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Research

Seed Mass, Viability, and Germination of Japanese Stiltgrass (*Microstegium vimineum*) under Variable Light and Moisture Conditions

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The success of Japanese stiltgrass as an invader may be due to its ability to respond to stochastic events (e.g., by sexual reproduction via chasmogamous [CH] flowers) and to maintain a beneficial genetic make-up (e.g., by self-fertilizing via cleistogamous [CL] flowers) when conditions are stable. This paper evaluates the importance of Japanese stiltgrass seed type (chasmogamous seeds, cleistogamous seeds, and seeds originating from forest-interior [F-I] plants) in terms of seed mass, viability, and germination across variable moisture regimes (three regions in West Virginia) and at two light levels (roadside and forest interior). Seeds from nine populations were sampled in three site types in 2005 and 2008 and stored at 5 C until testing in April 2009. Seeds were tested for viability using a dye test. Seeds were germinated under both constant and fluctuating day/night temperatures. Additional samples of CH and CL seeds collected in 2008 were tested for viability again in September 2010 for a measure of seed longevity. CL and F-I seeds were smaller in mass than CH seeds. Seeds from the drier sites were smaller in mass than seeds from the more mesic sites. CL seeds, followed by F-I seeds, were less viable than CH seeds in 2005 and 2008. CL and F-I seeds had lower germination rates than CH seeds for each site type in 2005, but germination rates of the seed types did not differ in 2008. Differences in seed longevity for 2008 seeds were lower for CL compared to CH seeds, but only in the drier sites. Japanese stiltgrass' longer-lived and larger CH seeds from the roadsides may ensure population survival over the long term. Younger CL and F-I seeds differ less from CH seeds in terms of germination than older seeds, which may help Japanese stiltgrass to maintain populations under relatively stable conditions in the short term.

Nomenclature: Japanese stiltgrass; *Microstegium vimineum* (Trin.) A. Camus.

Key words: Chasmogamy, cleistogamy, Japanese stiltgrass, light, *Microstegium vimineum*, moisture regime, seed longevity, seed viability.

High seed viability and longevity improve the fitness of short-lived individuals, such as annuals (Chambers 1989; Rees 1994). Prolific production of viable seeds with a transient seed bank is a reproductive strategy of early-successional, ruderal species (Grime 2002) as well as many invasive exotic species (Rejmánek and Richardson 1996). Likewise, cleistogamy (self-fertilization in an enclosed flower; Campbell et al. 1983), may add to a plant's fitness by ensuring progeny that can maintain desirable traits (Lu 2002). Chasmogamy (open flowers that are more likely to be out-crossed) allows a plant some genetic flexibility if environmental conditions are stochastic (Rees 1994). Consequently, if environmental conditions are relatively stable, one would predict that a plant would benefit more from seeds of CL flowers (hereafter called CL seeds) than seeds of CH flowers (hereafter called CH seeds), as long as such seeds are highly viable and long-lived. In contrast, if environmental stochasticity is common, chasmogamy should be favored over cleistogamy. Indeed, there is some evidence that CH seeds may have greater fitness than CL seeds, which may suffer from inbreeding depression. For instance, CH seedlings of jewelweed (*Impatiens capensis*

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Interpretive Summary

The differences in viability of the three seed types (CH, CL, and F-I) revealed in this study add support for focusing management efforts on Japanese stiltgrass populations in which CH seed production is highest, such as those along roadsides or in open areas exposed to higher light levels. The relative importance of the CH seeds suggests that Japanese stiltgrass has adapted to less-stable conditions (i.e., seeds that are a product of out-crossing are of more use than seeds that are product of self-fertilization). Nonetheless, Japanese stiltgrass produces large amounts of the less-viable CL seeds, which may enable it to maintain populations during stable conditions in the short term. Control treatments such as mowing (prior to flowering) stiltgrass populations in higher-light areas, such as roadsides, is a way to minimize CH seed output. Although any remaining upper CL flowers may transform into CH inflorescences, the amount of CH seeds produced should still be reduced. Treatment with herbicide of the roadside Japanese stiltgrass populations may better ensure that the forest interiors are not receiving a steady supply of CH seeds with comparatively high viability. The influence of regional moisture variation on Japanese stiltgrass seeds is less defined than the effects of light, but may be related to drought-induced seed dormancy of CH seeds. Seed longevity of both CL and F-I seeds is shorter in drier environments compared to seed longevity of CH seeds, which indicates that removal of Japanese stiltgrass populations that produce the most viable CH seeds may be even more effective in drier environments than in mesic environments. It is conceivable that after removing roadside populations of Japanese stiltgrass, small forest populations could eventually go extinct, especially under drier conditions.

Meerb.) were found to be competitively superior to CL seedlings (Waller 1984).

