CONGELOSI, ALEXANDRA M., M.S. Altitudinal Variation in *Microstegium vimineum*, an Invasive Plant Species. (2013)
Directed by Dr. Elizabeth P. Lacey. 64pp.

Invasive plant species are becoming a major concern because they have been found to have negative effects such as decreasing biodiversity and inhibiting growth of native species. *Microstegium vimineum*, also known as Japanese stiltgrass, is such an invasive species. Because ***M. vimineum*** has spread quickly to many variable environments in the United States and because it is also an annual plant species, it is possible that this invasive grass has undergone contemporary evolution. I hypothesized that ***M. vimineum*** is undergoing contemporary evolution via local adaptation. With growth chamber and field experiments, I specifically tested the hypothesis that high-altitude populations have adapted to their cool environments by having higher germination, seed number, and reproductive effort at cool temperatures, and that the opposite is true for low-altitude populations. I also tested two hypotheses regarding the selective advantage of cleistogamy, the resource allocation hypothesis and the optimal genotype hypothesis.

To test the hypothesis, I measured germination, flower phenology, total seed number, chasmogamous and cleistogamous seed number, percent cleistogamous/total seed number, and reproductive effort of plants from populations sampled along an altitudinal gradient. Plants were grown outside and in two growth chambers set at two temperatures. For the growth chamber experiment, I found that altitude affected germination, total seed number, and percent cleistogamous seeds. Temperature also affected germination, total seeds, cleistogamous seed number, and percent cleistogamous

seeds. Low-altitude populations had higher germination and seed number in warm temperatures and high-altitude populations had higher germination and seed number in cool temperatures. There was an altitude by temperature interaction for total seeds, number cleistogamous seeds, and percent cleistogamous seeds. For the field experiment, altitude had an effect on germination, number of chasmogamous seeds, and reproductive effort per plant. As altitude increased, germination and number of chasmogamous seeds decreased, and reproductive effort increased. My results are consistent with the hypotheses that *M. vimineum* shows phenotypic variation associated with altitude and temperature and has undergone contemporary evolution in North Carolina.

ALTITUDINAL VARIATION IN *MICROSTEGIUM VIMINEUM*, AN INVASIVE PLANT SPECIES

by

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A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2013

Approved by

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Dedicated to my parents and my brother, who have always been supportive of my dreams.

APPROVAL PAGE

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ACKNOWLEDGMENTS

I would like to thank my advisor, Elizabeth Lacey, for her guidance and support over the past few years. I would like to thank the rest of my committee for their support as well. I am grateful for several people who helped with my project: Katherine Allison, Kathleen Brown, Haley Gotwald, and Freddy Herrera. Lastly, I would like to thank my family and friends, who have kept me sane during stressful times.

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CHAPTER I

INTRODUCTION

Invasive plant species are becoming a major concern because they have been found to have negative effects such as decreasing biodiversity and inhibiting growth of native species as well as altering ecosystem functions (D'Antonio et al. 1998; Mack et al. 2000; Ellstrand et al. 2010). An example of an invasive species is *Microstegium vimineum* (Trin.) A. Camus, commonly known as Japanese stiltgrass and Nepalese browntop. It is an annual grass that was first documented in the United States in Tennessee in 1919 (Fairbrothers and Gray 1972; Warren et al. 2011). Since its arrival, it has spread throughout the eastern part of the United States causing detrimental effects to native plant species (Flory 2010; Flory and Clay 2010; Simao et al. 2010). Because *M. vimineum* has spread quickly to several variable environments and because it is also an annual plant species, it is possible that this invasive grass has undergone contemporary evolution. I have examined whether or not *M. vimineum* has undergone contemporary evolution since its arrival in the U.S.

Contemporary evolution is evolution that occurs within a few hundred generations or less (Kinnison et al 2007). It is synonymous with the terms "rapid evolution" and "short-term evolution" (Cody and Overton 1996). In general, most studies that test contemporary evolution use an invasive species as a study organism. This is because

during times of significant environmental change, such as an introduction of a new species, selection is estimated to be at its strongest (Reznick and Ghalambor 2001; Lande 2009). Many studies comparing native versus introduced populations show evidence of contemporary evolution. For example, some introduced populations have evolved longer wings (Huey et al. 2000), larger body size and lighter pigments (Johnston and Selander 1964) with increasing latitude. Plant studies have found enhanced growth, reproduction, survival, and dispersability in introduced populations compared to native (Cody and Overton 1996; Lavergne and Molofsky 2007; Flory et al. 2011; Hodgins et al. 2011). Other plant studies have found that crop-wild hybrids of *Raphanus* have evolved earlier flowering time, longer leaves at flowering, and have produced more seeds per plant than the native wild lineages (Campbell et al. 2008; Ridley and Ellstrand 2009).

Additional support for contemporary evolution comes from studies that have looked at population differences in the introduced range. Populations of *Ambrosia artemisiifolia* show population divergence in reproductive allocation (calculated as the ratio of reproductive parts to total biomass), with the high altitude and/or latitude populations having greater reproductive allocation (Chun et al. 2011). Other studies have shown that northern populations of *Impatiens glandulifera* and *Lythrum salicaria* flower earlier and have lower aboveground biomass than do southern populations (Kollmann and Banuelos 2004; Colautti et al. 2010). Colautti et al. (2010) accounted for this relationship as a trade-off between vegetative size (at time of first flower) and days to first flower. Altitudinal studies of populations in native ranges have shown similar trends in plant size and flower phenology (Clausen et al. 1948; Alexander et al. 2009). For

example, Clausen et al. (1948) found that an alpine race at 3350 m was the shortest-stemmed and flowered the earliest compared to all other climatic races of *Achillea millefolium*. In addition, subalpine races of *A. millefolium* had shorter and more slender stems compared to all other climatic races. In contrast, introduced populations of *Buddleja davidii* showed no evidence for local adaptation (Ebeling et al. 2011). The researchers suggested that phenotypic plasticity rather than local adaptation might be the cause of *B. davidii's* invasive spread. Phenotypic plasticity is the ability of a genotype to change its phenotype in response to different environments (Bradshaw 1965).

Data from a previous study suggest that contemporary evolution has occurred in *M. vimineum* (native to eastern Asia) after being introduced into North America. Native and introduced populations were found to phenotypically differ in biomass production and survival (Flory et al. 2011). Introduced populations of *M. vimineum* differed in flower phenology, total biomass, above-ground biomass, and root biomass along a latitudinal gradient (Novy et al. 2013). These phenotypic differences suggest local adaption with respect to climatic differences. Local adaptation is a process in which a population genetically changes over time in ways that increase fitness in response to its local environment. This raises a few questions: Do we see phenotypic differences along an altitudinal gradient? Do these differences in altitude parallel with latitudinal differences? Do these altitudinal differences reflect local adaptation? I conducted experiments that help test the hypothesis that *M. vimineum* has undergone local adaptation in response to temperature associated with altitude change since its arrival into

the United States. There is little information on *M. vimineum* with respect to germination, reproduction, and phenotypic plasticity.

Based on previous literature, I am testing several hypotheses concerning differences among populations along an altitudinal gradient.

- Hypothesis 1: High-altitude populations have adapted to their locally cool environment by increasing germination and seed number, having earlier reproduction, and increasing reproductive effort at cool temperatures. Likewise, low-altitude populations are locally adapted to their warm environment by increasing germination, increasing seed number, and reproductive effort, as well as having earlier reproduction at warm temperatures.
- Hypotheses 2-3: The second hypothesis is based on the observation that in many cleistogamous (CL) plant species, CL production is temperature-dependent (Uphof 1938) and that cleistogamy is less costly compared to chasmogamy. CL flowers go through self-fertilization, while chasmogamous (CH) flowers allow for out-crossing (Lord 1981). The ability of *M. vimineum* to self-fertilize may be considered advantageous because it takes a smaller amount of energy compared to outcrossing due to CH flowers being larger than CL flowers (Campbell et al. 1983; Cheplick 2008; Warren et al. 2011). Therefore, when a plant is under environmental stress, cleistogamy is favored over chasmogamy (Campbell et al. 1983). This trend has been observed in several mixed-mating plants including *Viola praemorsa* and *Collomia grandiflora* (Albert et al. 2011, Jones et al. 2013).

(2) Thus, the "reproductive assurance hypothesis" states that high-altitude populations have a greater proportion of CL seeds compared to CH seeds at warm temperatures. Likewise, low-altitude populations have a greater proportion of CL seeds at cool temperatures (Figure 1a). Alternatively, plants living in an optimal environment should produce more CL seeds in order to maintain those favorable alleles in the local environment (Lu 2002). (3) Thus, the "optimal alleles hypothesis" states that high-altitude populations have a greater proportion of CL seeds at low temperatures, and low-altitude populations have a greater proportion of CL seeds at warm temperatures (Figure 1b).

Testing the hypothesis that a species has undergone local adaptation is a two-step process. The first step involves comparing the phenotypes of the different populations, and the second step involves testing if those phenotypic differences are genetically based. I will complete the first step by comparing populations of introduced *M. vimineum* along an altitudinal gradient. If I find that the populations do not phenotypically differ in a common garden experiment, then I can rule out local adaptation. If I find that populations do phenotypically differ from one another, then my results will be consistent with local adaptation. However, I cannot rule out environmentally induced maternal effects. Environmentally induced maternal effects are effects a maternal environment can have on the phenotype of its offspring (Roach and Wulff 1987; Lacey and Herr 2005).

