The Relative Risk of Invasion: Evaluation of *Miscanthus × giganteus* Seed Establishment

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The sterile hybrid, giant miscanthus, has emerged as a promising cellulosic bioenergy crop because of its rapid growth rate, high biomass yields, and tolerance to poor growing conditions; these are traits that are desirable for cultivation, but also have caused concern for their contribution to invasiveness. New seed-bearing lines of giant miscanthus would decrease establishment costs for growers, yet this previously unresearched propagule source increases fears of escape from cultivation. To evaluate the consequences of seed escape, we compared seedling establishment among seven habitats: no-till agricultural field, agricultural field edge, forest understory, forest edge, riparian, pasture and roadside; these were replicated in Virginia (Blacksburg and Virginia Beach) and Georgia (Tifton), USA. We use a novel head-to-head comparison of giant miscanthus against five invasive and three noninvasive species, thus generating relative comparisons. Overall seed germination was low, with no single species achieving germination rates >37%, in all habitats and geographies. However, habitats with available bare ground and low resident plant competition, such as the agricultural field and forest understory, were more invasible by all species. Giant miscanthus seeds emerged in the roadside and forest edge habitats at all sites. Early in the growing season, we observed significantly more seedlings of giant miscanthus than the invasive and noninvasive species in the agricultural field. Interestingly, overall seedling mortality of giant miscanthus was 99.9%, with only a single 4 cm (1.58 in) tall giant miscanthus seedling surviving at the conclusion of the 6-mo study. The ability to make relative comparisons, by using multiple control species, was necessary for our conclusions in which both giant miscanthus and the noninvasive control species survival (≤1%) contrasted with that of our well-documented invasive species (≤10%). Considering the low overall emergence, increased propagule pressure may be necessary to increase the possibility of giant miscanthus escape. Knowledge gained from our results may help prepare for widespread commercialization, while helping to identify susceptible habitats to seedling establishment and aiding in the development of management protocols.

Nomenclature: Giant miscanthus, *Miscanthus × giganteus* J. M. Greef and Deuter ex Hodk. and Renvoize.

Key words: Bioenergy, biofuel, controlled introduction, giant miscanthus, habitat susceptibility, invasibility.

The pursuit of renewable fuel sources has sparked a growing interest in the cultivation of large-statured grasses for the production of bioenergy (Lewandowski et al. 2003). Many of the rhizomatous perennial grasses bear a suite of desirable traits for large-scale production such as rapid growth and biomass accumulation, minimal input requirements, and the ability to grow on marginal land. The very characteristics that make biofuels desirable crops are also shared with many invasive plants (Barney and DiTomaso 2008; Raghu et al. 2006).

Candidate feedstocks include native species such as switchgrass, *Panicum virgatum* L., and several exotic species such as giant reed, *Arundo donax* L.; napier grass, *Pennisetum purpureum* Schumach.; and giant miscanthus, *Miscanthus × giganteus* J. M. Greef and Deuter ex Hodk. and Renvoize. As evidence of the invasion concern, the California Department of Food and Agriculture preemptively added *P. virgatum* to their noxious weed list, thus preventing intentional planting or distribution, only to later remove the listing (DiTomaso et al. 2013). *Arundo donax* has landed on several state noxious weed lists (Quinn et al. 2013) because of its impact in riparian areas (Bell 1997; Katibah 1984). *Miscanthus × giganteus,* a sterile hybrid of the two documented weed species *M. sinensis* Anderss. and *M. sacchariflorus* (Maxim.) Franch., is a leading bioenergy crop in the United States and Europe (Quinn et al. 2010). Despite some risk assessments

Management Implications

There is tremendous concern about exotic bioenergy crops escaping cultivation and becoming invasive species. One such crop, the sterile hybrid *Miscanthus* × *giganteus*, possesses many desirable agronomic traits, but is expensive to plant. Newly developed fertile lines add a previously under-researched source of wind-dispersed propagules, increasing the chance for establishment outside cultivated fields. Contrary to previous studies of *M.* × *giganteus* drought tolerance using vegetative propagules, we found much lower survival of seedlings in dry environments. Of the seven habitats we examined, those with more bare ground and low competition from resident vegetation were more susceptible to invasion. Low competition environments and adequate seed to soil contact is important for seed germination. While *M.* × *giganteus* did not exhibit the same ability to establish (one of 16,000 introduced seeds survived 6 mo) as other well-known invasive species we examined, the ability of this species to produce as many as 2.5 billion spikelets ha^{-1} yr^{-1}, increases the chance of successful establishment. Based on our findings, *M.* × *giganteus* is less likely to be problematic in conventional agricultural fields subject to tillage or herbicide applications. Scouting areas near production fields or along transport routes, especially those with low resident plant competition, may help detect young plants when management is relatively easy. Low light environments did not deter seedling emergence of *M.* × *giganteus,* and therefore should not be overlooked. Young seedlings of *M.* × *giganteus* appear to have high seedling mortality rates, especially when subjected to environmental stress. However, identification of even small populations, at an early age, could be critical for effective eradication as once plants become established and develop extensive rhizomes, management is likely to be much more difficult.

suggesting a low invasion risk (Barney and DiTomaso 2008), *M.* × *giganteus* produces abundant biomass, has few pests, and requires few inputs after the establishment year (Lewandowski et al. 2003). Some characteristics are reminiscent of Herbert Baker's (1965) ''ideal weed'', and many traits are shared by some of the world's most damaging invasive species (Raghu et al. 2006).

Despite the concerns regarding invasive species, bioenergy crops are likely to be rapidly adopted and cultivated. To meet growing demands and federal mandates, projections indicate that up to 60 million ha of land could be put into dedicated bioenergy production in as little as ten years (Field et al. 2008; Robertson et al. 2008). The Southeastern United States is likely to play a large role in this emerging industry, as many feedstocks are well suited to this geographic region, with its long growing seasons and ample idle land from a shrinking tobacco market (Perlack et al. 2005). While there is considerable agronomic literature available for *M.* × *giganteus* (Heaton et al. 2010; Jorgensen and Muhs 2001; Lewandowski et al. 2000), very little addresses the invasive potential of cultivation at such a large scale. *Miscanthus* × *giganteus* was first introduced to Europe in the 1930's and more

recently to the US (Lewandowski et al. 2003), allowing relatively little time for investigation of potential consequences of large scale cultivation. Currently, there are no reports of naturalized populations despite the relatively cosmopolitan introduced distribution of this species (Lewandowski et al. 2003). However, while the spread of pests and pathogens can increase rapidly, it may take decades before plant populations become problematic (Barney and DiTomaso 2008; Crooks and Soule 1999; Mack et al. 2002). With the relatively recent introduction of *M.* × *giganteus* to the United States, it may be too early to make conclusions regarding its ability to naturalize outside of cultivation. Thus, we have an opportunity to conduct research now, providing us with valuable information to prevent and manage the invasion risk.

