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Effects of soil water content and nitrogen supply on the productivity of *Miscanthus* × *giganteus* Greef et Deu. in a Mediterranean environment

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Abstract

Miscanthus × *giganteus* is one of the most promising biomass crops for non-food utilisation. Taking into account its area of origin (Far East), its temperature and rainfall requirements are not well satisfied in Mediterranean climate. For this purpose, a research was carried out with the aim of studying the adaptation of the species to the Mediterranean environment, and at analysing its ecophysiological and productive response to different soil water and nitrogen conditions. A split plot experimental design with three levels of irrigation (I_1, I_2 and I_3 at 25%, 50% and 100% of maximum evapotranspiration (ETm), respectively) and three levels of nitrogen fertilisation (0 kg ha^{-1}: N_0, 60 kg ha^{-1}: N_1 and 120 kg ha^{-1}: N_2 of nitrogen) were studied. The crop showed a high yield potential under well-watered conditions (up to 27 t ha^{-1} of dry matter). *M.* × *giganteus*, in Mediterranean environment showed a high yield potential even in very limited water availability conditions (more than 14 t ha^{-1} with a 25% ETm restoration). A responsiveness to nitrogen supply, with great yield increases when water was not limiting, was exhibited. Water use efficiency (WUE) achieved the highest values in limited soil water availability (between 4.51 and 4.83 g l^{-1}), whilst in non-limiting water conditions it decreased down to 2.56 and 3.49 g l^{-1} (in the second and third year of experiment, respectively). Nitrogen use efficiency (NUE) decreased with the increase of water distributed (from 190.5 g g^{-1} of I_0 to 173.2 g g^{-1} of I_2); in relation to N fertilisation it did not change between the N fertilised treatments (N_1 and N_2), being much higher in the unfertilised control (177.1 g g^{-1}). Radiation use efficiency (NUE) progressively declined with the reduction of the N fertiliser level (1.05, 0.96 and 0.86 g d.m. MJ^{-1}, in 1994, and 0.92, 0.91 and 0.69 g d.m. MJ^{-1}, in 1995, for N_2, N_1 and N_0, respectively).

Keywords: *Miscanthus* × *giganteus*; Mediterranean environment; Soil water content; Nitrogen application; WUE; RUE; NUE

1. Introduction

Miscanthus is a gramineous rhizomatous species originating from Far East, introduced to Europe as ornamental plant (Jones and Walsh, 2001). An hybrid which recently has raised interest is *Miscanthus* × *giganteus*

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Greef et Deu., firstly recognised in 1935 in Japan, from where it was introduced to Denmark, and it was named *Miscanthus sinensis* “Giganteus” hort. (Greef and Deuter, 1993).

The interest aroused towards this species, considered as one of the most promising biomass crops for non-food utilisation (energy, pulp of cellulose, compost, etc.) (Stander, 1989) has induced, since 1989, a German oil company (Veba Oil Company) and the European Union, to carry out and support research projects on this species. In particular, positive aspects have been indicated on:

- its great productivity (Heaton et al., 2004) and radiation use efficiency (Van der Werf et al., 1993; Beale and Long, 1995), higher than other C_4 crops, at temperate climate conditions;
- its perennial habit, reducing the soil tillage;
- its low environmental impact, since requirements of nitrogen fertilisation (Schwarz et al., 1994; Lewandowski and Heinz, 2003) and herbicides and pesticides applications, are fairly reduced;
- its potential capacity on reducing erosion processes in sloping soils (Foti et al., 2001; Cosentino et al., 2004);
- the low nitrates leaching (Christian et al., 1997).

The high transplanting costs are joined to the impossibility of producing seeds due to the sterility of the hybrid. Genetic breeding programs aim at searching other hybrids within the same genus and of selecting fertile cultivars (Greef and Deuter, 1993; Lewandowski et al., 2003).

Miscanthus is often harvested in late winter-early spring, when the biomass has reached a low moisture content avoiding added costs for biomass drying. During winter, nevertheless, the crop loses a considerable part of product which could be retrieved with an early harvest before winter (Ercoli et al., 1999).

According to experiments carried out in different sites of the European Union (Van der Werf et al., 1993; Foti et al., 1996), the *Miscanthus* production reaches the highest yield in the second to fifth year, depending on the site (Clifton-Brown et al., 2002; Jones and Walsh, 2001). In relation to its origins, the natural crop growing conditions are characterised by mild temperatures and heavy rainfall well distributed throughout the year (Greef and Deuter, 1993).

Taking into account its thermal requirements, in Mediterranean environment it could be grown in summer time, making, thus, necessary the use of water for irrigation (Foti et al., 1996).

Since the irrigation water is not always available in semi-arid areas, within the framework of the European AIR3 CT92-0294 “Miscanthus productivity network” project, a research aiming at studying the crop adaptation to new environmental conditions, which is the main step for recognising new cultivation areas, and the eco-physiological, biological and productive response of the crop to different soil water and nitrogen availability, was carried out.

Table 1
Soil characteristics of the field site in the top 0–50 cm

Soil characteristic	Value
Sand (%) (Gattorta method; Lotti and Galoppini, 1980)	49.27
Loam (%) (Gattorta method; Lotti and Galoppini, 1980)	22.43
Clay (%) (Gattorta method; Lotti and Galoppini, 1980)	28.30
pH (in water solution)	8.6
Total calcareous (%) (gas-volumetric method; AOAC, 1990)	15.24
Organic matter (%) (Walkley and Black method; AOAC, 1990)	1.40
Total N (‰) (Kjeldahl method; AOAC, 1990)	1.00
P_2O_5 availability (ppm) (Ferrari method; AOAC, 1990)	5
K_2O availability (ppm) (Dirks and Sheffer method; AOAC, 1990)	244.8
Field capacity at −0.03 MPa (%)	27
Wilting point at −1.5 MPa (%)	11

2. Materials and methods

The research was carried out between 1993 and 1996 at Catania (Sicily, 10 m a.s.l., 37°25′N Lat., 15°30′E Long.), on a vertic xerochrepts soil (Soil taxonomy, USDA) whose characteristics are reported in Table 1.

Micropropagated plantlets of *M.* × *giganteus* provided by Piccoplant (Oldenburg, Germany) were transplanted in the field on 10 June 1993, adopting a 4 plants m^{-2} density.

In a split-plot experimental design with three replicates, the following factors were studied:

- three levels of maximum evapotranspiration (ETm) restoration: 25% (I_0), 50% (I_1) and 100% (I_2);
- three levels of nitrogen fertilisation: 0 kg ha^{-1} (N_0), 60 kg ha^{-1} (N_1) and 120 kg ha^{-1} (N_2) of nitrogen.

