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Miscanthus × *giganteus* productivity: the effects of management in different environments

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Abstract

Miscanthus × *giganteus* is a C_4 perennial grass that shows great potential as a high-yielding biomass crop. Scant research has been published that reports *M.* × *giganteus* growth and biomass yields in different environments in the United States. This study investigated the establishment success, plant growth, and dry biomass yield of *M.* × *giganteus* during its first three seasons at four locations (Urbana, IL; Lexington, KY; Mead, NE; Adelphia, NJ) in the United States. Three nitrogen rates (0, 60, and 120 kg ha^{-1}) were applied at each location each year. Good survival of *M.* × *giganteus* during its first winter was observed at KY, NE, and NJ (79–100%), and poor survival at IL (25%), due to late planting and cold winter temperatures. Site soil conditions, and growing-season precipitation and temperature had the greatest impact on dry biomass yield between season 2 (2009) and season 3 (2010). Ideal 2010 weather conditions at NE resulted in significant yield increases ($P < 0.0001$) of 15.6–27.4 Mg ha^{-1} from 2009 to 2010. Small yield increases in KY of 17.1 Mg ha^{-1} in 2009 to 19.0 Mg ha^{-1} in 2010 could be attributed to excessive spring rain and hot dry conditions late in the growing season. Average *M.* × *giganteus* biomass yields in NJ decreased from 16.9 to 9.7 Mg ha^{-1} between 2009 and 2010 and were related to hot dry weather, and poor soil conditions. Season 3 yields were positively correlated with end-of-season plant height ($\hat{\rho} = 0.91$) and tiller density ($\hat{\rho} = 0.76$). Nitrogen fertilization had no significant effect on plant height, tiller density, or dry biomass yield at any of the sites during 2009 or 2010.

Keywords: bioenergy, biomass feedstock, biomass yield, environment effect, *Miscanthus*, N fertilization, plant growth

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Introduction

Concerns about worldwide energy supply and national, environmental, and economic security have resulted in a search for alternative energy sources. In response, several herbaceous crops are being studied as potential biomass feedstocks that can be utilized as energy sources. One of these crops, *Miscanthus* × *giganteus*, a sterile and rhizomatous perennial C_4 photosynthetic plant (Lewandowski *et al*., 2000), has potential to produce substantial dry biomass yields (Heaton *et al*., 2004), and exhibits efficient conversion of solar radiation to biomass, efficient use of nitrogen and water, and no reported commercial pest and disease problems (Beale & Long, 1995; Beale *et al*., 1999). This long-lived perennial grass is a cross between *Miscanthus sinensis* and *Miscanthus sacchariflorus* (Hodkinson & Renvoize, 2001) as confirmed by Hodkinson *et al*. (2002) and Swaminathan *et al*. (2010). *Miscanthus* × *giganteus* was first collected in Japan in 1935 (Hodkinson *et al*., 2002) and was initially planted as a landscape ornamental in Europe and the USA, and later as a bioenergy feedstock in Europe.

From the time of planting, 3–5 growing seasons are required before *M.* × *giganteus* is considered fully established and capable of achieving ceiling biomass yields

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(Miguez *et al.*, 2008) that have reached nearly 40 Mg ha^{-1} peak biomass yields in some European locations (Miguez *et al.*, 2008). In a quantitative review of *M.* × *giganteus* production literature (Heaton *et al.*, 2004), mature stands from across Europe produced mean peak biomass yields of 22 Mg ha^{-1} when averaged across N rates and precipitation levels. Harvestable yields up to 25 Mg ha^{-1} have been reported for areas between central Germany and southern Italy (Lewandowski *et al.*, 2000).

Miscanthus × *giganteus* is typically harvested during the winter or early spring once significant drying of stems and leaves and translocation of nutrients from above ground plant tissue to rhizomes has occurred. There is a tradeoff between harvesting early at the end of the growing season or late during the winter or in the early spring. End of season or 'peak biomass' harvests have higher yields, but higher moisture levels and greater amounts of undesirable minerals, particularly nitrogen. Waiting to harvest until winter or early spring reduces yields, but the need for fertilization is reduced and feedstock quality is improved (Beale & Long, 1997; Lewandowski *et al.*, 2000; Clifton-Brown & Lewandowski, 2002; Heaton *et al.*, 2009). Waiting to harvest until winter or early spring is the recommended practice and results in approximately a 33% reduction from peak biomass yields (Lewandowski *et al.*, 2003), lost primarily from drying tissue and senesced leaves that have dropped from the stems.

Study of *M.* × *giganteus* as a bioenergy feedstock in the United States has increased over the past decade, but relatively little information regarding plant growth, biomass yield ,and the plant's response to agronomic treatments, such as applications of nitrogen fertilizer, has been published. At present, *M.* × *giganteus* biomass yields in the United States have been reported in only four publications from IL and KS (Heaton *et al.*, 2008; Dohleman & Long, 2009; Propheter & Staggenborg, 2010; Propheter *et al.*, 2010). Propheter *et al.* (2010) reported mean harvestable biomass yield increases of 2.7–11.8 Mg ha^{-1} and 4.0–13.7 Mg ha^{-1} from season 1 to season 2 for Manhattan, Kansas and Troy, Kansas, respectively. Heaton *et al.* (2008) and Dohleman & Long (2009) reported mean *M.* × *giganteus*, biomass yields ranging from 17.9–34.6 Mg ha^{-1} in Illinois from mature stands.

Currently, several studies are taking place in the Midwest, Great Plains, and Atlantic coast regions of the United States that will expand the number of *M.* × *giganteus* evaluation sites and provide valuable data that can be used to identify the optimal growing region for the crop. Such data will also provide valuable information about establishment success, responses to new and varying environments, growth patterns from season to season, and response to N fertilizer. Because there are limited US sites growing *M.* × *giganteus*, little information is available to suggest a suitable growing region for the grass.

It is believed that *M.* × *giganteus* should be grown in temperate climates, as a frost period is needed to mark the end of the growing season and the beginning of dormancy (Pyter *et al.*, 2009). *Miscanthus* × *giganteus* possesses winter hardiness traits obtained from *M. sinensis* (Clifton-Brown & Lewandowski, 2000), however, there are still concerns about the crop's ability to withstand harsh winter environments (low and fluctuating winter temperatures) (Clifton-Brown & Lewandowski, 2000). In northern Europe, testing has shown that *M.* × *giganteus* rhizomes are severely affected by temperatures <−3.4 °C (Clifton-Brown & Lewandowski, 2000). Additionally, late-spring frosts have proven to negatively affect emerging and young *M.* × *giganteus* tillers (Farrell *et al.*, 2006). The risk of growing this crop in some colder environments may be confined primarily to the establishment years because mature stands of *M.* × *giganteus* have survived winters in IL with air temperatures lower than −26 °C (−15°F) (personal observations).