Grower adoption of the sterile *M.* × *giganteus* 'Illinois' clone has been slow as vegetative propagules are both costly and require specialty equipment that may not be available to many growers (Heaton et al. 2010; Lewandowski et al. 2000). With the goals of lower cost of plant establishment, and yields equivalent to or higher than existing vegetative materials; a fertile variety of *M.* × *giganteus,* known as 'PowerCane'™, has been developed (Mendel Biotechnology, personal communication). This is one of several fertile *M.* × *giganteus* lines in development.

A seeded *M.* × *giganteus* may be more economically attractive to growers. However, seeded *M.* × *giganteus* dramatically increases the propagule number produced by this species, which subsequently raises the risk of escape from cultivation. The sterile 'Illinois' clone, relying on comparatively fewer and less mobile vegetative propagules, had been regarded by the qualitative Australian weed risk assessment as being a low risk species (Barney and DiTomaso 2008). However, propagule pressure has proven to have a substantial role in the invasion process (Lockwood et al. 2005; Rejmánek et al. 2005). The number of propagules produced annually by *M.* × *giganteus,* or other candidate crops, create the potential for an ongoing source and accumulation of successfully established individuals (Davis 2009). Establishment rates are not static and the system in which propagules disperse is not homogeneous, especially over time. Once propagules are established, the new seedlings must produce propagules of their own, which face the same set of biotic and abiotic barriers as the parent, which is a function of species traits and habitat characteristics. For example, even though sterile *M.* × *giganteus* 'Illinois' has a limited ability to disperse, barring major disturbance events, the robust rhizomes likely allow for an increased chance of persistence once established. In contrast, new wind-dispersed seeds greatly enhance the ability of this species to disperse and increase the probability of escape outside the cultivated boundary, not only in habitats surrounding agricultural fields, but along transport routes as well (Barney and DiTomaso 2010).

Cultivation of the fertile lines of M. × giganteus 'PowerCane' could greatly increase the likelihood of escape beyond the production field. A single M. × giganteus plant can produce >100 inflorescences after the second year of growth, with each inflorescence producing an average of 1,270 spikelets (L. Smith, unpublished data), totaling over 2.5 billion spikelets ha^{-1} yr^{-1}. Additionally, each spikelet is subtended by a ciliate lemma (Gleason 1952) for wind-assisted dispersal. Recently, Quinn et al. (2011a) showed that 77% of the anemochorous spikelets of M. × giganteus 'Illinois' landed within 50 m of the source with 4% landing between 300 and 400 m. These spikelets, however, were infertile as they lacked an embryo, and therefore yielded a lighter fruit (~0.35 mg). Data from the same study for M. sinensis, in which 95% and 0.4% of spikelets dispersed within 50 m and between 300 and 400 m respectively, may be a closer match as spikelet weights for fertile M. × giganteus and M. sinensis are similar (~ 0.8 to 1.0 mg) (L. Smith, personal observation). This indicates that the risk of off target spread is not confined to the local agricultural field, but habitats adjacent to production fields, transport routes, and storage sites will be most at risk for invasion.

It is possible that fertile M. × giganteus will disperse not only from cultivated fields, but along the supply chain as well. Post-harvest transport from the field and at storage sites, without proper management practices, could increase the number of habitats exposed to propagules. A diverse landscape matrix will be found between the field and conversion facility, and each habitat will be uniquely susceptible to invasion from a propagule (i.e., forest is different than a pasture). The success of establishment outside of cultivation will therefore be directly linked to the propagule pressure, species traits, and ecosystem or habitat invasibility at a given time (Barney and Whitlow 2008; Lockwood et al. 2005; Rejmánek et al. 2005). A recent demographic model suggests that fertile M. × giganteus has a much greater probability of spread than sterile M. × giganteus (Matlaga and Davis 2013). This model is interesting because it requires an estimate of seedling survival, although no current published data exist. The probability of M. × giganteus invasion in this model directly hinges on the probability to germinate and establish and will ultimately be the difference between population growth or decline. For this very reason it is imperative that we gain a better understanding of the ability of M. × giganteus to germinate and establish in numerous habitats within the southeastern United States.

To empirically address the invasion potential of M. × giganteus 'PowerCane' spikelets, we use a novel methodology to relativize the establishment stage invasion potential across a range of relevant habitats. We selected diverse habitats based on a range of light, moisture, competition, and disturbance characteristics. This sample is intended to capture a range of potential habitats that are likely to be encountered in the Southeast during cultivation and transport, allowing us to determine habitats that may be most susceptible to invasion. Our methodology also includes several known invasive species in the United States, (positive controls) as well as noninvasive species (negative controls). These species were selected specifically for the geographic location and habitats used in this study. Through this design, we can make relative comparisons of Miscanthus × giganteus 'PowerCane' along an invasion gradient in which all species have some probability of being invasive across a diverse range of habitats. The objective of our study is to compare the emergence and establishment potential of M. × giganteus 'PowerCane' across a range of habitats and geographies relative to known invasive and noninvasive controls. Specifically, we aim to: (1) evaluate the ability of M. × giganteus 'PowerCane' to germinate and establish in any one of seven habitats likely to be encountered during cultivation and transport in the Southeast; and (2) determine if 'PowerCane' emergence and establishment rates are more comparable to known invasive or noninvasive species in each respective habitat and geographic location.

Materials and Methods

Site Selection. In January 2012, we initiated a controlled introduction of M. × giganteus 'PowerCane' spikelets, in which we evaluated emergence and establishment in seven habitats in three geographic locations. Seven habitats were included in Blacksburg, Virginia, USA (37°20′ N, 80°58′ W): agricultural field (no-till Roundup Ready® soybean [Glycine max (L.) Merr.] field), agricultural field edge, forest understory, forest edge, pasture, riparian and roadside (Table 1). The forest understory, forest edge, pasture and roadside habitats were replicated in Virginia Beach, Virginia (36°85′ N, 75°97′ W) and in Tifton, Georgia (31°40′ N, 83°60′ W). The agricultural field edge was only replicated at the Virginia Beach site, while the agricultural field and riparian sites were only established in Blacksburg. We understand this limits broader application in these two habitats. However, because of the proprietary nature of the 'PowerCane' spikelets, we were restricted to Virginia Tech or Mendel property, which were habitat-limited. It should be noted that other important ecological studies have been carried out using a single location (Von Holle 2005; Von Holle and Simberloff 2005), but we limit broad applicability of our results appropriately. The study was also established in Lafayette, Indiana, Schochoh, Kentucky, and Memphis, Tennessee for a much broader sampling of geographic locations. However, because of circumstances beyond our control, those sites were eradicated shortly after the first data collection.

