



## Water Use Efficiency and Biomass Partitioning of Three Different *Miscanthus* Genotypes with Limited and Unlimited Water Supply

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*Miscanthus* species, which are C<sub>4</sub> perennial grasses, have a high biomass potential but yields at many sites in Europe can be limited by insufficient water supply and plant survival is endangered under extreme summer drought. A pot experiment was conducted to measure the influence of reduced water supply on the water use efficiency (WUE) and biomass partitioning of three *Miscanthus* genotypes (*M. x giganteus*, *M. sacchariflorus*, and a *M. sinensis* hybrid) in a controlled environment. The experiment consisted of three phases (phase 1 = 0–20 d; phase 2 = 21–39 d; phase 3 = 40–54 d) punctuated by destructive harvests. In phase 1, soil moisture was non-limiting. In the second and third phases, lowered soil moisture contents induced water deficits. Air vapour pressure deficit (VPD) was  $0.49 \pm 0.05$  kPa. Water deficits caused leaf senescence in *M. x giganteus* and *M. sacchariflorus*, but not in the *M. sinensis* hybrid. Green leaf conductances were lowest in *M. sinensis* under water deficit, indicating stomatal regulation. Water use efficiency for whole plants of each genotype ranged from 11.5 to 14.2 g dry matter (DM) kg<sup>-1</sup> H<sub>2</sub>O but did not differ significantly between genotypes or water treatments under the conditions of this experiment. However, differences in dry matter partitioning to the shoot (the harvestable component) resulted in genotypic differences in WUE, calculated on a harvestable dry matter basis, which ranged from 4.1 g DM kg<sup>-1</sup> H<sub>2</sub>O for *M. sacchariflorus* to 2.2 g DM kg<sup>-1</sup> H<sub>2</sub>O for *M. x giganteus*.

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**Key words:** *Miscanthus sinensis*, *Miscanthus sacchariflorus*, *Miscanthus x giganteus*, water use efficiency, biomass, C<sub>4</sub> plants, drought.

### INTRODUCTION

*Miscanthus* spp., which are C<sub>4</sub> perennial, rhizomatous grasses, have been identified as key potential biomass crops due to high yield and quality (Huisman, 1995; Lewandowski and Kicherer, 1997; Walsh, 1997). Although water use efficiencies of C<sub>4</sub> crops are often higher than for C<sub>3</sub> crops (Long, 1983), availability of water will often dictate the maximum yield achievable by *Miscanthus* at a site (Schwarz *et al.*, 1994). Water limitation is relevant especially in Southern Europe where, due to high temperature and irradiation, there are potentially high productive sites for C<sub>4</sub> crops. However, during summer drought, individual shoots, or even the whole crop, can die (Tayebi and Basch, pers. comm.). Since irrigation of biomass crops is unlikely to be economic, it is important to identify genotypes that optimize the use of water in different climatic regions, and those which are tolerant of water stress. Indigenous *Miscanthus* has a wide geographical distribution in tropical to temperate East-Asia (Numata, 1974). Genotypes originating from different climates vary in their optimal requirements for temperature and water supply.

Considerable intraspecific variation in strategies of response to water deficit have been recorded for other C<sub>4</sub> grasses such as maize and sugar cane (Blum and Sullivan,

1986; Inman-Bamber and De Jager, 1986). These strategies include: (1) avoidance of damaging water deficits; (2) stress-tolerance adaptations that enable the plant to continue functioning in spite of plant water deficits; and (3) mechanisms that enable the plant to optimize the utilization of water for dry matter production (Jones, 1992). Although some plant species may adopt only one of these strategies, a single plant genotype can display all three depending on the duration of the drought. For the genus *Miscanthus*, Weng (1993) reported differences in osmotic adjustment among four ecotypes when exposed to water stress, and found a relationship between the annual rainfall at a location and the degree of osmotic adjustment. This is consistent with the second strategy, since osmotic adjustment enables the plant to continue to function at lower water potentials. However, Weng (1993) did not attempt to calculate the water use efficiency (WUE) of these clones either from leaf gas exchange measurements or from the relationship between whole plant dry matter gain and transpiration. Therefore the importance of the third strategy, which is vital for achieving high productivity of *Miscanthus*, was not ascertained by Weng (1993). To date, quantitative estimates of WUE appear to exist only for the genotype *Miscanthus x giganteus* Greef et DEU (Greef and Deuter, 1993); these include measurements made in the field (Beale *et al.*, 1999) and in the glasshouse using large pots (Jacks-Sterrengerg, 1995).

To support the selection of *Miscanthus* genotypes for different ecological regions of Europe, a knowledge of

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WUE, and the strategies employed when water stress occurs, is essential. The aim of this work was to investigate whether differences in WUE exist between different genotypes of *Miscanthus*. WUE responses to water deficit were investigated in pot trials using three genotypes (*M. x giganteus*, *M. sacchariflorus* and a newly-bred *M. sinensis* hybrid) that showed high productivity traits in field trials in Southern Germany (Lewandowski *et al.*, 2000).

## MATERIALS AND METHODS

### Genotype acquisitions

*M. x giganteus* (Greef and Deuter, 1993) is a vigorous clone that has been used extensively in field trials (Walsh, 1997). It may be a natural hybrid of *M. sinensis* and *M. sacchariflorus* (Hodkinson *et al.*, 1997). It is triploid and therefore sterile, and has a chromosome number of 57.

*M. sacchariflorus* is a tall species when grown in central Europe. The genotype used was selected from a seed population, obtained from Japan in 1992, by the plant breeding company TINPLANT (Magdeburg, Germany) (Deuter and Abraham, 1998). This genotype is tetraploid and the chromosome number is 76.

A *M. sinensis* hybrid which is similar to a commercial genotype 'Goliath' was selected (by TINPLANT) from the cross pollination of several *M. sinensis* genotypes, and was selected as the most vigorous plant from a seed mix. The chromosome number is 57 and it is triploid. All genotypes were established in tissue culture. Propagated plants were planted in field trials at the experimental station of the University of Hohenheim (near Stuttgart, South Germany) in May 1997. Measured yields in late November in 1997 and 1998 were not significantly different among the three genotypes in the second ratoon (Lewandowski and Clifton-Brown, unpubl. res.).

