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Keywords

axillary bud, bioenergy crop, biomass, establishment, meristem, planting, rhizome, vegetative propagation

Disciplines

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Comments

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Abstract

The sterile triploid *Miscanthus × giganteus* is capable of yielding more biomass per unit land area than most other temperate crops. Although the yield potential of *M. × giganteus* is high, sterility requires all propagation of the plant to be done vegetatively. The traditional rhizome propagation system achieves relatively low multiplication rates, i.e. the number of new plants generated from a single-parent plant, and requires tillage that leaves soil vulnerable to CO₂ and erosion losses. A stem-based propagation system is used in related crops like sugarcane, and may prove a viable alternative, but the environmental conditions required for shoot initiation from stems of *M. × giganteus* are unknown. A study was conducted to investigate the effect of temperature, illumination and node position on emergence of *M. × giganteus* shoots. Stems of *M. × giganteus* were cut into segments with a single node each, placed in controlled environments under varied soil temperature or light regimes and the number of emerged shoots were evaluated daily for 21 days. At temperatures of 20 and 25 °C, rhizomes produced significantly more shoots than did stem segments ($P = 0.0105$ and 0.0594 , respectively), but the difference was not significant at 30 °C, where 63% of stems produced shoots compared to 80% of rhizomes ($P = 0.2037$). There was a strong positive effect ($P = 0.0086$) of soil temperature on emergence in the range of temperatures studied here (15–30 °C). Node positions higher on the stem were less likely to emerge ($P < 0.0001$) with a significant interaction between illumination and node position. Planting the lowest five nodes from stems of *M. × giganteus* in 30 °C soil in the light resulted in 75% emergence, which represents a potential multiplication rate 10–12 times greater than that of the current rhizome-based system.

Keywords: axillary bud, bioenergy crop, biomass, establishment, meristem, planting, rhizome, vegetative propagation

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Introduction

Growing global awareness of the negative implications of fossil fuel use has led to an increased interest in alternative energy. Biofuels from perennial dedicated energy crops are increasingly expected to replace petroleum, and emerging legislation in the United States will require their use [Perlack & Stokes (leads), 2011]. Among dedicated energy crops there are particularly high hopes for the large, warm-season grass *Miscanthus × giganteus* Greef et Deu. ex Hodkinson et Renvoize (Hodkinson & Renvoize, 2001). *Miscanthus × giganteus* is expected to make major contributions to biomass supply (Heaton *et al.*, 2008; Hastings *et al.*, 2009; Bauen *et al.*, 2010; Li *et al.*, 2010; Sang & Zhu, 2011), biofuel production (Hayes & Hayes, 2009; Lemus & Parrish, 2009; Reijnders, 2010; Solomon, 2010), soil carbon sequestration (Anderson-Teixeira *et al.*, 2009; Lal, 2009; Blanco-Canqui, 2010), the economy (Scheffran & Bendor, 2009; Bocqueho & Jacquet, 2010; Jain *et al.*, 2010; James *et al.*, 2010) and

climate change mitigation (Georgescu *et al.*, 2009, 2011; Hillier *et al.*, 2009; Rowe *et al.*, 2009; Smeets *et al.*, 2009).

Although *M. × giganteus* has not been improved through breeding, this naturally occurring hybrid exhibits many ideal traits needed in a dedicated energy crop (Lewandowski *et al.*, 2000; Jones & Walsh, 2001; Heaton *et al.*, 2004). Key among these traits is biomass yield; *M. × giganteus* yields substantially more biomass per unit area than switchgrass (*Panicum virgatum* L.) (Heaton *et al.*, 2008) and corn (*Zea mays* L.) (Dohleman & Long, 2009) in the US Corn Belt making it more economically viable than other perennial grasses (Khanna *et al.*, 2008).