Species Selection. The primary species of interest is a fertile M. × giganteus ('PowerCane' hereafter), which was

Table 1. Habitat selection for seed introduction in three geographic locations. Exotic invasive (positive) and noninvasive (negative) control species were selected based on location and habitat characteristics. Metrics for site characteristics ($\pm$ SE), with the exception of pH, which was measured only in January 2012, were averaged over eight data collection events from March to August 2012. *Miscanthus* $\times$ *giganteus* 'PowerCane' was introduced to all sites and categories.

Location	Habitat	Positive control	Negative control	Site characteristics				
				Moisture	Light availability	Vegetative cover	Bare ground	Soil pH
				%	PAR	——— % ———		
Blacksburg, VA	agricultural field (no-till soybean)	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	17.85 (0.95)	1298 (20)	14 (1)	86 (1)	6.41 (0.06)
	agricultural field edge	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	15.46 (0.83)	1297 (20)	80 (1)	15 (1)	5.68 (0.13)
	forest edge	*M. sinensis* *M. vimineum*	*A. gerardii* *F. rubra*	20.71 (0.99)	242 (37)	67 (2)	19 (1)	6.25 (0.13)
	forest understory	*M. sinensis* *M. vimineum*	*A. gerardii* *F. rubra*	20.26 (1.30)	160 (45)	16 (1)	17 (1)	4.59 (0.04)
	pasture	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	21.06 (1.04)	1168 (44)	94 (<1)	4 (<1)	6.08 (0.03)
	riparian	*M. sinensis* *P. arundinacea*	*A. gerardii* *P. virgatum*	27.46 (1.55)	291 (55)	94 (1)	6 (1)	5.52 (0.11)
	roadside	*M. sinensis* *S. halepense*	*A. gerardii* *F. rubra*	23.06 (1.22)	1055 (45)	59 (2)	2 (<1)	6.64 (0.27)
Virginia Beach, VA	agricultural field edge	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	37.99 (7.01)	1317 (21)	96 (<1)	4 (<1)	5.51 (0.04)
	forest edge	*M. sinensis* *M. vimineum*	*A. gerardii* *F. rubra*	27.76 (5.84)	141 (43)	41 (1)	2 (<1)	5.01 (0.04)
	forest understory	*M. sinensis* *M. vimineum*	*A. gerardii* *F. rubra*	26.68 (4.41)	58 (17)	4 (1)	30 (1)	4.92 (0.03)
	pasture	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	21.69 (1.82)	1313 (15)	91 (1)	9 (1)	5.65 (0.05)
	roadside	*M. sinensis* *S. halepense*	*A. gerardii* *F. rubra*	28.99 (4.87)	1325 (25)	92 (<1)	8 (<1)	6.19 (0.39)
Tifton, GA	forest edge	*M. sinensis* *S. arundinaceus*	*A. gerardii* *F. rubra*	18.50 (1.43)	592 (151)	86 (2)	9 (1)	6.55 (0.04)
	forest understory	*M. sinensis* *S. arundinaceus*	*A. gerardii* *F. rubra*	15.51 (1.06)	121 (50)	12 (2)	17 (1)	5.06 (0.04)
	pasture	*M. sinensis* *S. halepense*	*A. gerardii* *P. virgatum*	11.94 (1.67)	1128 (120)	70 (2)	31 (2)	4.89 (0.04)
	roadside	*M. sinensis* *S. halepense*	*A. gerardii* *F. rubra*	16.79 (1.71)	1041 (112)	74 (2)	14 (1)	5.23 (0.05)

tested in each location against two exotic invasive species (hereafter positive controls) and two noninvasive controls (hereafter negative controls) all with similar life histories (Table 2), the identity of which varied among sites. This methodology allows us to compare 'PowerCane' against a gradient of controls to relativize our otherwise isolated results. We have the ability to compare 'PowerCane' in relation to our negative and positive controls in each habitat and geographic location, thus determining placement on an invasive gradient in each habitat.

The two positive controls for each location were chosen from among: Maiden grass, *M. sinensis*; johnsongrass, *Sorghum halepense* (L.) Pers.; tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort.; reed canarygrass, *Phalaris arundinacea* L.; and Japanese stiltgrass, *Microstegium vimineum* (Trin) A. Camus. Weedy populations of all positive controls are well documented in the geographic regions of this study (Barnes et al. 1995; Holm et al. 1977; Jakubowski et al. 2011; Quinn et al. 2011b), and receive a high risk rating from the Plant Protection and Quarantine

Table 2. Species, common name, genotype and source of seed used in the introduction experiment. A total of 250 seeds of each of two exotic invasive (positive) and two noninvasive (negative) controls along with 'PowerCane' were added to each enclosure. The germination rate of each species was tested prior to commencement of the study under ideal conditions.

Species	M. × giganteus	M. sinensis	S. halepense	S. arundinaceus	M. vimineum	P. arundinacea	A. gerardii	P. virgatum	F. rubra
Common name	'PowerCane'™	silvergrass	johnsongrass	tall fescue	Japanese stiltgrass	reed canarygrass	big blue stem	switchgrass	red fescue
Accession	OP MBX-45:46:56	09s0337op	—	Matador GT	naturalized (Blacksburg, VA)	Palaton	'Suther' PNC Ecotype	Cave-in-rock	subsp. commutata
Source	Mendel BioEnergy Seeds	Mendel BioEnergy Seeds	Azlin	Turf-Seeds, Inc.	VT Lab collection	Outsidepride. com	Ernst Seeds	Ernst Seeds	Silver Lawn
Control type	unknown	positive	positive	positive	positive	positive	negative	negative	negative
Germination %	94	94	29	20	89	71	48	57	80

Weed Risk Assessment Model (Koop et al 2011; L. Smith, unpublished manuscript). The two negative controls were chosen from among: big blue stem, *Andropogon gerardii* Vitman; switchgrass, *Panicum virgatum,* and red fescue, *Festuca rubra* L. These negative controls are native to North America and are not considered highly competitive or weedy in comparison to our positive controls.

We used *M. sinensis* as a positive control in all habitats as *M. × giganteus* shares half its genetic makeup with *M. sinensis,* and because *M. sinensis* is considered a moderately aggressive invasive weed (Quinn et al. 2010), especially in the Mid-Atlantic and Southeastern United States. Thus, it is an ideal positive control for this study. We used *A. gerardii* as a negative control in all habitats as this species is frequently noted to have poor seedling establishment and low seedling vigor (Smart et al. 2003). Otherwise, we varied the species comprising positive and negative controls depending on the habitat and geography. For example, *M. vimineum,* a common understory invader (Rauschert et al. 2009), was our positive control in the forest understory, and *P. arundinacea,* a documented wetland invader (Jakubowski et al. 2011), was the positive control in the riparian site.