My Experimental Questions

I conducted two common garden experiments, a growth chamber experiment and a field experiment. In my growth chamber experiment I answered the following questions.

(1) Do populations sampled along an altitudinal gradient show differences in percent germination, flowering phenology, total seed production, reproductive effort, and percent CL seeds/total seeds? I also tested the effects of cold-stratification on seed germination.
(2) Does temperature affect percent germination, flowering phenology, total seed production, reproductive effort, and percent CL seeds/total seeds?
(3) Do populations along an altitudinal gradient differ in phenotypic plasticity with respect to temperature?

For my field experiment, I answered the following questions.

(1) Do populations show differences in germination, total seed production, reproductive effort, and percent CL seeds/total seeds?
(2) Do phenotypes of field-grown populations differ from those of growth chamber-grown populations with respect to the above traits?
(3) Do populations show evidence of a "home-field advantage?" "Home-field advantage" is a term used to describe the greater reproductive success of the locally derived population compared to others, presumably due to that population being locally adapted to that specific area.

Study Species

M. vimineum (Trin.) A. Camus (Poaceae) is an annual C4 grass that thrives in moist environments (such as flood plains or sewer line sites), but it can tolerate drier areas (Barden 1987; Fairbrothers and Gray 1972). This grass is native to Asia, where it grows in diverse areas, including riparian zones and forest borders (Chen and Phillips 2007). It is invasive in the United States, covering 25 states and Puerto Rico (USDA 2013). It germinates in the spring and goes through its vegetative growth in the summer.

Percent germination can vary. Percent germination (~ 87%) was found to be relatively high in one growth chamber experiment, in which introduced and native populations were germinated (Flory et al. 2011). However, another growth chamber study showed that percent germination differed significantly between introduced populations (range = ~ 32-80%) (Droste et al. 2010). Warren et al. (2012) found that only 38% germinated in various field plots and leaf litter treatments. They concluded that percent germination decreased by 22-57% due to leaf litter (Warren et al. 2012). Huebner (2011) found that chasmogamous seeds had higher percent germination than cleistogamous and forest interior seeds (neither CH nor CL, but a separate seed type) in 2005, but there was no difference in germination in 2008. I have not found any previous literature that discusses differences in stratification.

In autumn this grass reproduces (Barden 1987; Hunt and Zaremba 1992; Redman 1995). An individual tiller can produce 70-90 seeds, which can lead to several hundred seeds per parent plant (Gibson et al. 2002). *M. vimineum* seeds can be dormant for many

years in the soil (Barden 1987; Gibson et al. 2002). Seeds are thought to be dispersed through many modes, including epizoochory, water and human activities (Flory 2010).

M. vimineum produces chasmogamous (open) and cleistogamous (closed) flowers. Species that produce CL flowers usually produce CH flowers as well. Both of these floral forms can be present simultaneously (Lloyd 1984; Masuda et al. 2001; Redbotorstensson and Berg 1995; Schoen 1984). Cleistogamy is immensely common in the Poaceae and has been reported in hundreds of species. *M. vimineum*'s CH flowers and seeds have been reported to be found only on the terminal raceme, while CL flowers and seeds are found on axillary racemes (Cheplick 2007). A previous study showed that CH allocation on the terminal raceme was greater for shaded areas than for sunny areas and that allocation to CL declined from terminal stalks to reproductively immature sub-terminal stalks (Cheplick 2006). Huebner (2011) showed that cleistogamous and forest interior seeds are smaller than chasmogamous seeds. She also showed that seeds that came from drier sites were smaller in size than seeds that came from more moist sites.

There is little information about phenotypic plasticity in *M. vimineum*. Novy et al. (2013) compared introduced populations along a latitudinal gradient, from South Carolina to Connecticut, in a common garden experiment. However, they found no evidence of phenotypic plasticity for flower phenology and biomass (total, above-ground, and root). Flory et al. (2011) also conducted a common garden experiment and observed no evidence of phenotypic plasticity in vegetative traits for any of the native and introduced populations. However, in a greenhouse experiment the degree of plasticity in

specific leaf area (SLA) and biomass production in response to shade and drought varied across seven southern Indiana populations (Droste et al. 2010).

Published studies have shown that introduced populations differ along a latitudinal gradient (Novy et al. 2013) and differ from native *M. vimineum* populations (Flory et al. 2011), and introduced populations from southern Indiana vary in degree of plasticity for two vegetative traits (Droste et al. 2010). However, no study has yet compared introduced populations along an altitudinal gradient, compared populations with respect to germination and reproduction, or compared population differences in temperature response.

CHAPTER II

MATERIALS AND METHODS

Seed Sources

Cleistogamous seeds were collected during the month of October 2011 from five sites in North Carolina along an altitudinal gradient: Greenville (17m), Raleigh (107m), Greensboro (220m), North Wilkesboro (293m) and Boone (1006m) (Table 1). In 2012, cleistogamous seeds were collected again to sample populations between 220 meters and 1006 meters. The populations sampled were Greensboro, Lewisville (250m), North Wilkesboro, Berry Mountain (360m), Pure Gas Station (568m) and Falcon Ridge (836m). These populations were sampled on November 4, 2012 and then on December 11, 2012.

Experimental Design

Growth Chamber Experiment:

Germination

I conducted four germination experiments in growth chambers (Table 2). I tested the December and November collections for germination with varying lengths of time between collection date and germination. For the December collection, the length of time between collection and germination was three months. While for the November collection, the length of time was one and then four months.

I began the first germination experiment February 24, 2012 by germinating four hundred seeds per population (except 100 seeds for Boone), from the October 2011 collection. Seed samples for Boone were lower than other populations because my collection contained few seeds. Half of the seeds were stratified (200 per population, 50 for Boone) to see if stratification would affect germination. Stratification was accomplished by placing seeds in a refrigerator at 5^{o}C for four weeks. After stratification, 10 seeds were randomly assigned to each of 20 petri dishes per stratification treatment (5 seeds per 10 dishes per stratification treatment for Boone). The 40 petri dishes per population were randomly assigned to one of two Percival growth chambers. (Total dishes = 4 populations x 2 stratification treatments x 10 replicate dishes x 2 temperatures + 1 population x 2 stratification treatments x 5 replicate dishes x 2 temperatures = 180 dishes). The warm growth chamber was set at 25^{o}C, 12 hr day/20^{o}C, 12 hr night and the cool growth chamber was set at 15^{o}C, 12 hr day/10^{o}C,12 hr night. These temperatures were chosen to resemble temperatures found in *M. vimineum*'s natural setting in North Carolina for the month of March, which is when *M. vimineum* often germinates in the field (Figure 2). The cool temperature treatment resembled the monthly mean temperature for the coldest site (Boone) and the warm temperature treatment resembled the monthly mean temperature for the warmest site (Greenville). Light levels for the center of cool and warm chambers were 178.73 μmol/m^2/s and 176.65 μmol/m^2/s, respectively. Seeds were germinated in 60 x 15 mm petri dishes containing a water-moistened filter paper. Filter paper was kept moist until the experiment ended. Total germination was recorded daily for 19 days (end date = March 15, 2012).

All other germination tests were conducted using unstratified seeds collected in 2012. For experiment two, I began the germination of November 4th seeds on December 1st (Table 2). Germination was recorded for two weeks. On March 1st, 2013, I germinated seeds collected on November 4th (Experiment 3) and December 11th (Experiment 4). For the November collection, eight seeds per population were randomly assigned per petri dish (6 populations x 1 collection date x 4 replicate dishes x 2 temperatures = 48 dishes). For the December collection, eight seeds per population were also randomly assigned per dish (5 populations x 1 collection date x 4 replicate dishes x 2 temperatures = 40 dishes). Total germination was recorded daily for 19 days (end date = March 22, 2013). Chamber conditions for all experiments were the same, except that for the third experiment, the light level for the center of the cool chamber was 118.05 μmol/m^2/s and 112.03 μmol/m^2/s for the warm chamber. Cool and warm chambers were switched between the first and third experiments.

Growth and Reproduction

I used plants from the first germination experiment to examine growth and reproduction of *M. vimineum* at two temperatures. Twenty unstratified seedlings per temperature/ per population (10 from Boone) were kept. Seedlings were transplanted (one per pot) to 6.5 x 6.5 x 5.5 cm pots on March 20, 2012 and placed back into the growth chambers. Unfortunately, a few weeks later, the cool growth chamber overheated and all plants in that chamber died. Therefore, on May 16, 2012, the warm-chamber plants were transplanted to 10.5 x 10.5 x 12.5 cm pots to provide more room for adventitious growth. Then on May 30, 2012 (97 days from start of germination), plants

were randomly assigned to a warm or cool temperature chamber. Sample sizes per population per chamber were: Cool: BO=3, NW=10, GSO=9, RA=9, GV=9; Warm: BO=3, NW=9, GSO=10, RA=8, GV=10. Temperatures were reset to resemble mean monthly temperatures found in *M. vimineum*'s natural setting (in North Carolina) for July for Greenville and Boone (warm: 32^{o}C, 16 hr day/20^{o}C, 8 hr night; cool: 25^{o}C, 16 hr day/15^{o}C, 8 hr night) (Figure 1a). Plants were watered till saturation every other day and were fertilized with ½ strength Hoaglands solution only once at transplanting. Light levels for cool and warm chambers were 182.24 μmol/m^{2}/s and 180.36 μmol/m^{2}/s, respectively.