### Preparation of plant material

Plants were sampled from the field trials on 3 Sept. 1998. Rhizome pieces with a fresh weight of  $10 \pm 1$  g were prepared by cutting. The rhizome propagules were stored in sand at 2–5°C for 23 d to break cold dormancy. On 26 September, 30 rhizomes of each genotype were planted in individual pots and partitioned into six treatments using random numbers.

### The pot system

Pots were filled with 1.1 l of a loess sub-soil taken from the C-horizon (Wippenhausen near Weihenstephan, Germany). This is a suitable soil for pot experiments since it undergoes minimal shrinking and cracking when dried, and is easily wetted even when air dry (Buljovic, pers. comm.). The relationship between gravimetric soil water content and soil moisture tension for this soil was determined by thermocouple psychrometry (Wescor C-52 and HR 33T dewpoint microvoltmeter, Logan, USA). The measurement cups were filled at the same bulk density as in the experimental pots ( $1.3 \text{ g dry soil cm}^{-3}$ ). Water was

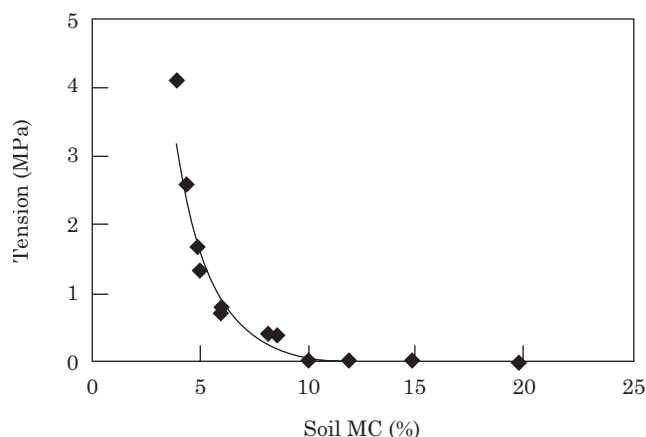


FIG. 1. Relationship between gravimetric soil moisture content (MC %, g H<sub>2</sub>O/100 g dry soil %) and soil water tension for the soil used in this experiment, determined by thermocouple psychrometry. The fitted curve is a negative exponential.

added until the soil exceeded 30 % moisture content. The soil was allowed to dry by evaporation to a pre-determined weight on an analytical balance. When the desired weight had been reached, the cup was sealed with parafilm and held for at least 6 h before transfer to the psychrometer chamber to ensure uniformity in water distribution. Dewpoint was measured, and by reference to saline standard solutions, the water potential was calculated in MPa. Since it was found that water potentials of soil with moisture contents above 12 % were close to zero, it can be concluded that water supply to the plants at gravimetric soil moisture contents above 12 % were non-limiting (Fig. 1).

Nutrient status in the soil was adjusted by addition of a nutrient solution providing 100 mg kg<sup>-1</sup> N, 50 mg kg<sup>-1</sup> P, 200 mg kg<sup>-1</sup> K, 2 mg kg<sup>-1</sup> Fe, 10 mg kg<sup>-1</sup> Zn, and 10 mg kg<sup>-1</sup> Ca, which, in combination with rhizome reserves, was estimated from previous pot experiments to give the plants an ample nutrient supply throughout the experiment.

Water was supplied to each pot through two irrigation drippers (Ecodrip, Lego, Israel) permanently connected to a funnel (a 50 ml syringe without a plunger) into which water was added daily using a wash bottle (Fig. 2). A parafilm membrane was stretched over the top surface of the pot to prevent evaporation from the soil. A highly-absorbent capillary cloth (Schlecker, Germany), covered with a 30 µm nylon membrane (Zürich Bolting Cloth, Rüschlikon, Switzerland) to prevent roots growing into the capillary cloth, prevented water loss from the bottom of the pot. Rapid equilibration of soil moisture content following watering was assisted by the slow irrigation drippers, small pot size, soil texture and by sealing of the top surface and the lining of the pot (Fig. 2). A preliminary test of the pot system showed that roots were evenly distributed throughout the soil, and were not concentrated in a ball around the drippers. Soil moisture content differences at 14 h after watering were typically less than 1 %. Adjacent blank pots (plant-free) were used to estimate evaporation losses. Aluminium foil covered the outer surface of the pots to reduce pot heating during the period of illumination.

