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Propagation method affects *Miscanthus* × *giganteus* developmental morphology☆

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ABSTRACT

The Illinois clone of *Miscanthus* × *giganteus* has many traits of an ideal biomass crop, including sterility, which significantly limits invasive potential. However, this sterility necessitates vegetative propagation, a time and labor intensive process that currently challenges the crop's adoption. Traditionally propagated by rhizome segments, *M.* × *giganteus* can also reproduce by stems like its relative, sugarcane. Previous work indicates, however, that non-traditional propagation of *M.* × *giganteus* can affect developmental morphology of resultant plants in the field. We investigated the effect of stem propagation on developmental morphology (part I, this paper), and survival and yield (part II), of field-grown *M.* × *giganteus* (Illinois clone) plants at three sites in Iowa, USA during the second and third year of growth. Although stem propagation affected morphology compared to traditional rhizome propagation, the differences were less pronounced than reported for hormone-aided micropropagation. Observed differences (and similarities) between stem and rhizome propagated plants were consistent between different growing environments and years, despite extreme weather. Rhizome propagated plants had larger basal circumferences (146.2 cm vs. 134.7 cm on average, $P = 0.0107$), but stem propagated plants had more stems per plant (38 vs. 33 on average, $P = 0.0492$) suggesting that these two propagation techniques result in plants with different growth strategies but may achieve similar yields. Though small, these differences persisted consistently throughout the duration of this experiment, suggesting morphological differences may be maintained over time in mature stands of *M.* × *giganteus*.

1. Introduction

In an effort to reduce petroleum use, the United States Congress legislated that 136 billion L of biofuels be used by 2022, 79.5 billion L of which must come from non-starch sources, e.g., advanced biofuels, (Energy Independence and Security Act, 2007). In 2012, the United States produced 50.4 billion L of ethanol (Renewable Fuels Association, 2013). Although a substantial increase over 2007 production of 24.6 billion L (Renewable Fuels Association, 2013), only 6.6 billion L of the mandated non-starch biofuel were produced in 2012 (US EPA, 2013), limited in part by the lack of cheap, abundant, non-food feedstocks. The energy crop *Miscanthus* × *giganteus* (Greef et Deu. Ex Hodkinson et Renvoize) (Hodkinson and Renvoize, 2001) (hereafter referred to simply as '*M.* × *giganteus*') has been identified as a leading candidate to not only provide this needed feedstock (Heaton et al., 2008), but also multiple ecosystem services such as reduced nitrate leaching and reduced nitrous oxide emissions (Smith et al., 2013).

Field trials to date have shown the Illinois clone of *M.* × *giganteus* to be particularly productive in the Midwestern United States (Dohleman et al., 2012; Kiniry et al., 2013), but predictions of expected productivity in this region are limited by a paucity of field data on growth, development and yield over a range of geographic, environmental and temporal conditions (Nair et al., 2012). We aim to address this knowledge gap by assessing *M.* × *giganteus* growth and developmental morphology (this paper, Boersma & Heaton Part I) as well as survival and yield (Boersma & Heaton Part II) during the three-year establishment period in three distinct growing regions in Iowa, USA, a leading agricultural state for which no *M.* × *giganteus* field data have yet been published. In addition to providing this needed primary data, we specifically investigate competing methods of plant propagation suspected to differentially affect *M.* × *giganteus* productivity.

Although *M.* × *giganteus* exhibits many traits of an ideotypic bioenergy crop (Jones and Walsh, 2001; Heaton et al., 2004;

Abbreviations: RP, rhizome plants; SP, stem plants; GDD, growing degree days; AGDD, accumulated growing degree days.

☆ Part 1 of a 2-part series on the effects of *Miscanthus* × *giganteus* propagation method on growth and productivity.

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Somerville et al., 2010), propagation of this highly productive hybrid is particularly challenging due to its triploid genome and inherent sterility ($2n = 3x = 57$) (Greef and Deuter, 1993; Hodkinson et al., 2002; Nishiwaki et al., 2011). This sterility, although ideal for minimizing invasive potential (Raghu et al., 2006; Barney and Ditomaso, 2008; Gutterson and Zhang, 2009), necessitates vegetative propagation, currently an expensive process challenging the economics of *M.* × *giganteus* (Khanna et al., 2008; James et al., 2010).

Producing many plants vegetatively takes much longer than reproduction by seed, a concern since a massive number of *M.* × *giganteus* plants must be grown to significantly offset fossil fuel use. A European review of *M.* × *giganteus* propagation indicated 500 million plants would be required to meet 25% of renewable energy goals in the UK (Atkinson, 2009). In the US, to offset just 20% of petroleum use would require a staggering 180 billion plants at currently prescribed planting densities of 15,000 plants ha^{-1} (Heaton et al., 2008). For comparison, this is similar to the number of corn (*Zea mays* L.) plants needed to plant 2.6 million ha, or roughly half the 2011 Iowa corn crop (Iowa Department of Agriculture and Land Stewardship, 2011). Because it does not fit into the seed industry model for arable crops, Atkinson (2009) highlighted that an efficient and cost effective propagation system is urgently needed for *M.* × *giganteus* to achieve its potential at a commercial scale.

