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# Miscanthus sacchariflorus – biofuel parent or new weed?

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## Abstract

The perennial grass triploid *Miscanthus* × *giganteus* is a promising renewable bioenergy feedstock in the United States and Europe. Originating from eastern Asia, this species is a sterile hybrid cross between *M. sinensis* and *M. sacchariflorus*. While research has begun to examine the impacts of *M. sinensis* and triploid *M. × giganteus* on the landscape, *M. sacchariflorus* has been largely overlooked in the peer-reviewed literature. This review article discusses the origin, uses, distribution, and invasive potential of *M. sacchariflorus*. *M. sacchariflorus* is capable of producing high yields (10.7 t DM ha<sup>-1</sup> yr<sup>-1</sup>), generally does not reproduce by seed, and can be challenging to establish due to poor cold tolerance, likely due to the limited germplasm used in evaluations. However, *M. sacchariflorus* has abundant and aggressively spreading rhizomes, which underscores its invasive risk. In the United States, it is listed as escaped from cultivation in at least eight states, primarily in the Midwest, although it is likely that not all populations have been reported. As such, it is essential to generate a comprehensive dataset of all known *M. sacchariflorus* populations and monitor any continued spread of this species.

## Keywords

biofuel, biomass, invasive species, *Miscanthus sacchariflorus*, *Miscanthus x giganteus*, weed

## Disciplines

Agricultural Science | Agriculture | Agronomy and Crop Sciences | Plant Breeding and Genetics | Weed Science

## Comments

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## REVIEW

# *Miscanthus sacchariflorus* – biofuel parent or new weed?

CATHERINE L. BONIN, EMILY A. HEATON and JESSICA BARB

*Department of Agronomy, Iowa State University, Ames, IA, 50011, USA***Abstract**

The perennial grass triploid *Miscanthus* × *giganteus* is a promising renewable bioenergy feedstock in the United States and Europe. Originating from eastern Asia, this species is a sterile hybrid cross between *M. sinensis* and *M. sacchariflorus*. While research has begun to examine the impacts of *M. sinensis* and triploid *M. × giganteus* on the landscape, *M. sacchariflorus* has been largely overlooked in the peer-reviewed literature. This review article discusses the origin, uses, distribution, and invasive potential of *M. sacchariflorus*. *M. sacchariflorus* is capable of producing high yields (10.7 t DM ha<sup>-1</sup> yr<sup>-1</sup>), generally does not reproduce by seed, and can be challenging to establish due to poor cold tolerance, likely due to the limited germplasm used in evaluations. However, *M. sacchariflorus* has abundant and aggressively spreading rhizomes, which underscores its invasive risk. In the United States, it is listed as escaped from cultivation in at least eight states, primarily in the Midwest, although it is likely that not all populations have been reported. As such, it is essential to generate a comprehensive dataset of all known *M. sacchariflorus* populations and monitor any continued spread of this species.

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**Introduction**

As concerns rise over the price of oil and the amount of greenhouse gases (GHG) emitted by burning fossil fuels, emphasis has been placed on developing plant-based, renewable energy sources. With the passage of the Energy Independence and Security Act (EISA) of 2007, the United States established an aggressive agenda to reduce dependency on fossil fuels and foreign oil. While corn grain is the primary biofuel feedstock in the United States, research on resource use, GHG displacement, and ecological impacts suggests that grain ethanol is a poor long-term choice (Donner & Kucharik, 2008; Searchinger *et al.*, 2008; Davis *et al.*, 2009). Instead, second-generation (2G) biofuels that are nonfood crops and require fewer inputs, including *Miscanthus* species, may alleviate the problems posed by corn grain biofuels.

Originating from Southeast Asia, *Miscanthus* species are native to a diverse set of habitats from Siberia to Polynesia, and from sea level to over 3000 m in elevation (Clifton-Brown *et al.*, 2008). There are 14 species of *Miscanthus* (ten in Asia, four in Africa) growing in a variety of environments, including dry, wet, or saline conditions in grasslands, mountainsides, riverbanks, and disturbed areas (Hodkinson *et al.*, 1997; Chen & Renvoize, 2006). *Miscanthus* species possess a C<sub>4</sub> photosynthetic pathway with high levels of water, light,

and nitrogen-use efficiency, but unlike other C<sub>4</sub> species, some species of *Miscanthus* are also adapted for growth in cooler climates (Yan *et al.*, 2012). Three taxa in particular, *M. sacchariflorus*, *M. sinensis*, and triploid *M. × giganteus*, are of primary interest as bioenergy feedstocks with triploid *M. × giganteus* being the most commonly cultivated form.

