

Productivity of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus x giganteus* Greef et Deuter) as energy crops: growth analysis

Nicoletta Nassi o Di Nasso, Neri Roncucci, Federico Triana, Cristiano Tozzini, Enrico Bonari Land Lab, Scuola Superiore S. Anna, Pisa, Italy

Abstract

The growing interest in bioenergy crops is leading to the development of new research aims. In fact, there is a lack of knowledge of most of these crops in terms of suitability to specific environmental conditions and of biotic and abiotic influences. The objective of our study was to compare giant reed (Arundo donax L.) and miscanthus (Miscanthus × giganteus Greef et Deuter), two promising lignocellulosic energy crops in Southern Europe, in terms of productivity, through growth analysis, in order to understand environmental and/or management constraints to crop development. Our research was carried out in 2009, in San Piero a Grado, Pisa (Central Italy; latitude 43°41' N, longitude 10°21' E), on a seven-year-old crop, in loam soil characterised by good nutrient and water availability. Results confirmed high yields in both species, about 40 t/ha/yr in miscanthus and 30 t/ha/yr in giant reed, achieved in the second half of October. Different growth strategies were noted as miscanthus developed a greater number of stems per square meter and higher stems, although it showed minor basal stem diameter and leaf area changes. In addition, the physiological difference between crop pathways (C3 in giant reed vs C4 in miscanthus) in a non-limiting environment allowed miscanthus to perform better. As a result, the choice of the proper crop has to be made in order to obtain maximum yield levels, minimising external inputs and optimising the land use.

Introduction

Increased concern for the negative impact of fossil fuels on the environment, particularly of greenhouse gas emissions, has increased the urge to find energy alternatives such as the use of biomass (Amougou $et\ al.$, 2010). Among bioenergy crops, perennial rhi-

Correspondence: Nicoletta Nassi o Di Nasso, Land Lab, Scuola Superiore Sant'Anna di Pisa via Santa Cecilia 3, 56127, Pisa, Italy. E-mail: n.nassiodinasso@sssup.it

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zomatous grasses such as giant reed (Arundo donax L.) and miscanthus (Miscanthus x giganteus Greef et Deuter) seem promising owing to their high productivity and longevity even under temperate and cold conditions, and to their low nitrogen fertilisation requirements (Heaton et al., 2004; Angelini et al., 2009; Smith and Slater, 2010). During the last two decades, several field trials have been carried out on miscanthus and giant reed under different environments, in order to investigate their potential yield. As reported by Lewandowski et al. (2003) and Zub and Brabcourt-Hulmen (2009), miscanthus dry matter (DM) yields without irrigation range from 10 to 15 t/ha in North and Central Europe to more than 30 t/ha in South Europe, reaching top values of 49 t/ha under no limiting conditions in France. Variable dry matter yields are also reported for giant reed: irrigated experimental plots in Greece achieved 26 t/ha DM within the first four years of the trial, showing high variability owing to genetic and environmental issues (Christou et al., 2001). On the other hand, Lewandowski et al. (2003) reported yields of over 30 t/ha DM in South Europe. Direct comparisons between miscanthus and giant reed have been performed in two studies conducted in Southern and Central Italy. Mantineo et al. (2009) documented maximum yields of 27 and 39 t/ha DM for miscanthus and giant reed, respectively, in three-year-old crops. On the other hand, Angelini et al. (2009) reported an average yield of 29 t/ha/yr and 38 t/ha/yr DM for miscanthus and giant reed, respectively, in a 12-year field trial without irrigation. However, further research is needed to improve knowledge on biomass accumulation dynamics and how abiotic and biotic factors influence productivity. For this reason, growth analysis (Hunt, 1982) provides a useful framework to assess productivity in energy cropping systems. Plant growth analysis suggests an explanatory, holistic and integrative approach for interpreting plant form and function. It has the advantage of using simple primary data such as weight, leaf area and plant partitioning to investigate processes involving the whole crop (Hunt, 2003). In fact, crop dry matter is the product of coupled morphological and physiological yield components, which represent, respectively, the capacity of the crop to acquire growth limiting resources and the efficiency with which those resources are utilised to synthesise dry matter (Heggenstaller et al., 2009). The objective of our study is to compare, under the same environment, two species of the Poaceae family, miscanthus and giant reed, characterised by similar morphology and different physiology (C3 and C4 pathways for giant reed and miscanthus, respectively); thus, to investigate, through growth analysis, their biomass accumulation strategies.