Flowering began on June 4th, 2012, after which I recorded flowering every other day. A plant was counted as flowering if I saw at least two or more CH flowers on the terminal raceme. I wrapped paper funnels around tillers to catch mature CH seeds falling from plants. On June 22 2012, temperatures were reduced in both growth chambers to bring the plants into fall conditions (warm chamber: 27^{o}C, 10 hr day/20^{o}C, 14 hr night; cool chamber: 20^{o}C, 10 hr day/10^{o}C, 14 hr night). On June 30, 2012, the temperatures were lowered again (warm chamber: 25^{o}C, 10 hr day/20^{o}C, 14 hr night; cool chamber: 15^{o}C, 10 hr day/10^{o}C, 14 hr night). And on July 27 2012, the temperatures were reduced one final time (warm chamber: 15^{o}C, 10 hr day/10^{o}C, 14 hr night; cool chamber: 10^{o}C, 10 hr day/5^{o}C, 14 hr night). These temperatures resembled the temperatures found in *M. vimineum*'s natural setting for the months of September through October, in Greenville and Boone (Figure 2). Once plants had died, all CH seeds per plant were collected as

well as CH seeds that had fallen to the bottom of the growth chamber. In general, the Raleigh plants germinated, reproduced, and died first.

Plants were harvested from August 27th to October 3rd. I harvested above-ground biomass per plant and dried it at 60°C for 72 hours. Chasmogamous (CH) seeds were removed from tillers and vegetative and seed biomasses were weighed to the nearest hundreth gram and hundredth microgram, respectively. Total aboveground biomass per plant was the sum of the vegetative and seed biomass per plant. Seed number per plant was determined by counting and weighing 100 seeds per plant per seed type. Any additional seeds per plant were then weighed to estimate total seed number. Overall total seed number, seed biomass, and percent CL/total & CH/total seeds per plant were then estimated from these values.

Catching mature CH seeds in the paper funnels was difficult. I recovered many CH seeds from the bottom of the growth chambers and in trays at the end of the experiment. Therefore, to reduce the loss of CH seeds I began to collect seeds before they had fully matured (swollen) and turned brown. Therefore, reproductive effort was measured as the ratio of total seeds (#) to total aboveground biomass (mg).

Field Experiment:

On March 11, 2012, unstratified seeds per population from the 2011 collection were scattered onto a common garden plot in a shady, woodland area at 2314 River Run Rd. Browns Summit, NC 27214. ***M. vimineum*** is found naturally near this site. The area is moist due to a drainage basin that flows from a resident's backyard.

I surrounded eighteen 0.25-meter plots (3 replicate plots per population and 3 controls), with metal flashing to make sure seeds were not washed away by rain during germination and cleared plots of the thick leaf litter so that the seeds would be in contact with the soil and have a greater probability of germination (c.f. Schramm and Ehrenfeld 2010). Seeds were scattered on top of each plot (100 seeds for each of 3 replicate plots per population, 33 seeds per plot for Boone). I then spread a small handful of dried hay on top of the seeds to help retain moisture during germination. Three control plots received no *M. vimineum* seeds to account for any *M. vimineum* seeds that might have originally been at the site before the start of the experiment.

Germinating tree seedlings were removed from each plot (about 1-2 per plot) before the start of the experiment, as well as throughout the entire experiment. By April 16, 2012, *M. vimineum* seedlings had emerged. Seedlings were counted at two times for each plot (April 16, 2012 and April 29, 2012). On October 25th, 2012, when one plant had begun to turn brown and brittle, I began collecting CH seeds from that individual plant. As additional plants in the common garden started to wither, I collected those CH seeds associated with each withering plant. When an individual plant died (completely brown and dry), I harvested that plant and separated it into vegetative and reproductive parts. By November 20th, 2012, all of the plants in the experiment had died and were harvested. Field plants were processed in the same way as growth chamber plants. I collected data on germination, aboveground biomass, total seed production, percent CL/total seeds, and reproductive effort per plot.

CHAPTER III

STATISTICAL ANALYSIS

All analyses were performed with SAS 9.2. Logistic regression analysis (PROC LOGISTIC) was used to analyze the binary response of seed germination and percent CL seeds/total seeds. Logistic regression does not produce exact values, but rather odds ratios that estimate the probability of a particular event occurring relative to the odds of that event not occurring. Explanatory variables for the growth chamber experiments were stratification (germination only), temperature, altitude, test date (germination only) and all interactions. All of the explanatory variables were considered categorical, except for altitude (continuous). The first model for unstratified seeds alone, analyzed the binomial response of germination against temperature, altitude, date and all interactions. Because the 3-way interaction was significant, I analyzed the data separately by collection date. I also examined effects of altitude for some temperatures separately. For the field experiment, altitude was the only explanatory variable.

A linear regression model (PROC GLM) was used to analyze the remaining reproductive data: total seed production, number of CL seeds, number of CH seeds, reproductive effort, and days to flower. Reproductive effort was logit transformed due to unequal variance and distribution. Linear regression was used to analyze the effect of altitude on reproductive effort in the field. Thermal plasticity for each trait was

calculated by subtracting the trait value in the cool chamber from the trait value in the warm chamber.

CHAPTER IV

RESULTS

Germination

For all germination experiments (except experiment 2), the first day of germination was approximately four to five days after the start of each experiment (Figure 3). Most seeds had germinated within two to three weeks. Germination was delayed approximately 3-7 days in the cool chamber. In general, final germination was higher in the warm chamber, compared to the cool, except for the Falcon Ridge population at 836m (Figure 3). No seeds germinated in the second experiment (seeds collected on November 4th, 2012 and germinated on December 1st, 2012).

Stratification significantly lowered final percent germination for the October 2011 collection (Table 3; Figure 4; Appendix Figure C1). Final germination was approximately 8-23% higher for unstratified seeds. There was also evidence of an interaction between stratification and altitude (Figure 4). This is better explained by population differences, which were much stronger than any altitudinal trend. Greenville (17m) had the greatest decrease in germination and therefore the highest plasticity. Raleigh (107m) had the lowest decrease in germination.

Data from all germination experiments for only unstratified seeds showed statistically significant evidence that percent germination differed with altitude and

temperature (Table 4). For low-altitude populations (< 500m), the odds of germination were 37-79% lower in the cool chamber compared to the warm chamber (Figure 5). For high-altitude populations (> 700m), the odds of germination between cool and warm temperatures did not differ. In general, the temperature by altitude relationship shows that as altitude increases, germination plasticity becomes more negative. As altitude increased, greater germination at warm temperature changed to greater germination at cool temperature. There was also a significant three-way interaction between altitude, temperature, and collection date, which implied that the altitude by temperature interactions was influenced by experiment date (Figure 5). Because of this strong interaction, I analyzed the data separately by date.

For the 2011 collection, stratified seeds were removed from the analysis in order to be consistent with the 2012 collection analyses. The 2011 collection showed there was statistical evidence that temperature moderately affected final percent germination (Table 5). The odds of germination in the cool chamber were 26.3% lower than the odds of germination in the warm chamber (Figure 6A,D). There was also a significant altitude effect. For every 100 meter increase in altitude, the odds of germination decreased by 15.6%. In the warm treatment, low-altitude populations had higher final percent germination than did high-altitude populations (Figure 6D). However, in the cool treatment, there was no such trend. There was no evidence of a statistically significant altitude by temperature interaction (Fig 6A). However, there is a trend for the higher altitude populations to decline in percent germination from cool to warm temperature in

contrast to the lower altitude populations, which show an increase in percent germination (Figure 6D).

For the third and fourth experiments (seeds collected in 2012), final germination was lower for the December 11th collection than for the November 4th collection at both warm and cool temperatures (Figures 3B-F). For the December 2012 collection, there was no evidence that altitude or temperature affected germination, and there was no significant temperature by altitude interaction (Table 5; Figure 6C,F). However, the highest altitude population, Falcon Ridge (836m), and also North Wilkesboro (293m) had negative slopes where probability of germination was higher at the cool temperature than at the warm. The highest altitude population (Falcon Ridge) had the most negative temperature-sensitive plasticity (Appendix Table C1; germination plasticity = - 0.3339).

For the November, 2012 collection, there was strong statistical evidence that temperature affected germination (Table 5). All populations showed high final percent germination in the cool chamber (Figure 3D). Falcon Ridge (836 m) reached 96.875% final percent germination in the cool chamber, but in the warm chamber, germination was significantly lower (65.63%; lsmeans p = 0.0027). There was also convincing statistical evidence of an altitude by temperature interaction (Table 5). In the warm chamber, as altitude increased by 100 meters, the odds of germination decreased by 42.6%. In the cool chamber, as altitude increased by 100 meters, the odds of germination increased by 22.4%. In general, low-altitude populations (< 500m) had approximately the same final percent germination across temperatures (Figure 6B,E). However, the probability of

germination for Falcon Ridge (836m) and North Wilkesboro (293m) decreased in the warm chamber. Due to this strong interaction, I analyzed the data separately by date and temperature (Appendix Table C2). I found that the warm chamber was significantly affected by altitude, but the cool chamber was not.

Reproduction

Plants from warm and cool chambers began flowering six days after they were assigned to warm and cool chambers. Most of the plants that flowered first were from Raleigh (Appendix Figure C2). In addition, I found that CH flowers were produced not only on the terminal raceme, but also on the axillary racemes. Cool chamber plants turned purple in color as time progressed, and these same plants produced purple anthers.