In addition to being highly productive, *M. × giganteus* is a naturally sterile allotriploid ($2n = 3x = 57$) (Greef & Deuter, 1993; Hodkinson *et al.*, 2002; Nishiwaki *et al.*, 2011). Sterility is an important trait for a dedicated energy crop to minimize its potential to become invasive or weedy (Raghu *et al.*, 2006; Gutterson & Zhang, 2009). Although sterility does not guarantee a lack of invasiveness, it strongly inhibits it, and *M. × giganteus* has been shown to pose less of a risk of becoming invasive

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outside of its native range than fertile *Miscanthus sinensis* (Andersson) varieties, switchgrass or giant reed (*Arundo donax* L.) (Barney & Ditomaso, 2008; Quinn *et al.*, 2010; Gordon *et al.*, 2011).

While sterility limits the invasive potential of *M. × giganteus*, it also makes propagation and establishment more challenging (Clifton-Brown & Lewandowski, 2000; Lewandowski *et al.*, 2000; Hocking *et al.*, 2008; Pyter *et al.*, 2010), resulting in high planting costs that reduce profitability and limit adoption by farmers (Khanna *et al.*, 2008; James *et al.*, 2010). Currently the majority of *M. × giganteus* propagation and establishment is achieved using a rhizome-based system (Atkinson, 2009). Hand harvests of *M. × giganteus* rhizomes have been shown to yield relatively low numbers of rhizomes for propagation, with first year stands showing a multiplication rate of 7–10 and second year stands yielding 25–30 rhizomes per parent plant (Pyter *et al.*, 2009). In addition to a relatively low return, harvesting rhizomes requires digging up the parent stand, which reverts it back to a 'year one' stand and leaves exposed soil vulnerable to CO₂ losses and erosion.

An alternative to rhizome propagation is stem propagation. Other cane grasses such as bamboos (Ramana-yake & Yakandawala, 1997; Shirin & Rana, 2007), teosinte (*Zea diploperennis* Iltis, Doebley & Guzman) (Zale *et al.*, 2008) and giant reed (Wijte *et al.*, 2005) have been shown to propagate from aerial stem segments. Sugarcane (*Saccharum officinarum* L.), a closely related species to *M. × giganteus*, is propagated entirely by stems on a commercial scale (James, 2004). Could a stem propagation system be appropriate for *M. × giganteus*?

A stem propagation system could minimize disturbance to soil and parent stands and offer higher multiplication rates than achieved through rhizome propagation. For example, we have observed that a typical *M. × giganteus* plant in Iowa, USA has 20 stems with 10 nodes (with axillary buds) per stem by the end of its first growing season. A multiplication rate of 200 would be possible if each of these nodes were able to generate a new plant, 20 times greater than published rhizome multiplication rates (Atkinson, 2009; Pyter *et al.*, 2009). Multiplication rate will be essential in determining how long it will take to establish enough *M. × giganteus* to meet the legislated biofuel goals. At recommended planting rates of 12 000–16 000 plants ha⁻¹ (Atkinson, 2009), 192 billion plants will be required to offset just 20% of current US gasoline use (Heaton *et al.*, 2008).

While no primary, peer-reviewed research articles have investigated *M. × giganteus* stem propagation methods, Atkinson (2009) called for research and stressed its potential importance to *M. × giganteus* production in the United Kingdom. In a published meeting

abstract, Hong & Meyer (2007) suggested that stem propagation may be possible, but the success of the system is variable. They found that node position on the stem had an effect on rooting success but only evaluated four of the ca. 16 nodes that can be found on a mature *M. × giganteus* stem. Although Hong & Meyer (2007) did not present values for the number of new plants generated in their experiment, they did raise the possibility that stem propagation of *M. × giganteus* is feasible and should be investigated.

