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Light Response of Native and Introduced *Miscanthus sinensis* Seedlings

David P. Matlaga, Lauren D. Quinn, Adam S. Davis, and J. Ryan Stewart*

The Asian grass *Miscanthus sinensis* (Poaceae) is being considered for use as a bioenergy crop in the U.S. Corn Belt. Originally introduced to the United States for ornamental plantings, it escaped, forming invasive populations. The concern is that naturalized *M. sinensis* populations have evolved shade tolerance. We tested the hypothesis that seedlings from within the invasive U.S. range of *M. sinensis* would display traits associated with shade tolerance, namely increased area for light capture and phenotypic plasticity, compared with seedlings from the native Japanese populations. In a common garden experiment, seedlings of 80 half-sib maternal lines were grown from the native range (Japan) and 60 half-sib maternal lines from the invasive range (U.S.) under four light levels. Seedling leaf area, leaf size, growth, and biomass allocation were measured on the resulting seedlings after 12 wk. Seedlings from both regions responded strongly to the light gradient. High light conditions resulted in seedlings with greater leaf area, larger leaves, and a shift to greater belowground biomass investment, compared with shaded seedlings. Japanese seedlings produced more biomass and total leaf area than U.S. seedlings across all light levels. Generally, U.S. and Japanese seedlings allocated a similar amount of biomass to foliage and equal leaf area per leaf mass. Subtle differences in light response by region were observed for total leaf area, mass, growth, and leaf size. U.S. seedlings had slightly higher plasticity for total mass and leaf area but lower plasticity for measures of biomass allocation and leaf traits compared with Japanese seedlings. Our results do not provide general support for the hypothesis of increased *M. sinensis* shade tolerance within its introduced U.S. range compared with native Japanese populations.
Nomenclature: Eulaliagrass; *Miscanthus sinensis* Anderss.
Key words: Biomass allocation, light response, *Miscanthus sinensis*, phenotypic plasticity.

There is growing interest in developing biomass crops for energy production, with research underway to evaluate the economic benefits (Clifton-Brown et al. 2001; Khanna et al. 2008) and producers poised to begin large-scale production. However, many fundamental aspects of the invasion biology of candidate species remain unknown and, if determined, may be valuable in preventing biomass production from exacerbating the invasive species problem. Candidate biofeedstocks share key traits with invasive species (e.g., rapid growth, clonal spread, etc.), and many are known to naturalize or invade outside their native range (Barney and Ditomaso 2008; Chimera et al. 2010; Raghu et al. 2006). Rhizomatous perennial grasses are recognized as ideal for biomass production because of characteristics such as efficient photosynthesis and low nutrient requirements (Heaton et al. 2004), characteristics that contribute to their success as invaders as well (D'Antonio and Vitousek 1992). *Miscanthus sinensis* Anderss. is a tall (up to 3 m) rhizomatous perennial grass native to southeast Asia (Koyama 1987; Ohwi 1964) currently being evaluated for biomass production in the United States and Europe (Clifton-Brown et al. 2001). *Miscanthus sinensis* was first introduced to the United States from Japan in the late 1800s for use in the ornamental trade (Favretti and Favretti 1997). By 1913, *M. sinensis* was found outside of cultivation in the eastern United States (Britton and Brown 1913), and by 1950, naturalized populations were noted as a "nuisance" in natural areas (Hitchcock 1950).

Today, *M. sinensis* is one of the most widely planted ornamental grasses in the United States (Wilson and Knox 2006). More than 100 horticultural varieties are commercially available (Grounds 1998). It is classified as an invasive species by regional invasive plant councils in the Southeast (SE-EPPC 2010) and New England (IPANE 2010) and by the U.S. Forest Service (USDA Forest Service 2006). Naturalized *M. sinensis* populations have been documented in 27 U.S. states, primarily in the eastern

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* First and third authors: Postdoctoral Researcher and Ecologist, Global Change and Photosynthesis Research Unit, USDA Agricultural Research Service, N-319 Turner Hall, 1102 S Goodwin Avenue, Urbana IL, 61801; second author: Postdoctoral Researcher, Energy Biosciences Institute, University of Illinois, 1206 W Gregory Drive, Urbana, IL 61801; fourth author: Assistant Professor, Department of Crop Sciences, University of Illinois, 1201 S Dorner Drive, Urbana, IL 61801. Corresponding author's E-mail: dmatlaga@illinois.edu

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Management Implications

Eulaliagrass (*Miscanthus sinensis*), an Asian species under consideration for biomass production in the Midwest, has escaped ornamental plantings in the United States to form naturalized populations. Evidence suggests that U.S. populations are able to tolerate relatively shady conditions, but it is unclear whether U.S. populations have greater shade tolerance than the relatively shade-intolerant populations within the species' native range in Asia. Increased shade tolerance could result in a broader range of invaded light environments within the introduced range of *M. sinensis*. However, results from our common garden experiment do not support the hypothesis of increased shade tolerance in introduced U.S. populations compared with seedlings from native Asian populations. Our results do demonstrate that for both U.S. and Japanese populations under low light conditions, *M. sinensis* seeds germinate and seedlings gain mass and leaf area; therefore, land managers should carefully monitor or eradicate *M. sinensis* within these habitats.

