Fertility and Reproductive Pathways in Diploid and Triploid *Miscanthus sinensis*

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Abstract. Miscanthus sinensis Andersson is a popular ornamental grass and has additional potential as a bioenergy crop. In some regards, the ability of *M. sinensis* to withstand a broad range of climatic and cultural conditions is desirable, but its propensity to rapidly colonize open and disturbed environments has allowed it to naturalize and become weedy in some regions in the United States. Considering the value of this crop, the development and documentation of infertile clones would be desirable. Triploid plants were evaluated for male and female fertility using pollen viability staining and seed set and germination, respectively. Pollen viability staining, seed set, and seed germination from triploid plants were reduced compared with diploids but varied considerably within each cytotype. Overall, relative female fertility of individual triploids clones [(% seed set × % germination for triploid)/(% seed set × % germination for diploid control)] was reduced substantially and ranged from 49% to 0.7%. Additionally, the reproductive pathways of triploid plants were examined by evaluating the 2C genome sizes of progeny derived from open pollination. The limited progeny arising from open-pollinated triploids were predominantly aneuploids with 2C genome sizes intermediate between diploids and triploids. There was no clear evidence of apomixis, selfing, or triploid × triploid fertilization events observed among triploid parents. Formation of unreduced gametes was rare for both ploidy levels (≈1%). The considerable reduction in female fertility in some triploid clones combined with the limited production of primarily aneuploid progeny provides highly infertile alternatives to existing diploid cultivars.

The genus *Miscanthus* Andersson contains ≈20 species of perennial C4 grasses predominantly from Asia (Glowacka and Jezowski, 2009; Hodkinson et al., 2002). Genetic diversity is high within *Miscanthus* and has allowed some species to evolve populations adapted to highly diverse environments (Heaton et al., 2010). *Miscanthus* spp. have long been recognized for their potential as ornamentals, and maiden grass or Japanese silver grass (*M. sinensis*) has been grown in the United States since being introduced in the late 19th century

(Quinn et al., 2010). *Miscanthus sineensis* is native to China and Japan and, like many *Miscanthus* spp., is notable for its high water use efficiency and the ability to fix nitrogen through a bacterial symbiotic relationship (Eckert et al., 2001; Stewart et al., 2009). Several hundred years of cultivation has given rise to over 100 *M. sinensis* cultivars, featuring a range of heights, foliage types, and variegations (vertical or horizontal banding) (Darke, 2007). In North America, *M. sinensis* is one of the most widely planted of all ornamental grasses as a result of its colorful and dense inflorescences, durability, and capacity to thrive in USDA hardiness zones 4 to 9 (Meyer and Tchida, 1999; Wilson and Knox, 2006). The merits of *M. sinensis* have led to over 50 new selections introduced within the past 25 years (Wilson and Knox, 2006). More recently, *M. sinensis* has shown promise as a potential crop for bioenergy applications, particularly in northern latitudes where less cold-hardy grasses are unreliable (Glowacka and Jezowski, 2009; Heaton et al., 2010).

Miscanthus sinensis is strictly a clump-forming grass, which spreads naturally over short distances by wind-blown seeds (Darke, 2007; Stewart et al., 2009). Naturalization of this species, predominantly in open or disturbed environments, can be significant and problematic (Barney and DiTomaso, 2008; Meyer and Tchida, 1999). The first documented naturali-

zations in the United States were reported in Washington, DC, New York, and Florida in 1913 (Quinn et al., 2010). Wilson and Knox (2006) noted that *M. sinensis* has since naturalized in at least 25 states, of which many include it on invasive watch lists. Although *M. sinensis* is an early successional, pioneer species that typically colonizes open disturbed sites (Ohtsuka et al., 1993; Stewart et al., 2009; Tsuyuzaki and Hase, 2005), it can be a prolific weed in managed landscapes such as roadsides and pastures (Hirata et al., 2007).