What environmental factors favor successful production of *M. × giganteus* from stem nodes? In general, temperature is a strong regulator of plant growth and development in perennial, rhizomatous grasses. For example, McIntyre (1967) found reducing the growth temperature of quackgrass (*Agropyron repens* L. Beauv) from 27 to 10 °C strongly influenced both the number of axillary buds that developed as well as the type of organ, aerial stem or rhizome, that they produced. In giant reed, a grass similar to *M. × giganteus* in that it spreads predominately by rhizomes, but can also reproduce from stem segments (Bell, 1997); Wijte *et al.* (2005) found that 100% of stem node segments could successfully produce roots at relatively cool temperatures (10–22.5 °C). Although naturalized to many warm regions of the world, giant reed uses the C₃ photosynthetic pathway and these cool temperatures should support its growth. By contrast, *M. × giganteus* is productive at cool temperatures but uses the C₄ photosynthetic pathway (Beale *et al.*, 1996; Naidu & Long, 2004) and could conceivably require warmer temperatures for shoot initiation from stem axillary buds. Smit (2011) found emergence from sugarcane stem pieces to increase linearly with temperature, with best emergence at 30 °C and no emergence below 18 °C.

Light is another environmental factor that may strongly control shoot initiation, but is yet to be investigated in *M. × giganteus*. The timing, quantity and quality of light can all influence both the germination of seeds and the development of new shoots in grasses (Lambers *et al.*, 1998; Barnes, 2007). McIntyre (1967) found that reducing the time quackgrass buds was exposed to light from 18 to 9 h strongly promoted shoot growth at the expense of rhizome development, although the intensity of light had no effect. In sugarcane, increasing the amount of light that reached the base of existing stalks increased production of new shoots (Bonnett *et al.*, 2005). Bonnett *et al.* (2005) also observed that edge plants and plants adjacent to lodged plants showed increased tillering.

Given that stem propagation of *M. × giganteus* is possible and could improve crop production and the economics of this crop, the present study was conducted to answer three questions: How does (i) temperature,

(ii) illumination, and (iii) node position influence emergence of *M. × giganteus* shoots from stem nodes?

Materials and methods

Plant material

The Illinois clone of *M. × giganteus* (Caveny Farm, Monticello, IL, USA) was used in both experiments described below. Plants were greenhouse-grown in Ames, IA (42°03' N, 93°64' W) in 15 cm diameter pots with 1.85 L of potting media (Sunshine® LC1 mix; Sun Gro® Horticulture, Vancouver, BC, Canada) under natural ambient sunlight which varied daily and seasonally, and 8 h of supplemental light (500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plants were grown under a 35/25 °C day/night temperature regime. Although the room was well-ventilated, the plants were not kept under windy conditions. Plants were watered every other day as needed and fertilized weekly with Peters® Excel 15-5-15 NPK (Scott's – Sierra Horticultural Products Company, Marysville, OH, USA).

Experiment 1: Temperature and propagule effects

The effect of soil temperature (15, 20, 25, or 30 °C) on shoot emergence from *M. × giganteus* stem nodes was examined in two controlled environment chambers (Conviron® CMP3244, Conviron®, Winnipeg, MB, Canada). The experiment was conducted in a split-plot incomplete block design with two of the four possible temperatures considered in each incomplete block. Each soil temperature (15, 20, 25, 30 °C) was replicated three times, and each temperature was paired with every other temperature, i.e. all possible combinations of two temperatures were considered (Table 1).

A single stem (Fig. 1a) was randomly selected from each of 10 pots of *M. × giganteus*. Stems used in this experiment came from plants with 5–17 nodes per stem and 3–22 stems per plant. Leaves were removed from the stem to reveal individual nodes (Fig. 1b) and the first five nodes were excised as single node segments with the node, and its associated axillary bud, centered on the segment (Fig. 1c). The node seg-

ment closest to the soil surface was considered 'node one' and numbering progressed upward toward the shoot apex. A single rhizome was also harvested from each plant for a total of 10 stems \times (5 nodes + 1 rhizome) = 60 propagules in each block. Propagules were planted horizontally just below the soil surface in ca. 918 cm³ pots filled with potting media (Sunshine® LC1 mix; Sun Gro® Horticulture). Stem segments were completely covered by potting media, such that no green tissue was visible. Pots were directly placed in a controlled environment chamber with 16 h/8 h light/dark periods and an average photosynthetic photon flux density of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Given there was a range of parent plant size and stage, randomization was done at an individual propagule level ensuring that nodes 1–5 and the rhizome from an individual parent plant were distributed randomly among the treatments. Emergence of new shoots was assessed daily for 21 days.