mid-Atlantic, but also as far west as California and as far north as Ontario, Canada (USDA NRCS 2010). Relatively large populations can be dense (15,075 individuals ha^{-1}), can extend to more than a hectare, and may exclude native vegetation (Quinn et al. 2010). Within its native and introduced range, *M. sinensis* is found in open areas, in sites with frequent disturbance (e.g., mowing and burning), and along roadsides (Meyer 2004; Stewart et al. 2009). Additionally, but only in its introduced range, *M. sinensis* is described as shade tolerant (Miller 2003). Horton et al. (2010) showed that within the secondary forest understory, *M. sinensis* maintains high rates of photosynthesis and positive daily carbon gains. Hence, the concern among land managers is that *M. sinensis* could spread through the understory of primary forests (Horton et al. 2010). Shade-tolerant exotics, although relatively uncommon, pose a threat to forests because their abundance could increase over successional time (Martin et al. 2009). For example, a study documenting the abundance of invasive plants 40 yr after land abandonment in Puerto Rico found that shade-intolerant species declined but the shade-tolerant rose apple, *Syzygium jambos* (L.) Alston, persisted or increased in abundance (Lugo 2004).

A central goal of invasion biology is to understand the causes and consequences of genetic and phenotypic differences among conspecifics between native and introduced ranges (Richards et al. 2006). When populations within introduced and native ranges remain connected through repeated introductions, there are often no genetic differences between ranges (Bossdorf et al. 2005). Conversely, when native and introduced populations are isolated from one another, the genetic-shift hypothesis posits that evolutionary processes can create genetic divergence, causing phenotypic differences between regions (Blossey and Notzold 1995; Pritchard 1960). Genetic divergence can result from several mechanisms, including founder effects and postintroduction natural selection (Ellstrand and Elam 1993; Siemann and Rogers 2001) and, in the case of escaped ornamentals, horticultural selection (Kitajima et al. 2006). A population genetics study has compared six introduced (U.S.) *M. sinensis* populations with five native (Japanese) populations (Quinn et al., in press). Both regions were found to have high levels of heterozygosity and genetic diversity. Patterns of genetic variability between regions were distinct with lower allelic diversity among U.S. populations (Quinn et al., in press).

Shade tolerance is the relative ability of a plant to survive and perform under low light conditions (Valladares and Niinemets 2008). A plant's ability to tolerate shade is influenced by a suite of functional traits that determine light capture (Reich et al. 2003). The "carbon gain hypothesis" argues that trait expression that maximizes light capture and minimizes respiration costs improves shade tolerance (Givnish 1988). Comparing mean values of traits that maximize light capture in a specific environment is one approach to understanding differences in shade tolerance between plants (Reich et al. 1998). Carbon gain can be maximized by traits including high specific leaf area (SLA; leaf area per unit of leaf mass) and leaf area ratio (LAR; leaf area per unit of whole plant mass) and greater allocation to foliage than belowground tissue (root to shoot ratio, RSR; belowground mass per aboveground mass) (Reich et al. 2003; Valladares and Niinemets 2008). Alternatively, comparing phenotypic plasticity is an approach that addresses a different aspect of shade tolerance. Plasticity is the capacity of a genotype to express different phenotypes under different environmental conditions and is an important means to cope with heterogeneity for sessile organisms like plants (Sultan 2000). Generally, light heterogeneity is higher within the forest understory where long periods of diffuse light are interrupted by bright sunflecks and small gaps compared with the relatively homogeneous light intensity found in open areas with high light areas (Ogren and Sundin 1996; Valladares et al. 1997).

Plasticity may extend ecological breadth, allowing plants to express advantageous phenotypes in a broader range of environments (Richards et al. 2006 and references therein). It has long been thought that phenotypic plasticity promotes invasiveness (Baker 1965). Genetic divergence between introduced and native populations sometimes results from evolution of greater phenotypic plasticity in introduced populations (Kaufman and Smouse 2001; Parker et al. 2003). Studies explicitly comparing phenotypic plasticity between introduced and native conspecifics are relatively scarce, and the results are mixed; some studies indicate a trend of greater plasticity among introduced populations (e.g., Caño et al. 2008; Hua et al. 2011; Kaufman and Smouse 2001; Lavergne and Molofsky 2007), whereas others do not (e.g., DeWalt et al. 2004; Williams et al. 2008).

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Table 1. Locations and habitat description, including mean (± SE) canopy cover, of the U.S. and Japanese *Miscanthus sinensis* populations included in greenhouse common garden experiment. More information for U.S. populations can be found in Quinn et al. (2010).

		Latitude	Longitude		Elevation
Location	Code	°N	°W	Habitat	m
United States					
North Carolina	NC	35.5341	82.5468	Regenerating Eastern pine (*Pinus strobus*) forest	679
Kentucky	KY	37.8034	83.6628	Forest opening cleared before 1978 surrounded by mixed oak forest	391
Ohio	OH	39.4760	81.3045	Grazed pasture	228
Japan					
Tomakomai	TOM	42.6709	141.7641	Former wetland drained in 1972, naturally colonized by *M. sinensis*	3
Sugadaira	SUG	36.5238	138.3496	Open area adjacent to forest	1,335
Nishinasuno	NIS	36.9244	139.9519	Open area adjacent to grazed pasture and forest	315
Kawatabi	KAW	38.7704	140.7538	Forest opening maintained by fire, grazing, and cutting	540

In this study, we test the hypothesis that *M. sinensis* appears more shade tolerant in its introduced than native range because of genetic differences in light response at the seedling stage. Functional traits and phenotypic plasticity are compared for seedlings grown across an artificial light gradient from *M. sinensis* seed collected in Japan and the United States. We hypothesized that increased shade tolerance by seedlings would be observed in introduced U.S. populations. We expected increased tolerance to be manifested in traits that are thought to maximize carbon gain while minimizing carbon costs. Specifically, we expected to observe higher SLA and LAR and lower RSR, as well as increases in indicators of overall performance, such as total leaf area and growth. Additionally, we expected to observe increased phenotypic plasticity in introduced U.S. populations, as manifested by reaction norms with steeper slopes and higher plasticity index values for any measures of seedling size, growth, or biomass allocation, compared with Japanese populations.