Beyond the need to identify the optimal *M.* × *giganteus* growing regions in the United States, additional research is needed to determine its response to applied N. The literature shows varied response to N applications (Lewandowski *et al.*, 2000; Heaton *et al.*, 2004; Miguez *et al.*, 2008). For example in Italy over 4 years *M.* × *giganteus* responded favorably to applied N up to 200 kg N ha^{-1} (Ercoli *et al.*, 1999). At Rothamsted, England, *M.* × *giganteus* grown for 14 years on a silty clay loam did not respond to annual applications of 60 and 120 kg N ha^{-1} (Christian *et al.*, 2008). Across 14 years there was only a 5% difference in biomass yield between N treatments. In their study Christian *et al.* (2008) suggested that annual applications of 7 kg P ha^{-1} and 100 kg K ha^{-1} were important for soil maintenance. Aside from site differences, a major difference between these two studies is that the Italian study was harvested at peak biomass, while the English study was harvested in winter, the recommended harvest timing. In general it appears that yield response to applied N will occur on a site-by-site basis. It has also been suspected that some N may be made available from biological N-fixation (Davis *et al.*, 2010). Other factors that may impact N response include soil type and quality (i.e., texture, bulk density, rooting depth), percent soil organic matter which influences the amount of annual N mineralization, harvest timing, status of other soil nutrients, and the length of time since planting.

As part of the Sun Grant/U.S. Department of Energy Regional Biomass Feedstock Partnership, *M.* × *giganteus* was planted in replicated trials in four locations through the central region of the eastern half of the United States. The locations ranging from west to east were Mead, NE; Urbana, IL; Lexington, KY; and Adelphia, NJ. This experiment began in 2008 and will be ongoing for 5 or more years, allowing sufficient time for the crop to reach maturity at each site. This experiment involves various investigators from multiple institutions, and multiple aspects of production and sustainability are being evaluated. The overall objective of this long-term experiment is to evaluate the potential to geographically expand *M.* × *giganteus* production as a bioenergy feedstock beyond Illinois and Kansas. This study focuses on aboveground *M.* × *giganteus* material during the first 3 years at IL, KY, NE, and NJ. The specific objectives of this study were to: (1) establish *M.* × *giganteus* at these four locations and assess its overwintering capability, and (2) collect morphological, growth, and biomass yield data to assess the impact of temperature and precipitation, season of growth, and N rate on the productivity of *M.* × *giganteus* at each of these locations.

Materials and methods

Crop establishment

Miscanthus × *giganteus* rhizomes obtained from the Chicago Botanic Garden (Glencoe, IL, USA) were used to develop a demonstration planting at the University of Illinois Landscape Horticulture Research Center (Urbana, IL, USA) in 1988. This planting continues to the present and, since 2001, has supplied rhizomes used to plant more than 4 ha of bioenenergy fields at various sites in Illinois, USA.

In fall 2007, *M.* × *giganteus* rhizomes were harvested from a field planting at the University of Illinois, Urbana, IL, USA. Propagation took place in University of Illinois greenhouses where rhizomes of approximately 25 g were planted into 9 cm square pots during winter and spring 2008, in artificial soil mixes and grown in the greenhouse. In early-to-mid summer 2008, 1200 potted plants were shipped to each location for hardening and transplanting.

This study was conducted at four university field sites: University of Illinois Urbana-Champaign (Urbana, IL, 40°06′20″ N, 88°19′18 W), University of Kentucky (Lexington, KY, 38°07′45″ N, 84°30′08 W), University of Nebraska-Lincoln (Mead, NE, 41°10′07″ N, 96°28′10″ W), and Rutgers, The State University of New Jersey (Adelphia, NJ, 40°13′31″ N, 74°14′54 W). Three 1 m deep soil cores from each plot at each location were collected in 2008 on 17 July in IL, 5 August in KY, 14 August in NE, and 3 September in NJ. Each core sample was split into five segments: 0–10, 10–20, 20–30, 30–50, and 50–100 cm depths (Table 1).

At each location, twelve 10 m × 10 m plots comprised of 100 *M.* × *giganteus* plants per plot were transplanted at a density of 1 plant m^{-2}, with 5 m alleys between the plots. Since planting, the gaps between plants are closing due to the rhizomatous growth habit of *M.* × *giganteus*. Transplanting dates were 24 July, 20 June, 18 June, and 19 June, respectively, in IL, KY, NE, and NJ. Late planting at the IL site occurred due to the time required to propagate additional *M.* × *giganteus* plants. The plots at each location were arranged in a randomized complete block design with four replicates. Three N treatments (0, 60, and 120 kg ha^{-1}) were applied at each location in each replicate each year beginning with the establishment year (2008). During the planting year, irrigation and mechanical weed control were provided where necessary at each location to promote establishment of *M.* × *giganteus*. In spring 2009, the percent winter survival at each location was determined, and dead plants were replaced with potted plants at that time so that each plot contained 100 live plants. Percent winter survival was measured again in each plot at each location in spring 2010.

Plant measurements

Throughout the second (2009) and third (2010) growing seasons, plant growth and morphological measurements were collected at KY, NE, and NJ. These data were not collected in IL until 2010 due to 2009 replanting. Emergence date was determined in the spring when the first 10 plants in each plot had emerged. Date of full-headed flowering (R3) (Moore *et al.*, 1991) was determined when approximately 50% of the plants in each plot were fully flowered. Average plant height was measured in KY, NE, and NJ and was determined by measuring the height of the tallest point of the leaf on the tallest stem from five randomly selected plants in each plot each month in 2009 and 2010. In 2009 and 2010, average tiller density (tillers m^{-2}) was determined by counting the number of tillers per plant on at least five random plants in each plot after the end of the growing season. These sampling dates are shown in Table 2. In 2010 when tiller density measurements were collected, the average number of phytomers per tiller and average tiller diameter were also determined. This was done by randomly selecting a total of 10 representative tillers from each of five plants. Tiller diameter was determined by measuring each tiller at the center of the first full internode above the ground level of the tiller. Phytomer number was determined by recording the number of nodes on each tiller. Average tiller diameter and phytomer number were determined by first calculating the average within plants and then across all plants within a plot.

In 2009 and 2010, dry biomass yield estimates were obtained at each location after the end of the growing season. Harvest dates for 2009 are shown in Table 2. Harvesting at all locations in 2009 and 2010, with exception of NE, employed the following protocol. A representative plant in each plot was randomly selected, avoiding plants on the border rows of the plot. A 1 m^{-2} quadrat was centered on the middle of the plant and all standing tillers within the meter-squared area were cut at ~10 cm above the ground. No ground litter was included in the sample. After harvesting the first plant, the 1 m^{-2} quadrat was flipped directly to the north and the adjacent meter-square area (i.e., single plant) was harvested. If the adjacent sampling area was on the edge of the plot, the quadrat was flipped to the south and that adjacent meter-square area was harvested. This process was repeated a second time by randomly selecting a

Table 1 Selected soil variables from each site sampled during summer 2008 at five soil depths (0–10, 10–20, 23–30, 30–50, and 50–100 cm)

