Volunteer Establishment of *Miscanthus × giganteus* Vegetative Propagules: Implications for Biofuel Production

THESIS

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By

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Abstract

Criticisms regarding the efficiency of first generation biofuel feedstock have prompted the development of sustainable alternatives in the form of dedicated perennial biomass crops. Perennial species are appealing due to high productivity and environmental plasticity, traits that are also consistent with invasive flora. While they present a viable alternative to maize-based ethanol, widespread cultivation of perennial biomass crops may provide opportunities for dissemination into novel environments. Of the top candidates being considered for production, *Miscanthus × giganteus* is believed to pose little risk, owing primarily to its putative sterility. Vegetative reproduction however, does not necessarily limit widespread invasion, as seen in giant reed (*Arundo donax*). In fact, little is known about the invasive potential of *M. × giganteus* even though production has already begun in the Midwest. In order to determine the establishment potential of *M. × giganteus*, vegetative propagules (plug plantlets, rhizomes, and stem cuttings) were placed under 5 cm of soil (control), underneath mulch, or on top of the soil surface (directly exposed to desiccation). Separate experiments were performed to assess the effect of competition and water addition on these treatments. The relative contribution of these factors on survival (the presence of above ground growth), shoot height, and shoot number were evaluated. Under field conditions, *M. × giganteus* plug plants and rhizomes generated shoots and continued to do so through a second year of growth; however, there was significant variation in overall performance, with plug plants being somewhat more successful (greater survival and shoot height) than rhizomes. Stem cuttings initially generated shoots across all planting
treatments, but by mid-August all had died. *M. × giganteus* is more likely to establish from vegetative propagules when buried under 5 cm of soil or underneath mulch than when on the soil surface. Survival of surface planted propagules was low, but shoot height increased when neighboring plant density was high. Although water addition promoted shoot height early on in the growing season, it had no effect on final survival or shoot height. There is evidence, however, to suggest that water availability increased shoot production. *M. × giganteus* established under environmental conditions propagules may encounter upon escape. This is significant considering central Ohio was experiencing abnormally dry conditions throughout the duration of this experiment. This study is among the first to demonstrate the establishment potential of *Miscanthus × giganteus* vegetative fragments.
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Introduction

Recent trends in international oil prices, energy security, and rising greenhouse gas emissions have led to increased interest in the development of renewable fuel sources. In an attempt to combat growing energy problems, Congress approved the Energy Policy Act of 2005, establishing the Renewable Fuel Standard (RFS). Designed to offset the annual production of petroleum-based fuel, the RFS became the first federal mandate requiring a minimum volume of renewable fuel blended into transportation fuels (Malmedal 2007). The Energy Independence and Security Act (EISA) of 2007 followed, reinforcing the commitment to renewable fuels by expanding and extending annual volume standards first set by the RFS (Sissine 2007). Since the inception of the RFS, first generation biofuel, produced from sugars and vegetable oils derived from food crops (specifically maize-based ethanol), has remained the dominant bioenergy product (USEIA, 2012).

Although these petroleum-based blends are supported through existing infrastructure, with nearly 50.3 billion liters (10% of gasoline consumed) blended into gasoline in the U.S. alone (USEIA, 2012), there are several limitations inherent in the production process (Tillman et al. 2009; Fernando et al. 2010; Georgescu et al. 2011). First, a threshold exists above which production would threaten the nation’s food supply (Solomon et al. 2007, Mitchell 2008). Second, first generation feedstock require significant inputs in the form of pesticides, fertilization, irrigation, and soil cultivation (Solomon et al. 2007). Third, production may alter the ‘ecosystem carbon balance’ (Qin et al. 2012). This can occur either indirectly or directly.
For example, diverting food crops to biofuel production may indirectly influence land use changes (and exacerbate associated environmental impacts), as natural ecosystems are converted to farmland to meet existing (and growing) demand. This creates a ‘biofuel carbon debt’, in which the carbon (stored in soil and plant biomass) emitted from land conversion is not offset by replacing fossil fuels with maize-based fuel (Fargione et al. 2008). First generation biofuels also provide little direct aid in balancing the global carbon budget (Lobell & Field 2007; Fargione et al. 2008; Searchinger et al. 2008; Naik et al., 2010). This is due in large part to the nutrient cycling inefficiencies and inorganic fertilizer requirements of first generation feedstock (Crutzen et al. 2008). Davis et al. (2009) demonstrated that corn agro-ecosystems emit from 956 to 1899 g more CO$_{2eq}$ m$^{-2}$ y$^{-1}$ (including CO$_2$, N$_2$O, CH$_4$), when compared to alternatives with less N demand. Considering that N$_2$O has a 100-yr average global warming potential that is 296 times greater than an equal mass of CO$_2$, the addition of significant quantities of N-fertilizer may do more to enhance warming rather than offset its effect (Crutzen et al., 2008). As a result the industry has begun to move beyond maize-based ethanol and shift its focus towards the development of sustainable alternatives that maximize energy output while reducing production costs (Glaser & Glick 2012).

Second-generation biofuel is produced from sustainable, non-food source biomass. Dedicated cellulosic energy crops, specifically perennial grasses (*Arundo donax* L., *Panicum virgatum* L., *Phalaris arundinacea* L., *Phragmites australis* (Cav.) Trin. Ex Steud., and two species in the genus *Miscanthus*: *M. sinensis* Anderss. and *M. × giganteus* Greef et Deu ex Hodkinson et Renvoize), have emerged as some of the most promising sources for second generation biofuels (Glaser & Glick 2012). The attraction lies in their ability to reduce potential
conflicts with food supplies, limit agricultural inputs and production costs, and reduce indirect and direct carbon debts associated with production (Naik et al. 2010). Perennial species can easily accomplish this due to several key aspects of their biology and life history. Many perennial species are typified by high nitrogen use efficiency, C₄ photosynthetic pathway, tolerance to drought, salinity, and low-fertility soils, efficient use of water, rapid growth rate, low pest and disease pressure, longevity, and enhanced competitive ability (Barney & DiTomaso 2008; Gelfand et al. 2012). These traits also facilitate growth on marginal (low productivity) soils, thereby limiting the need for conversion of highly productive natural systems (i.e. rainforests and grasslands) to cropland (Campbell et al. 2008; Fargione et al. 2008; Gelfand et al. 2012). To this end, second generation biofuel offers distinct advantages over first generation feedstock. In fact, recent revisions to the RFS (RFS2) reflect the desire to develop alternatives to maize-based ethanol and require increases specifically in the cellulosic biofuel category from 250 million to 1 billion gallons by 2013, with additional annual increases set to 16 billion gallons by 2022 (USEISA, 2012).