For the seeds that I could assign to an individual plant, there was statistical evidence of a temperature effect for total seeds per plant (Table 6). There was also suggestive evidence of an interaction between temperature and altitude. For the cool treatment alone, as altitude increased 100 meters, total seed number per plant increased by 26 seeds (Figure 7; Figure 8A). In contrast in the warm treatment as altitude increased 100 meters, total seed number decreased by 44 seeds (Figure 7; Figure 8C).

Across both temperatures, *M. vimineum* produced more CH (chasmogamous) than CL (cleistogamous) seeds (Figure 8A,C). Due to a suspected loss of more CH seeds from the Raleigh population, I repeated analyses with the Raleigh population removed. I found results did not change (Figure 8B,D). I suspected a greater loss of CH seeds from Raleigh because these plants were the first to flower. When I first began collecting

mature seeds, I attempted to let seeds ripen (fully swell and turn brown) before collection. This method resulted in the loss of many seeds, more likely from Raleigh plants. The estimated total number of CH seeds I was able to capture are as follows cool: 19,766; warm: 31,724. The estimated number of seeds that I could not assign to a plant are: cool chamber = 1,508,287; warm chamber = 1,409,426. No CL seeds were lost because they were retained in leaf sheaths until removed after harvest.

For the number of CL seeds, there was a significant effect of temperature and a significant interaction between altitude and temperature (Table 7). In the cool chamber, the number of CL seeds per plant increased by 12.03 seeds with every 100 meter increase in altitude (Figure 8A). In the warm chamber, number of CL seeds per plant decreased by 16.5 seeds with every 100 meter increase (Figure 8C; Appendix Figure C3b). Greenville had the highest level of plasticity (Appendix Table C3; CL seed plasticity = 307.18).

Altitude and temperature statistically affected percent CL seeds (Table 8; Figure 9; Appendix Figure C4). There was also evidence of population differences in temperature-sensitive plasticity. For low-altitude populations (<500m), the odds of producing CL seeds at the cool temperature were approximately 38% lower than the odds of producing CL seeds at the warm temperature. However, for Boone (1006m), there was no significant difference in percent CL seeds between warm and cool temperatures.

There was no evidence that temperature or altitude affected reproductive effort (Appendix Figure C5) (temperature: $p = 0.1104$; altitude: $p = 0.5347$). Furthermore,

there was no evidence that altitude or temperature affected flower phenology (Appendix Figure C2).

Field

For the field experiment, most seeds germinated by April 29th, 2012. There was strong statistical evidence of an altitudinal effect on percent germination per plot; seed germination was adjusted by number of seeds sown (Table 9). For every 100 meter increase in altitude, the odds of germination decreased by 9.1% (Figure 10). The population with the highest percent germination was Raleigh at 80% for April 16th and 83% for April 29th. The Boone population had the lowest percent germination at 59% for April 16th and 63% for April 29th.

Altitude also affected the number of CH seeds per plant (Table 9), but the effect was rather low (0.014 seeds with every 100 m increase in altitude, Figure 11). Altitude had no effect on the number of CL seeds or on total seeds per plant. In contrast to the growth chamber experiment, the field plants also produced more CL seeds per plant compared to CH seeds. There was strong statistical evidence that altitude affected reproductive effort per plant (Table 9; Figure 12). For every 100 meter increase in altitude, reproductive effort per plant increased by 0.737 #seeds/ug. There was no evidence of an altitudinal effect on percent CL seeds ($p = 0.6373$).

CHAPTER V

DISCUSSION

Species can become invasive for several reasons. One reason is that certain species have locally adapted to their introduced range. Previous studies have compared introduced *M. vimineum* populations along a latitudinal gradient for flower phenology and biomass (Novy et al. 2013) and in smaller geographic regions for specific leaf area (SLA) and survival (Droste et al. 2010). Other studies have compared populations with respect to survival and vegetative traits (Flory et al. 2011) and phenotypic plasticity for SLA and survival with regard to shade and water conditions (Droste et al. 2010). These studies found that populations showed phenotypic differences for these particular traits and evidence that is consistent with local adaption and phenotypic plasticity. However, none of these previous experiments has examined germination, reproduction output, or temperature-sensitive phenotypic plasticity in these traits. My experiment has addressed all of these topics.

My germination and reproductive data provide evidence consistent with the hypothesis of population differentiation along an altitudinal gradient and local adaptation in response to temperature. Low-altitude populations (<500m) phenotypically differed in response to temperature. They showed higher germination and seed number than did the high-altitude populations in warm temperatures. All populations reached high

germination in the warm chamber, except for the two highest altitude populations, Falcon Ridge (836m) and Boone (1006m). Low-altitude populations made more total seeds, more CH and CL seeds, and percent CL seeds than did the high-altitude populations in the warm chamber, but made fewer seeds than did the high-altitude populations in the cool chamber. Of these traits, total seed number, number of CL seeds, and percent CL seeds/total showed evidence of temperature-sensitive phenotypic plasticity. For percent CL seeds/total, low altitude populations had greater plasticity than did the high altitude population.

My results are consistent with previous papers in which introduced populations of *M. vimineum* show phenotypic differences (Novy et al. 2013), and evidence of phenotypic plasticity (Droste et al. 2010). However, I found new information that shows that introduced populations phenotypically differ in reproduction output. In addition, I found that populations show plastic responses to temperature for germination and seed production.

The growth chamber data on percent CL seeds per plant are more consistent with the "optimal alleles hypothesis" than the "reproductive assurance hypothesis". The pattern observed for all seed types was that low-altitude populations produced more seeds at warm temperature and fewer at cool temperature. High-altitude populations produced more seeds (both CH and CL) at cool temperatures and fewer at warm. Therefore, populations increased the percentage of CL seeds in their "home thermal" environment, promoting "optimal or presumably adapted" alleles. Yet, there was the large loss of CH

seeds throughout all of the populations that could not be assigned to parent plants, which could have altered the ratio of seeds. If I were to repeat the experiment, I would have each plant population assigned to an individual tray. That way any CH seeds that fell would fall into a known tray and therefore, seeds could be properly assigned.

When the field and growth chamber data are combined, however, data are more consistent with the "reproductive assurance hypothesis" than with the "optimal alleles hypothesis". There was no statistical evidence of an association between altitude and percent CL seeds (none for Boone (1006m) plants) in the field plots. CH seed production was so low in all populations that population differences are not biologically meaningful. Field-grown plants were shorter, had less above-ground biomass, and lower seed production than did growth-chamber plants. These observations suggest that my particular field site was not an optimal location for growth and reproduction. None of the field-grown plants produced CH flowers on axillary racemes. My growth chamber experiment showed that CH flowers can be produced on axillary racemes, not just from the terminal raceme, as reported by Cheplick (2007). These data suggest that water and light allocation are greater "stress factors" than is temperature, and that when water and light are limiting, axillary racemes do not emerge from leaf sheaths. Thus, under stress potentially CH flowers revert to CL flowers, which ensures some seed production. I also casually noted that non-experimental plants growing in a sunlit ditch close to my field plots did produce CH flowers from axillary racemes.

While completing my growth chamber experiment, I made three interesting observations that I had not read about in previous literature. Results of the germination experiments two through four suggested that seeds need an after-ripening period before they will germinate. When I tried to germinate seeds within one month of collection, there was no germination, but when I waited at least three months, germination occurred. Also, I observed the highest germination in seeds that germinated four months after collection. Second, plants grown in the cool but not warm chambers produced anthocyanin and therefore, turned purple in color. Many anthers were also found to be purple. Novy (2012) found similar results in regards to anthocyanin production. In a common garden experiment (under one common temperature), Novy (2012) compared introduced and native populations of *M. vimineum* and found that North American populations produced reddish-brown anthers, but the Japanese/Chinese populations produced yellow anthers. Novy (2012) also found that both native and introduced populations produced anthocyanic (purple) surface roots. Third, my results for the stratification test show that unstratified seeds had higher percent germination than stratified seeds. I found no other studies that have compared stratification treatments in *M.vimineum* seeds. Stratification in summer annuals is usually essential to break dormancy (Baskin and Baskin 1998).

To test for local adaptation, two steps must be completed. The first step is to compare phenotypes of populations and find if they differ. The second step, if populations do phenotypically differ, is to determine if those differences are genetically based. I have completed the first step and found that populations along an altitudinal

gradient do phenotypically differ. The next step is to test for maternal effects, which might alternatively explain my results, and genetic differentiation. Reciprocal transplants across diverse altitudes would also test if *M. vimineum* has locally adapted in its introduced range and would shed more light on *M. vimineum*'s rapid evolution. A third possible explanation is that when *M. vimineum* was introduced from Asia to the U.S. that the Asian plants from high altitudes or a cool climate were introduced into the North Carolina mountains, whereas Asian plant from low altitudes or a warmer climate were introduced into the piedmont and the coastal plain of North Carolina. The likelihood of such an occurrence seems very low. Regardless, my results provide evidence that certain populations of *Microstegium vimineum* phenotypically differ along an altitudinal gradient in North Carolina, which is consistent with local adaptation.

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APPENDIX A

TABLES

Table 1. Description of source populations.

Description of all source populations of *M. vimineum* in the state of North Carolina. Population altitudes are also shown.

