Ecology and niche characterization of the invasive ornamental grass *Miscanthus sinensis*

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ABSTRACT

The recent trend in bioenergy feedstock development focuses on the use of large-statured perennial grasses that pose a relatively high risk of becoming invasive species due to the similarity in desirable agronomic traits with those of many of our worst invaders. Thus, it would be prudent to evaluate the potential ecological benefits and consequences of widespread cultivation of potentially invasive species. *Miscanthus sinensis* and its sterile daughter species, *Miscanthus* × *giganteus*, are two prominent bioenergy feedstock candidates due to their low input requirements and significant biomass production in a broad range of growing conditions. Despite being an extremely popular ornamental grass, and naturalizing in over half of US states, little is actually known about the biology, ecology and niche requirements of *M. sinensis*. Thus, the objective of our research is to characterize extant *M. sinensis* populations, and evaluate the niche requirements, especially in terms of the commonly limiting resources of light and soil moisture. In order to better assess the risk of *M. sinensis* (and subsequently *M.* × *giganteus*) cultivation, we surveyed 18 naturalized populations across the east coast to characterize habitat preferences, population structure, and plant performance across a latitudinal gradient. We found the vast majority of *M. sinensis* populations occurred in areas of high and low resource availability (e.g. soil nutrients and light) along roadsides and forest edges, with outlier individuals found in forest understories. We conducted a greenhouse study to compare shade and soil moisture tolerance among common ornamental cultivars and naturalized populations, where we found enhanced plant growth and vigor in naturalized biotypes compared to ornamentals across varying levels of shade. We also found that both naturalized and ornamental biotypes were not significantly affected by soil moisture stress, and thus express significant drought tolerance. Finally, we investigated the temperature and moisture requirements of *M. sinensis* seeds and determined a base temperature of approximately 8°C, as well as variable moisture and time to germination requirements between varieties and seed sources. These basic ecological studies will help refine and support future evaluations and weed risk assessments of both *Miscanthus sinensis* and *M.* × *giganteus,* which is critical in prevention of major ecological invasions.

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“If I have seen further it is by standing on the shoulders of giants.”
– Isaac Newton

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CHAPTER 1

Natural history survey of the ornamental grass *Miscanthus sinensis* in its introduced range

ABSTRACT

Miscanthus sinensis is a perennial grass native to Asia, but since its introduction to the United States in the late 19th century, it has become both a major ornamental crop and invasive species. Previous studies of the ecology of *M. sinensis* in both its introduced and native ranges have suggested that it may be occupying a novel ecological niche in the introduced range. *Miscanthus sinensis* and its daughter species, *Miscanthus* × *giganteus*, are under evaluation as bioenergy crops; therefore, characterization of the ecology and environmental niche of *M. sinensis* is essential to mitigate the risk of fostering future invasion in the US. In July 2011, we surveyed 18 naturalized *M. sinensis* populations spanning the US distribution, covering a 6° latitudinal gradient from North Carolina to Massachusetts. *Miscanthus sinensis* populations ranged in size from 3 to 181,763 m^2 with densities between 0.0089 and 2.2 individuals m^{-2}, and strongly favored highly disturbed and unmanaged habitats such as roadsides and forest edges. Population size and individual plant morphology (i.e., tiller height, basal diameter, and tiller number) were not affected by soil characteristics and nutrient availability, though increased canopy cover reduced population size ($p < 0.0001$). Plant size and vigor were not significantly affected by low light availability, which supports previous suggestions of enhanced shade tolerance of *M. sinensis*. In summary, *M. sinensis* can tolerate a broad range of climatic conditions, light availability, and nutrient availability in the eastern US, suggesting risk of further invasion beyond its current distribution in the US.

Keywords: biofuel (or bioenergy), invasive, roadside

Introduction

Miscanthus sinensis (Maiden Grass, Chinese Silvergrass) is a C_4 perennial grass native to eastern Asia, but is a wildly popular ornamental grass in the US. The earliest known introduction of *M. sinensis* to the United States was from Japan to Asheville, North Carolina in 1893 and later to Washington, DC in 1894 (Anon 1984; Quinn et al. 2010). In 1907 the Biltmore Nursery in Asheville, NC had at least four varieties available for sale via mail order catalog (Alexander 2007), and by 1913 *M. sinensis* had naturalized in New York, Florida, and Washington DC (Britton and Brown 1913). *Miscanthus sinensis* had "become thoroughly established in many places" in West Virginia as early as the 1940's (Core 1941). Despite escapes, the ornamental grass industry has boomed in the last 20 years largely due to their pleasing aesthetic and low input costs.

As a result of multiple introductions from Asia, traditional breeding programs, and commercialization, there are now over 100 named cultivars of *M. sinensis* available on the market (Darke 2007; Quinn et al. 2010). *Miscanthus sinensis* has become one of the most popular and highly recommended ornamental grasses in the US (Maynard 2012). As recently as 2008, sales of *M. sinensis* totaled nearly $40 million in the state of North Carolina alone (Trueblood 2009). Currently, *M. sinensis* has naturalized in at least 25 states (EDDMapS 2012), and its known distribution continues to expand as more populations are added to public databases. Of the 25 states with naturalized populations, 85% are east of the Mississippi River; however, occurrences have also been recorded in western states such as California and Colorado.

New interest in *M. sinensis* has arisen for use as a potential bioenergy feedstock itself, as well as breeding germplasm for novel lines of high biomass producing crops (e.g., *Miscanthus* × *giganteus)* (Zub and Brancourt-Hulmel 2010). Candidate bioenergy crops must possess a litany of agronomic characteristics to fulfill both economic and production requirements, which include perenniality, C_4 photosynthesis, few pests, rapid growth, high water-use efficiency, and tolerance of poor growing conditions (Barney and DiTomaso 2010; Raghu et al. 2006). Unfortunately, these traits are shared with many of the most problematic weeds and invasive species (Raghu et al. 2006); thus, bioenergy crops pose a potential invasion risk. *Miscanthus sinensis* is known to express many of these desirable traits, including C_4 photosynthesis, rhizome production, rapid growth, and tolerance to cold and frost (Stewart et al. 2009; Zub and Brancourt-Hulmel 2010; Zub et al. 2012). These traits contribute to its immense popularity as an ornamental, as *M. sinensis* cultivation requires relatively few inputs. However, these same traits may have contributed to the success of *M. sinensis* beyond its planted boundaries.

Before large-scale cultivation of any bioenergy crop begins, it is important to understand and assess the potential agricultural, ecological, and economic risks that may come with introducing exotic species to novel ecosystems (Barney and DiTomaso 2010; Yokomizo et al. 2012). Previous comparisons of *M. sinensis* populations from the native and introduced (i.e., US) ranges have suggested that it may be shifting into a novel ecological niche in the introduced range due to variation in climate and edaphic factors (Quinn et al. 2011b). Invasive species often perform better and grow larger in novel ecosystems for a variety of reasons, including release from pathogens, predators, and competition (Firn et al. 2011; Thébaud and Simberloff 2001). The potential of an ecological niche shift raises the importance of understanding the history, general ecology and habitat preferences of *M. sinensis* as a precaution to introducing the next major invasive species.

The goal of this study was to investigate the natural history and ecology of *Miscanthus sinensis* in the eastern United States. More specifically, our objective was to characterize the ecological niche of *M. sinensis* by identifying habitat, soil, and climate characteristics associated with both individual and population level characters of established populations.

Materials and Methods

Study Sites

Eighteen locations were visited in the eastern US in the summer of 2011 from North Carolina to Massachusetts (latitude 35.2690° N to 41.5815° N, longitude 70.5256° W to 82.5651° W). Sites were chosen from a database we compiled of *M. sinensis* escapes collected from an email survey of Master Gardeners, Master Naturalists, Invasive Plant Councils, Exotic Pest Plant Councils, and Departments of Natural Resources. The eighteen sites we visited were chosen based on our ability to travel to and collect samples from as many sites as possible over a two-week period (to reduce phenological variation), while representing the latitudinal and geographic range of naturalized *M. sinensis* populations in the Eastern US. Although more sites would have been desirable, nearly the entire latitudinal range of *M. sinensis* in the eastern US was sampled, and therefore our sample can be considered a reasonable representation of the variation among populations.

Table 1.1 Locations, sampling dates, and habitat descriptions for all surveyed sites

State	City	Site	Latitude	Longitude	Est. Population Area	Est. Population Density	Habitat Description
			° N	° W	hectares	plants m^{-2}	
NC	Henderson	NC-01	35.2690	82.4102	0.903	1.4	Roadside, railroad, open field
NC	Asheville	NC-02	35.5505	82.5651	0.911	0.9	Forest understory
NC	Mars Hill	NC-04	35.9410	82.5585	0.419	1.4	Roadside
NC	Mars Hill	NC-03	35.9542	82.5640	0.245	0.8	Forest understory, trailside
VA	Martinsville	VA-02	36.7505	79.7340	18.176	1.8	Roadside, open field, forest edge
VA	Amherst	VA-01	37.5622	77.0147	1.587	1.6	Forest edge, roadside
MD	Cromwell	MD-04	39.4040	76.5623	0.654	1.2	Roadside, forest edge
MD	Fallston	MD-03	39.5080	76.3825	0.541	1.1	Open field, forest edge
MD	Monkton	MD-02	39.5998	76.6046	1.323	2.2	Forest understory, forest edge
PA	Fort Washington	PA-01	40.1197	75.2235	1.107	0.6	Forest edge, trailside
PA	Quakertown	PA-02	40.4152	75.3133	1.286	0.9	Railroad, forest edge
NY	Heckscher State Park	NY-02	40.7093	73.1488	4.909	0.8	Trailside, open field, forest edge
NJ	Great Swamp	NJ-02	40.7153	74.4875	0.000280	0.7	Forest edge
NY	Seatuck	NY-01	40.7168	73.4083	0.902	0.5	Open field
NJ	Bernardsville	NJ-01	40.7329	74.5763	0.196	0.4	Roadside, forest understory
RI	Charlestown	RI-01	41.3732	71.5938	0.691	0.0012	Forest interior
CT	Quaker Hill	CT-01	41.3974	72.1145	0.146	0.0089	Roadside, forest edge
MA	Falmouth	MA-01	41.5815	70.5256	0.0302	0.060	Open field

At each location we recorded several environmental variables adjacent to *M. sinensis* individuals to broadly characterize the habitat. At each of five randomly placed sampling points within the *M. sinensis* population we recorded percentage of tree canopy cover using fisheye lens photography and WinSCANOPY© (Regent Instruments Inc.) software or a convex spherical densiometer (Forestry Suppliers Inc.). We also collected bulk soil samples from the top 15cm of the soil, which were subsequently air-dried, homogenized, and analyzed for pH (1:1, *v:v*), organic matter (%), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), and boron (B) (in mg/kg) using Mehlich-1 extractant and supernatant analyzed by inductively coupled plasma atomic emission spectrometer (ICP-ES). GPS points were collected around the perimeter of each population, and these were subsequently analyzed in a geographic information system (GIS) to estimate total population spatial extent. GPS data were also used to collect environmental and climate information from WorldClim databases in GIS software (ArcMap10, ESRI), including elevation, temperature, and precipitation. To estimate population density, we counted the total number of *M. sinensis* individuals in five 10 by 1m belt transects randomly located throughout each population. We estimated plant density over each population by extrapolating the average density per transect (individuals 10 m^{-2}) to the total area of the population.

To characterize the population at each location, we randomly selected several *M. sinensis* individuals for measurement of morphological characters [n = 20 for most sites, except MA-01 (n = 18), CT-01 (n = 13), RI-01 (n = 8), and NJ-02 (n = 2), where only that number of plants were found]. For each *M. sinensis* individual we recorded three subsamples of tiller height (soil surface to the uppermost collar) and tiller diameter, the total number of tillers, and basal diameter recorded in two orthogonal directions. Finally, photosynthetically active radiation (PAR) was recorded using an AccuPAR LP-80 PAR/LAI ceptometer (Decagon Devices, Inc., Pullman, WA) above and below the *M. sinensis* canopy. Basal area was calculated as $\pi \times (0.5 * \text{average diameter})^2$, and tiller density as tiller number/basal area.

Finally, we scouted for extant *M. sinensis* individuals and/or populations (naturalized and ornamental plantings) by driving approximately 1.6 km away from each site in all available directions different directions to scout roadsides. The approximate number of individuals and the GPS location were recorded for each “satellite” population we located.

Data Analysis

To describe the full range and variation of naturalized *M. sinensis* populations in the US, summary statistics were generated from the morphological characters, demographics and soil data collected. Soil characteristics, including pH, macronutrients (P, K, Ca, Mg), micronutrients (Mn, Fe, Cu, B, Zn), and organic matter, were ordinated using principal components analysis (cross-products matrix derived from correlation). Climate and geography characteristics, such as elevation, maximum average temperature of the warmest month, minimum average temperature of the coldest month, precipitation of the driest month, and precipitation of the wettest month, were derived from spatial analysis (ArcMap10, ESRI) were also ordinated using principal components analysis (from correlation). Principal component analyses enable us to linearize the multivariate soil and climatic data we collected, enabling us to identify which individual characteristics contribute (through loading values to each principal axis) most to our measured population responses (Abella et al. 2012). All soil and climate characteristics were Z-score transformed prior to principal components analysis. Linear fixed effect regressions (JMP9, SAS Inc., Cary, NC) were performed to determine how *M. sinensis* population size and morphology were correlated with the first and second principal components from the above edaphic and climatic analyses. Further linear fixed effects models compared the same morphological characteristics to canopy cover, using latitude as a covariate. The morphological characters plant height, basal area, tiller number, and population size were transformed using the best Box-Cox transformation. The Martinsville, VA site was determined to be a statistical outlier using Grubb's test for outliers (α = 0.05), and was subsequently omitted from population size models.