Experiment 2: Illumination and node position effects

The effect of the presence or absence of illumination on emergence of *M. × giganteus* stem nodes was examined in a single controlled environment chamber (Conviron® CMP3246; Conviron®). The experiment was conducted in a split-plot incomplete block design with each illumination treatment replicated three times. Each block considered a single illumination treatment, light or dark, and the order of blocks was completely randomized. The dark treatment had a light/dark period of 0 h/24 h and the light treatment had a light/dark period of 16 h/8 h with an average photosynthetic photon flux density of 507 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The controlled environment temperature was set to hold a constant soil temperature of 30 °C that was confirmed with a thermometer on the soil surface.

A single stem (Fig. 1a) was randomly selected from a population of stems with 10–16 nodes in each of 10 pots of *M. × giganteus*. Leaves were removed from the stem to reveal individual nodes (Fig. 1b), and the basal 10 nodes were excised as single node segments with the node, and its associated axillary bud, centered on the segment (Fig. 1c).

Propagules were planted horizontally just below the surface in ca. 918 cm³ pots filled with potting media (Sunshine® LC1

Table 1 Experimental layout for temperature experiment

	Run											
	1		2		3		4		5		6	
Temperature (°C)	30	25	20	15	15	25	25	20	20	30	30	15
Propagules	1	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	2	2	2	2	2	2	2	2	2
	3	3	3	3	3	3	3	3	3	3	3	3
	4	4	4	4	4	4	4	4	4	4	4	4
	5	5	5	5	5	5	5	5	5	5	5	5
	R	R	R	R	R	R	R	R	R	R	R	R

Each propagule was replicated five times and completely randomized within each temperature treatment. Propagule number refers to node position, and 'R' refers to a rhizome.

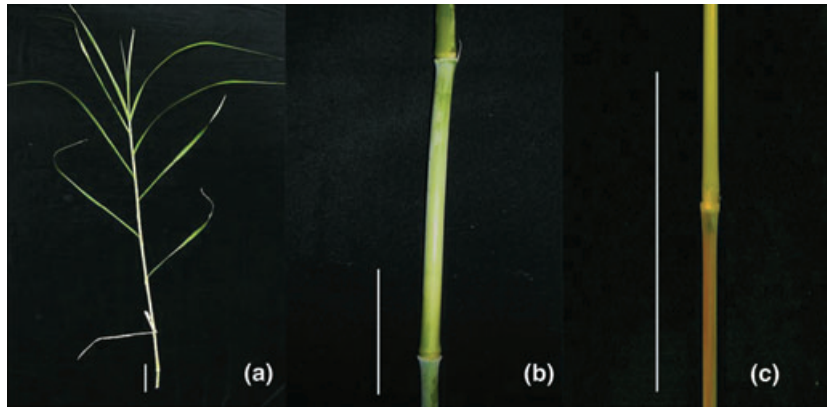


Fig. 1 (a) *Miscanthus × giganteus* stem with attached leaves. (b) Leaves were removed to expose nodes and associated axillary buds to be used in experiments. (c) Stem segments were cut to single node segments for use in temperature and illumination experiments. White bar indicates 8 cm in each panel.

mix; Sun Gro® Horticulture). Stem segments were completely covered by potting media, such that no green tissue was visible. Pots were then randomly placed in a controlled environment chamber set with appropriate illumination conditions. Emergence was evaluated daily for 21 days.

Data analyses

Data were analyzed using PROC GLIMMIX adjusted for binary data (SAS software version 9.2; SAS Institute Inc., Cary, NC, USA). Fisher's least significant difference test was used for pairwise comparisons and one-way or two-way ANOVA was used to determine the differences between main effects. Contrast statements were used to compare group means where appropriate. There was no effect of block in either experiment so the experiment was analyzed as a split-plot design. Temperature and illumination were tested using the whole plot error and propagule and node position were tested using the subplot error in each experiment. In each experiment, the date of initiation was included in the model as a continuous covariate to determine the effect of time over the course of the experiment.