Materials and Methods

Experimental field

A field trial set in 2003, comparing miscanthus $(M. \times giganteus)$ and giant reed (A. donax), was used for the experiments. The trial





was conducted at the Enrico Avanzi Interdepartmental Centre for Agro-Environmental Research of the University of Pisa (CIRAA), located in the Pisa coastal plain (Central Italy; latitude 43°40' N, longitude 10°19' E; 1 m above mean sea level and 0% slope). The soil was a typical Xerofluvent, representative of the lower Arno River plain, and it was characterised by a shallow water table (Table 1). The experimental design was a randomised block with three replications (plots 7 m x 7 m each). Tillage was conducted in the autumn of 2002, after wheat harvesting, and consisted of medium-depth ploughing (30-40 cm). Seedbed preparation was conducted in spring, immediately before planting. For both crops, establishment was performed using rhizomes with a couple of buds weighing 500 g. The rhizomes were planted at 10-20 cm of soil depth, at 0.50×1 m spacing (20,000 plants/ha). Fertiliser were distributed at a rate of 100 kg N/ha, 100 kg P₂O₅/ha and 100 kg K₂O/ha. Nitrogen fertiliser was applied in the establishment year 50% as preplant and 50% as side dressing, when plants were 0.30-0.40 m tall. The following years, P2O5 and K2O fertiliser were applied during winter, while N was applied in spring at the beginning of growth. Plots were kept weed-free by hoeing. No crop diseases were detected during the experimental period and irrigation treatment was never necessary.

Plant sampling and measurements

For the purpose of this study, field measurements and meteorological data collection were carried out during 2009, corresponding to the crops seventh year of growth. Daily changes in air temperature, daily rainfall and daily global incoming radiation were recorded along the growing cycle at the nearby weather station. The mean thermal time in growing degree days (GDD) was calculated for each year with the NOAA method, above a base temperature of 10°C and a maximum cut-off temperature of 30°C as:

GDD =
$$_{S1}\sum_{}^{S2}(T_{m}-b_{0})$$
 [1]

where T_m was the mean daily temperature, b_0 the base temperature, and S1 and S2 were the growth start and the harvest time, respectively, expressed in Julian days.

Crop sampling began 30 days after crop re-growth. A sampling area of 1 $\rm m^2$ was selected randomly every two weeks from each plot, and all plants were clipped at 5 cm above ground level. Border plants in the outer rows were not included in the harvested area. Primary data comprise above-ground (leaves and stems) dry biomass (W), plant height, basal stem diameter and shoot number. Leaf area index (LAI) was estimated by means of a plant canopy analyser (SunScan). Dry weight of all plant material was measured after drying for 4-6 days in a forced-air dryer at $60^{\circ} \rm C$.