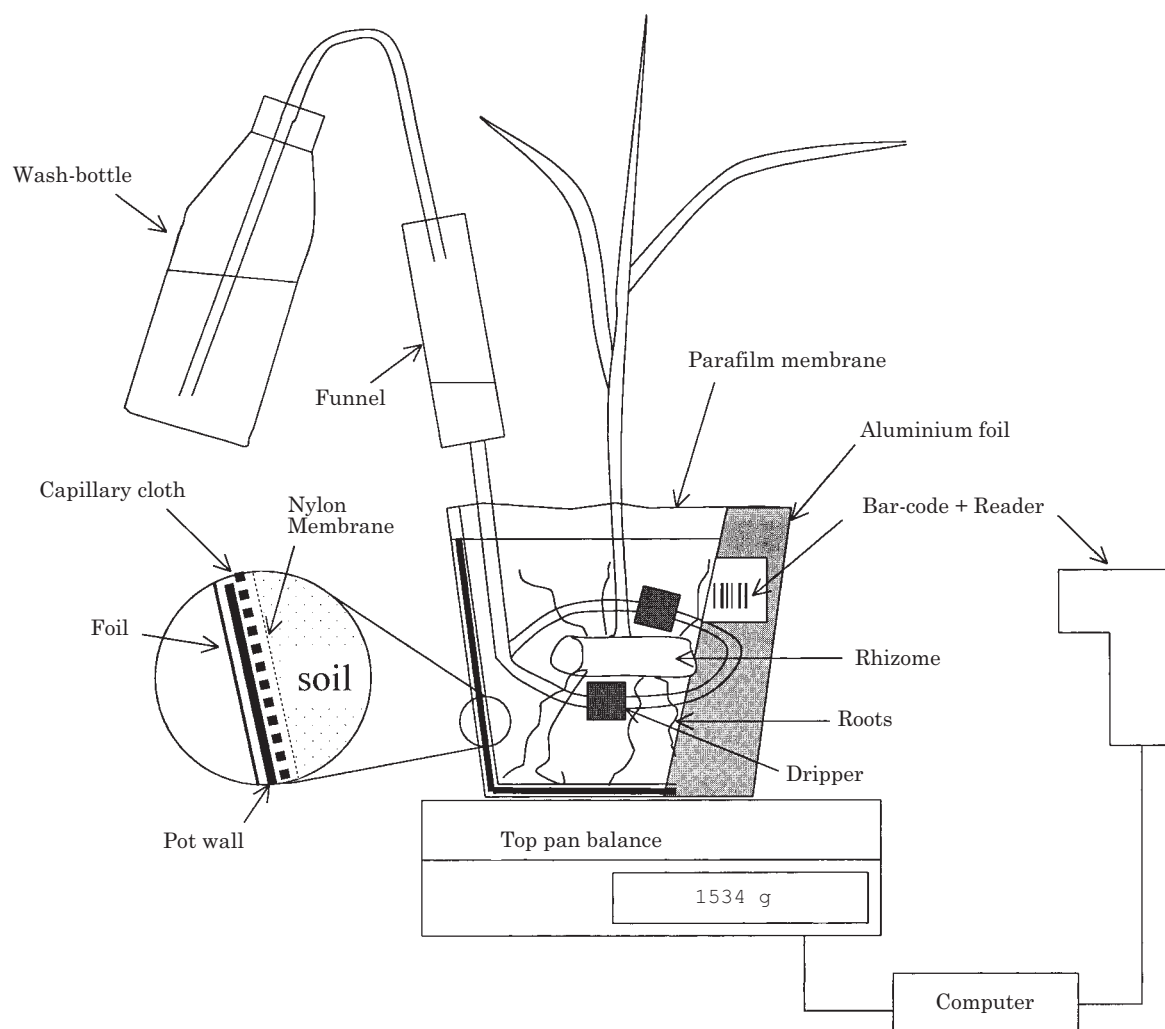


FIG. 2. The system employed to provide controlled water supply to the plants. See text for full explanation.

### Growth conditions

Plants were grown throughout the experiment in a controlled environment with an air temperature of  $21 \pm 1.5^\circ\text{C}$ , and a relatively small air vapour pressure deficit (VPD) of  $0.49 \pm 0.05$  kPa (relative humidity of  $81 \pm 1\%$ ) to moderate the transpiration rate and prevent large diurnal changes in soil moisture content. Radiation intensity was  $700 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD at plant height over a photoperiod of 16 h. The temperature of the soil in the pots was  $26^\circ\text{C}$  during the day and  $19.5^\circ\text{C}$  during the night. The 90 pots in this experiment were arranged in five blocks, each containing one replication of each of the six treatment combinations between genotype (*M. x giganteus*, *M. sacchariflorus* and *M. sinensis*), water supply (WS1, WS2, WS3) and time of harvest (H1, H2, H3). Plants had sufficient space to prevent shading of smaller plants by larger ones. The experiment consisted of three phases, delimited by harvests on days 19 (20 October), 39 (10 November) and 54 (26 November) from the start of the experiment. At each harvest date, five plants per treatment were harvested.

Moisture content in the soil was determined gravimetrically and non-destructively. Each pot carried a bar coded label which was read by a hand-held laser scanner connected to a PC (Fig. 2) in which all of the particulars for the pot were recorded (weight of empty pot, weight of dry soil, fresh weight of the rhizome) and the desired soil water content. The amount of water transpired was calculated daily from weights recorded before and after watering to the programmed water content. The position and block of each plant was changed daily at weighing to minimize effects deriving from spatial variation of conditions in the climate chamber.

As the fresh weight of the growing plants increased during the first two phases of the experiment, this additional fresh weight was not included in the weight of the pot system when watering. Consequently, targeted and actual soil water contents diverged during the experiment. Since it was not easy to compensate for this effect during the experiment, compensation for plant weight increases were made only once, after the harvest on day 39 of the experiment. At the end of the experiment, the fresh weight could be estimated

by linear interpolation of harvest data for each individual plant for every day of the experiment and used to recalculate a more accurate soil moisture content in each pot for each day of the experiment.

#### Water supply treatments

The time-courses of moisture content for the water supply treatments WS1, WS2 and WS3 are shown in Fig. 3. At the end of the third phase, gravimetric soil moisture contents were 16–18 %, 9 % and 6 %, corresponding to no-limitation, mild water limitation and strong limitation.

#### Leaf area

Leaf length, from the ligule to the leaf tip, and leaf width, midway between the ligule and the tip, were measured for each leaf using a graduated ruler every second day on the single shoot emerging from each pot. Subsequently, total green leaf area was calculated following the empirically-derived relationship:

$$\text{area (cm}^2\text{)} = 0.74 \times \text{length (cm)} \times \text{width (cm)} \quad (1)$$

(Clifton-Brown, 1997). The coefficient (0.74) agrees closely with general estimates for maize (Stewart and Dwyer, 1999). Senescing leaf area was deducted from the total expanded leaf area by measuring reductions in leaf length and width and applying eqn (1). It was assumed the drought had a minimal effect on the coefficient.

#### Plant transpiration

Plant transpiration was derived from the quantity of water added daily to the pots minus losses estimated from blank pots. Using the measured green leaf area for each plant, the mean daily transpiration rate ( $E$ ) was calculated in units of  $\text{kg m}^{-2} \text{s}^{-1}$ . Leaf temperature was measured using a fine copper-constantan thermocouple (0.15 mm diameter) held by a spring clip in contact with the mid-point of the abaxial surface of the last fully expanded leaf. The saturation vapour pressure of the air ( $e_a$ ) and at the leaf [ $e_s(T_s)$ ] was estimated from air and leaf temperatures, respectively (see Clifton-Brown and Jones, 1999). Leaf conductance, which consists of stomatal and boundary layer conductance in series, was calculated using the following equation:

$$g_l = E / [(0.622\rho_a/P)(e_s(T_s) - e_a)] \quad (2)$$

where  $\rho_a$  is the density of the air, and  $P$  is air pressure. Equation (2) gives values of  $g_l$  in units of  $\text{mm s}^{-1}$ , which were converted to  $\text{mmol m}^{-2} \text{s}^{-1}$  (Jones, 1992).