Although several perennial species represent an ‘ideal’ dedicated biomass crop (Heaton et al. 2004), they possess traits that are common to many invasive species (Barney & DiTomaso 2008). In fact, many of these species comprise a distinct functional group of invasive plants, collectively referred to as large-statured invasive grasses, whose life history traits facilitate effective colonization and rapid growth in novel habitats (especially those who have undergone anthropogenic modification) (Lambert et al. 2010). Several candidate crops are non-native either within the proposed range of cultivation or throughout the United States, and a few have a history of invasion and naturalization (i.e. Arundo donax, Phalaris arundinacea, Phragmites
australis, and Miscanthus sinensis) (Barney & DiTomaso 2008). Considering that many of these plants are currently in early stages of development, breeding programs will likely focus on maximizing productivity by enhancing traits that lead to greater biomass. Therefore, although they may present a highly efficient alternative, the cultivation of dedicated perennial crops may pose a significant threat to natural communities (if escape to surrounding areas is possible). In response, several research groups have attempted to assess the potential risks associated with specific candidate species by utilizing variants of Weed Risk Assessment (WRA), originally developed by Dr. Paul Pheloung at the Western Australian Department of Agriculture (Barney & DiTomaso 2008, Gordon et al., 2011). WRA is a semi-qualitative prescreening tool designed to assess weediness by scoring plants on a series of 49 questions related to biology, life history, climatic requirements, and association with other invasive flora (Barney & DiTomaso 2008). Of the top candidates, Miscanthus × giganteus yielded an “accept” in analyses conducted for both Florida (Gordon et al. 2011) and California (Barney & DiTomaso 2008), and is believed to pose little risk of becoming invasive (Barney & DiTomaso 2008).

Miscanthus × giganteus is a naturally occurring sterile allotriploid (3n = 57) hybrid cross between diploid Miscanthus sinensis Anderss. (2n = 38) and tetraploid Miscanthus sacchariflorus Maxim. (4n = 76) (Adati and Shiotani 1962; Greef and Deuter 1993; Linde-Laursen 1993; Hodkinson et al. 2002; Swaminathan et al. 2010). The parent species are perennial C₄ rhizomatous grasses native to Japan, and while they are primarily found in different habitats, there are localized areas in which their ranges overlap (Watanabe and Maruyama, 1977). M. × giganteus was initially discovered in 1935 in Yokohama, Japan by Danish plant collector Aksel Olsen (Nielsen, 1990), and has since been introduced to Europe and North America (Greef and
Clones of *M. × giganteus* currently being propagated for commercial production, such as the well-known “Illinois clone”, are derived from this original plant (Rayburn *et al.* 2008; Swaminathan *et al.* 2010). The occurrence of several other natural triploid plants has also been documented (Honda 1939; Hirayoshi *et al.* 1957; Adati 1958; Nishiwaki, *et al.* 2010), as well as the discovery of novel triploid genotypes (Dwiyanti *et al.* 2012). However, to our knowledge none have been exploited for biofuel production.

*M. × giganteus* is an attractive candidate for biofuel production due to its ability to tolerate cold climates (Naidu *et al.* 2003; Farrell *et al.* 2006), high nitrogen use efficiency (Beal and Long 1997, Lewandowski & Smith 2006), high productivity at about 30 t ha⁻¹ to 60 t ha⁻¹ (Clifton-Brown *et al.*, 2001, Heaton *et al.*, 2008), and potential to fix carbon dioxide at an estimated 5.2 to 7.2 t C/ha/yr (Clifton-Brown *et al.*, 2007). Its perennial nature may reduce annual costs associated with planting. Lewandowski *et al.* (1995) estimated that commercial plantings could last between 15–20 years; in fact, several European stands have reported high productivity for well over 20 years. Comparisons among alternatives in Illinois demonstrate that *M. × giganteus* can produce more than twice the biomass on less than half the projected acreage requirements for both corn and switchgrass (Heaton *et al.* 2008). Therefore, *M. × giganteus* could potentially supply all of the cellulosic fuel required by EISA (2007) on the same amount of land currently devoted to maize-based ethanol (Heaton *et al.* 2008). Lack of seed set dramatically reduces the risk of widespread invasion and is one of the reasons the crop yielded an “accept” on the WRA (Heaton *et al.* 2004). In fact, *M. × giganteus* has been under development in Europe for the past 30 years with almost no reports of escape (Lewandowski et al). The only
documented case occurred in Germany, in which single plants established from garden compost (Jorgenson 2010, Brennenstuhl 2008).

Although *M. × giganteus* scored relatively high on the WRA, there are several criticisms regarding both the utility of such measures in predicting weediness in novel environments and the variability among repeated screenings (Cousens 2009, Hulme 2012). Despite being considered a useful first step, WRA should not replace field evaluations nor serve as the sole influence for policies regarding the regulation of biofuel production. In addition, past “good behavior” does not guarantee similar performance in the future, especially in novel habitats and under different management regimes. This is especially important in the case of *M. × giganteus*, considering that neither WRA nor ample field evaluations have been conducted throughout the proposed region of cultivation. Although *M. × giganteus* may have yielded an “accept” in both California and Florida, that may not be the case in the central part of the country where the USDA has already begun assisting the production of 200,000 acres within Ohio, Missouri, Arkansas, and Pennsylvania as part of the Biomass Crop Assistance Program (USDA-FSA, 2011). Considering both the amount of land required to meet proposed production needs (an estimated 22 to 61 million hectares), as well as the infrastructure required to support such a system, opportunities for escape during transportation and cultivation are appreciable (Raghu et al. 2006). In fact, cultivation alone has been shown to facilitate widespread invasion of other horticultural species (Mack 2000). Therefore, it is important to determine whether this region might be susceptible to invasion.

