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Does propagation method affect yield and survival? The potential of *Miscanthus* × *giganteus* in Iowa, USA☆

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ABSTRACT

As a sterile hybrid, *Miscanthus* × *giganteus* must be vegetatively propagated. Previous work has shown that propagation method may negatively impact not only yield of *M.* × *giganteus*, but also winter survival. However, these studies only considered rhizome and micropropagated *M.* × *giganteus*. Recently, stem propagated plants have also become available to the US market. Similar to micropropagation, these propagules do not rely on rhizomes to produce planting stock, but little is known about the yield potential or survival of stem propagated plants in the field. Here we addressed these questions in a replicated, side-by-side comparison of rhizome and stem propagated plants at three sites in Iowa, USA. We found no propagule related differences in above- or belowground biomass, establishment losses or winter losses of *M.* × *giganteus*. Yields averaged 24.7 (±3.5) Mg ha^{-1}. Though *M.* × *giganteus* productivity frequently peaks in the third year after planting, second year yields in Iowa were not significantly different than third. Additionally, winter mortality was very low, averaging only 1.2% during the first two winters. Establishment mortality, however, was significantly greater ($P < 0.0001$) and averaged 23.7%. We found *M.* × *giganteus* is productive in Iowa, with yields similar or higher than other US trials, and that stem propagated *M.* × *giganteus* performed very similarly to rhizome propagated *M.* × *giganteus*. While much research has been conducted on cold tolerance and winter survival in *M.* × *giganteus*, future research should also address establishment losses to reduce planting costs, the major upfront expense in *M.* × *giganteus* production.

1. Introduction

Biomass productivity of clonally propagated crops may be affected by differences in vegetative planting material, such as culms, rhizomes or tubers. Variability in clonal propagules can lead to variability in the field even with consistent post-planting management, and has been demonstrated in dicots and grasses alike (Nieves et al., 2003; Baker, 2012; Campos et al., 2012). The grassy biomass crop *Miscanthus* × *giganteus* (Greef et Deu. Ex Hodkinson et Renvoize) can be propagated by rhizome, stem or micropropagation (Lewandowski, 1998; Meyer and Hong, 2011; Boersma and Heaton, 2012). Here we will use abbreviations similar to Lewandowski (1998) when referring to plants established from differing propagation methods. Specifically, plants propagated by stems will be referred to as SP,[1] and rhizome plants will be abbreviated as RP.[2] Although SP are a readily available planting stock in the US that exhibit many advantages over conventional rhizome propagation as highlighted in Boersma and Heaton (2012, 2014), the impact of this new propagation method on subsequent yield of field grown plants has not yet been examined.

Different propagation methods can impact tiller size and number in *M.* × *giganteus* (Lewandowski, 1998; Boersma and Heaton, 2014), attributes that have been shown to affect yields in several *Miscanthus* species (Jezowski et al., 2011) as well as in switchgrass (*Panicum virgatum* L.) (Boe and Beck, 2008). European trials showed consistent yields from RP and micropropagated *M.* × *giganteus* (Clifton-Brown et al., 2007), but the effect of stem propagation has not yet been tested. Furthermore, earlier EU trials used a different *M.* × *giganteus* clone than the Illinois clone now commonly used in the US. Because morphology differs subtly in plants of this clone when propagated from stems instead of rhizomes (Boersma and

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1 SP – stem plants.
2 RP – rhizome plants.

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Heaton, 2014), it may be possible that stem propagation affects also yields, and ultimately adoption, of this novel bioenergy crop.

Planting stock differences may also affect winter survival in the first year after planting. Previous work has shown that micropropagated *M.* × *giganteus* plants can have very high winter mortality rates, up to 100% (Clifton-Brown, 1997; Schwarz et al., 1998; Clifton-Brown et al., 2007) while other trials have reported no differences in winter survival between micropropagated plants and RP (Lewandowski, 1998). None of these studies considered SP.

An often overlooked aspect of *M.* × *giganteus* survival is establishment loss, that is, the number of plants that emerge and die or fail to emerge at all shortly after planting. Winter survival and cold tolerance are more frequently investigated (Clifton-Brown and Lewandowski, 2000; Farrell et al., 2006; Purdy et al., 2013), whereas initial establishment success is not evaluated or given as a side note with little or no statistical evaluation. When mentioned, however, establishment losses as high as 12% have been reported (Pyter et al., 2010), but establishment losses as low as 0% have also been assumed for cost analyses of *M.* × *giganteus* (Khanna et al., 2008). Accurately anticipating establishment mortality is critical to farmers and commercial suppliers of *M.* × *giganteus* because it determines initial planting densities and thus establishment cost, as well as final plant populations and thus biomass yield. Commercial plantations often assume a 20–30% loss in the first year, but it is not clear when those losses occur (Dean Tiessen, Personal Communication). Here we evaluate both winter and establishment losses in determining survival in a newly planted *M.* × *giganteus* stand.