Growth analysis

The analytical method used in this study follows those proposed by Hunt (1982), Yusuf *et al.* (1999) and Heggenstaller *et al.* (2009) to examine giant reed and miscanthus cropping systems. Primary crop data were first transformed to logarithms to stabilise variance (Heggenstaller *et al.*, 2009) and subsequently they were fitted to clarify the relationship between measurements and time, expressed by Julian days. The Gompertz function:

$$f(t) = p \exp(-\exp(c(d-t)))$$
 [2]

was used to describe the above-ground dry biomass of giant reed and miscanthus, where t is time reported as Julian days, c is the relative growth rate at the inflection point, d is the X-axis value at the inflection point and p is the plateau. In the case of above-ground dry biomass data, curve fitting was performed without considering the senescence phase. Quadratic polynomial curves were used for fitting leaf area and LAI data as reported by Yusuf $et\ al.\ (1999)$ and Heggenstaller $et\ al.\ (2009)$. The Gompertz and the polynomial curves

were fitted with the R software version 2.10.1 (2009) (DRC package). A test of lack-of-fit at the 95% level was performed for each growth curve, indicating the suitability of the applied models to our primary data (Knezevic *et al.*, 2007). Additionally, fitted functions were used to estimate instantaneous crop growth efficiency and assimilative capacity.

The relationship between primary biomass data and time may be written as:

$$\ln W = f_w(t)$$
 [3]

where W represents the above-ground dry yield in g/m² and g/stem, respectively. Statistical differences between miscanthus and giant reed growth curves were analysed using the F-test ANOVA. Following this, three growth parameters were computed: crop growth rate, absolute growth rate and net assimilation rate.

Crop growth rate (CGR; g/m² land area per day) is defined as the change rate of the total crop biomass over time, and is equal to the first derivate of Eq. 3;

$$CGR = \exp [f_w(t)] \times f_w'(t)$$
 [4]

Absolute growth rate (AGR; g/stem/day) is the increment in weight per time unit if f(t) is the total dry weight per stem; AGR is then the first derivate of Eq. 3;

$$AGR = \exp \left[f_w(t) \right] \times f_w'(t)$$
 [5]

Net assimilation rate (NAR; g/m^2 leaf area per day), defined as the dry biomass increment in relation to leaf surface, measures the photosynthetic efficiency of leaves. It is calculated as:

$$NAR = f'(t)/L_A$$
 [6]

where L_A is the green leaf area and f'(t) is the first derivate of Eq. 3. In addition, data on plant height, basal stem diameter and number of shoots per unit area were analysed for each sampling date using one-way ANOVA (R software). An arcsin transformation was applied to all data expressed as fractions before performing ANOVA.

Radiation use efficiency (RUE, g/MJ) was determined as the slope of the relationship between above-ground biomass per square meter and cumulated intercepted photosynthetic active radiation (PAR_{in}) in MJ/m^2 . For each crop, incident PAR (IPAR) and transmitted PAR (PAR_i) were measured using the Sunscan instrument corresponding to each biomass sampling. Daily intercepted PAR was thus calculated

$$PARin = RAD \cdot 0.5 \cdot \left(\frac{PAR_{in} - PAR_{t}}{PAR_{in}} \right)$$
 [7]

assuming daily incident PAR (*IPAR*) as 50% of total daily solar radiation (RAD) (Monteith and Unsworth, 1990).

Table 1. Physical and chemical soil properties (soil horizon 0-30 cm sampled in March 2009).

Sand (2-0.05 mm; %)	41.0
Silt (0.05-0.002 mm; %)	38.5
Clay (<0.002 mm; %)	20.5
рН	8.2
Organic matter (%)	2.0
Total nitrogen (Kjeldahl method; g/kg)	1.1
Assimilable phosphorus (Olsen method; mg/kg)	6.2
Exchangeable potassium (Dirks and Scheffer method; mg/kg)	138.8
CE (conducibility; μS)	68.3



Results and Discussion

Monthly mean air temperature along with monthly rainfall is shown in Figure 1. Air temperature increased from March to August with maximum values over 30°C in July and August. Total annual rainfall in 2009 reached 1032 mm, about 200 mm higher than the total rainfall average of the 1990-2008 period. This was mainly because of the abundant rainfall falling during winter and spring. A dry period, as defined by Bagnouls and Gaussen (1953), was recorded during late spring and summer (Figure 1).