Reproductive biology of *M. sinensis* has been studied in both wild populations and cultivars of diploid plants. Stewart et al. (2009) documented seed set (percentage of florets setting seed) among wild populations in Japan between 3% and 84% depending on plant density as well as genetic and environmental factors. Among cultivated varieties, seed set and germination have been shown to vary as a function of location and genotype (Meyer and Tchida, 1999; Wilson and Knox, 2006). Cultivars of *M. sinensis* are generally regarded as self-incompatible, and effective pollination and seed set requires different genotypes in close proximity (less than 4 m) with ample pollen load and overlapping bloom times (Chiang et al., 2003; Stewart et al., 2009). Pollen grains of *Miscanthus* are trinucleate, which is generally indicative of rapid germination on stigmas and short-lived viability (Friedman and Barrett, 2011). There are, however, no published reports on male (pollen) fertility for *Miscanthus* sp.

Miscanthus sp. have a base chromosome number of 19 and *M. sinensis* varieties and cultivars have been reported as diploid with $2n = 2x = 38$ (Rayburn et al., 2009). Hybridizations between species as well as across ploidy levels have been documented, including *M. ×giganteus* ($2n = 3x = 57$), a naturally occurring triploid hybrid between diploid *M. sinensis* and tetraploid *M. sacchariflorus* (Glowacka and Jezowski, 2009). As a result of the inability of three chromosome sets to divide equally during meiosis, triploid plants often suffer from reduced fertility or complete sterility (Linde-Laursen, 1993; Ramsey and Schemske, 1998). For example, *M. ×giganteus* is widely reported to be highly infertile (Rayburn et al., 2009). However, in some cases, triploids can have limited fertility resulting from formation of apomictic embryos, unreduced gametes, and the union of aneuploid gametes (Lim et al., 2003; Lo et al., 2009; Ramsey and Schemske, 1998; Sears, 1953). Determination of 2C genome size of progeny from triploid parents can elucidate the reproductive pathway by which seedlings originated (Doležel and Bartos, 2005).

Development of new triploid *M. sinensis* may produce infertile plants with low reproductive potential. Infertile forms of *M. sinensis* would be valuable alternatives for landscape and bioenergy applications. Researchers at the North Carolina State University, Mountain Crop Improvement Laboratory™ (MCI Laboratory), Mills River, NC, have developed a population of triploid *M. sinensis*, of which 18 clones were selected for having desirable ornamental and/or growth characteristics. Unlike

the full sterility documented in *M. ×giganteus*, some of these triploid *M. sinensis* were observed to produce both pollen and low numbers of seeds. To evaluate the reproductive potential of these plants, it is necessary to evaluate and document the fertility among each of the selected clones. Therefore, the objectives of this study were to evaluate selected triploid clones of *M. sinensis* for male and female fertility, examine the reproductive pathways of intraploid and interploid matings, and determine the 2C genome sizes of progeny derived from these crosses.

Materials and Methods

Plant material. The taxa used in this study consisted of five diploid (*2x*) and 18 triploid (*3x*) clones of *M. sinensis* (Table 1). Diploid plants included four commercially available cultivars and one hybrid developed at the MCI Laboratory. Tetraploid plants were developed by hybridizing *M. sinensis* 'Variegatus' with 'Strictus' followed by in vitro polypoid induction using procedures similar to Herbert et al. (2010). Triploid plants were generated by crossing these autotetraploids with selected diploids and subsequently recovered by embryo rescue. Triploid taxa selected for this study were chosen from 700 genotypes after 2 years of field trials.

Experimental design. Divisions of each genotype were propagated in 1-gallon pots and field-planted in mid-May 2010 using a randomized complete block design consisting of five replicated blocks. Each block contained 38 plants, including one of each triploid clone and four (subsamples) of each diploid clone to ensure adequate pollination. Plants were spaced on 46-cm centers in cultivated (mounded) rows with 1.2 m between rows and irrigated as needed throughout the summer using drip irrigation.

Evaluating male fertility. Male fertility was assessed by estimating the viability of fresh pollen with acetocarmine staining. Pollen was collected over a 2-week period in late Aug. 2010. Sampling was conducted randomly within blocks, one block at a time. Inflorescences were collected between 0800 and 1000 HR, placed in a sealed plastic bag, and immediately brought into the laboratory for assessment. Fresh pollen was dusted onto a microscope slide by gently shaking the inflorescence. Pollen grains were stained by adding one to two drops of 1% acetocarmine to the microscope slide and incubated for 30 min at room temperature (23 °C). Pollen grains that were well-stained and well-formed were scored as viable with 100 or more grains scored per replicate. Pollen viability was calculated by number of grains scored as viable/number of grains counted.