Symbols	Source Populations	Collection Time	Geographic Region	Altitude (m)	Description
GV	Greenville	October 2011	Coastal Plain	17	Phil Carroll Nature Preserve, Pitt County, NC: along dirt road to the Preserve, mesic, shaded, little leaf litter
RA	Raleigh	October 2011	Piedmont	107	1805 Dorton Road, Raleigh, 27607, Wake County, NC: moist area near a creek, sun and shade, abundant leaf litter
GSO	Greensboro	October 2011, November 2012	Piedmont	235	Haw River State Park, Guilford County, NC: north side of lake, very moist area due to water from drainage pipe, shaded, scattered leaf litter
LW	Lewisville	November 2012	Piedmont	250	US-421 S. Lewisville, 27023, Forsyth County, NC: shallow ditch area, partially shaded, no leaf litter
NW	North Wilkesboro	October 2011, November 2012, December 2012	Piedmont	293	Veterans of Foreign Wars campground, Wilkes County, NC: backside of baseball field, moist area in ditch, mostly sunny, no leaf litter
BM	Berry	November	Mountains	360	Berry Mountain Park,

	Mountain	2012, December 2012			Wilkes County, NC: just past entrance gate, shaded area, partial leaf litter
PG	Pure Gas	November 2012, December 2012	Mountains	568	4220 Blowing Rock Blvd. Lenoir, 28645, Caldwell County, NC: gas station, moist area along creek, mix of sun and shade, no leaf litter
FR	Falcon Ridge	November 2012, December 2012	Mountains	836	7489 Falcon Ridge Rd. Lenoir, 28645, Caldwell County, NC: housing development, mesic, shaded area, partial leaf litter
BO	Boone	October 2011	Mountains	1006	Old US 421 S and Coolwoods Drive, Watauga County, NC: moist area adjacent to small ditch, partly sunny, abundant leaf litter

Table 2. Experimental design for germination experiments.

Basic experimental design for all germination experiments.

Experiment #	Collection Date	Start of Germination Experiment	Population No.	Time from Collection to Germ Experiment
1	Oct 2011	Feb 24th, 2012	5	4 mo.
2	Nov 4th, 2012	Dec 1st, 2012	6	1 mo.
3	Nov 4th, 2012	Mar 1st, 2013	6	4 mo.
4	Dec 11th, 2012	Mar 1st, 2013	5	3 mo.

Table 3. Germination results for the October 2011 collection.

Parameter estimates and p-values for the germination experiment with seeds from the October 2011 collection. Estimate values shown are not valid until they are exponentiated (odds ratio). Therefore, both estimate values and odds ratios are shown. However, only the odds ratios should be used for interpretations. Interpretation of Alt*Temp: The odds of germination in the cool chamber increases by 7% (1- 1.07) for every 100m increase in altitude.

Parameter	Estimate	Odds Ratio	P- value
Temperature	-0.4827	---	< 0.0001
Altitude	-0.0012	---	< 0.0001
Stratification	0.6652	---	< 0.0001
Alt*Temp	0.0007	1.07	0.0049
Alt*Strat	-0.0005	0.951	0.0417

Table 4. Results for when all germination experiments were combined.

Parameter estimates and p-values for all germination experiments combined. All seeds analyzed were unstratified. Estimate values shown are not valid until they are exponentiated (odds ratio).

Parameter	Estimate	Odds Ratio	P- value
Temperature	-0.4788	---	0.0456
Altitude	-0.0014	---	0.0026
Collection Date	0.9675	---	0.0084
Temp*Date	- 0.9625	---	0.0081
Alt*Temp	0.0013	---	0.0060
Alt*Date	- 0.0006	---	0.5016
Alt*Temp*Date	0.0015	1.002	0.0126

Table 5. Germination results for experiments 1, 3, and 4.

Parameter estimates and p-values for germination experiments 1, 3, and 4 (unstratified seeds only). Estimate values shown are not valid until they are exponentiated (odds ratio). Therefore, both estimate values and odds ratios are shown. However, only the odds ratios should be used for interpretations. Interpretation of October 2011 temperature: The odds of germination in the cool chamber are 26.3% (1- 0.737) lower than the odds of germination in the warm chamber. Due to a significant interaction for the November 2012 collection, slope values were calculated for each temperature across altitude.

Date (# exp)	Independent Factors	Estimate	Odds Ratio	P- value
Oct. 2011 (1)	Temperature	- 0.3043	0.737	0.0734
	Altitude	- 0.0017	0.844	< 0.0001
	Alt*Temp	0.0003	1.000	0.4376
Nov. 2012 (3)	Alt-Cool	0.00202	1.224	<0.0001
	Alt-Warm	- 0.00555	0.574	0.0073
	Alt*Temp	0.00757	2.132	0.0017
Dec. 2012 (4)	Temperature	0.4837	1.622	0.1659
	Altitude	- 0.0008	0.923	0.2045
	Alt*Temp	- 0.0002	1.000	0.7998

Table 6. Total seed production at warm and cool temperatures.

Parameter estimates and p-values for total seeds produced at warm and cool temperatures. Estimate values need to by multiplied by 100 in order to interpret correctly. Interpretation of Cool-Altitude: For every 100m increase in altitude, total number of seeds produced increased by 26.19 seeds. Due to a significant interaction, slope values were calculated for each temperature across altitude.

Parameter	Estimate	P- value
Temperature	-456.308	0.0009
Altitude	-0.4422	0.6512
Altitude*Temperature	0.70	0.0804
Cool-Altitude	0.2619	0.0009
Warm-Altitude	-0.4422	0.0009

Table 7. Number of CL seeds produced at warm and cool temperatures.

Parameter estimates and p-values for number CL seeds produced at warm and cool temperatures. Estimate values need to by multiplied by 100 in order to interpret correctly. Interpretation of Cool-Alt: For every 100m increase in altitude, number CL seeds produced increased by 12.02 seeds. Due to a significant interaction, slope values were calculated for each temperature across altitude.

Parameter	Estimate	P- value
Temperature	-207.50	<0.0001
Altitude	-0.16495	0.7045
Altitude*Temperature	0.28523	0.0174
Cool-Altitude	0.12028	<0.0001
Warm-Altitude	-0.16495	<0.0001

Table 8. Percent CL seeds produced at warm and cool temperatures.

Parameter estimates and p-values for percent CL seeds produced at warm and cool temperatures. Estimate values shown are not valid until they are multiplied by 100 exponentiated (odds ratio). Interpretation of Warm-Alt: For every 100m increase in altitude, the odds of producing percent CL seeds decreases by 0.7%. Due to a significant interaction, slope values were calculated for each temperature across altitude.

Parameter	Estimate	Odds Ratio	P- value
Temperature	- 0.4856	---	< 0.0001
Altitude	- 0.00007	---	< 0.0001
Altitude*Temperature	0.000481	1.049	< 0.0001
Cool-Altitude	0.000411	1.042	0.1938
Warm-Altitude	- 0.00007	0.993	< 0.0001

Table 9. Effect of altitude on germination and reproduction in the field.

Parameter estimates and p-values for effect of altitude on germination and reproductive traits in the field experiment. The transformed estimate shown is the estimate value multiplied by 100 and exponentiated. Interpretation of % Germination: For every 100m increase in altitude, the odds of germination decreases by 9.1%.

Trait	Estimate	Transformed Est.	P- value
% Germination	-0.00095	0.909	0.0001
# CL Seeds	- 0.00021	---	0.6953
# CH Seeds	- 0.00014	---	0.0331
Total Seed #	- 0.00035	---	0.5347
Reproductive Effort	0.00737	---	0.0007

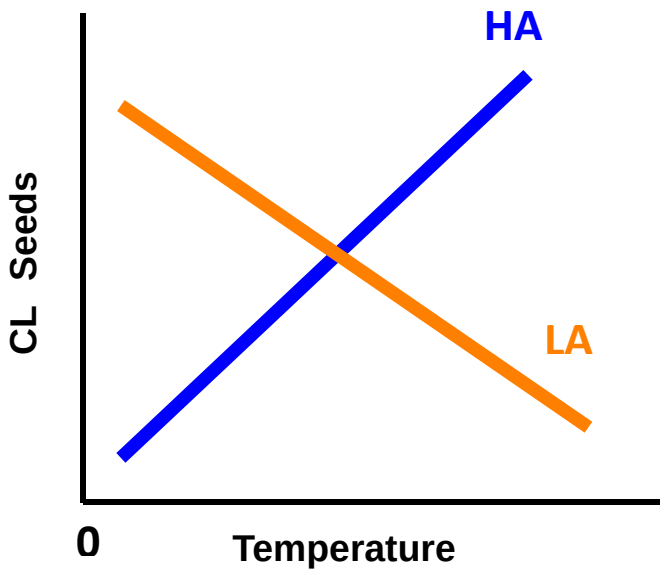
APPENDIX B

FIGURES

Figure 1. Allocation of CL seeds in response to temperature.

Diagrams that show how plants can allocate CL seeds in response to temperature, according to previous literature. A) Stressful environment, B) Optimal environment.

A.



B.