Above-ground dry yield

As previously described, owing to heterogeneous variances, the primary data were transformed to natural logarithms. However, to avoid complicated language, the transformed units will no longer be designated as such in the discussion (Yusuf *et al.*, 1999). This is an accepted practice in the growth analysis literature (Hunt, 1982) and is appropriate, because the transformation is done only for statistical reasons and does not alter the interpretation of data.

Both species showed very similar biomass accumulation trends, with a growing cycle starting by the end of March and ending at the beginning of November. During this period, the two crops needed different amounts of thermal units for completing their cycle, about 2000°C and 3000°C for miscanthus and giant reed, respectively. A lack-of-fit test at the 95% level was not significant for any primary data set indicating that the Gompertz model was correct for describing miscanthus and giant reed biomass accumulation (Figures 2 and 3) (Knezevic *et al.*, 2007). Table 2 reported the estimated coefficients

describing giant reed and miscanthus biomass accumulation, highlighting significant differences between the species on dry yield accumulation expressed as production per square meter and per stem. For both species, biomass accumulation was described by three sub-phases: an early accelerating phase, a linear phase and a saturation phase. The maximum above-ground dry yield values were achieved

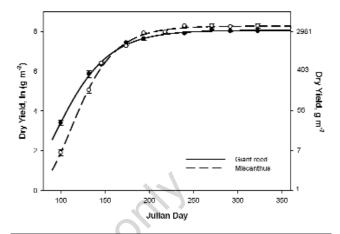


Figure 2. Mean predicted dry yield per ground area as a function of Julian day for giant reed and miscanthus crops during the 2009 growing season. • and O indicates observed values (±SD) for giant reed and miscanthus, respectively.

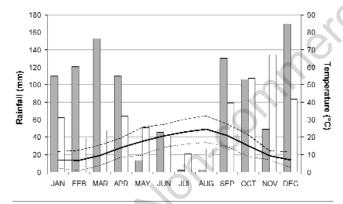


Figure 1. Meteorological data of the study site (Pisa, 43° N, 10° E), expressed by a Bagnouls–Gaussen diagram. A month is considered *dry* when the value of the average monthly rainfall (R) is equal to or less than twice the monthly average temperature (T) (R≤2T). Grey and white columns refer to 2009 monthly rainfall and 1992-2008 average monthly rainfall, respectively; unbroken line represents monthly average air temperature; dotted lines show maximum and minimum air temperatures.

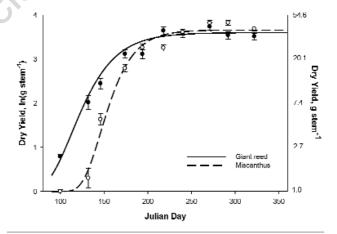


Figure 3. Mean predicted dry yield per stem as a function of Julian day for giant reed and miscanthus crops during 2009 growing season. ● and ○ indicate observed values (±SD) for giant reed and miscanthus, respectively.

Table 2. Estimated coefficients and standard errors for dry yield response to Julian day fitted with a Gompertz function° for giant reed and miscanthus crops in 2009 in Pisa (Central Italy).

Variables	Species	Coefficient (±SE)			P	Significance
		p	c	d		
Dry Yield, g/m²	Giant reed	8.06 (±0.052)	$0.030\ (\pm0.003)$	95(±4.0)	0.028	*
	Miscanthus	$8.27 (\pm 0.007)$	$0.035\ (\pm0.004)$	111(±3.1)		
Dry Yield, g/stem	Giant reed Miscanthus	$3.60 (\pm 0.062)$ $3.66 (\pm 0.072)$	$0.036 (\pm 0.006)$ $0.048 (\pm 0.006)$	123(±3.0) 146 (±4.6)	0.000018	***

oGmpertz function expressed as ln(DY)=p*exp[-exp(c*(d-JD)]; NS not significant; *, **, ** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.





for both crops by the second half of October (about 4000 g/m 2 vs 3100 g/m 2 in miscanthus and giant reed, respectively) (Figure 2). Regarding the above-ground dry yield accumulation per stem (Figure 3), giant reed showed higher values than miscanthus until the beginning of August, when the species accumulated 36 and 38 g per stem (plateau) for giant reed and miscanthus, respectively.