Less than optimal, but stable, environmental conditions may also influence plant fitness by forcing selection for plant traits more amenable to such conditions. Doing so may initially favor CH seeds until the best traits are selected, at which point cleistogamy would once again be favored as long as environmental conditions remained constant (Brown 1952; Lu 2002). Alternatively, stressful or limiting environmental conditions may negatively impact flower production, such that less-costly CL seeds are favored over costly CH seeds (Campbell et al. 1983). Several studies (e.g., peanut grass [*Amphicarpum purshii* Kunth], Cheplick and Quinn 1982); Texas needlegrass [*Stipa leucothrica* Trin. and Rupr], phlox [*Collomia grandiflora* Dougl. Ex Lindl.], Minter and Lord 1983; and jewelweed [*Impatiens pallida* Nutt.], Bennington and McGraw 1995) support the hypothesis that cleistogamy is favored over chasmogamy under stressful environmental conditions.

Invasive exotic plant species may be successful at rapidly colonizing both disturbed and undisturbed habitats for several reasons, including a lack of predators (Keane and Crawley 2002), novel weapons (Callaway and Ridenour 2004), prolific seed production (Simberloff 2009), and the ability to grow under a wide range of environmental conditions (Geng et al. 2007). The importance of an invader's reproductive strategy and capacity under different environmental conditions has been evaluated in less detail as a determinant of invasion success. Milbau and Stout (2008) compared 65 invasive plant species in Ireland using 44 variables including introduction history, habitat, and biological characteristics. They found that flower type was the third most important variable (after ornamental introduction and a hermaphroditic reproductive strategy) predicting invasion success, with cleistogamy negatively correlated with invasion success. Milbau and Stout (2008) assumed the negative effect of cleistogamy was due to the increased inbreeding depression often associated with selfing. The success of cheat grass (*Bromus tectorum* L.), an obligate CL selfer in North America, thus, may appear to be somewhat of an anomaly, though populations in Canada appear to benefit from increased genetic diversity due to multiple introduction events (Valliant et al. 2007). Other invasive plants, including Chinese lespedeza [*Lespedeza cuneata* (Dum. Cours.) G. Don.; Woods et al. 2009], the native deer tongue grass ([*Dichanthelium clandestinum* (L.) Gould]; Bell and Quinn 1985), and this paper's focus species, Japanese stiltgrass [*Microstegium vimineum* (Trin.) A. Camus]; Tanaka 1975) are able to produce both CL and CH flowers, which may increase their ability to invade a wide range of environments.

Japanese stiltgrass is an invasive, annual grass that is especially productive in mesic environments (Barden 1987; Redman 1995) and is drought intolerant, despite being a C_4 plant (Barden 1987; Webster et al. 2008). Though Japanese stiltgrass is both CH and CL, it is predominantly CL (Cheplick 2005a). Seeds persist in the seed bank for about 3 yr (Barden 1987). Although this species is shade tolerant (Horton and Neufeld 1998; Winter et al. 1982), light conditions of 5 to 18% full sunlight limit both its growth (plant height) and reproductive capacity (Claridge and Franklin 2002; Cole and Weltzin 2005). Gibson et al (2002) found that Japanese stiltgrass' CH and CL flowering is restricted to high-light environments, such as roadsides, in times of moisture stress. Japanese stiltgrass seeds found in forest interiors (hereafter called F-I seeds) tend to come from plants of small stature (typically less than 7 cm [2.8 in]), each with a single inflorescence that is partially CH and partially CL and with seeds that are smaller in size than roadside CH seeds. Roadside plants are commonly over 1 m (39.4 in) in height (Huebner 2010a). Unlike many other plants growing under less than optimal conditions, growth in a forest interior does not result in a higher ratio of CL flowers to CH flowers for Japanese stiltgrass compared to plants grown in high-light conditions (Cheplick 2005a, 2007, 2010). However, there is some evidence of adaptation to F-I conditions. Under high-light conditions, plants originating from F-I seeds grew more rapidly than plants originating from seeds produced under high-light conditions (Cheplick 2008). This finding suggests that seeds originating from low-light conditions may be just as fit as seeds originating from high-light conditions.

The goal of this research was to evaluate Japanese stiltgrass' CL and CH seed mass, viability, and germination under variable moisture and light conditions. I addressed three questions: (1) How do mass, viability, and germination of seeds originating from F-I plants (i.e., F-I seeds) and CH and CL seeds originating from roadside plants differ across variable moisture regimes? (2) How do seed mass, viability, and germination differ under two light levels (roadside vs. the forest interior)? And (3) how do seed mass, viability, and germination differ with seed age?

Materials and Methods

Study Species. Japanese stiltgrass was accidentally introduced to Knoxville, TN in 1919 from Asia (Fairbrothers and Gray 1972) and it was first documented in West Virginia in 1956 (Huebner 2003). This species is currently listed as a noxious weed in four states and is included on at least 11 Northeastern, Mid-Atlantic, Southeastern, and Midwestern state invasive plant lists in the United States (USDA NRCS; West Virginia Department of Agriculture 2008). Negative native species and community impacts attributed to Japanese stiltgrass include decreases in native plant diversity (Flory and Clay 2010; Oswalt et al. 2007); decreases in soil microarthropod (McGrath and Binkley 2009) and arthropod diversity (Simao et al. 2010); and changes in soil pH, mineralization, and nitrification (Kourtev et al. 1999; Ehrenfeld et al. 2001) as well as changes in soil phosphorus (McGrath and Binkley 2009).