After transplanting and until the autumnal vegetative quiescence, the soil water content was kept at a good level in order to allow a good plant establishment. The irrigation treatments were differentiated from the second year.

The nitrogen fertilisation was carried out supplying 60 kg ha^{-1} of N, as ammonium sulphate, at the spring crop re-growing (in both fertilised treatments) and the further 60 kg ha^{-1} at the beginning of jointing (only in N_2 treatment).

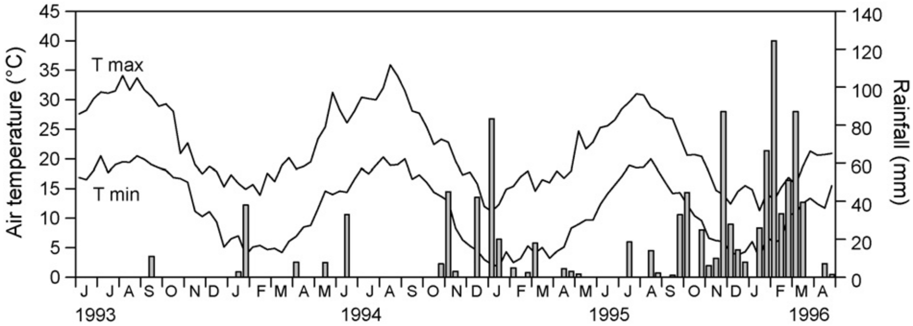

Fig. 1. Changes in maximum and minimum air temperature (mean 10-day values) and rainfall (total 10-day values).

The water was distributed by means of a drip irrigation system. The irrigation was determined on the basis of the maximum available water content in the first 0.6 m of soil, where most of the root is expected to grow, calculated by means of the following formula (Doorembos and Pruitt, 1977):

$$V = 0.66(\mathrm{FC} - \mathrm{WP})\Phi D \quad (1)$$

where V = water amount (equal to 63.3 mm); 0.66 = fraction of readily available soil water permitting unrestricted evapo-transpiration, calculated according to Foti et al. (2003); FC = soil water at field capacity, equal to 27% of dry soil weight (Table 1); WP = soil water at wilting point, equal to 11% of dry soil weight (Table 1); Φ = apparent volumetric mass, equal to 1.2 kg m^{-3}; D = equal to 0.6 m, which is the soil depth were the bulk of roots is expected to develop (Beale et al., 1999).

The irrigation was carried out when the sum of daily ETm (maximum evapotranspiration), calculated as follows, corresponded to V:

$$\mathrm{ETm} = E_0 \mathrm{Kp}\,\mathrm{Kc} \quad (2)$$

where ETm = daily maximum evapotranspiration (mm); E_0 = evaporation of class "A" pan (mm); Kp = pan coefficient, equal to 0.80 in semi-arid environment (average relative humidity 40–70%, low wind speed, fetch 1 m) (Doorembos and Pruitt, 1977); Kc = crop coefficient, ranging between 0.4 and 0.7 from plant emergence to beginning of jointing, between 0.7 and 1.1 from beginning to end jointing, equal to 1.1 from end jointing to flowering, and between 1.1 and 0.7 from flowering until October.

The crop coefficients adopted refer to those of C_4 plants (e.g. sorghum and corn) for the Mediterranean environments (Doorembos and Pruitt, 1977). According to the experimental irrigation treatments, the total amount of water distributed with each irrigation was: 15.8 mm for 25% ETm, 31.7 mm for 50% ETm, 63.4 mm or 100% ETm. Details are reported in Foti et al. (2003). In 1995 a prolonged lack of irrigation water in July and August did not allow the full ETm restoration, negatively affecting the crop growth and development in all treatments.

The following meteorological variables were recorded: air temperature, air relative humidity, rainfall, global solar radiation, class "A" pan evaporation, using a data logger (CR10, Campbell Scientific, USA) located 100 m apart from the experimental field. Meteorological data were those of a typically Mediterranean environment (Fig. 1). The temperatures, in the 3 years, reached the maximum value (30–35 °C) in July and August. The daily evaporation in these months was 8–10 mm. The minimum air temperature at the end of March, when the crop started re-growth at the second and third year of experiment, was about 5 °C in all the 3 years.

Rainfall during the growing period (from June to November), negligible in 1993 (11 mm), was heavier in the following years especially in the third one (81 and 241.3 mm, respectively in 1994 and 1995).

During the same period, starting from the transplanting year, the date of flowering (when at least 70% of plants in the plot were flowered) and plant height from soil to inflorescence, a month after flowering, were recorded; moreover, the following traits were also measured monthly during the growing season:

- leaf area;
- aboveground dry biomass.

In order to estimate aboveground biomass and leaf area, a stratified random sampling of stems on five plants per each plot was collected. A 20% of total stems per plant were selected according to the frequency distribution.

For aboveground dry biomass estimation, plant samples were dried at 60 °C in a thermo-ventilated oven until constant weight was achieved.

Harvest was carried out on February 1995 and March 1996. The final yield was obtained, after removal of lateral plants in the plot, by harvesting 24 m^2 of plot. Dry matter yield was estimated by calculating the moisture content in leaves and stems, dried as above described.

For each water treatment, the crop water use (CWU) was determined by means of water balance calculation along the period between plant emergence up to the last irrigation (end of October), adopting the following formula:

$$\mathrm{CWU} = I + P \pm \Delta C \qquad (3)$$

where CWU = crop water use (mm); I = water supplied by means of irrigation (mm); P = precipitation (mm); ΔC = difference between soil water content at plant emergence and soil water content after the last irrigation (end of October) (mm).

Soil water content was measured gravimetrically. Soil samples were collected up to 0.8 m depth every 0.2 m in three replicates for each treatment, before and 1 day after each irrigation treatment, throughout the whole growing season. Soil samples were dried in oven at 105 °C and weighed until constant weight was reached.

For the second and third year, the crop water use efficiency (WUE), expressed as ratio between dry biomass production at final harvest and water used by the crop, was calculated.

In the third year only (1995), leaf nitrogen content was determined along the growing season. To this purpose, the dried samples were milled to 0.5 mm particle size. Nitrogen content was determined by means of Kjeldahl method (AOAC, 1990). In correspondence to maximum dry biomass cumulated (November), nitrogen content was determined as above described, in samples of leaves and stems. Nitrogen use efficiency (NUE), which indicates the total biomass produced per unit of N uptake and is expressed as ratio between dry matter production and nitrogen content ($\mathrm{g\,g^{-1}}$), was calculated according to Beale and Long (1997).