Little is known about the performance of *Miscanthus × giganteus* outside of cultivation; however, both parent species (*M. sinensis* and *M. sacchariflorus*) have histories of escape and
naturalization throughout the United States (Quinn et al. 2010; USDA NRCS 2010). Although extremely rare, fertile seeds of $M \times giganteus$ have been reported (Nielsen 1987). In fact, sterility is not always inevitable in alloploids (Gray et al. 1991, Ramsey & Schemske 1998), as there are various ‘natural’ mechanisms capable of restoring fertility (Rieseberg & Willis 2007, Yu et al. 2009). Although the production of viable seeds facilitates dispersal, it is certainly not the only means of invasion. Vegetative (clonal) reproduction of dense rhizome systems, through fragmentation or lateral expansion, can also be advantageous to plant invasion (Kolar and Lodge 2001; Lloret et al. 2005), as carbohydrates stored within these structures (Decruyenaere and Holt 2001) enhance productivity during early season establishment (Quinn 2007).

Giant reed ($Arundo donax$ L.), for example, an aggressive invader of riparian habitats throughout southwestern United States, spreads vegetatively (seeds are rarely fertile) through rhizomes and stem fragments (Dudley 2000). Although rhizomatous spreading is a possibility (albeit unlikely considering $M. \times giganteus$ spreads laterally at a pace of only 10 cm per year (Matlaga et al. 2012)), the more widespread and most likely means of invasion is through transportation of live material (falling off a truck), or during cultivation (movement through irrigation ditches and waterways near tilled field margins) and disposal (compost piles) (Jorgenson 2010). Considering that vegetative propagules will likely be introduced into the environment in large quantities for commercial production, there is a critical need to conduct experimental introductions into semi-natural areas to assess the ability of vegetative material to become established throughout the proposed range of cultivation and to determine what factors favor establishment and growth.
To date, several studies have attempted to assess the invasive potential of vegetative material, as well as propagule characteristics and environmental conditions that may facilitate invasion. Mann *et al.* (2012b) have demonstrated that whole culms and fragments of *M. × giganteus* were able to produce shoots and roots in both soil and standing water in a greenhouse experiment conducted at the University of California, Davis. In the same study, they documented high survival rates for rhizomes that were buried (50-100%) and surface planted (80%), suggesting that vegetative material has the potential to become established upon escape. Matlaga *et al.* (2012) demonstrated the ability of rhizome fragments to survive in field conditions in Illinois. Rhizome survival in this study was also positively correlated with fragment size and age, suggesting that larger fragmented rhizomes originating from an older mother plant pose a greater threat of becoming established. Barney *et al.* (2012) documented poor survival of both *Miscanthus × giganteus* transplants (< 11%) and rhizomes (< 20%) in dry land habitat in central California. In contrast, the moist lowland riparian habitat supported giant miscanthus (5-19% survival) transplants. Mann *et al.* (2012a) similarly reported that *M. × giganteus* tolerated flood conditions but not mild to severe drought conditions (greenhouse experiment). Both authors concluded that moist lowland habitats or those that experience limited moisture stress are most at risk of invasion by *Miscanthus × giganteus*.

Here we assess the invasive potential (establishment, shoot height, shoot number, survival) of *Miscanthus × giganteus*, using vegetative material currently under development for commercial production. Our objectives were (1) to determine whether vegetative propagules (plugs, rhizomes, and stems) could establish within the proposed range of production, and (2) to
determine what conditions (propagule type, planting method, competition, and water availability) facilitate this process.

Methods

Plant Material & Site Description

In order to mimic spillage from spring and summer planting, cultivation, and live material transportation, Miscanthus × giganteus plug plants (plantlets vegetatively produced from rhizomes, with a dominant meristem), rhizomes, and stem fragments (with at least one leaf node from 1 year old plants) were used in this study. Plug plants and rhizomes (~15 g rhizome segments) were purchased from New Energy Farms, a leading biofuel company based in Canada, and whole plants (for stem materials) were purchased from Earthly Pursuits, a wholesale retailer in Maryland. The “Illinois clone” of M. × giganteus was used in both experiments described below. Therefore results presented here represent a test of one genotype over a range of conditions.

To determine the ability of vegetative propagules to establish under different environmental conditions, two experiments (hereafter referred to as Cohort 1 and Cohort 2) were established on agricultural land at The Ohio State University’s Waterman Agriculture and Natural Resources Laboratory in Columbus, OH (40º N, 83º W). The soil at this site is a Crosby silt loam (fine, mixed mesic Aeric Ochraqualf in the USDA classification, and a Stagnic Luvisol in the FAO classification).
Cohort 1

In early June 2011, a randomized block factorial design experiment was established using three propagule types of Miscanthus × giganteus: rhizomes, plugs, and stem sections. Three planting methods were imposed, one of which served as a control, while the remaining two mimicked contrasting modes of escape: loss of live material during transportation and improper disposal. Therefore propagules were placed either on the surface (loss of live material), underneath 5 cm of mulch (improper disposal), or buried 5 cm deep (control).

Two levels of competition, referred to as low and high, were assigned to each combination of propagule type and planting method, in order to simulate two environments that vegetative material might encounter upon dispersal. Both competition treatments mimicked an environment that had undergone a recent disturbance. Prior to initiation of the experiment, plots were surface tilled, removing all vegetation. After propagules were placed in the field, competition from local weeds remained unhindered throughout the experiment. High competition treatments had switchgrass as an additional competitor. Three single-shoot, clonal switchgrass plants of the “Kanlow” variety were placed equidistant from each other about 30.5 cm away from the focal propagule. Kanlow, a lowland-ecotype, warm-season, perennial bunchgrass native to the United States, was selected because its growth characteristics resemble those of M. × giganteus. Three-year-old Kanlow plants from a nearby field were selected to obtain cuttings. Plantlets (~ 45.7 cm) were propagated from rhizome cuttings in early May 2011 and remained in the greenhouse on a 29/18 °C day/night cycle for three weeks prior to transplantation. After transplantation, competition from local weeds remained unhindered for the duration of the experiment, as in the low competition treatment. Nearly all switchgrass
transplants survived. The few that did not were replaced within three weeks of the initial transplant.

We used a randomized block field design. There were a total of 5 blocks with 4 of each treatment combination per block (i.e. 4 plugs on the surface in the low competition treatment) and 20 replicates per treatment combination (3 propagule types x 3 planting methods x 2 competition treatments x 20 replicates = 360 total propagules; see Table 1 for summary of the experimental design). Propagules were placed in plots with an area of 0.5m$^2$ and were situated exactly 2 m apart from one another.