Study Area. Nine sites within West Virginia were selected based on the presence of a contiguous roadside population of Japanese stiltgrass adjacent to a closed-canopy upland forest of 70 or more years of age and evidence of Japanese stiltgrass establishment and spread into the forest (Huebner 2010a, b). Three replicate sites types were located in three regions representing different moisture regimes: (1) the Fernow Experimental Forest (38°53′N, 79°17′W) of the Monongahela National Forest (MNF) of the Allegheny Plateau (hereafter called Fernow), (2) Cooper's Rock State Forest and the West Virginia University Forest (39°39′N, 79°47′W) also located in the Allegheny Plateau (Cooper), and (3) the Seneca Rocks area (39°2′N, 79°42′W) of the Cheat-Potomac Ranger District of the MNF in the Ridge and Valley Province (R-V). Annual precipitation averages approximately 160 cm for both the Fernow and Cooper sites, and about 79 cm annually for the R-V sites (Clarkson 1964). The highest average annual temperature was approximately 23 to 25 C for the Fernow and Cooper sites, but 30 C for the R-V sites (National Climatic Data Center 2008). The Fernow and Cooper sites are in mixed-mesophytic forests whereas the R-V sites are in oak-dominated forests. Despite being similar in climate and plant species, the Cooper sites tend to have a less species-rich understory than the Fernow sites, with the R-V sites being the least diverse. Light conditions averaged 140 µmol m^{-2} s^{-1} (7 to 10% of full sunlight) along the roadsides and 25 µmol m^{-2} s^{-1} (about 1 to 2% of full sunlight) within the forest interiors. Light was measured in 2005, 2006, and 2007 using instantaneous light meters (noting sunflecks) and averaged over 3 yr. The differences in plant height and seed production of the F-I plants occur in as short a distance as 5 m into the forest interior from the canopy edge (Huebner 2010a), showing that light levels even within a forest-edge effect zone (about 40 m; Matlack 1994; Saunders et al. 1999) are low enough to impact growth and reproduction of Japanese stiltgrass.

Seed Collection. Separate seed collections were made at each of the nine sites (resulting in three replicates of each treatment type combination) from (1) CH inflorescences from roadside Japanese stiltgrass populations, (2) CL inflorescences from roadside populations, and (3) partially CH and partially CL inflorescences from the F-I plants. F-I plants occurred 5 m or more beyond the forest canopy edge (Huebner 2010a). The seeds of the F-I plants were partially enclosed and partially exposed and were not separated into CH and CL seed types, because the accuracy of doing so was questionable. In terms of their response to different light levels, the comparisons of interest are between the F-I seeds and the CH and CL seeds from the more robust roadside plants. These comparisons were made by arbitrarily classifying F-I seeds as a third seed type. Approximately 150 plants were sampled from each of the three seed types at each site. Because of the plant size differences between the roadside (0.5 to 1.5 m) and F-I plants (3 to 15 cm), fewer seeds were collected from each F-I plant than from each roadside plant. The F-I plants typically had only one terminal inflorescence, and the entire plant containing all the seeds was taken. The entire CH inflorescence was taken and the entire stem containing CL seeds was taken from each sampled roadside plant. All internodes per plant were sampled because seed viability of other CL grass species varies by internode position, with seeds from lower internodes being less viable (Cheplick 1996). Mixed CH and CL inflorescences on the roadside plants, which were uncommon, were not sampled. Seeds were collected from every site in 2005 and 2008 in late September. Florets, which are composed of the seed, palea, lemma, and glumes, were removed from the inflorescences; mixed within each replicate seed type, site type, and year; and then dried at room temperature for a week. The florets, hereafter referred to as seeds, were then refrigerated in airtight plastic bags at 5 C until viability tests and germination trials were conducted April and May 2009 for the seeds collected in 2005 and 2008 and in August and September 2010 for a second evaluation of the older 2008 seeds (none of the 2005 seeds were still viable). Six sets of 100 seeds were counted from each site type, seed type, and year (2005 and 2008), and their mass was determined.

Viability Tests. Three sets of 100 seeds from each of the three replicate site types, three seed types, and 2 yr were imbibed in water overnight and cut (cross-section just below the top of the embryo or about one-third of the way up from the base of each caryopsis). The lower section containing most of the embryo was then soaked in 1% 2,3,5-triphenyl-tetrazoliumchloride solution overnight at room temperature (Chambers 1989; Porter et al. 1946). Seeds were evaluated for carmine-stained embryos under a dissecting microscope. If the embryo was fully and evenly stained, the seed was considered viable. Seeds with unstained or splotchy, partially stained embryos were labeled as not viable. Viability of the CH and CL seeds from each site type was also compared for two storage times using 2008 seeds evaluated in April and May 2009 and 2008 seeds evaluated 18 mo later in September 2010.