Materials and Methods

Seed Collection and Experimental Design. Mature *M. sinensis* panicles were collected from naturalized populations in the United States and native populations in Japan by local collaborators. Because we were interested in understanding whether *M. sinensis* undergoes a genetic shift during naturalization, we chose to focus only on large (and thus likely old) U.S. populations to avoid sampling recent ornamental escapes. In the United States, three populations were chosen for study (Table 1). In Japan, four populations in grasslands, spanning the natural latitudinal gradient of *M. sinensis* in the country, were selected. Ten mature panicles were collected from each of 20 haphazardly located plants at least 10 m apart from one another. Seeds from the same plant will be referred to henceforth as being of the same maternal family. Panicles were collected between August and November 2009 from three U.S. (OH: collection date October 19, NC: September 23, and KY: November 9) and five Japanese (KAW: September 26, NIS: October 18, TOM: September 9, SUG: September 7, and ASO: August 25) populations (see Table 1 for location abbreviations). Panicles were mailed to the Energy Biosciences Institute (Urbana, IL), where they were stored at 4 C until the time of planting.

Because of permit restrictions, it was necessary to conduct our study indoors; therefore, our experiment was conducted in the University of Illinois Plant Care Facility greenhouse (40°6′N, 88°12′W). Four benches located in one room of the greenhouse were designated as spatial replicates. Each bench (replicate) contained all four light treatments: high light (unshaded bench), medium-high light (30% shade cloth), medium-low light (50% shade cloth), and low light (70% shade cloth; Table 2). Shade cloth was supported on PVC structures (79 by 79 cm and 56 cm tall). Each light treatment occupied a 1.5- by 1.5-m bench section, and the placement of treatments on a bench was randomly assigned. Within each replicate, each treatment contained two Ray Leach Cone-tainer racks (Hummert International, Springfield, MO 65803) with 160 Ray Leach Cone-tainers (3.8 cm diam, 21 cm depth; 20 maternal families by eight populations). Populations were randomly ordered within the Cone-tainer rack. On February 10 and 11, 2010, Cone-tainers were prepared to begin the experiment. Each Cone-tainer was filled to 2 cm below its top with a peat/perlite soil mix (510 Metro-Mix, Sun Gro Horticulture, Bellevue, WA 98008). Thirty seeds from each maternal family were placed on the soil surface

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Table 2. Mean (± 1 SE) irradiance levels in the four light treatments.

Light treatment	Type of shade cloth	Irradiance at solar noon				Irradiance after sundown	
		Cloudless sky		Cloudy sky			
		PAR	Relative trans.	PAR	Relative trans.	PAR	Relative trans.
	%	μmol m^{-2} sec^{-1}	%	μmol m^{-2} sec^{-1}	%	μmol m^{-2} sec^{-1}	%
High	None	1,713 ± 60	100	1,145 ± 44	100	602 ± 21	100
Medium high	30	1,060 ± 58	61 ± 7	733 ± 42	64 ± 6	363 ± 10	60 ± 3
Medium low	50	770 ± 52	45 ± 6	549 ± 32	47 ± 5	313 ± 8	52 ± 3
Low	70	428 ± 36	25 ± 5	263 ± 25	23 ± 5	156 ± 6	26 ± 2

[a] Abbreviations: PAR, photosynthetically active radiation; Relative trans., transmittance relative to the high-light treatment.

and covered with a 0.5-cm layer of soil. Beginning 2 wk after planting, seedlings were thinned biweekly, leaving only the largest seedling. Cone-tainers were watered daily throughout the experiment and fertilized weekly beginning 3 wk after planting (Plantex General Purpose Fertilizer, 20N-20P-20K, 250 ppm, Plant Products, 314 Orenda Road, Ontario, Canada L6T 1G1). Ambient light illuminated the room, except between 7:00 A.M. and 9:00 P.M., when high-pressure sodium vapor lamps (1000 W), located approximately 2.5 m above the benches, were used when irradiance levels fell below 700 W m^{-2}.

Dependent Variables. Light availability was quantified 6 wk after planting, the approximate midpoint of the experiment. To quantify the relative light levels between treatments, instantaneous photosynthetically active radiation (PAR) was measured using the external sensor on a ceptometer (LP-80 AccuPAR, Decagon Devices, Court Pullman, WA 99163). To capture the full range of light variation, measurements were taken at solar noon under a cloudless sky, solar noon under a cloudy sky (with lights illuminated), and after sundown but before the supplemental lighting was turned off (Table 2). Three PAR measurements were recorded in each light treatment per replicate. These measurements demonstrate that we were able to create a gradient of light availability, with relative transmittance levels (relative to open bench space within the greenhouse) of 100% (high light), 60% (medium-high), 50% (medium-low), and 30% (low) and relatively low levels of PAR variability within treatments (Table 2). Because artificial lights were used in our experiment, direct comparisons with natural light conditions were not possible. However, our goal was not to mimic natural light levels but to create a large, consistent light gradient.