Our results disagree with those of Angelini *et al.* (2009) who reported higher dry yield in giant reed rather than in miscanthus (38 vs 29 t/ha/yr). However, our data could have been influenced by the shallow water table owing to the closeness of the study site to the Arno River. In fact, in the Mediterranean environment, if water availability is not a limiting factor, a C4 species such as miscanthus would be able to optimise its biomass accumulation with respect to a C3 species such as giant reed. This finding is confirmed by the 2009 rainfall distribution, which reached over 500 mm from January to June, and by the total rainfall level of 2008 (about 1000 mm), which was above the annual average.

Crop growth rate (CGR) curves (Figure 4) highlighted a higher daily growth rate in giant reed than in miscanthus until the end of May. From then onwards, we observed opposite results with the highest values in miscanthus. Maximum CGR for all crops occurred simultaneously at the middle of June. The maximum CGR achieved by miscanthus (maximum value 48 g/m²/d) was consistently greater than that achieved by giant reed (maximum value 33 g/m²/d). Our CGR values were in agreement with those reported by Heggenstaller et al. (2009) for a C4 crop such as maize, while giant reed CGR values were higher than those recorded by Massantini et al. (1985) in wheat and alfalfa in the same environment. On the other hand, the maximum absolute growth rate (AGR) was achieved earlier in giant reed (maximum value 420 mg/d at the 150° Julian day) than in miscanthus (maximum value 600 mg/d at the 174° Julian day) (Figure 5). This seems to be related to a different crop growth pattern in these crops. In fact, as reported by Table 3, during the whole growing season, giant reed showed significantly higher values of basal stem diameter than miscanthus (11.1 mm vs 9.3 mm) and a significantly lower number of stems in the early season. Furthermore, during the growing season. miscanthus stem number, as reported also by Christian et al. (2008) and Heaton et al. (2008), was quite stable, while giant reed showed continuous new stems sprouting from rhizomes (Spencer et al., 2005; Thornby et al., 2007) (Table 3). In addition, plant height was slightly higher in miscanthus (334 cm vs 286 cm) and biomass partitioning data highlighted a leaf percentage reduction in both species from May

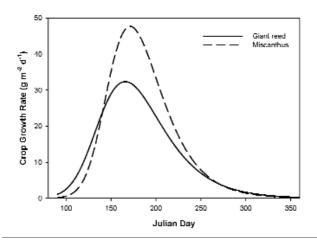


Figure 4. Mean predicted crop growth rate as a function of Julian day for giant reed and miscanthus crops during 2009 growing season.

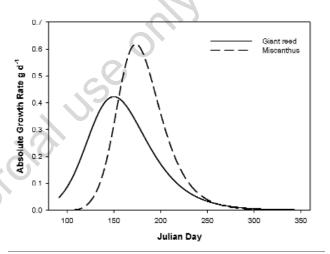


Figure 5. Mean predicted absolute growth rate as a function of Julian day for giant reed and miscanthus crops during 2009 growing season.

Table 3. Basal stem diameter, plant height, number of stems per square meter, leaf and stem partitioning during 2009 for giant reed and miscanthus.

Julian	Basal stem o	diameter, mm	Stem he	ight, cm	N° ste	m/m2	Leaf	, %	Sten	n, %
day	GR	M	GR	M	GR	M	GR	M	GR	M
132	11.6 a	8.1 b	92 a	56 b	50 b	98 a	32.5 b	39.5 a	67.5 a	60.5 b
194	11.0 a	9.7 b	236 b	273 a	78 b	92 a	37.5 a	23.5 b	62.5 b	76.3 a
240	11.3 a	10 ab	276 b	292 a	64 b	96 a	35.6 a	20.4 b	64.4 b	79.6 a
322	10.5 a	9.54 ab	286 ab	334 a	80 a	90 a	26.1 a	17.6 b	73.9 ab	82.4 a

For each parameter and date, values followed by different letters on the same row are significantly different (P=0.05) according to the Least Significant Difference's Test (LSD). GR, giant reed; M, miscanthus.

