta, 10 Mg ha<sup>-2</sup> of poultry waste, and 30 Mg ha<sup>-2</sup> of their mixture). We adopted a randomised block design with six replicates. The main properties of the manures used in the experiment are summarised in Table 1.

Table 1. C and nitrogen (N) content of the animal manures included in the experiment.

	Organic C (% DM)	Total N (% DM)
Bovine excreta	14.62	2.02
Poultry waste	5.92	0.63

The measurement of CO<sub>2</sub> emissions from the soil was based on the method proposed by Isermeyer (1952). We covered a portion of soil surface by a glass bell-jar (driven into the soil at 2 cm deep) under which we collocated a glass jar containing 20 ml of NaOH (0.5 N), to trap CO<sub>2</sub> emitted by the soil. At each sampling moment, the bell-jars were opened and rapidly reclosed after removing the NaOH jars. The quantification of CO<sub>2</sub> lost were performed by titration with a HCl solution (0.5 N). At fixed intervals (8, 10, 13, 17 and 32 days after soil application), the jars were removed and NaOH replaced. Data were analysed through ANOVA and the Bonferroni post-hoc test was applied to separate significantly different means.

## Results

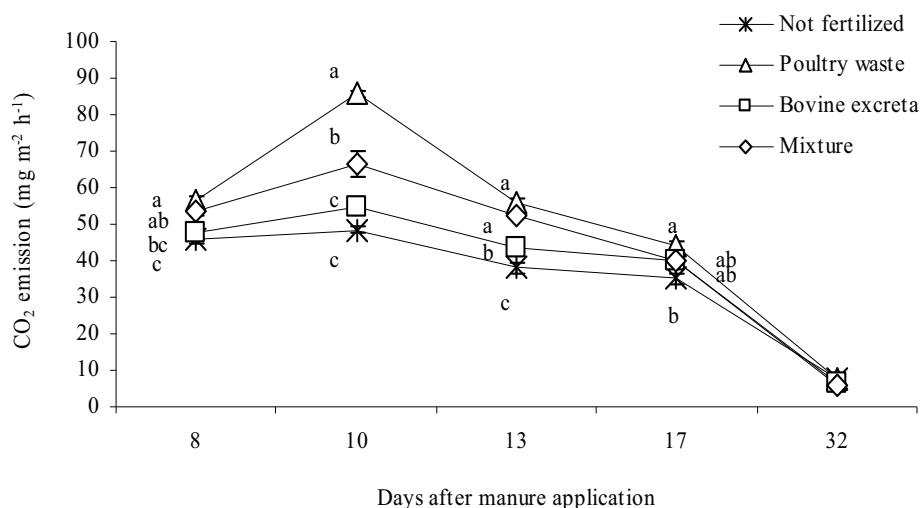
CO<sub>2</sub> cumulative emissions are shown in Table 2.

Table 2. CO<sub>2</sub> cumulative emissions (g m<sup>-2</sup>) at day 32 after manures application.

	CO <sub>2</sub> emissions
Bovine excreta	13.1 c
Poultry waste	16.4 a
Mixture	14.2 b

Although the absolute amount of C applied to the soil was the highest for the bovine excreta, the addition of the poultry waste generally induced a higher microbial activity than the other treatments. This behaviour could be related to the different chemical composition: the poultry waste had, in fact, a lower C to N ratio with respect to the other manures (Velthof et al. 2003). As expected, the mixture showed an intermediate trend.

Figure 1. CO<sub>2</sub> hourly emissions (g m<sup>-2</sup> h<sup>-1</sup>).



It is likely that the poultry waste had a higher content of labile C compounds than the other manures, due to the different diet, that is soybean-maize meal based for poultry, and therefore poorer of fibre and more recalcitrant C compounds than that of bovines (Melo et al. 2008). The dynamics of CO<sub>2</sub> emission (shown in Figure 1) presented a first phase of increase (from day 8 to day 10) with a higher slope for poultry waste than for other manures, thus confirming the elevated availability of readily decomposable C compounds in this material (Bertora et. al. 2008).

## Conclusions

The poultry waste, due to its chemical composition, induced a higher microbial activity, after its soil incorporation, than the bovine excreta.

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# Carbon Losses in the Short Period Following Corn to Alfalfa Conversion

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The greenhouse gas mitigation potential for cropped soils results from adapting locally in a changing climate, the best agronomic practices and land uses aimed at increasing agroecosystem's C stocks (i.e. Soil Organic Carbon SOC) and at lowering N<sub>2</sub>O and CH<sub>4</sub> soil emissions. The best option (considering efficacy, cost and additional benefits) to mitigate greenhouse gas is the use of SOC sequestration activities which are based on increasing soil carbon inputs, and/or slowing decomposition processes of crop residues and SOC itself. A variety of management practices have been developed and tested to maintain, or possibly increase crop production and soil organic matter levels. These options include reduced or no-tillage, the addition of organic amendments, improved crop nutrition, and crop rotations that include legumes. Drinkwater et al. (1998) reported that rotations with legumes can maintain higher SOC levels than continuous cropping systems with non-leguminous row crops. Gregorich et al, 2001, found greatest difference in soil C levels between alfalfa-corn rotation and monoculture corn systems. Cropping with deep-rooted alfalfa was the key factor in maintaining higher levels of SOC, particularly at depths below the plow layer. This is attribute to the higher legume derived aromatic SOC compounds below the plow layer; these C compounds are more resistant and so they contributed to an annual increase of SOC that could range from 40 to 85 gC m<sup>-2</sup> y<sup>-1</sup> as reported by Drinkwater (1998) and Gregorich (2001) respectively. More detailed studies, based on ecosystem C balance, are required on land use change from monoculture corn (*Zea Mays* L.) to rotation maize with alfalfa (*Medicago sativa* L.). This study investigates the effects on Net Ecosystem Production (NEP) and Net Biome Production (NBP) one year after conversion from monoculture corn to rotation corn with alfalfa. The additional objective of this study is to make an estimation of Global Warming Potential considering N<sub>2</sub>O emissions as affected by the crop rotation change.

## Methodology

The study area is located in Beano (Italy; 46°00'N 13°01' E). The soil is loamy gravelled and has a SOC content of 48.4±8.5 MgC ha<sup>-1</sup> in the 0-0.3m depth level. During the previous three decades it was conventionally farmed with irrigated monoculture of corn. At the beginning of the experiment (December 2006) the study area was divided north-south wise into two blocks to obtain an eastern area of 8.6 ha and a western area of 4.7 ha. The East field was cropped with corn, applying a moldboard plough at 0.35 m on December 2006 (thereafter continuous-corn, C-C). The West field was cropped with hay alfalfa crop (*Medicago sativa* L.), after plough at 0.35m on February 2007 (thereafter corn-alfalfa, C-A). An eddy-covariance (EC) flux tower was installed in each field (C-C and C-A) to assess Net Ecosystem Exchange (NEE). The measurement height was changed from 2.5 m up to 4.5 m depending on the crop canopy height. Each EC system consists of a sonic anemometer (Young, USA) and an open path infra-red gas analyser (Li7500, Licor, USA). In both fields were installed two weather stations equipped with sensors required by Euroflux protocol. Twelve intensive measurement plots (IMP) (10x10 m<sup>2</sup>) were established, corresponding to a factorial design of 2 crops x 2 tillage (tillage and no-tillage) replicated in 3 randomized blocks. The IMPs were used to study crop growth and biomass partitioning, soil respiration, belowground C deposition, N<sub>2</sub>O emissions and crop residue decomposition. Soil respiration was performed in each IMP every two hours using three automated soil respiration chambers (closed dynamic system; see a detailed description of the system in Delle Vedove et al., 2008). To estimate heterotrophic soil respiration (Rh) during growing season, one chamber was installed on root exclusion cylinder after sowing. In the present paper we report only data pertaining the

tilled treatments of corn (C-C) and the alfalfa (C-A). The main management operations for the C-C and C-A plots are reported in Table 1.

### Results

In both sites, irrigation maintained soil water content close to field capacity throughout the growing season. The peak leaf area index was 4.0 and 5.0 for C-C and C-A, respectively. Corn yields (September, 6<sup>th</sup>), recorded in the IMPs, were similar to average yields obtained in the footprint area ( $11.0 \pm 0.57 \text{ Mg ha}^{-1}$  of dry matter vs. of  $11.2 \pm 0.49 \text{ Mg ha}^{-1}$ ) and total corn residues, recorded after 2007 harvest, were  $14.1 \pm 4.4 \text{ Mg ha}^{-1}$ . The C-A yield was  $18.0 \pm 1.0 \text{ Mg ha}^{-1}$  of dry matter (sum of five harvests from May, 21<sup>st</sup> to October, 1<sup>st</sup>; Table 1).