**Cohort 2**

In June 2012 a second randomized block factorial design experiment was established in the field adjacent to Cohort 1. The same three propagule types and planting methods were used, and a water treatment was added (3 propagules x 3 planting methods x 2 water treatments x 20 replicates = 360 total propagules). Competition was not included as a factor in this study. All weeds were removed from the plot prior to planting, but were thereafter unhindered throughout the course of the experiment. Water supplementation began in June 2012 and was maintained throughout the growing season. Plots were manually watered each week during June, July, August, and September via a 1.25 cm diameter hose. Approximately 2.36 L (volume per square ft for 1 inch of rain) of water were added per week, which equated to approximately twice the long-term monthly average rainfall for these months (Table 2). Plots that did not receive additional water were exposed only to ambient weather conditions. These treatments will hereafter be referred to as wet and dry plots (see Table 1 for summary of the experimental
design). Propagules were placed in plots with an area of 0.5m$^2$ and were situated exactly 2 m apart from one another.

Data Collection & Analysis

Survival (characterized as the presence of one or more live shoots) and height were recorded twice, on July 31 and September 31 for both cohorts. Survival and height recorded on July 31 are referred to respectively as % emergence and early height, while measurements taken on September 31 are referred to as % survival and final height. The number of shoots per plant was recorded once at the end of the growing season (Sept 31) for both Cohorts 1 and 2.

Logistic regression was used to analyze emergence and survival for both experiments as a function of propagule type, planting method, competition, water addition, and their interactions. Main and interaction effects were compared using odds ratios. Odds ratios measure the partial effect of each variable on the odds of survival and were calculated by taking the exponential of the estimate of each parameter. Odds ratios $> 1$ indicate positive effects on survival and ratios, $< 1$ indicate negative effects. The significance of these ratios was calculated using Fisher’s exact test.

A three way fixed effects analysis of variance (Anova) was used, with propagule type, planting method, and competition as independent variables, to analyze early and final height, as well as the number of shoots per plant, for Cohort 1. Height measurements for the presence of multiple shoots in each plot were averaged to give a single plot value. The same methods were used to analyze early and final height, as well as the number of shoots per plant for Cohort 2, this time with propagule type, planting method, and water addition as independent variables.
Dependent variables were checked for normality and homoscedasticity and transformed as necessary. In both years and cohorts, height measurements required a log 10 transformation, as the distribution violated assumptions of normality. Main effect and interaction means were compared using Tukey HSD tests. All analyses were performed with JMP v9 (SAS, Cary, NC). All values are presented as untransformed means and standard deviations, in figures and tables.

Results

Propagule Type

Emergence, survival, and shoot height differed significantly among propagule types (Tables 3, 4, 5, & 6: Fig 1 & 2). Plugs significantly outperformed rhizomes and stems in Cohort 1 and Cohort 2. Rhizome survival was intermediate compared to both plugs and stems. Plug survival averaged across all treatments was between 73-79%, while mean shoot height ranged from 22.2-52.6 cm. Rhizome survival averaged across all treatments was between 57-64% and shoot height ranged from 13.4-15 cm. Stems had generated shoots (~2.54 cm) when recorded at July 31 for both Cohort 1 and 2, but all died by end of both growing seasons.

Differences in percent emergence and survival rates were greatest during the first growing season for Cohort 1 (Fig 1: A & B). By the end of year 1 (September 31, 2011) all rates were nearly halved when compared to % emergence (July 31 2011). Differences among emergence and survival rates for both plugs and rhizomes the second year of growth for Cohort 1 (Fig 1: C & D) as well as cohort 2 (Fig 1; E & F) were slight. There was no significant difference ($X^2 = 0.14; p = 0.7074$) in overall survival between years (Cohort 1), with slightly higher rates
for propagules in year 2 (56.7%) than year 1 (43.3%). Unlike survival, there is a significant
difference in overall propagule height between years 1 and 2 ($X^2 = 9.37; p < 0.01$).

**Planting Method**

Planting method and its interaction with propagule type had significant effect on
emergence, survival, and shoot height (Tables 3, 4, 5 & 6: Fig 1 & 2). Propagules that were
either buried or underneath mulch performed equally well, and in most cases these treatments
were not significantly different from one another. Propagules that were buried under 5 cm of soil
averaged 53-55% survival with average shoot height ranging from 21.9-34.4 cm, while
propagules that were placed under mulch had similar survival rates averaging between 47%-53%
and average shoot height between 18-19.7 cm. Propagules placed on the surface demonstrated
the poorest performance, with only 30-37% survival and average shoot height ranging between
11.8-14.7 cm.

Each propagule type had varying responses to planting methods. Rhizomes displayed
relatively little variability across the three planting methods. For example, survival rates for
rhizomes averaged 62.5% when buried, 68% when under mulch, and 52% when on the surface.
Plugs, however, demonstrated the greatest variability due to planting methods with 100%
survival when buried, 80% when under mulch, and 41.5% when surface planted. Therefore, there
was a significant interaction between propagule type and planting method for plugs with those
buried or under mulch more likely to survive than those on the surface. Alternatively we did not
observe an interaction with planting method for rhizomes, as all treatment levels were equally
likely to survive. Therefore the success of the buried and mulch treatments were largely driven
by plug performance. Again all stem cuttings regardless of planting method became desiccated and died by the end of the first growing season.

Height followed a similar pattern with greater variability between plugs than rhizomes. For example, average shoot height for rhizomes averaged 12.7 cm when buried, 17.3 cm when under mulch, and 12.7 cm when on the surface. Plugs however, had an average shoot height of 38.5 cm when buried, 21.3 cm when under mulch, and 13.5 cm when surface planted. Therefore like survival there was a significant interaction with planting method for plugs on shoot height, while rhizome shoot performance was not significantly different between planting methods. Survival rates increased slightly in year 2 (Cohort 1) for both buried and mulch treatments. However, shoot height for plugs underneath mulch and buried rhizomes was halved in the second growing season. This reduction was statistically significant ($X^2 = 5.39; p = 0.0209$).

**Competition**

Competition had no significant main effect on survival or height, either initially or throughout the entirety of the experiment. Competition did, however, have a significant interaction with planting method at the end of the first growing season (Table 4). Propagules that were placed on the surface, in the presence of high competition, had significantly greater height than those in the low competition treatment (Fig 3).

**Water Addition**

Experimental water addition had no main effect on survival; however, there was a significant interaction between water addition and propagule type on % emergence (Fig. 4).
Stems performed significantly better in plots that had been given additional water. Water addition also had no main effect on the final height of propagules, but the addition of water was a significant factor for early height (Table 6). Propagules that received additional water had greater mean height as compared to propagules that did not. The number of shoots per plant was significantly related to the addition of water (F = 548.9 p < 0.0001). Plants in the wet plot had a greater number of shoots than those in the dry plot (Fig. 5).