Moreover, in the transplanting year (1993), between 13.00 and 13.30 p.m., by means of a Line Quantum Sensor (LI-COR Inc., Lincoln, NE), the solar radiation (PAR) at the soil level, inside the crop and over the crop canopy, was recorded. Thus, the rate of solar radiation intercepted by the crop was calculated, according to the following formula:

$$\mathrm{PAR_{int}} = \frac{\mathrm{PAR_i} - \mathrm{PAR_s}}{\mathrm{PAR_i}} \qquad (4)$$

where $\mathrm{PAR_{int}}$ = rate of PAR intercepted by the crop; $\mathrm{PAR_i}$ = incident PAR ($\mathrm{W\,m^{-2}}$); $\mathrm{PAR_s}$ = PAR at the soil ($\mathrm{W\,m^{-2}}$).

The relationship between LAI and the rate of intercepted radiation was studied, as described by an asymptotic equation $y = 1 - e^{-k\,\mathrm{LAI}}$, calculated according to the formula of Monsi and Saeki (1953), where k represents the extinction coefficient. This formula was used to calculate the radiation intercepted by the crop in 1994 and 1995, considering a linear behaviour of LAI between subsequent sampling dates.

Finally, in the second and third year, the relationship between the intercepted solar radiation throughout the growing season and the aboveground dry biomass cumulated during the same period, was calculated by means of the correlation coefficient and the linear regression technique. The regression coefficient (b) of the relationship was considered as an estimation of the crop radiation use efficiency (RUE).

The data of biological and productive characteristics were analysed by a two-way ANOVA (Snedecor and Cochran, 1989), using CoStat Version 6.003 (CoHort Software) related to the split-plot experimental design in field. The percentage values of leaves and stems were previously arcsine transformed. With significant difference, the Student–Newman–Keuls (SNK) method for the means separation was applied.

3. Results

3.1. Phenology

The crop attained the flowering stage in the second and third year. In 1994, in non-limiting soil water availability, the crop flowered between the end of August and the beginning of September (Table 2). The increase of water deficit (I_0) delayed flowering up to the end of September.

In 1995, probably because of the irregular water supply, flowering occurred almost a month later than the previous year, between 3 October (I_2N_2) and 12 October (I_0N_2), confirming, as far as water stress effect is concerned, observations of the previous year.

3.2. Biometric traits

With respect to the average plant height recorded a month after flowering in the second year, a more remarkable irrigation, than fertilisation, effect, was observed (Table 3). Indeed, irrespective of the nitrogen levels, a +49% plant height increase (from 206.0 to 306.4 cm, in I_0 and I_2, respectively) due to the higher water availability, was observed; the effect of the increased nitrogen fertilisation was less significant (+11% in N_2 compared to N_0).

Table 2
Date of plant flowering in *Miscanthus* × *giganteus* in relation to the studied treatments

Treatment	Date of flowering	
	1994	1995
I_0N_0	22/9	4/10
I_0N_1	25/9	10/10
I_0N_2	24/9	12/10
I_1N_0	29/8	6/10
I_1N_1	5/9	7/10
I_1N_2	6/9	6/10
I_2N_0	29/8	3/10
I_2N_1	30/8	2/10
I_2N_2	29/8	5/10
Average	8/9	6/10
Nitrogen effect		
N_0	6/9	4/10
N_1	10/9	6/10
N_2	10/9	8/10
Average	9/9	6/10
Irrigation effect		
I_0	24/9	9/10
I_1	3/9	6/10
I_2	29/8	3/10
Average	8/9	6/10

Table 3
Plant height in *M.* × *giganteus* in relation to the studied treatments

Treatment	Plant height (cm)	
	1994	1995
I_0N_0	202.7	161.0
I_0N_1	207.0	208.0
I_0N_2	207.0	236.5
I_1N_0	253.0	169.7
I_1N_1	261.3	251.3
I_1N_2	273.0	269.2
I_2N_0	274.0	178.5
I_2N_1	311.0	270.7
I_2N_2	334.3	274.3
Average	258.2	224.4
Nitrogen effect		
N_0	243.2 a	169.7 a
N_1	260.2 ab	243.3 b
N_2	271.4 b	260.0 b
Average	258.3	224.3
Irrigation effect		
I_0	206.0 a	201.8 a
I_1	262.4 b	230.1 b
I_2	306.4 c	241.2 b
Average	258.3	224.4

Values followed by the same letter do not differ at $P \leq 0.05$ by SNK.

In the third year, the differences among water treatments were less consistent since the most irrigated treatments (I_2 and I_1) were penalised by the lack of water for irrigation. Conversely, a strong effect of nitrogen supply emerged: while the two fertilised treatments did not significantly differ in plant height (260.0 and 243.3 cm, respectively, for N_2 and N_1), the N_0 control was much more affected by the lack of fertilisation, reaching a plant height of 169.7 cm (the 30% less than plant height measured in N_0 in the second year).

A similar trend was observed for leaf area index (LAI) recorded in the second year, which, as well as the previous trait, was more strongly affected by water than nitrogen availability (Fig. 2). With regard to the LAI recorded in August, I_2 treatment compared to I_0, showed a 77% increase (from 5.3 to 9.4), whereas the nitrogen supply determined a +24% LAI increase (6.8 and 8.4, in N_0 and N_2, respectively).

In the third year, I_2 treatment exhibited a LAI value 60% higher than that of I_0 control. The reduced nitrogen supply determined a significant decrease of LAI, especially in 1995. Maximum LAI values, in the second and third year, were observed during flowering phase, after which the canopy started to senesce.

The number of stems per square meter in the second and third year, after the re-growing progressively declined until jointing phase, keeping an almost constant number thereafter (Fig. 3). The differences among water treatments appeared negligible, whereas significant differences among nitrogen treatments (133.4 and 169.5 stems m^{-2} in the second year, 99.3 and 124.3 stems m^{-2} in the third year, respectively, in N_0 and N_2 during August measurements) were observed.

3.3. Productive traits

The total aboveground dry biomass progressively increased from re-growth, up to a maximum value recorded in autumn, at full plant flowering phase, occurred in “middle October”, in the second year, and between “end October–beginning of November”, in the third year. Afterwards, due to the loss of leaves and the probable assimilates translocation towards roots, the amount of biomass decreased (Fig. 4). At well-watered conditions (100% ETm restoration) and irrespective of nitrogen treatments, the biomass attained a maximum value of 34.5 t ha^{-1} in the second year, 80% higher than the stressed control at the same date of measurement (October). Less pronounced, as for biometric traits, was the response to the increasing nitrogen supply, with a total dry biomass 21% higher in N_2 than N_0 at the same measurement date.