Table 1 – Crop management details, grain and grass yields for the two land uses during 2007 (C-C=Cont. Corn; C-A = Alfalfa following Corn; standard deviations (n=3) are also reported.

Site	Tillage	Sowing	Yield <sup>1</sup>	NEP <sup>2</sup>	Rh <sup>2</sup>	NPP <sub>EC</sub> <sup>2</sup> NEP+Rh	Ch <sup>2,3</sup>	NBP <sup>2</sup> NEP-Ch
C-C	Dec 15 '06	Apr 4	11.0±0.6	425	574±11	999±11	506±26	- 81±26
C-A	Feb 21 '07	Mar 2	18.0±1.0	646	587±10	1233±10	828±47	- 182±47

<sup>1</sup>) Units are  $\text{Mg ha}^{-1}$  of dry biomass; <sup>2</sup>) Units are  $\text{gC m}^{-2} \text{ year}^{-1}$  (Dec 2006 to Nov 2007); <sup>3</sup>) Ch Carbon of harvested material assuming a C content 0.43 and 0.46 for corn grain and harvested alfalfa.

The Surface Residual Energy Balance analysis confirmed a good performance of EC systems (data not shown). The accuracy of flux approach was tested, for both C-C and C-A treatments, fitting Net Primary Production ( $\text{NPP}_{\text{EC}}$ ), computed as the sum of cumulated NEP and Rh fluxes, with seven growth-analysis data of above and belowground crop biomass. (C-C: slope 1.07, intercept = 0.19,  $R^2 = 0.92$ ; C-A: slope 0.97, intercept = -0.20,  $R^2 = 0.99$ ).

As a consequence of longer green canopy duration, C-A showed on yearly basis, a higher cumulated NEE (or NEP) than C-C ( $-646 \text{ gC m}^{-2}$  vs.  $-425 \text{ gC m}^{-2}$ , respectively). On yearly basis, C-C and C-A summed a similar Rh ( $574 \pm 11 \text{ gC m}^{-2}$  and  $587 \pm 10 \text{ gC m}^{-2}$ , respectively). The not significant difference in Rh between C-C and C-A ( $-13 \text{ gC m}^{-2}$ ) let the hypothesis that annual C losses from soil (SOC + residues) were similar even if an higher soil  $\text{CO}_2$  efflux was observed after spring plowing operations for C-A due to higher soil temperature.

In terms of net C balance (NBP), C-C was almost neutral ( $-81 \text{ gC m}^{-2} \text{ yr}^{-1}$ ), while C-A was a source of C ( $-182 \text{ gC m}^{-2} \text{ yr}^{-1}$ ) resulting in  $\Delta\text{NBP} = -101 \text{ gC m}^{-2} \text{ yr}^{-1}$ . This decrease in C (i.e. SOC) following the land use change is mainly due to the higher harvest registered in the C-A. The  $\Delta\text{NBP}$  results are relevant as they are in contrast with the few researches reporting high soil C sequestration after corn conversion to alfalfa. To estimate the GWP associated with the land use change, an N budget was performed according to IPCC 2006 revised guidelines for GHG inventories. This budget showed that the conversion from C-C to C-A decrease  $\text{N}_2\text{O}$  emissions ( $4.0 \text{ kg N}_2\text{O ha}^{-1} \text{ yr}^{-1}$ ). In spite of that, computing an overall GHG balance, in terms of  $\text{CO}_2$  equivalent, the conversion from C-C to C-A increases the GWP by  $251 \text{ gCO}_2 \text{ equ. m}^{-2} \text{ yr}^{-1}$ .

### Conclusions

According to the preliminary results described above, the change from a monoculture corn to corn-alfalfa rotation resulted in a net loss of carbon and in an increase of the overall GHGs budget. However, monitoring in the next years is required for final conclusions.

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# Fine-Scale Spatial Variability of Soil Organic Carbon and Related Environmental Variables in a Protected Area of Sicily, Italy

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The institution of Natural Reserves has promoted, in Italy, the conservation and the environmental improvement of several areas and their physical and biological factors. Agriculture, forestry and every human activity are regulated to preserve their high ecological and naturalistic value. Land use, in particular, must follow careful rules to preserve the soil fertility and to limit the factors of landscape degradation. Maps of soil organic carbon (SOC) or soil organic matter (SOM) are of interest for agricultural management, resulting a very important soil fertility parameter, as well as in environmental policy related to the terrestrial sequestration of atmospheric carbon. Thus, a better understanding of the distribution of soil organic carbon (SOC) pool is necessary in order to manage soil fertility and to predict its potential responses to land use change. Geostatistics is widely used to map SOC at any scale level assessing also the statistical uncertainty. At fine scale level geostatistical methods that utilize spatially correlated secondary information increase the quality of the maps of SOC distribution (Simbahan, 2006). In fact, whereas are significant correlations between the target variable and secondary data hybrid techniques generally result in more accurate local prediction (Goovaerts, 1999; McBratney et al., 2000). The goals of this study were: i) to assess the SOC spatial variability in the Natural Reserve of S. Ninfa (Italy) and ii) to quantify the relationships among soil C, land use and some environmental variables.

## Methodology

The study area, located in Western of Sicily, ranges from an altitude of 400 and 663 m a.s.l. The climate is Mediterranean semiarid. Land use are represented by: vineyards, arable lands, horticultural crops, olive groves, pastures, shrubs and woodlands (Eucalyptus and Pines). A set of soil samples were collected to quantify SOC in 0 to 20 cm ("A" layer) and in 20 to 40 cm ("B" layer) depth. Sampling scheme were transects oriented in West-East direction with a sampling pass of 200 meters along each transect. Total sampling points were 154 uniformly distributed in the study area. At every sampling points two soil core were collected and spatially georeferenced by GPS. Air-dried soil samples were analysed for SOC using the wet oxidation method with potassium dichromate.

Spatial variability was analysed by geostatistical procedures for every depth. To improve the spatial interpolation quality at fine scale a series of ancillary variables were acquired including several simple terrain indices derived from DEM and from recent landsat ETM+ images (NDVI). Recent land use and soil cover were mapped to be compared with SOC spatial distribution.

## Results

Experimental variograms (Fig. 1) showed a long-range variability of SOC data. A nested model (nugget + exponential) was used to fit the experimental variograms and ordinary block kriging procedure was used to produce SOC interpolated maps for A and B layer (Fig. 2).

High nugget values in variograms indicate that the sampling scheme used not resolved most of the spatial variability in SOC. This suggests the possibility to increase the number of sample points trying to resolve the spatial variability at short range.

Maps show a variability structure similar in both depths. Spatial regression between SOC map values of A and B layer showed a significant value ( $R^2=0.61$ ;  $p<0.001$ ). Comparing these with the land use map, the highest values of SOC was found in correspondence of pastures and the lowest in vineyards at lower quote but direct correspondences were not found between land use pattern and SOC distribution. No direct spatial correlations were found between SOC distribution and other environmental variables. While comparing SOC maps with the soil map, the distribution pattern of SOC, mainly for the B layer, seems to follow the soil pattern. In fact, clustering the map data of SOC by soil groups, the relative variability decrease remarkably in both A and B layer. The highest values of SOC were found for Mollic Gypsic Cambisols followed by Haplic Cambisols, the lowest values were for Haplic Regosols.

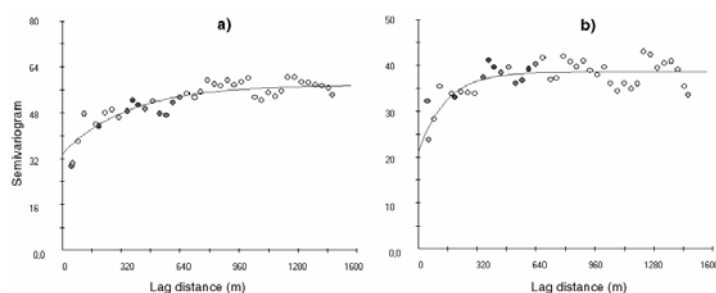


Fig. 1 – Experimental variograms and fitted models: a) A soil layer; b) B soil layer.

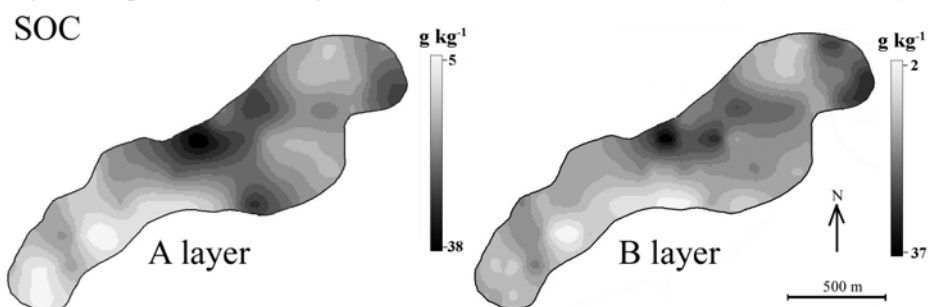


Fig. 2 – Maps of SOC at the two investigated depths.