Germination tests were performed on all species in a greenhouse setting under ideal conditions prior to this study to determine the percent of viable, nondormant seed. Therefore, all reported germination values in this field study are based on the mean number of viable seed recorded during the greenhouse germination study (Table 2), unless otherwise mentioned.

Establishment. Within each habitat we constructed five 3.75 by 1.5 m enclosures using eight 50 cm-tall wooden stakes and aluminum window screen (30.5 cm high) to prevent off-site seed movement. Each enclosure was subdivided, in a linear pattern, into six 0.25 by 0.25-m quadrats spaced >0.5 m from the window screen boundary and 0.25 m from each other.

Each of the six quadrats within an enclosure was randomly assigned 250 seeds from five species ('Power-Cane', two positive controls, and two negative controls), except the two *Miscanthus* species, which were forced to one of the opposite ends of the enclosed plot to reduce misidentification at the seedling stage. In addition to the five plant species, one 0.25 by 0.25-m quadrat was left unplanted to account for potential resident individuals of the sown species. Seeds were surface-broadcast on January 28, 2012, coinciding with time of naturalized *M. × giganteus* spikelet dispersal or shortly thereafter (Quinn et al. 2010).

At the time of seed sowing, two pooled 2.5 by 30.5-cm soil samples were taken within the boundary of each of the five replicated enclosures at each habitat in all three locations. Soil samples were submitted to the Virginia Tech

Soil Testing Laboratory for analysis of soil pH and macro- and micronutrients. We began recording seedling emergence biweekly beginning February 15 at Tifton, GA and March 21, 2012 at Blacksburg and Virginia Beach, VA. Volumetric soil moisture was recorded bimonthly using a TH300 soil moisture probe (Dynamix, Inc. Houston, Texas, USA), in which three subsamples were taken in each of the enclosures. An AccuPAR LP-80 PAR/LAI ceptometer (Decagon Devices, Inc., Pullman, Washington, USA) was used to collect photosynthetically active radiation (PAR), with three subsamples per enclosure recorded bimonthly. Plant morphology data, including height (ground to tallest collar), number of culms, and number of flowering culms were collected on a monthly basis. The plant community in each quadrat was also assessed for percent cover of resident vegetation, as well as percent bare ground at monthly intervals.

During this study all habitats were managed as would be expected for that site. For example, the roadside was mowed in accordance with Department of Transportation schedules in Blacksburg, VA, and carried out at the other two locations at the same time for consistency. Pastures were mown at the same time as the surrounding habitat determined by research farm technical staff at the Kentland Research Farm in Blacksburg, VA, and again carried out in the other two locations at a similar time. The agricultural field in Blacksburg, VA received a 4% preplant application of glyphosate (Gly-4 Plus®, Universal Crop Protection Alliance, Eagan, Minnesota, USA) on May 4, 2012 and was planted with Roundup Ready soybeans (Monsanto, St. Louis Missouri, USA) on May 11, 2012. Soybeans were planted in 76-cm rows with the 0.25 by 0.25-m quadrats in each enclosure falling between two rows. The agricultural field edge, riparian, forest understory and forest edge received no management.

On August 15, after 6 mo of observation, the experiment was terminated. All introduced plants were eradicated from within the enclosures to minimize the chance of future spread of the introduced seeds. Scouting in all geographic regions and habitats followed in the spring and summer of 2013. Any seedlings deemed to be introduced from this study were removed by hand weeding.

Statistical Analysis. Our data were zero-inflated because of low emergence and establishment rates common in germination/establishment studies (Martin et al. 2005). To meet all criteria for parametric testing, a two-step process was used for analysis. First, we created two sets of data, one based on actual numeric counts of all emerged seedlings within a plot, and one based on overall presence or absence of our target species in each plot. Data at the time of maximum and final emergence events for each replicated enclosure were evaluated for presence or absence of species across locations. To further examine the influence of habitats at each geographic location, we also analyzed each location separately. Data were analyzed using nominal logistic regression with species designation (positive and negative controls), habitat, block and location as independent variables. Second, all zero data points were removed, leaving only numeric data for emerged individuals. Then, the maximum and final emergence percentages, while controlling for germinability of each species (Table 2), were Box Cox Y transformed to normalize the data (Box and Cox 1964). A mixed effects model was used including location and enclosure as random components and habitat and species as fixed effects. A linear regression was used to test for correlation between the availability of bare ground, light availability, soil moisture and soil pH and nutrient availability in each 0.25 by 0.25-m quadrat of the enclosure and the number of emerged individuals at the maximum emergence event. Again, all numeric data were Box Cox Y transformed to meet assumptions. All data from this study were analyzed using JMP 10 statistical software (SAS Institute, Cary, North Carolina, USA).

Results

Emergence for all species and locations was universally low, with no single species achieving emergence rates >37%. Emergence (P = 0.0067) and the maximum number of seedlings (P < 0.0001) varied across the geographic sites (Table 3). However, because of the very low number of mature plants and seedlings we observed at the final data collection on August 15, location did not have a significant effect on final establishment (P = 0.2291). The maximum number of seedlings (P = 0.0019) also varied among habitats with the highest overall emergence observed in the agricultural no-till soybean field (14.8%) (until herbicide application) and the forest understory (14.3%). Lowest emergence for all species categories occurred in the pasture (5.1%), forest edge (5.8%) and roadside (5.3%) (Figure 1). Differences in habitat characteristics such as vegetative cover and percent bare ground (Table 1) influenced seed emergence and likely explains some of the variation among the seven habitats. The percent bare ground was positively correlated with emergence for positive controls (R^2 adjusted = 0.1103; P = 0.0013) and 'PowerCane' (R^2 adjusted = 0.2358; P = 0.0038) across all locations, but not correlated with the emergence of negative controls (P > 0.05). Vegetative cover was negatively correlated with seedling emergence for positive (R^2 adjusted = 0.0641; P = 0.0124) and negative controls (R^2 adjusted = 0.0564; P = 0.0157), but not a significant factor in the emergence of 'PowerCane' (P > 0.05). Light availability, soil moisture and nutrient availability were not correlated with 'PowerCane' seed emergence.

We also evaluated each location separately to further assess factors that may contribute to the success of

Table 3. Regression of seedling emergence, assessing presence/absence or numerical counts at the maximum and final data collection events, from March to August 2012. A single exotic invasive (positive) and noninvasive (negative) control species was also selected in each habitat for further analysis with 'PowerCane'.