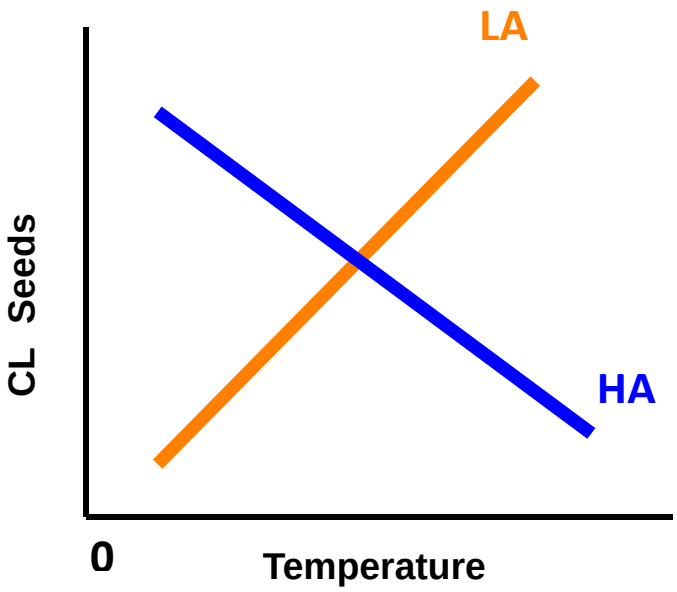
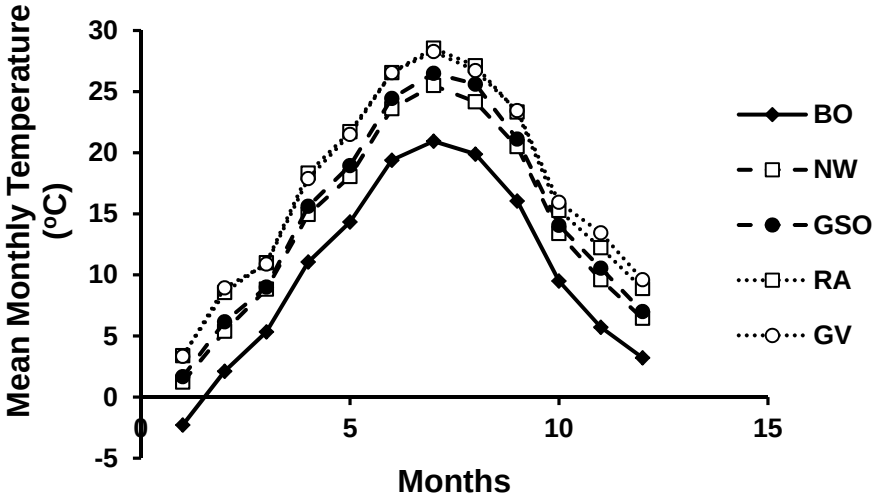


Figure 2. Monthly temperatures for populations in 2011-2012 in North Carolina.

A) Mean monthly temperatures for the year 2011. All five populations of *M. vimineum* collected in North Carolina in 2011 are represented. B) Mean monthly temperatures for the year 2012. Eight out of the nine populations of *M. vimineum* collected in North Carolina in 2012 are represented. LW is not present due to lack of information from NOAA (http://www.ncdc.noaa.gov/cdo-web/search#t=secondTabLink). Refer to Table 1 for symbol key.

A.



B.

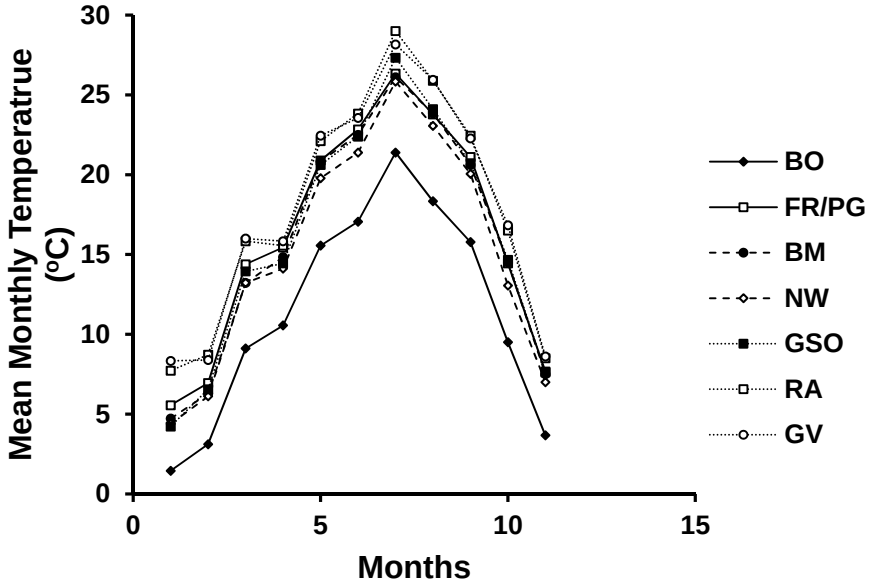


Figure 3. Percent germination at warm and cool temperatures.

Mean percent germination of *M. vimineum* populations shown by altitude (meters above sea level) for the: 2011 collection in the (A) warm and (B) cool chambers, November 2012 collection in C) warm and D) cool chambers, December 2012 collection in E) warm and F) cool chambers. Temperature treatments were averaged over stratified and unstratified seeds. Vertical bars equal ± 1 SE. Standard errors were so small that they are not visible in figure.

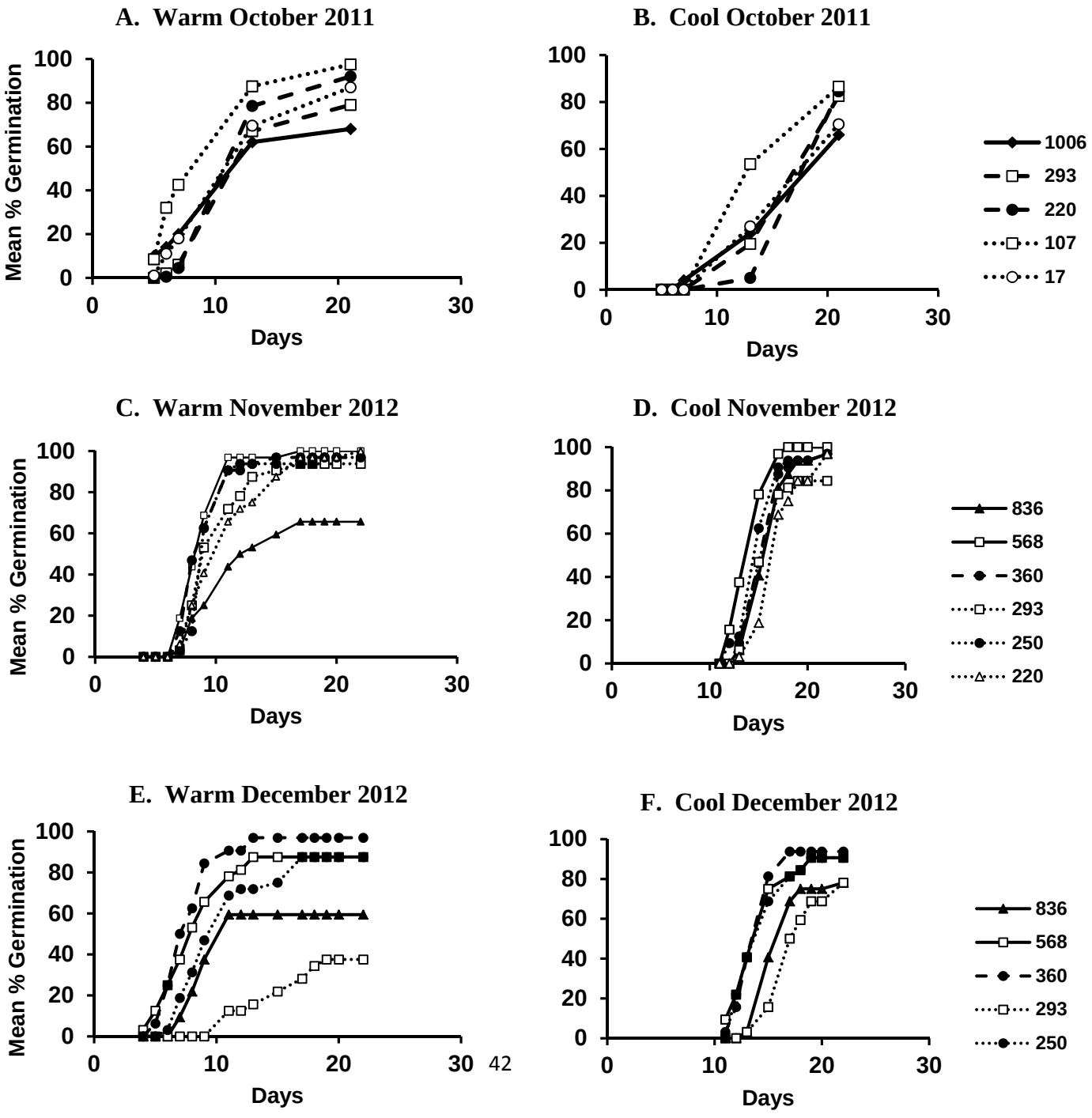


Figure 4. Final percent germination for stratified and non-stratified seeds.

Final percent germination per *Microstegium vimineum* seed samples collected in 2011 for unstratified and stratifed seeds. Stratification treatments were averaged over warm and cool temperatures. Vertical bars equal ± 1 SE. Standard errors were so small that they are not visible in the figure.