Germination Trials. One hundred seeds from each site type, seed type, and year (2005 and 2008) were evenly placed on moist paper towels in 10-cm by 5-cm shallow plastic containers and covered tightly with plastic wrap. The containers were placed in a growth chamber with a constant temperature of 21 C under 500 μmol m^{-2} s^{-1} of light for 12 h and no light for 12 h and humidity of 70% during the day and 50% at night. Very few 2008 seeds germinated under these conditions, so the trials were stopped after evaluating six (two from each site type) of the nine sites of each seed type. A second trial was run using all seed and site types for 2008 and only CH and CL roadside seeds from three (one from each site type) of the nine sites for 2005; the daylight temperature was 25 C for 12 h and the nighttime temperature was 15 C for 12 h, with all other variables the same as in the previous trial. Due to a lack of additional 2005 seeds, only three of nine sites and only CH and CL seeds were evaluated; no F-I seeds were evaluated at these fluctuating temperatures. In both germination trials, flats were evaluated for moistness (adding water as needed) and germination every 2 d over 2 wk, and germinated seeds were removed. Seeds that did not germinate within the 2 wk were tested for viability using the tetrazolium test described above for the 2005 constant-temperature and the 2008 fluctuating-temperatures germination trials. The 2-wk period was chosen because little, if any, germination occurred after this time period.

Seed Longevity. The differences in viability and germination between seeds stored in a refrigerator from collection date in 2005 until evaluation in 2009 and seeds stored under the same conditions since collection in 2008 until evaluation in 2009 may have been due to seed age. However, differences in viability may also have been due to environmental conditions associated with each collection year. Consequently, differences in seed viability over time in storage were determined by subtracting the average seed viability for each site and seed type of 2008 seeds tested in April and May 2009 from the average seed viability for each site and seed type of 2008 seeds tested in September of 2010.

Data Analyses. Seed mass (six replicates) was compared across seed type (CH, CL, and F-I), the three site types (Fernow, Cooper, and R-V), and the two collection years (2005 and 2008) using a three-way ANOVA (Proc GLM in SAS[1]) after conducting a log-normal transformation. Normality and constant variances assumptions were met. A repeated measures analysis was not used because the same plants were not sampled for seeds year to year. A Tukey adjusted post-hoc comparison of the least square means was conducted for multiple comparisons.

Seed viability and germination were analyzed using a two-way ANOVA (Proc GLM) in terms of seed and site types. For the 2005 seed viability and the 2005 and 2008 seed germination data, an arcsine square root transformation was used to meet normality and constant variances assumptions. For the 2008 seed viability data, an arcsine square root transformation of the cubed data was used. The years were evaluated separately because of the small number of replicates (three) in each seed and site type. Viability of seeds that did not germinate was also analyzed using a two-way ANOVA (Proc GLM) with an arcsine square root transformation for both 2005 and 2008 data. A Tukey adjusted post-hoc comparison of the least square means was conducted for multiple comparisons.

Differences in seed viability over time (seed longevity) were evaluated using a generalized linear model with a normal distribution and an identity link function. Although the normality assumption was met, the constant variances assumption was only marginally met and the data means were restricted between 0 and 1. Generalized linear models are recommended when it is not reasonable to assume data are normally distributed, data means are restricted to a range of values (e.g., 0 and 100), or variances are not likely to be constant for all observations. Transformations to fix such problems are inferior to using actual data distributions, if suitable distributions can be found (Bolker et al. 2008; SAS 9.1 2007), which was not the case for analyses of seed mass, seed viability, and seed germination percentages. Goodness of fit for each model was assessed by determining (1) whether or not the scaled deviance divided by the degrees of freedom was close to 1 and (2) whether or not the log likelihood values differed significantly from the model with the intercept only as well

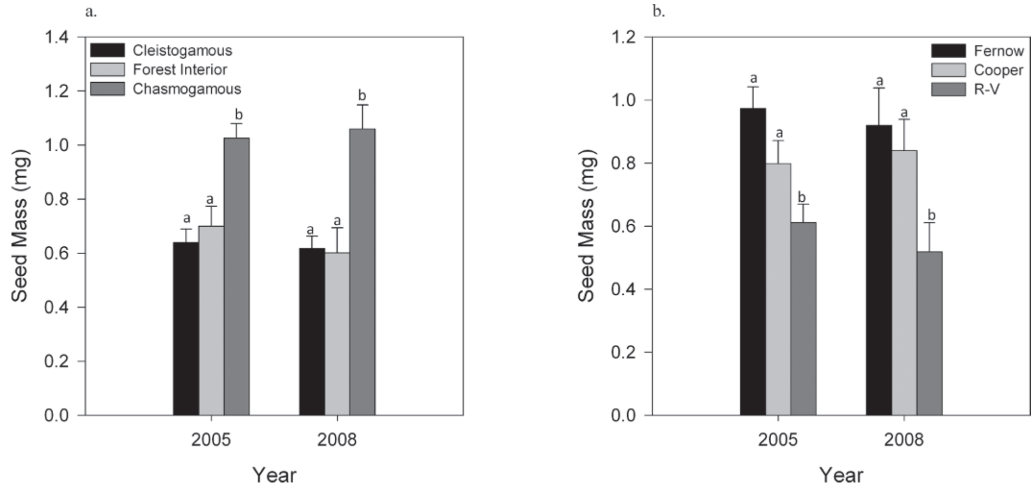

Figure 1. Mass of seeds collected from (a) three seed types and (b) three site types in 2005 and 2008. Statistical differences were determined using log-normal transformed data subjected to a three-way ANOVA. The years did not differ significantly nor was there a seed type, site type, and year interaction. Bars with different letters differ significantly at $P < 0.05$.

as with other distributions, including gamma, Poisson, and negative binomial.