*Miscanthus* species were initially brought to Europe from Asia for ornamental purposes in the 1930s, although others suggest that *M. sacchariflorus* was introduced into Europe in the late 1800s by the Russian botanist Maximowicz (Lewandowski *et al.*, 2000; Sacks *et al.*, 2012). Records of *Miscanthus sinensis* suggest that it was present in the United States before 1900 and is planted at the Biltmore Estates (Hitchcock, 1901; 1917; Quinn *et al.*, 2010). The original hybrid of *M. × giganteus* is reported to have originated in southern Japan and was then brought to Denmark in 1935. (Linde-Laursen, 1993; Hodkinson *et al.*, 2002). The basic chromosome number of the *Miscanthus* genus is  $x = 19$ , but ploidy level varies both within and among species. *Miscanthus* × *giganteus* is a sterile allotriploid ( $2n = 3x = 57$ ), originating from the interspecific hybridization of *M. sacchariflorus* and *M. sinensis* (Linde-Laursen, 1993; Hodkinson *et al.*, 1997, 2002; Rayburn *et al.*, 2009; Swaminathan *et al.*, 2010). Evidence from numerous studies strongly suggests that the maternal *M. sacchariflorus* parent in this cross was an allotetraploid, but genomic tests have been unable to definitely resolve the three genomes in this species due to high homology (Hodkinson *et al.*, 1997, 2002).

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While triploid *M. × giganteus* is taller than both parental species, its rhizomes are of intermediate length (Hodkinson *et al.*, 1997). Yield trials in Europe show that triploid *M. × giganteus* routinely outperforms its parental lines by 1.7–6 t DM ha<sup>-1</sup> yr<sup>-1</sup> (Gauder *et al.*, 2012). In the United States, 3-year yields average 30 t DM ha<sup>-1</sup> yr<sup>-1</sup>, with annual maximum yield at one site of 60 t DM ha<sup>-1</sup> (Heaton *et al.*, 2008). A large majority of the triploid *M. × giganteus* that is currently grown as a dedicated biofuel crop is descended from a single clone, which may limit the productivity of this species in different climates and poses a risk for problems with pests and disease (Nishiwaki *et al.*, 2011). As such, new genotypes with improved yield under diverse growing conditions and increased cold tolerance are needed. These new genotypes of triploid *M. × giganteus* with varied ecological adaptations must either be collected from regions where sympatric populations of *M. sacchariflorus* and *M. sinensis* naturally exist (Nishiwaki *et al.*, 2011; Dwiyantri *et al.*, 2013) or by resynthesizing the hybridization of *M. sacchariflorus* × *M. sinensis* using conventional techniques (Chae *et al.*, 2013).

Concerns about the invasive potential of biofuel crops necessitate the critical analysis of potential feedstock options. Many of the same plant traits that are beneficial in a biofuel feedstock species are also present in invasive species, including perennial growth, high yield, rapid growth, and tolerance of drought and soil disturbance (Raghu *et al.*, 2006; Cousens, 2008). A weed risk assessment (WRA) performed in the United States on triploid *M. × giganteus* determined that this species was 'acceptable' due to its failure to produce viable seed under the conditions tested. In contrast, *M. sinensis*, which does produce large quantities of seed, 'failed' and is listed as an invasive species in several US states (Barney & Ditomaso, 2008). Comprehensive studies of the invasive potential *M. sinensis* are already underway to further describe any risks posed by this species (Stewart *et al.*, 2009; Quinn *et al.*, 2010). Less, however, is understood about *M. sacchariflorus* and whether it poses any risk as an invasive species. This article reviews the current knowledge of *M. sacchariflorus*, its distribution in the United States, and discusses its invasive potential.