Evaluating female fertility. Female fertility was assessed by determining percentage of seed set and percentage of viable seeds based on germination. Whole inflorescences (up to 10 per replicate) were collected in Oct. 2010 and stored in large paper bags at room temperature until dry (8 weeks). For each replicate, spikelets were randomly sampled from different inflorescences. Up to 1000 individual florets were examined per replicate (or until 100 seeds were extracted). Seed set was calculated by number of seeds counted/number of florets examined.

Seeds collected during the seed set experiment were germinated one block at a time. Seeds were dehulled, placed on blotter paper (SDB3.5; Anchor Paper Co., St. Paul, MN), and moistened with distilled H_2O in plastic petri dishes (100 mm × 15 mm). Petri dishes were sealed with parafilm and kept at 23 ± 2 °C with a 16-h photoperiod of 30 µmol·m^{-2}·s^{-1} (400 to 700 nm) provided by cool-white fluorescent lamps. Germination was recorded after 4 weeks using radical emergence as the criterion. Total germination was calculated as seeds germinated/total seeds. Overall female fertility (percent viable seed set) was determined by: {[seed set (%) × seed germination (%)]/100}. Additionally, relative female fertility (overall female fertility of each clone in relation to the diploid control with the highest overall female fertility) was determined as: [(% seed set × % germination for given clone)/(% seed set × % germination for diploid control)].

Determining 2C genome size and ploidy level. Flow cytometric analysis was used to determine the 2C genome size and ploidy level of all parents and offspring. Young leaf tissue (≈0.5 cm^2) was finely chopped within 0.4 mL of nuclei extraction buffer along with *Pisum sativum* L. 'Ctirad', an internal standard with a known 2C genome size of 8.75 picograms (pg) (Doležel et al., 1998). Nuclei were filtered using a 50-µm nylon filter and stained using 1.4 mL 4′, 6-diamidino-2-phenylindole (DAPI) staining buffer. Genome size (DNA content of the nonreplicated chromosome complement) was analyzed with a Partec PA-II flow cytometer (Partec, Münster, Germany). Holoploid, 2C genome size was calculated as: 2C = genome size of standard × (mean fluorescence value of sample/mean fluorescence value of standard).

Evaluating reproductive pathways. From each germination trial (for three complete blocks), six germinated seedlings were chosen at random and moved to a fresh petri dish.

Table 1. Ploidy level, 2C genome size, pollen viability, seed set, seed germination, overall fertility, and relative fertility based on diploid control ('Zebrinus') of selected *Miscanthus sinensis* genotypes.