Discussion

Propagule Type

This study is among the first to demonstrate the establishment potential of Miscanthus × giganteus vegetative fragments. Under field conditions, M. × giganteus plug plants and rhizomes have the ability to generate shoots in central Ohio, with plug plants being somewhat more successful than rhizomes. Stems cuttings initially generated shoots across all planting treatments, but by mid-August all had died. These results are consistent with Pyter et al. (2007), who reported success in propagating new plants from stem cuttings in the greenhouse; however, these plants fail to survive transplanting to the field. Several studies have also examined the relationship between stem node viability and seasonality (Atkinson 2009, DEFRA 1992, Hong & Meyer 2007). For example, Mann et al. (2012b) recorded the highest stem node survival in the spring, intermediate survival in early summer, and the lowest survival in late summer, in a greenhouse study. In fact, the mere act of cutting whole culms into fragments has been shown to reduce stem node viability (Atkinson 2009, DEFRA 1992, Hong & Meyer 2007, Mann et al.)
Therefore stem cuttings do not appear to present a significant risk at this time, as viability in a field setting has not been demonstrated.

While work on both the agronomic and invasive potential of rhizome fragments has begun, research on the performance of plug plants is fairly limited. Boersma & Heaton (2011) noted that although plugs are limited by a smaller rhizome system, there was no significant difference in the establishment or survival of rhizome fragments and plug plants grown under ideal agronomic conditions. However, it is important to note that plugs received regular irrigation for the first two weeks of their experiment, while rhizomes did not. In fact, results from several studies have demonstrated that plug plants require two weeks of regular irrigation following planting to allow successful establishment and growth, whereas rhizome fragments are generally self-sufficient (Caslin et al. 2011). Caslin et al. (2011) therefore suggested that rhizomes would fair better than plug plants under dry or adverse field conditions.

In contrast, we found a significant difference favoring plug plants, in terms of survival under conditions propagules might face upon escape (i.e. lack of regular water availability). In fact, for the duration of this experiment (especially the summer of 2012) Columbus was reported to be abnormally dry by the U.S. Drought Monitor maintained by the National Oceanic and Atmospheric Administration (NOAA 2012). This suggests that plugs may be more resilient to adverse conditions than previously believed. This is especially important considering that plug plants offer considerable economic and production advantages over rhizome propagation. Propagation of plugs is both faster and less labor intensive than rhizomes, which requires the excavation of ‘mother plants’. In addition, rhizome segments send up their shoots in a more uneven fashion, preventing growers from utilizing mechanical weed control, thereby limiting
them to the more costly use of herbicide. Therefore plugs may soon replace rhizomes as the dominant propagation method and may therefore have increased probability of disseminating into the environment.

Lower viability of rhizomes, as compared to plug plants, may also be explained by factors related to planting date. Atkinson (2009) noted that effective rhizome growth throughout the growing season, as well as the following year, requires early spring establishment. This study began in June rather than the optimal planting time of March to April (although propagation can occur as late as June and still be successful). Matlaga et al. (2012) initiated a similar field experiment in Illinois from late May to late June and documented poor rhizome fragment survival of only 9% on average (across all fragment weights). Early season establishment enables larger rhizome systems to develop and facilitates the growth of a more robust plant with greater drought tolerance (Caslin et al. 2011). In addition, soil moisture content is greater during spring compared to summer months (when the soil surface dries rapidly) within this region. This is important considering rhizomes will not establish at soil moisture contents below 40% (Caslin et al. 2011). Spring planting may therefore allow greater aboveground performance during early establishment. Thus we cannot definitively conclude that plug plants pose more of a threat to native communities than rhizome fragments. However, there is evidence here to suggest that survival of rhizome fragments and plug plants is possible under abnormally dry conditions.

We also demonstrated that survival could be maintained through a second year of growth. This is consistent with anecdotal evidence that suggests that if a M. × giganteus plant lives through the first winter, it is highly likely to survive through subsequent seasons (Caslin et al. 2011). However, long-term viability of up to five years or more should be assessed before we
can conclude whether or not survival documented in this study is truly maintained. Survival rates for both plug plants and rhizomes increased slightly (not statistically significant) in the second growing season. Whether this was due to a lack of above ground development on the plants part or growth being to small to detect is difficult to determine. However, it affirms the need for repeated site monitoring around field edges, as it may take some time to easily identify escaped plants.

Shoot height among surviving plants varied as a function of propagule type, with plug plants amassing nearly twice the height of rhizomes. These results vary slightly from Boersma & Heaton (2011), who found no significant difference between above ground performance of rhizomes and plug plants grown under ideal agronomic conditions. Again, differences between plug plant and rhizome shoot height may be due to the same factors that affected survival. Unlike survival, there was a difference in overall height between the first and second growing seasons. This result was largely driven by the plug control treatment, which amassed nearly double the height of any other combination of propagule type and planting method. Again, considering growing conditions in this study were unfavorable, this suggests that plug plants may not be as negatively affected by environmental conditions such as those encountered upon escape within this region.

As a final note on propagule type, this study did not assess other propagule characteristics known to facilitate establishment such as age and size. Future experiments should expose a variety of age and size classes (and any other variable characteristics) to similar conditions, to better predict what type of propagule poses the greatest threat. For now, care should be taken when handling both rhizomes and plug plants.
Planting Method

*M. × giganteus* is more likely to establish when buried under 5 cm of soil or under mulch than when left on the soil surface. These planting methods were positively related to survival and height when considered across all treatment conditions and years. These conditions would be expected after heavy rainfalls, which dislodge soil and increase movement of debris. If fragmentation occurs or if live material is present (e.g. lost during transportation or cultivation), such conditions might lead to rapid establishment. Caslin et al. (2011) noted that experimental mulch additions significantly aided establishment and growth of *M. × giganteus*. Care should therefore be taken during transportation and disposal of live material. Propagules placed on the surface demonstrated the poorest performance. However, Atkinson (2009) noted that propagule viability decreases rapidly if exposed to ambient conditions.