### Genetics of *Miscanthus sacchariflorus*

The primary and secondary centers of diversity for *Miscanthus* are in China and Japan, respectively. In China, *M. sacchariflorus* is typically diploid ( $2n = 2x = 48$ ) and very rarely polyploid, while in Japan the opposite is found and most forms of *M. sacchariflorus* are commonly tetraploid and diploid forms are absent (Sacks *et al.*, 2012). Meiotic pairing and nuclear and

chloroplast sequence data have demonstrated that some of the tetraploid forms of *M. sacchariflorus* in Japan are actually allopolyploids derived from the interspecific hybridization of *M. sinensis* and diploid *M. sacchariflorus* (Adati & Shiotani, 1962; Hodkinson *et al.*, 1997; Lledó *et al.*, 2001; Dwiyantri *et al.*, 2013), and according to Sacks *et al.* (2012) should instead be identified as *M. × giganteus*.

In lieu of this recommendation from Sacks *et al.* (2012), 'triploid *M. × giganteus*' will be used, henceforth, when referring to an allotriploid originating from the interspecific hybridization of the genomes of *M. sacchariflorus* and *M. sinensis*, and 'tetraploid *M. × giganteus*' will be used to describe a purported *M. sinensis*/*M. sacchariflorus* allopolyploid, which in some cases was historically identified as 'tetraploid *M. sacchariflorus*'. If the parentage and ploidy is not known, the original designation of '*M. sacchariflorus*' will be used. Diploid *M. sacchariflorus* and tetraploid *M. × giganteus* (i.e. *M. sinensis*/*M. sacchariflorus* allopolyploids) have also been observed along the Korean peninsula, but further work is needed to confirm the genetic background of these forms (Lledó *et al.*, 2001; Sacks *et al.*, 2012). In general, future efforts are needed to correct misidentified forms of *M. sacchariflorus* and determine if actual auto-tetraploid forms of *M. sacchariflorus* exist (Dwiyantri *et al.*, 2013). Once the ploidy and parentage of these taxa are correctly identified, the seed set, gamete viability, and compatibility of each should be evaluated to assist with ongoing efforts to develop improved clones of triploid *M. × giganteus*.

### Uses of *Miscanthus sacchariflorus*

In addition to serving as parental material for triploid *M. × giganteus*, *M. sacchariflorus* has also been used as an ornamental species and as a source of pulp for papermaking. Also known as Amur silver grass or silver banner grass, *M. sacchariflorus* grows 1.5–3 m tall, with silvery-white panicles (20–25 cm), and leaves that change to a rust color, adding visual interest in autumn (Trinklein, 2006; Pudelska, 2008). The stem of a tetraploid *M. sacchariflorus* (Sac-5) is relatively thick, averaging about 0.9 mm in diameter, but has relatively sparse stand densities of 42–55 shoots m<sup>-2</sup>, compared to an average stem density of at 66–76 shoots m<sup>-2</sup> for triploid *M. × giganteus* (Gauder *et al.*, 2012). *Miscanthus sacchariflorus* 'Robustus' has been sold by nurseries and can be found in American and European gardens (Sacks *et al.*, 2012). Recommended uses for *M. sacchariflorus* include stream bank soil stabilization and as an ornamental species near water (Trinklein, 2006). Inflorescences of this species are also used in winter bouquets (Pohl, 1959).

In China, stands of *M. sacchariflorus* may be harvested annually for 30+ years and used in the cellulose industry for making paper products (Clifton-Brown *et al.*, 2008). This species is one of the primary non-wood papermaking materials in China due to its rapid growth, large biomass production, and relative ease in cooking (a part of the papermaking process) and pulping (Cappelletto *et al.*, 2000). Compared to common reed (*Phragmites australis*), *M. sacchariflorus* generates less ash and produces more pulp at a lower cooking temperature and shorter cooking time (Hu *et al.*, 2004). *M. sacchariflorus* by itself is generally not suitable for pulp and papermaking, but the incorporation of 20–25% pine groundwood pulp makes the native *M. sacchariflorus* pulp suitable for papermaking (Young, 1997). The paper produced by this mixture has good mass, high opacity, and suitable ink absorbency, and may be used in rotary and offset printing processes (Visser *et al.*, 2001). *M. sacchariflorus* has also been examined as a partial substitute in the production of composite boards (Park *et al.*, 2012; Liao *et al.*, 2013). Other potential uses for *Miscanthus* include as a construction and building material, for bioremediation of heavy metal polluted areas, as a component of composting, and as feedstock for fermentation (Visser *et al.*, 2001).