Though private industry is developing improved methods for *M.* × *giganteus* rhizome propagation, traditional rhizome propagation is still the predominant method of *M.* × *giganteus* clone regeneration. This system has inherent drawbacks, however, and other options are needed to meet the growing *M.* × *giganteus* demand (Atkinson, 2009). For example, traditional rhizome propagation requires intensive excavation of existing fields, leaving them susceptible to erosion and CO_2 losses. Harvesting rhizomes also significantly impacts the productivity of parent *M.* × *giganteus* plantations by essentially restarting them as first-year stands. Further, obtaining field-grown rhizomes of consistent size and quality requires significant post-harvest quality control, typically done by intensive manual inspection and sizing of each rhizome to ensure compatibility with planting equipment.

The disadvantages of rhizome propagation led some European researchers to rely on micropropagation of *M.* × *giganteus* using tissue culture, but this system had its own problems, including altered plant morphology and reduced winter hardiness following planting as described by Lewandowski (1998). By contrast, grasses such as bamboos (*Bambusoideae* spp.) and giant reed (*Arundo donax* L.) that are difficult to propagate from seed have instead been propagated using stems (Ramanayake and Yakandawala, 1997; Wijte et al., 2005; Shirin and Rana, 2007). In sugarcane (*Saccharum officinarum* L.), which is very closely related to *M.* × *giganteus*, stem propagation is standard practice in commercial production (James, 2004).

A system utilizing aboveground stems for propagation has been suggested (Hong and Meyer, 2007; Atkinson, 2009) and demonstrated for *M.* × *giganteus* (Meyer and Hong, 2011; Boersma and Heaton, 2012). Boersma and Heaton (2012) found stem propagation may be up to 12 times more prolific than rhizome propagation. While specialized planting equipment for *M.* × *giganteus* rhizomes is limited in the US, the physically uniform plants generated from stem propagation are compatible with commercially available transplanting equipment widely used in the vegetable and tobacco industries. Further, propagating *M.* × *giganteus* from aerial tissues avoids disturbance of both soil and the perennating rhizome system. However, although alternative propagation methods such as stem propagation and micropropagation may seem advantageous compared to traditional rhizome propagation, it is possible they could negatively impact *M.* × *giganteus* morphology, yield and survival in the field.

Hereafter, when referring to plants propagated from different methods we will use abbreviations similar to Lewandowski (1998). Plants that arise by rhizome propagation will be referred to as RP and plants that are generated from stem propagation will be referred to as SP.

Propagation method can change *M.* × *giganteus* growth and development (Lewandowski, 1998). In Germany, fields established from small, micropropagated plants had more, but thinner, stems than RP generated through the traditional method, and these differences persisted throughout the three-year establishment phase (Lewandowski, 1998). The growth and morphology of *M.* × *giganteus* established from SP during the critical first three years of growth is currently unknown. Are the vegetatively propagated SP interchangeable with the more familiar RP? Or, are there lasting differences in the development and appearance of SP, as with micropropagated plants?

To assess the influence of propagation method on *M.* × *giganteus* developmental morphology, we used a multi-site, field-based approach. Our objectives were to determine:

(1) If propagation method influences developmental morphology of *M.* × *giganteus*.
(2) If differences between RP and SP are consistent within years and sites.
(3) If differences between RP and SP are maintained between years, and throughout the two years of growth following the planting year.

2. Materials and methods

2.1. Field description

Field trials were established in spring 2009 at three Iowa State University research farms in Northwest, Central and Southwest Iowa, USA (Table 1). Cropping history of the three sites was similar: glyphosate-resistant soybean [*Glycine max* L. (Merr.)] rotated annually with corn; soybeans were grown in 2008.

2.2. Plant material

Miscanthus × *giganteus* (Illinois clone) rhizomes were harvested from Caveny Farm fields (Monticello, IL, USA) in October 2008. Rhizomes were sorted and selected based on the criteria that each 7–12 cm segment of rhizome had a minimum of one visible axillary bud (Fig. 1). Rhizome segments were harvested, handled and stored similarly to Pyter et al. (2010). Rhizomes were stored in plastic containers and kept cool with moist paper towels and ice. Half of the sorted rhizomes were randomly selected for rhizome propagation and transported to Iowa State University, Ames, IA, USA, where they were stored at 5 °C with moistened paper towels until planting. The remaining rhizomes were shipped to Speedling Inc. (Sun City, FL, USA), where new plants were established and used to generate SP using a proprietary method similar to that described by Boersma and Heaton (2012). Two weeks prior to planting, resultant SP (Fig. 2) were shipped to the Iowa State University Agricultural Engineering & Agronomy Farm (Boone, IA, USA) and cold stored at 7 °C until planting in May 2009 (Table 1).

2.3. Plot establishment and maintenance

Field sites were tilled prior to planting in 2009 to ensure a good seed bed for establishment. Eight plots were established at each site in a completely randomized design with 4 replicates ($n = 4$) of each propagule (directly planted rhizomes and SP). At each site, a pointed metal bar was used to open holes in the soil into which either a rhizome or SP was placed, along with 350 mL of