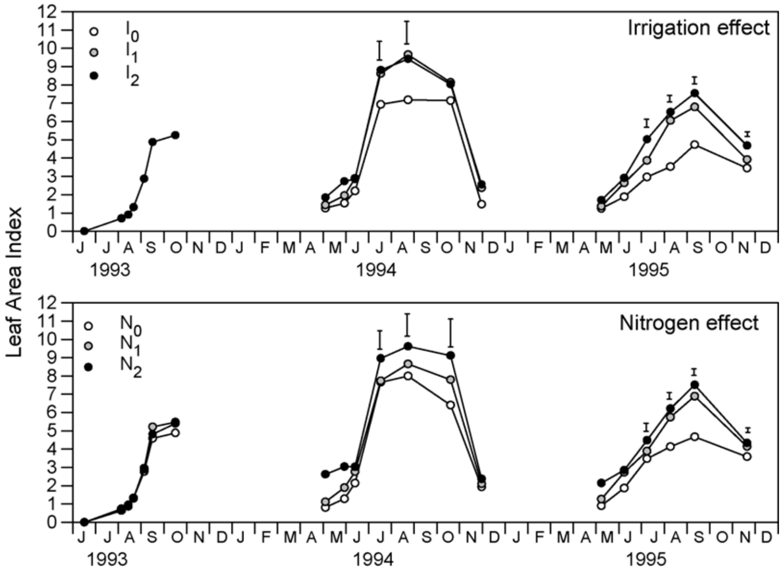

Fig. 2. Mean effect of irrigation and nitrogen on LAI course in *Miscanthus* × *giganteus*. Vertical bars, if present, indicate LSD ($P \leq 0.05$).

In the third year, the highest average dry biomass production (20.9 t ha^{-1}) was observed in November. The yield difference between the well watered (I_2) and the less watered (I_0) treatment, was less consistent (−30%) than that of the previous year. Furthermore, the crop was significantly affected by the absence of nitrogen supply, keeping, compared to the previous year, yield levels in N_0 lower than those of fertilised treatments (16.3, 24.9 and 27.4 t ha^{-1} in N_0, N_1 and N_2, respectively).

The final yield, in the year of seedlings transplanting, was in average 4.73 t ha^{-1} whose 60.2% were leaves and 39.8% stems (Table 4). In the subsequent year, the yield varied in relation to the studied factors, with significant increases with increasing water and nitrogen supply. No significant interaction was highlighted by ANOVA between the two experimental factors.

The total dry biomass, in the best water and nitrogen conditions, reached 27 t ha^{-1}, whereas in the less watered and N fertilised treatment it attained 17.4 t ha^{-1}. Yield partitioning was not significantly affected by the experimental factors, being subdivided in 77% of stems and 22% of leaves.

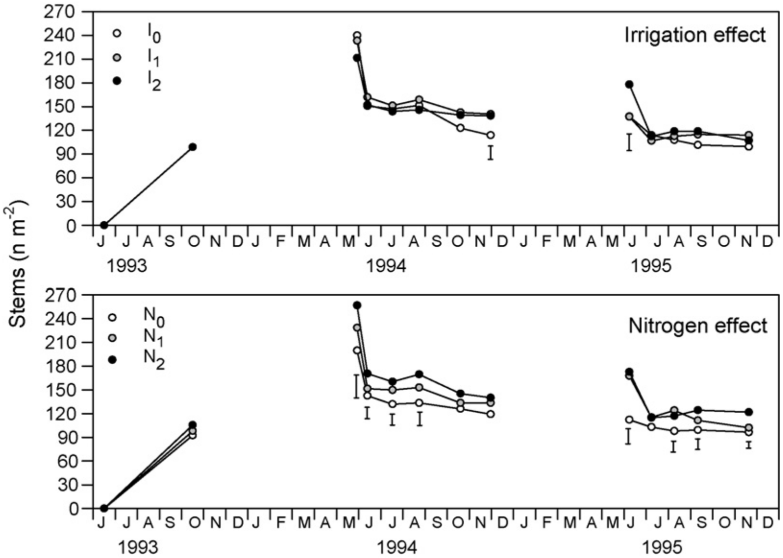

Fig. 3. Mean effect of irrigation and nitrogen on number course of stems m^{-2} in *M.* × *giganteus*. Vertical bars, if present, indicate LSD ($P \leq 0.05$).

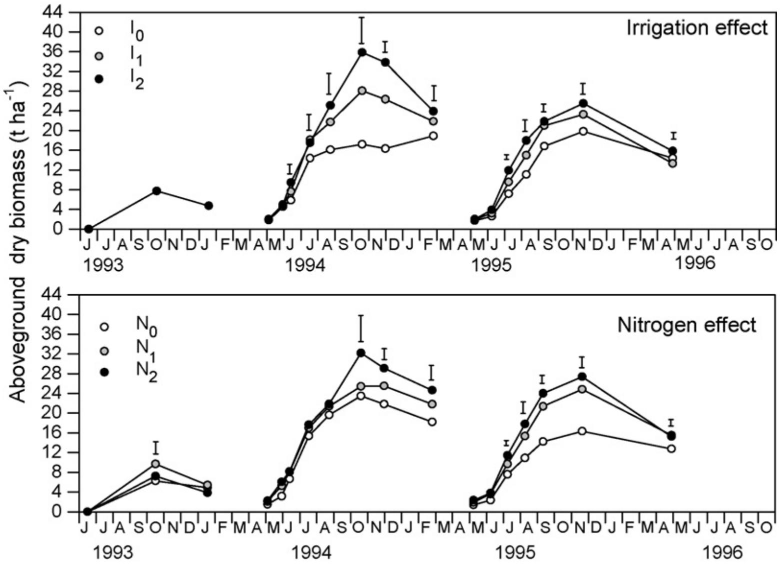

Fig. 4. Mean effect of irrigation and nitrogen on aboveground biomass course in *M.* × *giganteus*. Vertical bars, if present, indicate LSD ($P \leq 0.05$).

The reduced water availability for irrigation occurred at the third year determined a slight difference between the well watered and fertilised treatment and the low input treatment (18.2 against 14.5 t ha^{-1}, respectively). Irrespective of nitrogen availability, no significant water effect was observed, whereas in the average of irrigation treatments a significant effect of nitrogen was recorded between N_0 (14.8 t ha^{-1}) and N_1 and N_2 treatments (16.9 and 16.6 t ha^{-1}, respectively) (Table 4).

The biomass water content at harvest, in all experimental treatments, was always less than 16%.