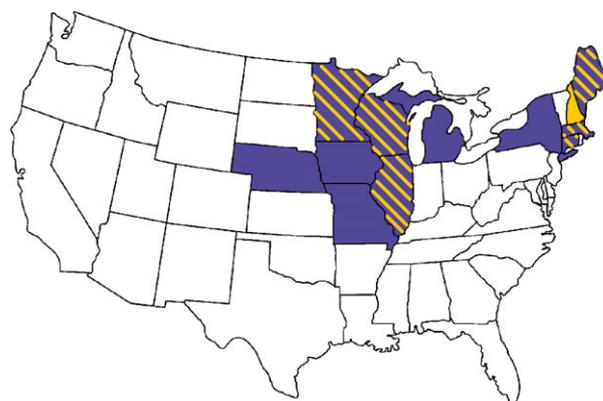
*Miscanthus sacchariflorus* also has potential as a bioenergy crop, particularly in colder climates where the overwintering survival of more tropical  $C_4$  species is low. Genotypes of *M. sacchariflorus* from the northern portion of its range show improved cold tolerance, as evidenced by its greater overwintering survival rate in northern China (latitude of about 44° N), as compared to *M. sinensis* (Yan *et al.*, 2012). Although not as high-yielding as triploid *M. × giganteus*, *M. sacchariflorus* still produces large amounts of biomass. In Germany, the average 14-year yield for *M. sacchariflorus* is 10.7 t DM ha<sup>-1</sup> yr<sup>-1</sup> (Gauder *et al.*, 2012), which is comparable to the average US corn grain yield from 2000 to 2010 (i.e. 9.3 t ha<sup>-1</sup> yr<sup>-1</sup>) (USDA-NASS, 2013). Tissue analysis shows that *M. sacchariflorus* has a lower lignin content (i.e. 20% vs. 24% in triploid *M. × giganteus*) and a total polysaccharide content of nearly 65%, suggesting that a greater proportion of biomass may be converted to biofuel (Kim *et al.*, 2012b). With a combination of high yields, lower lignin, and improved cold tolerance, *M. sacchariflorus* is well suited as a biofuels feedstock in some climates and environmental conditions.

### Distribution in the United States

According to the Biota of North America Program (BONAP), *M. sacchariflorus* is currently present in 11 US states, as well as in eastern Canada (Kartesz, 2011). Based on herbarium records, it is likely that this species

originally escaped cultivation in the Midwest along the Mississippi River in Iowa, Wisconsin, and Illinois in the late 1940s and 1950s (Ada Hayden Herbarium, 2013; Illinois Natural History Survey, 2013; University of Wisconsin-Stevens Point, 2013). Since then, *M. sacchariflorus* has spread throughout other Midwestern states and to the East Coast (New York Botanical Garden, 2013). Records from the New York Botanical Garden's C.V. Starr Herbarium suggest that some of the East Coast populations may be descendants of plants introduced to New York from DuPage County, Illinois from 1941 to 1958 by J. A. Steyermark (New York Botanical Garden, 2013). While there are confirmed populations as far east as Maine, most of the naturalized *M. sacchariflorus* populations are located in the Midwest.

Attempts to document the presence of *M. sacchariflorus* in the United States have produced variable results. One web-based mapping system, EDDMapS, recorded the presence of *M. sacchariflorus* at 273 sites in seven states (EDDMapS, 2013). This differs from BONAP in that it does not include IA, MI, MO, NE, or NY, but has records from an additional state, VT (Fig. 1). Based on these conflicting results, as well as *M. sacchariflorus* field mapping work in central and eastern Iowa, it is clear that a comprehensive map of *M. sacchariflorus* populations is lacking. Without a resource such as this, it is difficult to understand the extent to which *M. sacchariflorus* has expanded in the United States since its introduction. Furthermore, a more inclusive map would also assist in determining whether this species is at risk of being invasive and allow scientists to make recommendations for its management, if warranted.



**Fig. 1** Current distribution of *Miscanthus sacchariflorus* in the United States, generated from combined data of BONAP (Kartesz, 2011) and EDDMapS (EDDMapS, 2013). States depicted in blue represent those solely found in BONAP records, while the state in yellow represents the one solely in EDDMapS records. Striped states are where both mapping programs have recorded *M. sacchariflorus*.