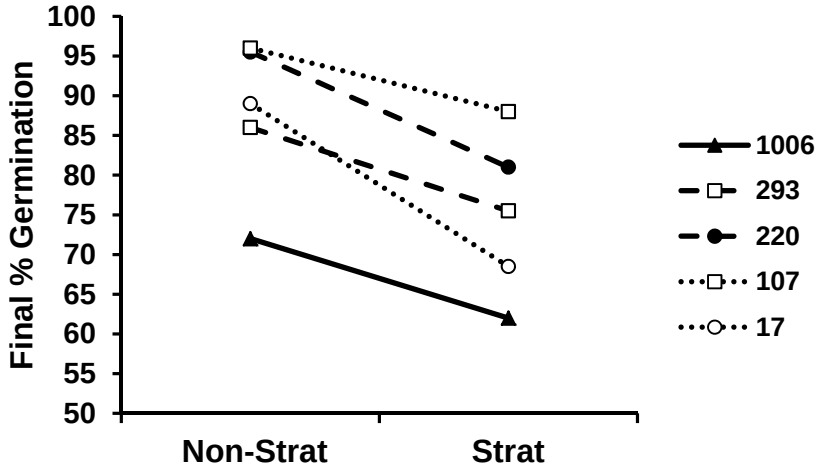


Figure 5. Germination plasticity trends across altitude.

Germination plasticity values for *M. vimineum* samples collected in October 2011 (solid circle, solid line), November 2012 (open box, dashed line), and December 2012 (solid triangle, dotted line). Positive values mean that populations had greater germination in the warm chamber. Negative values mean that populations had greater germination in the cool chamber.

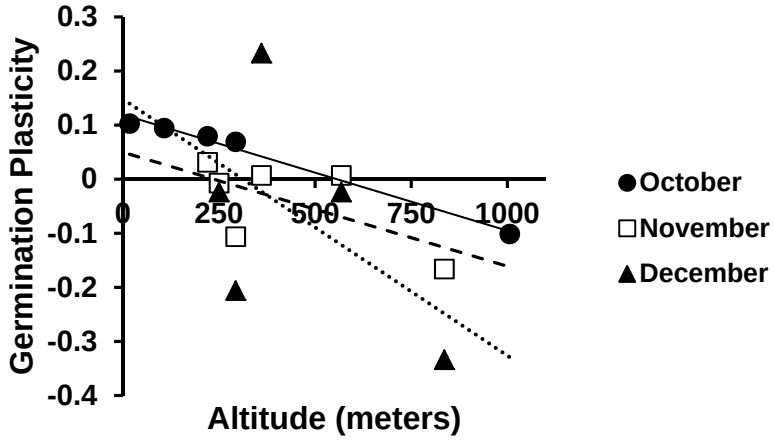
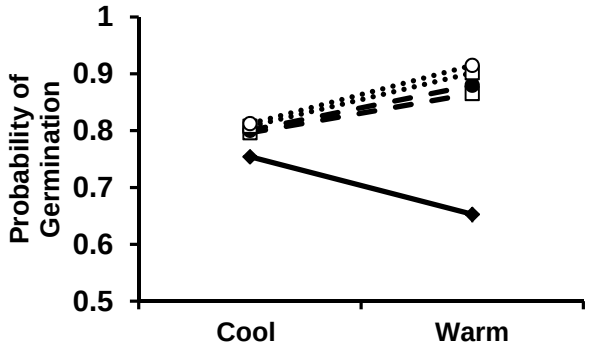


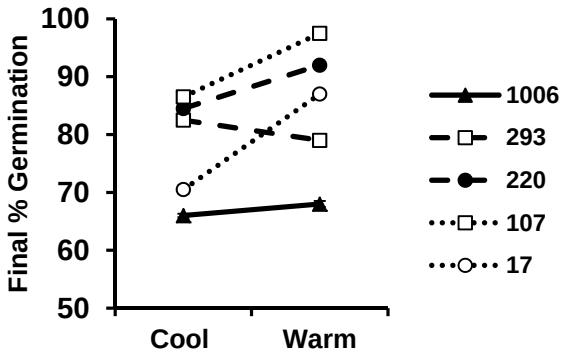
Figure 6. Probability of germination at warm and cool temperatures.

Germination probability per *Microstegium vimineum* population at cool and warm temperatures based on logistic regression odds ratios. Seeds were collected in (A) October 2011, (B) November 4th 2012, and (C) December 11th 2012. Final percent germination per population at cool and warm temperatures for seeds collected in (D) October 2011, (E) November 4th 2012, and (F) December 11th 2012. For seeds collected in October 2011, the experiment began on February 24th. 2012. For seeds collected in 2012, the experiment began on March 1st, 2013.

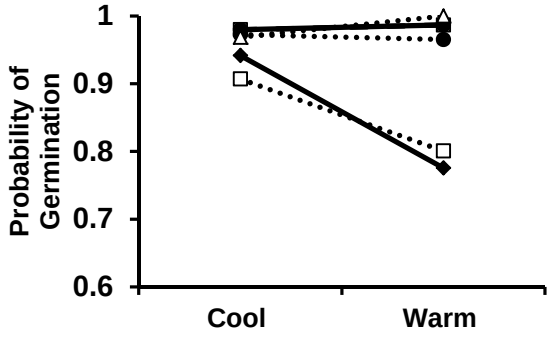
A. Oct 2011



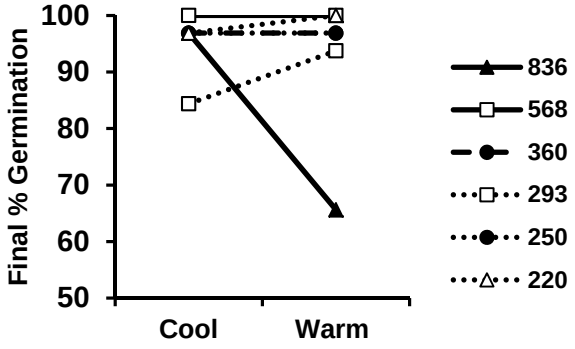
D.



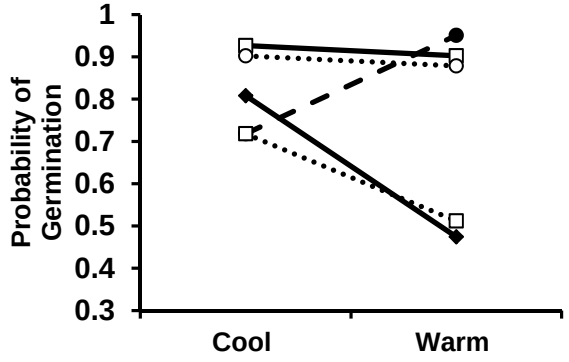
B. Nov 2012



E.



C. Dec 2012



F.

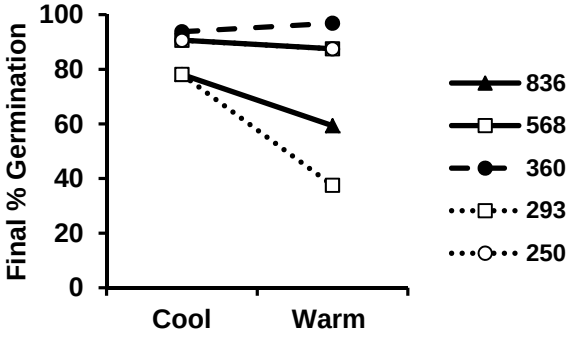


Figure 7. Estimated total seed number per plant at warm and cool temperatures.

Estimated total seed number per plant per population at cool and warm temperatures. Vertical bars equal ± 1 SE. Warm: solid diamond, solid line. Cool: open box, dotted line.

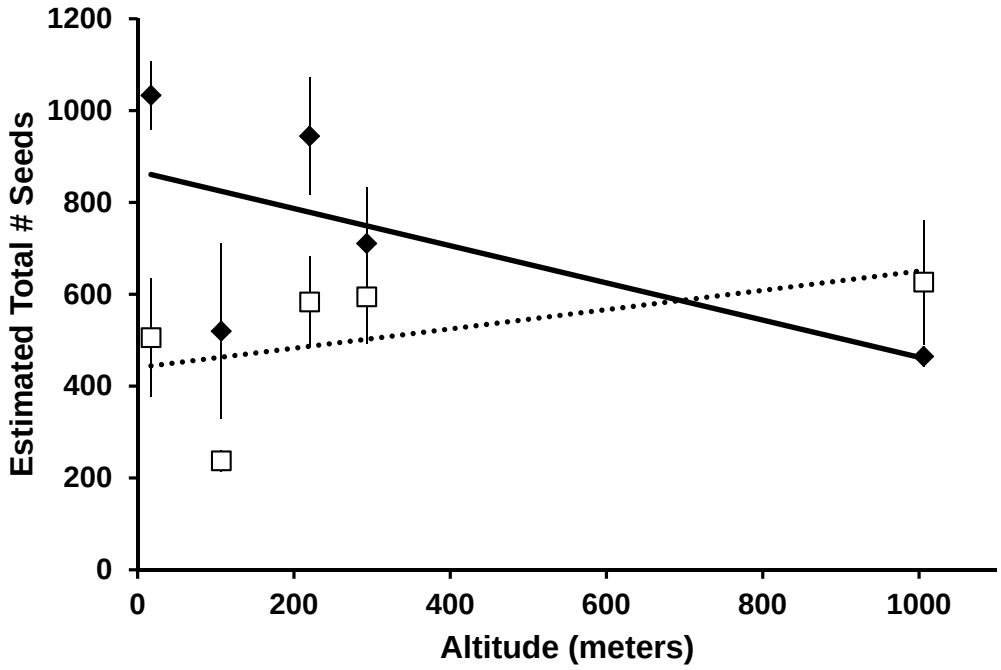


Figure 8. Estimated number of seeds produced per plant at warm and cool temperatures.