Seedlings were censused 6 and 12 wk after planting. During each census, the length of all leaves of each seedling were measured. In the second census, seedlings from 11 maternal families per population were randomly selected for destructive biomass measurements. To determine the allometric relationship between leaf length and leaf area, the leaf area of three fresh leaves per seedling were measured, the approximate smallest, largest, and median leaves, using a LI-COR 3000 leaf area meter (LI-COR, Lincoln, NE 68504). To estimate biomass of different seedling components, we separated leaf blades, leaf petioles, and roots; dried each at 60 C for 48 h and measured their mass. Leaf petioles were not included in estimates of leaf area and leaf mass. The allometric relationships between leaf length and leaf area were determined for each light treatment–population combination and used these relationships to estimate the area of each leaf in the larger sample.

The effects of light treatment and source region on *M. sinensis* seedlings was evaluated for measures of overall seedling size (leaf number [number of leaves per seedling], total leaf area [LA_{Total}], and total mass), growth (leaf area relative growth [RGR_{LA}]), leaf traits (average individual leaf area [$LA_{Individual}$] and leaf length/area ratio), and biomass allocation (LAR, RSR). LA_{Total} and $LA_{Individual}$ were calculated per seedling. RGR was calculated as [ln(total leaf area at census 1) − ln(total leaf area at census 2)]/time between censuses (days) (Evans 1972). Leaf length/area ratio was estimated per seedling, but only for the subset of plants that were destructively sampled and, therefore, had direct measurements of their leaf areas. We calculated two variables from the biomass measurements: SLA and RSR.

Data Analysis. Linear mixed effects models were used to compare the effects of the light gradient and source region on seedling size, growth, and biomass allocation. Light gradient, source region, and their interaction term were treated as fixed effects, whereas a nested random factor, replicate{region{population{maternal family}}}, represented the spatial structure of our experimental design. LA_{Total}, $LA_{Individual}$, seedling mass, LAR, RSR, and leaf length/area ratio were square root–transformed for analyses; transformations of other variables were not necessary to achieve normality. Light treatments were treated as a continuous variable in analyses, using the relative transmittance values calculated at solar noon on a cloudless day (Table 2).

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Table 3. Results from mixed models testing for the effects of region (U.S. vs. Japan), light intensity, and their interaction on the size, growth, leaf traits, and biomass allocation of *Miscanthus sinensis* seedlings. See Appendix for explanation of abbreviations.

	Region		Light intensity		Region × light intensity	
Variation source	*F*	P	*F*	P	*F*	P
Size						
Leaf number	9.80	0.0492[a]	335.51	< 0.0001[b]	18.74	< 0.0001[c]
LA_{Total}	9.18	0.0500[a]	36.20	< 0.0001[b]	6.57	< 0.0001[c]
Total mass	3.85	0.1445[d]	186.72	< 0.0001[e]	6.65	0.0109[f]
Growth						
RGR	4.90	0.1137[a]	215.83	< 0.0001[b]	6.60	0.0103[c]
Leaf traits						
$LA_{Individual}$	12.98	0.0367[a]	91.03	< 0.0001[b]	14.82	< 0.0001[c]
Leaf length/area ratio	24.38	0.0159[d]	44.99	< 0.0001[e]	0.79	0.3733[f]
Biomass allocation						
SLA	0.25	0.6178[d]	89.31	< 0.0001[e]	2.00	0.1596[f]
LAR	1.29	0.0380[d]	237.05	< 0.0001[e]	8.38	0.0044[f]
RSR	0.01	0.9761[d]	94.14	< 0.0001[e]	0.39	0.7451[f]

[a] $df = 1, 3$.
[b] $df = 1, 1{,}174$.
[c] $df = 1, 1{,}174$.
[d] $df = 1, 3$.
[e] $df = 1, 143$.
[f] $df = 1, 143$.

Genetic variation for plasticity in a dependent variable (and trait) is typically inferred from a statistically significant interaction between genetic origin (region) and environmental factor (Richards et al. 2006). When a significant region by light treatment interaction was detected, contrasts were performed to identify in which segment of the light gradient slopes differed significantly between U.S. and Japanese seedlings. Preliminary analyses revealed that population latitude lacked explanatory power and was therefore not included in further analyses.

Phenotypic plasticity was quantified using the relative distance plasticity index (RDPI; Valladares et al. 2006), which uses pairwise distances (absolute differences in trait measures) between individuals of the same maternal family grown in different environments to calculate an overall plasticity score per maternal family for a specific trait. RDPI scores were calculated across all light treatments to evaluate overall plasticity and between each segment of the light intensity gradient. The RDPI scores ranges from 0 (no plasticity) to 1 (maximum plasticity). A RDPI score was quantified for each maternal family within each replicate for all dependent variables. Linear mixed effects models were used to compare RDPI scores for the dependent variable between regions, by using the source region as the fixed effect, and the nested random factor, replicate{region{population}}. All analyses were performed in the *nlme* package of R (v.2.10.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Miscanthus sinensis seedlings responded to the light intensity gradient with substantial differences in measures of seedling size, growth, and leaf traits, as well as biomass allocation between high– and low–light intensity ends of the continuum (Table 3). Some measures of plant size increased with light intensity, but individual variables differed in the magnitude of the response (Figures 1 and 2). Seedlings grown in high light had nearly twice as many leaves and more than three times more mass than seedlings grown in low light (Figures 1a and 2a). LA_{Total} was greatest in the intermediate light treatments (Figure 1b). Individual leaves varied, in terms of their size and shape, across the light intensity gradient. Across low and intermediate light intensities, $LA_{Individual}$ was relatively constant, but in high light intensity, it was markedly lower (Figure 1c). Conversely, the leaf length–to–area ratio was relatively constant across high and intermediate light intensities, but it was markedly higher, indicating narrower leaves, in