The objective of this study was to provide necessary survival and yield potential information to the scientific and industrial community. We evaluated the two most abundant planting stocks in the US: RP and SP. These planting stocks have not been compared in side-by-side field trials reported in the peer-reviewed literature. In addition, there have been no reported yields or survival statistics for *M.* × *giganteus* in Iowa, which has been the nation's biofuel leader for nearly two decades (USDA, 2013), using more than half of harvested corn (*Zea mays* L.) grain to produce nearly 30% of all US ethanol (Iowa Corn Growers Association, 2013). To address the above unknowns, we used a field-based approach to evaluate the following field performance characteristics:

(1) yield potentials of SP and RP;
(2) establishment losses of SP and RP and
(3) winter losses of both propagation methods.

2. Materials and methods

2.1. Site and plant material

Field management, experimental layout, weather conditions and plant materials are described in Boersma and Heaton (2014). Briefly, whole rhizomes or SP were hand planted in a replicated completely randomized design ($n = 4$) at three sites in Iowa (Northwest, Central and Southwest) in May 2009. Plots were 10.7 m × 10.7 m with 0.76 m between and within plant rows.

2.2. Biomass measurements

2.2.1. Aboveground

Following a killing frost in the fall of 2010 and 2011, two randomly selected 0.5 m^2 quadrats were centered over a single randomly selected plant in each plot and any stems within the quadrat were cut 10 cm above the soil surface. Stems may have been from adjacent plants, but were cut if the base of the stem fell within the quadrat. The entire quadrat sample was immediately weighed. A grab sample (~1 kg) of each quadrat sample was taken and dried at 60 °C to a constant mass to determine the moisture content of each sample. After drying, leaves were separated from stems at the collar (leaf sheath remained with stem portion) and the mass of each organ recorded in order to calculate stem to leaf ratios.

Table 1
Harvest dates of *M.* × *giganteus* at three Iowa State University research farms during the 2010 and 2011 growing seasons. See Boersma and Heaton (2014) for plot descriptions and details.

Year	Site	Harvest date
2010	Northwest	11 November
	Central	9 November
	Southwest	10 November
2011	Northwest	9 November
	Central	10 November
	Southwest	14 November

2.2.2. Belowground

In each of the same 0.5 m^2 quadrats that were cut for aboveground biomass sampling, soil and rhizome/root complexes were excavated to 20 cm, then stored at 5 °C before processing. Samples were thoroughly washed to remove soil and dried to a constant mass at 60 °C. After recording this mass, a subsample of each rhizome/root complex was taken and separated into rhizome and root fractions. When samples were separated to a point where only very small fragments remained, they were sieved through a 4 mm screen. Ultimately subsamples were sorted into four fractions: rhizomes, roots, residual soil and fragments. Each portion was weighed and this proportion was applied to the quadrat sample mass to estimate total belowground biomass to 20 cm.

2.3. Survival

2.3.1. Establishment losses

In mid-July 2009, approximately two months after planting, plants were counted and establishment losses calculated. The plants that had not yet emerged (rhizomes) or had died (SP) were filled in with plants from the edges of each respective plot, ensuring replacement was with plants of the same developmental stage, from the same propagation technique and from within the same environment (Boersma & Heaton, 2014). All plots then had 100% survival upon completion of the first growing season.

2.3.2. Winter losses

During July 2010 and 2011, plant populations were again assessed and winter losses calculated as a percentage of the plants remaining from the previous year (Table 1).

2.4. Statistical analyses

Yield measurements taken from the same plots were averaged within a growing season. PROC GLIMMIX (SAS 9.2, SAS institute Cary, NC, USA) was used to conduct an analysis of variance (ANOVA). The model for these analyses included year and propagation technique as fixed variables and site as a random variable. The statistical model was also adjusted for repeated measures at the plot level. Where appropriate, preplanned contrasts within growing seasons were made with a confidence level of $\alpha < 0.05$.

3. Results

3.1. Total aboveground yields

Propagation method did not affect aboveground yields during the second and third years of *M.* × *giganteus* growth during