Results

Temperature and propagule effects

This experiment investigated the effect of soil temperature on the emergence of *M. × giganteus* shoots from different vegetative propagules, i.e. stem node segments vs. rhizome segments. Only the first five nodes from the base of the plant were considered. Averaged over the range of temperatures tested, the number of *M. × giganteus* shoots that emerged differed significantly depending on the propagule type planted, i.e., stem segments or rhizome ($P = 0.0230$, Table 2).

Specific contrast statements revealed that among stem segments, there was no significant effect of node posi-

Table 2 Overall ANOVA table for temperature and propagule effects

Source	df	F	P-value
Propagule	5	2.65	0.0230
Temperature	3	9.61	0.0086
Propagule × Temperature	15	0.78	0.6961
Date	1	1.07	0.3418

df, degrees of freedom.

tion on emergence ($P = 0.2499$). Averaged over temperatures, 51.7% rhizomes emerged, which was significantly higher than the 30% emergence rate observed from stem segments ($P = 0.0015$).

Temperature had a strong effect on emergence rates of both stem segments and rhizomes ($P = 0.0086$, Table 2). The low overall average of stem emergence was driven largely by the relatively low emergence at 15 °C. While only 4% of stem nodes produced shoots at 15 °C, 63% emerged at 30 °C, which did not differ significantly from the 80% emergence for rhizomes at the same temperature ($P = 0.2037$, Fig. 2). Also, rhizome and stem emergence did not differ significantly at 15 °C ($P = 0.1738$); therefore, the overall difference between rhizome and stem node emergence observed in the main effect was driven by the difference in emergence at 20 and 25 °C ($P = 0.0105$ and 0.0594, respectively, Fig. 2).

Illumination and node position effects

Illuminated stem segments exhibited a 58% emergence rate. Although this was higher than the 48% emergence rate from nonilluminated stems, the main effect of illumination was not significant ($P = 0.6809$, Table 3).

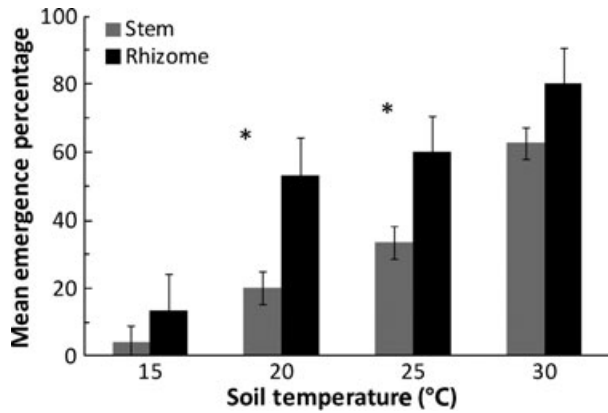


Fig. 2 Mean emergence of shoots from propagules of *Miscanthus × giganteus*. Stem segments and rhizomes were planted horizontally and placed in controlled environment chambers set to maintain soil temperatures as indicated. Emergence was assessed daily for 21 days. Stem emergence shown was averaged over node positions 1–5, and means were calculated from 75 stem segments for each temperature. Rhizome emergence was calculated from 15 rhizomes for each temperature. Error bars indicate standard error of the mean, and asterisk indicates significant difference within a temperature treatment ($P < 0.06$).

Table 3 Overall ANOVA table for light and node position effects

Source	df	F	P-value
Light	1	0.19	0.6809
Node	9	13.07	<0.0001
Light × Node	9	2.41	0.0108
Date	1	1.07	0.1233

df, degrees of freedom.

Consistent with the temperature analysis of Experiment 1, the effect of node position was not significant when considering only the first five nodes ($P = 0.1712$). However, expanding our scope to consider 10 nodes indicated a significant effect of node position on emergence success ($P < 0.0001$, Table 3, Fig. 3). Node positions above ‘node four’ showed a negative relationship between increasing node position and emergence, i.e., nodes higher on the stem exhibited a lower emergence rate (Fig. 3). The majority of new shoots came from the lower five nodes. Contrast statements showed that the difference in emergence success of nodes 1–5 (75%) and 6–10 (26%) was highly significant ($P < 0.0001$).