At harvest, the fresh and dry weight of the leaf lamina, culm and leaf sheaths, root and rhizome fractions were determined destructively on five plants per treatment. Dry weight was determined after drying the sample at 80°C to constant weight.

Mean daily water use efficiency was calculated for all plants within a genotype from regression of water trans-

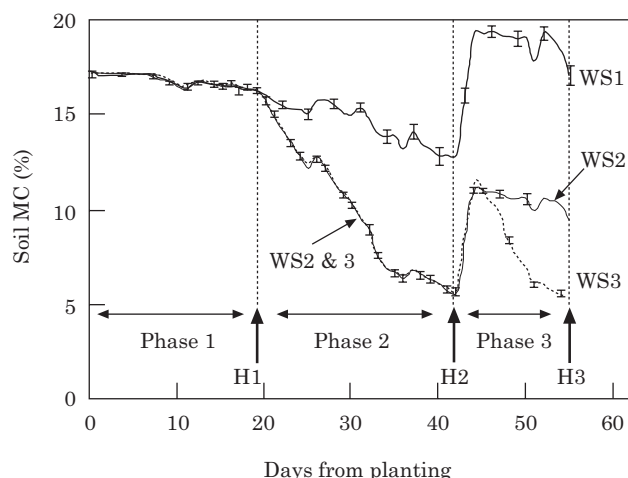


FIG. 3. Time-course of mean daily soil moisture content (MC %, g  $\text{H}_2\text{O}/100 \text{ g dry soil %}$ ) for the duration of the experiment for three water supply treatments (WS1, WS2 and WS3, see text). 'H' indicates the time of harvests (H1, H2 and H3). Values are means of five determinations  $\pm$  s.e.

pired by the plants (lost only through the leaf area) against the dry matter (DM) accumulation of the whole plant or of the shoot which forms the 'harvestable' biomass of this plant.

$$\text{WUE} = \text{g DM produced/kg H}_2\text{O transpired} \quad (3)$$

#### Statistics

Biomass data were analysed by analysis of variance and the Scheffe *post-hoc* test (Data Desk, 4.1, Ithaca). Regression lines for the calculation of mean WUE were constrained to pass through the origin and General Linear Models procedure was used to test differences of the regressions (analysis in SAS v6.2; SAS, 1987).

## RESULTS

#### Leaf area

At the end of the third phase, 54 d after planting the rhizomes, plant leaf areas of *M. sacchariflorus* and *M. x giganteus* were similar under WS1, but the leaf areas of the *M. sinensis* plants were lower (Fig. 4). The lower soil moisture contents under WS2 and WS3 during phases 2 and 3 (Fig. 3) resulted in lower leaf areas in *M. sacchariflorus* and *M. x giganteus* than under WS1 (Fig. 4). However, the leaf area of *M. sinensis* was not influenced systematically by water supply, with the result that plants in the WS3 treatment had similar areas to plants receiving more water (Fig. 4). Senescence, indicated by the amount of brown leaf area, occurred in *M. x giganteus* and *M. sacchariflorus*. Both the proportion and the total amount of brown leaf area were higher in plants receiving WS2 and WS3 than WS1. *M. sinensis* was completely



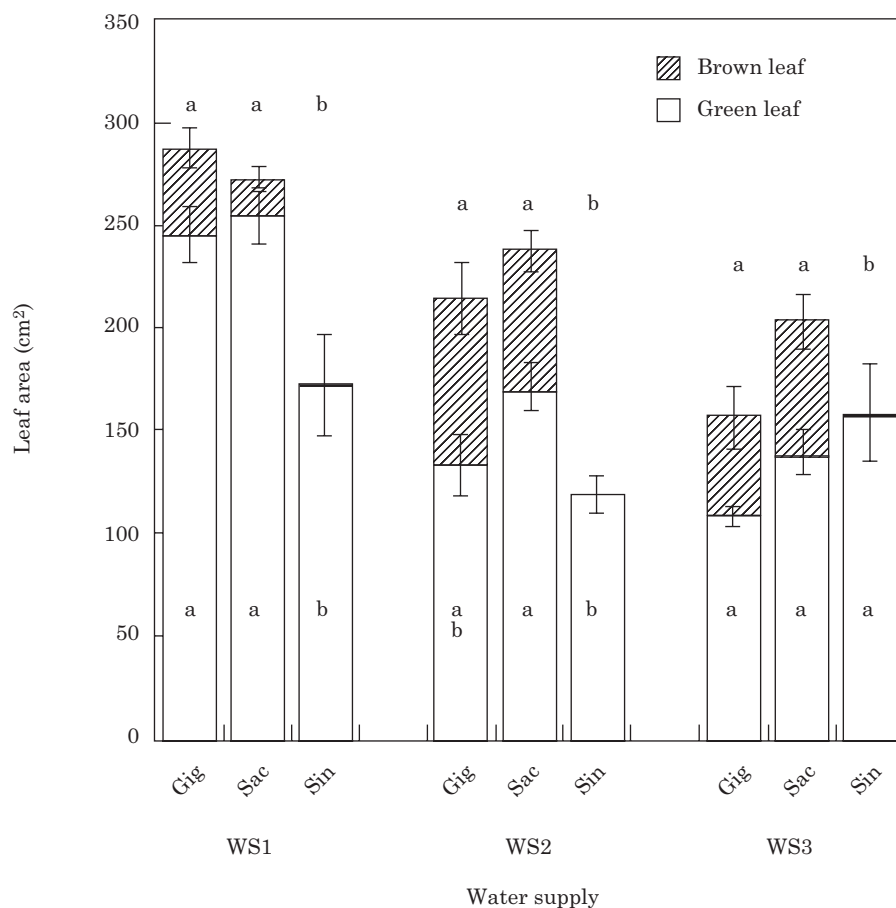


FIG. 4. Green and brown leaf area (cm<sup>2</sup> per plant) on day 53 (before the third harvest) for *M. x giganteus* (Gig), *M. sacchariflorus* (Sac) and *M. sinensis* (Sin) growth under the water supply regimes WS1, WS2 and WS3, illustrated in Fig. 3. Values are means of five determinations  $\pm$  s.e. Different lower case letters (a, b) within bars (green leaf area) and above bars (brown leaf area) indicate differences between genotypes within the water treatments which are significant at  $P < 0.05$  (Scheffé test).

resistant to senescence, and all expanded leaf area remained green irrespective of water supply (Fig. 4).