Location	Depth (cm)	% Sand	% Silt	% Clay	pH	CEC ($cmol_c\ kg^{-1}$)	% SOM	Total (%) C	Total (%) N	Extractable ($mg\ kg^{-1}$) P	K	Ca	Mg	S	BD ($g\ cm^{-3}$)
Urbana, IL	0–10	55	30	16	5.7	10.6	1.9	1.14	0.11	39.1	110	1390	154	15.8	1.52
	10–20	54	30	15	5.9	10.7	1.9	1.11	0.11	47.1	144	1613	154	17.1	1.69
	20–30	52	32	16	6.0	10.9	1.9	1.08	0.11	39.5	130	1686	177	17.4	1.66
	30–50	36	43	21	6.0	12.0	1.6	0.75	0.08	11.3	75	1722	325	13.4	1.59
	50–100	11	53	35	6.5	20.9	1.2	0.41	0.05	1.4	126	2659	840	11.4	1.64
Lexington, KY	0–10	9	64	27	5.1	18.6	4.7	2.49	0.31	322.2	229	1860	214	45.5	1.38
	10–20	9	63	28	5.8	16.2	3.2	1.64	0.21	302.8	117	2122	174	25.2	1.56
	20–30	8	60	32	5.9	16.0	2.4	1.08	0.15	321.7	91	2145	144	16.3	1.57
	30–50	10	52	38	6.0	17.8	1.8	0.66	0.10	383.4	92	2405	133	12.5	1.71
	50–100	14	38	48	5.9	25.6	1.8	0.46	0.09	391.8	106	3453	146	17.3	1.83
Mead, NE	0–10	4	59	36	6.1	22.4	5.1	2.99	0.33	108.3	667	3053	610	19.6	1.23
	10–20	4	58	39	6.7	22.6	3.6	1.98	0.24	74.7	672	3082	634	16.2	1.39
	20–30	4	55	42	6.7	23.3	3.2	1.70	0.20	60.3	614	3098	715	14.9	1.40
	30–50	3	54	43	6.9	23.8	2.5	1.18	0.14	28.4	575	3006	834	13.7	1.45
	50–100	4	56	40	7.0	23.2	1.4	0.53	0.07	24.9	414	2861	879	11.6	1.53
Adelphia, NJ	0–10	52	35	13	5.3	9.5	2.1	1.23	0.11	219.2	119	800	154	19.3	1.34
	10–20	53	35	12	5.5	9.6	2.2	1.24	0.12	220.5	95	874	158	18.1	1.52
	20–30	53	35	13	5.5	9.1	2.0	1.14	0.11	202.3	92	816	155	17.4	1.57
	30–50	55	30	15	5.5	5.8	1.1	0.48	0.04	43.4	69	627	177	15.4	1.68
	50–100	70	15	16	5.4	5.0	0.8	0.24	0.03	14.7	70	643	141	27.9	–*

CEC, cation exchange capacity; SOM, soil organic matter; BD, bulk density.
*A restrictive soil feature ranging between 50 and 80 cm precluded the calculation of bulk density at this NJ site.

Table 2 Sampling dates from the 2009 and 2010 growing seasons for plant height, tiller density, and biomass yield harvest

Location	Plant height measurements	Tiller density measurements	Biomass yield harvest
2009 Growing season			
IL			January 2010*
KY	24 April, 18 May, 19 June, 27 July, 4 September	4 September	16 March 2010*
NE	10 May, 10 June, 15 July, 17 August, 15 September	15 September	1 April 2010*
NJ	15 May, 17 June, 22 July, 20 August, 18 September, 23 October	23 October	16 December 2009
2010 Growing season			
IL		29 November 2010	29 November 2010
KY	9 April, 10 May, 11 June, 17 July, 9 August, 14 September, 12 October	11 November	11 November 2010
NE	28 April, 26 May, 28 June, 28 July, 24 August, 27 September, 26 October, 22 November	2 December	2 December 2010
NJ	17 May, 17 June, 15 July, 18 August, 24 September, 13 October, 2 November	9 December	9 December 2010

*Harvesting for the 2009 growing season in Illinois (IL), Kentucky (KY), and New Jersey (NJ) actually occurred early in 2010 before the start of the 2010 growing season.

second representative *M.* × *giganteus* plant in each plot. This resulted in a total of four harvested plants, each representing a 1 m^{-2} area from each *M.* × *giganteus* plot. Fresh weight was determined by weighing a subsample, and dry biomass ($Mg\ ha^{-1}$) was determined by calculating the percent moisture of a subsample dried in the oven at 60 °C for at least 48 h. The

NE plots were harvested at a height of ~10 cm using a mechanical forage plot harvester (Carter MFG Co., Inc. Brookston, IN, USA) by harvesting one row of plants (10 plants) for a total area of 10 m^{-2} in each plot. A subsample from each plot was weighed and dried to calculate percent moisture and determine dry biomass yield.

Statistical modeling and analysis

Data from IL were not statistically analyzed with the other locations because this location (as of 2010) possessed a mixture of 2- and 3-year-old plants, and the stand was nonuniform. When applicable, mean values from IL were reported in the results to provide some information regarding this location, and these mean values were calculated using the MEANS procedure of SAS [SAS Institute (2007) *SAS/STAT 9.2 Users's Guide*. SAS Institute, Cary, NC, USA].

Dry biomass yields were analyzed in the MIXED procedure of SAS while tiller density, which follows a Poisson distribution (aka count data), was analyzed in the GLIMMIX procedure of SAS. Locations (KY, NE, and NJ) and years (2009 and 2010) were combined to create six environments: KY-2009, KY-2010, NE-2009, NE-2009, NE-2010, NJ-2009, and NJ-2010. Environments, blocks, and subsamples were considered random effects, while nitrogen rate was declared a fixed effect. The mixed model was described as follows:

$$y_{ijkl} = \mu + e_i + b_{j(i)} + \alpha_k + e\alpha_{ik} + \text{error}_{j(i)k} + s_{ijkl},$$

where dry biomass yield or tiller density (y_{ijkl}) depends on the lth random subsample of the kth nitrogen rate in the jth block nested in the ith environment, having an intercept (μ), and being influenced by the random environment (e_i), random block ($b_{j(i)}$), random interaction between environment and nitrogen rate ($e\alpha_{ik}$), and fixed nitrogen rate (α_k) effects. The model assumes that e_i, $b_{j(i)}$, and $e\alpha_{ik}$ are independent normal random variables with expectations zero and respective variances σ_e^2, $\sigma_{b(e)}^2$, and $\sigma_{e\alpha}^2$, and that the errors ($\text{error}_{j(i)k}$) and subsamples (s_{ijkl}) have means of zero and common variances. Environments were considered random to account for different weather and other environmental conditions at each site which could not be controlled. Significance of random effects were calculated using the COVTEST option in the MIXED and GLIMMIX procedures of SAS. Best linear unbiased predictions of random effects (i.e., means of random effects) and their interactions were calculated using estimate statements with appropriate degrees of freedom and standard errors. Residuals were examined for normality and the assumption of common variances by inspection of residual plots.

The nonlinear function used to model the increase in *M.* × *giganteus* plant height throughout the growing season was the logistic growth function,

$$f(x) = \frac{\text{asym}}{1 + \exp^{-((x-\text{xmid})/\text{scal})}}. \quad (1)$$

Here, $f(x)$ is *M.* × *giganteus* plant height (meter units) measured throughout the growing season and x is the day of year (DOY). Three parameters describe the shape and spread of the function: (1) asymptote (asym) or maximum height achieved by the crop, (2) scale (scal) or the elapsed time between the crop achieving half and three quarters of it maximum height, and (3) inflection point (xmid) or DOY at which the crop achieves half of its maximum height. A nonlinear mixed model was used to implement the logistic growth function and investigate the effects of environment, N rate, and there interaction. This was accomplished by considering asym, scal, and xmid for each environment and each N rate as fixed components, and individual plots (experimental units) as random. The modeling process followed principles in Pinheiro & Bates (2000) and was implemented with 'nlme' package (Pinheiro *et al.*, 2009) of R statistical software (R Core, v. 2.12.1, 2010). Residuals were checked for patterns by plotting standardized residuals against their fitted values. Parameter estimates and their 95% confidence intervals were obtained using the 'summary' and 'interval' functions of R (R Core, v. 2.12.1, 2010). Prediction plots and all other graphics were obtained using the 'graphics' and 'lattice' packages of R (R Core, v. 2.12.1, 2010).