Table 1.2 Elevation and climate data for eighteen *M. sinensis* populations surveyed. All data derived from WorldClim database using ArcMap10. Abbreviations: Tmax: maximum average temperature of warmest month; Tmean: mean temperature across all months; Tmin: minimum average of coldest month; precip = precipitation. Precipitation of the wettest and driest months (mo.) are cumulative across those months. Annual precipitation is cumulative across all months.

Site	Elevation (m)	Tmax (°C)	Tmean (°C)	Tmin (°C)	Annual precip. (mm)	Precip. wettest mo. (mm)	Precip. driest mo. (mm)
NC-01	664	29.0	13.0	-3.6	1474	148	111
NC-02	664	29.1	12.7	-4.2	1158	116	83
NC-04	1022	26.1	10.4	-6.3	1321	130	94
NC-03	1140	25.2	9.8	-6.5	1390	136	100
VA-02	296	30.4	13.2	-4.0	1150	115	83
VA-01	137	31.4	14.3	-3.1	1101	117	76
MD-04	109	30.8	12.6	-5.1	1151	114	80
MD-03	122	30.2	12.2	-5.6	1154	113	76
MD-02	179	29.4	11.5	-6.1	1128	113	76
PA-01	52	29.9	11.9	-5.4	1130	113	73
PA-02	158	29.1	10.5	-7.0	1154	112	74
NY-02	3	27.8	11.3	-4.5	1144	111	84
NJ-02	75	29.3	10.5	-7.6	1235	123	77
NY-01	0	28.1	11.4	-4.5	1118	109	81
NJ-01	172	28.5	9.8	-8.3	1266	124	79
RI-01	15	26.2	10.0	-5.9	1153	116	77
CT-01	48	27.3	9.9	-7.2	1224	120	86
MA-01	9	25.7	9.9	-5.7	1167	118	75

Table 1.3 Summary statistics for soil characteristics and canopy cover across all sites surveyed (n = 18).

	P	**K**	**Ca**	**Mg**	**Zn**	**Mn**	**Cu**	**Fe**	**B**	**pH**	**organic matter**	**canopy cover**
	------------------------------- mg/kg --										------------------ % --------------	
Min	3.0	31.5	316.5	17.0	1.50	6.80	0.35	10.7	0.2	4.22	3.9	0.16
Med	6.5	73.5	665.0	124.5	4.00	21.2	0.80	21.4	0.4	5.93	7.65	38.02
Mean	16.0	81.6	835.7	140.0	6.84	26.2	0.96	32.7	.04	5.92	10.4	52.09
Max	93.0	167.5	1771.0	379.0	37.6	78.7	2.2	137.9	0.8	7.27	49.1	100.0

RESULTS

Miscanthus sinensis populations are distributed over a large geographic range representing a wide variety of climatic conditions and habitats (Tables 1.1 and 1.2). The latitudinal gradient of populations spanned approximately 6° from 35.55° in North Carolina to 41.58° in Massachusetts. Invaded habitats fell into several categories (Table 1.1), including forest understories, forest edges, open fields, and road and railroad rights-of-way. Of these categories, forest edge (n = 10) and roadsides (n = 8) were the most common. Sources of population origin (e.g., ornamental planting) were indeterminable from the surveys of surrounding areas as it was impossible to estimate which came first. The average estimated population size was 9,437 individuals with an average population density of 86 individuals 100 m^{-2}.

As expected, climate conditions at each site correlated with latitudinal position, with minimum and maximum mean temperatures generally decreasing for northern populations (Table 1.2). The maximum average temperature experienced by any population was 31.4°C (VA-01; Martinsville, VA), while the minimum average temperature was -8.3°C (NJ-01; Bernardsville, NJ). In the principal component analysis of climate conditions, the first principal component accounted for 53% of variance while the second principal component accounted for 32% (85% overall, Figure 1.1).

Characteristics exhibiting the highest loadings included annual precipitation (loading = 0.96), precipitation during the wettest month (0.92), precipitation during the driest month (0.88), and elevation (0.81) in the first principal component. The annual mean temperature (0.93), minimum temperature of the coldest month (0.86), and maximum temperature of the warmest month (0.59) exhibited the highest loadings for the second principal component of climatic conditions.

Edaphic conditions measured from bulk soil samples were highly variable among sites (Table 1.3). The pH ranged between 4.22 and 7.27 with a mean of 5.29. Macronutrients such as P (3 Mg kg^{-1} to 93 Mg kg^{-1}), K (31.5 Mg $kg^{-1}$to 167.5 Mg kg^{-1}) and Ca (316.5 Mg kg^{-1} to 1771 Mg kg^{-1}) had the most variation of any nutrient characteristic, while organic matter content ranged from near zero to almost 50% (Table 1.3). In the principal component analysis of soil properties, the first principal component accounted for 33% of the variance, and the second principal component accounted for 19% (52% overall, Figure 1). Properties and characteristics exhibiting the highest loadings on the first two components included Ca (loading = 0.94) and pH (0.78) for component 1, and Cu (0.77), Mn (-0.62), and organic matter (0.59) for component 2.

The linear fixed effects model of the second principal component of climate characteristics was positively correlated with population size (R^2 adjusted = 0.237; p = 0.0273). All other models of principal components and morphological responses were non-significant ($p > 0.05$). Finally, we found that canopy cover does not significantly affect tiller height (p = 0.1331), basal area (p = 0.4499), tiller number (p = 0.0755), or population size (p = 0.1613).

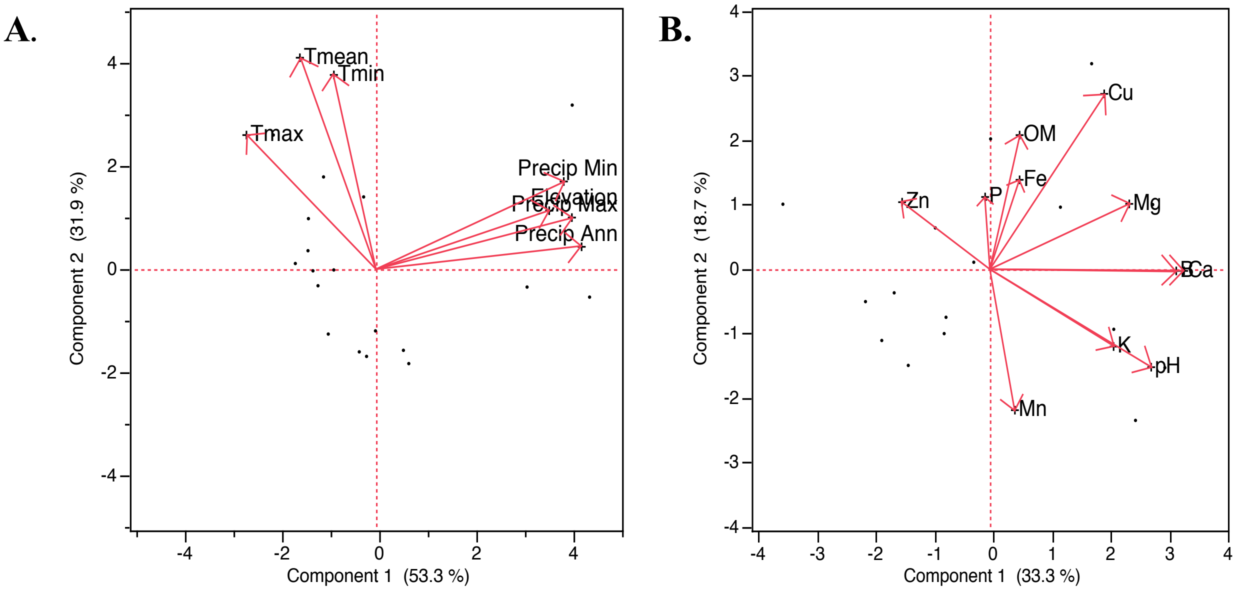

Figure 1.1 Principal components 1 and 2 (variationaccounted for by each component) of A) climate characteristics and B) soil characteristics for the 18 naturalized *Miscanthus sinensis* populations. Abbreviations. Tmax: average maximum temperature of the warmest month. Tmin: average minimum temperature of the coldest month. Tmean: average annual temperature. Precip_Min: average precipitation of the driest month. Precip_Max: average precipitation of the wettest month. Precip_Ann: average annual precipitation.

DISCUSSION

In its native range, *M. sinensis* commonly forms vast grasslands, where it tolerates fire disturbances, grazing, and forage harvesting (Osawa 2011; Stewart et al. 2009). In the introduced range, *M. sinensis* appears to establish in a variety of habitats defining a broad ecological niche, which include highly-disturbed, anthropogenic habitats (e.g., rights-of-way), as well as unmanaged forest edges and more rarely forest interiors. By far the most common habitat types in this survey were roadsides (n = 8) and forest edges (n = 10), and often a combination of the two. This is likely a result of the frequent disturbance along roadsides and the wind-dispersed spikelets of *M. sinensis* (Quinn et al. 2011a). One of the key questions regarding invasive species, and especially *M. sinensis*, is the source of the naturalized populations. Are they plantings gone wild? Ornamental hybrids from neighboring houses or commercial properties? We attempted to identify potential source (or satellite) populations within 1.6 km of the surveyed populations, and were generally unsuccessful. While some ornamental plantings and small cohorts were discovered, it is not possible to attribute them as the propagule source for the sites surveyed, or vice versa. The distance between many of these "satellite" populations (or plantings) and the original surveyed population often exceeded 800m (data not shown), which is far beyond the distance *M. sinensis* spikelets have been empirically shown to disperse (Quinn et al. 2011a). It is important to note that our survey techniques for these "satellite" populations were very limiting and only sampled a few points. Genetic characterization would be needed to begin to address this source-sink issue.

Surveyed populations were present in both highly disturbed and low-maintenance sites with high and low resource availability. Invaded habitats covered a broad latitudinal range, low and high elevations (0 to 1140m), acidic soils, and a wide range of macro- and micronutrient availability. Our findings are consistent with previous assertions that *M. sinensis* can tolerate stressful and/or marginal environments in both the native and introduced ranges (Ezaki et al. 2008; Quinn et al. 2011b; Stewart et al. 2009).

The large latitudinal range covered in this study exhibits the broad climatic tolerance of *M. sinensis*, which is consistent with climate niche model predictions (Barney and DiTomaso 2011). Several populations occurred in regions with minimum temperatures below freezing (Table 1.2), supporting previous suggestions of cold tolerance (Stewart et al. 2009; Zub et al. 2012). Populations were significantly smaller in colder areas, but present nonetheless, which is likely due to the shorter growing season and exposure to lower minimum temperatures. Aside from climatic conditions, this may also be related to population age and time since establishment, combined with limited reproductive output in the shortened growing season. Smaller population sizes may result in limited reproductive output and seed set; however, we found that seed collected from each population along the distribution was viable under lab and greenhouse conditions, regardless of populations size and/or location (Dougherty, unpublished data). The largest populations in this study were found near areas of the earliest introductions of *M. sinensis* (i.e., Asheville, NC and Washington, DC), suggesting that, with time and propagule pressure, there may be population size increases in the Northeast in the future. In fact, our multivariate analysis of climatic factors shows that the vast majority of populations surveyed cluster together according to temperature variables rather than variables associated with precipitation (Figure 1a). In addition, climate principal component two (loadings: Tmean 0.93, Tmin 0.86, Tmax 0.59) was positively correlated with population size (R^2 adjusted = 0.237; p = 0.0273). This implies that temperature, much more than precipitation, is a greater driver of the establishment and expansion of *M. sinensis* populations. Previous studies support this notion, often suggesting that *M. sinensis* is a drought tolerant species (Quinn et al. 2010; Stewart et al. 2009); therefore, precipitation wouldn't be expected as a primary limitation to population performance.

In the native range, *M. sinensis* is an early successional species, and is often found as one of the first pioneer plants to establish in extremely acidic (pH < 3.0) volcanic ash-based soils (Stewart et al. 2009). Although such extreme conditions weren't found during our survey, populations were established in soils with a wide range of pH (4.22 - 7.27) and equally wide ranges of macro- and micro-nutrient availability (Table 1.3). Multivariate analysis of soil characteristics shows most populations do not cluster according to any soil variables, including pH, macronutrients, micronutrients, and organic matter (Figure 1b). Regressions comparing plant morphology and soil principal components were all non-significant ($p > 0.05$). This suggests that soil resources are not a limiting factorin the establishment and success of *M. sinensis* populations.