#### Biomass

Although the fresh weights of the rhizome propagules were similar ( $10 \pm 1$  g), the starting dry weight of *M. x giganteus* (2.64 g DM per plant) was lower than that of *M. sacchariflorus* and *M. sinensis* (3.4 and 3.6 g DM per plant), indicating differences in rhizome dry matter content. From the original rhizome used for propagation, *M. x giganteus* and *M. sacchariflorus* more than quadrupled the dry matter biomass between planting and the third harvest (Fig. 5) in WS1. Biomass accumulation in *M. sinensis* over the same period was lower than for *M. x giganteus* and *M. sacchariflorus* but water supply treatments had less effect on biomass production. In *M. x giganteus* and *M. sacchariflorus* grown under WS1, root growth constituted the greatest proportion of the biomass gain but, in *M. sinensis*, rhizome growth was proportionally greater. The lower soil moisture contents under WS3 caused a significant reduction in the root biomass of *M. x giganteus* and *M. sacchariflorus*, but not of *M. sinensis*. Shoot biomass was

greater in *M. sacchariflorus* than *M. x giganteus* and *M. sinensis* in all treatments. The lower water supply treatments (WS2 and WS3) caused similar reductions in shoot biomass in the faster-growing genotypes *M. x giganteus* and *M. sacchariflorus* but had no detectable influence on shoot production in *M. sinensis*. Averaged over the three water supply treatments, the ratio of above-ground to below-ground dry matter partitioning was highest for *M. sacchariflorus* (0.44:1), intermediate for *M. sinensis* (0.35:1) and lowest for *M. x giganteus* (0.21:1).

#### Leaf conductance

Mean green leaf conductance ( $g_l$ ) over all water supply treatments, over 5 d before the third harvest, was 50 % higher in *M. x giganteus* than in *M. sacchariflorus* and *M. sinensis* (Fig. 6). In treatment WS3,  $g_l$  was significantly reduced in all genotypes, but the lowest  $g_l$  was in the *M. sinensis* genotype. This was 28 % of the  $g_l$  for *M. x giganteus* and 50 % of the  $g_l$  recorded for *M. sacchariflorus*. The reduction of  $g_l$  between WS1 and WS3 was 80 % in *M. sinensis*, but only 57 and 59 % in *M. x giganteus* and *M. sacchariflorus*, respectively.

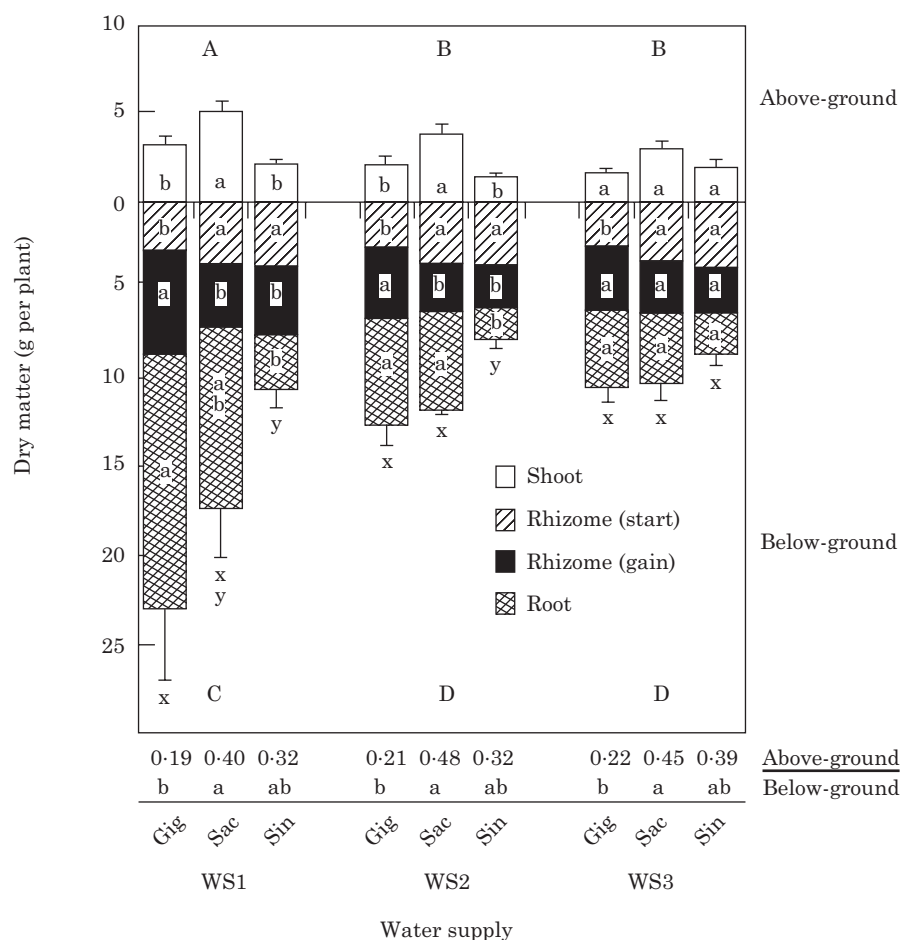


FIG. 5. Dry matter partitioning for plants of *M. x giganteus* (Gig), *M. sacchariflorus* (Sac) and *M. sinensis* (Sin) at the final harvest (harvest 3, day 54) grown under the different water supply regimes WS1, WS2, and WS3 illustrated in Fig. 3. Below-ground biomass is partitioned into rhizome [initial weight on day 0 (start), and gain over 54 d indicated (gain)] and root. Values are means of five determinations  $\pm$  s.e. The ratio of above-ground to below-ground dry matter gain is shown. Different lower case letters indicate differences in mean dry matter between genotypes within a water treatment (a, b) for each plant fraction [shoot, rhizome (start), rhizome (gain) and root] or for the sum of all below-ground fractions (x, y) at  $P < 0.05$  (Scheffé test). Different upper case letters (A, B above-ground; C, D below-ground) indicate differences between water treatments ( $P < 0.05$ , Scheffé test).