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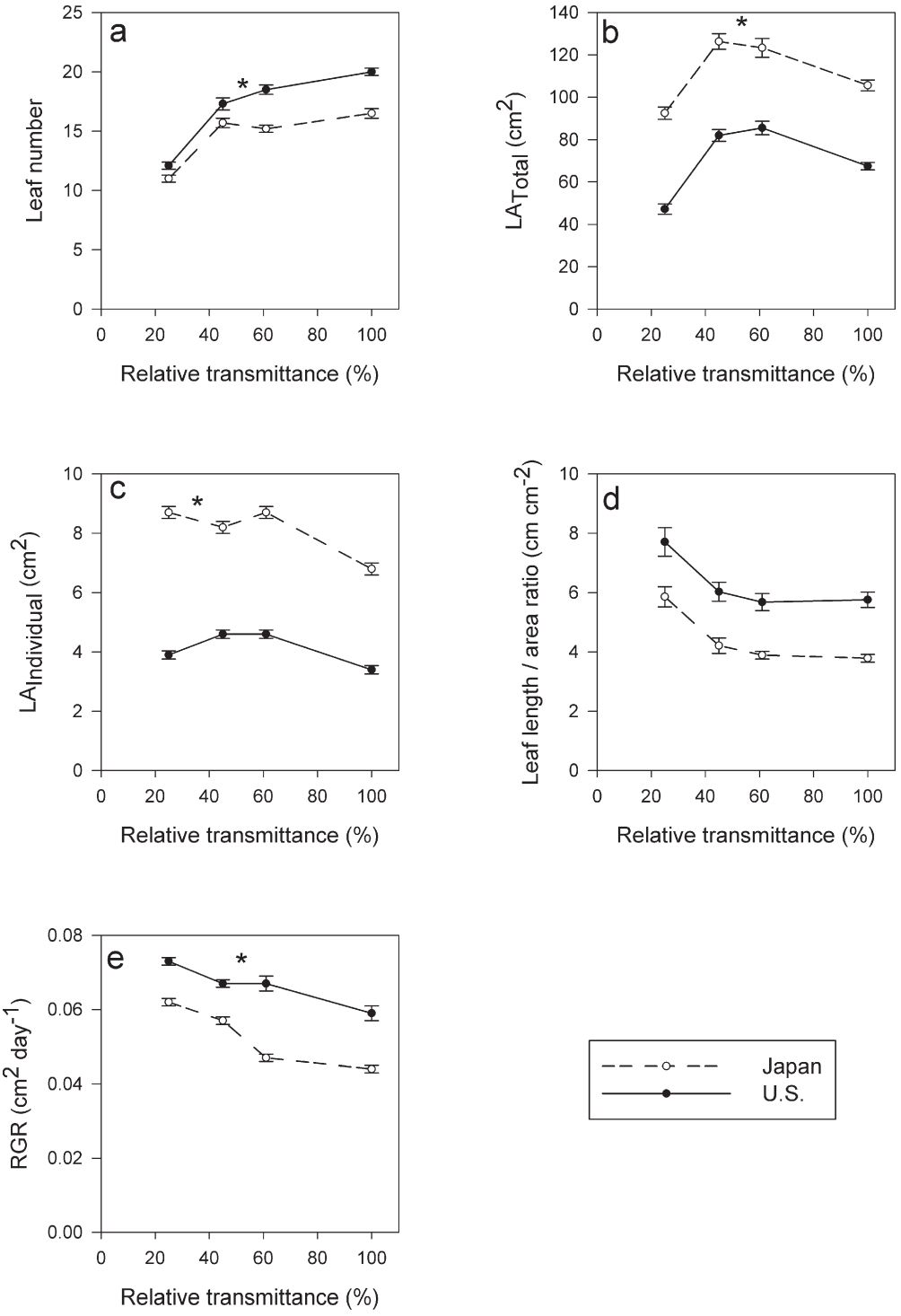


Figure 1. Norms of reaction for seedling size—(a) leaf number, (b) total leaf area (LA_{Total}), leaf traits, (c) average individual leaf area ($LA_{Individual}$), (d) leaf length-to-area ratio—and growth (e.g., relative growth [RGR]) of *Miscanthus sinensis* grown from seed collected in populations from its introduced range (U.S., filled circles) and native range (Japan, open circles) under four light intensity treatments. Relative transmittance, displayed on *x*-axis, was quantified at solar noon on a cloudless day (Table 4). Significant differences ($P < 0.05$) in slope between regions are indicated (*) for specific segments of the light intensity gradient. See Appendix for explanation of abbreviations.