Since *M. sinensis* is occasionally found in low light environments, it is increasingly important to understand its shade tolerance. Recent studies have shown that *M. sinensis* can maintain high photosynthetic rates even at extremely low light levels (Horton et al. 2010). However, individuals from native and introduced populations have not shown significant differences in biomass accumulation and total leaf area (Matlaga et al. 2012). In this survey, sites had canopy covers ranging from < 1% to 100% (Table 1.3), suggesting a broad range of potential tolerance. The vast majority of surveyed populations established in canopy covers < 50% (n = 10); compared to only 30% of populations under canopy cover > 85% (n = 5). This may suggest enhanced shade tolerance in the introduced range; however, population size was not significantly correlated with canopy cover. As mentioned previously, the average population size from this survey was 9,437 individuals (n = 17), compared to 11,844 individuals in populations with canopy cover <50% (n = 9) and 2,446 individuals in populations with canopy cover > 85% (n = 5). Even so, canopy cover did not significantly affect *M. sinensis* morphology. It does appear that *M. sinensis* populations can establish in low-light environments; however, more empirical work must be done to determine shade tolerance.

Potential for Future Spread and Conclusions

As mentioned previously, Quinn et al. (2011b) found variation in the environmental tolerances between *M. sinensis* populations in the native and introduced ranges, and proposed that it may be occupying a novel ecological niche in the introduced range. Our survey provides evidence in support of this claim. *Miscanthus sinensis* populations in highly disturbed, high-canopy habitats across the eastern United States are quite different from the open grasslands of *M. sinensis* in the native range (i.e., Japan and eastern China). *Miscanthus sinensis* is currently naturalized in 25 states, with population sizes ranging from a handful of individuals to hundreds of thousands, all capable of producing vast amounts of wind-dispersed spikelets. While not all of these spikelets will be viable, seed collected from each population in this survey was germinable in laboratory conditions (Dougherty, unpublished data). A fully mature *M. sinensis* individual can produce >100 flowering panicles per year (Quinn et al. 2011a), with approximately 1,800 spikelets panicle^{-1}. Hypothetically, if we apply these numbers to our average population size (9,437 individuals), assuming each individual produces approximately half of the 100+ potential panicles with 1,800 spikelets panicle^{-1}, the average naturalized *M. sinensis* population is capable of producing over 8.4 billion spikelets year^{-1}. Although these numbers may seem daunting, the amount of actual fertilized and viable spikelets likely varies significantly among individuals and populations, which has yet to be examined.

Currently, it is not uncommon to find populations of *M. sinensis* that span well over 50 miles alongside North Carolina interstate highways. This already intense propagule pressure, combined with increasing horticultural demand and potential bioenergy crop status, could result in tremendous spread and propagation well beyond the current distribution. Populations established along roadsides, interstate highways, and railroads could push populations into forest interiors and conservation areas, especially given results that indicate a tolerance for low light conditions (Horton et al. 2010; Matlaga et al. 2012).

Miscanthus has broad climatic tolerance and minimal soil requirements for establishment. Although it currently tends to establish in low-value areas such as roadsides and forest edges, horticulturists and bioenergy stakeholders should proceed with caution in order to prevent further spread and naturalization of *M. sinensis* or its derivatives in its introduced range. Further work on *M. sinensis* should explore shade tolerance and soil moisture requirements for establishment to further clarify its ecological niche. There can be significant variation in the reproductive output (i.e., seed set) between ornamental cultivars (Meyer and Tchida 1999). Therefore, it would be pertinent to compare a range of ornamental cultivars and naturalized individuals in order to identify any high or low-risk ornamental varieties.

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CHAPTER 2

A hydrothermal time model predicts germination of the invasive ornamental grass *Miscanthus sinensis*

ABSTRACT

Following transport to a new area, propagule establishment is the first, and arguably most important, step in the invasion process. Information about the germination requirements of an invasive plant could be critical for range prediction, and subsequent management efforts. The C_4 perennial grass *Miscanthus sinensis* is an important ornamental grass and potential bioenergy crop in the United States. This study aimed to generate hydrothermal time models for *M. sinensis* seed germination as a predictive tool for evaluation of invasive potential.

The hydrothermal time model combines three essential parameters for seed germination – water availability, temperature, and time, as a way to quantitatively evaluate germination requirements. Controlled laboratory experiments were performed to develop a hydrothermal time model for *M. sinensis* using seed from two naturalized accessions and one commercial cultivar. Seeds from each population were placed in solutions of varying water potential (0.0 MPa, -0.25 MPa, -0.50 MPa) along a temperature gradient (7.6 °C – 37.8 °C) for a total of 60 treatment combinations per seed lot. We determined that *Miscanthus sinensis* has a base temperature of 8.3 ± 0.8 °C, and was tolerant of all water potential treatments at temperatures above this threshold. The $\psi_{b(50)}$ varied between seed sources, suggesting population-level adaptations may control tolerance to water potential. Hydrothermal models fit at each temperature were greatly improved when θ_{HT} was allowed to vary. Data from this model could be combined with geographical information system (GIS) models to develop a predictive model of high and low risk areas for *M. sinensis* potential germination and establishment.

INTRODUCTION

Development of predictive models that identify locations and conditions susceptible to invasion are essential tools for management and control of invasive species (Rejmánek 2000). Climatic conditions such as temperature and water availability are two of the most accurate predictors of a species growth and establishment (Graziani and Steinmaus 2009; Woodward 1987), and therefore are the primary components of many ecological niche models (e.g., Barney and DiTomaso 2011). These factors are also the primary drivers of species distribution, growth, fecundity, and seed germination (Sutherst 2003). For example, Barney and DiTomaso (2011) found that access to a constant water source could increase the ecoregion range of several invasive species by nearly 20%, especially in the drier regions of the Western US. The predictive power of these models is especially important in invasion ecology, where the best management practice is prevention of germination and, subsequently, establishment. In order to prevent establishment of an invasive species, one must determine where, and under what conditions, the species of interest is most likely to thrive—which begins with the germination of propagules (Theoharides and Dukes 2007).

The thermal time and hydrotime models utilize temperature and water availability, respectively, to predict seed germination based on the accumulation of time spent above either a base temperature, T_b, or base water availability, ψ_b (Bradford 1990). These base values represent the minimum temperature and water availability at which germination can occur. These values vary among species, and act as critical constraints on the climatic and geographic range of a species (Bradford 1990). Gummerson (1986) proposed that combining thermal time and hydrotime into a single predictive hydrothermal time model could better explain seed germination of a specific population or species, which is described by:

$$\theta_{\mathrm{HT}} = (T - T_{\mathrm{b}})(\psi - \psi_{\mathrm{b(g)}})t_g \qquad (1)$$

where θ_{HT} is the hydrothermal constant (MPa-degree-hours) for the population, the base temperature T_b , the observed water potential ψ , the base water potential ψ_b, and t_g is the time to germination of a population fraction g (Bradford 1990; Gummerson 1986). In this model, base water potential and germination time vary by germination fraction, while hydrothermal time and base temperature are assumed constant for a population. The base water potential is assumed to follow a normal distribution within a population, with mean $\psi_{b(50)}$ and standard deviation $\sigma_{\psi b}$. Once the constant parameters are identified, repeated probit analysis of cumulative germination data yields a hydrothermal time-course curve via the equation:

$$\mathrm{Probit}(g) = \frac{\psi - (\theta_{HT}/(T - T_b)t_g) - \psi_{b(50)}}{\sigma_{\psi b}} \qquad (2)$$

Probit transformation linearizes a cumulative normal distribution of germination that facilitates development of the model (Bauer 1998; Bradford 1990; Christensen 1996).

The accuracy and assumptions of the hydrothermal time model have often been questioned (Kebreab and Murdoch 1999). Model fit is poor when seeds are incubated at a low ψ range (Bradford 1990; Dahal and Bradford 1994), and several studies have found violations of model assumptions such as constant T_b (Kebreab and Murdoch 1999, Welbaum and Bradford 1991), $\psi_{b(50)}$ (Dahal and Bradford 1994), and θ_{HT} (Kebreab and Murdoch 1999) within a seed population. Because the hydrothermal model is still in its infancy, additional species and seeds lots must be studied to validate the assumptions of the model (Bradford 1995). This study focuses on development of the hydrothermal time model for the invasive ornamental grass *Miscanthus sinensis*.

Miscanthus sinensis is an ornamental grass native to East Asia that has been repeatedly introduced to the US since the late 19th century, and subsequently naturalized across the eastern seaboard (Dougherty et al. 2013; Quinn et al. 2011a). Traits such as C_4 photosynthesis, broad environmental tolerance, rapid growth, and widespread phenotypic variation make *M. sinensis* not only an important invasive species, but also a candidate crop for bioenergy and biofuel production (Quinn et al. 2011a, 2011b). *Miscanthus sinensis* forms a clumping growth habit with flowering tilers up to 2m (Stewart et al. 2007). The flowers occur in panicles comprising a group of racemes 10 to 20 cm long, with the seeds 3 to 5 mm long, and feature long callus hairs grouped at the base of the glume (Koyama 1987). Currently, *M. sinensis* is capable of tolerating climatic conditions in approximately 56% of ecoregions in North America, and, with a constant water source, that range could increase to as much as 64% (Barney and DiTomaso 2011). Phenotypic and physiological differences between naturalized accessions and ornamental cultivars of *M. sinensis* are often great, and can lead to varying levels of tolerance to environmental factors such as light and water availability (Dougherty and Barney 2013). *Miscanthus sinensis* has naturalized in over half of US states, yet we have only a basic understanding of its biology and ecology (Dougherty et al. 2013). Certain varieties of *M. sinensis* are capable of producing up to 160,000 spikelets per individual, which are capable of wind-dispersing distances as great as 400m (Meyer and Tchida 2007; Quinn et al. 2010). Therefore, investigation of its seed biology is an important step to better understand the ecology and invasive potential of *M. sinensis*.

The objective of this study was to determine the temperature and moisture requirements for *M. sinensis* seed germination under the parameters of the hydrothermal time model. We hypothesize that differences in sensitivity to low temperature and moisture will vary among accessions, likely according to habitat type and location.

MATERIALS AND METHODS

Experimental Design

Our goal was to represent variation in both ornamental and naturalized varieties. Due to a lack of ornamental seed availability, the selection of cultivated seed was reduced to a commercial strain from Jelitto Seed Company (Schwarmstedt, Germany). In total, three lots of *Miscanthus sinensis* seeds were collected for this study in November 2011 – two naturalized US accessions and one commercial cultivar (Jelitto). The naturalized accessions were a roadside, forest edge site in Loch Raven, Maryland (MD) and an open field site in Heckscher State Park, New York (NY). More information on these accessions can be found in Dougherty et al. (2013a). After initial drying, seeds were stored in a dark cold room at 4° C for approximately 200 days before trial one, and 400 days before trial two.

Seeds were incubated at 1 of 20 constant temperatures on a thermal gradient table (trial one, 7.6 – 32.6 ° C, trial two, 8.6 – 37.9 ° C) at one of three water potentials (0, -0.25, -0.50 MPa). Twenty seeds were placed in a 60 mm Petri dish on two layers of germination paper saturated with 4 mL of either deionized water or polyethylene glycol

(PEG 8000) solution. Concentrations of PEG for each temperature were determined according to Michel (1983) and tested with a WP4 Potentiameter (Decagon Devices, Inc., Pullman, WA). To adjust for changes in water potential due to seed water uptake, saturation of the germination paper, and evaporation, seeds were transferred to fresh PEG solutions every 3-5 days (Cheng and Bradford 1999; Christensen 1996). The design was a full factorial of temperature and water potential treatments. There were three replicates of each accession at each water potential treatment within a single temperature - for a total of 9 replicates per accession, and 27 replicates per temperature. Petri dishes were completely randomized within each temperature, and sealed with parafilm to reduce moisture loss due to evaporation. Each trial was allowed to run for five weeks, and germination was recorded at the earliest time of visible radicle emergence. At the end of the five-week period, seeds remaining in all replicates were transferred to fresh water (0 MPa) solutions and incubated at 25 ° C for two weeks to test for viability. Seeds that germinated during this time were considered viable and, to account for differences in dormancy, cumulative germination percentages were calculated based on the total number of viable seeds, rather than the full number of seeds sown (Grundy et al. 2000).

Parameter estimation for the hydrothermal time model

Germination data from trial one and two were pooled for each accession, giving a total of twenty temperatures and three water potentials. Germination rate (GR(*g*)) of each population fraction (e.g. 10, 20, 50 % of total germinated seeds) was determined with probit analysis of germination time courses for each *M. sinensis* seed variety at every temperature, water potential, and replicate in JMP10 (SAS Inc, Cary, NC). Germination rate for the g subpopulation ($GR_{(g)}$) was calculated as the inverse of the time, in hours, required for g percent of the population to germinate (Steinmaus et al. 2000). Parameters of the hydrothermal time model are estimated from algebraic relationships between temperature, water potential, and GR(g) (Zambrano-Navea et al. 2013). The linear function of $GR_{(50)}$ on suboptimal temperatures was used to estimate the base temperature (T_b) of the population, estimated as the x-intercept of this regression (Bradford 1990; Dahal and Bradford 1994). The inverse slope of the linear regression of the 10, 20, 30, 40, 50, 60, and 70% subpopulations was used to determine thermal time (θ_T). Base water potential ($\psi_{b(50)}$) and its deviation ($\sigma_{\psi b}$) were determined by repeated probit regression of cumulative germination percentage on:

$$\psi - \theta_{HT}/((T - T_b)t_g) \tag{3}$$

from (Cheng and Bradford 1999). The best fit linear regression line gives $\psi_{b(50)}$ as probit $=$ 0, and $\sigma_{\psi b}$ as the inverse of the slope according to Bradford (1992).