A significant interaction of illumination treatment and node position was observed in this experiment ($P = 0.0108$, Table 2, Fig. 4). Illuminated conditions resulted in more shoots emerging from lower nodes than under nonilluminated conditions, whereas upper

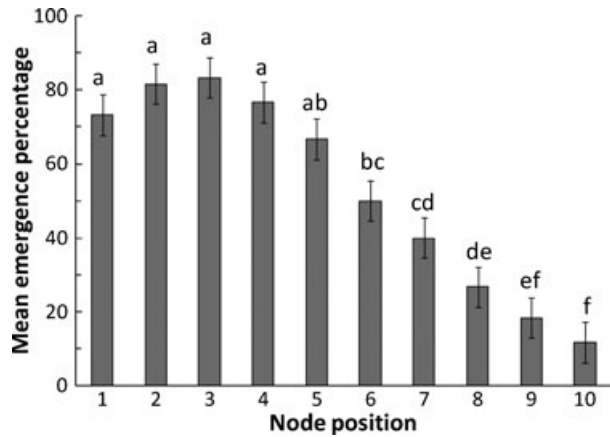


Fig. 3 Node position effect on shoot emergence from stem segments of *Miscanthus × giganteus*. Stem segments were planted and grown in illuminated or nonilluminated controlled environments with a soil temperature of 30 °C. Node position numbering begins at the lowest node and increases toward the shoot apex. Emergence was observed daily for 21 days. Means were calculated from 60 stem segments, and error bars indicate standard error of the mean. Means with the same letter are not statistically different at $P = 0.05$.

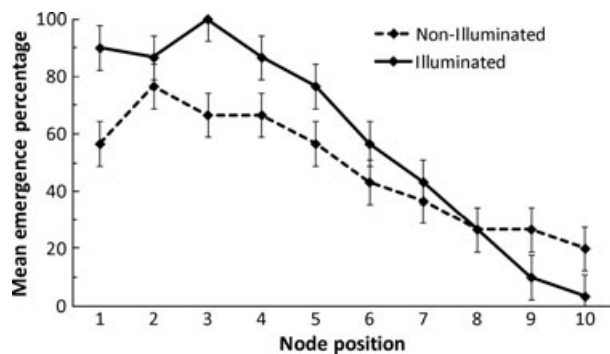


Fig. 4 Mean emergence of illuminated or nonilluminated stem segments over node positions. Each illumination treatment was replicated three times. Stem segments were grown in controlled environments set to maintain a soil temperature of 30 °C. Emergence was assessed daily for 21 days. Points are the mean of 30 stem segments at each node position, and error bars indicate standard errors of the means.

node emergence was consistently lower for both illumination conditions.

Discussion

The main finding of this study is that *M. × giganteus* can be propagated from stem nodes under controlled conditions, especially those from the bottom portion of the stem. Three specific questions were addressed in this study and each is now considered.

*How does soil temperature influence emergence of *M. × giganteus* shoots from stem segments?*

Here, we showed that *M. × giganteus* stem segments exhibited higher shoot emergence with warmer soil temperatures. Although investigating shoot initiation, not root initiation, our study is generally consistent with the finding that giant reed increased rooting percentage at higher temperatures (Wijte *et al.*, 2005). However, giant reed was shown to exhibit 100% rooting at the relatively cool temperature of 17.5 °C after 40 days (Wijte *et al.*, 2005), whereas we found a mean of 77% of *M. × giganteus* stem nodes tested had emerged after 21 days at the considerably warmer temperature of 30 °C. This rate is higher than has been reported for teosinte under a similar growing temperature (26 °C). Zale *et al.* (2008) reported 54.5% and 37.5% of planted stem nodes produced plants for *Zea diploperennis* and *Zea perennis* (Hitc.) Reeves and Magelsdorf, respectively (Zale *et al.*, 2008).