Results and Discussion

Seed Mass. Seed mass differed among seed types ($F_{2,17}$ = 23.81, $P < 0.0001$) and site types ($F_{2,17}$ = 14.60, $P < 0.0001$), but not year ($F_{1,17}$ =1.38, $P = 0.24$); there was no significant three-way interaction ($F_{12,17}$ = 0.57, $P = 0.86$). A separate model with the two-way interaction (no year) was not significant and showed similar results. A comparison of the least square means showed that the seed mass of CL and F-I seeds was smaller than the mass of CH seeds for both years (Figure 1a). Likewise, seeds from the R-V sites were smaller in mass than seeds from both of the Allegheny Plateau sites in 2005 and 2008 (Figure 1b).

These CH and CL seed mass results are similar to those found by Cheplick (2005a). The seed masses recorded in this study may be slightly smaller than those in Cheplick's (2005a) study, because Cheplick's seeds were all from a mesic, fertile site in New Jersey, and my seed type comparison includes seeds from the drier R-V sites. Like Japanese stiltgrass seeds, Chinese lespedeza's CH seeds are larger in mass than its CL seeds. The difference in size for lespedeza was attributed to significantly greater out-crossing in the CH flowers (Woods et al., 2009). Japanese stiltgrass' CH flowers are more likely to be out-crossed, especially if they are located in open areas with high wind turbulence, such as alongside roads (Dupont et al. 2006; Van de Water et al. 2007), but our results cannot confirm this. The fact that seeds from drier sites were smaller than seeds from more mesic sites and that CL and F-I seeds were smaller in size than CH seeds may indicate that smaller seeds are inferior to larger seeds in terms of seed viability, germination, and longevity. Stanton (1984) and Winn (1985), working with (*Raphanus raphanistrum* L.) and heal-all (*Prunella vulgaris* L.), respectively, both showed a positive correlation between seed mass and germination for wild radish. Stanton's (1984) study also showed that plants originating from larger seeds grew more rapidly and produced more flowers than those originating from smaller seeds. My study does not address fitness beyond viability and germination, but it is of interest to note that smaller Japanese stiltgrass seeds (e.g., those from drier environments and the CL and F-I seeds) may produce plants that are less fit than plants coming from CH seeds.

Seed Viability. In 2005, seed viability differed among seed types ($F_{2,8}$ = 24.64, $P < 0.0001$) and site types ($F_{2,8}$ = 6.01, $P = 0.011$). There was no significant interaction between site and seed type ($F_{4,8}$ = 0.50, $P = 0.74$). A comparison of the least square means showed that the 2005 CL and F-I seeds both were less viable than the CH seeds (Figure 2a). The 2005 Cooper seeds were less viable than the R-V seeds (Figure 2b). In 2008, seed viability did not differ significantly among site types ($F_{2,8}$ = 0.11, $P = 0.89$), but did differ among seed types ($F_{2,8}$ = 6.02, $P = 0.010$) (Figures 2b and 2a, respectively). There was no significant interaction between seed and site types in 2008 ($F_{4,8}$ = 0.34, $P = 0.85$). A comparison of the least square means showed that CH seeds had a higher viability than both CL and F-I seeds in 2008 (Figure 2a).

CH seeds were more viable than CL and F-I seeds, no matter the seed age. However, F-I seeds were not less viable than CL seeds, which suggests that seed type differences may be due more to seed size (with the CL seeds being the smallest of the three seed types, followed by the F-I seeds) or the effects of being out-crossed vs. self-fertilized, rather than a stress response to a lack of light. Moreover, the more