Table 4. Estimated coefficients and standard errors for LAI response to Julian day fitted to a quadratic function° for giant reed and miscanthus crops in 2009 in Pisa (Central Italy).

Variables	Species	Coefficient (±SE)			P	Significance
		p				
LAI	Giant reed	3.36	0.041	-0.000084	0.053	NS
	Miscanthus	3.42	0.044	-0.000091		

[°]Quadratic function expressed as ln(LAI)=a+b*(JD)+c*(JD)²; NS, not significant; *, ***, **** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.





to November, with significantly lower leaf percentage in miscanthus than in giant reed (Table 3) (Ercoli et al., 1999; Spencer et al., 2005; Burner et al., 2009; Angelini et al., 2009). We may state that our data outlined a different growth strategy in the two species: at the beginning of the growing season, giant reed develops a progressively thicker stem than miscanthus, also with a lower density per ground area, determining higher yield per square meter. From June, miscanthus growth rate becomes higher (probably owing to an environmental condition favourable to its C4 pathway) (see Figures 4 and 5) and it is characterised by higher stem density, higher yield per square meter and slightly lower yield per stem. In addition, at the end of the growing season, miscanthus flowering may have partially contributed to achieving higher yield. In fact, in the Mediterranean environment where the experiment was carried out, only the miscanthus crop attains flowering at the end of September. A high temperature and low photoperiod probably regulate this phase (Cosentino et al., 2007), as it does not occur in the colder regions of Central and North Europe.

Leaf area and leaf photosynthetic efficiency represent two fundamental factors driving crop growth (Hunt, 1982). A lack-of-fit test at the 95% level was not significant for LAI measured data, indicating that the quadratic function model was correct for describing miscanthus and giant reed LAI (Figure 6). Table 4 reports the estimated coefficients describing giant reed and miscanthus LAI, highlighting the absence of significant differences between the species. For miscanthus and giant reed, maximum LAI was achieved 10 weeks following maximum CGR (Figure 6). However, during the whole growing season, miscanthus LAI showed the highest values, related to its higher stem density. The maximum LAI recorded was about 6.6 and 5.8 for miscanthus and giant reed, respectively. For both species, LAI increased greatly from vegetative re-growth until the third week of August. The canopy closure (LAI ≥5) was observed at the beginning and at the end of July for miscanthus and giant reed, respectively. In addition, LAI maintained high values (>5) throughout the summer months until the end of October. Our LAI results are in agreement with those reported by Cosentino et al. (2007) and Danalatos et al. (2007) for miscanthus crops cultivated without irrigation in the Mediterranean area, while Clifton-Brown et al., 2002 reported slightly lower LAI values, probably owing to the lower radiation and temperature that characterised their study site. With the aim of evaluating the yield increase in relation to leaf area and, consequently, to estimate indirectly the photosynthetic efficiency of miscanthus and giant reed, we took into account the net assimilation rate (NAR), expressed as the daily biomass accumulated per unit of leaf area (Figure 7). Seasonal patterns in NAR were similar for both crops: it began at a low level, increased to a maximum point relatively early in growth and then declined to a very low level by the end of the growing season. Giant reed NAR was higher than that for miscanthus until the end of May while in the following part of the growing season, we observed an opposite trend. Maximum NAR was higher in miscanthus than in giant reed (12.3 vs 11.0 g/m²/d), and for both crops occurred two weeks before maximum CGR. The radiation use efficiency (RUE) was evaluated for both crops (Figure 8). Therefore, in our trial, where radiation, temperature and water availability were not limiting factors during the summer period, miscanthus seemed to optimise radiation increasing its yield with a higher growth rate than giant reed. Our results confirmed the observations reported by Squire (1990) on the radiation use in C3 and C4 species with a lower value in giant reed than miscanthus (2.0 vs 2.7 g/MJ). Miscanthus RUE showed a value of 2.7 g_{DY}/MJ, in agreement with those of Clifton-Brown and Lewandowski (2000) and Hastings et al. (2008) (values from 2.35 to 4.1 g/MJ) but higher than those of Price et al. (2004) and Cosentino et al. (2007) (0.8 g/MJ). To our knowledge, giant reed RUE has not yet been described and it was not possible to compare our results with other data. However, our results were similar to those reported for other C3 species such as sunflower and wheat (Kiniry et al., 1989).