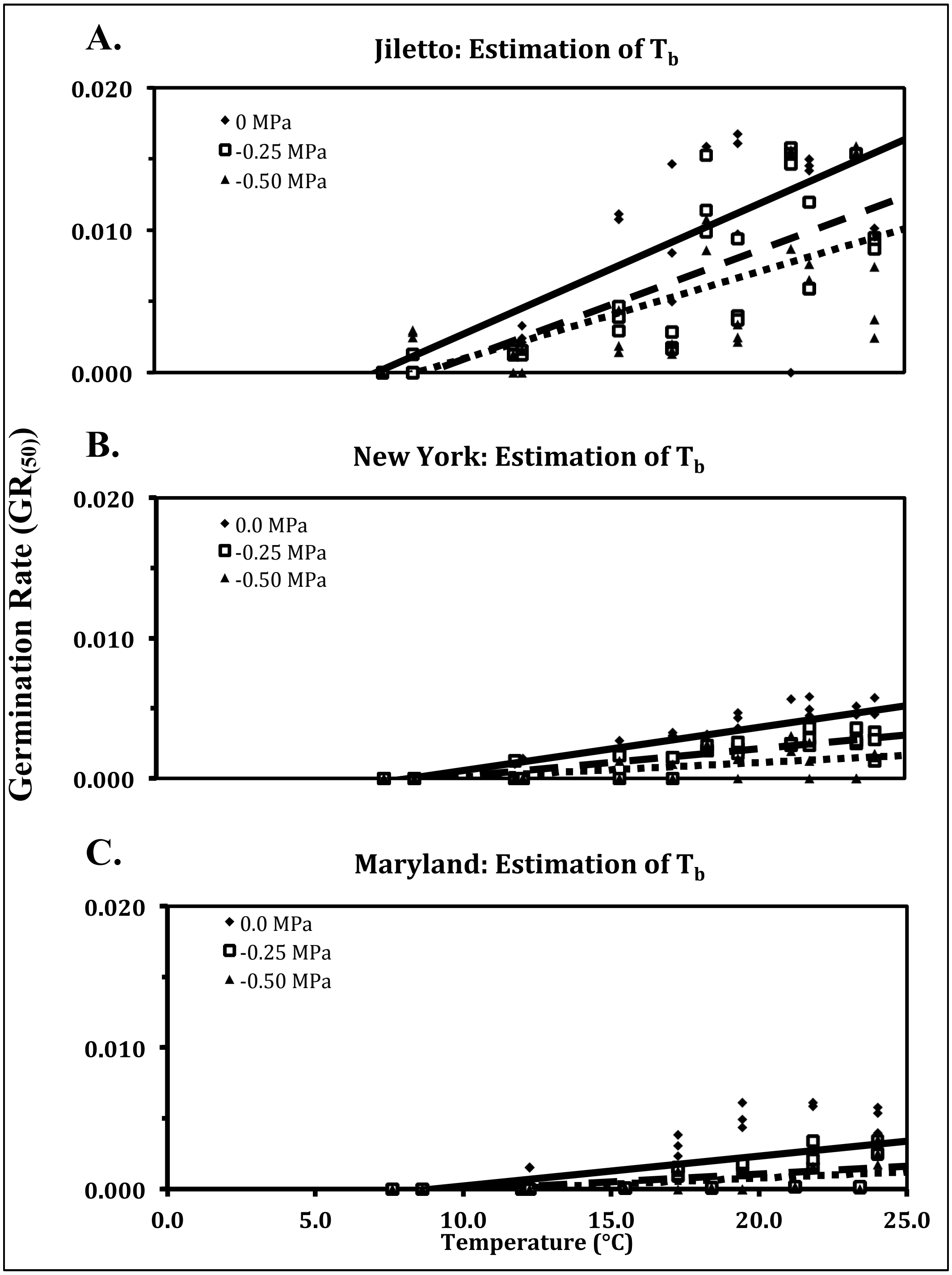

Figure 2.1 Estimation of T_b of all cultivars and accessions by regression of $GR_{(50)}$, calculated as the inverse of the time required for 50% germination on suboptimal temperatures. X-intercept of regression lines are equal to estimate of T_b. Solid line = 0.0 MPa. Dashed line = -0.25 MPa. Dotted line = -0.50 MPa.

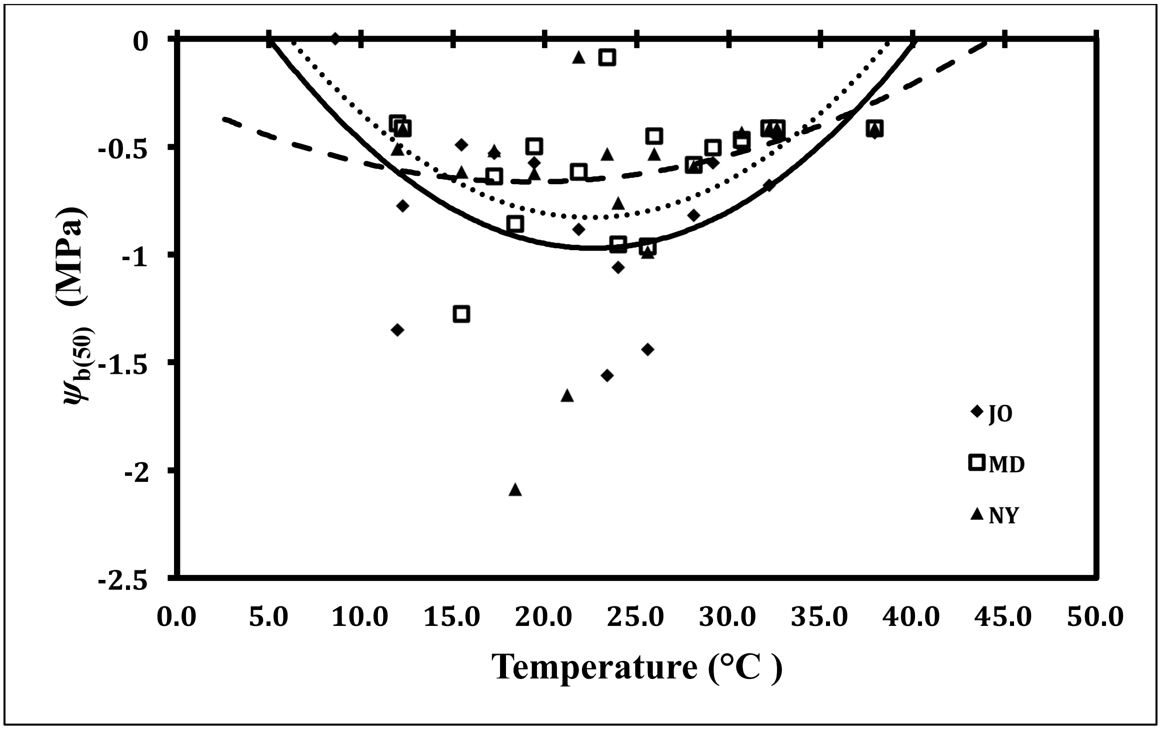

Figure 2.2 Values of $\psi_{b(50)}$ versus temperature. Exhibits the variation with temperature of $\psi_{b(50)}$ within a seed lot. Solid line = Jiletto (JO). Dashed line = Maryland (MD). Dotted line = New York (NY).

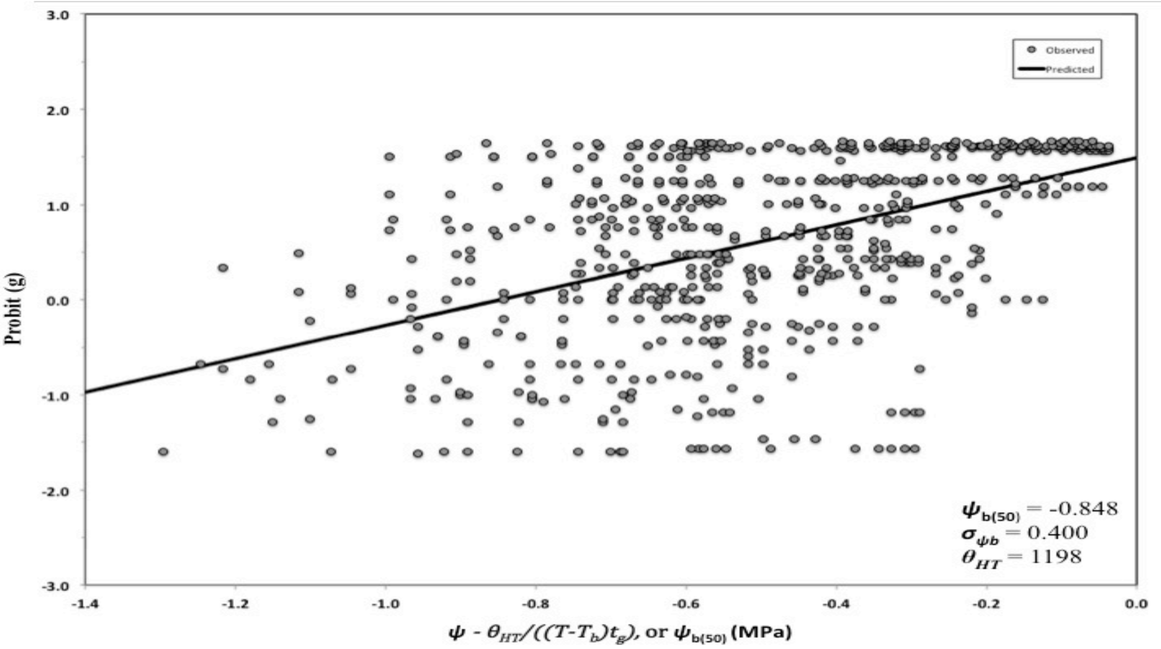

Figure 2.3 Repeated probit regression using best fit of Jiletto (JO) germination percentages at all temperatures on estimations of $\psi_{b(50)}$ with constant θ_{HT}. Inverse of slope is $\sigma_{\psi b}$.

Testing the assumptions of the hydrothermal time model

The hydrothermal time model is based on Gummerson (1986) and Bradford (1990), which gives Eq. (1). Cumulative germination progress can be predicted under the assumptions of this model according to Eq. (2) (Cheng and Bradford 1999; Dahal and Bradford 1994).

Although variation does exist between subpopulations of a seed lot, the hydrothermal constant for the 50% subpopulation ($\theta_{HT(50)}$) was used to develop the predictive models of germination progress for simplicity (Dahal and Bradford 1994; Wang et al. 2005). We tested the predictability of the hydrothermal model under the following assumptions:

(1) MODEL 1 (M1) - All parameters of the model were assumed constant within a seed population (Gummerson 1986). The base temperature of the 50% subpopulation at 0 MPa was used as the common T_b, and the common θ_{HT} was estimated as the inverse slope of $1/\theta_{T(50)}$ on ψ (Gummerson 1986; Wang 2005):

$$\frac{1}{\theta_{T(50)}} = \frac{\psi - \psi_{b(50)}}{\theta_{HT(50)}} \qquad (3)$$

(2) MODEL 2 (M2) - T_b, $\psi_{b(50)}$, and $\sigma_{\psi b}$ assumed constant, while θ_{HT} was allowed to change with temperature and estimated using Eq. (1).

The power of these models was tested using the root-mean-square-error (RMSE) between the observed and predicted values (Wang et al. 2006). Finally, the means of cumulative germination percentages and germination rates for each population, temperature, and water potential were compared with ANOVA in JMP10 (SAS Inc, Cary, NC).

Table 2.1 Summary of total germination and viability percentages by water potential for all varieties and accessions.

Germination (%)				
Variety	**0 MPa**	**-0.25 MPa**	**-0.50 MPa**	**TOTAL**
Jiletto	90 ± 15	75 ± 29	59 ± 35	74 ± 30
New York	55 ± 13	34 ± 21	19 ± 17	36 ± 23
Maryland	60 ± 10	41 ± 24	27 ± 24	43 ± 24

Viability (%)				
Variety	**0 MPa**	**-0.25 MPa**	**-0.50 MPa**	**TOTAL**
Jiletto	88 ± 29	75 ± 36	57 ± 42	74 ± 37
New York	76 ± 38	60 ± 41	43 ± 41	63 ± 42
Maryland	82 ± 35	54 ± 40	45 ± 44	62 ± 42

Table 2.2 Hydrothermal parameter estimates when assumed constant for a seed source.

Population	$\boldsymbol{\theta_{HT}}$	$\mathbf{T_b}$	$\boldsymbol{\psi_{b(50)}}$	$\boldsymbol{\sigma_{\psi b}}$
	MPa ° h	**°C**	**MPa**	
New York	1700	8.5	-0.64	0.10
Maryland	1187	9.0	-0.66	0.58
Jelitto	1198	7.4	-0.85	0.40

Table 2.3 θ_{HT} values when allowed by vary by temperature

Temperature (°C)	**New York**	**Maryland**	**Jelitto**
15.5	2163	1513	714
19.4	1766	1827	684
24	2112	1985	1122
28.1	2501	2288	1526
30.7	11576	4490	2988
Mean	4024	2421	1407

Table 2.4 ANOVA results of cumulative germination and germination rates by variety, temperature, and water potential. All treatments were fixed effects. Significant values are in **bold**.