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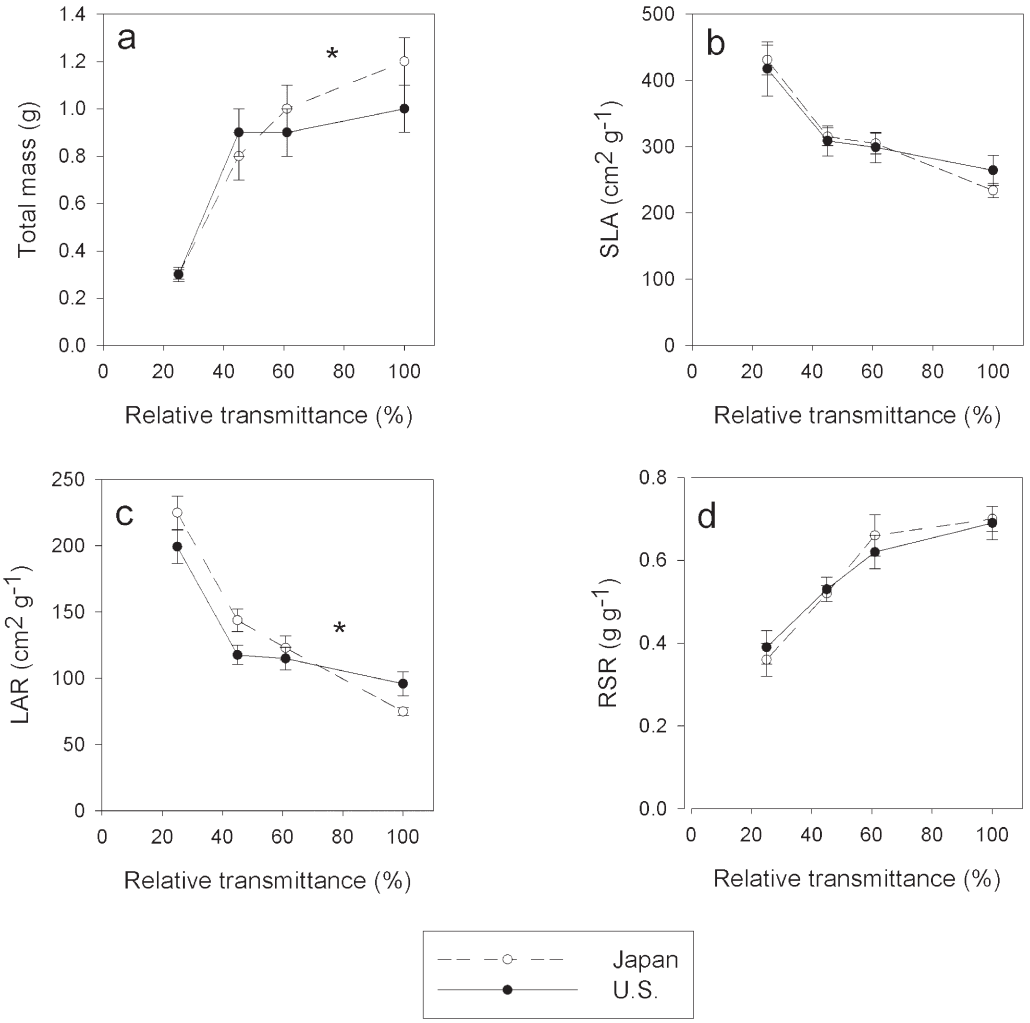


Figure 2. Norms of reaction for seedling biomass allocation—(a) total mass, (b) specific leaf area [SLA], (c) leaf area ratio (LAR), (d) root to shoot ratio (RSR)—of *Miscanthus sinensis* grown from seed collected in populations from its introduced range (U.S.; filled circles) and native range (Japan; open circles) under four light intensity treatments. Relative transmittance, displayed on *x*-axis, was quantified at solar noon on a cloudless day (Table 4). Significant differences ($P < 0.05$) in slope between regions are indicated (*) for specific intervals of the light intensity gradient. See Appendix for explanation of abbreviations.

seedlings grown under low light intensity (Figure 1d). Seedling RGR had a negative response to light intensity, with greatest leaf area accrued on seedlings grown under low light intensity (Figure 1e). Light intensity treatments affected patterns of biomass allocation, with SLA and LAR declining with increasing light intensity (Figures 2b and 2c). The ratio of seedling aboveground-to-belowground biomass shifted across the light intensity gradient, with increasing RSR, and therefore higher belowground investment, at high light levels.

Measures of seedling size, leaf traits, and LAR differed significantly between regions (U.S. vs. Japanese) (Table 3). *Miscanthus sinensis* seedlings from U.S. populations had a greater number of leaves (Figure 1a) but smaller $LA_{Individual}$ (Figure 1b) than seedlings from Japanese populations. United States seedlings had just over half the LA_{Total} of Japanese seedlings (Figure 1c). Seedlings from U.S. populations had narrower leaves, indicated by a greater leaf length–to–area ratio, than seedlings from Japanese populations (Figure 1d). Leaf area increased faster, indicated by higher RGR, among seedlings from the United States compared with Japanese populations (Figure 1e). There were significant differences in LAR between regions, with Japanese seedlings having higher LAR in low light and lower LAR in high light, compared with U.S. seedlings (Figure 2c).

Reaction norms differed between regions (U.S. vs. Japanese), as indicated by significant region by light treatment interactions for several measures of seedling size, growth, leaf traits, and biomass allocation (Table 3).