	Logistic regression of presence/absence data				Mixed model regression for numeric data		
	df	Maximum emergence	Final establishment	Select +/− (max emerg)	df	Maximum emergence	Select +/− (max emerg)
Location	2	**0.0067**	0.2291	0.1297	2	**<0.0001**	**0.0001**
Rep	4	0.7185	0.6653	0.4966	4	0.2965	0.1676
Species designation	2	1.000	1.000	1.000	1	0.0526	0.9228
Habitat	6	**<0.0001**	**0.0099**	**<0.0001**	5	**0.0019**	**0.0002**
Species designation × habitat	12	0.0863	0.9777	**0.0044**	11	0.1123	0.1676
Blacksburg, VA							
Rep	4	0.2916	0.8483	0.6467	4	0.8814	0.5939
Species designation	2	1.000	1.000	1.000	1	0.5144	—
Habitat	6	**<0.0001**	1.000	**<0.0001**	4	**0.0002**	**0.0003**
Species designation × habitat	12	0.2916	0.9896	**0.0098**	10	**0.0481**	**0.0321**
Virginia Beach, VA							
Rep	4	**0.0003**	0.7804	**0.0210**	4	0.2448	0.1063
Species designation	2	**0.0002**	1.000	**<0.0001**	1	0.3887	0.2208
Habitat	4	**0.0010**	1.000	**0.0109**	1	0.8966	0.3889
Species designation × habitat	8	**0.0092**	1.000	**0.0200**	5	**0.0266**	0.0854
Tifton, GA							
Rep	4	0.1493	0.3455	**0.0306**	4	0.8969	0.8695
Species designation	2	1.000	1.000	1.000	0	—	—
Habitat	3	**<0.0001**	**0.0045**	**<0.0001**	1	0.6331	0.9262
Species designation × habitat	6	**0.0395**	1.000	0.1103	3	0.9690	0.8907

'PowerCane' seedling establishment. We observed the highest overall emergence rates in Blacksburg, VA, with 61% of the 175 individual quadrats having at least one seedling. Again, habitat was an important factor in emergence with the greatest number of seedlings in the forest understory and agricultural soybean field (Figure 2). At maximum emergence, positive and negative controls performed equally in all habitats. 'PowerCane' did, however, have higher (21.6%) maximum emergence, than the positive (15.5%) and negative (12.5%) controls in the agricultural no-till soybean field. However, after 6 mo, no 'PowerCane' seedlings remained in any of the habitats in Blacksburg, VA, and with the exception of a single *A. gerardii* (negative control) seedling in the agriculture field edge, only positive control seedlings remained (Figure 2). For example, *M. vimineum* had higher percent emergence in the forest edge and forest understory than any other single species introduced to those habitats (Table 3). *Microstegium vimineum* quadrats had an average of 80.6 (36.%) and 22.2 (10.0%) seedlings in the forest understory and forest edge respectively, while, 'PowerCane' produced a maximum of 31.4 (8.7%) seedlings per quadrat in the forest understory and no 'PowerCane' seedlings were ever observed in the forest edge. The closely related *M. sinensis* was the only positive control species found in the agriculture field edge, with 2.1% of seedlings at the conclusion of this study (Figure 2).

Percent emergence of 'PowerCane' was lower at the Virginia Beach location with seedlings observed only in the agriculture field edge (3.7%) and pasture (1.8%) habitats (Figure 2). In contrast, positive and negative controls germinated in all habitats in Virginia Beach, VA. Maximum emergence of the positive and negative controls was significantly greater than 'PowerCane' at the pasture habitat while no measurable differences were found between any of the species designation categories in the agriculture field edge (Figure 2; Table 3). As in Blacksburg, VA, the only seedlings remaining at the end of the study in Virginia Beach were positive controls, with *M. vimineum* again producing the largest maximum and final number of seedlings in the forest edge (4.5% maximum and 0.7% final) and forest understory (22.1% maximum and 3.7% final). *Sorghum halepense* was the only remaining species at the agriculture field edge and roadside habitats.