## Conclusions

The geostatistical analysis confirmed and quantified a high spatial variability of SOC in the study area. However the relatively high nugget effect indicates that the sampling scheme used not resolved most of spatial variation in SOC. A optimised second sampling step to thicken the spatial data could be useful to improve the spatial resolution of the SOC maps. Many studies have shown strong influence of terrain indices on the spatial variation of SOC (Mueller and Pierce, 2003). However, no direct correlation was found, in this study, between SOC, recent land use and considered environmental variables, rather its spatial structure seems more affected of soil genetic pattern.

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# Modelling Crop Rotations from Field to Regional Scale: a Rural Planning Oriented Approach

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Crop simulation models are powerful tools both in research and in rural planning. Applications depend on the availability of input data and on their suitability to model requirements. In agroecosystem simulations oriented to rural planning (e.g. leaching evaluation at regional scale), multi-year crop rotations are needed. Crop rotations can be retrieved by Common Agricultural Policy (CAP) databases, where single crops and surface areas are yearly recorded at the cadastral level. However, simulations at the regional scale require an abatement of the very large number of rotations reconstructed from the crops series over the same field in a given time window in order to alleviate time-consuming simulation runs and to better explain model outputs. Moreover, the lack of information over a part of the total agricultural area must be filled using the available data in order to run complete geographical simulations. An approach aimed at upscaling crop rotations from the field to the regional scale and reducing the number of crop rotations while maintaining the representativeness of local agricultural systems and the linkage to the geographic features is proposed. The approach takes also into account that cropping systems simulations will be carried out over long time periods (decades), therefore requiring repeated rotations cycles.

## Methodology

Although some available tools allow to generate new crop rotations maintaining crops spatial and temporal patterns (e.g. LandsFACTS; Castellazzi et al., 2008), codified systems aimed at grouping similar rotations or assigning known rotations to fields without crop information are still lacking. A number of agronomic, combinatory and computational rules were implemented in a hierarchical framework on a 5-year series dataset in the following order:

- i) crops were grouped according to the type or phenology (e.g. orchards, grain legumes, vegetables); poorly represented crops (e.g. berries, fiber crops) were classified as “other”;
- ii) agronomically meaningless crop series were excluded from the analysis, as they were considered as errors in the original database (e.g. ABACA when A = alfalfa or vineyard or orchard);
- iii) specific grouping criteria were selected for rotations nesting perennial field crops: 4, 3 or 2 contiguous years of meadows were considered rotational meadows, classifying as “contiguous” also the 1<sup>st</sup> and the 5<sup>th</sup> year in repeated cycles; non contiguous years of meadow, regardless of their position within the series, were treated as annual grassland. When a single year of alfalfa occurred at the 1<sup>st</sup> or 5<sup>th</sup> year, or when 2 contiguous years of alfalfa occurred in the first two or in the last two years of the series, 3 years of alfalfa were assumed, according to the usual agronomic practices; other rotations including alfalfa in different position within the series and without at least 3 years of continuous alfalfa were excluded;
- iv) non rotational perennial crops (vineyards, orchards, permanent meadows) were separately treated: 5 years of meadows were considered as permanent meadows; 5 years of vineyards/orchards, or crops series showing at least the 5<sup>th</sup> year of vineyard/orchard were considered as non rotational perennial crops; other series with vineyards/orchards were excluded from the analysis;
- v) crops classified as “other” in rule i) were excluded from the analysis, owing to their low representativeness;
- vi) all rotations with 4 years of the same crop and 1 year of a different crop were grouped together, regardless of their order in the sequence (AAAAB = AAABA = AABAA = ABAAA = BAAAA);
- vii) the minimum repeating window in the 5-year crop series was identified; therefore 2-, 3-, 4- and 5-

year crop rotations were reconstructed; as alfalfa is a perennial crop usually lasting 3 years, 6- and 7-year crop rotations were also reconstructed when 1 year of alfalfa occurred at the 1<sup>st</sup> or at the 5<sup>th</sup> year and when 2 years of alfalfa occurred at the first or at the last two years of the crop series, respectively, as in the following examples: ABABA = BABAB = 2-year crop rotation AB; ABCAB = CABCA = BCABC = 3-year crop rotation ABC; ABCDA = ... = DABCD = 4-year crop rotation ABCD; ABCDE = BCDEA = ... = EABCD = 5-year crop rotation ABCDE; AACDE = CDEAA = 6-year crop rotation AAACDE when A = alfalfa; ABCDE = EABCD = 7-year crop rotation ABCDEEE when E = alfalfa; viii) in the minimum repeating window, rotations having the same crops in different order (crop permutations; e.g. ABCDE vs. ACBDE; ABCAB vs. ACBAC) were grouped together when nitrogen leaching simulated using the CropSyst model (Stöckle et al., 2003) did not demonstrate significant differences among rotations according to a one-way ANOVA ( $\alpha=0.05$ ) carried out on a selected extreme condition datasets, considering rotation cycles as replicates; ix) only rotations accounting for more the 90% of the investigated surface area were considered; x) owing either to the lack of data in the CAP database or to the discard of information following the application of previous rules, crop rotation of a number of cadastral units over the whole area remained unknown. Rotations were therefore assigned to the blank cadastral units taking into account the relative surface area ( $S_r$ ) of the known rotations over a regular grid (1000 x 1000 m), therefore keeping the spatial distribution variability; for each grid element the number of blank cadastral units to be linked to each known rotation was calculated as  $N_r = N_t S_r$ , where  $N_t$  is the total number of blank cadastral units for each grid element; according to  $N_r$ , crop rotations were randomly assigned to blank cadastral units.

## Results and conclusions

Using a 5-year dataset (2002-2006) from the Friuli Venezia Giulia (FVG) CAP database, 8261 different geographically referred crop rotations accounting for 141,973 ha, representing 66% of the total agricultural area, were reconstructed. These rotations were distributed on 238,301 cadastral units, while rotations were unknown in 115,583 units; the cadastral units had an average surface area of 0.6 ha, therefore showing a high fragmentation level. The implementation of the established rules led to a progressive decrease of the initial number: i) to v): 6089 (-5.5% of the rotation surface area); i) to vii): 3365; i) to viii): 1537; i) to ix): 153. Non rotational perennial crops, continuous crops, 2-, 3-, 4-, 5-, 6- and 7-year crop rotations were reconstructed. The final 153 crop rotations maintain most of the actual land use representation (Fig.1 and Tab.1) and can be used to run complete geographical models simulations.

CROP	step i) 8261 c.r.	step viii) 1537 c.r.	step ix) 153 c.r.
maize	55%	56%	60%
soybean	12%	13%	12%
barley	4%	4%	3%
permanent meadows	4%	4%	5%
vineyard	4%	5%	5%
alfalfa	5%	5%	4%
wheat	5%	5%	4%
others	11%	9%	7%

Tab. 1. Surface area percentage of the main crops in rotation during the different steps of the model implementation; c.r. = crop rotations

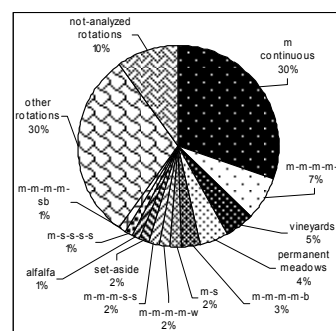


Fig. 1. Share of reconstructed crop rotations over the FVG agricultural area; m= maize; s= soybean; b = barley; w = wheat.

## Acknowledgement

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# Effects of Elevated Atmospheric CO<sub>2</sub> Concentration (FACE) on Nitrogen Dynamics in a Typical North German Crop Rotation

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Increasing atmospheric CO<sub>2</sub> concentrations [CO<sub>2</sub>] will have positive growth effects on arable crop production – without consideration of other climate change effects - as determined in Free Air Carbon Dioxide Enrichment (FACE) studies (Kimball et al 2003). Elevated [CO<sub>2</sub>] was also shown to affect crop tissue composition (carbon (C) : nitrogen (N) ratio) and crop water relationships which might have effects on soil N turnover. Only very limited information exists on the effect of elevated [CO<sub>2</sub>] on N dynamics in crop rotations under FACE conditions (e.g. Prior et al. 1997). However, as nitrogen and carbon turnover are closely linked to each other changes in N turnover may have repercussions on C turnover in arable soils affecting soil fertility and carbon storage.