Pearson correlation coefficients and their respective P-values were calculated using the CORR procedure of SAS to evaluate the linear association of dry biomass yield, end of season plant height, tiller density, phytomers per tiller, and tiller diameter among environments during the 2010 growing season. End of season plant height measurements were determined by selecting the last set of plant height (between September and November) measurements collected in each environment. For each variable, the mean value for each plot was determined using the MEANS procedure of SAS, resulting in 12 observations, n = 12 (4 blocks × 3 N rates) for assessing the effect of environment on these variables. In addition, these variables were correlated with accumulated thermal time and growing-season precipitation (April through September). Matrix scatter plots were obtained using the SGSCATTER procedure of SAS to visually assess correlations among environments within a growing season.

Results

Site growing conditions

Selected soil-data variables from each site were averaged and summarized (Table 1). At IL, the soil is classified as a very deep, well-drained Wyanet silt loam (loamy, mixed, active, mesic Typic Argiudolls). The upper 30 cm of soil at this site is dominated by a sandy loam that transitions into a silty clay loam at the 50–100 cm depth (Table 1). The water table at this site ranges between 61 and 107 cm in depth. Organic matter levels are relatively low ranging from 1.9% at the 0–10 cm depth down to 1.2% at the 50–100 cm depth (Table 1). This is an atypical site for east-central IL; typical soils for this area are usually Drummer (very deep, poorly drained, silty clay loam soils) or Flanagan (very deep, somewhat poorly drained, silt loam soils) series. At KY, the soil is classified as very deep, well-drained Maury silt loam (fine, mixed, active, mesic Typic Paleudalfs) with a water table deeper than 200 cm.

Percent organic matter levels at this site range from 4.7% at the 0–10 cm depth down to 1.8% at the 50–100 cm depth. At NE, the soil is classified as a very deep well-drained Tomek silt loam (fine, smectitic, mesic Pachic Argiudolls), however, this specific site is dominated by a silty clay loam soil texture (Table 1). The water table at this site is greater than 200 cm in depth and percent organic matter levels range from 5.1% at the 0–10 cm depth down to 1.4% at the 50–100 cm depth. At NJ, the soil is a Holmdel sandy loam (fine-loamy, mixed, active, mesic Aquic Hapludults) with a relatively high water table ranging between 15 and 91 cm in depth. Percent organic matter levels at this site range from 2.1% at the 0–10 cm depth down to 0.8% at the 50–100 cm depth. At this site there is a restrictive soil layer or a bedrock layer between 50 cm and 80 cm in depth, depending on the plot.

Weather data from stations near to Urbana, IL; Lexington, KY; Mead, NE; and Adelphia, NJ were obtained from the Midwestern Regional Climate Center, a cooperative program of the Illinois State Water Survey and the National Climatic Data Center, or directly from the National Climatic Data Center. Data from each location were collected from stations nearby Urbana, IL; Lexington, KY; Mead, NE; and Adelphia, NJ, respectively, with station name and cooperative identification number, Urbana, IL (118740), Lexington Bluegrass AP, KY (154746), Mead 6S, NE (255362), and Hightstown 2W, NJ (283951). Monthly weather data for 2008, 2009, and 2010 are summarized in Tables 3 and 4 and Figures 1 and 2. Accumulated thermal time (aka growing-degree days) was calculated with a base temperature of 0°C as has been done in other studies (Miguez *et al*., 2008, 2009; Hastings *et al*., 2009).

Winter survival

The percent of *M.* × *giganteus* plants that survived the first winter was very dependent upon the site. Because only 25% of the *M.* × *giganteus* plants survived the first winter in IL, replanting was required to bring the plots

Table 3 Total monthly precipitation (mm) at each location during 2008, 2009, and 2010, and their 30-year normal averages

	IL				KY			
Month	2008	2009	2010	30-Year normal	2008	2009	2010	30-Year normal
January	59	17	31	48	112	110	76	85
February	151	43	41	51	146	65	41	83
March	72	67	74	82	160	61	29	112
April	76	176	53	93	150	121	59	93
May	154	145	87	122	112	153	253	121
June	163	112	212	107	91	132	117	116
July	200	160	95	119	87	192	154	122
August	20	143	42	111	55	115	15	96
September	207	20	81	82	36	150	15	79
October	75	223	28	71	39	147	31	69
November	33	100	98	88	64	24	113	87
December	124	96	65	70	153	102	63	102
Annual	1336	1302	906	1043	1205	1372	966	1166
	NE				NJ			
January	6	7	23	12	69	71	67	95
February	10	12	17	13	110	15	110	70
March	17	8	41	47	83	47	229	100
April	118	41	102	70	62	99	67	100
May	151	30	68	106	116	112	82	112
June	251	165	249	101	107	187	78	100
July	95	67	183	84	89	159	75	126
August	26	185	64	85	39	172	20	123
September	110	39	148	73	178	100	77	109
October	129	110	6	55	90	119	82	87
November	45	0	0	40	84	64	50	93
December	30	67	0	18	151	166	74	95
Annual	987	732	902	704	1177	1311	1012	1211

IL, Illinois; KY, Kentucky; NE, Nebraska; NJ, New Jersey.

Table 4 Average minimum and maximum air temperature (°C) at each location during 2008, 2009, and 2010, and their 30-year normal averages