We saw a different trend at the Tifton, GA location, with significantly greater presence of negative controls in
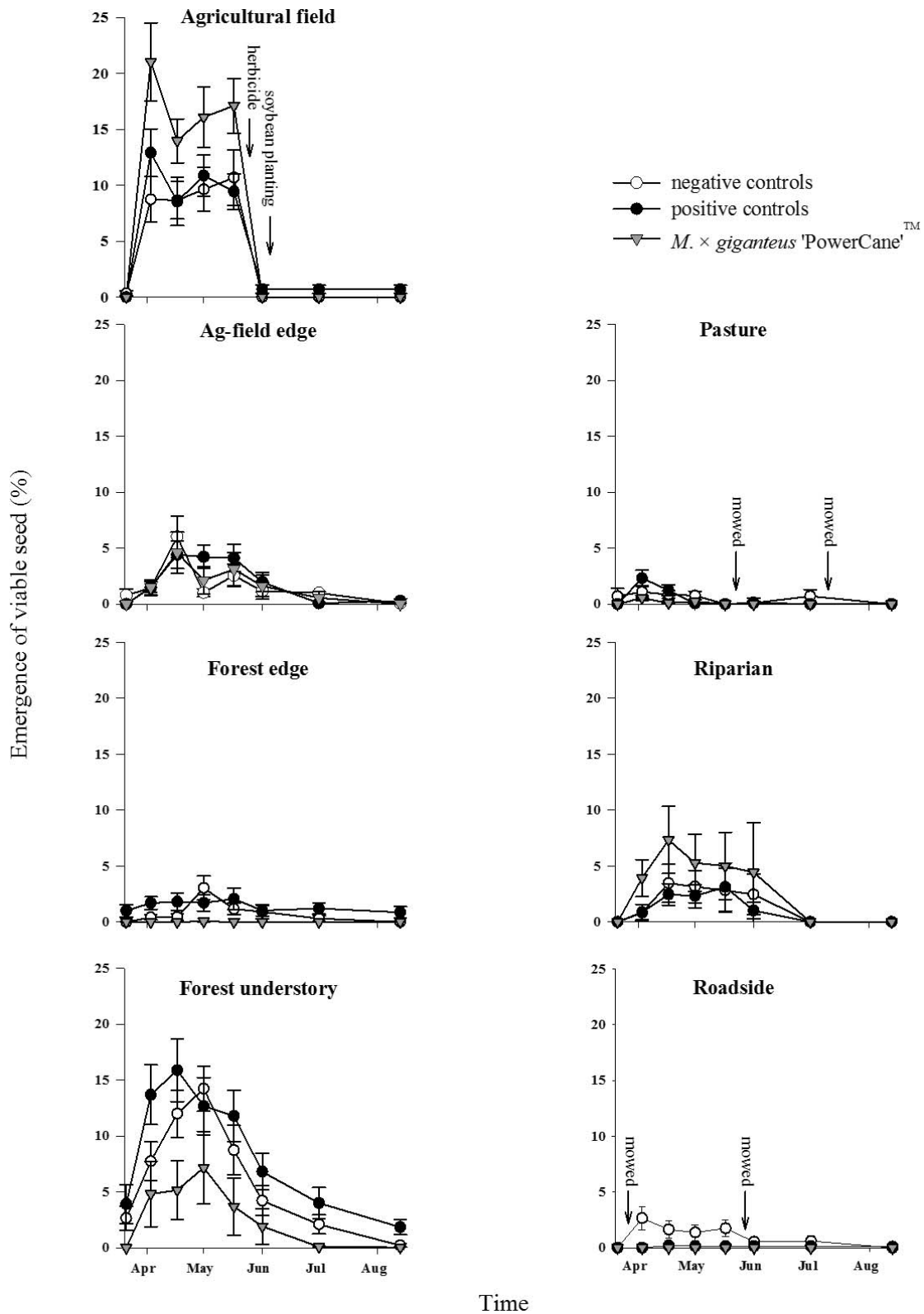
Figure 1. Mean percent emergence, of viable seed, observed across three geographic locations at each of the seven habitats. Data were recorded bimonthly from March 15 through June 1 2012 and monthly thereafter.
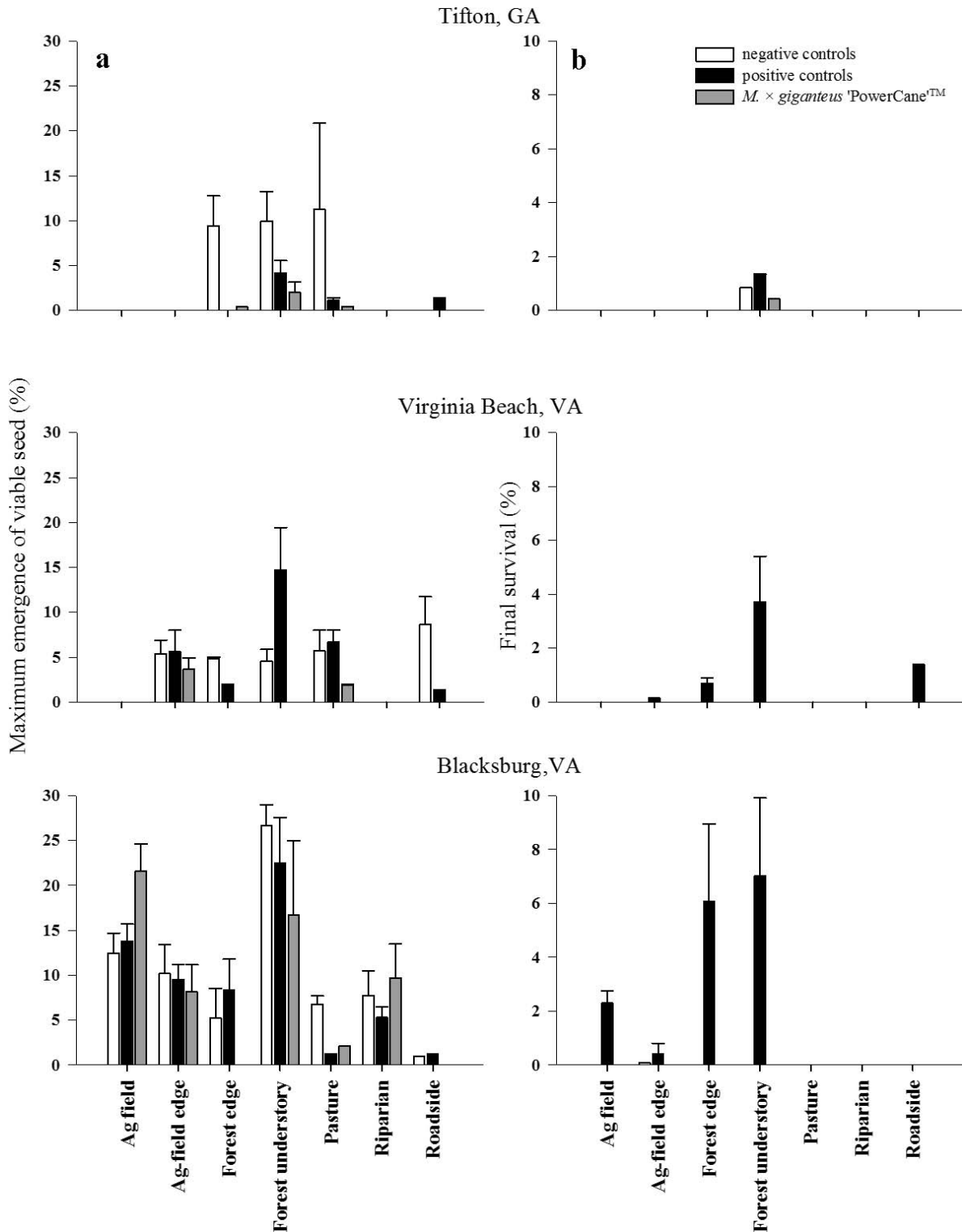
Figure 2. Mean percent emergence observed at the (a) maximum and (b) final data collection events. Timing of the maximum emergence event was variable depending on the species, location and habitat. The final data collection event, recording the final percentage of surviving seedlings, occurred on August 15,2012. Lack of error bars indicates the presence of either a single seedling or all observed seedlings were found within a single replicate.

the forest edge, forest understory and pasture habitat quadrats than 'PowerCane', or our positive controls (Figure 2). However, because of the low overall emergence at this site, we are unable to detect any statistical differences between the maximum observed values of the species designations in this study (Table 3). *Festuca rubra* was the dominant negative control in the forest edge (13.5% maximum observed emergence) and forest understory 10.75% maximum observed emergence), but was only present in six of ten subplots, while *A. gerardii* was the dominant negative control in the pasture (11.25% maximum observed emergence) but only found in two of the five replicated sub-plots. Also deviating from the other two locations is the low final observation of positive controls and the comparatively high presence of negative controls (15) as well as a single 'PowerCane' seedling in the forest understory (Figure 2). Of the 16,000 'Power-Cane' spikelets introduced across all locations, habitats, and replications, only one remained at the conclusion of this study, and it was only 4 cm tall.