Treatment	Cumulative Germination (%)	Germination Rate
	p-value	**p-value**
Variety	0.3666	**0.0230**
Temperature (Temp)	**<.0001**	0.7680
Variety*Temp	**0.0165**	0.1620
Water Potential (MPa)	**0.0008**	**0.0297**
Variety*MPa	0.5906	**0.0199**
Temp*MPa	**0.0378**	0.4187
Variety*Temp*MPa	0.3146	0.5902

RESULTS

Germination of *M. sinensis* seeds varied by accession, temperature, and water potential (Tables 2.1, 2.4). The commercial cultivar Jelitto had the highest overall germination at approximately 74 ± 30 %, followed by Maryland and New York, which germinated at 43 ± 24 % and 36 ± 23 % across all temperatures and water potentials, respectively. The Jelitto cultivar had the overall shortest time to 50% germination, the lowest base temperature, and the lowest $\psi_{b(50)}$ of any population (Table 2.2). Variety ($p = 0.0362$) and water potential ($p = 0.0425$) had the greatest affect on germination rate, which was defined as the inverse of the time required for 50% of the seed lot to germinate (Table 2.4). Changes in temperature had no effect on the rate of germination, but was positively correlated with increases in cumulative germination ($p < 0.0001$). Decreases in water potential at all temperatures resulted in a drop in germination percentage (Table 2.1).

Estimation of the base temperature was slightly different (± 1.2 °C) between water potentials within a variety, but was not significant ($p = 0.18$). Overall, the base temperature among accessions varied between 7.4°C (Jelitto) and 9.0 °C (Maryland) with an overall mean of 8.3 ± 0.8 °C (Figure 2.1). The repeated probit regression approach of estimating the base water potential generated $\psi_{b(50)}$ values of -0.64 ± 0.10, -0.66 ± 0.58, and -0.85 ± 0.40 MPa for New York, Maryland, and Jelitto, respectively. These values follow the approximately normal distribution of water potential on temperature on both the probit regression line (Figure 2.3), as well as the variation found from regressions of $GR_{(50)}$ on ψ (Figure 2.2).

Hydrothermal time models of cumulative germination under the two sets of assumptions (M1 and M2) were tested at several temperatures. Overall, the average RMSE of M1 models were nearly double that of M2 models (data not shown), which suggests that modifying θ_{HT} at each temperature greatly increases the performance of the model. Model prediction under both assumptions was greatest at moderate temperatures (22 – 26 °C), but M2 models were far greater predictors outside this range (Figures 2.4 and 2.5). At 19.4 °C, M2 models had an average RMSE of 0.855, compared to 2.509 for M1 models. Predictive models for the Maryland accession were poor at all temperatures and water potentials (Figures 2.4 and 2.5). The mean hydrothermal constants, θ_{HT}, across temperatures were 4024, 2421, and 1407 MPa ° h for New York, Maryland, and Jelitto, respectively. The New York population had the greatest variation in θ_{HT}, ranging from 2163 MPa ° h at 15.5 °C and 11576 MPa ° h at 30.7 °C.

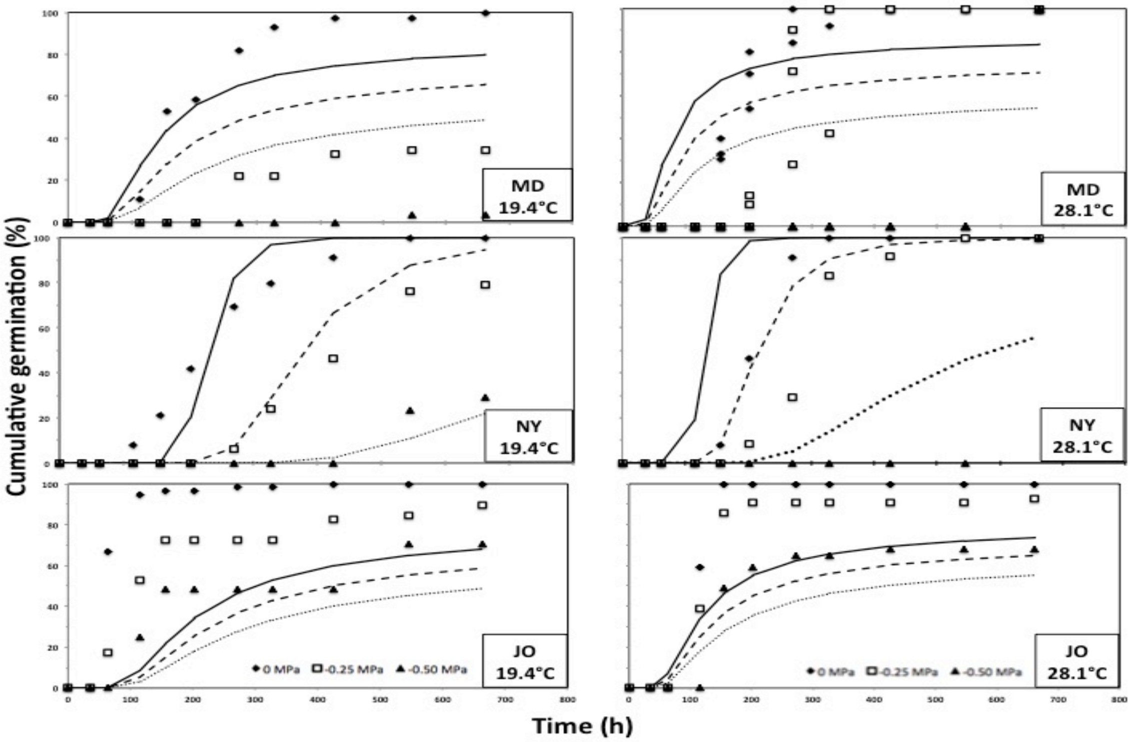

Figure 2.4 Cumulative germination models generated under M1 assumptions at 19.4°C and 28.1°C – all parameters constant. MD = Maryland. NY = New York. JO = Jelitto. Prediction lines: solid = 0.0 MPa, dashed = -0.25 MPa, dotted = -0.50 MPa.

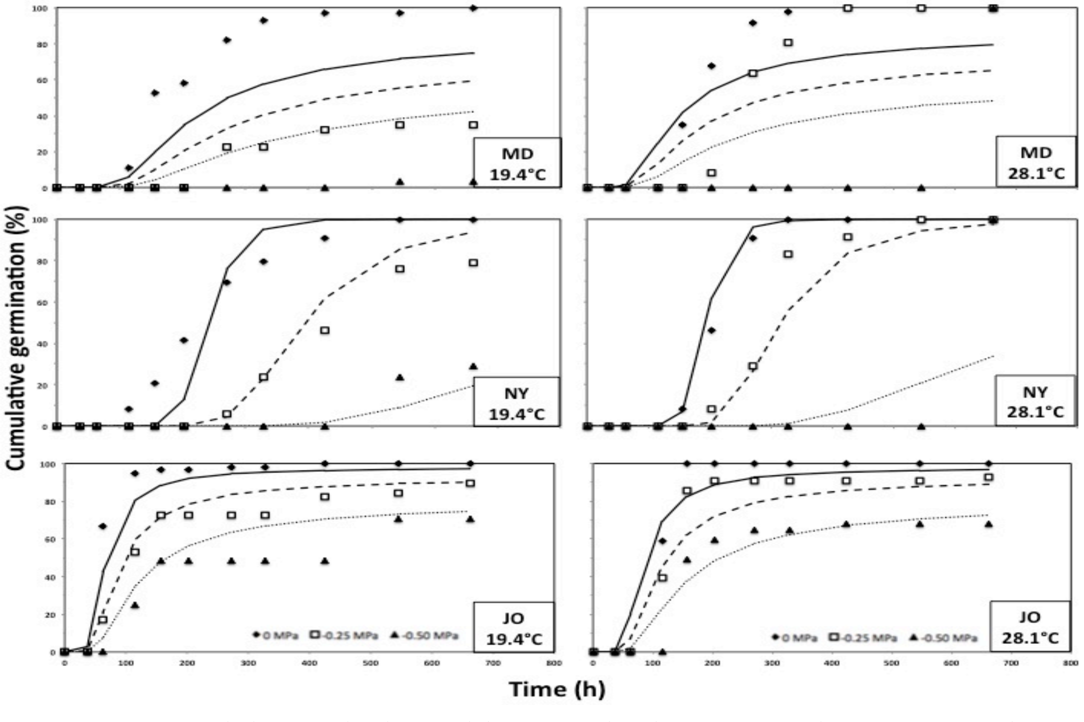

Figure 2.5 Cumulative germination models generated under M2 assumptions at 19.4°C and 28.1°C - θ_{HT} allowed to vary with temperature. MD = Maryland. NY = New York. JO = Jelitto. Prediction lines: solid = 0.0 MPa, dashed = -0.25 MPa, dotted = -0.50 MPa.

DISCUSSION

The rate and percentage of germination for *M. sinensis* seeds increased in response to an increase in temperature and water availability. Growth rate increased linearly from the base temperature (7.73 – 9.24 °C) to the optimal temperature of approximately 26-28 °C. This temperature range agrees with previous reports of optimal threshold estimates for *M. sinensis* between 10 °C and 30 °C (Barney and DiTomaso 2011). The variation in hydrothermal parameters is likely due to genetic differences between cultivars and accessions. Another possible source of variation are physical attributes of the seeds. The seeds of *M. sinensis* are typically surrounded by a glume (or "hull"), which provides a physical layer of protection from the elements, which, in this study, may have slowed water uptake in New York and Maryland seeds. Jiletto seeds were de-glumed, and thus are directly exposed to the treatments of temperature and water availability, which may explain the increased germination rates and low base temperature. The Jiletto cultivar also has a much lower $\psi_{b(50)}$ than the naturalized varieties, which again is due to the dehulling process. The lack of this extra physical barrier around the embryo leads to direct contact with the water and PEG solutions and subsequently likely greater osmotic uptake.

Without the advantage of previous studies of *M. sinensis* seed germination requirements, we tested various assumptions of the hydrothermal time model. Several species, such as tomato (Dahal and Bradford 1994) and winterfat (Wang 2004), do not behave under the assumptions of the hydrothermal model, such as a constant θ_{HT} for all temperatures, or constant T_b under all water potentials. Therefore, we generated multiple hydrothermal time models under different assumptions in order to identify the conditions that best predict cumulative germination of *M. sinensis*. Although the parameters of the hydrothermal model are proposed to be constant for a seed lot (Gummerson 1996), we found that the best fit models for prediction of *M. sinensis* seeds allow for variation of θ_{HT} among temperatures. Cumulative germination models at 19.4°C and 28.1°C for each seed lot under M1 assumptions (i.e., all parameters constant) often under-estimated germination at 0 MPa and over-estimated at -0.25 and -0.50 MPa (Figure 2.4). Model fit for M1 was slightly greater at higher temperatures, but still underperformed models fit under M2 assumptions (Figure 2.5). Models fit to the Maryland accession were generally poor regardless of assumptions, which points to innappropriate parameter estimation more so than model error (Bauer 1998; Zambrano-Navea et al. 2013).

As one would expect from varieties of the same species, the θ_{HT} values derived for *M. sinensis* were closely related when operating under the assumption of constant θ_{HT} at all temperatures (Table 2.2). The Maryland and Jelitto accessions were seperated by only 9 MPa ° h. In contrast, when allowed to vary by temperature, the difference between θ_{HT} values of the same two accessions was never less than 700 MPa ° h (Table 2.3). Because the base temperature values for these three lots are so similar, differences in θ_{HT}, according to Eq. (1), must stem from differences in θ_{H}, or hydrotime. Differences in θ_{H} indicate high plasticity among accessions during germination, as has been shown in *Bromus tectorum* (Allen and Meyer 2002). The New York lot had far greater θ_{HT} values at each temperature, which may represent local adaptation. Because it is located further north than the Maryland variety, it would be an advantage to delay germination until later in the season, when damaging frost events and winter storms are less likely. More accessions are needed to evaluate these location-based inferences, but germination adaptions are common among accessions, especially in species that cover a broad geographic range, as is the case for *M. sinensis* (Allen and Meyer 2002; Bradshaw 1965; Dougherty et al. 2013).

Evidence from this study shows seed germination of *M. sinensis* does not follow the basic assumptions of the hydrothermal time model. The temperature requirements for different cultivars and accessions are similar; however, water availability thresholds decreased germination rates between seed lots and led to major differences in $\psi_{b(50)}$ and $\theta_{HT.}$ The M1 model (Figure 2.4), which assumes all parameters are constant, had poor model fit compared to the M2 model (Figure 2.5), which supports previous findings in other species (Alvarado and Bradford 2002; Wang et al. 2005). Other germination studies have found that variation in ψ_b can lead to poor model fit, especially across a large temperature gradient (Bradford 2002). Graziana et al. (2009) have suggested that when variation in ψ_b becomes too great within a population, using only $\psi_{\mathrm{b}(50)}$ could lead to errors depending on how it is employed. Currently, the dormancy and after-ripening requirements of *M. sinensis* seeds are unknown, so inferences of model fitness based on these principles cannot be made.

Although our results do not completely support the hydrothermal time model, the temperature and water potential parameters estimated from this study may still be useful in development of other ecological models for *M. sinensis*, such as climate niche modeling (Graziani and Steinmaus 2009). *Miscanthus sinensis* generally flowers in mid-to-late Fall, and subsequently sheds thousands of propagules, depending on the variety, before and during the winter months (Madeja et al. 2012). Therefore, development of a seed emergence model for the germination of *M. sinensis* after the overwintering period could be an invaluable tool to land managers and property owners in order to control and prevent further invasion. Models based on the thermal requirements of *M. sinensis* and similar grass species have already been developed (Clifton-Brown et al. 2011), and could be greatly refined with the addition of biologically relevant parameters such as $\psi_{b(50)}$, $\sigma_{\psi b}$, and θ_{HT} that are developed within the confines of the hydrothermal model.