### Water use efficiency

WUE using all plants (H1, H2 and H3) for total biomass gain (above- and below-ground) was  $13 \text{ g DM kg}^{-1} \text{ H}_2\text{O}$  across all water supply treatments, and it did not differ significantly among genotypes (Fig. 7A). Water supply treatment influenced whole plant WUE only in *M. sinensis*, increasing from 10.3 to 14.8 between WS1 and WS3 ( $P = 0.04$ ) (Fig. 7A).

When WUE was calculated for shoot production only, *M. sacchariflorus* had the highest WUE, *M. sinensis* intermediate and *M. x giganteus* the lowest values (Fig. 7B). As with WUE calculated for the whole plant, the values for *M. sinensis* only increased with reduced water supply.

When the biomass gain data from the 30 plants of each genotype (all treatments and three harvests combined) and their respective quantities of transpired water were plotted against each other, it was found there was no significant difference between the water use efficiency of the three

genotypes based on whole plant biomass gain (Fig. 8A). However, when analysed on the basis of shoot dry matter gain, *M. sacchariflorus* was significantly more efficient than *M. sinensis* and *M. x giganteus* (Fig. 8B).

## DISCUSSION

### Methods

In order to expose plants to water stress in a controlled way, they must be grown in containers, and recent evidence has shown that small pots need not affect the calculation of water use efficiency (e.g. for maize: Ray and Sinclair, 1998). From a series of experiments with grasses, Thomas (1986) concluded that the method of growing plants in relatively small volumes of soil, which are returned frequently to some pre-determined water content, is preferable to using culture solutions because of unknown effects of osmotica such as polyethylene glycol (reviewed in Dracup *et al.*,

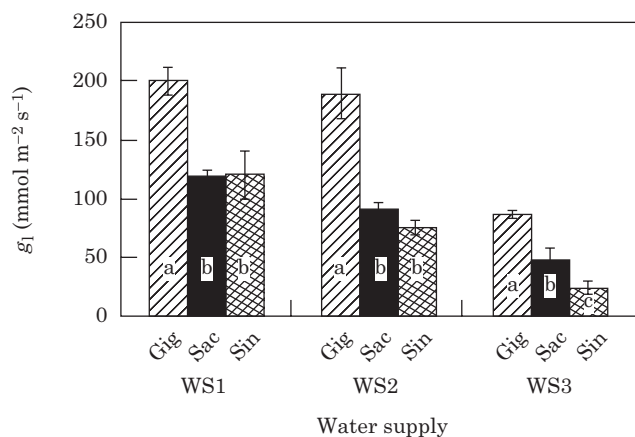


FIG. 6. Values of mean leaf conductance  $g_1$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) for the green leaf area of plants of *M. x giganteus* (Gig), *M. sacchariflorus* (Sac) and *M. sinensis* (Sin) grown at three levels of water supply WS1, WS2, WS3 illustrated in Fig. 3. The measurements were taken between days 48 and 53. Values are means of five determinations  $\pm$  s.e. Different letters indicate differences between  $g_1$  within water treatments which are significantly different at  $P < 0.05$  (Scheffe test).

1986); and also that small pots are preferable to large pots filled with soil because of the amount of space required to replicate treatments adequately. Replicates are especially important in rhizome-grown plants, which tend to be rather more variable than seed-grown crop plants such as maize. A disadvantage of smaller pot sizes is the rapid rate at which the soil water content will decrease when plants have substantial leaf area, and increases in plant biomass change the total mass of a small pot-plant system proportionally more than a large one. Therefore, biomass has a more pronounced effect on soil moisture content in the small pot.

In this experiment, the small pot sizes allowed an adequate degree of replication (five per treatment) in *M. x giganteus* and *M. sacchariflorus*; however, the more variable *M. sinensis* data (Fig. 5) suggest that more replication would have been beneficial. To control heterogeneity in vigour, deriving from differences in the size of the initial rhizome propagule, the initial rhizome fresh weights at planting of all plants were  $10 \pm 1$  g (earlier work had shown that a minimum fresh weight of 8 g was necessary for *M. x giganteus*; Clifton-Brown, 1997).

The influence of increasing biomass was not corrected for in the first and second phases of the experiment. During the second phase, it can be seen that soil moisture contents in WS1 (which ideally should have been at a constant level) fell by almost 5% (from 17 to 12%). However, the moisture content remained above levels that cause water stress according to Fig. 1. We recommend that, in future experiments using similar small pots, harvests for fresh weight compensation be carried out at intervals of 15 d.

#### Reaction of different genotypes to water stress

Differences in biomass partitioning were observed among genotypes within this experiment. Limiting water supply increased the proportion of shoot growth in all three genotypes. This does not agree with observations made on other species (e.g. maize, soybean, cotton and squash)