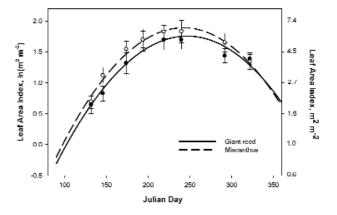


Figure 6. Predicted function and observed values (±standard deviation) of giant reed and miscanthus leaf area index in 2009 at Pisa (Central Italy). ● and ○ indicate observed values (±SD) for giant reed and miscanthus, respectively.

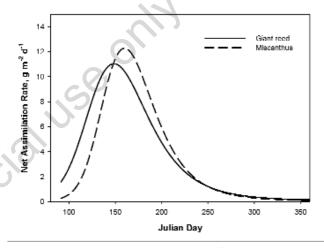


Figure 7. Predicted net assimilation rate of giant reed and miscanthus during the 2009 growing season at Pisa (Central Italy).

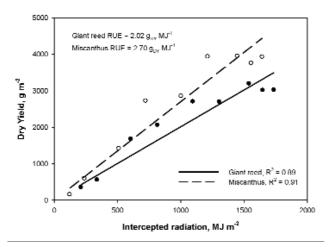


Figure 8. Radiation use efficiency of giant reed and miscanthus during the 2009 growing season at Pisa (Central Italy). ● and ○ indicate observed values for giant reed and miscanthus respectively.





Conclusion

This study compared the productivity of two perennial rhizomatous species, miscanthus and giant reed. Contrary to our outlook based on previous results, giant reed did not yield higher biomass than miscanthus. This suggests that, during the summer period in an environment where water availability, temperature and solar radiation are not limiting factors, a mature miscanthus crop can reach an aboveground dry yield about 40 t/ha/yr, against the over 30 t/ha/yr of giant reed. Growth analysis results suggest that a combination of physiological and morphological factors, in addition to the environmental condition, seems to drive the observed differences between these species. Potential morphological differences include the number of stems per unit area, plant height and basal stem diameter, while physiological differences can be related mainly to the different crop pathway (C3 vs C4). In fact, miscanthus and giant reed showed different growth strategies: miscanthus, from the beginning of the growing season, produced an elevated number of thin stems along with high LAI values during the whole summer period, which allowed the optimisation of solar radiation through the C4 pathway. In addition, the environmental conditions of the study site at the end of September drove miscanthus to flower, leading to higher plant height values than giant reed and, consequently, to a greater yield per ground area. On the other hand, giant reed showed a lower but continuously increasing number of thick stems during the growing season; its LAI was lower and the growth rate decrease observed during the summer period might be a result of the high temperatures, which could have limited the C3 pathway. Hence, our results confirmed that yield is strongly related to environmental conditions. For this reason, the choice of the proper crop has to be made in order to guarantee the sustainability of the cropping systems through the optimisation of land use, the reduction of external inputs and the achievement of adequate yield levels. Further studies are necessary to investigate the performances of these species in marginal land, in which water and nutrient availability can represent a limiting factor.

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