	IL								KY							
	Average minimum (°C)				Average maximum (°C)				Average minimum (°C)				Average maximum (°C)			
Month	2008	2009	2010	30-Year normal	2008	2009	2010	30-Year normal	2008	2009	2010	30-Year normal	2008	2009	2010	30-Year normal
January	−8.6	−12.3	−10.1	−8.8	1.8	−2.4	−3.4	0.0	−4.4	−6.5	−5.8	−4.4	4.7	2.6	1.7	4.4
February	−7.9	−5.7	−8.0	−6.1	0.7	4.7	−0.4	3.1	−2.3	−2.1	−5.6	−2.4	6.2	8.5	2.1	7.3
March	−1.1	0.1	1.1	−0.9	8.5	12.6	11.9	9.7	1.2	3.4	2.9	2.2	12.3	14.3	13.4	12.9
April	4.7	5.0	7.6	4.4	16.6	16.3	21.3	16.8	6.8	7.4	8.5	6.7	18.2	18.7	22.0	18.4
May	8.9	11.4	12.5	10.6	20.4	23.3	23.7	23.1	10.5	13.1	14.4	12.0	22.0	23.1	24.3	23.3
June	17.2	18.1	18.6	15.8	28.6	29.2	29.0	28.1	17.4	17.9	19.5	16.8	29.0	28.3	30.1	27.9
July	17.6	16.0	19.4	18.0	28.8	26.2	30.5	29.6	18.2	17.6	20.3	19.1	30.2	26.8	30.8	29.9
August	16.5	15.8	18.8	16.9	28.0	26.9	31.3	28.4	17.3	17.9	19.4	18.3	30.0	27.9	31.8	29.2
September	14.1	13.9	12.9	12.4	25.3	24.7	26.3	25.3	15.5	15.6	14.2	14.4	28.4	24.9	28.8	25.6
October	6.1	5.3	6.2	6.1	19.2	14.4	20.8	18.4	7.4	6.9	7.4	8.0	20.6	16.6	22.2	19.4
November	−0.6	2.7	−0.7	0.3	8.9	13.1	12.1	9.7	0.9	3.7	2.1	2.9	11.2	14.1	14.4	12.5
December	−7.8	−6.1	−8.8	−5.6	1.3	1.9	−1.4	2.7	−3.3	−2.4	−5.8	−2.0	7.6	5.8	0.8	6.8
	NE								NJ							
January	−12.9	−12.4	−13.4	−12.4	−1.6	0.4	−5.6	−0.7	−3.9	−7.6	−4.9	−5.8	6.4	1.3	4.1	3.7
February	−11.6	−8.4	−11.7	−9.1	−0.4	6.5	−2.7	2.8	−3.4	−4.8	−5.0	−4.8	7.4	7.2	3.4	5.1
March	−5.0	−4.2	−1.8	−3.0	9.6	10.2	8.8	9.4	−0.2	−1.1	2.4	−0.4	11.2	10.4	13.4	10.1
April	0.7	1.1	5.2	3.4	13.4	16.1	20.3	16.7	5.7	5.4	6.3	4.1	18.1	17.4	20.1	16.1
May	7.9	9.1	8.9	9.8	21.5	23.7	21.4	22.8	8.6	10.6	10.8	9.4	20.8	22.1	24.9	22.0
June	14.9	15.0	15.9	15.3	27.8	27.3	28.4	28.6	16.1	14.7	17.1	14.4	29.4	24.9	30.0	26.8
July	17.3	14.8	18.4	17.9	29.9	27.2	29.6	30.8	18.4	15.7	19.8	17.3	31.0	28.8	32.3	29.4
August	15.2	14.3	17.4	16.4	29.4	27.5	31.1	29.3	14.4	17.6	18.4	16.4	28.9	29.5	29.9	28.4
September	10.4	10.3	11.8	11.1	24.6	24.1	25.5	25.3	13.4	11.7	14.1	12.2	25.6	23.7	27.8	24.6
October	4.2	1.8	3.8	4.1	18.2	12.4	21.7	18.5	3.7	5.4	7.4	5.7	18.6	17.0	19.5	18.4
November	−2.2	−0.7	–	−2.9	10.1	13.3	–	8.6	1.3	4.8	0.9	1.4	10.9	14.4	13.3	12.3
December	−12.3	−12.5	–	−9.3	−0.3	−1.9	–	1.3	−3.0	−3.1	−5.4	−2.9	7.8	6.3	4.1	6.4

IL, Illinois; KY, Kentucky; NE, Nebraska; NJ, New Jersey.

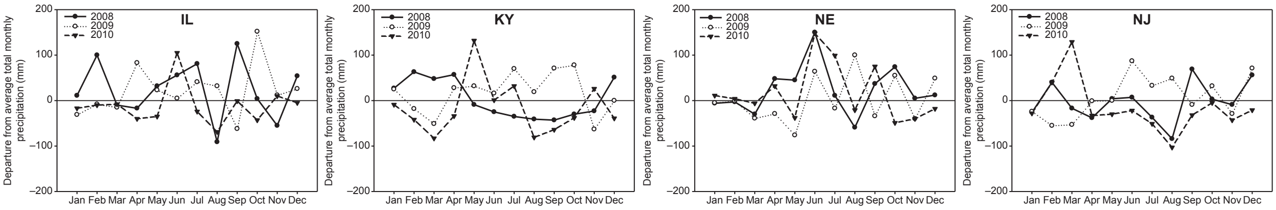

Fig. 1 Departure from average for total monthly precipitation (mm) at each location during 2008, 2009, and 2010. Departures were calculated as the difference between total monthly precipitation and monthly 30-year normal averages.

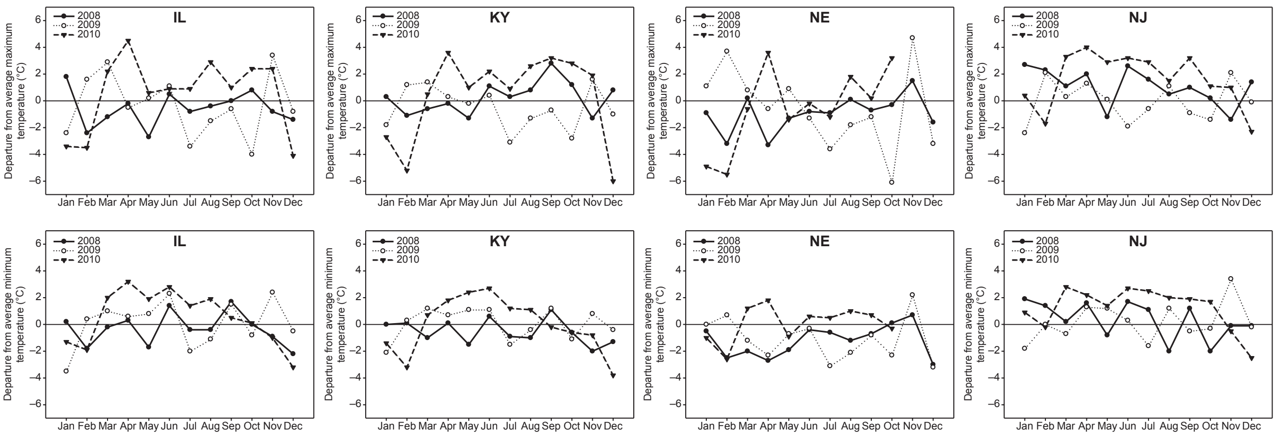

Fig. 2 Departure from average minimum and maximum air temperature (°C) at each location during 2008, 2009, and 2010. Departures were calculated as differences between monthly average minimum and maximum temperatures and 30-year normal averages.

to a fully planted status during the 2009 season. The other three sites had adequate to excellent winter survival. Survival in KY, NE, and NJ was 99%, 79%, and 100%, respectively. Poor winter survival at IL was related to late transplanting on 24 July 2008. In addition to being planted late, winter temperatures at IL dropped to −27 °C in the middle of January 2009. Percent winter survival at the beginning of the 2010 season was near 100% at each site.

Growing season conditions

Kentucky had more growing-season precipitation (April through September) than either NE or NJ in 2009 (Table 3). In 2010, early season precipitation in KY was much higher than normal, but August and September were very dry, with precipitation levels 81 and 64 mm less than normal, respectively (Table 3). In addition, temperatures were above normal each month from April through October (Table 4). In 2010, accumulated thermal time in NE (3402) was at least 1000 units lower than KY (4531) and NJ (4601) (Table 5), while growing-season precipitation (April through September) was at least 200 mm greater in NE (814 mm) than in KY (613 mm) and NJ (399 mm) (Table 3).