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Table 4. Mean (± 1 SE) relative distance plasticity index (RDPI) scores calculated for *M. sinensis* seedlings grown from seed collected in invasive Unites States (U.S.) and native Japanese populations. Overall RDPI score was calculated across all light intensity treatments. RDPI scores were calculated for the three segments of the light intensity gradient: low end, low and medium-low light intensity; middle, medium-low and medium-high light intensity; and high end, medium-high and high light intensity. See Table 2 for a description of light treatments. Significant differences (Sig.) between regions are indicated by asterisks.[a]

				Segment of light intensity gradient		
	Overall			Low end		
Variable	Japan	Sig.	U.S.	Japan	Sig.	U.S.
Size						
Leaf number	0.147 ± 0.009	NS[b]	0.162 ± 0.009	0.085 ± 0.002	NS[b]	0.100 ± 0.014
LA_{Total}	0.153 ± 0.013	**[b]	0.212 ± 0.016	0.122 ± 0.0135	NS[b]	0.142 ± 0.021
Total mass	0.388 ± 0.025	*[c]	0.465 ± 0.021	0.287 ± 0.043	NS[c]	0.376 ± 0.055
Growth						
RGR	0.134 ± 0.007	*[b]	0.112 ± 0.010	0.119 ± 0.011	NS[b]	0.086 ± 0.019
Leaf traits						
$LA_{Individual}$	0.130 ± 0.017	NS	0.136 ± 0.010	0.101 ± 0.009	NS	0.090 ± 0.015
Leaf length/area	0.160 ± 0.013	NS[b]	0.153 ± 0.010	0.141 ± 0.011	NS[b]	0.130 ± 0.014
Biomass allocation						
SLA	0.366 ± 0.007	NS[c]	0.401 ± 0.012	0.182 ± 0.015	NS[c]	0.160 ± 0.012
LAR	0.276 ± 0.007	*[c]	0.231 ± 0.012	0.132 ± 0.018	NS[c]	0.100 ± 0.013
RSR	0.311 ± 0.025	NS[c]	0.355 ± 0.021	0.287 ± 0.043	NS[c]	0.276 ± 0.055

[a] Abbreviations: NS, no significant difference; LA_{Total}, total leaf area; RGR, relative growth; $LA_{Individual}$, average individual leaf area; SLA, specific leaf area; LAR, leaf area ratio; RSR, root to shoot ratio.

[b] $df = 1, 116$.

[c] $df = 1, 52$.

* $P < 0.05$.

** $P < 0.01$.

However, the segment of light gradient in which reaction norms differed between regions was not consistent across variables (Table 3; Figures 1 and 2). Reaction norms for leaf number, LA_{Total} and RGR differed between regions at intermediate light levels (Figures 1a, 1b, and 1e). Reaction norms differed between regions for total mass and LAR at the high-light end of the gradient (Figures 2a and 2c). It also differed between regions for $LA_{Individual}$ at the low-light end of the gradient (Figure 1c).

Comparisons of RDPI scores between U.S. and Japanese populations revealed higher plasticity of leaf number, LA_{Total}, and total mass for U.S. seedlings compared with those from Japanese populations (Table 4). Greater plasticity among Japanese populations was found for $LA_{Individual}$ and the biomass allocation variables SLA and LAR (Table 4). The majority of the significant differences in RDPI scores detected between regions were located at the high end of the light intensity gradient. Additionally, two of the significant differences in RDPI between regions were found in the middle of the light intensity gradient, but RDPI scores did not differ between regions at the low end of the light gradient.

Discussion

Genetically determined differences in ecological breadth between native and introduced ranges can be the result of local adaptation or increased phenotypic plasticity (Bossdorf et al. 2005; Richards et al. 2006). *Miscanthus sinensis* is thought to occupy a shadier environments within its introduced range in the United States, where it has been described as shade tolerant and is found in both high-light areas and in the shaded understory of secondary forests (Horton et al. 2010; Miller, 2003) compared with its native range in Japan, where it is found primarily in open areas (Meyer 2004; Stewart et al. 2009). We tested the hypothesis that *M. sinensis* is more shade tolerant in the United States than Japan because of genetic differences in the light response at the seedling stage. Results from our experiment demonstrate differences in seedling morphology and light response of *M. sinensis* seedlings between ranges, indicating a genetic difference. However, our results of *M. sinensis* functional traits and phenotypic plasticity do not illustrate a compelling overall pattern that supports the

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Table 4. Extended.

Segment of light intensity gradient					
Middle			High end		
Japan	Sig.	U.S.	Japan	Sig.	U.S.
Size					
0.173 ± 0.016	NS[b]	0.207 ± 0.017	0.210 ± 0.014	*[b]	0.251 ± 0.014
0.185 ± 0.025	**[b]	0.288 ± 0.021	0.134 ± 0.019	*[b]	0.199 ± 0.018
0.485 ± 0.047	NS[c]	0.542 ± 0.045	0.526 ± 0.048	*[c]	0.577 ± 0.045
Growth					
0.150 ± 0.009	**[b]	0.085 ± 0.008	0.186 ± 0.011	NS[b]	0.155 ± 0.012
Leaf traits					
0.111 ± 0.014	NS	0.110 ± 0.012	0.135 ± 0.012	*[b]	0.101 ± 0.008
0.131 ± 0.014	NS[b]	0.138 ± 0.012	0.129 ± 0.012	NS[b]	0.131 ± 0.018
Biomass allocation					
0.322 ± 0.022	NS[c]	0.319 ± 0.024	0.434 ± 0.014	*[c]	0.347 ± 0.025
0.304 ± 0.021	*[c]	0.239 ± 0.024	0.474 ± 0.015	*[c]	0.397 ± 0.025
0.475 ± 0.046	NS[c]	0.542 ± 0.044	0.559 ± 0.058	NS[c]	0.567 ± 0.045

hypothesis of increased shade tolerance within the United States, for reasons discussed below.