	Ploidy level (x)	2C genome size (pg)[z]	Pollen viability (%)[y]	Seed set (%)	Germination (%)	Overall fertility (%)[x]	Relative fertility[w]
M. sinensis 'Gracillimus' 2004-256	2	3.97 ± 0.03[v]	73.4 ± 5.0 abcdef	50.2 ± 6.2 c	44.6 ± 4.7 d	24.8 c	44.8 c
M. sinensis 'Superstripe' 2006-238	2	4.02 ± 0.01	77.0 ± 25 a	69.8 ± 2.5 b	71.2 ± 3.4 ab	49.7 ab	91.0 ab
M. sinensis 'Variegatus' 2004-257	2	3.94 ± 0.05	75.0 ± 3.1 abc	84.4 ± 2.7 a	52.9 ± 3.5 d	43.9 b	79.4 b
M. sinensis 'Zebrinus' 2004-259	2	4.08 ± 0.02	74.0 ± 3.3 abcde	74.4 ± 2.1 b	73.4 ± 4.8 a	55.3 a	100.0 a
M. sinensis H2006-026-006	2	4.01 ± 0.00	76.4 ± 3.3 ab	66.3 ± 2.9 b	75.3 ± 3.3 a	49.9 ab	91.4 ab
M. sinensis H2008-025-002	3	5.68 ± 0.02	72.2 ± 4.9 abcde	23.0 ± 4.6 eh	38.8 ± 5.8 d	9.1 de	16.5 de
M. sinensis H2008-026-010	3	5.82 ± 0.01	60.4 ± 6.4 efghi	7.9 ± 1.5 hijk	31.7 ± 7.4 ghij	2.8 de	5.1 de
M. sinensis H2008-027-003	3	5.91 ± 0.05	60.0 ± 4.2 fghi	16.5 ± 1.9 e	23.2 ± 1.3 ij	3.8 de	6.8 de
M. sinensis H2008-036-025	3	5.62 ± 0.01	55.6 ± 2.4 ghi	7.3 ± 1.2 hijk	35.4 ± 4.9 efghij	2.4 de	4.3 de
M. sinensis H2008-061-001	3	5.65 ± 0.02	62.0 ± 2.6 defghi	52.1 ± 4.6 c	50.8 ± 5.9 cdefg	27.2 c	49.2 c
M. sinensis H2008-063-011	3	5.47 ± 0.02	53.3 ± 4.5 ghi	5.6 ± 3.7 hijk	31.4 ± 9.9 fghj	2.5 de	4.4 de
M. sinensis H2008-064-009	3	5.73 ± 0.02	55.2 ± 3.8 hi	32.6 ± 6.8 de	23.0 ± 8.2 ij	9.6 de	17.3 de
M. sinensis H2008-065-003	3	5.81 ± 0.02	63.3 ± 2.8 cdefghi	24.9 ± 1.3 eg	69.0 ± 8.1 abc	17.0 cd	30.7 cd
M. sinensis H2008-065-006	3	5.80 ± 0.01	59.2 ± 6.7 fgh	12.9 ± 1.0 fghijk	50.8 ± 5.7 cdefg	6.5 de	11.8 de
M. sinensis H2008-067-021	3	5.62 ± 0.03	65.4 ± 3.3 abcdefgh	27.6 ± 4.5 ef	16.0 ± 2.7 j	4.3 de	7.8 de
M. sinensis H2008-071-002	3	5.77 ± 0.01	50.3 ± 6.5 i	15.8 ± 3.2 fghijk	42.2 ± 3.7 defghi	6.5 de	11.7 de
M. sinensis H2008-071-009	3	5.65 ± 0.03	54.5 ± 4.3 ghi	20.1 ± 3.7 efghij	48.3 ± 3.1 defgh	9.9 de	18.5 de
M. sinensis H2008-072-001	3	5.89 ± 0.04	62.3 ± 0.8 cdefghi	12.6 ± 2.8 fghijk	52.5 ± 9.0 cdef	6.4 de	11.6 de
M. sinensis H2008-084-002	3	5.80 ± 0.01	70.5 ± 7.0 abcdef	20.9 ± 8.1 efghi	21.3 ± 7.0 ij	6.4 de	11.9 de
M. sinensis H2008-087-001	3	5.60 ± 0.02	52.0 ± 5.3 hi	8.9 ± 1.0 ghijk	54.8 ± 8.5 cde	5.1 de	8.9 de
M. sinensis H2008-091-004	3	5.86 ± 0.02	64.6 ± 5.9 bcdefgh	0.7 ± 0.1 k	56.3 ± 7.1 bcd	0.4 e	0.7 e
M. sinensis H2008-130-001	3	5.87 ± 0.01	58.6 ± 3.0 fghi	6.3 ± 1.9 ijk	27.5 ± 5.1 ij	1.7 de	3.1 de
M. sinensis H2008-131-001	3	5.91 ± 0.01	67.2 ± 3.3 abcdefg	45.9 ± 7.5 cd	28.4 ± 5.9 hij	12.7 cde	23.0 cde

[z]2C DNA values represent the mean value of four subsamples conducted for each taxon.
[y]Mean separation within columns by Fisher's least significant difference at $P \leq 0.05$.
[x]Calculated as (seed set × seed germination)/100.
[w]Calculated as overall fertility/(0.546), where 54.6 is the percentage fertility of 'Zebrinus', a diploid control.
[v]Values are means ± 1 SEM.