3.4. Water use and water use efficiency

The crop water consumption, calculated with the balance method (Table 5), in the best conditions of water availability and supply as those of 1994, was 932.9 mm; in 1995, because of the already recalled reduced water availability during summertime, the crop totally con-

Table 4
Total dry biomass at the final harvest and partitioning in *M.* × *giganteus*

Treatment	Dry biomass (t ha^{-1})				Leaves (% of total d.m.)				Stems (% of total d.m.)			
	I_0	I_1	I_2	Average	I_0	I_1	I_2	Average	I_0	I_1	I_2	Average
First year (January 1994)												
N_0			4.9	4.9 a			60.7	60.7 a			39.3	39.3 a
N_1			5.4	5.4 a			59.2	59.2 a			40.8	40.8 a
N_2			3.9	3.9 a			60.7	60.7 a			39.3	39.3 a
Average	–	–	4.73				60.2				39.8	
Second year (February 1995)												
N_0	17.4	18.0	19.3	18.2 a	24.1	24.5	21.3	23.3 a	75.9	75.5	78.7	76.7 a
N_1	18.2	21.9	25.4	21.8 b	23.9	22.1	21.0	22.3 a	76.1	77.9	79.0	77.7 a
N_2	21.1	25.8	27.0	24.6 b	22.9	22.9	21.4	22.4 a	77.1	77.1	78.6	77.6 a
Average	18.9 a	21.9 b	23.9 b		23.6 a	23.2 a	21.3 a		76.4 a	76.8 a	78.7 a	
Third year (March 1996)												
N_0	14.5	12.3	14.6	13.8 a	22.3	28.0	24.2	24.8 b	77.7	72.0	75.8	75.2 a
N_1	17.0	15.2	18.6	16.9 b	20.8	22.3	23.3	22.1 ab	79.2	77.7	76.7	77.9 ab
N_2	15.6	16.0	18.2	16.6 b	20.6	23.3	15.8	19.9 a	79.4	76.7	84.2	80.1 a
Average	15.7 a	14.5 a	17.1 a		21.2 a	24.5 b	21.1 a		78.8 b	75.5 a	78.9 b	

Average values followed by the same letter do not differ at $P \leq 0.05$ by SNK.

Table 5
Water balance (mm) and aboveground water use efficiency (WUE) in *M.* × *giganteus* in the second and third year of experiment

Year	Treatment	Water supplied, I (mm)	Rainfall, P (mm)	ΔC[a] (mm)	CWU, $I+P+\Delta C$ (mm)	WUE ($g\,l^{-1}$)
1994	I_0	215.5	56.7	+119.5	391.7	4.83
	I_1	440.9	56.7	+60.0	557.6	3.92
	I_2	881.7	56.7	−5.5	932.9	2.56
1995	I_0	76.5	151.4	+120.0	347.9	4.51
	I_1	146.9	151.4	+70.0	368.3	3.93
	I_2	287.9	151.4	+51.7	491.0	3.49

[a] Difference between soil water content at plant emergence and soil water content at the end of October, measured at 0.8 m of depth.

sumed 491.0 mm. With the 25% ETm restoration (I_0), the crop used 391.7 and 347.9 mm, respectively, in 1994 and 1995.

With the increase of water supply, the crop reduced the water use efficiency (Fig. 5). Therefore, WUE was lower in watered treatments (I_1 and I_2) compared to the 25% ETm restoration treatment (I_0). The WUE value recorded in fully irrigated treatment was lower in 1994 ($2.56\,g\,l^{-1}$) compared to the 1995 one ($3.49\,g\,l^{-1}$), whereas in I_0 treatment it was 4.83 and 4.51 $g\,l^{-1}$, respectively, in 1994 and 1995.

3.5. Nitrogen concentration and nitrogen use efficiency

Nitrogen concentration of leaves was determined in samples of leaves collected in 1995. At the beginning of the growing season (May), nitrogen concentration was quite high, reaching the maximum level (>2.0 g 100 g^{-1} d.m.). Thereafter, leaf nitrogen concentration progressively declined (Fig. 6). The effect of water treatment was more evident than that of nitrogen, being leaf nitrogen concentration in I_0 always significantly lower than I_1 and I_2, when excluded the last sampling (of November) when almost the same minimum N concentration was achieved in all water treatments (0.68% on average). With respect to N-fertilisation effect, in both fertilised treatments (N_2 and N_1) the same amount of the nutrient was uptaken by leaves along the whole growing season (from 2.02% of May to 0.76% of November). In plots which did not receive N with fertilisation, only in the samplings of August and September a leaf nitrogen amount significantly lower than N_2 and N_1 was measured.

The N concentration of the whole plant (leaves + stems) in November, when the maximum biomass was attained, irrespective of nitrogen supply, did not exhibit any significant difference, ranging

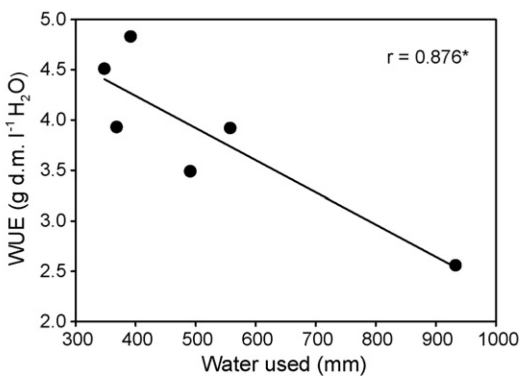

Fig. 5. Relationship between water used (irrigation + rainfall + soil water difference between beginning and end of irrigation period) by the crop during the growing season (April–October) and water use efficiency (WUE) in *M.* × *giganteus*. Data of 2 years and with different water supply.

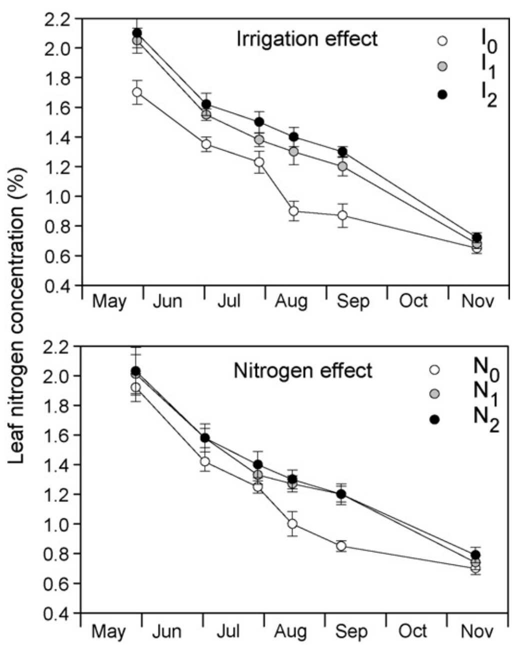

Fig. 6. Mean effect of irrigation and nitrogen on leaf nitrogen content course in *M.* × *giganteus* (year 1995). Vertical bars represent the standard error.

from 0.52% of I_0 to 0.58% of I_2. Irrespective of water treatment, the nitrogen concentration did not significantly differ between the fertilised treatments (0.62% and 0.63% in N_1 and N_2, respectively), while it was significantly lower in the unfertilised control (0.56%) (Fig. 7).