Mann et al. (2012b) documented slightly higher survival rates of vegetative fragments buried under 5 cm of soil (50-100%) and surface planted (80 %) than those observed in this study. However, rhizome segments in Mann et al. (2012b) were kept under controlled conditions in a greenhouse including 20-35% soil moisture, 29/18° C day/night cycle, 28 to 69 % humidity, a soilless media consisting of 50 % washed sand, 50 % sphagnum peat moss, and no competition. Plants in this study were exposed to competition, poor soil quality, and severe weather conditions. In addition, the duration of their experiment was much shorter (a little over a month). Propagules placed on the surface in this study were directly exposed to desiccation and lacked the appropriate rooting depth (5 cm) to achieve substantial growth.
Survival rates associated with propagules that were both buried and under mulch increased slightly between years, while those placed on the surface decreased slightly. Therefore, long-term viability for propagules exposed directly to desiccation on the surface may be significantly reduced over time. As such, long-term viability needs to be evaluated before we can conclude whether surface planted propagules present a serious threat to native communities. Again, observed increases may be due to factors mentioned previously. These results demonstrate the need for proper methods related to transportation and disposal of live material.

**Competition**

Overall, there was no significant difference on survival or shoot height between the two levels of competition imposed in this experiment. The higher level of competition, did, however appear to favor greater shoot height of propagules placed on the surface, suggesting that facilitation rather than competition took place (Holmgren *et al.* 1997). Facilitation, being the positive effect of neighboring plants on either establishment or growth (Clements *et al.* 1926, Connell and Slatyer 1977) as opposed to the negative effects observed (on establishment and growth) when plants compete for limiting resources (Clements *et al.* 1929). Bertness and Callaway (1994) hypothesized that facilitation is more important under harsh or adverse conditions (similar to the ones in this study), while competitive interactions increase in importance as conditions improve. One such model applicable to this study, the light-water model, postulates that under dry conditions the benefits derived from increased water availability, underneath a shaded canopy, exceed the costs associated with limited light availability (Holmgren *et al.* 1997). It is possible that switchgrass plants present before local
weeds had become fully grown provided this environment however, the magnitude and longevity of this effect cannot be determined from our data. Although we cannot definitively conclude that neighboring plants facilitate surface planted propagule growth (specifically because soil moisture content and light availability were not evaluated), these results warrant further review. There is evidence here to suggest that if propagules landed on the surface during escape, that dense vegetation may facilitate growth in dry conditions.

Mean shoot height documented in this study is generally not comparable to the plant’s agronomic potential of 0.5-1 m in the first year and nearly 2.5-3.5 m in subsequent seasons. There were, however, a few individual plants of both propagule types that achieved comparable height, but the majority of surviving plants yielded very little growth (in terms of shoot height). Caslin et al. (2011) notes that weed control within the first three years is essential for successful stand development. In fact, fertilizer use within that time frame is not recommended to prevent the growth of local weeds, as *M. × giganteus* is only able to outcompete native vegetation after three years of successful growth (Caslin et al. 2011). Since this experiment did not impose a ‘no competition’ treatment, we cannot address whether competition directly affected growth and our ability to discuss how aggressive *M. × giganteus* is in comparison to other native or invasive plants is limited. Future research should therefore aim to introduce a no competition control plot along with comparisons to native (i.e. switchgrass) and known invasive species (i.e. giant reed) with similar growth patterns, in a field setting. Results from that experiment may allow us to better predict how aggressive of an invader *M. × giganteus* may be.
Water Availability

Water addition had no significant effect on survival of vegetative propagules. Ambient conditions did not impede vegetative establishment. This is significant considering this region was experiencing abnormally dry conditions. Thus, our data confirm that *M. × giganteus* is tolerant of such conditions. Emergence rates for stem cuttings were significantly greater in plots that received additional water. Although all stems had become desiccated and died by the end of the season, they appear to be the only propagule to respond favorably (in terms of emergence) to water availability, at least for a time. Therefore, plugs and rhizomes are more resilient to dry conditions than stems. Future research on the invasive potential of stem fragments should be directed at assessing the relationship between water availability and survival, as there appeared to be some early benefits of water availability. This is especially important if whole culm fragmentation occurs during spring and summer months or if stand cutting becomes a useful cultivation tool. Water addition appeared to favor shoot height early in the season, as well. These results are consistent with experimental trials, which demonstrate that water availability, during early establishment, promotes aboveground development throughout the season (Caslin *et al.* 2011).

There is also evidence to suggest that soil moisture differences at rooting depth may have contributed to shoot production differences between treatments. Water availability may therefore aid competitive growth in *Miscanthus × giganteus*. In fact, Caslin *et al.* (2011) reported that greater biomass can be achieved with increased water availability, with losses of up to 90 kg of biomass per hectare for every millimeter of soil water lost. Mann *et al.* (2012a) also demonstrated significant reductions to *M. × giganteus* biomass in both mild and severe drought
conditions. Water availability in this study appeared to promote aboveground biomass production, especially early in the season. *Miscanthus × giganteus* may therefore be more successful at invading areas with low moisture stress.

**Management Recommendations**

*Miscanthus × giganteus* may help reduce energy prices, slow climate change, and improve energy sustainability. However, taking precautions when handling live material is recommended. Growers should continue to apply best management practices as outlined in “Planting and Managing Giant Miscanthus as a Biomass Energy Crop, Technical Note No. 4 – July 2011”. These include: establishing and maintaining a barrier around a *M. × giganteus* stands to monitor and manage any spread, continued monitoring of surrounding field areas during planting (especially after a severe storm event) and after stands are no longer productive (considering vegetative growth was sometimes difficult to detect in this study), confirming that vegetative material is properly secured during transportation or at any point propagules are outside field margins, after planting and harvesting inspect all equipment and remove any live residual material from planters or tillers, and finally disposing of live material properly (do not place live material in mulch piles or unmonitored habitats) by burning or desiccation. Experimental field studies designed to assess the risks associated with *M. × giganteus* should continue throughout the proposed range of production, as this study provides evidence the vegetative material can establish under conditions propagules may encounter upon escape in central Ohio.
References


Appendix A: Tables and Figures
Figure 1. Effects of propagule type (plug, rhizome, or stem) and planting method (buried, mulch, or surface) on percent emergence and percent survival for Cohort 1 (A-D) and Cohort 2 (E, F). N = 40 because data were pooled from the two competition treatments for Cohort 1 and the two watering treatments for Cohort 2. Values with different superscripts are significantly different at P < 0.05.
Figure 2. Effects of propagule type (plug, rhizome, or stem) and planting method (buried, mulch, or surface) on plant height (cm) for Cohort 1 (A-D) and Cohort 2 (E, F). N = 40 because data were pooled from the two competition treatments for Cohort 1 and the two watering treatments for Cohort 2. Means + 1 SE are shown. Values with different superscripts are significantly different at P < 0.05.
Figure 3. Effects of planting method (buried, mulch, or surface) and competition (low vs. high) on plant height (cm) for surviving plants at the end of Year 1 for Cohort 1. Data from the three propagule types were pooled for ease of presentation. Mean ± 1 SE are shown, see Appendix 1 for sample sizes. See Table 4 for Anova showing a significant interaction between planting method and competition.