## Methodology

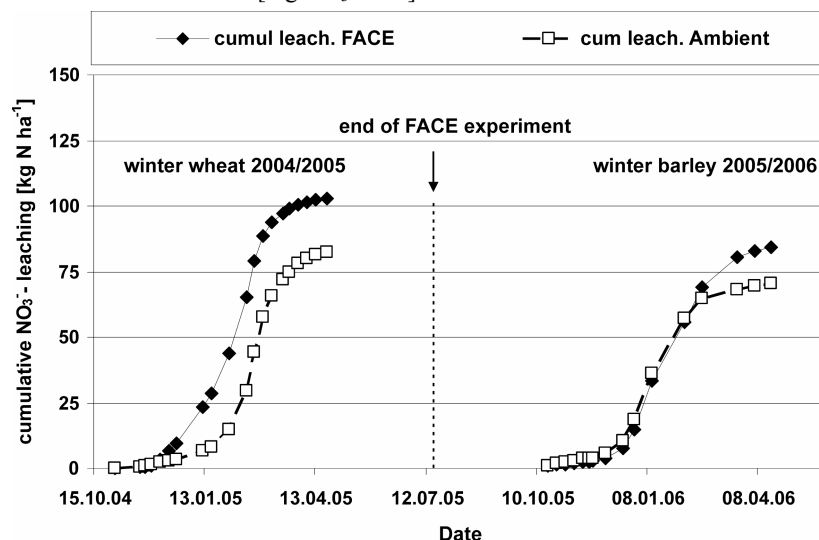
A FACE experiment was set-up for the investigation of the effect of elevated [CO<sub>2</sub>] on an agro-ecosystem planted with a typical North German crop rotation (winter barley, sugar beet, winter wheat), repeated twice in the years 1999-2005 (Weigel et al. 2005). Treatments included 4 experimental rings (20 m diameter) with two CO<sub>2</sub> fumigation levels: 2 rings without CO<sub>2</sub> enrichment (ambient 380 ppm [CO<sub>2</sub>]) and two rings with CO<sub>2</sub> enrichment (550 ppm [CO<sub>2</sub>]). Each ring was split in two halves and supplied with 2 levels of N fertilization (appropriate, 50% of appropriate). Crop roots were sampled by soil coring during the vegetation period of winter wheat 2005 and separated by hand washing (root biomass, root length densities, C:N ratios). Soil mineral N in the plough layer was determined monthly throughout the 6 years experimental campaign. In the second winter wheat growing season (2004/2005) and in the year after completion of the FACE experiment (winter barley 2005/2006) soil water suction cups were installed (35 cm and 80 cm) in the ring halves with appropriate N supply for the determination of N concentrations in soil solution. Soil N leaching was calculated using the crop growth modelling environment developed at the agronomy department of Kiel University (Kage and Stützel 1999), based on measured crop biomass and LAI values. Evapotranspiration was calculated with the Penman-Monteith equation modified for ambient and FACE conditions.

## Results

Elevated [CO<sub>2</sub>] increased above ground biomass by ca. 10% and root biomass by about 20 % at times of maximum root expansion as compared to ambient [CO<sub>2</sub>] conditions. In addition, [CO<sub>2</sub>] enrichment resulted in an increased C:N ratio in the roots and led to higher soil water levels during the growing seasons. However, the increase in C:N ratio was mainly observed in the N treatment with appropriate N-supply and was of the same magnitude as the effect of reduced N-supply. Throughout the 6 year experimental period only few significant – although always positive - effects of [CO<sub>2</sub>] enrichment on mineral N contents in the plough layer were observed. However, incidences of increased mineral N content under FACE conditions were more often found towards the end of the experimental period.

**Tab. 1** Nitrate concentrations in two sampling depths in the FACE experimental site, Braunschweig, under FACE (550 ppm [CO<sub>2</sub>]) and ambient conditions (380 ppm [CO<sub>2</sub>]) (winter wheat) and on the same plots without CO<sub>2</sub> treatment (winter barley, post-FACE), sufficient N supply treatment

30 cm soil depth	Winter wheat 2004/2005		Winter barley 2005/2006	
	FACE	Ambient	FACE plots	Ambient plots
Mean concentration [mg NO <sub>3</sub> <sup>-</sup> -N l <sup>-1</sup> ]	74.50	122.49	123.94	69.27
Median concentration [mg NO <sub>3</sub> <sup>-</sup> -N l <sup>-1</sup> ]	49.66	134.55	50.46	21.98
80 cm soil depth				
	FACE	Ambient	FACE plots	Ambient plots
Mean concentration [mg NO <sub>3</sub> <sup>-</sup> -N l <sup>-1</sup> ]	87.52	106.67	100.98	103.11
Median concentration [mg NO <sub>3</sub> <sup>-</sup> -N l <sup>-1</sup> ]	78.83	147.03	89.06	111.87



**Fig. 1:** Cumulative nitrate leaching calculated for the FACE experimental site, Braunschweig, under FACE (550 ppm [CO<sub>2</sub>]) and ambient (380 ppm [CO<sub>2</sub>]) conditions (winter wheat) and on the same plots without CO<sub>2</sub> treatment (winter barley, post-FACE), sufficient N supply treatment, based on NO<sub>3</sub><sup>-</sup> concentrations in 80 cm soil depth

Mean and median NO<sub>3</sub><sup>-</sup>-concentrations in soil solution (Tab. 1) showed no clear trend with regard to the effect of elevated [CO<sub>2</sub>] on N-turnover: while both parameters were higher for both depths in the ambient treatment in winter wheat, the opposite was the case in the post-FACE period in winter barley (30 cm sampling depth). Nitrate leaching (Fig. 1) was considerably higher in the last year of FACE experimentation (winter wheat) under both [CO<sub>2</sub>] conditions than in the post-FACE period (winter barley). This was mainly due to the high amount of sugar beet residues (leaves) and higher amount of percolation water in this year. However, NO<sub>3</sub><sup>-</sup>-leaching was higher in both years for the experimental sites with [CO<sub>2</sub>] enrichment. Slightly higher amounts of percolation water under FACE conditions (winter wheat) did only contribute to a small degree to this effect. The main reasons were higher NO<sub>3</sub><sup>-</sup>-concentrations in the FACE plots during the main percolation periods in both years.

## Conclusions

The results indicate that effects of elevated [CO<sub>2</sub>] on plant tissue composition (increased C:N ratio) had no downscaling effect on N turnover in the agro-ecosystem studied. In contrast, higher amounts of residual crop roots and plant residues due to enhanced crop growth under elevated [CO<sub>2</sub>] resulted in increased mineral N contents in the plough layer and higher N leaching losses in the main percolation periods. The findings suggest that there was no N-limitations to C-turnover under elevated [CO<sub>2</sub>] in the studied conventionally managed agro-ecosystem with mineral N supply.

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# Carbon balance of field grown apple (*Malus domestica*) trees

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To be ecologically sustainable, productive tree ecosystems should be managed in order to enhance their potential to fix atmospheric carbon (C). The exchange of CO<sub>2</sub> between ecosystem and atmosphere reflects the balance between photosynthesis from plants (GPP or Gross Primary Production) and respiration by plants, animals and microbes (RE or Ecosystem Respiration). This balance is called Net Ecosystem Exchange (NEE) or Net Ecosystem Productivity (NEP = -NEE) and it is equal to the Net Primary Production (NPP) minus the heterotrophic component of RE (Rh). Therefore distinguishing the autotrophic and heterotrophic components of soil respiration and estimate the belowground C inputs from plants are crucial steps to determine if ecosystems are C-sources or sinks.

## Methodology

In this study we investigated an apple orchard planted in 1996/1997 in the experimental farm of the University of Bologna in Cadriano, in the Po Valley. Trees are apple (*Malus domestica*) cv. Mondial Gala on M9 rootstock, planted 1x3,8 m (2632 trees per hectare) in 1,8 m wide tree rows with no-residual herbicide treatment and a 2 m wide grass stripes between the tree rows. The climate is sub-Mediterranean with dry summers and two peaks of precipitation in spring and fall. The mean annual temperature is 13°C, the mean annual precipitation is ~700 mm rain year<sup>-1</sup>. The soils are alluvial, fertile silty clay loam (sand 18%, silt 50%, clay 32%) Trees were irrigated in the row and had an annual input of NPK equal to 80-15-100 Kg ha<sup>-1</sup>. The orchard is managed according to integrated production system guidelines.

Allometric relationships between tree size and biomass were obtained after destruction of three plants in winter 2005. Aboveground productivity of trees (ANPP) was assessed on three field grown apple trees by measuring the biomass of abscised leaves, thinned and harvested fruits, by measuring shoots lengths and diameters and estimating tree framework biomass increase. Increases in stem and branches were estimated with repeated measures of length and diameter in the same points, then using the allometric equations between volume and biomass.