Nitrogen use efficiency, calculated at the same date of measurement, using the nitrogen concentration of total aboveground biomass (leaves + stems), decreased with the increase of water supplied (from 190.5 g g^{-1} of I_0 to 173.2 g g^{-1} of I_2). In relation to N fertilisation, a higher NUE was calculated in the unfertilised control (177.1 g g^{-1}, against 161.8 and 157.4 g g^{-1} of N_1 and N_2, respectively).

3.6. Intercepted solar radiation

During the transplanting year, the rate of solar radiation intercepted by canopy was measured up to maximum value of green LAI (Fig. 8). It was 23%, in the average of the three nitrogen treatments, at the beginning of August, and reached a value >80% at the end of the same month, when LAI was 3. The interception of almost total incident solar radiation (>95%) occurred at middle October, when LAI reached its maximum values. According to the relationships between LAI and the rate of intercepted radiation, an extinction coefficient of 0.56 was estimated (Fig. 9).

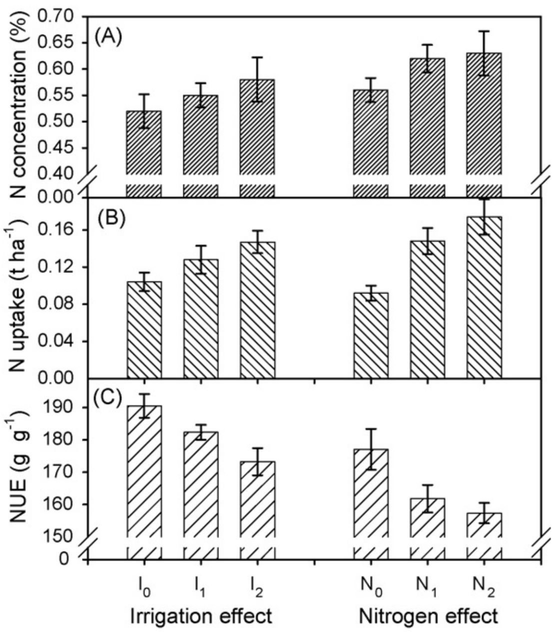

Fig. 7. Mean effect of irrigation and nitrogen on nitrogen concentration (A), nitrogen uptake (B) and nitrogen use efficiency (NUE) (C) in the aboveground dry biomass (stems + leaves) in *M. × giganteus* at the sampling date of November. Vertical bars indicate LSD ($P \leq 0.05$).

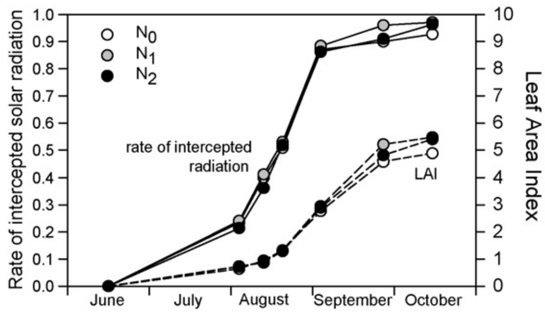

Fig. 8. Course of rate of solar radiation intercepted by canopy and LAI in relation to the nitrogen fertilisation treatments in *M. × giganteus* (year 1993).

3.7. Radiation use efficiency

A linear relationship between global intercepted solar radiation and aboveground dry biomass cumulated throughout the crop growing season is shown in Figs. 10 and 11. The soil water availability greatly affected the slope of the linear regression, which represents the radiation use efficiency. At the highest soil water restoration, the RUE values were highest in the best N fertilisation conditions (120 kg ha^{-1}), progressively decreasing with the reduction of the fertiliser level (1.05, 0.96 and 0.86 g d.m. MJ^{-1} in 1994, and 0.92, 0.91 and 0.69 g d.m. MJ^{-1} in 1995).

The relationship between the intercepted radiation and biomass, in I_0 and I_1 of 1994, was not linear, but it showed two different steps. In the first one, up to 1500 MJ m^{-2} of solar radiation intercepted, with still good soil water conditions, RUE maintained high values (>1.0 g d.m. MJ^{-1}) with no differences among the studied treatments. In the second step, when soil water deficit

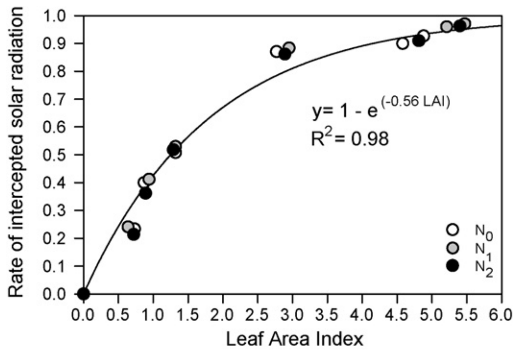

Fig. 9. Relationship between LAI and rate of solar radiation intercepted by canopy in *M. × giganteus*.

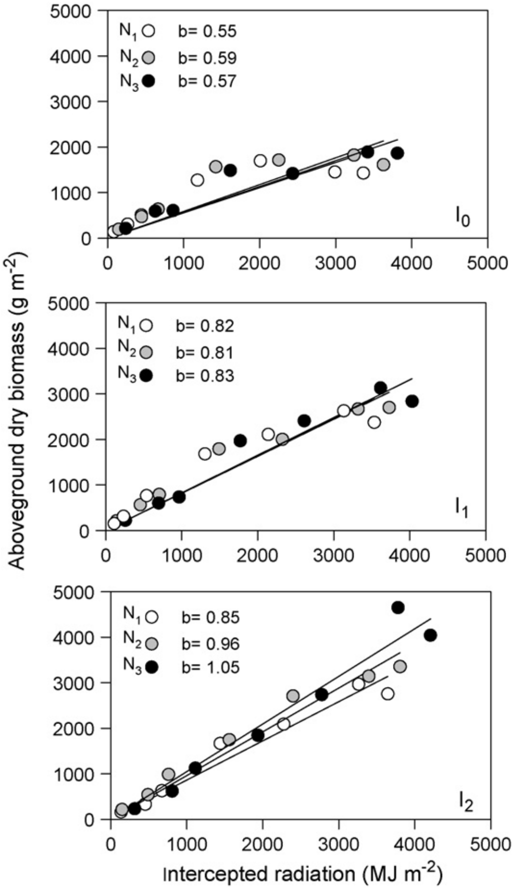

Fig. 10. Radiation use efficiency (RUE) calculated during the crop growing season in relation to the studied treatments in *M.* × *giganteus* (year 1994).