Plant height

The N rate × environment interaction was found nonsignificant for the asym (P = 0.1206), xmid (P = 0.5919), and scal (P = 0.9343) parameters. The N rate main effect was also nonsignificant for the asym (P = 0.4418), xmid (P = 0.8802), and scal (P = 0.5290) parameters, whereas environment was significant for each parameter (P < 0.0001). Plant height increases throughout the growing season showed very similar patterns among different environments and N rates (Fig. 3). The estimated asym attained in the different environments ranged between 3 and 3.79 m, and there were significant increases in the asym in the KY and NE environments from 2009 to 2010 (Table 6). The parameters xmid and scal were quite different from environment to environment ranging from 143.5 to 171.5 for xmid and 15.38 to 24.23 for scal. These differences were environment dependent and appear related to the length of the growing season and weather conditions in each environment.

Biomass yield

The environment × N rate interaction did not contribute significant variation. Biomass yield was not affected by

Table 5 Season length, accumulated thermal time, average emergence date, and flowering date(s) for each season and location

Location	Season length*	Accumulated thermal time†	Emergence date‡	Flowering date(s)§
2009 Growing season				
KY	193 days (8 April–18 October)	3783	31 March	18 September
NE	172 days (15 April–4 October)	3196	26 April–1 May	23 September
NJ	185 days (17 April–19 October)	3459	27 April	~25–30 September
2010 growing season				
IL	196 days (10 April–23 October)	3911	10 April	1 October
KY	217 days (27 March–30 October)	4531	2 April	Did not flower
NE	167 days (19 April–3 October)	3402	2 week April	27 September
NJ	218 days (28 March–1 November)	4601	11 April	30 October¶

*Season lengths were calculated as the number of days between the last frost in the spring to the first frost in the fall. One exception is in KY in 2010, where a late frost on 19 April was not used as the beginning of the growing season since there had already been 2–2 weeks of above-freezing weather since the previous frost on 27 March. In this case, 27 March was marked as the last frost in the spring.

†Accumulated thermal time was calculated with a base temperature of 0 °C, by determining the average of the minimum (when greater than 0 °C) and maximum daily (no limit) temperatures, and summing these values across time. In calculating accumulated thermal time for individual days, if the average temperature for that day did not exceed 0 °C, no thermal time was accumulated

‡Emergence date was determined in the spring when approximately the first 10 plants in each plot had emerged.

§Date of full-headed flowering was determined when approximately 50% of the plants in each plot were fully flowered.

¶Only plots that flowered at Adelphia, NJ in 2010 were those plots applied with 0 kg N ha^{-1} and some that received 60 kg N ha^{-1}. When it occurred flowering took place on 30 Oct. First fall frost was 1 November.

N rate (P = 0.3938), but did differ by environment (P = 0.0611). There was a significant decrease in biomass yield at NJ (P < 0.0001) from 2009 to 2010 (Table 7), representing a 42.6% yield decrease. From 2009 to 2010, biomass yields at KY increased slightly (P = 0.1755) and 75.6% in NE ($P \leq 0.0001$) (Table 7). In 2009, there were no yield differences among the KY, NE, and NJ environments ($P \geq 0.2289$). In 2010, however, there were significant differences in biomass yields among the KY, NE, and NJ environments with yields in NE greater than yields in KY and NJ ($P \leq 0.0001$), and KY yields greater than NJ yields ($P \leq 0.0001$). Mean biomass yields at IL in 2009 were 1.1, 3.8, and 4 Mg ha^{-1} for 0, 60, and 120 kg N ha^{-1}, respectively, while 2010 mean biomass yields increased to 14.8, 16.1, and 16 Mg ha^{-1} for 0, 60, and 120 kg N ha^{-1}, respectively.

Tiller density

There was no significant tiller-density response to applied N in any of the environments and the only significant effect was the environment main effect (P = 0.0624). There were significant increases in tiller densities in KY ($P \leq 0.0001$) and NE ($P \leq 0.0001$) from 2009 to 2010 (Table 7). To put this in perspective, tiller densities in 2010 were approximately 52% and 56% greater than in the 2009 seasons for KY and NE, respectively (Table 7). Tiller densities were relatively constant (P = 0.7820) between the NJ-2009 and NJ-2010 environments. Mean tiller densities at IL in 2010 were 37.3, 32.3, and 37.6 tillers m^{-2}, respectively, for 0, 60, and 120 kg N ha^{-1}.

Correlations among variables

In 2010, there was a strong positive correlation between end-of-season plant height and dry biomass yield ($\hat{\rho} = 0.94$) (P < 0.0001). There were also strong positive correlations between dry biomass and tiller density ($\hat{\rho} = 0.87$) (P < 0.0001), and between plant height and tiller density ($\hat{\rho} = 0.88$) (P < 0.0001). There were moderate positive correlations between plant height and tiller diameter ($\hat{\rho} = 0.59$) (P = 0.0002), and between tiller density and tiller diameter ($\hat{\rho} = 0.63$) (P < 0.0001). These relationships are summarized with matrix scatter plots in Figure 4 and suggest that higher biomass yields were primarily related to higher tiller densities and taller plant heights. NE stands out in the consistency of the relationships among these variables, having the highest biomass yields, highest tiller densities, and tallest plant heights in 2010. New Jersey tended to have the lowest biomass yields and tiller densities and shorter plant heights, with KY lying between NE and NJ. Tiller diameter and phytomers per tiller did not produce any noteworthy relationships to biomass yield. In 2010, accumulated thermal time had strong-to-moderate negative correlations on biomass yield ($\hat{\rho} = -0.83$) (P < 0.0001), plant height ($\hat{\rho} = -0.89$) (P < 0.0001), tiller density ($\hat{\rho} = -0.78$) (P < 0.0001), and phytomer number