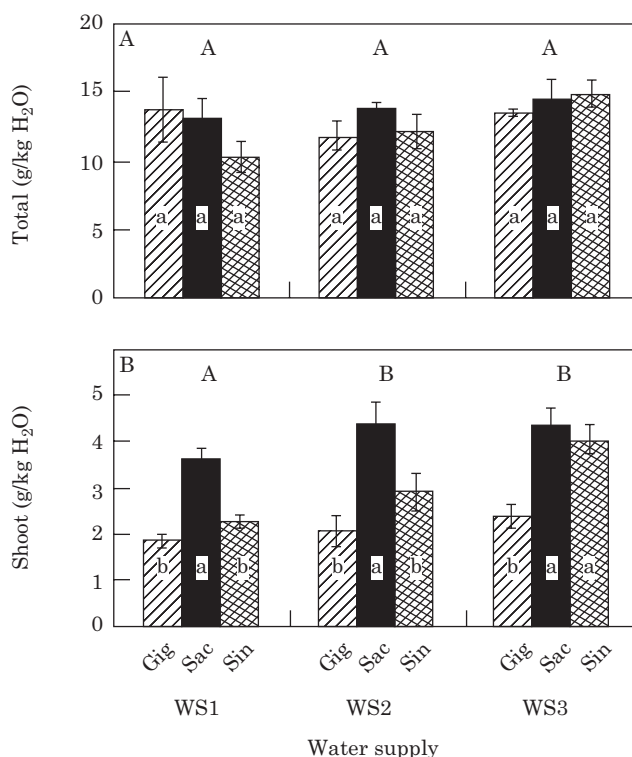


FIG. 7. Water use efficiency ( $\text{g H}_2\text{O kg}^{-1} \text{ plant DM}$ ) for the whole plant (A) and the shoot fraction (B) of the genotypes *M. x giganteus* (Gig), *M. sacchariflorus* (Sac) and *M. sinensis* (Sin) at three levels of water supply WS1, WS2, WS3. Plants were harvested 54 d from the start of the experiment. Values are means of five determinations  $\pm$  s.e. Different lower case letters indicate differences between the WUE of genotypes in a given water treatment ( $P < 0.05$ , Scheffe test). Different upper case letters indicate differences between water treatments ( $P < 0.05$ , Scheffe test).

where water stress led to stimulation of root growth and the suppression of shoot growth (Spollen *et al.*, 1993).

Reducing the soil moisture content by controlled re-watering to a desired soil moisture deficit produced symptoms of water stress. The senescence of green leaf area in *M. x giganteus* and *M. sacchariflorus* and the lack of senescence in *M. sinensis* in the treatments with lower soil moisture contents reflect differential responses to water stress that have been observed in the field during summer drought (Clifton-Brown, unpubl. res.). Where stress or damage do not intervene, senescence is a controlled process involving signalling that is related to increased transcription of enzymes involved in tissue salvaging (Buchanan-Wollaston, 1997). In this experiment, whereas leaf area expansion in *M. x giganteus* and *M. sacchariflorus* was faster than in *M. sinensis*, senescence of older leaves occurred more rapidly in the faster-growing genotypes. As soil moisture content was reduced (Fig. 7) the *M. sinensis* plants experienced the same soil water content as the other genotypes, but with a smaller leaf area. Since the constant environmental conditions in the climate chamber would have produced a constant boundary layer conductance throughout the experiment, changes in  $g_1$  must be mainly caused by changes in stomatal conductance. Therefore, the present results suggest that the 'stay green' mechanism in *M. sinensis* may

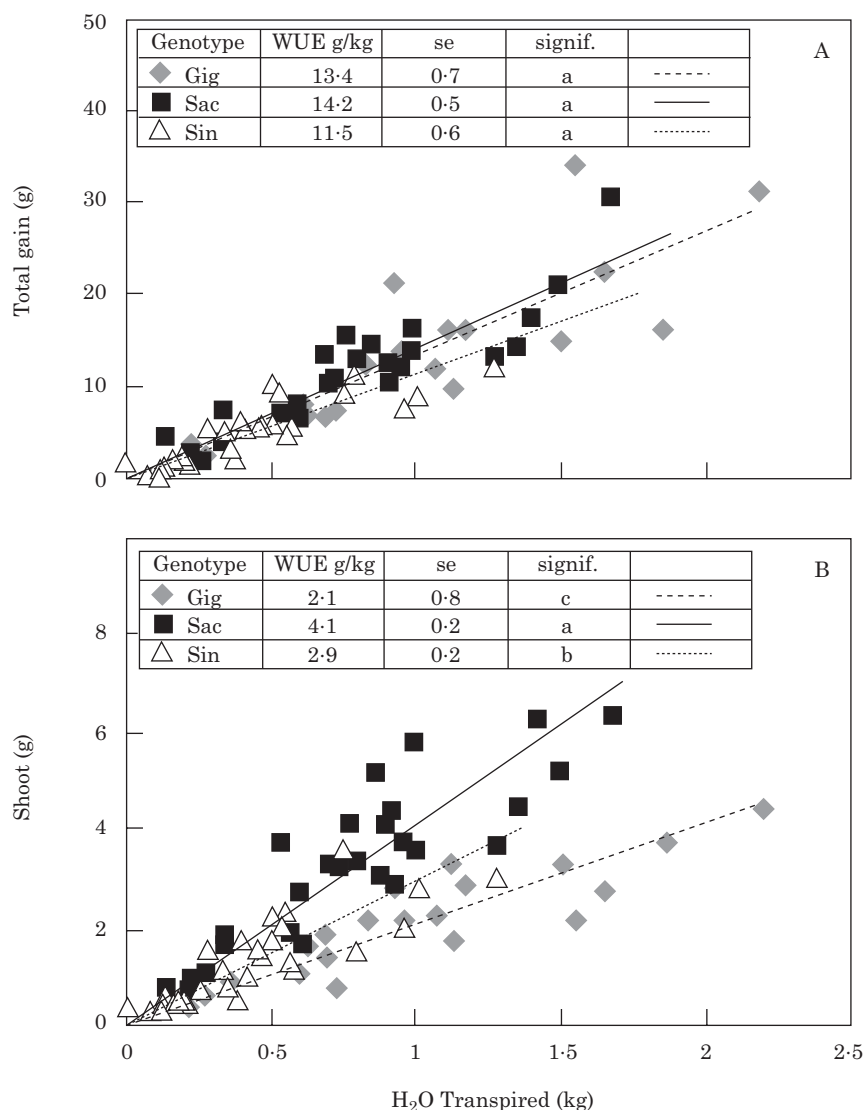


FIG. 8. Water use efficiency ( $\text{g H}_2\text{O g}^{-1} \text{DM}$ ) for the whole plant (A) and the shoot fraction (B) derived from the regression of the individual water use of the plants of the genotypes *M. x giganteus* (Gig), *M. sacchariflorus* (Sac) and *M. sinensis* (Sin), against biomass from all three harvests shown in Fig. 3. Linear regression results are tabulated. Different letters in the columns 'signif.' indicate significance at  $P < 0.05$  (SAS, General Linear Models).

be related to stomatal closure at low soil moisture content. Since *M. x giganteus* and *M. sacchariflorus* do not control leaf conductance as effectively as *M. sinensis* when water is limiting, it is possible that *M. sinensis* could be grown without irrigation at drier sites than *M. x giganteus* and *M. sacchariflorus* with less risk of drought-induced death. Complete senescence of leaf area in non-irrigated *M. x giganteus* in Portugal is rapid whereas, under the same conditions, *M. sinensis* stays green longer; when soil moisture conditions improve, *M. sinensis* plants can resume growth without the requirement to produce new shoots (Tayebi and Basch, pers. comm.).