Belowground Net Primary Productivity (BNPP) was calculated measuring root biomass increment and estimating root respiration and root turnover. Coarse root increase was estimated with an allometric relationship between diameter below grafting point and root biomass obtained by roots excavation in 2004. Fine root density, was assessed by soil coring in February 2006 and 2007. C input to the soil by roots (*Litter<sub>root</sub>*) was estimated with a carbon budgeting approach (TBCA, *Total Belowground Carbon Allocation* approach) derived from that used in forest ecosystems (Raich and Nadelhoffer 1989; Giardina and Ryan 2002). This approach is based on the fact that if losses for erosion or leaching are negligible, C fixed from the plants and sent to the soil, must either be respired by microbes or roots, generating the total soil CO<sub>2</sub> efflux (*R<sub>soil</sub>*), or stored in soil as organic matter, litter layer or root biomass. This could be described by this equation:

$$TBCA = R_{soil} - Litter_{leaf} + \Delta soilC + \Delta C_{root} + \Delta litter C \quad (1)$$

Where  $Litter_{leaf}$  is C input from aboveground litter,  $\Delta soilC$ ,  $\Delta C_{root}$  and  $\Delta litterC$  are changes in SOM, root biomass and litter layer respectively. TBCA can be also solved for the plant components of the system as the sum of  $\Delta C_{root}$  plus  $Litter_{root}$  and root respiration ( $R_r$ ) allowing to calculate  $Litter_{root}$  from this equation:

$$Litter_{root} = R_{soil} - R_r - Litter_{leaf} + \Delta litterC + \Delta soilC \quad (2)$$

$R_r$  was estimated with the *trenching method*, a root exclusion approach that consists in severing all roots around the perimeter of a treatment plot in order to disrupt aboveground input from trees (Hanson et al. 2000). The trenched plots (50x50x50cm) were established by digging trenches between contiguous plants in the row. Before filling back the trenches, each plot was lined with a geotextile canvas that prevents external root ingrowths but permits gas and solutes flow. In each trenched plot two PVC collars were inserted in the soil for a total of 8 collars to measure microbial respiration ( $R_h$ ). Total soil respiration ( $R_{soil}$ ) was measured in 8 identical collars, placed outside the trenched plots. The study was carried out in the tree row because previous studies outlined that the major part of tree roots are concentrated there by the effect of localized fertilization and irrigation. Four trenched plots (two for each side of the row) were established in May 2005. During 2006 soil respiration in trenched and control was measured monthly, twice a day, with an infrared gas analyzer EGM 4 (PP Systems, UK) connected to a closed dynamic chamber SRC 1 (PP Systems, UK),  $R_r$  was then calculated as difference. Mean values of representative days for each month were used to estimate annual values of  $R_{soil}$  and  $R_h$ .

## Results

NPP of trees was equal to 7.92 Mg C/ha: ANPP accounted for roughly 68 % of NPP, while BNPP accounted for the remaining 32%. The largest fraction of C was partitioned to the fruit; approximately 30 % of NPP and about 42% of ANPP. The flux of C belowground averaged 3.53 Mg C/ha and about one third was accounted by root turnover. Annual root respiration was roughly 1 Mg C/ha accounting for 43% of  $R_{soil}$  and responding to changes in soil temperature and moisture. But her behaviour in springtime and fall seems clearly to depend on physiological processes occurring in the plant.

## Conclusions

Although one third of Net Ecosystem Productivity (NEP) was exported from the system as harvested fruits, the latter resulted an important C sink. The significance of the C flux to the roots could be explained either as an effect of the alternate bearing (fruit production of 2006 was half than previous year) or as a consequence of optimal growth conditions. However, C balance of the orchard have to accounts also for CO<sub>2</sub> emissions by farm operation and, in our case, of the herbaceous component of the system, the correct estimation of those, being important to close the balance of whole productive ecosystem.

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# Carbon balance in tree ecosystems: a productive orchard compared to an artificial forest

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The role of tree ecosystems in the future climate scenarios proposed by scientists is likely to be very important. The enhanced productivity induced by increased temperature and [CO<sub>2</sub>] will result in an augmented belowground carbon allocation (BCA) that will result in a major carbon (C) flux in terrestrial ecosystems (Giardina et al. 2005). The exchange of CO<sub>2</sub> between ecosystem and atmosphere reflects the balance between photosynthesis from plants (GPP or Gross Primary Production) and respiration by plants, animals and microbes (RE or Ecosystem Respiration). This balance is called Net Ecosystem Exchange (*NEE*) or Net Ecosystem Productivity (*NEP* = -*NEE*) and it is equal to the Net Primary Production (*NPP*) minus the heterotrophic component of RE (*R<sub>h</sub>*). The rate at which C accumulates in the soil is a balance among the inputs of C from vegetation (rhizodeposition, and litterfall) and losses due to microbial respiration and leaching. Therefore distinguishing the autotrophic and heterotrophic components of soil respiration and the response of these fluxes to increases in soil temperature is a crucial step to understand the processes regulating soil organic matter turnover and to determine if ecosystems are C sources or sinks.

## Methodology

During 2006, we measured the NPP and its partitioning above- and belowground in two typical tree ecosystems of the Po valley (Northern Italy): a fifteen year old, mixed hardwood plantation and a twelve year old apple tree orchard. The first is established on a former agricultural land near the town of Nonantola (44°41'N, 11°02'E) with a density of 1100 trees per hectare and the most represented species are pedunculate oak (*Quercus robur* L.) and narrow-leaved ash (*Fraxinus oxycarpa* Bieb.); soil has a clay content of around 60%. The second is situated in the experimental farm of the University of Bologna in Cadriano (44°33'N, 11°21'E) and was planted with *Malus domestica* (cv. Mondial Gala on M9 rootstock) in 1,8 m wide, weeded tree rows spaced by 2 m wide grass stripes (2632 trees per hectare). Both sites have sub-humid climate (averaged annual precipitation and temperature are 700 mm and 13,7 - 13°C).

In both systems an estimation of Aboveground Net Primary Production (ANPP) was obtained by inventory. Belowground net Primary Production (BNPP) was estimated in part by allometry (coarse root increase) in part by direct measurement (fine root density increase). C input to the soil by root (*Litter<sub>root</sub>*) was estimated with a carbon budgeting approach (TBCA, *Total Belowground Carbon Allocation* approach) derived from that used in forest ecosystems (Raich and Nadelhoffer 1989; Giardina and Ryan 2002). This approach is based on the fact that if losses for erosion or leaching are negligible, C fixed from the plants and sent to the soil, must either be respired by microbes or roots, generating the total soil CO<sub>2</sub> efflux (*R<sub>soil</sub>*), or stored in soil as organic matter, litter layer or root biomass. This could be described by this equation:

$$TBCA = R_{soil} - Litter_{leaf} + \Delta soilC + \Delta C_{root} + \Delta litter C \quad (1)$$

Where  $Litter_{leaf}$  is C input from aboveground litter,  $\Delta soilC$ ,  $\Delta C_{root}$  and  $\Delta litter C$  are changes in SOM, root biomass and litter layer respectively. TBCA can be also solved for the plant components of the system as the sum of  $\Delta C_{root}$  plus  $Litter_{root}$  and root respiration ( $R_r$ ) allowing to calculate  $Litter_{root}$  from this equation:

$$Litter_{root} = R_{soil} - R_r - Litter_{leaf} + \Delta litter C + \Delta soil C \quad (2)$$

Root respiration was estimated with the *trenching method*, consisting in a root exclusion approach that consists on severing all roots around the perimeter of a treatment plot in order to disrupt aboveground input from trees (Hanson et al. 2000). Six trenched plots (50x50x50cm) were established in the forest and four in a tree row in the orchard in April-May 2005. During 2006  $R_h$  in trenched plots and  $R_{soil}$  in control plots nearby were measured monthly, twice a day, with an infrared gas analyzer EGM 4 (PP Systems, UK) connected to a closed dynamic chamber SRC 1 (PP Systems, UK),  $R_r$  was then calculated as difference. Annual integration of soil CO<sub>2</sub> fluxes were obtained with an empirical exponential model depending on soil temperature in the forest, and by using mean values of representative days for each month in the orchard.

### Results

In the forest NPP was equal to 9.66 Mg C/ha: ANPP accounted for about 45% of NPP, while BNPP accounted for the remaining 55%, resulting higher than ANPP. In the orchard, NPP of trees was equal to 7.92 Mg C/ha, ANPP accounting for about 68 % of NPP, BNPP accounting for the remaining 32%. Root respiration was roughly 1 Mg C/ha in both sites, but accounted for 43% of  $R_{soil}$  in the orchard and 22% in the forest.  $Litter_{root}$  in the forest was almost double than in the orchard, accounting for the 61% of BNPP in the first and for the 50% in the second.

### Conclusions

Despite the big difference in standing biomass and C stocks between the two systems NPP was similar, and in both sites BNPP was comparable with ANPP conditioning the net ecosystem productivity. Although NEP was higher in the orchard due to the fruit production, the C sink potential of two ecosystems was comparable. However, C balance of the orchard have to accounts also for CO<sub>2</sub> emissions by farm operation and, in our case, of the herbaceous component of the system, the correct estimation of those, being important to close the balance of whole productive ecosystem.