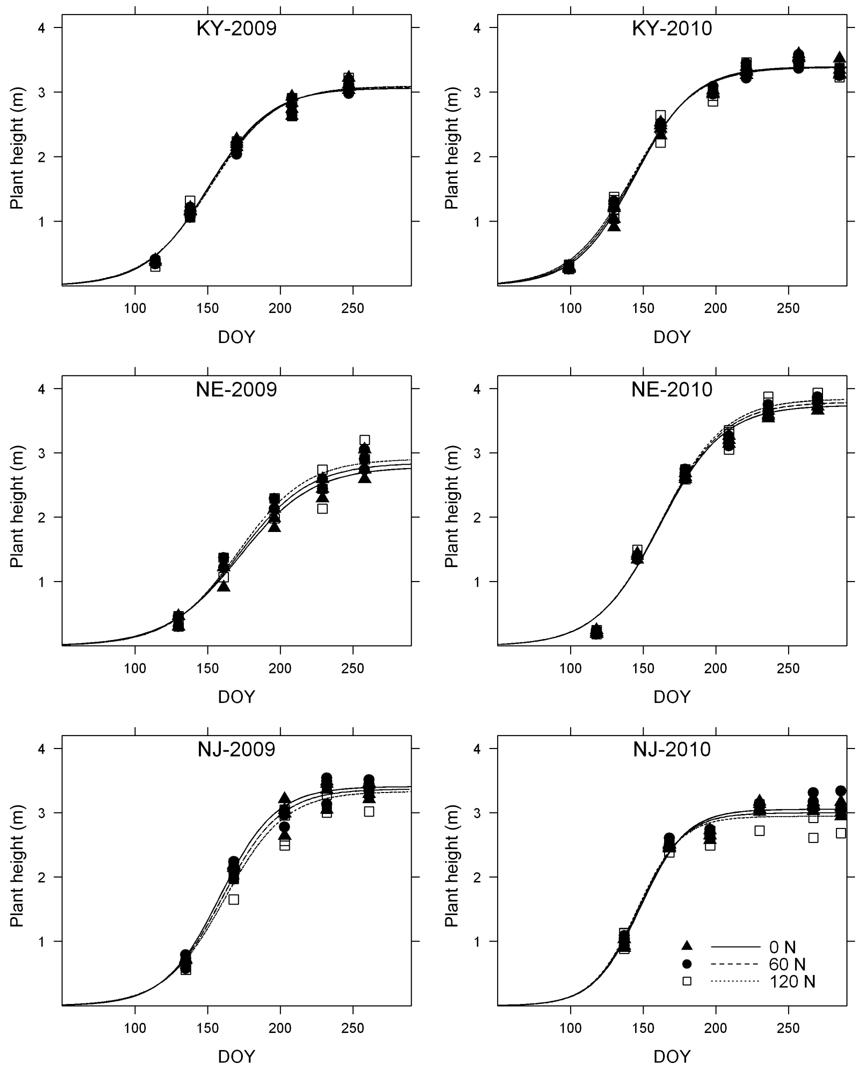

Fig. 3 *Miscanthus* × *giganteus* plant height (m) plotted against day of year (DOY) and fit with logistic growth functions for each N rate (0, 60, 120 kg N ha^{-1}) in each of six environments (KY-2009, KY-2010, NE-2009, NE-2009, NE-2010, NJ-2009, and NJ-2010).

($\hat{\rho} = -0.74$) ($P < 0.0001$), whereas growing-season precipitation (April through September) had strong-to-moderate positive correlations with biomass yield ($\hat{\rho} = 0.95$) ($P < 0.0001$), plant height ($\hat{\rho} = 0.96$) ($P < 0.0001$), tiller density ($\hat{\rho} = 0.91$) ($P < 0.0001$), and stem diameter ($\hat{\rho} = 0.65$) ($P < 0.0001$).

Discussion

The percent of *M.* × *giganteus* plants that survived the first winter was dependent upon the site and was related to late planting and cold winter temperatures. In IL, extreme winter temperatures during the 2008–2009 winter reduced the *M.* × *giganteus* population by 75%. In Europe, rhizomes from newly planted *M.* × *giganteus* were affected by temperatures less than −3.4 °C (26°F) which killed 50% of the rhizomes exposed to these temperatures (Clifton-Brown & Lewandowski, 2000). Clifton-Brown & Lewandowski (2000) also suggest that larger plants tend to overwinter better than *M.* × *giganteus* plants that are shorter, have high rhizome moisture content, and that do not begin dormancy until the first fall frost occurs. Generally, winterkill is an issue during the establishment year as mature stands of *M.* × *giganteus* have survived IL winter temperatures lower than −26 °C (−15°F) (personal observations).

The growing conditions were adequate for *M.* × *giganteus* growth in KY, NE, and NJ environments in 2009, and the dry biomass yields at each location in 2009 were slightly higher than second season biomass yields reported in a Kansas, USA, study (Propheter & Staggenborg, 2010). In NJ, shallow, sandy soils likely contributed to the low yields (Table 1). Tiller densities at each site in 2009 were similar, and it was

Table 6 95% Confidence intervals (upper and lower) of the parameters estimates of the logistic growth function for each environment

Parameter	Environment	Lower	Estimate	Upper
asym	KY-2009	2.99	3.08	3.17
	KY-2010	3.19	3.39	3.59
	NE-2009	2.62	2.85	3.07
	NE-2010	3.59	3.79	3.99
	NJ-2009	3.17	3.37	3.57
	NJ-2010	2.80	3.00	3.20
xmid	KY-2009	149.1	151.5	153.8
	KY-2010	138.3	143.5	148.7
	NE-2009	165.4	171.5	177.6
	NE-2010	156.7	161.7	166.8
	NJ-2009	155.1	160.2	165.4
	NJ-2010	141.8	146.8	151.9
scal	KY-2009	19.71	21.75	23.79
	KY-2010	16.42	20.86	25.30
	NE-2009	19.08	24.23	29.37
	NE-2010	17.53	21.84	26.15
	NJ-2009	15.31	19.82	24.33
	NJ-2010	10.96	15.38	19.81

asym, maximum height (m); xmid, day of year at which crop achieves half of its maximum height; scal, time (in terms of days) between half and three quarters maximum height; KY, Kentucky; NE, Nebraska; NJ, New Jersey.

Table 7 Dry biomass yield (Mg ha^{-1}) and tiller density (number tillers m^{-2}) estimated for each environment at each of three N rates (kg N ha^{-1}) and their means averaged across N rates

	N rate (kg N ha^{-1})				
Environment	0	60	120	Mean*	*P*-value†
Dry biomass (Mg ha^{-1})					
KY-2009	16.5‡	17.6	17.1	17.1	0.1755
KY-2010	18.2	19.4	19.5	19.0	
NE-2009	15.7	15.9	15.2	15.6	<0.0001
NE-2010	26.8	28	27.7	27.4	
NJ-2009	15.2	17.9	17.6	16.9	<0.0001
NJ-2010	9.5	10	9.3	9.7	
Number tillers m^{-2}					
KY-2009	38.8	37.4	38.5	38.2	<0.0001
KY-2010	58.8	56.8	58.4	58	
NE-2009	45.2	43.6	44.8	44.6	<0.0001
NE-2010	70.4	67.9	69.8	69.3	
NJ-2009	44.7	43.2	44.3	44.1	0.7820
NJ-2010	44.0	42.4	43.6	43.3	

*Mean values are averaged across N rate.
†*P*-values for contrast statements comparing mean environment values within a location (KY-2009 vs. KY-2010, NE-2009 vs. NE-2010, and NJ-2009 vs. NJ-2010).
‡No contrasts among N rates were made because the environment × N rate and N rate effects were nonsignificant for both dry biomass and tiller density.
KY, Kentucky; NE, Nebraska; NJ, New Jersey.

expected that tiller densities and biomass yields would increase from 2009 to 2010 at each site (Miguez *et al.*, 2008), but this was not the case (Table 7).

Dry biomass yields at KY increased only slightly from 2009 to 2010 and this could be attributed to abnormal weather conditions in 2010. The crop emerged on 2 April (Table 5), and a frost on 19 April caused damage. Exposure of newly emerging shoots to freezing temperatures can reduce *M. × giganteus* biomass yields (Farrell *et al.*, 2006). Early season precipitation in KY was much higher than normal while conditions in August and September were very dry, and temperatures were above normal during the entire growing season (Table 4). Generally, high summer temperatures should not have a negative impact on this C_4 crop, but when combined with limited precipitation, it appears to have caused the *M. × giganteus* plants to go dormant earlier than normal and not flower in 2010 (Table 5), thus limiting their biomass production.