Of the three strategies in response to water deficit outlined in the Introduction (Jones, 1992), *M. sinensis* utilized avoidance. Damaging water deficits were avoided by reduced green leaf conductance and the utilization of water was optimized. Meanwhile, *M. x giganteus* and

*M. sacchariflorus* employed the second strategy i.e. they continued to function in spite of plant water deficits and lost green leaf area by senescence, which indicates a lack of adaptation to drought.

#### Water use efficiency

When the plants were droughted, *M. sinensis* was the only genotype in which WUE rose (Fig. 7). This can be attributed to the low leaf conductance in this genotype (Fig. 6), as discussed above. The higher partitioning to the shoot in *M. sacchariflorus* resulted in a significantly higher above-ground WUE than in *M. x giganteus* (Fig. 8B). However, under the conditions of this experiment the different physiological responses to increasing water deficit (senescence, stomatal closure) had no significant influence on the overall WUE of the genotypes when all treatments



were combined [range: 11.5 to 14.2 g DM kg<sup>-1</sup> H<sub>2</sub>O (Fig. 8A)]. It is interesting that the whole plant WUE of the slowest-growing genotype (*M. sinensis*) was not higher than the faster ones, since it has often been observed that slow growth rate is associated with highest WUE (Jones, 1993). Although the stomatal closure of *M. sinensis* at low soil moisture contents caused an increase in WUE, these improvements were too small to increase the overall WUE, calculated from all treatments and harvest times.

Values of WUE for shoot biomass production obtained in this experiment ranged from 2.1 to 4.1 g DM kg<sup>-1</sup> H<sub>2</sub>O for *M. x giganteus* and *M. sacchariflorus*, respectively (*M. sinensis* was intermediate). This is quite similar to the value for 2-year old *M. x giganteus* grown in large pots in a glasshouse (3.3 g DM kg<sup>-1</sup> H<sub>2</sub>O; Jacks-Sterrenberg, 1995). Both of these values of WUE for *M. x giganteus* grown in pots are much lower than the values reported recently by Beale *et al.* (1999) for an irrigated mature crop in the field (7.8 g DM kg<sup>-1</sup> H<sub>2</sub>O).

These large differences between pot and field determinations can be explained as follows. Firstly, WUE is dependent on VPD since this influences the gradient in water vapour pressure difference between inside the leaf and air (Jones, 1992). Secondly, in the present experiment, plants were in the young vegetative stage, during which *M. x giganteus* invested 81 % (Fig. 5) of its biomass below-ground. As the crop matures in the field, the ratio of above- to below-ground partitioning alters. For example, Beale and Long (1995) found that the below-ground fraction of a *M. x giganteus* stand was only 40 % of the biomass. Biomass partitioning to the roots and rhizomes probably continues to decline in the third and fourth growing seasons. It can be assumed that the 5-year-old crop studied by Beale *et al.* (1999) was mature and had no net root/rhizome growth. When WUE was calculated for the total biomass gain in our experiment and normalized for VPD, the values from the present experiment were close to those found in the field [6.6 here compared with 7.8 g DM kg<sup>-1</sup> H<sub>2</sub>O for Beale *et al.* (1999)].

#### Relevance to field conditions

It is not known if small pot experiments, such as those reported here, are capable of revealing WUE traits which influence field productivity of *Miscanthus* genotypes. It is certainly unreliable to use such estimates of shoot WUE when attempting to model crop growth under conditions of water limitation in the field, because of changes in harvest index as the crop matures. However, from the pot trial experiments made here, useful traits essential to performance under water deficit have been identified. This is important information for the selection of drought tolerant genotypes and genotypes suitable for different ecological regions of Europe.

Further research is required to establish if the total WUE values calculated here are really a reflection, though not an absolute measure, of the genetic potential for WUE within *Miscanthus* under field conditions. If this were so, then it would appear that, even when different transpiration strategies are employed by different genotypes, the WUE

is rather weakly affected. For improved performance under drought, other strategies such as increased depth of rooting may be more important. The data obtained in this experiment indicate that *M. x giganteus* and *M. sacchariflorus* develop larger rooting systems than *M. sinensis*, but since the pots were small it is not possible to assess the efficiency of different rooting strategies on production. A further experiment could be designed to measure rooting capacity (length, depth and intensity) during drought. Such an experiment would require several growing seasons in this rhizomatous genus, and would probably be best performed in the field.

In warm environments with ample water supply, *M. x giganteus* out-yields the *M. sinensis* hybrid genotype tested here (Tayebi and Basch, pers. comm.). In such environments other considerations, such as the length of the vegetative period of the mature crop, are important. For example in Portugal, the *M. sinensis* genotype flowers earlier (beginning of July) than *M. x giganteus* (late August) and *M. sacchariflorus* (late September) so that the period for biomass accumulation in *M. sinensis* is greatly shortened. Although *M. sacchariflorus* flowers later than the other two genotypes, and is very tall (approx. 4 m), the crop has a comparatively low stem population density, and therefore its yield is often lower than both *M. x giganteus* and the *M. sinensis* hybrid in the field (Lewandowski and Clifton-Brown, unpubl. res.).

## CONCLUSIONS

The *Miscanthus* genotypes studied differed in their response to water deficit. *M. sinensis* had a flexible water-saving strategy, responding to soil drying by reducing leaf conductance so that it could maintain green leaf area even when water was severely limiting. In contrast, leaf area in *M. x giganteus* and *M. sacchariflorus* was lost by senescence under water stress. Therefore *M. sinensis* would appear to be more suitable than the other two genotypes for biomass production in non-irrigated environments with frequent short to medium length summer droughts.

*M. sacchariflorus*, with the highest water use efficiency overall in this experiment, is a useful breeding partner for improving water use efficiency; however, due to its low stem density it will probably remain a breeding partner rather than a genotype for commercial biomass production.

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