After another 1 to 2 weeks of growth, 568 seedlings were subjected to flow cytometric analysis following the procedures described previously. Data from all studies were subjected to analysis of variance procedures and means separated using Fisher's least significant difference with SAS Version 9.1 (SAS Inst. Inc., Cary, NC).

Results and Discussion

The mean 2C genome size of the five diploid genotypes was 4.02 ± 0.03 (SEM) pg, whereas the mean for the 18 triploid genotypes was 5.75 ± 0.03 (SEM) pg (Table 1). The genome size of triploids was slightly less than 1.5 times that of the diploids, yet intermediate between diploid and tetraploid parents [tetraploids had a mean 2C genome size of 7.52 ± 0.04 (SEM) pg]. The decrease in 1Cx genome size (genome size of one complete set of chromosomes) with increasing ploidy level may indicate genomic downsizing, a phenomenon often observed after chromosome doubling (Buggs et al., 2009; Leitch et al., 2008; Soltis et al., 2003).

Male fertility. Pollen stainability in triploid plants ranged from 50% to 72% (60% average) compared with 73% to 77% (74% average) for the diploid controls (Table 1). Although acetocarmine staining is used routinely to estimate pollen viability, this dye indiscriminately stains DNA and cytoplasmic proteins and may stain pollen grains that are not fully functional (MacFarlane Smith et al., 1989; Slomka et al., 2010) and can thereby overestimate pollen viability. Pollen germination is a preferable method for more accurately assessing viability. However, like many grass species, pollen of *Miscanthus* is short-lived and efforts to develop reliable germination protocols have been unsuccessful.

Female fertility. Seed set was significantly lower in triploids compared with diploids ($P < 0.0001$). Seed set also differed within diploid cultivars and within triploid cultivars ($P < 0.0001$) (Table 1). Seed set data for diploid plants ranged from 50% for 'Gracillimus' to 84% for 'Variegatus', demonstrating that diploid cultivars can vary substantially in this trait. Among triploid plants, seed set varied considerable by genotype, ranging from 0.7% for H2008-091-004 to 52% for H2008-061-001. Of the 18 triploids used in this research, six had less than 10% seed set.

Miscanthus seed began germinating within 3 to 4 d after imbibing and after 4 weeks no further germination was observed. Similar to seed set, there was a significant overall reduction in germination in triploids compared with diploids ($P < 0.0001$). Furthermore, germination differed among both diploid and triploid cultivars ($P < 0.0001$) (Table 1). Germination in diploid plants ranged from 45% for 'Gracillimus' to 75% for H2006-026-006. Because we did not conduct post-germination viability tests, it is possible that some ungerminated seed was viable and dormant. In comparison, Wilson and Knox (2006) assessed both germination and post germination viability through tetrazolium staining. Their results estimated that germination of viable seed

for 'Gracillimus', 'Variegatus', and 'Zebrinus' ranged from 87% to 100%, 88% to 100%, and 94% to 100%, respectively, indicating that germination is a good indicator of viability. Furthermore, we observed higher germination in 'Variegatus' (53%) and 'Zebrinus' (73%) compared with Meyer and Tchida (1999) who reported a maximum of 16% and 60%, respectively. Germination among our triploid plants ranged from 16% for H2008-067-021 to 69% for H2008-065-003.

Overall female fertility reflects the percentage of viable seed produced among florets (Table 1). Overall female fertility ranged from 25% to 55% among diploids compared with 0.4% to 27% for triploids. The diploid cultivar Zebrinus had the highest overall female fertility, although it did not have the highest individual seed set or germination. Relative female fertility reflects overall female fertility relative to the most fertile diploid control, 'Zebrinus'. Relative female fertility also showed significant reductions in triploid plants with eight triploids having less than 10% relative fertility and four triploids having less than 5% relative fertility. The most significant reduction in fertility was observed with H2008-091-004, which had a relative fertility of 0.7% when compared with the diploid control.