According to the "carbon gain hypothesis" traits that maximize light capture and minimize respiration costs confer increased shade tolerance (Givnish 1988). From this hypothesis, we predicted that if *M. sinensis* from U.S. populations had evolved increased shade tolerance, they would maximize light capture at both the whole-plant level, with a greater fraction of biomass distributed to foliage (low RSR), and at the leaf level, with greater surface area per gram of leaf (higher SLA) and plant mass (higher LAR). We observed *M. sinensis* seedlings to have a strong light response, in which under shaded conditions, plants increased traits associated with light interception, increasing proportional allocation to foliage (low RSR) and producing thinner leaves, with greater surface area per mass (high SLA), similar to that of many other species (DeWalt et al. 2004; van Kleunen et al. 2011). However, we found that plants from U.S. populations did not allocate more biomass to foliage. Also, their leaves did not have greater surface area per leaf mass than those from Japanese populations, as indicated by similar RSR and SLA between regions. There was a slight, but significant, difference in the LAR reaction norm between regions, indicating higher LAR for U.S. populations, but only at the higher end of the light gradient. For LAR to confer increased shade tolerance, increased LAR in this parameter must be apparent under low light conditions. Our results are in contrast to Horton et al. (2010), who found that SLA of *M. sinensis* did not change across a natural light gradient. This difference could be due to the life stage under consideration. We studied seedlings, which might have greater plasticity for SLA compared with the adult plants investigated by Horton et al. (2010).

We also predicted that U.S. seedlings with increased shade tolerance would outperform Japanese populations under low light conditions. Results from our study provide mixed support for this prediction. Seedlings from Japan produced more than twice the leaf area across all light levels, compared with U.S. seedlings, which produced a greater number of leaves, but these leaves were on average 30% smaller, than those from Japanese seedlings. Consistent with our prediction, we did find higher RGR of U.S. compared with Japanese seedlings, even under low light conditions. The morphology of U.S. seedlings with many small leaves and narrow leaf blades, compared with Japanese seedlings, could be the result of horticultural selection. *Miscanthus sinensis* varieties, including the popular 'Gracillimus' variety, were selected for fine-textured leaves with narrow leaf blades by Ernst Pagels and introduced into the horticultural trade (Mary Meyer, personal communication). It has been shown that many, smaller leaves reduces self-shading compared with few, larger leaves (Takenaka et al. 2001). Therefore, the leaf display observed among U.S. seedlings could reduce self-shading and be advantageous under low light conditions.

We predicted that U.S. seedlings would display greater phenotypic plasticity than Japanese seedlings, in accordance

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with the long-standing idea that plasticity increases niche breadth and facilitates invasion (Bradshaw 1965; Richards et al. 2006) and that plasticity aids in coping with light heterogeneity in the shaded understory (Valladares et al. 1997; Valladares and Niinemets 2008). Our results showed significant differences in reaction norms and RDPI scores between regions for several variables, but these differences never occurred in the low light segment of our light gradient. Our results were mixed as to which region had greater plasticity depending on the variable under consideration. Greater plasticity was found for U.S. populations compared with Japanese populations for all measures of plant size (leaf number, LA_{Total}, and total mass). However, greater plasticity was found for Japanese seedlings compared with U.S. seedlings for growth (RGR), leaf traits ($LA_{Individual}$), and biomass allocation variables (SLA and LAR). These conflicting results are somewhat surprising, considering it is thought that plastic responses at lower levels of organization (e.g., leaves) influence higher levels of organization (e.g., whole plant; Valladares and Niinemets 2008). However, our results showing higher $LA_{Individual}$ in Japanese populations but higher LA_{Total} in U.S. populations instead suggest that plasticity at different levels of organization can vary independently.

Although our results do not suggest that seedlings in *M. sinensis* populations in the United States included in this study are more shade tolerant than those in Japan, this should not ease concerns about the potential of this species to invade forest understories. Our results demonstrate that seeds can germinate and seedlings can grow in low light conditions (low light treatment: 156–428 μmol m^{-2} s^{-1}, Table 2). Similar light levels were found within the understory of three floodplain forest sites in northeastern Illinois (11–495 μmol m^{-2} s^{-1}; Davis and Matlaga, unpublished data), a region where ornamental plantings of *M. sinensis* are common. When considered in the context of other work showing that the reproductive output of *M. sinensis* is only slightly reduced in low light (Horton et al. 2010), our findings suggest that shading might not prevent spread of this species into forest understories. Results of our study are limited by our artificial greenhouse conditions, which exposed individual plants to a relatively constant light environment. By contrast, light levels experienced by a forest understory plant are variable, especially for those exposed to sunflecks. Additionally, our results of *M. sinensis* performance might not be consistent with performance of adult plants. Field studies that quantify vital rates of all life cycle stages of *M. sinensis* across a range of light intensity are needed to understand whether populations in low light are viable over the long term. Additionally, we studied a limited suite of functional traits and cannot rule out the possibility that other traits might be responsible for the ability of *M. sinensis* to tolerate shaded conditions in the United States. Future studies investigating the influence of light intensity on the expression of a broader suite of traits will further our understanding of the demographic performance of this species within its invaded range.

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APPENDIX 1
Functional trait and population abbreviations.

SLA	leaf area per unit of leaf mass
LAR	leaf area per unit of whole plant mass
RSR	belowground mass per aboveground mass
RMR	root mass per whole plant mass
LMR	leaf mass per whole plant mass
PAR	photosynthetically active radiation
LA_{Total}	total leaf area
RGR	leaf area relative growth
$LA_{Individual}$	average individual leaf area
RPDI	relative distance plasticity index
EICA	evolution of increased competitive ability hypothesis

Population abbreviations:

NC	North Carolina
KY	Kentucky
OH	Ohio
TOM	Tomakomai
SUG	Sugadaira
NIS	Nishinasuno
KAW	Kawatabi

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