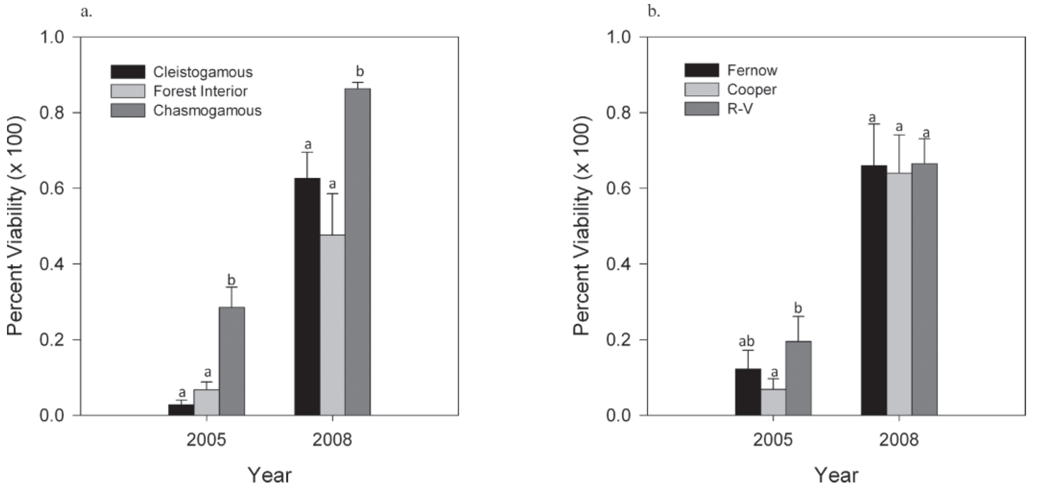

Figure 2. Viability of seeds collected from (a) three seed types and (b) three site types in 2005 and 2008. The data for 2005 were arcsine square root transformed and subjected to a two-way ANOVA. The data for 2008 were cubed, arcsine square root transformed, and subjected to a two-way ANOVA. Bars within each year grouping with different letters differ significantly at $P < 0.05$.

xeric sites did not produce less-viable seeds than seeds from more mesic sites. Indeed the R-V sites had seeds (from 2005) that were slightly more viable than seeds from the Allegheny Plateau sites. Thus, cleistogamy, at least in terms of seed viability, was not favored in the more stressful R-V sites, unlike in other plants with both CH and CL seeds (Cheplick and Quinn 1982; Minter and Lord 1983). Although I did not evaluate total seed abundances by seed and site type in this study, a related study did show that in 2006 (a year that had lower August precipitation amounts compared to 2005 and 2007), plants were shorter and had fewer CL inflorescences than plants located in the more mesic sites (Huebner 2010a). This reduction in seed production under drier conditions supports the findings of Gibson et al. (2002), and further supports the seed viability results that cleistogamy is not favored by Japanese stiltgrass under stressful conditions.

Japanese stiltgrass' CL inflorescences open in response to removal of the CH inflorescences (especially inflorescences closer to the stem apex; C. D. Huebner, unpublished data), suggesting that the CL flowers may have heteroblastic inflorescence development (Lord 1979) and are less mature than the CH flowers. Heteroblastic inflorescence development is further supported by the fact that Japanese stiltgrass' CL seeds in the upper internodes mature before the lower nodes (Cheplick 2005b) and may be more viable than seeds from lower internodes (Cheplick 1996). Less-mature seeds are more likely to be less viable compared to more-mature seeds; less-mature seeds may also be more likely to be negatively affected by stressful environments (Soeda et al. 2005). Likewise, a lack of light may slow seed maturation (Schemske 1977), so it is possible that F-I seeds are less mature than CH seeds.

Seed Germination. For 2005, the germination rate under constant 21 C differed among seed types ($F_{2,8} = 5.01$, $P = 0.019$) but not site types ($F_{2,8} = 2.05$, $P = 0.16$) (Figures 3a and 3b, respectively), and there was no significant interaction ($F_{4,8} = 0.57$, $P = 0.69$) between seed and site types. A comparison of the least square means showed that CH seeds from 2005 had higher germination percentages than CL seeds (Figure 3a). Seeds from 2008 germinated under constant 21 C temperature had such low germination rates (most being zero) for all seed and site types that tests of significance could not be performed. Comparing the same site types used within each germination trial under the two temperature conditions (constant vs. fluctuating), CH and CL seeds from 2005 that were germinated under a constant temperature of 21 C had higher germination rates (51% ± 21% SE and 27% ± 22% SE, respectively), but not significantly higher, than those germinated under the 25/15 C fluctuating day/night temperatures (32% ± 25% SE and 1% ± 0.58% SE, respectively). These results indicate that the 2005 seeds, after 3 yr of refrigeration, were no longer dormant but that fluctuating temperature was required to break dormancy of the younger 2008 seeds.

When seed and site types of the 2008 seeds were tested under fluctuating day/night temperatures, germination rates showed a marginally significant difference among site types ($F_{2,8} = 3.08$, $P = 0.071$) but no significant difference among seed types ($F_{2,8} = 0.03$, $P = 0.97$) (Figures 3b and 3a, respectively). There also was no significant interaction ($F_{4,8} = 0.57$, $P = 0.69$) between seed and site types in terms of percentage of germination for the 2008 seeds under fluctuating temperatures. A comparison of the least square means showed that Fernow