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- Raich J.W. and Nadelhoffer K.J., 1989. Belowground Carbon Allocation in Forest Ecosystems: Global Trends. *Ecology* 70: 1346-1354.

# Measuring the Carbon Balance of Olive Trees Using Chambers

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The measurement of the carbon and water balance of trees is fundamental to understand the processes leading to carbon assimilation and water use, but is difficult to measure at plant level. A convenient method for measuring the gas exchange of individual plants is the use of chambers. Most research at plant level on this subject has been done on small plants (especially in pots), due to the practical difficulties in the design, construction and operation of chambers large enough to enclose a whole tree. Unfortunately, the scaling up from small, young plants in pots to mature trees in the field is often speculative, and should be backed up by sound experimental tests on mature trees.

## Methodology

The chamber, of transient-state closed type (Reicosky 1990, Livingston and Hutchinson, 1995), is designed as a hexagonal prism, with a height of 4 m and a total volume of 41.6 m<sup>3</sup>, enough to enclose a mature tree of most of the cultivated species (Figure 1).

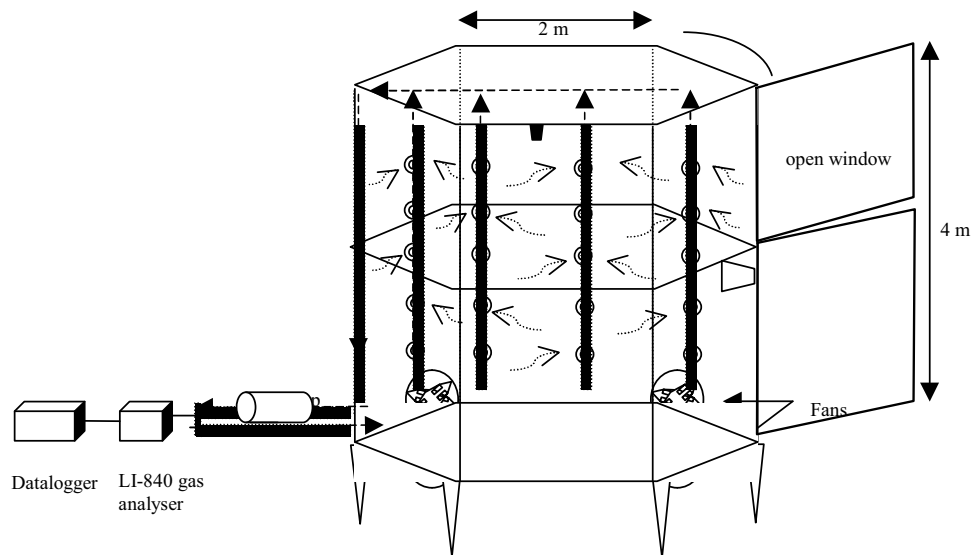


Figure 1

The bottom is made of thick polyethylene sealed around the tree trunk; all the walls are made of biaxially-oriented polyethylene terephthalate (Mylar®), chosen for its excellent broadband transmittance. The top, and four of the six vertical faces, are hinged windows that can be quickly

opened and closed. Four fans mix the air during the closing period (which in normal operating conditions does not need to exceed three minutes), maintaining the boundary layer similar to the environment outside the chamber. The air is aspirated from inside the chamber through many intake points, spatially distributed in the chamber volume, and circulated into a closed system. A subsample of this flow is passed through a gas analyser (LI-COR mod. LI-840), measuring CO<sub>2</sub> and water vapour concentration; the output is recorded by a datalogger. From the rate of change of the gas concentration when the chamber is closed, the whole-canopy net photosynthesis (or respiration) and transpiration rate can be calculated.

## Results

Figure 2 shows a typical measurement cycle during daytime, when photosynthesis exceeds respiration. The chamber enclosed a 12-year-old olive tree, on 24 August 2006 in Cordoba (Spain). When the chamber is closed, [CO<sub>2</sub>] begins to decrease and [H<sub>2</sub>O] to increase steadily. The rates of changes of gas concentration are constant and the scatter is minimal, allowing a precise calculation of the gas exchange rates. The increase in canopy temperature (measured with infra-red thermometers), although it was a clear summer day, is around one °C only.

In Figure 2 it is shown the diurnal course of the canopy gas exchange of the same olive tree. The maximum assimilation rate is reached in the morning; the CO<sub>2</sub> assimilation decreases with the increasing evaporative demand in the afternoon. The peak transpiration is reached in the first afternoon, and remains sustained until the evening.

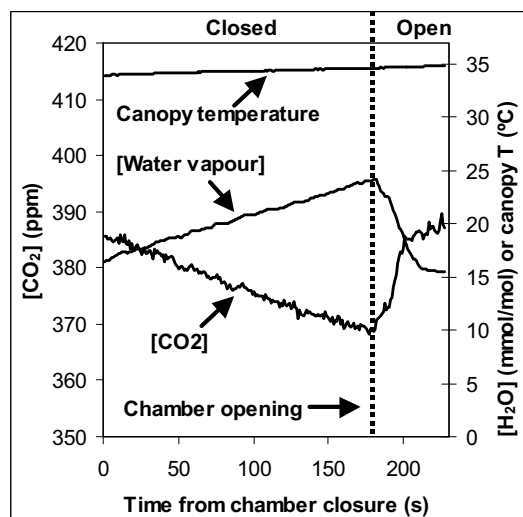


Figure 2

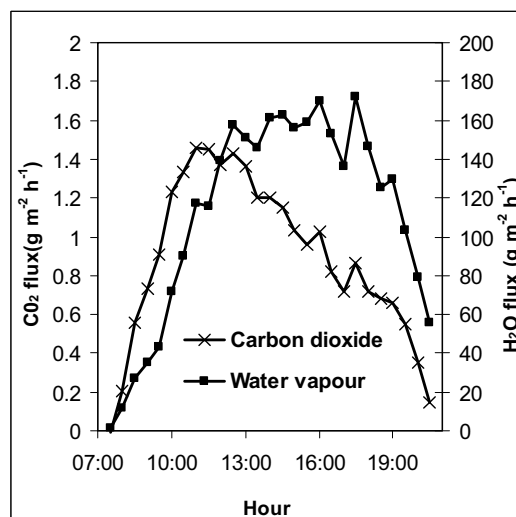


Figure 3

## Conclusions

The chamber design has demonstrated its capability for precise measurements of canopy gas exchange in large, mature trees. The preliminary results presented here suggest that this kind of chamber has a great potential for measuring directly the carbon and water exchange of whole mature trees in orchards without disturbance of the canopy micrometeorological environment.

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# Comparison of MODIS-Based Evapotranspiration Estimates with Eddy-Covariance Measurements on a Vineyard in North-Eastern Italy

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In a vineyard located in a typical wine growing area in North-Eastern Italy, an eddy covariance tower is providing continuous measurements of sensible heat, water vapour and carbon dioxide fluxes since summer 2005. The vineyard covers a flat, homogeneous and extensive area, and it is characterized by a high uniformity in management (cultivar, pruning, plant height) and soil profile. The plot is therefore very well suited for combined micrometeorological measurements and remote sensing observations. In this work, we tested the possibility of using data from the MODIS (*Moderate Resolution Imaging Spectroradiometer*) programme for estimating and monitoring vineyard evapotranspiration (ET).

## Methodology

**Study site** - The site location is at Negrizia di Ponte di Piave (45.74 N, 12.44 E; 11 m a.s.l), a village in the Veneto Region. The vineyard, which was planted in 1992, is 25 hectares wide, uniformly covered with the cultivar Carménère. Irrigation is performed via subirrigation, normally during a week within the first half of July.

**MODIS data collection and processing** - Belonging to the CarboItaly/FLUXNET network, the study site benefits of specially-processed and accurately geo-referenced MODIS products, which allow single-pixel time series analysis. The MODIS sensor flies onboard two NASA satellites: TERRA (launched in 1999, datasets tagged with “MOD”), and AQUA (launched in 2002, datasets tagged with “MYD”). Time series of the MOD/MYD13 products (Collection 5) were downloaded from [http://daac.ornl.gov/cgi-bin/MODIS/GR\\_col5\\_1/mod\\_viz.html](http://daac.ornl.gov/cgi-bin/MODIS/GR_col5_1/mod_viz.html).

MOD/MYD13 provides 16-day composite vegetation indices: the *Normalized Difference Vegetation Index* (NDVI) and the *Enhanced Vegetation Index* (EVI), at 250 m resolution.