*Iowa as a case study*

One of the earliest reports of feral *M. sacchariflorus* in Iowa was documented in 1951 by Hitchcock, who noted that it escaped cultivation in Clinton County, which borders the Mississippi River (Hitchcock, 1951). In 1959, Pohl stated that *M. sacchariflorus* was planted as an ornamental species at Iowa State University in the Herbaceous Garden, but was removed due to rapid vegetative spread (Pohl, 1959). While rhizomatous spread is the common method of expansion for *M. sacchariflorus*, Pohl also observed small discontinuous patches, which may have been colonized through seeds. We have noticed similar patches in Conservation Reserve Program (CRP) grasslands (Fig. 2).

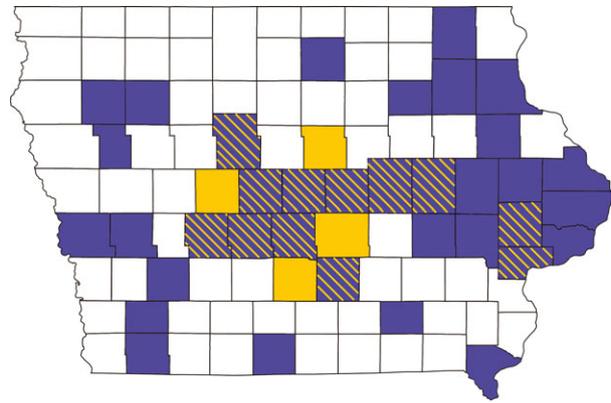
In 2012, a mapping effort of *M. sacchariflorus* in central and eastern Iowa identified nearly 100 populations in 15 counties, many along roadsides, in ditches, and near bodies of water. In several cases, escaped populations were located near residential plantings of *M. sacchariflorus*. Notably, populations were identified in four counties (i.e. Jasper, Greene, Hardin, and Warren) that were not previously recorded in the BONAP map as having *M. sacchariflorus* present (Fig. 3).

**Invasive potential***Vegetative spread and habitats*

Compared to *M. sinensis*, *M. sacchariflorus* is less preferred as an ornamental species due in large part to its potential for rapid vegetative spread. In a single year, the size of a patch of *M. sacchariflorus* may increase



**Fig. 2** Patches of *Miscanthus sacchariflorus* in Conservation Reserve Program (CRP) grasslands in Iowa. Two patches are noted by arrows. Photo credit: Doug Davenport, NRCS.



**Fig. 3** Current recorded presence of *Miscanthus sacchariflorus* in Iowa. Counties depicted in blue represent those solely found in BONAP records (Kartesz, 2011), while those in yellow represent additional counties found in a 2012 central Iowa survey. Counties that are striped represent an overlap between BONAP records and the 2012 survey.

almost six times, from 0.5 to 3 m<sup>2</sup>, compared to the more caespitose *M. sinensis*, which only doubles in size from 0.08 to 0.17 m<sup>2</sup> (Matumura *et al.*, 1985). Due to this growth pattern, stands of *M. sacchariflorus* tend to be larger but less dense than stands of *M. sinensis*. The aggressive spread of *M. sacchariflorus* contributes to the dramatic increase in coverage, as described by Matumura *et al.* (1986) where the average length of branched rhizomes without terrestrial shoots was nearly 15 cm (compared to 2 cm for *M. sinensis*), and the length between the branching points of rhizomes with terrestrial shoots was over 20 cm (compared to 5 cm for *M. sinensis*). This study also characterized *M. sacchariflorus* as having an average of 27 nodes, nine active buds, and 17 dormant buds per branched rhizome, which is critically important because rhizome buds are an important method for colonization after disturbances such as floods or plowing, as fragments can sprout and establish new stands (Deng *et al.*, 2013).

As *M. sacchariflorus* is typically found in mesic environments, areas near wetlands and water are at risk for invasion by escaped populations. Because this species is intolerant of flooding, the distribution of *M. sacchariflorus* is generally limited to the upper shoreline, due to low oxygen conditions and poor rhizome spread. Thus, rhizomes in drier areas are typically longer (i.e. 50% greater than 21 cm) than rhizomes growing in more flood-prone areas (i.e. 3–6 cm) (Yamasaki, 1990). Once escaped from cultivation, *M. sacchariflorus* readily spreads along waterways and along roadsides where mowing or plowing may further increase colonization (Fig. 4).