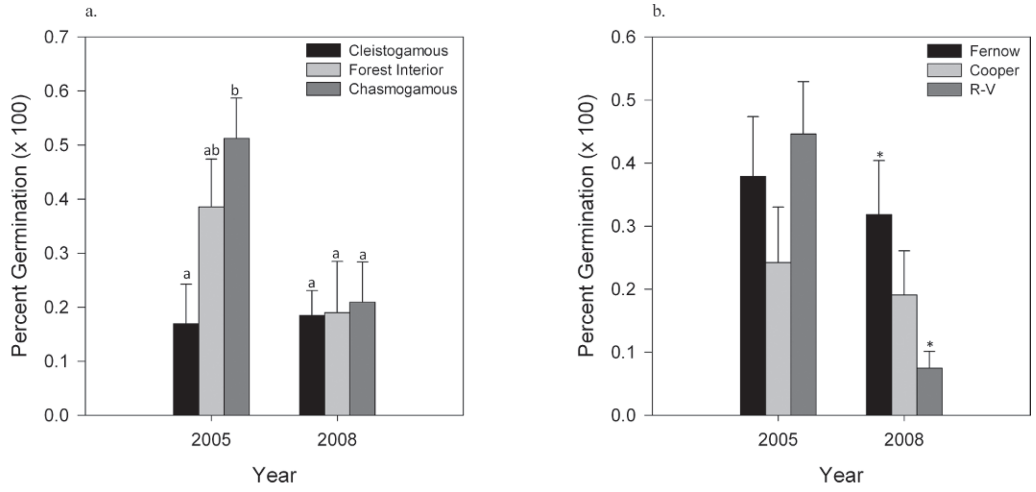

Figure 3. Seed germination percentages of seeds collected from (a) three seed types and (b) three site types in 2005 and 2008. The data for both 2005 and 2008 were arcsine square root transformed and subjected to a two-way ANOVA. Bars within each year grouping with different letters differ significantly at $P < 0.05$. Bars marked with * are marginally significantly different.

seeds from 2008 germinated at a marginally significant ($P = 0.058$) higher germination percentage than R-V seeds (Figure 3b). Thus, the germination percentages of the three seed types for the younger 2008 seeds did not differ, but they did for the older 2005 seeds, which suggests that a smaller proportion of the CL seeds, and to a lesser extent the F-I seeds, remain dormant compared with the CH seeds.

The 2005 seeds that did not germinate for each year under constant 21 C were tested for viability using tetrazolium in order to confirm that the 2005 seeds showed no apparent dormancy. There was no significant difference among seed types ($F_{2,8} = 1.09$, $P = 0.36$) or site types ($F_{2,8} = 1.12$, $P = 0.86$) (Figures 4a and 4b, respectively) nor was there a significant interaction ($F_{4,8} = 0.31$, $P = 0.86$) for the 2005 seeds. In contrast, the 2008 seeds that did not germinate under fluctuating temperatures did show differences in viability when comparing seed types ($F_{2,8} = 14.41$, $P = 0.0002$), but not site types ($F_{2,8} = 0.25$, $P = 0.79$) (Figures 4a and 4b, respectively), with no significant interaction ($F_{4,8} = 0.84$, $P = 0.52$). A comparison of the least square means showed that nongerminating 2008 CH seeds had higher viability percentages than both CL and F-I seeds (Figure 4a), suggesting that any Japanese stiltgrass seed bank development is mostly dependent on CH seeds. The older (2005) CH seeds were more likely to germinate than 2005 CL seeds (Figure 4a), which also supports the postulate that CH seeds are more likely to survive in a seed bank.

Because the R-V 2008 seeds showed marginally lower germination percentages than seeds from the Fernow

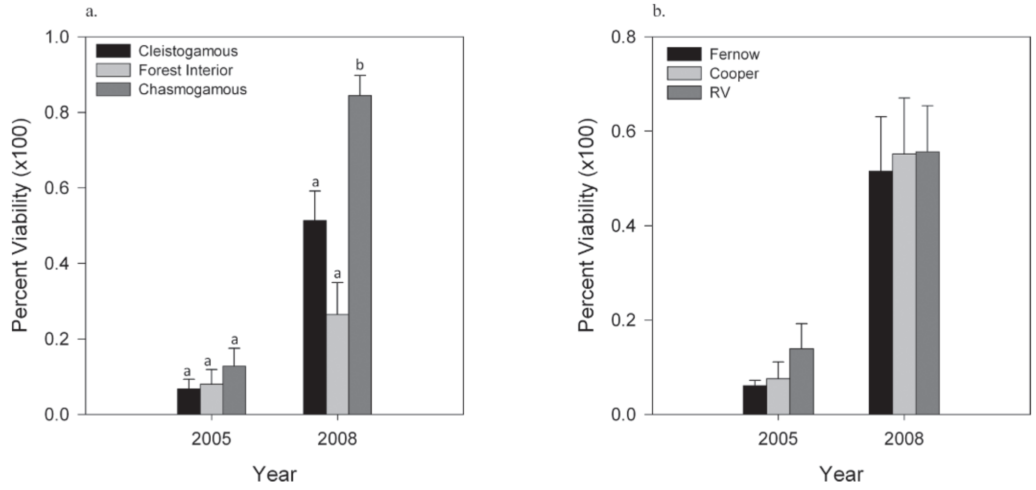

Figure 4. Viability of nongerminated seeds from germination trials of seeds collected from (a) three seed types and (b) three site types in 2005 and 2008. The data for both years were arcsine square root transformed and subjected to a two-way ANOVA. Bars within each year grouping with different letters differ significantly at $P < 0.05$.

sites (most mesic), and the 2008 R-V nongerminating (dormant) seeds had higher viability than seeds from the more mesic sites, R-V seeds may have a longer dormancy period than seeds from the more mesic sites or they may have different germination requirements for breaking dormancy. It is possible that increased impermeability of the seed coat during maturation under dry conditions may induce seed dormancy, suggesting a phenotypic response to drought stress (Geng et al., 2007; Kranner et al. 2010; Ozinga et al., 2004). If the seeds are a product of a maternal-plant phenotypic response, it would be interesting to know if a single drought event could trigger secondary dormancy. Moriuchi and Winn (2005) suggest that an annual's response to such a single event is more likely to be permanent during its lifetime, whereas a perennial is able to adapt to multiple changes in conditions. Thus, drought stress (as estimated by the regional moisture regimes) may affect Japanese stiltgrass seeds by inducing dormancy.