EVI was tested as predictor for ET using the procedure described by Nagler et al. (2005). This approach consists of fitting separately EVI and ground-measured daily maximum temperature (Tmax) against tower-measured ET. The two functions are then combined into one single expression, which is used as an empirical predictor for ET. An exponential model is used for fitting EVI while Tmax is best fitted by a sigmoidal curve:

$$ET = a * (1 - e^{-b*EVI}) * \frac{c}{1 + e^{\frac{-(T_{max}-X_0)}{d}}}$$

**Eddy Covariance measurements** – In this study we used measurements of water vapour fluxes and air temperature, which are recorded at 30 min intervals. In order to be compared with MOD/MYD13 product, daily ET and Tmax were calculated, then expressed as 16-day composite taking the average for each 16-day period.

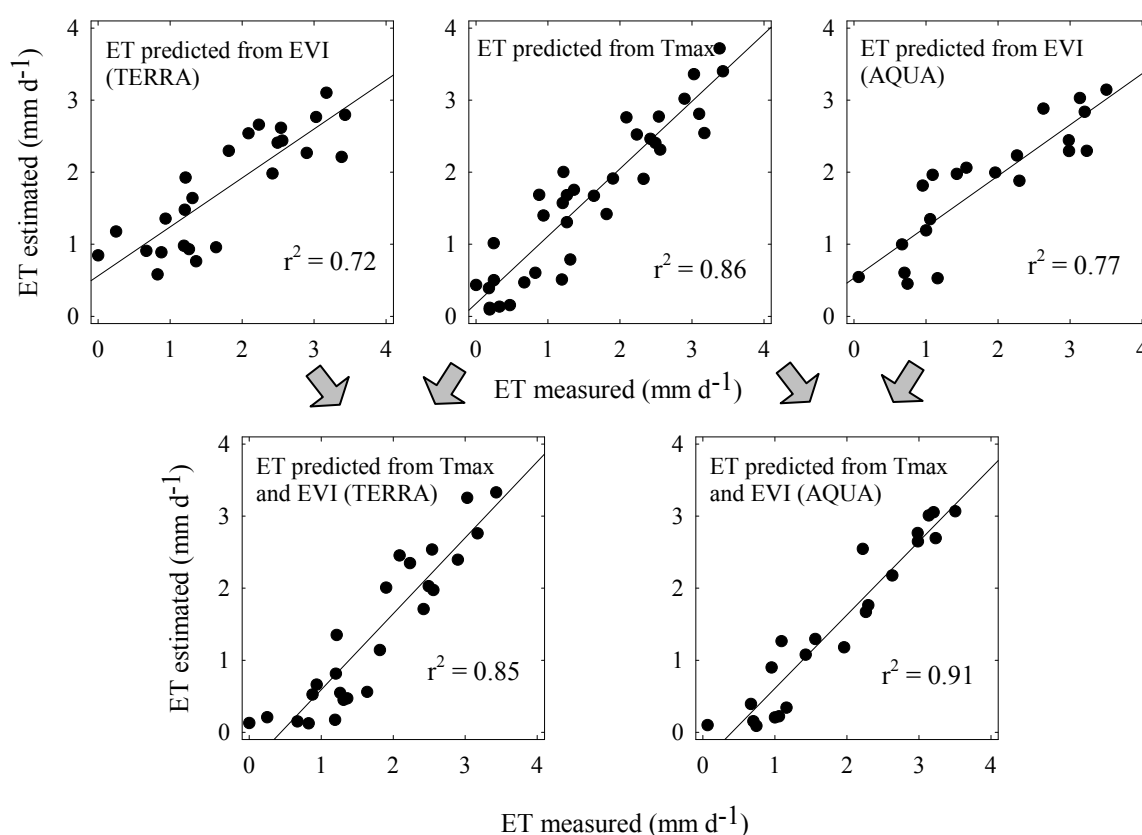
## Results

The analysis was carried out for the 2006-2007 period. Evapotranspiration predicted from Tmax was correlated with measured ET ( $r^2 = 0.86$ ). A lower correlation was found between ET predicted from EVI, with  $r^2 = 0.77$  and  $r^2 = 0.72$  for AQUA and TERRA respectively.

Combining EVI and Tmax regressions into one single function, improved the estimating ability only for the AQUA dataset, where we obtained an  $r^2 = 0.91$ , while for TERRA it was  $r^2 = 0.85$ , lower than the ET estimated from Tmax only.

This behaviour could be due to the different pass time of the two satellites: while TERRA passes over Italy at around 11.00h (local time), AQUA passes at around 13.00h, that is, in a moment coincident with the daily temperature peak. This probably improved the consistency between the two predictor variables.

**Figure 1** – Comparison between ET predicted from EVI and Tmax and from combined EVI-Tmax.



## Conclusions

Estimates of vineyard ET obtained through an empirical model based on MODIS EVI and ground measured Tmax, were compared to direct micrometeorological measurements of water flux. The two available EVI datasets showed a different consistency with measured data, with AQUA EVI having the highest correlation with measured ET.

The method is expected to provide more consistent results once the micrometeorological dataset will be extended in the next tower operational years.

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# Effects of Atmospheric CO<sub>2</sub> Concentration and Irrigation Scheduling on Sunflower: a Simulation Case-Study

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## Introduction

Atmospheric CO<sub>2</sub> concentration is today at 375 ppm and is projected to be in the range of 550-750 ppm by the end of this century (IPCC, 2007). Elevated CO<sub>2</sub>, associated climate change, may greatly increase growth and yield of most agricultural plants (Kimball *et al.*, 2002; Ainsworth and Long, 2005).

Crop simulation models are able to simulate the effects of several CO<sub>2</sub> concentrations on crop productivity (Tubiello *et al.*, 2002). In this study, EPIC model, that has been previously used from many authors on this topic (Easterling *et al.*, 1993; Brown and Rosenberg, 1999), has been chosen.

The EPIC model (Erosion Productivity Impact Calculator) was developed in the USA to investigate the relationship between erosion and soil productivity and was subsequently enhanced by the further addition of modules to improve crop, water quality and carbon cycle simulation (Sharpley and Williams, 1990; Williams *et al.*, 1990). Modifications were made in EPIC in order to account for the effects of elevated CO<sub>2</sub> on crop growth and yield (Stockle *et al.*, 1992a). The modifications consisted of incorporating functions that simulated the effects of changes in CO<sub>2</sub> concentrations and a vapour pressure deficit on crop radiation-use efficiency, leaf resistance, and transpiration. EPIC uses the concept of light-use efficiency in calculating photosynthetic conversion to biomass. The CO<sub>2</sub> algorithms incorporated in EPIC were achieved by Stockle *et al.* (1992b).

The aim of this study was to assess the simultaneous effects of elevated CO<sub>2</sub> concentrations and irrigation management, on sunflower (*Helianthus annuus* L.) grown in Southern Italy.

## Methodology

The simulation was performed for the period from 1952 to 2006, using a daily climatic data set collected in Foggia (Southern Italy). The Priestley-Taylor option was used to estimate potential evapotranspiration. Five CO<sub>2</sub> atmospheric concentrations (from the present value, 350 to 750, by 100 ppm) were simulated in sunflower submitted to three irrigation strategies: no irrigation (rainfed treatment = RF), 60 mm of irrigation (supplemental irrigation treatment = SI) at bud flower phenological phase and well automatic irrigation water amount (well-watered treatment = WW). Crop management dates were implemented in input files, according to local practice and crop parameters were obtained from previous validation and calibration studies (Ventrella and Rinaldi, 1999; Rinaldi, 2001). In this study the version no. 5300 of the i\_EPIC (Interactive) model was used.

The following output variables of the model were analyzed: seed yield, total biomass and water use efficiency ( $WUE_y = \text{seed yield}/ET_a$  and  $WUE_b = \text{total biomass}/ET_a$ , in kg m<sup>-3</sup>, with ET<sub>a</sub> as actual evapotranspiration).

## Results

The results indicated that sunflower seed and biomass yield increased with irrigation water amount, so far as the water use efficiency, while the greater number of water stress days (data not shown) was in rainfed treatment. The simulation with i\_EPIC model showed that irrigation is needed to reach high and economic sunflower yield in the Mediterranean region. Under elevated atmospheric CO<sub>2</sub> concentrations (750 ppm), EPIC simulated a significant increase (49 %) of seed and biomass production respect to the present CO<sub>2</sub> value. This increment was similar in RF and SI treatments, lightly higher in the WW condition. The interaction “[CO<sub>2</sub>] levels x irrigation treatments” showed as the positive effect of [CO<sub>2</sub>]

was higher in well watered conditions, especially for sunflower plant growth and seed yield. The response of water use efficiency to  $[\text{CO}_2]$ , on the contrary, was less influenced by irrigation scheduling and water availability (Figure 1).