Reproductive pathways. Genome sizes (2C) recorded from 568 progeny of open-pollinated diploid and triploid cytotypes provide insight into their reproductive pathways (Fig. 1). The majority (99%) of seedlings derived from open-pollinated diploid maternal parents were diploids with a mean 2C genome size of 4.13 ± 0.02 (SEM) pg. This value is slightly higher (≈ 0.11 pg) than the diploid parents measured at different times and under different conditions and may vary as a result of subtle variation in tissue chemistry and pigments that can interact with the DAPI fluorochrome (Doležel et al., 1998; Ochatt et al., 2011). Fertilization of diploid maternal parents with reduced pollen from triploids was rare; however, in four instances (1.4%), diploid seedlings displayed 2C genome size between 4.55 and 4.76 pg, suggesting diploid × triploid hybridization. One extreme outlier with 2C genome size = 6.15 pg ($\approx 3x$) was observed among the progeny from diploid maternal parents (0.4% of seedlings). This fertilization event was most likely the result of an unreduced (2n) gamete from one diploid parent pairing with a reduced (n) diploid gamete from a second diploid parent.

The majority of progeny from maternal triploids had 2C genome sizes intermediate between triploid and diploid parents with a mean of 4.67 ± 0.01 (SEM) pg (Fig. 1). We found no clear evidence of triploid × triploid hybridization (assuming random chromosome assortment in meiosis) or apomixis among triploids, which would result in progeny with 2C genome sizes of ≈ 5.8 to 6.3 pg. In some plant species, triploids are able to produce viable gametes that contain an exact haploid ($1x$) or diploid ($2x$) chromosome complement (Norrmann and Quarin, 1987; Sears, 1953). For example, odd ploidy levels are maintained in the triploid grass andropogon (*Andropogon ternatus* Spr.) through the formation of functional male gametophytes with two sets of chromosomes and female gametophytes with one set of chromosomes (Norrmann and Quarin, 1987). If gametes had complete sets of chromosomes, we would have expected to observe 3x and 4x (in addition to 2x) progeny, yet no evidence of this was found. Rather, seedlings from tiploid maternal parents displayed a normal distribution of genome sizes indicative of triploid × diploid hybridization after random chromosome segregation and aneuploid formation similar to *Andropogon gerardii* Vitman (Norrmann and Keeler, 2003) and vine cacti [*Hylocereus* (Berger)] hybrids (Tel-Zur et al., 2005). In three cases, seedlings had 2C genome sizes of 6.39, 6.71, and 7.32 pg, substantially greater than their triploid maternal
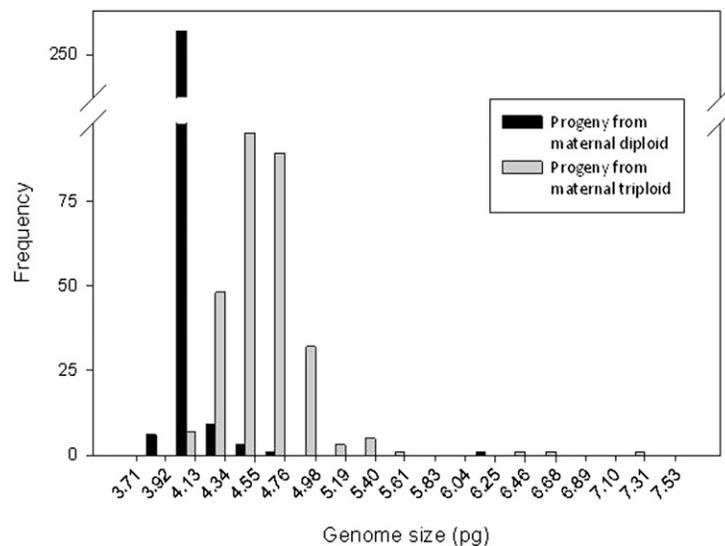

Fig. 1. Frequency distribution of 2C genome sizes of seedlings derived from open pollinated diploid and triploid *Miscanthus sinensis* genotypes. Gate width for columns was based on a 95% prediction interval for diploid plants calculated as the mean ± 1.96 SD = 0.21 pg.

parents. These three instances (1.1%) most likely represent the formation of unreduced gametes from one of the parents. The frequency of unreduced gametes among all progeny was 1.4%.