Lower yields at NJ in 2010 could be attributed to several environmental conditions and management activities. First, weather conditions were both warmer and drier than normal in 2010 (Tables 3 and 4). Growing-season precipitation and soil moisture availability have been shown to be extremely important for *M. × giganteus* to achieve its biomass yield potential (Heaton *et al.*, 2004; Richter *et al.*, 2008), whereas Clifton-Brown *et al.* (2002) reported that low precipitation or prolonged periods of drought are not suitable for growing *M. × giganteus*. In addition, the shallow and sandy soils at that site may have also contributed to the low yields as the rocky layer may have impeded *M. × giganteus* root development and restricted the plant's ability to obtain water at deep soil depths. In a mature stand of *M. × giganteus* in Germany, roots grew down to 250 cm, with almost half growing deeper than 90 cm (Neukirchen *et al.*, 1999). Finally, the harvest of 2009 biomass was delayed until early April 2010, at the time shoots were emerging which may have damaged some new shoots and affected the 2010 biomass yields.

In NJ in 2010, not all of the plots reached full maturity with plants in only half of the plots flowering (Table 5). The plants that flowered were in the 0 or 60 kg N ha^{-1} plots, and there was no flowering in the 120 kg N ha^{-1} plots. Nitrogen-fertilized plots of *M. × giganteus* remained vegetative longer than unfertilized plots (Himken *et al.*, 1997); a difference, however, in the yields of these plots was not observed

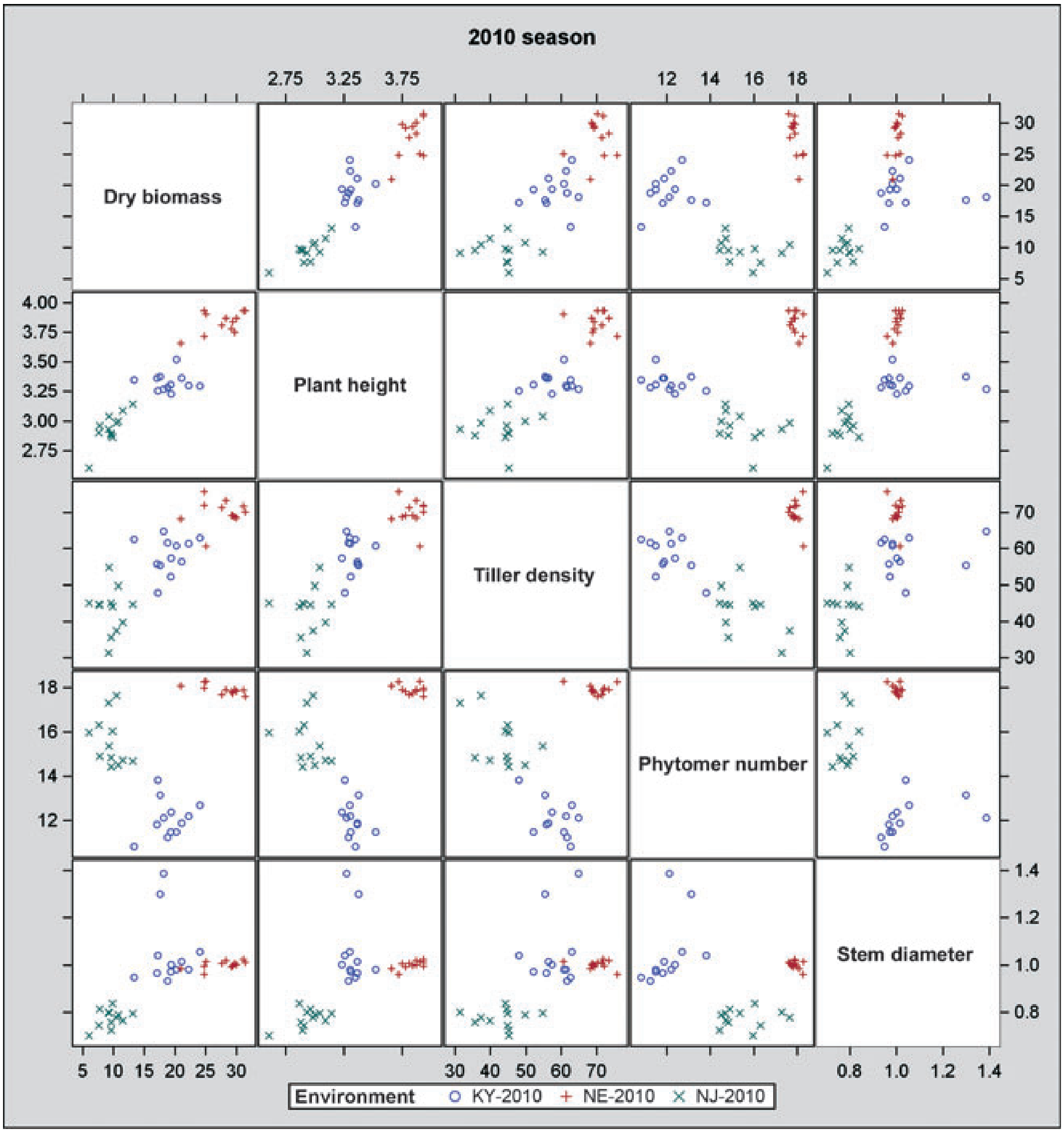

Fig. 4 Matrix scatter plots showing relationships between dry biomass yield (Mg ha^{-1}) end of season plant height (m), tiller density (number tillers m^{-2}), average number of phytomers tiller^{-1}, and average tiller diameter (cm) grouped by different 2010 environments: KY-2010, NE-2010, and NJ-2010.

once leaf senescence was complete and the plots harvested in winter.

In 2010, NE growing conditions were favorable for crop growth, and this is reflected in the high biomass yields which were highly correlated with end of season plant height (Table 7, Fig.4). Similar results relating biomass to plant height have been reported in production in central Italy (Angelini *et al.*, 2009). Between April and October 2010 in NE, there was approximately 43% (246 mm) more precipitation than normal and growing-season precipitation was highly correlated with biomass yield. Additionally, there were 25 days during the growing season at NE when air temperatures were 32.2 °C or higher. Optimal growing temperatures for *M.* × *giganteus* are between 30 and 35°C (Naidu *et al.*, 2003). The combination of warm temperatures and adequate precipitation spread throughout the growing season created ideal growing conditions.

Even though there was no effect of applied N on any of the measured variables in any of the environments, it does not suggest that *M.* × *giganteus* will not require N fertilizer in the future. Cadoux *et al.* (2011) recommended that fertilizer not be applied during the first 2 years after *M.* × *giganteus* is planted, unless it is planted in poor soils. This recommendation is supported by a recent meta-analysis reporting that there was little response to N fertilizer during *M.* × *giganteus* establishment years (years 1–3), but once the crop reached maturity, a response to N fertilizer was detected (Miguez *et al*., 2008). At this reporting, our study is still in its 'yield building' or establishment years (Clifton-Brown *et al*., 2007), however, N might be necessary as stands mature and, with added yields, more N is harvested from the system.

This study increases our understanding of how different environments impact *M.* × *giganteus* growth, development, and biomass yield. Not surprisingly, increases in biomass yield rely on growing conditions in which water is adequately available, especially when temperatures are high and/or soils where soils hold limited moisture. Also of importance, nitrogen fertilization had no significant effects on *M.* × *giganteus* biomass yield in season two or three at any site. Over time, this experiment will continue to shed important light on the capacity of *M.* × *giganteus* to provide stable and reliable biomass yields at these locations.

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