Due to differences in environment and ploidy level, variations in plant morphology such as stem diameter



**Fig. 4** Populations of *Miscanthus sacchariflorus* in Iowa can be found (a) along roadsides and farmland, as well as (b) escaped from ornamental cultivation in residential areas. Mowing of stands and soil disturbance contributes to its spread. Photo credit: Brent Berns and Josh Grindeland.

and leaf width may occur (Jensen *et al.*, 2013), but it is not certain whether these are a result of phenotypic plasticity or differences in ploidy level. While this variability could affect plant growth and invasive potential, information is currently lacking on how aggressive or potentially invasive each *M. sacchariflorus* subspecies (by ploidy level and/or location of origin) may be. Although some papers describe which type of *M. sacchariflorus* is tested (e.g., Clifton-Brown *et al.*, 2001; Gauder *et al.*, 2012), others may provide a collection location (e.g., Kim *et al.*, 2012a), or no information at all. More research needs to be done to determine if certain subspecies of *M. sacchariflorus* pose a greater invasion risk than others, and it is beyond the scope of this article to review the subject of subspecies in detail. As such, this article will generally not attempt to differentiate among *M. sacchariflorus* subspecies.

#### Sexual reproduction

The primary form of spread for *M. sacchariflorus* is vegetative. Flowering in Europe is infrequent and occurs later in the growing season compared to other *Miscanthus* species (Clifton-Brown *et al.*, 2001; Jensen *et al.*, 2013). However, in Iowa *M. sacchariflorus* flowers in late

July or early August, earlier than ornamental varieties of *M. sinensis*. The flowering cues of *M. sacchariflorus* are poorly understood, but it is thought that this species is a quantitative short-day species, where flowering is delayed by at least 50 days when subjected to long-day photoperiods (Jensen *et al.*, 2011, 2013). Flowering is also affected by temperature (Jensen *et al.*, 2011). Later flowering is an advantage in terms of biomass production and also reduces the chance of seed production. With flowering occurring in late summer or early autumn, and dormancy strongly induced in early autumn, viable seed production may not occur in cooler climates like those observed in parts of Europe (Sacks *et al.*, 2012). One study in the United Kingdom found that less than 20% of *M. sacchariflorus* plants had over 50% of their stems in flower and 6% or less of the plants surveyed completed flowering (Jensen *et al.*, 2011), while another study found that no plants flowered in United Kingdom (Clifton-Brown *et al.*, 2001).

In its native Japan, seed set of tetraploid *M. sacchariflorus* (i.e. likely tetraploid *M. × giganteus*) varies by location, but is generally poor, ranging from 0 to 55% (Nishiwaki *et al.*, 2011). Analysis of feral populations in Iowa in 2012 also showed poor seed production, with no seeds being produced by the seven populations examined (Allison Snow, personal communication). Meyer & Tchida (1999) examined *Miscanthus* germination across hardiness zones 4 through 7 and found that *M. sacchariflorus* is capable of producing viable seed, but at much smaller amounts than *M. sinensis*. All *Miscanthus* taxa, including *M. sacchariflorus*, are self-incompatible, and multiple genotypes are necessary for a successful seed set (Deuter, 2000; Sacks *et al.*, 2012). Likewise, population size and proximity to other *M. sacchariflorus* stands affect seed set, with small, isolated stands having lower seed fertility than larger and presumably more genetically diverse stands (Deuter, 2000). Therefore, it is possible that each of the Iowa populations consisted of a single genotype, which would negate the possibility of successful seed set. Work is currently underway to genotype plants from these populations to determine whether this is the case (Allison Snow, personal communication).

Establishment of *Miscanthus* from seeds under field conditions can be challenging. Seeds are small (i.e. 1000 seeds weigh ca. 450 mg), have few carbohydrate reserves, and require warm and moist conditions to germinate (Deuter, 2000; Clifton-Brown *et al.*, 2008). Seeds of *M. sacchariflorus* do not appear to have primary dormancy and moist-chilling does not improve germination (Washitani & Masuda, 1990). Studies on *M. sinensis* suggest that heat and low moisture are major limiting factors for the establishment of this species (Christian *et al.*, 2005). Although moisture is necessary to prevent

desiccation, *M. sacchariflorus* germination rates drop to zero under flooded conditions (Nishihiro *et al.*, 2004).

Although the incidence of interspecific hybridization between tetraploid *M. sacchariflorus* and *M. sinensis* appears to be rare in naturally sympatric regions in Japan (Nishiwaki *et al.*, 2011), it is not known whether the tetraploid *M. sacchariflorus* clones in this study were autotetraploid forms of *M. sacchariflorus* or complex interspecific allopolyploids of *M. sacchariflorus* and *M. sinensis* (i.e. tetraploid *M. × giganteus*). Therefore, although the risk of increased seed set and invasive spread from sexual reproduction among species appears to be low, additional efforts are needed to fully understand the potential for interspecific hybridization among different species of *Miscanthus* with special emphasis on the identification of the relevant plant materials.