Seed Longevity. Loss of viability over 18 mo in seeds collected in 2008 differed among site types ($\chi^2 = 8.66$, $P = 0.013$) but not seed types ($\chi^2 = 4.53$, $P = 0.10$); there was an interaction between seed and site types ($\chi^2 = 16.25$, $P = 0.0027$). The difference in viability between the two time periods was less in the Fernow and Cooper sites than in the R-V sites. Because of the interaction between seed and site type, seed longevity was evaluated separately within each site type in order to better define any potential effects of seed type. Loss of seed viability did not differ significantly among seed types within the Fernow sites, but it did for the Cooper ($\chi^2 = 7.21$, $P = 0.027$) and the R-V sites ($\chi^2 = 8.10$, $P = 0.017$). In the R-V sites, CL and F-I seeds tended to have a greater loss of seed viability over time than CH seeds. Only CL seeds had a greater loss of seed viability over time than CH seeds for the Cooper sites (Figure 5). Although there is evidence that CL and F-I seeds can produce individuals of equal or greater fitness (Bell and Quinn 1985; Cheplick, 2008), this study shows that fewer Japanese stiltgrass CL and F-I seeds are likely to be viable, especially in drier sites, compared to CH seeds as these seeds age. Thus, managing to reduce CH seed production may be the most cost-effective management strategy of Japanese stiltgrass.

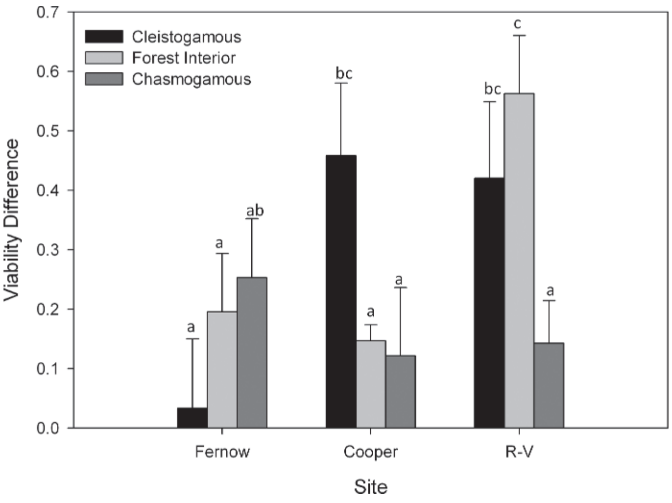

Figure 5. Seed longevity (differences in seed viability across time). Data were analyzed using a generalized linear model with a normal distribution and an identity link function. Bars within each site grouping with different letters differ significantly at $P < 0.05$.

Cold storage of seeds is not equivalent to seeds aging under field conditions. Soil seed pathogens and fluctuating temperatures likely have the effect of decreasing seed longevity more rapidly than refrigerated storage, possibly due in part to genetic weaknesses of the presumably inbred CL and F-I seeds. It is also possible that seeds aged in the field may exhibit shorter life spans in more mesic sites than drier sites, because pathogens could be more prevalent under such conditions. Seed dormancy, or lack thereof, under field conditions is also likely to vary with seed burial depth, litter depth, soil type, and site history (Granström 1987), making any possible patterns among seed and site types much more difficult to discern.

Conclusions

As an annual, Japanese stiltgrass' fitness is defined by its seed viability and longevity in addition to its overall seed production and survival in different environments. Establishment, survival, and spread of this species are more likely in mesic environments (Huebner 2010a and b), and these current findings suggest that most of this success may due to CH seeds, which are longer-lived and most likely to be the primary seed source of a Japanese stiltgrass seed bank. In contrast, CL and F-I seeds, either due to inbreeding effects or being less developmentally mature, may lose their viability more quickly than CH seeds, especially in drier environments. Although differences in site quality (more mesic sites compared with more xeric sites) may not negatively impact seed viability and only marginally impact germination of Japanese stiltgrass, seeds from the drier environments are significantly smaller in mass than seeds from the more mesic environments. Further research is needed to evaluate the effects of seed size on plant fitness as well as the long-term effects of smaller seed size on population growth. Being able to predict an invasive plant's fitness in different environments and over time increases our ability to forecast successful invasions in various habitats and to delineate the timeline likely required for successful restoration or rehabilitation of invaded sites.

Sources of Materials

[1] SAS for Windows, Release 9.1, SAS Institute, Inc., Cary, NC.

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