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CHAPTER 3

Response of naturalized and ornamental *Miscanthus sinensis* biotypes to soil moisture and light stress

ABSTRACT

The recent trend in bioenergy feedstock development includes the use of large-statured perennial grasses that pose a relatively high risk of becoming invasive species due to the similarity in desirable agronomic traits with those of many of our worst invaders. Despite being an extremely popular ornamental grass, and naturalizing in over half of US states, little is actually known about the ecology and niche requirements of *M. sinensis*. Previous studies on the biology and ecology of *M. sinensis* have suggested that enhanced tolerance to shade and drought conditions may be significant mechanisms for invasion in the United States.

We conducted a greenhouse study to compare shade and soil moisture tolerance among common ornamental cultivars and naturalized populations, where we found enhanced plant growth and vigor in naturalized biotypes compared to ornamentals across varying levels of light stress, from 5% to 100% light availability. We also found that both naturalized and ornamental biotypes were not significantly affected by soil moisture stress, and thus express significant drought tolerance. Significantly greater vigor and performance in naturalized biotypes compared to ornamental cultivars suggests naturalized populations have evolved enhanced shade tolerance, most likely from hybridization. These basic ecological studies will help refine and support future evaluations and weed risk assessments of both *Miscanthus sinensis* and *M.* × *giganteus,* which is critical in prevention of major ecological invasions.

INTRODUCTION

Invasive plants can have devastating effects on native species and ecosystem function (Vila et al. 2011), and bring excessive economic costs from management efforts (Simberloff et al. 2013). Ironically, the majority of these species have been introduced intentionally through the horticulture and landscaping industries in the U.S. (Dehnen-Schmitz et al. 2007; Reichard and White 2001). Over 80% of woody invasive species in the U.S. are horticultural in origin (Reichard and White 2001); not to mention that ornamental/horticultural species comprise approximately 60% of the Florida-IPC and California-IPC noxious/invasive plant lists (PERAL 2012). Breeding and selection of horticultural species often results in traits that may later confer escape and invasive potential, including broad environmental tolerance, pest resistance, and shade tolerance (Culley and Hardiman 2007; Kitajima et al., 2006). One such example, *Miscanthus sinensis*, is a perennial grass native to East Asia, and is one of the most popular ornamental species in the U.S. (Wilson and Knox 2006; Quinn et al. 2011a).

Originally introduced to the U.S. in the late 19th century at the Biltmore Estate in Asheville, North Carolina, it has since naturalized in over 25 U.S. states, primarily along the Appalachian corridor (Dougherty et al. 2013). *Miscanthus sinensis* is a large statured grass that currently has more than 100 ornamental cultivars commercially available (Grounds 1998), and as recently as 2009 retail sales of *M. sinensis* totaled nearly $40 million in the state of North Carolina alone (Trueblood 2009). Not only is *M. sinensis* a major ornamental species, but it has also been under evaluation as a candidate bioenergy crop along with its sterile daughter species *Miscanthus* × *giganteus*, due to its broad environmental tolerance and aboveground biomass yield potentials (Quinn et al. 2011b).

Miscanthus sinensis is currently classified as an invasive species by the U.S. Forest Service (USDA Forest Service 2006), as well as by several regional invasive plant councils (IPANE 2010; SE-EPPC 2010). Classification as an invasive species by these organizations includes all varieties and ornamental cultivars of *M. sinensis*, although little is known about their individual ecology and invasive potential among cultivars and accessions. Previous studies have found significant biological and ecological variation among ornamental cultivars of several species, including *Hydrangea macrophylla* (Reed 2002), *Ruellia tweediana* (Wilson and Mecca 2003), and *Berberis thunbergii* (Lehrer et al. 2006). Several of these studies found significant differences in seed production and biomass in as few as three varieties. Cultivated varieties (cultivars) of *M. sinensis* are bred to exhibit immense phenotypic variation, including tiller height, basal diameter, flowering time, flowering color, and leaf color. A recent survey of several naturalized *M. sinensis* populations across the Eastern U.S. also found tremendous phenotypic variation among invasive populations (Dougherty et al. 2013). It is therefore extremely likely that important differences may exist in the ecology and invasive potential among the greater than 100 ornamental cultivars of *M. sinensis* currently available contributing to the success of this ornamental outside cultivation.

The exact mechanism(s) by which *M. sinensis* has become a successful invader are still unknown. The most common habitats of naturalized *M. sinensis* populations are low-canopy areas such as roadsides and forest edges, where light and water availability are rarely limiting (Dougherty et al. 2013). Some of these habitats are high-disturbance and have poor resource availability, which suggests that *M. sinensis* may employ several traits to survive and establish. It has been suggested that naturalized populations in the

U.S. may be shade tolerant (Matlaga et al. 2012; Miller 2003). Still, many populations have been found encroaching into forest understories and other higher-canopy areas that reduce availability of both light and potentially soil moisture (Dougherty et al. 2013). It is areas such as these where tolerance to soil moisture stress and shade may be important traits for establishment and naturalization.

Shade tolerance has been defined as the ability of a plant to thrive and survive under low light conditions (Valladares and Niinemets 2008). Horton et al. (2010) found that *M. sinensis* individuals are capable of maintaining high photosynthetic rates within the natural light gradient (5-100% relative transmittance) of a forest understory. More recently, Matlaga et al. (2012) directly compared the morphology and light response of *M. sinensis* seedlings from the native and introduced ranges with mixed results, but did ascertain that seeds could germinate and seedlings could grow under shaded environments only 30% of full light. Aside from shade tolerance, *M. sinensis* has been claimed as a “drought tolerant” species as well (Quinn et al. 2011a, 2011b; Stewart et al. 2009), although empirical studies of this tolerance have generally used varieties bred specifically for bioenergy, rather than ornamental or naturalized varieties (Zub and Brancourt-Hulmel 2010).

A better understanding of the ecology and environmental tolerance of *M. sinensis* could add valuable insight to invasion ecology, the horticulture trade, and the bioenergy industry. By identifying traits and characteristics that may confer invasiveness, we can develop risk assessments and management protocols to mitigate further naturalization and spread of *M. sinensis*. In this study, we evaluate the shade and drought tolerance of three naturalized biotypes and seven ornamental cultivars of *M. sinensis*. We aim to evaluate claims of shade and drought tolerance in the introduced range and identify ornamental cultivars that may contribute to the invasive potential of *M. sinensis*. We predict that naturalized biotypes will have greater shade and drought tolerance than ornamental cultivars. We also hypothesize that there will be greater variation in shade tolerance among the ornamental cultivars than the naturalized biotypes, suggesting that certain cultivars may be more likely to naturalize than others.

MATERIALS AND METHODS

Cultivar Selection

Due to greenhouse space limitations, we could only accommodate seven ornamental and three naturalized varieties for this study. These accessions do not fully represent the entirety of variation in *M. sinensis*, but were chosen in order capture variation in several important life history traits. Ornamental varieties were selected based on phenotypic observations in naturalized populations (Dougherty et al. 2013), and reported reproductive output (Madeja et al. 2012) (Table 1). This meant choosing more upright and robust varieties such as 'Graziella' and 'Gracillimus', as well as shorter, bushier varieties such as 'Adagio'. We also included several variegated varieties such as 'Dixieland', 'Variegatus', and 'Zebrinus', as we commonly found variegated individuals within naturalized populations (Dougherty et al. 2013). Ornamental varieties were also selected based on varying reproductive output, which ranged from an average of 211 seeds individual^{-1} ('Variegatus') to 157,936 seeds individual^{-1} ('Autumn Light') (Madeja et al. 2012). All seven ornamental cultivars were purchased from Tidwell Nurseries (Greenville, GA) as 5" plugs. Naturalized varieties were selected from eighteen invasive populations of *M. sinensis* surveyed by Dougherty et al. (2013) in the summer of 2011. In contrast to the ornamental varieties, these accessions were propagated from seed. The populations chosen were from Long Island, New York (code – NY), Loch Raven, Maryland (MD), and Henderson, North Carolina (NC) (Table 1). These populations are distributed across the entire latitudinal gradient of *M. sinensis* populations in the eastern U.S. and occurred in areas of varying light availability.

Experimental Design

Ornamental *M. sinensis* transplants were planted immediately upon arrival into 12.5 by 12.5 by 14.5 cm pots with Metro-Mix 510 media (Sun Gro Horticulture, Bellevue, WA), and allowed to acclimate under greenhouse conditions (approximately 28 °C) for two weeks. Individuals from naturalized populations were propagated from seed collected in November 2011, which were individually sown into several 128-cell trays (3 by 3 by 5 cm cell^{-1}) with Metro-Mix 510 media and propagated for eight weeks under greenhouse conditions. This eight-week period allowed the naturalized biotypes to reach the approximate size of the ornamental cultivars. After the propagation period, naturalized individuals were randomly selected and transplanted into 12.5 by 12.5 by 14.5 cm pots as above ensuring that all *M. sinensis* individuals were approximately the same size at the beginning of the study.

This study was performed in a greenhouse at Virginia Tech (Blacksburg, VA). Four treatments of soil moisture availability were selected for this experiment: high (40% v/v or field capacity), medium high (30%), medium low (20%), and low (10%). The high, medium high, and medium low treatments were achieved using drip irrigation scheduled to maintain soil moisture ± 5% of the treatment target at all times.

Table 3.1 Summary and descriptions of ornamental and naturalized varieties.

Ornamental

Variety	**CODE**	**Mean Seed Set**[a] **(individual^{-1})**	**Description**[b]
'Adagio'	AD	27,078	1 meter, blooms in September-November, thin silver gray foliage, pink inflorescence turning white
'Autumn Light'	AL	157,936	2.1 – 3m, blooms in September, hardy variety, inflorescence bronze-red turning to silver
'Dixieland'	DX	785	1 – 1.2m, blooms in September, wide green leaves with white stripes, strong reddish inflorescence (dwarf form of *M. variegatus)*
'Gracillimus'	GC	3,146	1.5 – 1.8m, blooms in October, slender foliage, inflorescence bronze-red turning to silver
'Graziella'	GZ	90,569	1.5 – 1.8m, blooms in August, slender foliage, large white inflorescence
'Variegatus'	VR	211	1.8 – 2.1m, blooms in late September, white striped foliage, strong reddish inflorescence
'Zebrinus'	ZB	16,621	1.8 – 2.4m, blooms reddish in September-October, light green foliage, has horizontal yellow zebra-like bands

Naturalized[c]

Variety	**CODE**	**Latitude**	**Longitude**	**Habitat**
New York	NY	40.7093	-73.1489	open field and forest edge in conservation area
Maryland	MD	39.5598	-76.3825	roadside and forest edge
North Carolina	NC	35.2690	-82.4102	roadside and open field along railroad right-of-way

[a] Madeja et al. (2012) [b] Kurt Bluemel (2010) [c] See Dougherty et al. (2013) for more detailed descriptions of populations.

The low treatment (10% v/v) was a simulated acute drought in which individual pots would receive one irrigation event of approximately 1L biweekly. Soil moisture levels were monitored weekly with a TH300 soil moisture probe (Dynamax Inc., Houston, TX) and water potential values (MPa) were obtained for each treatment with a WP4 Dewpoint Potentiameter (Decagon Devices, Inc., Pullman, WA). The average water potential values were -0.02 MPa ± 0.02 (high), -0.12 MPa ± 0.04 (medium high) -0.50 MPa ± 0.08 (medium low), and -4.05 MPa ± 0.56 (low). In addition to the soil moisture availability treatments, we also imposed a series of light availabilities: 100% (high), 40% (medium), and 5% (low) relative transmittance using varying layers of 60% shade cloth (International Greenhouse Company, Danville, IL). Photosynthetically active radiation (PAR) levels were recorded between 12:00 PM and 3:00PM approximately five times over the first two weeks with an AccuPAR LP-80 PAR/LAI ceptometer (Decagon Devices, Inc., Pullman, WA) in each light treatment to confirm the levels of relative light transmittance. The high, medium, and low light treatments had PAR levels of 1209 ± 120 μmol m^{-2} s^{-1} (high), 488 ± 27 μmol m^{-2} s^{-1} (medium), and 60 ± 10 μmol m^{-2} s^{-1} (low). This study was a randomized complete block design, with the locations of the light availability treatments randomized within each soil moisture treatment, for a total of twelve treatment combinations. Five individuals from each *M. sinensis* accession were randomly placed within each treatment combination, except for the low soil moisture treatments, where only three individuals were used due to space limitations. At the beginning of the study, each of the individuals was watered to field capacity, and soil moisture treatments began to take effect after approximately seven days.

Data Collection

We collected plant morphology data, including tiller height, tiller number, and basal diameter prior to treatment initiation to account for starting size variation among the accessions. Individual survival was recorded biweekly until the termination of the experiment at sixteen weeks. Individuals were re-randomized within their respective light treatment at each data collection to reduce location effects within the greenhouse. After six weeks of treatment, photosynthetic data was collected from three individuals of each accession in each treatment using a LICOR XT6400 gas exchange system (LICOR). Two to three of the youngest, fully expanded leaves from each individual were subjected to varying levels of light from 0-1500 µmol m^{-2} s^{-1}.