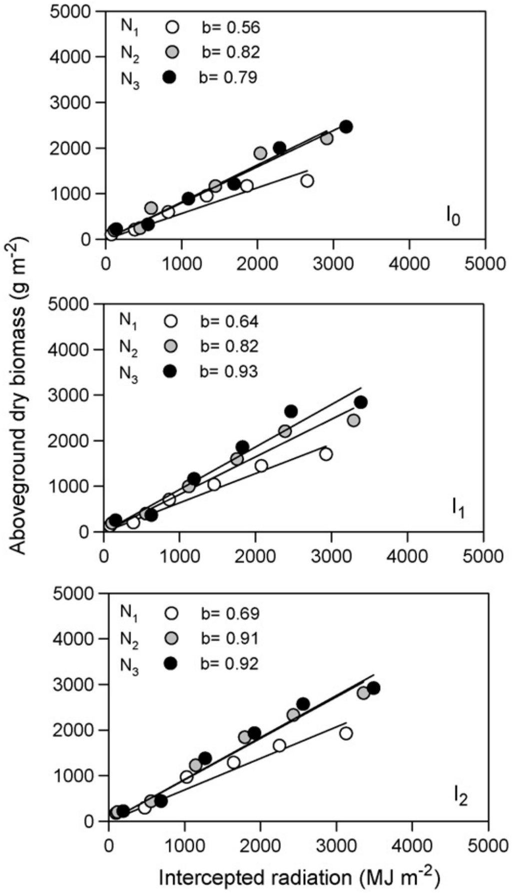

Fig. 11. Radiation use efficiency (RUE) calculated during the crop growing season in relation to the studied treatments in *M.* × *giganteus* (year 1995).

in the I_0 treatment increased, RUE steeply decreased down to a value close to zero.

4. Discussion

A methodological issue needs to be clarified before interpreting the data. In choosing the water regime for the experiment, the decision of not studying a treatment without irrigation supply was taken. The reason for that was related to the fact that, being a new plant for the Mediterranean environment and therefore having a physiological behaviour related to the environment of origin, there was a serious risk to lose the plantation because of the long dry and hot summer season in this environment (May–October), with a very high evaporative demand. Taking into account that *M.* × *giganteus* is a perennial crop, this would have affected the experiment in the subsequent years.

In the Mediterranean environment where the experiment was carried out, the crop attained the flowering between the end of August and the beginning of October. Seeds were also obtained but no fertile. High temperature and low photoperiod conditions probably regulate the flowering of the crop. The same species not always flowered in the colder regions of Central and North Europe (Beale et al., 1999; Clifton-Brown and Lewandowski, 2002). This may probably allow the crop to grow more in height.

The stem number attained values between 150 and 120 m^{-2} with the four plant density used in this experiment, respectively, for the second and the third year. These results are comparable to the findings of Danalatos et al. (1996) who in Greece, in an environment similar to the one of Sicily, using 1 plant m^{-2} density, obtained 60 stems m^{-2} from June onwards, and the findings of experiments carried out in Greece, where 30–40 stems per plant from May until October–November, were obtained with a plant density of 4 plants m^{-2} (Christian and Haase, 2001). Experiments carried out by Bullard et al. (1995) demonstrate as the number of stems increases up to a maximum ranging between 100 and 140 stems m^{-2}, values similar to our findings.

Leaf area index attained its maximum value (7–8) during flowering, steeply reducing thereafter during leaf senescence. According to Petrini et al. (1996), LAI values in both years showed a greater response to water than nitrogen availability.

Aboveground dry biomass achieved its maximum value in the second half of October in the second year of experiment, and during the first half of November in third one. Afterwards, it progressively decreased up to harvest, with a reduction of dry biomass from maximum value, of 33.4% in I_2 and 34.1% in I_1 in the second and third year, because of the detaching of dry leaves and, according to other perennial C_4 crops, probably for assimilate translocation towards the hypogeal part of the plant, to roots and above all to rhizomes (Hayashi et al., 1981; Long et al., 1988; Greef and Deuter, 1992). However, considering the long growing season (April–November) and the high solar radiation of the environment where the experiment was carried out, a higher aboveground biomass productivity of *M.* × *giganteus* might be expected, being a C_4 crop. It is likely that, since both thermal and photoperiod conditions of this environment determine a flowering initiation, the aboveground biomass accumulation ends and the crop stops its growth and translocates the photosynthates towards the rhizome.

According to the scientific literature, what observed in this experiment carried out in Mediterranean environment, where maximum values of aboveground biomass production were achieved in October and November and they subsequently decrease, is a common occurrence also in other pedoclimatic areas (Iwaki et al., 1975; Mutoh et al., 1985). In particular, in the environmental conditions of Central Europe, the maximum seasonal production was attained in middle September, with 10.6 and 29.7 t ha^{-1}, respectively, in the first and second year. Similarly, the highest biomass was achieved in September–October in *M.* × *giganteus* cultivated in UK (<30 t ha^{-1}) (Beale and Long, 1997) or in Denmark (<20 t ha^{-1}) (Jørgensen, 1997). In two localities of Central-Northern Italy (Pisa and Cervia), the maximum production values was reached during flowering (October); in particular, in the best water and nitrogen conditions, yields of 34.0 t ha^{-1} at Pisa, and 41.0 t ha^{-1} at Cervia, were recorded (Petrini et al., 1996). In an experiment carried out in Central Italy, the final dry biomass yield was affected by both water regime and nitrogen fertilisation: with no nitrogen supply, irrigation did not affect total dry biomass production, while the effect of irrigation increased with the increase of nitrogen fertiliser level (Ercoli et al., 1999).