One of the main advantages of *M. × giganteus* is high productivity at cool temperatures (Beale & Long, 1995). Rhizomes have indirectly been considered a key to this success, as they are generally able to initiate growth and supply an actively growing shoot at temperatures that would slow a maize seedling (Clifton-Brown & Jones, 1997; Naidu & Long, 2004). For example, it was found that rhizomes from *M. × giganteus* could initiate new shoot emergence at temperatures as low as 9 °C (Farrell *et al.*, 2006), when allowed 60 days for shoot emergence. Interestingly, here we found very little difference in growth of rhizomes and stem node pieces at cool temperatures (15 °C), where growth from either propagule was low (Fig. 1), or at warm temperatures (30 °C) where growth from both propagules was high, at 63% for stem nodes and 80% from rhizomes after 21 days. The significant differences between propagule types were seen at the moderate temperatures of 20 and 25 °C, temperatures typical of warming soils during early summer when *M. × giganteus* would be planted. One implication of this result is that a stem propagation system may require an intermediary greenhouse step to establish plants when soil temperatures are too cool in the early spring, which would need to be considered if this system were implemented on a commercial scale.

*How does illumination influence emergence of *M. × giganteus* shoots from stem segments?*

Grasses can respond to light both through tillering and seed germination (Lambers *et al.*, 1998; Barnes, 2007). Here, we showed that light impacts vegetative propagation from basal axillary buds of *M. × giganteus* stems which account for the majority of total emergence from

stem segments. This result is consistent with the finding that increased light reaching the base of stalks increases axillary bud outgrowth through tillering in sugarcane (Bonnett *et al.*, 2005).

*How does node position influence emergence of *M. × giganteus* shoots from stem segments?*

Hong & Meyer (2007) reported that in *M. × giganteus* the most basal node exhibited the greatest rooting success and rooting decreased for nodes 2–4; we found no significant difference in shoot emergence from nodes 1 to 5. However, when 10 nodes were considered we found a negative relationship between node position and emergence for nodes beyond 'node four'. Our working hypothesis for this result is the well-known inhibition of subsidiary axillary buds by the shoot apical meristem via auxin controls (Thimann & Skoog, 1933). Because the lowest five nodes on the stem behave very similarly to each other when responding to the light and temperature changes tested in this experiment, it could be that the lower nodes had reached some critical maturity stage, or distance from the shoot apical meristem, as indicated by the linear decrease in shoot emergence with increasing proximity to the shoot apical meristem.

Multiplication rate

To offset just 20% of US gasoline demand using *M. × giganteus*, will require an estimated 12 million ha of land (Heaton *et al.*, 2008). At recommended planting rates of 12 000–16 000 plants ha⁻¹ (Atkinson, 2009), this will require 192 billion plants. Quickly scaling up the limited *M. × giganteus* available in the United States to this level necessitates a higher annual multiplication rate than 10–30 that can be achieved with a traditional rhizome system. Here, we showed that under optimal conditions, a 75% emergence rate from stem nodes was possible. While field performance of plants generated under this system is still being evaluated, potential rates of multiplication are high: given that a typical first-year stand of *M. × giganteus* has 20 stems per plant, if five nodes per stem were used, a multiplication rate of 75 is achievable in the first season ($0.75 \times 20 \times 5$).

Although it is unknown whether stems may be planted directly into the field, our temperature experiment seems to indicate that in temperate climates an intermediary greenhouse step would be required to generate plugs that could then be transplanted to the field when soils warm in the spring. We anticipate these plugs would perform similarly to rhizome grown plants, but more investigation would be required to characterize the field performance of stem propagated plugs.

A stem propagation system has great potential to increase the multiplication rate of *M. × giganteus*, while avoiding soil disruption and impairment to the parent stand given proper management to account for nutrient removal from harvesting green stems. Using the first five nodes of each stem and growing them in warm soil under illuminated conditions may result in 10–12 times greater multiplication than the conventional rhizome-based system.

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