Figure 4. Effects of propagule type (plug, rhizome, stem) and watering treatment (dry vs. wet) on percent emergence (July 31, 2012). Data from the three planting methods were pooled for ease of presentation (N = 60). See Table 6 for logistic regression showing a significant interaction between propagule type and watering treatment.
Figure 5. Effects of watering treatment (wet vs. dry) on the final number of shoots per plant in Cohort 2. $F = 548.9$ $p < 0.0001$. Means $\pm$ 1SE; sample sizes are shown in Table 7 & 8.
Table 1. Summary of experimental design.

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration</strong></td>
<td>June 2011 – September 2012</td>
<td>June 2012 – September 2012</td>
</tr>
<tr>
<td><strong>Propagules</strong></td>
<td>Plug, Rhizome, Stem</td>
<td>Plug, Rhizome, Stem</td>
</tr>
<tr>
<td><strong>Planting Methods</strong></td>
<td>Buried, Mulch, Surface</td>
<td>Buried, Mulch, Surface</td>
</tr>
<tr>
<td><strong>Competition</strong></td>
<td>Two levels (Low and High)</td>
<td>One level (Low)</td>
</tr>
<tr>
<td><strong>Water Treatment</strong></td>
<td>One Level (ambient)</td>
<td>Two Levels (ambient, water addition)</td>
</tr>
<tr>
<td><strong>Replicates</strong></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>Blocks</strong></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total # of Propagules</strong></td>
<td>360</td>
<td>360</td>
</tr>
</tbody>
</table>

Table 2. Mean temperature and precipitation for Cohort 2 (Summer 2012), as well as the twenty-five year average for precipitation in the City of Columbus. Total precipitation accounting for water added each month is also displayed. Data taken from historical records available at weatherunderground.com and weather.com.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (Avg)</th>
<th>Actual Precipitation (total)</th>
<th>Precipitation (25 year Avg)</th>
<th>Precipitation (total w/treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>74 °F</td>
<td>2.43 in</td>
<td>3.71 in</td>
<td>6.43 in</td>
</tr>
<tr>
<td>July</td>
<td>81 °F</td>
<td>4.49 in</td>
<td>4.42 in</td>
<td>8.49 in</td>
</tr>
<tr>
<td>August</td>
<td>76 °F</td>
<td>1.71 in</td>
<td>3.15 in</td>
<td>5.71 in</td>
</tr>
<tr>
<td>September</td>
<td>66 °F</td>
<td>3.75 in</td>
<td>3.03 in</td>
<td>6.75 in</td>
</tr>
</tbody>
</table>

Table 3. Effects of propagule type (plug, rhizome, stem) and planting method (surface, mulch, buried) on the probability of emergence and survival for Cohort 1, based on logistic regression. Competition and block were removed from the analysis, as their effects were not significant in main effects or interactions.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>$X^2$</th>
<th>P</th>
<th>$X^2$</th>
<th>P</th>
<th>$X^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propagule</td>
<td>2</td>
<td>32.2</td>
<td>&lt; 0.01</td>
<td>189.3</td>
<td>&lt; 0.01</td>
<td>144.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Planting Method</td>
<td>2</td>
<td>20.9</td>
<td>&lt; 0.01</td>
<td>14.4</td>
<td>&lt; 0.01</td>
<td>138.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Propagule x Planting Method</td>
<td>4</td>
<td>14.7</td>
<td>&lt; 0.01</td>
<td>25.7</td>
<td>&lt; 0.01</td>
<td>192.6</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Table 4. Analyses of variance for effects of propagule type (plug rhizome, stem), planting method (buried, mulch, surface), and competition (low and high) on early and final height for Cohort 1 after log_{10} transformation. Block was removed from the analysis, as its effect was not significant in main effects or interactions. The three-way interaction between propagule, planting method and competition was also not significant at P > 0.05.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Early Height July 2011</th>
<th>F</th>
<th>P</th>
<th>Final Height September 2011</th>
<th>F</th>
<th>P</th>
<th>Final Height September 2012</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propagule</td>
<td>1</td>
<td>60.7</td>
<td>&lt; 0.01</td>
<td></td>
<td>15.9</td>
<td>&lt; 0.01</td>
<td></td>
<td>87.8</td>
<td>&lt; 0.01</td>
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</tr>
<tr>
<td>Planting Method</td>
<td>2</td>
<td>12.4</td>
<td>&lt; 0.01</td>
<td></td>
<td>5.5</td>
<td>&lt; 0.01</td>
<td></td>
<td>6.9</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Competition</td>
<td>1</td>
<td>0.7</td>
<td>&lt; 0.01</td>
<td></td>
<td>0.9</td>
<td>&lt; 0.01</td>
<td></td>
<td>0.2</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Propagule x Planting Method</td>
<td>3</td>
<td>8.4</td>
<td>&lt; 0.01</td>
<td></td>
<td>4.6</td>
<td>&lt; 0.01</td>
<td></td>
<td>175.3</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Planting Method x Competition</td>
<td>3</td>
<td>2.4</td>
<td>&lt; 0.01</td>
<td></td>
<td>5.5</td>
<td>&lt; 0.01</td>
<td></td>
<td>1.2</td>
<td>0.31</td>
<td></td>
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Table 5. Effects of propagule type (plug, rhizome, stem), planting method (surface, mulch, buried) and water addition on the probability of emergence and survival for Cohort 2, based on logistic regression. Block was removed from the analysis, as its effect was not significant in main effects or interactions. The three-way interaction between propagule, planting method and water addition was also not significant at P > 0.05.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Emergence July 2012</th>
<th>X^2</th>
<th>P</th>
<th>Survival September 2012</th>
<th>X^2</th>
<th>P</th>
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<tbody>
<tr>
<td>Propagule</td>
<td>2</td>
<td>24.2</td>
<td>&lt; 0.01</td>
<td></td>
<td>174.1</td>
<td>&lt; 0.01</td>
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<tr>
<td>Planting Method</td>
<td>2</td>
<td>21.5</td>
<td>&lt; 0.01</td>
<td></td>
<td>7.9</td>
<td>&lt; 0.01</td>
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<tr>
<td>Water Addition</td>
<td>1</td>
<td>2.4</td>
<td>0.12</td>
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<tr>
<td>Propagule x Planting Method</td>
<td>4</td>
<td>14.9</td>
<td>&lt; 0.01</td>
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<td>5.4</td>
<td>&lt; 0.01</td>
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<td>Propagule x Water Addition</td>
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<td></td>
<td>0.9</td>
<td>0.41</td>
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38
Table 6. Analyses of variance for effects of propagule type (plug rhizome, stem), planting method (buried, mulch, surface), and water addition on early and late height for Cohort 2 after log_{10} transformation. Block was removed from the analysis, as its effect was not significant in main effects or interactions. The three-way interaction between propagule, planting method and water addition was also not significant at P > 0.05.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Early Height July 2012</th>
<th>Final Height September 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Propagule</td>
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<td>61.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Planting Method</td>
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<td>&lt; 0.01</td>
</tr>
<tr>
<td>Water Addition</td>
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<td>9.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Propagule x Planting Method</td>
<td>4</td>
<td>7.9</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Table 7. Effects of propagule type and planting method on the height of surviving Miscanthus x giganteus propagules for Cohort 1 in 2011 and 2012. Means, SE, range, and N are shown. Values in the same group with different superscripts are significantly different at P < 0.05.