Propagule pressure is an appraisal of vegetative and gametic structures' differing abilities to advance naturalization of a species (Martínez-Ghersa and Ghersa, 2006). Assessing propagule pressure is an important measure of naturalization potential because unlike the majority of predictive factors, it has a consistent positive association with the establishment of a self-sustaining population (Colautti et al., 2006). Vegetatively, *M. sinensis* poses very little risk of spread as a result of its strong clumping habit. Native clumps in Japan had radial expansion rates on average of 2.4 cm^2/ year over a 9-year period, while at the same time, shoot density in the center of clumps declined year by year (Stewart et al., 2009). Gametic propagule pressure (seed) poses a far greater risk for naturalization of *Miscanthus* and several factors must be taken into consideration. Although our study shows that seed set of diploids can reach 84% with numerous nearby pollen donors, seed production in wild populations has been reported to be less than 20% among plants greater than 4 m apart (Stewart et al., 2009). However, seed set can be prolific with a mean of 1051 seeds per plant reported in the wild and a mean of 657 seeds (per three inflorescences) from cultivated plants in the United States (Meyer and Tchida, 1999; Stewart et al., 2009).

Decreasing fertility by reductions in pollen viability, seed set, and seed germination mitigates propagule pressure and the potential for plants to naturalize (see Anderson et al., 2006, for review). In particular, curbing fecundity to a point where more individuals are dying than are being established will inevitably lead to a decline in population growth (Jacquart and Knight, 2010; Ramula et al., 2008). For example, reducing fecundity and propagule survival by 60% was sufficient to cause population decreases in several invasive annual grasses (Tozer et al., 2008). In a study of scotch broom [*Cytisus scoparius* (L.) Link], Sheppard et al. (2002) estimated that a 62% reduction in seed set was needed to reverse dominance of this species in native grasslands. Similarly, populations of musk thistle (*Carduus nutans* L.) were estimated to begin decreasing in size pending a ≈69% decrease in seed production (Shea and Kelly, 1998). Populations of the prolific biennial weed, garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande], began declining when weevils reduced seed production by 49% (Davis et al., 2006). Overall, triploids examined in the present study had between 50% to 99% reductions in relative fertility compared with diploid controls. This represents a significant decline in fertility, which would reduce the potential for these plants to initially naturalize, particularly for highly infertile clones.

There is legislative precedence for exempting infertile cultivars from sales restriction of weedy species. For example, cultivars of butterfly bush (*Buddleia davidii* Franch.) documented to produce less than 2% viable seeds

are now considered effectively sterile by the Oregon Department of Agriculture (2011). A stipulation in the aforementioned criteria calls for the presence of abundant pollen donors to ensure legitimate results during testing. Our study included an abundance of diploid pollinators (100 plants) in close proximity to triploids. High levels of seed set among diploids confirmed favorable pollination conditions. In another example based on germination alone, Fine Line® buckthorn (*Rhamnus frangula* L. 'Ron Williams') is now being marketed as a non-invasive plant based on seed germination of 14% of that of the invasive parent species, *R. frangula* (Jacquart and Knight, 2010). Furthermore, assessment of invasive sacred bamboo (*Nandina domestica* Thunb.) suggests that some cultivars such as 'Firepower' have reduced dispersal potential, are unlikely to invade natural areas, and are suitable for landscape use (Fox et al., 2008). Favorable pollination and germination conditions in our study provided an effective evaluation of triploid reproductive potential.

In addition to reduced fertility of triploid clones of *M. sinensis*, the limited progeny derived from triploid maternal parents were predominantly aneuploids, which can exhibit (or in successive generations lead to) reduced fitness and fecundity (Ramsey and Schemske, 1998). The production of mostly aneuploidy progeny further supports use of low-fertility triploid *Miscanthus* as landscape plants. Despite triploid pollen being greater than 50% stainable with acetocarmine, data here suggest that successful *2x × 3x* and *3x × 3x* fertilization events were very rare. Therefore, this study provided optimal conditions for triploid fertilization as a result of an abundant supply of diploid pollinators. Seed production by triploid plants isolated from diploids may be extremely rare and will be examined in future experiments. This study concludes that some triploid *Miscanthus* display substantial reductions (greater than 95%) in fertility compared with diploid controls despite being phenotypically similar. Several of these clones with substantial reductions in fertility may provide desirable, noninvasive substitutes to highly fertile diploids.

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