Steady-state light curves were generated from photosynthetic data in SigmaPlot 11 (Systat Software Inc., San Jose, CA) from three individuals of each variety in each treatment combination. Photosynthetic data was fitted to the Von Bertalanffy growth equation:

$$p = a + L_\theta(1 - e^{-kt}) \tag{1}$$

where p is photosynthetic rate, a is dark respiration, L_θ is the maximum photosynthetic capacity, and k is quantum flux (Horton 1998).

Data Analysis

Plant morphology data from surviving individuals, including tiller height, basal diameter, tiller number, and leaf diameter, were analyzed with ANOVA fixed effect models in JMP10 (SAS Inc., Cary, NC). Because ornamental and naturalized varieties were initially transplanted at different stages of maturity and size (i.e., plugs and seeds), plant morphology data was analyzed as a total percent change over the entire sixteen-week trial rather than raw values. All morphological responses were log transformed before analysis to meet the assumptions of ANOVA. Treatment means were compared with Tukey's HSD ($p < 0.05$). At the end of the trial, individuals were assigned a binary mortality value, which was analyzed using logistic regression.

Steady-state light curve growth constants a, L_θ, and k from each variety were compared with ANOVA to identify physiological differences between varieties and their responses under varying treatment levels.

RESULTS

Mortality varied among variety, biotype, light availability, and soil moisture (Table 3.3). At the conclusion of the trial, seven varieties, six of which were ornamental, had no surviving individuals in at least one treatment combination. Only 'Graziella', Maryland, and New York varieties had at least one surviving individual in all treatment combinations, while 'Autumn Light', 'Variegatus', and 'Zebrinus' all had zero surviving individuals in the low light treatment, regardless of soil moisture level (data not shown). In total, 153 of 540 individuals did not survive, or approximately 28%. Light availability had a much stronger effect on mortality than soil moisture, with only 20% of individuals surviving the low light treatment. Mortality under soil moisture treatments was somewhat evenly spread between 18-31% (data not shown).

Like mortality, the morphological responses of surviving individuals were most affected by light availability (Table 3.2). As expected, tiller height, tiller number, basal diameter, and leaf diameter all decreased with light availability (Table 3.3). These morphological responses were generally lower in the low light treatment compared to the medium and high light levels, which performed similarly ($p > 0.05$). Naturalized varieties had greater gains in tiller height and leaf diameter than ornamental varieties under each soil moisture treatment; however, the response within a variety did not differ among soil moisture treatments (Figure 3.2). For example, gains in tiller height of ‘Gracillimus’ were equal across soil moisture treatments, but were lower than the Maryland, New York, or North Carolina varieties (Figure 3.1).

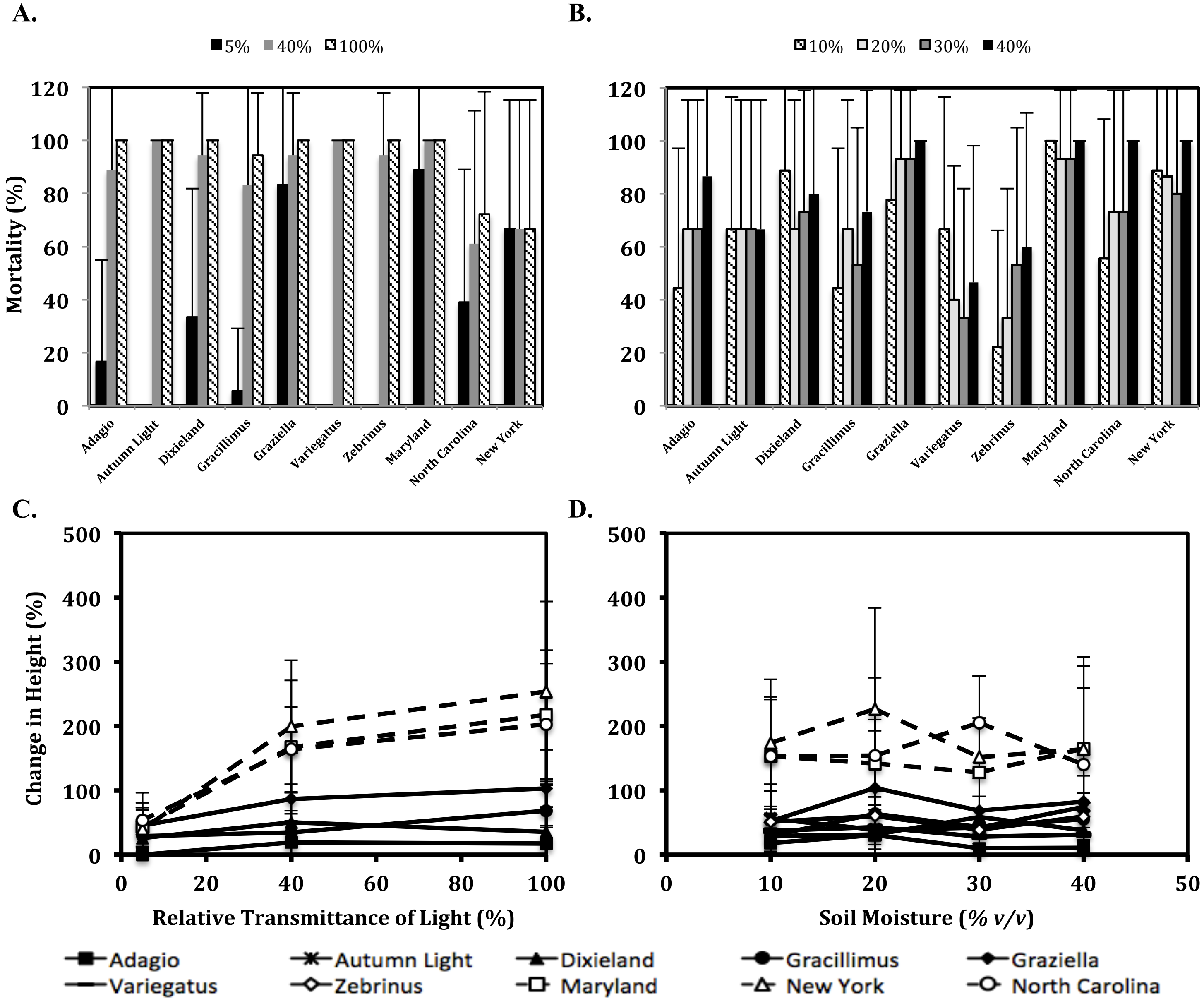
Naturalized varieties outperformed ornamental varieties in tiller height, basal diameter, and leaf diameter in all treatments (Table 3.2). Changes in tiller height of naturalized varieties in the low light treatment (5% relative transmittance) were equal to changes in tiller height of ornamental varieties in the high light treatment (100%; Figure 3.1). Subtle differences between varieties of the same biotype do exist; however, biotype (ornamental or naturalized) is clearly a more significant driver of morphological response to stress than variety alone (Table 3.2). Naturalized varieties were also more resilient to all levels of stress, accounting for less than 15% of mortalities (data not shown).

In addition to morphological responses, analyses of the steady-state light curves revealed significant physiological stress response differences among varieties and biotypes (Table 3.3). Respiration rate, maximum photosynthetic rate, and quantum capacity all varied among variety and light treatments ($p < 0.05$). Overall, naturalized varieties had higher dark respiration rates and quantum capacity, but ornamental varieties had higher maximum photosynthetic rates (Tables 3.3 and 3.4). Respiration rates were higher under the low light treatment than both the medium and high light treatments ($p = 0.0058$); however, maximum photosynthetic rate ($p < 0.0001$) and quantum capacity ($p <$

0.0001) were greater in higher light treatments as expected. Individuals exposed to the low soil moisture level treatment had significantly lower maximum photosynthetic capacities (p = 0.0226) than individuals at the medium low, medium high, and high treatments.

Table 3.2 ANOVA of steady-state light curve parameters from all varieties.

Variable	**A**		**L_θ**		**k**	
	μmol m^{-2} s^{-1}		μmol m^{-2} s^{-1}		slope	
	F	**P**	**F**	**P**	**F**	**P**
Variety[Biotype]	2.87	**0.005**	7.45	**<.0001**	3.61	**0.0006**
Biotype (B)	3.06	0.0818	18.72	**<.0001**	12.30	**0.0006**
Shade (ST)	5.29	**0.0058**	13.37	**<.0001**	24.45	**<.0001**
Moisture (MT)	2.57	0.0555	3.27	**0.0226**	0.41	0.7459
B*ST	1.87	0.1565	1.91	0.1514	7.58	**0.0007**
B*MT	0.07	0.9781	1.74	0.1599	0.40	0.7505



igure 3.1 **(A)** Average mortality of each variety under all light treatments**. (B)** Average mortality of each variety under all soil moisture reatments. **(C)** Total change in tiller height (%) of each variety under all light treatments. **(D)** Total change in tiller height (%) of each ariety under all soil moisture treatments. *Dashed lines represent naturalized varieties, solid lines represent ornamental varieties.

Table 3.3 ANOVA of morphological responses and logistic regression of mortality.

Variable	**Δ Height**		**Δ Tiller Number**		**Δ Basal Diameter**		**Leaf Diameter**		**Mortality**	
	F	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**ChiSq**	**P**
Variety[Biotype]	3.93	**0.0002**	2.30	**0.0203**	5.05	**<.0001**	5.23	**<.0001**	111.80	**<.0001**
Biotype (B)	164.66	**<.0001**	0.06	0.8083	19.63	**<.0001**	23.94	**<.0001**	28.86	**<.0001**
Shade (ST)	46.36	**<.0001**	8.96	**0.0002**	7.75	**0.0005**	8.62	**0.0002**	1.40e-4	0.9999
Moisture (MT)	1.17	0.3215	1.38	0.2479	3.35	**0.0191**	1.54	0.2049	1.50e-5	1.000
ST*MT	1.40	0.2139	2.62	**0.0167**	2.33	**0.0321**	1.50	0.1773	2.74	0.8402
B*ST	16.60	**<.0001**	4.21	**0.0156**	3.19	**0.0422**	3.68	**0.0261**	8.32e-6	1.000
B*MT	0.13	0.9426	0.31	0.8150	0.07	0.9975	1.71	0.1636	8.67e-6	1.000
B*ST*MT	1.11	0.3526	0.84	0.5384	0.48	0.8236	1.41	0.2087	2.62	0.8548

DISCUSSION

Repeated introductions and breeding for traits that increase horticultural value have given rise to many invasive species of ornamental origin, such as *Pyrus calleryana* (pear tree), *Berberis thunbergii* (Japanese barberry), *Ardisia crenata* (coralberry), and *Miscanthus sinensis* (Chinese silvergrass) (Culley and Hardiman 2007; Kitajima et al. 2006; Lehrer et al. 2006). Such traits include rapid growth, early flowering, increased flower number, broad environmental adaptability, and shade tolerance (Culley and Hardiman 2007; Kitajima et al. 2006). The tremendous genetic and phenotypic variation among cultivated varieties (cultivars) of ornamental species can lead to tremendous variation in the expression and magnitude of these traits (Conklin and Sellmer 2008; Kitajima et al. 2006; Lehrer et al. 2006). Empirical studies have found dramatic variation in the reproductive output and environmental tolerances between as few as three ornamental cultivars of a single species (Lehrer et al. 2006), which suggests that stress tolerance among the well over 100 phenotypically diverse ornamental varieties of *M. sinensis* likely exist.

We tested the hypotheses that invasive, naturalized varieties of *M. sinensis* exhibit greater tolerance to low light availability and soil moisture stress than ornamental varieties, and that ornamental varieties would vary more in their stress tolerance. Overall, our results demonstrate significant differences in plant morphology and survival between naturalized and ornamental varieties of *M. sinensis* in response to light and soil moisture stress. We found that naturalized varieties can tolerate extremely low light (60 ± 10 μmol m^{-2} s^{-1}) and soil moisture availability (-4.05 ± 0.56 MPa) to a greater degree than even the most tolerant ornamental varieties in terms of survival (Table 3.3), plant performance

(Figures 3.1 and 3.2), and ecophysiology (Table 3.4). Despite the limited sampling of all possible ornamental and naturalized *M. sinensis* varieties, our results support the hypothesis that naturalized populations have evolved enhanced shade tolerance in the US, and that certain ornamental varieties have greater environmental tolerance than others. However, tolerance to low soil moisture availability is a trait universal to both naturalized and ornamental varieties of *M. sinensis*.

In the US, *M. sinensis* generally naturalizes in high light areas such as roadsides and open fields, but populations have also been found in low light areas such forest edges, and to a lesser extent, understories (Dougherty et al. 2013). According to Horton and Neufeld (1998) successful invaders of forest understories tend to be shade tolerant, and express this tolerance through several morphological and physiological adaptations, such as increased leaf area ratio, survival, and ability to maintain photosynthetic rates. In our study, under each level of light availability, naturalized varieties grew taller and produced wider leaves than ornamental varieties (Table 3.3, Figure 3.1); however, all varieties performed significantly better in the high and medium light treatments than the low availability. Our results are consistent with the trait responses associated with shade tolerance, especially ability to survive (Horton et al. 2010; McAlpine and Jesson 2007; Spencer 2012). Although we did not directly measure leaf area ratio, leaf diameter did increase in lower light treatments; a trend associated with increased leaf area ratio. We hypothesized that ornamental varieties would have far greater mortality under shade stress than naturalized varieties that had the distinct advantage of natural selection over many generations under a range of canopy covers and light levels. Our results support this prediction overwhelmingly (Table 3.3), as naturalized varieties accounted for less than 15% of all mortality (n = 153). Not surprisingly, all mortality of naturalized varieties occurred under the low light treatment. In contrast, ornamental varieties suffered greater mortality under all light treatments; however, approximately 75% of ornamental mortality was under the low light treatment, compared to 16% and 9% in the medium and high light treatments, respectively.