#### *Survival of young Miscanthus plants*

Newly established *Miscanthus* plants are particularly susceptible to cold temperatures. The  $LT_{50}$  (i.e. temperature when 50% of plants die) of *M. sacchariflorus* was  $-3.4$  °C for rhizomes and  $-7$  °C for shoots (Clifton-Brown & Lewandowski, 2000; Farrell *et al.*, 2006). In Europe, the mortality of first-year *M. sacchariflorus* plants was 50–67% in the northern field sites (e.g. Sweden and Denmark), where soil temperatures dropped below  $-4.5$  °C (Clifton-Brown *et al.*, 2001). However, it should be noted that the *M. sacchariflorus* genotype (Sac-5) tested in this study originated in subtropical Asia, and it is expected that other genotypes originating from more northerly locales are expected to tolerate lower overwintering temperatures.

There are several possible reasons that first-year *M. sacchariflorus* plants have poor overwintering survival rates, as described by Christian *et al.* (2001). One hypothesis is that young plants lack the metabolic reserves in the rhizomes to survive the winter and initiate shoot production the following spring. Another possibility is that new plants do not attain a level of dormancy adequate to survive the winter so as the growing season ends, young plants may continue to produce shoots instead of going dormant. Finally, rhizomes may become damaged or die when temperatures drop below  $-5$  °C, but once established, the overall damage that winter temperatures have on stands is reduced. In older rhizomes, 75% of available reserves are in the form of carbohydrates and proteins, compared to only 65% in new rhizomes, and young rhizome reserves show a decrease after bud formation in November (Masuzawa & Hogetsu, 1977). Rhizome biomass and rooting depth increases substantially as plants age: in 7 years, roots extended to 2 m and rhizome biomass increased from 1.4 to 11.7 t ha<sup>-1</sup> (Riche &

Christian, 2001). Older rhizomes may thus be better able to cope with cold weather due to greater rhizome reserves, induced dormancy, and deeper rooted rhizomes that are more protected from cold weather.

#### **Agronomic implications and research needs**

In order for bioenergy cropping systems to be economically viable, a feedstock species must be highly productive while requiring relatively low inputs. Three taxa of *Miscanthus* meet this general parameter: *M. sinensis*, *M. sacchariflorus*, and triploid *M. × giganteus*. To date, the most frequently used species, triploid *M. × giganteus*, is a sterile hybrid capable of yields that exceed both its parent species, but this species has some ecological limitations and limited cold tolerance. Of additional concern is the fact that the triploid *M. × giganteus* is commercially planted in the United States and Europe is a single genotype, which could place the industry at risk for severe damage by pests and diseases. Because new genotypes of triploid *M. × giganteus* may help increase the ecological adaptation of this crop to diverse environments, it is important to maintain the germplasm collections of the two parental species of this interspecific hybrid, *M. sinensis* and *M. sacchariflorus*. Proper characterization of these materials using flow cytometry and available genetic markers is also needed, as part of these efforts (Lledó *et al.*, 2001; Kim *et al.*, 2012a; Slavov *et al.*, 2013; Zhou *et al.*, 2013).

#### **Conclusions**

As a parent of triploid *M. × giganteus*, *M. sacchariflorus* has great potential to provide additional genetic variation, but due to its invasive potential, this species should not be considered as a biofuel feedstock. Breeding efforts involving *M. sacchariflorus* need to be closely monitored and located away from habitats at high risk of invasion (e.g. wetlands and other mesic environments). Any vegetative spread of this species must be contained, and additional studies on the potential for viable seed production need to be performed. Additional research needs to be done to understand the requirements for successful *M. sacchariflorus* seed production, as this species would likely fail the WRA in conditions where large amounts of viable seeds are produced. Likewise, research examining gene flow among the three *Miscanthus* taxa in the United States should be conducted. Even without seed production, *M. sacchariflorus* poses an invasive threat because it has the potential to clonally take over parts of natural and managed landscapes. *M. sacchariflorus* may be useful as a source of genetic variation, but without careful management, it may also be a great invasive risk.

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