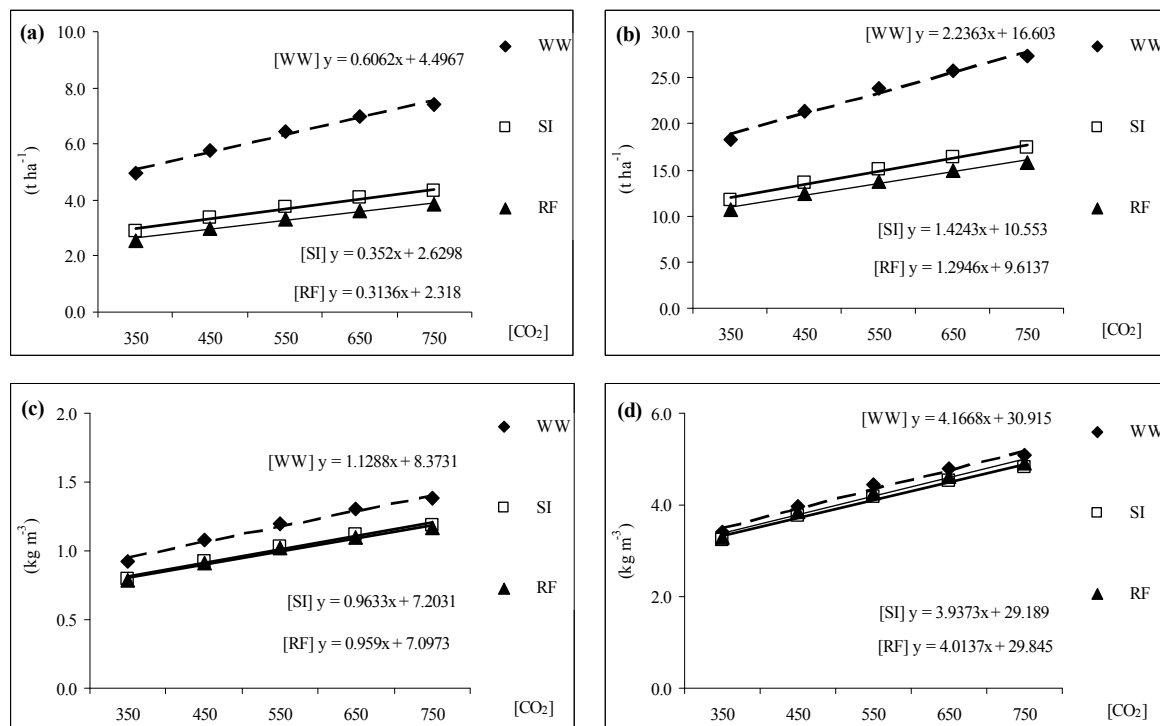


Figure 1 – Relationship between seed yield (a), total biomass (b),  $\text{WUE}_y$  (c),  $\text{WUE}_b$  (d) and the five  $[\text{CO}_2]$  levels, simulated by EPIC model on sunflower.

## Conclusions

The sunflower productivity has been simulated by i\_EPIC model with different  $\text{CO}_2$  concentration levels, showing a positive interaction of crop growth and seed yield with water availability.

This study demonstrated how i\_EPIC crop model can simulate climatic scenarios and forecast plant response to atmospheric  $\text{CO}_2$  variations and how crop management and, in particular, water management, can interact with carbon assimilation cycle. The simulation model, finally, can be an efficient and useful tool in irrigation scheduling, especially in water-limited situations.

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# Rotating Barley, Sugar Beet and Wheat under Elevated CO<sub>2</sub> Conditions: A Synopsis of the German FACE Experiment

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## Introduction

In current assessments of potential impacts of the anticipated climate change on future crop production the magnitude of the “CO<sub>2</sub>-fertilization” remains a matter of debate (Ainsworth et al. 2008). This is mainly due to the lack of appropriate field experiments. For example, information on how major arable crops in Europe might respond to future atmospheric CO<sub>2</sub> concentrations is mainly based on studies performed under conditions which hardly reflect the field situation. A free air carbon dioxide enrichment (FACE) experiment was carried over 6 years in an arable crop rotation at Braunschweig, Germany (Weigel et al. 2006). The objectives were to assess effects of elevated CO<sub>2</sub> and the interaction with nitrogen supply on crop performance (growth, biomass, yield and yield quality) and on related agro-ecosystem properties (water relations, soil carbon turnover and biodiversity). Key findings of the experiment will be presented and discussed in the context of results of other CO<sub>2</sub> enrichment studies.

## Material and Methods

The FACE experiment (2 replications per CO<sub>2</sub> treatment: 385/550 ppm; Fig.1) was applied to a rotation (1999-2005) consisting of winter barley (*Hordeum vulgare*), a ryegrass mixture as a cover crop, sugar beet (*Beta vulgaris*) and winter wheat (*Triticum aestivum*) which was repeated once. Agricultural management measures of the field (total 22 ha) were carried out according to local farm practices (mineral nitrogen fertilization; plough tillage). Irrigation was applied to keep the plant available soil water content above 50%. The soil is a luvisol of a loamy sand texture (69% sand, 24% silt, 7% clay) in the plough horizon with a pH of 6.5 and a mean organic matter (Corg) content of 1.4%. CO<sub>2</sub> supplied to the FACE rings was depleted in <sup>13</sup>C (δ<sup>13</sup>C = - 47‰). Nitrogen supply was restricted to 50% (N50) of adequate N in half of each of the FACE rings, resulting in a CO<sub>2</sub> × N split-plot design.



**Figure 1:** 20 m diameter FACE rings in a winter wheat field at Braunschweig, Germany; also shown are canopy chambers to measure CO<sub>2</sub>-/H<sub>2</sub>O-fluxes and shading devices to study CO<sub>2</sub>/radiation interactions

## Results and Discussion

Compared to ambient CO<sub>2</sub>, elevated CO<sub>2</sub> concentration (eCO<sub>2</sub>) markedly enhanced canopy photosynthesis (canopy carbon exchange rate; Burkart et al. 2007) of all crop species (up to ca. 41% for

sugar beet), while canopy evapotranspiration ( $E_c$ ) was reduced by  $eCO_2$  between ca. 3 - 20%. Due to reduced  $E_c$  under  $eCO_2$  higher soil moisture levels (field capacity) were observed during the canopy development. While green area indices of the cereal species were hardly enhanced, leaf area index of sugar beet was significantly reduced by  $eCO_2$  in both growing seasons. Above ground biomass and yield of cereals were stimulated by  $eCO_2$  by ca. 8 - 16% (barley; Manderscheid et al. 2008) and 15 - 16% (wheat), respectively. Accompanied by an enhanced senescence and a negative effect on leaf area index biomass and yield of sugar beet were stimulated by  $eCO_2$  by only ca. 7 - 8 % in the two growing seasons. The high  $CO_2$  treatment reduced grain N content of both cereals by ca. 3 - 15% compared to ambient air. For wheat a detailed analysis of grain protein fractions revealed marked changes of nearly all protein fractions under  $eCO_2$  (Wieser et al. 2008). Elevated  $CO_2$  did not affect variables of soil carbon turnover in a consistent manner. Root growth and soil respiration were only periodically stimulated by  $eCO_2$ . After 6 years soil microbial biomass remained unaffected by  $eCO_2$  and no  $CO_2$  induced changes in the soil carbon content could be detected. Concomitantly, a shift of the fungal/bacterial ratio of the microbial biomass in favour of bacteria was observed. Furthermore,  $eCO_2$  affected the ratio of functional groups of soil animals like collembolans and their C isotopic signatures (Sticht et al. 2008).

Overall, with respect to crop growth and yield cereals responded only moderately to  $eCO_2$ , while the sugar beet responses were unexpected. Table 1 summarizes important results of the  $eCO_2$  treatment (adequate N fertilization only).

**Table 1:** Summary of elevated  $CO_2$  effects on crop variables in the Braunschweig FACE experiment; note that no statistical significance is indicated; GAI/LAI = green area index/leaf area index

	relative $CO_2$ effect (%)					
	winter barley		sugar beet		winter wheat	
	2000	2003	2001	2004	2002	2005
<b>Canopy photosynthesis</b>	+ 18.1	+ 26.1	+ 41.5	+ 32.5	+ 36.9	+ 25.5
<b>Canopy evapotranspiration</b>	- 6.2	- 12.2	- 18.6	- 13.5	- 3.6	- 20.2
<b>Soil moisture</b>	+ 12.7	+ 19.0	+ 11.7	+ 22.5	+ 15.6	+ 11.0
<b>GAI / LAI</b>	(+ 11.7)	- 5.0	- 17.9	- 23.2	+ 6.1	+ 1.5
<b>Biomass</b>	+ 8.1	+ 17.6	+ 8.1	+ 6.6	+ 14.5	+ 15.2
<b>Yield</b>	+ 7.5	+ 16.4	+ 7.8	+ 7.1	+ 15.6	+ 15.8
<b>Grain N content</b>	- 15.1	- 10.8	---	---	- 3.0	- 13.9

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