A. Cohort 1 Year 1

<table>
<thead>
<tr>
<th>Survival (N = 40)</th>
<th>Early shoot height of survivors (cm)</th>
<th>Final shoot height of survivors (cm)</th>
<th>Number of Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SE</td>
<td>range</td>
</tr>
<tr>
<td>Plug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.38</td>
<td>6.4</td>
<td>cd</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.83</td>
<td>12.3</td>
<td>b</td>
</tr>
<tr>
<td>Buried</td>
<td>1.00</td>
<td>21.6</td>
<td>a</td>
</tr>
<tr>
<td>Rhizome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.53</td>
<td>6.1</td>
<td>cd</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.60</td>
<td>6.8</td>
<td>cd</td>
</tr>
<tr>
<td>Buried</td>
<td>0.58</td>
<td>6.6</td>
<td>c</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0</td>
<td>3.5</td>
<td>de</td>
</tr>
<tr>
<td>Mulch</td>
<td>0</td>
<td>2.9</td>
<td>d</td>
</tr>
<tr>
<td>Buried</td>
<td>0</td>
<td>3.6</td>
<td>de</td>
</tr>
</tbody>
</table>

Continued
Table 7 continued

<table>
<thead>
<tr>
<th>Survival (N = 40)</th>
<th>Early shoot height of survivors (cm)</th>
<th>Final shoot height of survivors (cm)</th>
<th>Number of Shoots</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SE</td>
<td>range</td>
</tr>
<tr>
<td><strong>Plug</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.33</td>
<td>9.3c</td>
<td>1.1</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.85</td>
<td>11.2bc</td>
<td>0.5</td>
</tr>
<tr>
<td>Buried</td>
<td>1.00</td>
<td>40.3a</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Rhizome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
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<td>9.8c</td>
<td>0.9</td>
</tr>
<tr>
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<td>14.7b</td>
<td>1.4</td>
</tr>
<tr>
<td>Buried</td>
<td>0.65</td>
<td>4.4d</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Stem</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mulch</td>
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</tr>
<tr>
<td>Buried</td>
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</tr>
</tbody>
</table>

Table 8. Effects of propagule type and planting method on the height of *Miscanthus x giganteus* propagules in Cohort 2, without vs. with additional water. Measured July 31 2012 (early height) and September 31, 2012 (final height). Means, SE, range, and N are shown. Values in the same group with different superscripts are significantly different at P < 0.05.

### a. Without water addition

<table>
<thead>
<tr>
<th>Survival (N = 20)</th>
<th>Early shoot height of survivors (cm)</th>
<th>Final shoot height of survivors (cm)</th>
<th>Number of Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SE</td>
<td>range</td>
</tr>
<tr>
<td><strong>Plug</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.45</td>
<td>4.9cd</td>
<td>0.8</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.80</td>
<td>13.6ab</td>
<td>2.7</td>
</tr>
<tr>
<td>Buried</td>
<td>1.00</td>
<td>21.1a</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Rhizome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.55</td>
<td>4.0cd</td>
<td>0.3</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.65</td>
<td>7.3bcd</td>
<td>1.7</td>
</tr>
<tr>
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<td>0.55</td>
<td>7.5bc</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Stem</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0</td>
<td>3.1cd</td>
<td>0.3</td>
</tr>
<tr>
<td>Mulch</td>
<td>0</td>
<td>2.8d</td>
<td>0.2</td>
</tr>
<tr>
<td>Buried</td>
<td>0</td>
<td>3.3cd</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Continued
Table 8 continued

<table>
<thead>
<tr>
<th>Survival (N = 20)</th>
<th>Early shoot height of survivors (cm)</th>
<th>Final shoot height of survivors (cm)</th>
<th>Number of Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SE</td>
<td>range</td>
</tr>
<tr>
<td><strong>Plug</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
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<td>8.9^{cd}</td>
<td>2.6</td>
</tr>
<tr>
<td>Mulch</td>
<td>0.85</td>
<td>15.7^{ab}</td>
<td>1.6</td>
</tr>
<tr>
<td>Buried</td>
<td>1.00</td>
<td>20.8^{a}</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Rhizome</strong></td>
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<td></td>
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</tr>
<tr>
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<td>6.6^{cd}</td>
<td>1.0</td>
</tr>
<tr>
<td>Mulch</td>
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<td>9.7^{bc}</td>
<td>1.4</td>
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<tr>
<td>Buried</td>
<td>0.70</td>
<td>7.8^{cd}</td>
<td>1.4</td>
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<td><strong>Stem</strong></td>
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<td></td>
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<tr>
<td>Surface</td>
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<td>3.9^{cd}</td>
<td>0.3</td>
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<tr>
<td>Mulch</td>
<td>0</td>
<td>3.8^{d}</td>
<td>0.3</td>
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<tr>
<td>Buried</td>
<td>0</td>
<td>3.9^{cd}</td>
<td>0.4</td>
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</tbody>
</table>