While a small number of total seedlings remained at the end of this study (205), not all are likely to have permanently established. The small size and lack of flowering culms, may suggest that seedling mortality would continue to increase. Again, because of the low final number of seedlings, and that only positive controls remained in the Blacksburg and Virginia Beach, VA locations, statistical analyses were not possible for final height, culm number, and inflorescence presence. Despite morphological differences between species selected for this study, the remaining positive controls were almost three times as tall as the few negative controls observed at the final data collection in mid-August (data not shown). *Sorghum halepense* seedlings grew rapidly and remaining plants in Blacksburg and Virginia Beach, VA achieved a mean height of 118 cm and produced an average of 12.2 culms, and 4.5 inflorescences. While no *M. vimineum* plants flowered during this study, plants reached a mean height of 20.1 cm and produced an average of 3 culms. *Miscanthus sinensis* seedlings remained only in the Blacksburg, VA agriculture field edge and Tifton, GA forest understory and attained an average height of 12 cm and never produced more than a single culm. Similarly, *S. arundinaceus*, also remained in the Tifton, GA forest understory at a mean height of 13 cm. As mentioned earlier, aside from a single 10 cm *A. gerardii* seedling at Blacksburg, VA, the 12 other individual negative controls remaining at the completion of this study were found in the forest understory at Tifton, GA consisting of 11 *F. rubra* (14 cm) and one *A. gerardii* (18 cm).

Discussion

We observed low emergence and establishment for all species across all habitats and locations over the course of

the study. However, our introduction size of 250 seeds (or 4,000 seed m^{-2}), which is approximately equivalent to four mature 'PowerCane' inflorescences m^{-2}, is very small compared to the propagule potential of a *M.* $\times$ *giganteus* production field, which could total more than 2.5 billion spikelets ha^{-1} yr^{-1} (250,000 spikelets m^{-2} within the field). Conversely, depending on location and distance from the production field this may be considered a high seed density, as the number of seeds per unit area drops exponentially from the source (Quinn et al. 2011a). The contribution of biotic factors such as soil fungi and seed predators to seedling mortality is unknown, but is not entirely unlikely (Dalling et al 2011). Environmental factors, such as temperature and precipitation, likely played a role in the low emergence and survival rates we observed in Tifton, GA and possibly Virginia Beach, VA as well. According to the United States Drought Monitor Archives (2013), Tift County, Georgia experienced "exceptional drought" April 17 through May 29, 2012 and remained under moderate drought for the duration of the experiment. Although, later in the growing season, this drought was punctuated by severe rain events in Tifton, GA, as we observed periodic deposits of debris in and around the habitat enclosures. These deposits were also observed in the low lying forest and forest edge habitats at Virginia Beach, VA following rain events, potentially damaging young seedlings or smothering them with heavy wet leaf litter. Interestingly, further analysis of weather conditions from the National Climatic Data Center (2013) suggests that, despite drought conditions, monthly precipitation was within one standard deviation of the ten year average for all locations with the exception of April at Virginia Beach, Virginia. This suggests that the precipitation during this study was not unusual for these regions, but still may have contributed to lower than expected germination, especially for the positive controls.

Previous studies have indicated that *Miscanthus* spp. have a broad range of environmental tolerances (Heaton et al. 2010), including drought tolerance. *Miscanthus sinensis* is reportedly the most drought tolerant member of the genus, with the ability to effectively reduce leaf conductance and maintain leaf area at very low soil moisture (Clifton-Brown and Lewandowski 2000). *Miscanthus* $\times$ *giganteus* also shares this drought tolerance to a degree, but soil water availability has been reported as the single most limiting factor determining biomass accumulation (Richter et al. 2008), and reduces the probability of establishment (Barney et al. 2012). Many of the drought tolerance experiments involving *Miscanthus* spp. have been carried out on mature plants or from large vegetative propagules (Barney et al. 2012; Clifton-Brown and Lewandowski 2000; Clifton-Brown et al. 2002; Dougherty 2013). Subsequent greenhouse studies suggest that young seedlings of *Miscanthus* spp. are sensitive to both drought and

overwatering (L. Smith, personal observation). It is possible that at the fragile seedling stage, both *Miscanthus* spp. in this study showed less drought tolerance than previously observed in older, larger plants. Further data are needed for such conclusions, though this may in part explain why we observed greater numbers of seedlings at the Blacksburg location (162% more seedlings than Virginia Beach and Tifton combined), which did not experience drought conditions in the summer of 2012 (United States Drought Monitor Archives 2013).

Many factors influence propagule establishment, including propagule quality, timing, abiotic factors, and the receiving habitat (Barney and Whitlow 2008; Lockwood et al. 2005; Rejmánek et al. 2005). *Microstegium vimineum* (positive control) met expectations for its ability to establish in the shaded forest edge and forest understories. *Sorghum halepense*, however, underperformed as one of the Southeastern United States most notorious weeds (Anderson 1969; Holm et al. 1977). The *S. halepense* seed lot we used had low germination rates and may have been characterized by predominately under ripe seeds. Additionally, many of the *S. halepense* seeds that did germinate, did so much later in the summer than the other species. At this time competition for light and particularly space may have been greater than in early spring when the other species germinated. Despite the low number of *S. halepense* seedlings observed in this study, once germinated, plants grew rapidly and flowered within 2 mo of initial observation. Once established, *S. halepense* not only produces copious amounts of seed (an average of 28,000 seeds plant^{-1}) but spreads rapidly from expansive creeping rhizomes (Warwick and Black 1983). The numerous inflorescences and rapidly expanding number of culms in such a short time would likely contribute to its persistence and eventual spread in the observed habitats.

Riparian habitats are well-documented sites of invasion success because of the periodic disturbance caused by flooding which can scour banks and leave gaps in vegetative cover (Ellenberg 1988; Pyšek and Prach 1994; Walker et al. 1986). Between March and August, the riparian habitat in Blacksburg, VA experienced a 10% increase in vegetative cover and a decrease in light availability of 700 PAR (832 to 33 μmol photons m^{-2} s^{-1}). The riparian site, initially dominated by mosses and spring ephemerals, later gave way to many tall and competitive species such as *Rubus* spp. and *Verbesina alternifolia* (L.) Britton ex Kearney. While we initially saw some of our highest emergence rates in the riparian location for all species, especially 'PowerCane', it is possible that the increased dominance of resident vegetation and reduction in light availability by mid-June, resulted in the high mortality at this site.

To add context to our study, we selected species that exhibit different traits and tolerances to the geographic locations and habitats. The native *A. gerardii* and *P. virgatum* are notoriously difficult to establish, even in a cultivated setting (Lewandowski et al. 2000; Lewandowski et al. 2003). Previous studies indicate that *P. virgatum* establishment in cultivation may be so low that populations are undetectable in the establishment year, especially when weed pressure is high (Hintz et al. 1998; Lewandowski et al. 2003). Our results agree with this conclusion, as emergence of both *P. virgatum* and *A. gerardii* was negatively correlated with increasing resident vegetation. We observed greater emergence rates from some of the negative controls, notably *F. rubra*, than our well-documented weedy species. This was not entirely unexpected as *F. rubra* is a common turfgrass species (Beard 1973), and would therefore need to be competitive in order to meet landscape and recreational expectations. However, we selected this variety of turfgrass because we expected higher levels of germination than our other negative controls, but we assumed that its environmental tolerance to the selected habitats may be lower than both *A. gerardii* or *P. virgatum*. *Festuca rubra* is a cool season grass known to thrive in cool humid regions with tolerance to low light (Beard 1973), which does not necessarily coincide with the high light environments and southern geography. In many cases *F. rubra* emergence alone increased the average negative control maximum emergence rate to a value greater than that of 'PowerCane' and the positive controls (Figure 2), further supporting the notion that invasive potential spans a continuum.