The water use efficiency in this experiment ranged between 2.56 and 4.83 g d.m. l^{-1} H_2O. These values were comparable to those obtained in other field experiments carried out in the same environment, with other C_4 crops such as sweet sorghum (from 4.52 to 6.10 g l^{-1}) (Cosentino, 1996). Previous experiments carried out with *M.* × *giganteus* gave similar results (from 2.88 to 3.57 g l^{-1}) (Foti et al., 1996). Few experiments on this topic have been already carried out both in laboratory and open field in Central Europe (Beale et al., 1999; Clifton-Brown and Lewandowski, 2000), where, however, the obtained values of WUE were considerably higher (between 7.8 and 13.4 g d.m l^{-1} H_2O) than those calculated in the present experiment. The different response between the two environments is likely to be ascribed to the effect of the vapour pressure deficit basically quite low in Central Europe (daily maximum VPD rarely exceeding 2.0 kPa) and almost doubled in Mediterranean area (often reaching 4.0 kPa) (Beale et al., 1999; Foti et al., 2003). The normalisation of the Mediterranean WUE values, obtained multiplying WUE by the average of daily maximum VPD along the growing season, brings towards comparable values (range of 6.4–12.1 g kPa kg^{-1}).

The water use efficiency was negatively and linearly ($r = -0.876^{*}$) related to the water consumed by the crop (Fig. 5): with the increasing of water used the efficiency declines. However, in experiment carried out in laboratory, Clifton-Brown and Lewandowski (2000) found a linear relationship between water added and biomass produced with plants grown with different levels of water supply (equivalent to I_0, I_1 and I_2 of the present experiment). In field conditions it may be possible that the lower WUE at higher water supplies is due to an overestimation of water transpired by the crop. In high soil water content conditions (I_2) the water used by the crop exceeded the need of the crop itself (‘luxury consumption’) and this could explain the correspondent lower WUE: the highest the water supply, the highest the water used.

Beale et al. (1999), in field experiment conditions, found in *M.* × *giganteus* differences in WUE between rainfed and irrigated conditions (9.2 against 7.8 g d.m. l^{-1} H_2O) with higher values at the lowest soil water conditions. The experiment shows (a) an increase in water loss due to an increase in evaporation in the best-irrigated treatment and (b) an increase in productivity which did not match the increase in water loss, explaining the lowest WUE of the crop in well watered conditions.

According to what reported by Clifton-Brown et al. (2002), it seems that *M.* × *giganteus* does not have a flexible water saving strategy as *M. sinensis* does, which reduces stomatal conductance in response to soil drying and allows the plant to maintain a green leaf area. However, in measurements carried out in the present field experiment in Mediterranean environment, a reduction of leaf transpiration and stomatal conductance at low soil water conditions has been observed (Foti et al., 2003), but this reduction of stomatal aperture does not immediately affect leaf net photosynthesis, which still maintains high (between 34 and 40 μmol CO_2 m^{-2} s^{-1}) even if stomatal conductance almost halved its values for 1.0–0.6 cm s^{-1}. This may explain the higher productivity of the crop with a reduction of water consumed.

Relationships between leaf transpiration and net photosynthesis also demonstrate a high level of CO_2 exchange at leaf transpiration ranging from 20 and 12 μg H_2O cm^{-2} s^{-1} (Cosentino et al., unpublished data).

The leaf nitrogen concentration showed a declining pattern along the growing season. Inversely than dry matter accumulation, the mineral content decreased more during autumn. This is a general effect in plants, which can be explained partly by the photosynthates 'diluting' the mineral pool during the main reproductive season and partly by retranslocation of minerals to storage or reproductive organs when the crop begins to ripen (Jørgensen, 1997). Plant nitrogen concentration did not significantly differ between treatments which received the nutrient by fertilisation (60 and 120 kg ha^{-1} of N). The values of N concentration are comparable to those obtained by Beale and Long (1997) and Jørgensen (1997). The reduced N availability determined a lower N uptake in N_0 treatment, correlated to a yield decline, also as consequence of a reduced photosynthetic activity and RUE, as observed in other experiments carried out in a *M.* × *giganteus* crop cultivated in Sicily (Cosentino et al., unpublished data). Conversely, no difference was highlighted between the two fertilised treatments, for N concentration and total N uptaken by the crop, with consequent no difference in yield. Indeed, NUE did not change between N_1 and N_2 treatments (which received 60 and 120 kg ha^{-1} of N, respectively), but it was much higher in the unfertilised control. In other words, the increase in nitrogen applied with fertiliser does not ameliorate the yield but, conversely, it raises the environmental impact of the fertilisation. Therefore, an accurate nitrogen balance may allow any fertiliser waste.

A similar NUE value (160 g g^{-1}), corresponding to a 60 kg ha^{-1} nitrogen fertilisation, was reported for *M.* × *giganteus* cultivated in UK, while higher NUE values (from 179 to 540 g g^{-1}) at the same N fertilisation level were obtained in other locations of North-Central Europe (UK, Belgium, Denmark) (Christian and Haase, 2001).

In the environment where the present experiment was carried out, 60 kg ha^{-1} of N, at least in 1995, have been adequate.

The low N content has important environmental implications for crops devoted to the production of source of renewable energy. The quantities of N oxides emitted on combustion that can lead to acid rain depend, in part, on the nitrogen content of the fuel (Beale and Long, 1997).

Radiation use efficiencies (ε) of C_4 species can be much higher than that of C_3 species (Squire, 1990), with a consequence that annual biomass production can be greater. The results obtained in this experiment indicate that in well-watered conditions ε for above ground production attained values higher than 1.0 g MJ^{-1} of global radiation.

PAR constitutes approximately 45% of global radiation, therefore, the values in the current paper need to be more than doubled in order to be compared with other results. In Germany, Schwarz et al. (1994) reported that establishment-year yields of *M. sinensis* "Giganteus" grown on 11 sites were 0.1–3.7 t ha^{-1}. In the third-season, ε averaged 1.27 g d.m. MJ^{-1} (0.32–1.98 g d.m. MJ^{-1} of PAR). In the Netherlands, Van der Werf et al. (1993) reported mature standing crop yields of the fourth season of a *M. sinensis* "Giganteus" crop, equivalent to 21.8 t ha^{-1}, 73% of which was stem. The crop density was also 2 plants m^{-2} and RUE ε for this crop was 2.56 d.m. MJ^{-1} m^{-2} of PAR.

5. Conclusions

In conclusion, it can be pointed that *M.* × *giganteus*, in Mediterranean environment showed a high yield potential in terms of biomass also under limited water availability conditions (more than 14 t ha^{-1} of dry biomass with a 25% ETm restoration); in good soil water condition, the crop attained a yield of 27 t ha^{-1} of dry matter.

The perennial character of the crop may ensure, compared to annual biomass species, energetic cost saving due to the lower crop management (soil tillage) and inputs required and to a higher erosion control capacity.

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