We also found significant differences in physiological responses to light availability between varieties and biotypes. Plants grown in low light environments often adapt by decreasing respiration rate (a) and maximum photosynthetic rate (L_θ) while increasing quantum capacity (k) as a way to conserve energy and maximize photosynthetic efficiency (Horton and Neufeld, 1998). Our results show that both biotypes follow these trends under low light availability, although ornamental varieties exhibited greater maximum photosynthetic rates (Tables 3 and 4). Even with higher maximum photosynthetic rates, ornamental varieties did very poorly in terms of morphological gains and survival, which suggests that maximum photosynthetic rate is not an appropriate indicator of shade tolerance. Quantum capacity was greater in naturalized varieties, which means they can reach maximum photosynthetic capacity more efficiently and in less time. This trait is likely the most important physiological adaptation in naturalized varieties, and a vital reason for greater survival and performance relative to ornamental varieties. Our results are consistent with the physiological responses of other invasive grasses such as *Microstegium vimineum* (Horton and Neufeld 1998), as well as previous studies of naturalized *M. sinensis* (Horton et al. 2010). These exotic C_4 grasses have formed invasive populations in the eastern U.S., and may exhibit enhanced shade tolerance within these populations. Generally C_4 plants do not adapt well to low light environments relative to C_3 species, but recent evidence suggests that C_4 species such as *M. vimineum* may actually have a competitive advantage in temperate understory habitats (Horton 1998).

Tolerance to low soil moisture availability and drought conditions, like in *Panicum virgatum* (switchgrass), can enhance naturalization potential across a broad range of habitats (Barney et al. 2009). Differences in tolerance to soil moisture availability have been found between native and invasive species (McAlpine et al. 2008; Schumacher et al. 2008), as well as between cultivars of the same species (Prunty 1981), and we expected to identify similar differences in performance in *M. sinensis*.

Miscanthus sinensis is anecdotally considered a drought tolerant species (Quinn et al. 2010; Quinn et al. 2011, Stewart et al. 2009), although little empirical evidence exists to support this notion. Clifton-Brown and Lewandowski (2000, 2002) have found that *M. sinensis* is the most drought-tolerant of the *Miscanthus* genus, but we question how the species has responded to artificial cultivation and whether or not naturalized varieties have an increased drought tolerance relative to ornamental varieties. Our results show that soil moisture did not have a significant effect on nearly any morphological or physiological response (Tables 3.3 and 3.4). Basal diameter decreased under the low and medium-low soil moisture treatments, although a significant interaction effect between light and soil moisture treatments ($p < 0.0001$) reveal that this significant decrease is more likely linked to the combination of treatments rather than soil moisture alone. Differences in basal diameter between varieties are possibly a result of artificially selected traits such as growth habit. Mortality in the low treatment (32%) was only slightly higher than in the high treatment (19%). Overall, naturalized varieties were more tolerant to soil moisture stress than ornamental varieties, but regardless of biotype, there were no differences in mortality among the soil moisture availability treatments. This is in stark contrast to switchgrass, which experienced decreases in biomass, tiller height, specific leaf area, and survival across a similar water potential gradient of 0.0 to -4.0 MPa in a study from Barney et al. (2009). Low soil moisture availability has been shown to decrease growth and survival of other invasive species under low light availability (Schumacher et al. 2008); a trend which our results did not support for *M. sinensis*. Mortality and changes in morphology did not vary between soil moisture treatments (Table 3.2). These trends support our prediction that *M. sinensis* is not only drought tolerant, but significantly more drought tolerant in naturalized varieties.

Finally, we also predicted that the response of ornamental *M. sinensis* varieties to light and water stresses would fall along a continuum—some ornamental varieties would be more shade and drought tolerant than others, and subsequently be of higher risk for invasion. Our results show the 'Graziella' variety possesses the greatest tolerance to shade and soil moisture stress and performs most like the naturalized varieties. We also found that 'Variegatus' and 'Zebrinus' are the least tolerant to these stresses and subsequently pose the lowest risk of invasiveness. It is important to note that our results only represent seven of over 100 ornamental varieties of *M. sinensis* and thus the recommendations of invasive risk are relative only among these seven varieties. Because significant differences in shade tolerance exist between a small fraction of the total number of *M. sinensis* varieties, it is logical to assume that other ornamental varieties may be even more, or less, shade tolerant. Future studies of ornamental *M. sinensis* should include evaluations of a much more diverse selection of varieties. Previous studies have also found that shade tolerance does not always pass through to progeny, since some offspring of shade tolerant parents are often shade intolerant (McAlpine and Jesson 2007). *Miscanthus sinensis* is also an obligate outcrosser, which means hybridization within and between naturalized populations may select for or against enhanced shade tolerance. It's also more than likely that naturalized varieties may be hybridizing with certain ornamental varieties if given the right circumstances. We suggest that future studies examine the reproductive output of *M. sinensis* under low light stress and identify the long-term consequences, including seed set, germination, and inheritance of shade tolerance.

In conclusion, *M. sinensis* does express shade tolerant traits such as increased leaf diameter, high photosynthetic efficiency, and most importantly, survival, in low light environments. These characteristics, combined with broad tolerance to drought stress, enhance the ability of *M. sinensis* to establish and naturalize in the eastern US in habitats of varying light and water availability. Artificial selection and breeding of ornamental varieties cause not only phenotypic variation, but also differences in response to environmental stress and potential invasiveness, including shade and drought tolerance. The vast majority of naturalized populations of *M. sinensis* are found in high light areas such as roadsides and forest edges, but many populations are also found in forest understories and habitats with low light and water availability. Identifying ornamental varieties of high and low risk for potential invasion is an essential step in management and control of *M. sinensis*. Repeated introduction of the most shade and drought tolerant ornamental varieties adds genetic fuel to the invasion fire, which could accelerate the expansion of *M. sinensis* populations into forest understories and other low light areas.

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CHAPTER 4

Conclusions on the ecology and invasive potential of *Miscanthus sinensis*

Theoharides and Dukes (2007) identified four stages that a nonindigenous species must go through to become a successful invader - transport, colonization, establishment, and landscape spread. These stages take years, often decades, to progress though, and a successful invader must possess a unique suite of characteristics to survive and advance through each stage. One of the primary objectives of this research was to piece together the history and ecology of *M. sinensis* from its introduction to its current state. Because of its prevalence as an ornamental species, and long, well-documented introduction history, *Miscanthus sinensis* an excellent case-study for the aforementioned invasion process. Therefore, we have the unique oppurtunity to describe in some detail the progress of the invasion of *M. sinensis* in the introduced range.

The first stage of the invasion process is long-distance transport. This can be accomplished in one of many ways, but the end goal is usually intercontinental movement of a species into a new region (Theoharides and Dukes 2007). *Miscanthus sinensis* was first introduced to the United States from Japan in the late 19th century as an ornamental species (Quinn et al. 2011a). First described at the Biltmore Estate nurseries in 1892, it was found in gardens in New York and Washington by 1910. By 1912, *M. sinensis* and several other *Miscanthus* species were listed in the Biltmore Nursery mail-order catalog, which made *M. sinensis* available all over the country (Dougherty et al. 2013; Quinn et al. 2010). In order to accomplish the transport stage, a species must have traits that enable it to either survive a long-distance journey if it is being transported accidentally, or, in the case of *M. sinensis*, it must have traits of value such as aesthetics and/or potential crop use. *Miscanthus sinensis* is a large-statured grass that forms tall, flowering tillers which come in many different shapes and colors. These flowering tillers are a major reason *M. sinensis* reached the first stage of invasion, but also wound up establishing in its introduced range, and progressing to the second stage of invasion – colonization.

Although many species have been transported and introduced across international borders, only a handful will successfully colonize and enter the second stage of invasion. Broad environmental tolerance and the ability to survive under different climate conditions is an essential trait for colonizing in the introduced range. In its native range, *M. sinensis* forms vast grasslands in open canopies that stretch between subtropical and subarctic climates in China, Japan, and Taiwan (Stewart et al. 2007). The entire eastern US south of Maine falls within this latitudinal range, making the transition to its introduced range rather easy. Not only is *M. sinensis* tolerant to a broad range of climatic conditions, but it is also able to establish and survive in extremely poor soil conditions (Ezaki et al. 2008; Stewart et al. 2007). In the US range, we found that *M. sinensis* populations can establish in soils with pH < 4.5 (Chapter 1, Table 3) and tolerate both high and low levels of macro- and micro-nutrients. Both naturalized and ornamental varieties can be considered drought-tolerant and can grow at normal rates in soil moistures as low as 10% v/v (Chapter 3, Table 2). These traits enable *M. sinensis* to survive even in periods of low resource availability, which is one of the major bottlenecks to the invasion process (Davis et al. 2000).

Once colonized, the next invasion stage is establishment, which is defined as forming self-sustaining, expanding populations (Theoharides and Dukes 2007). For *M. sinensis*, this process likely takes form through the escape from an ornamental planting to a roadside or forest edge just a short distance away. As mentioned earlier, one of the key traits to the early success of *M. sinensis* were its tall, colorful flowering tillers. These tillers are also the key to its transition from colonization to establishment for two reasons. The first reason is that each flowering tiller, depending on the variety, is capable of producing between one and 1,800 spikelets per panicle (Quinn et al. 2010). As discussed in Chapter 1, this means the average naturalized population of *M. sinensis* can produce over 8.4 billion spikelets a year. This heavy propagule pressure is one of the biggest assets to the establishment and expansion of *M. sinensis* populations. The second reason the flowering tillers are key to the transition from colonization to establishment is the spikelets/seeds themselves. Quinn et al. (2010) found that the hairy spikelets of *M. sinensis* and other *Miscanthus* species are capable of wind-dispersing at least 400m from the source individual. This combination of long-distance dispersal and propagule pressure give *M. sinensis* incredible invasion potential. When scouting for small cohorts and escaped individuals surrounding naturalized populations of *M. sinensis*, we discovered gaps between individuals of over 800m (Chapter 1). This evidence is entirely circumstancal and requires genetic work for confirmation, but it leads to interesting questions about the true dispersal potential of *M. sinensis*.

This brings us to the last stage of the invasion process, landscape spread. The line between establishment and landscape spread is blurred, and many of the same processes occur in both stages, but at varying levels of scale. During this time, *M. sinensis* is filling a new ecological niche within a new ecological community, and thus is prone to interspecific and resource competition, predation, and disturbance events (Theoharides and Dukes 2007). To complete its evolution to a fully naturalized invader, *M. sinensis* must utilize special traits to command resources and space. Our shade tolerance and soil moisture study found that naturalized individuals can not only tolerate, but thrive in low light conditions. This is a tremendous advantage for *M. sinensis*, and possibly one of the reasons that several populations have been found creeping into forest understories and other high canopy areas. These "shade-tolerant" individuals can grow rapidly, even under heavy shade, and maintain relatively high rates of photosynthesis (Chapter 2). Although decades of natural selection may have had a role in the development of this trait, the fact that *M. sinensis* is an obligate outcrosser means that various levels of hybridization may be occuring within and between naturalized and ornamental populations. This hybridization may explain the high amount of phenotypic variation in *M. sinensis* populations, and could also be a prominent reason for its expansion all over the east coast.

It has been over a hundred years since the first introduction of *M. sinensis* to the US in Asheville, North Carolina (Quinn et al. 2010), and it has advanced through all the stages of invasion to become a very successful and prominent invader. *Miscanthus sinensis* remains a prominent ornamental species today, and repeated introductions through horticulture are only adding to the invasive problem. Once a species has progressed through the stages of invasion, it is almost certainly going to remain a permant fixture in our ever-changing ecosystems. From this point, we must turn to control and management practices in an effort to prevent future expansion of the invasion front. For *M. sinensis*, that means identifying ornamental cultivars that are of high risk for invasion or hybridization with naturalized individuals. In this project we investigated the relative shade and soil moisture tolerance of several ornamental cultivars, of which we identified 'Gracillimus' and 'Graziella' as high risk varieties, and 'Variegatus' and

‘Zebrinus’ as low risk (Chapter 3). We also looked at the seed biology of *M. sinensis* and developed hydrothermal time models to predict the time at which *M. sinensis* is most likely to germinate under certain conditions. Creating predictive models and identifying areas of future risk for invasion are going to be instrumental steps in mitigating the invasive potential of *M. sinensis* in the eastern US.

This project strove to characterize the ecology and niche characterization of *M. sinensis* in the introduced range. We identified its most common habitats along roadsides and forest edges, while also discovering its putative future habitats in forest understories. We discovered that many naturalized individuals exhibit an increased shade tolerance over ornamental varieties, and that some ornamental cultivars may have more invasive potential than others. And we built the background for future predictive modeling and seed biology by estimating temperature and moisture parameters of the hydrothermal time model. This work is only preliminary in understanding the complete ecology and invasive potential of *M. sinensis* in the US, but it provides a solid foundation to be built upon.

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