Through the course of this study, 'PowerCane' emergence was at times, and in select habitats, greater than that of both the positive and negative controls (Figures 1 and 2). In the end, 'PowerCane' performance and final establishment (only one seedling remained) are not only lower than our positive controls, but also lower than the few remaining negative controls. This is not surprising as the congeneric *M. sinensis* emergence and establishment was also very low, with only a small number (14) of seedlings remaining. Other studies indicate that first year survival of *Miscanthus* spp. may be low. Matlaga et al (2012) observed establishment year survival of sterile *M. × giganteus* to be as low as 24% using various sized rhizome propagules. Another agronomic study, which surface broadcast *M. sinensis* seed, yielded 3% survival 12 wk after sowing (Christian et al 2005). However, *M. sinensis* is a documented weedy species, which has escaped cultivation since its introduction in 1893 (Anonymous 1984; Quinn et al. 2010). Yet not all cultivars of *M. sinensis* appear to be equally successful, as some have shown to produce almost no viable seed, while others exhibit germination rates >80% (Meyer and Tchida 1999). Again we are aided by the nature of our design, which suggests *M. sinensis* and 'PowerCane' emergence and establishment was similarly low in the seven habitats. Both *Miscanthus* spp. produce

spikelets with ciliate lemma that prevent good seed to soil contact, which also likely reduced the germination ability. We intended to simulate a natural dispersal event by surface broadcasting the seeds, as opposed to sowing or planting the seeds, which surely would have increased the germination rate. With our knowledge that some cultivars of this congeneric species have the ability to persist outside ornamental cultivation, we should use caution before discounting the ability of any fertile M. × giganteus cultivars to establish in habitats away from the cultivated field.

Barney and Whitlow (2008) argue that we frequently define artificial boundaries or define units of analysis for systems that are more appropriately, and in reality, a continuum. Exotic plant species are frequently classified as invasive, when in reality it is populations, in a given region or habitat that are invasive (Bauer 2006). Thus, we put the label of invasive on species as a whole, when in fact it is the interaction of species and habitats that result in invasions (Barney and Whitlow 2008). Some species will inherently perform better in shaded forest understories while others are better adapted to disturbed roadsides, as all invasive species are not invasive in every habitat within their introduced range (Barney and DiTomaso 2010). As mentioned earlier, 250 seeds may have been too few seeds to introduce given the known seed yield of several species in this study. Had we not used multiple species to make relative comparisons, we may have drawn vastly different conclusions. The low emergence and high mortality of 'PowerCane' would have likely suggested that growth outside of cultivation would be unlikely. Our results suggest that 'PowerCane' performance in these habitats is more closely related to that of P. virgatum or A. gerardii. However, the low overall emergence for our positive control species as well, suggests that the likelihood of establishment, for all species in this study, would likely increase as propagule pressure increases (Lockwood et al. 2005). Species such as S. halepense and M. sinensis can produce several thousand seeds per plant (Quinn et al. 2011a; Warwick and Black 1983). It has been inferred that differences in propagule pressure influence observed results of invasion history in different habitats (Kempel et al. 2013; Levine 2000). 'PowerCane' seed production from mature plants is similar to that of M. sinensis (L. Smith, unpublished data), although further study is required to assess seed viability and longevity. The seed coat of 'PowerCane' is reportedly only one cell layer thick (Mendel Biotechnology, personal communication) and has low nutrient reserves (Lewandowski et al. 2003). Studies have shown that even in a cultivated setting, mortality may be high, especially if good seed-to-soil contact is not achieved (Christian et al 2005). While species such as M. vimineum and S. halepense were able to establish at low propagule numbers, the innumerable Miscanthus seeds that would be produced in a cultivated agricultural setting suggest that some seedlings may disperse and establish.

Our results suggest that establishment may be difficult to achieve as a suitable site with low competition and appropriate environmental conditions (high site invasibility) may be important for M. × giganteus at this stage. Further study or replication may help to determine the climatic limitations of M. × giganteus seedlings. However, if propagule pressure is high enough, some individuals may survive as high level of propagules have been shown to overcome an environment that is perceived to have low invasibility (Davis 2009; Williamson 1996). Conversely, it may take only a small number of propagules for establishment in a favorable habitat, given the vast landscape and certainty of some available open space. Therefore, to prevent what is likely the inevitable accidental introduction of some 'PowerCane' or other fertile M. × giganteus propagules, it is imperative that we use data of this kind to design appropriate management strategies. The greatest number of germinated seedlings were observed in the agricultural field (Figure 1), but were effectively eradicated with conventional agricultural practices. It is likely that the absence of a winter crop and the availability of bare soil allowed for early seedling recruitment. However, as long as agricultural fields are not left fallow it seems unlikely that seedlings will persist under normal management strategies such as tillage and herbicide applications. The small, light seed and ciliate lemma (Gleason 1952), seem to interfere with the ability of the seed to make the important seed to soil contact required for germination of 'PowerCane', especially when organic matter or leaf litter is present. Quinn et al. (2011a) suggest that most Miscanthus spp. seed dispersal will occur within 50 m to the parent plant, making scouting habitats near large cultivated areas of fertile Miscanthus spp. or open storage areas an important management strategy. As previously stated, if 95% of filled seed disperse within 50 m (Quinn et al 2011a), the remaining 5% still yields a very high number of potential escapes when considering the billions of seed produced in a production field. Areas of high disturbance or with minimal vegetation are undoubtedly the most susceptible to seed establishment.

It is important to remember that many other staple crops, also members of the Poaceae Family, are already cultivated at a similar scale. Some of these species such as rye, Secale cereale L., oats, Avena sativa L. rice, Oryza sativa L., etc., do produce weedy escapes (DiTomaso and Healy 2007), but the agricultural and economic benefits outweigh the perceived impacts to natural, anthropogenic or agricultural ecosystems (Martin et al. 2006). The use of 'PowerCane' or other fertile M. × giganteus cultivars could improve grower adoption but the invasive potential and ecosystem impacts of widespread cultivation still require further evaluation. We have attempted to evaluate the early

establishment phase of invasion, which is a small part of the cyclical process. The knowledge gained from our results, may help prepare for widespread commercialization, while helping to identify susceptible habitats. The ability to rapidly detect new populations at an early stage and provide a targeted response will greatly enhance in the development of methods for improved stewardship.

Acknowledgments

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