Total, CH, and CL seeds per plant produced from October 2011 *Microstegium vimineum* collection grown at cool (A, B) and warm (C, D) temperatures. Raleigh seeds were removed from the analyses shown in B and D. Total seeds (solid circle, solid line), CH seeds (solid diamond, dashed line), and CL seeds (open box, dotted line). Vertical bars equal ± 1 SE.

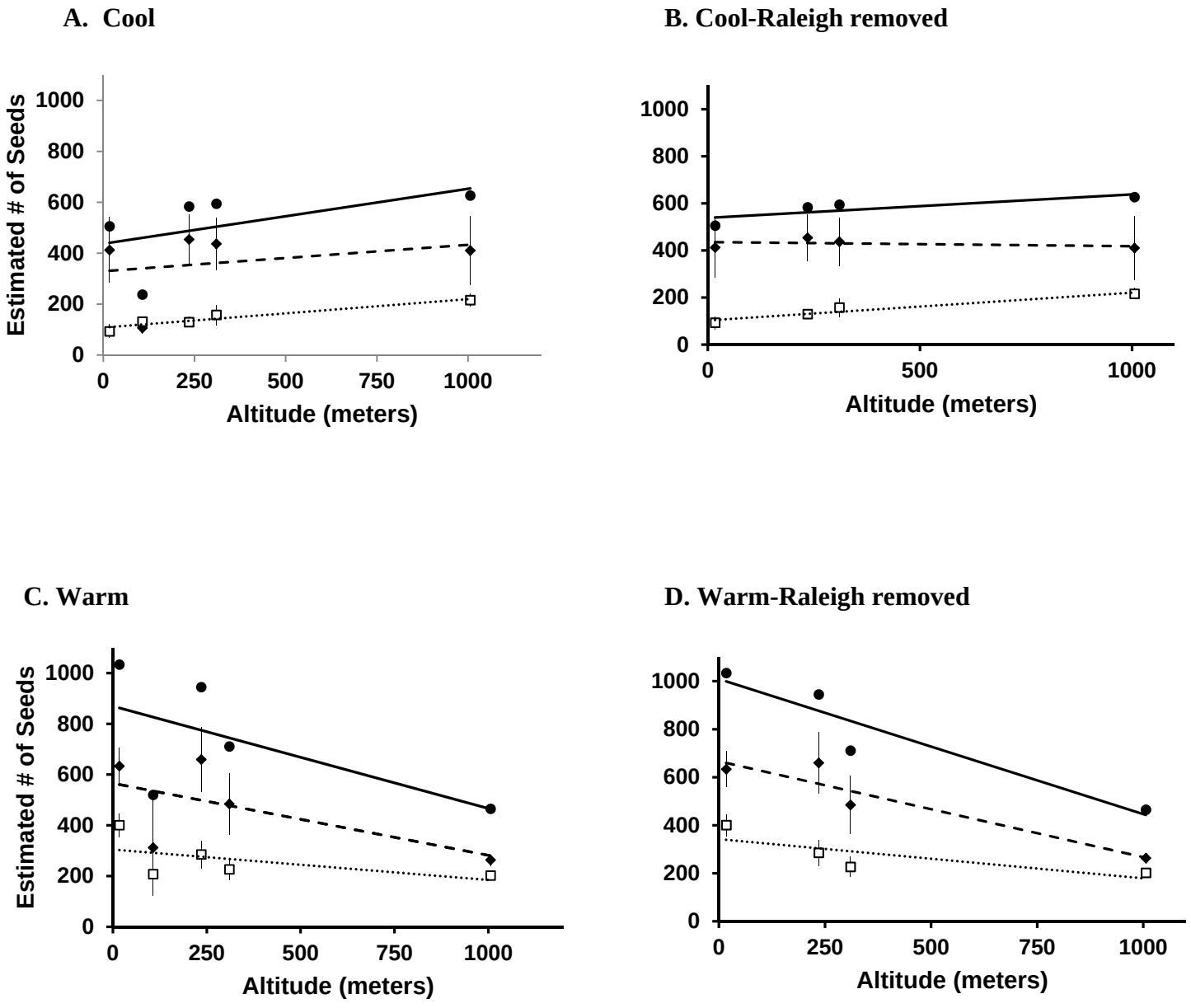


Figure 9. Probability of producing percent CL seeds at warm and cool temperatures.

Percent CL probability per *Microstegium vimineum* population at cool and warm temperatures based on logistic regression odds ratios.

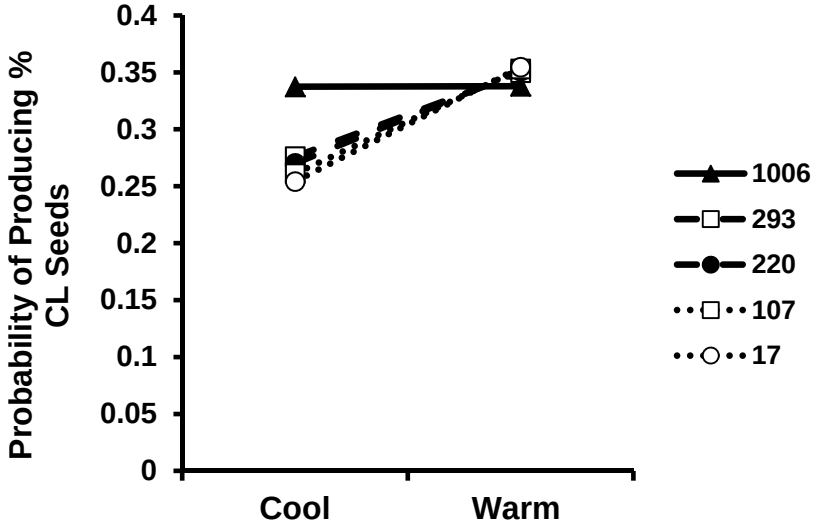


Figure 10. Percent germination for the field.

Mean percent germination per field plot for *Microstegium vimineum* for April 16th and April 29th, 2012. April 16th: open boxes, dotted line. April 29th: solid diamonds, solid line. Germination was adjusted for number of seeds sown per plot. Vertical bars equal ± 1 SE.

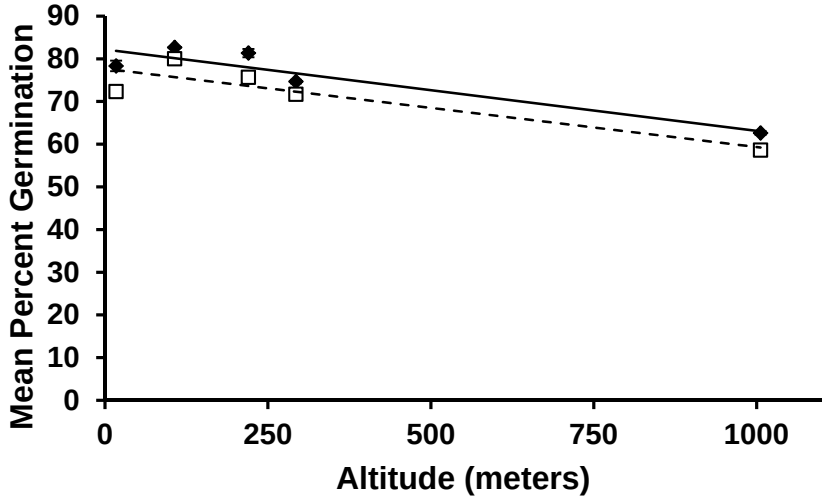


Figure 11. Number of seeds per plant for the field.

Number of *Microstegium vimineum* CH, CL, and total seeds produced per plant in field plots in Brown Summit, North Carolina. Parent plants were grown from seeds collected in 2011 and germinated on March 11, 2012. Vertical bars equal ± 1 SE. Total seeds: solid circle, solid line; CH seeds: solid diamond, dashed line; CL seeds: open square, dotted line.

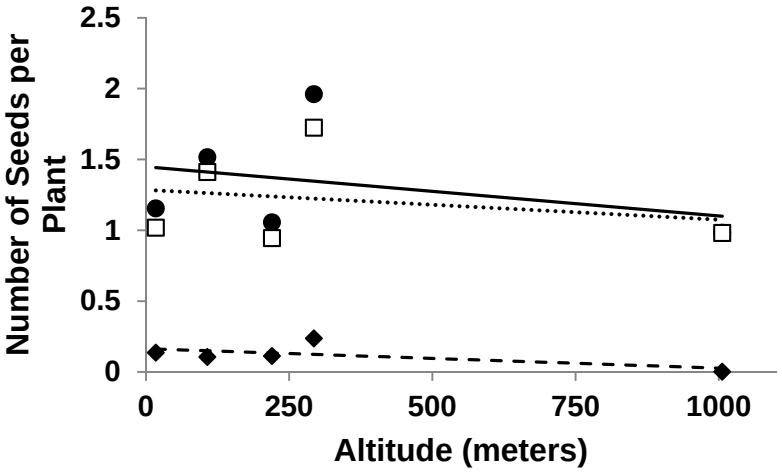
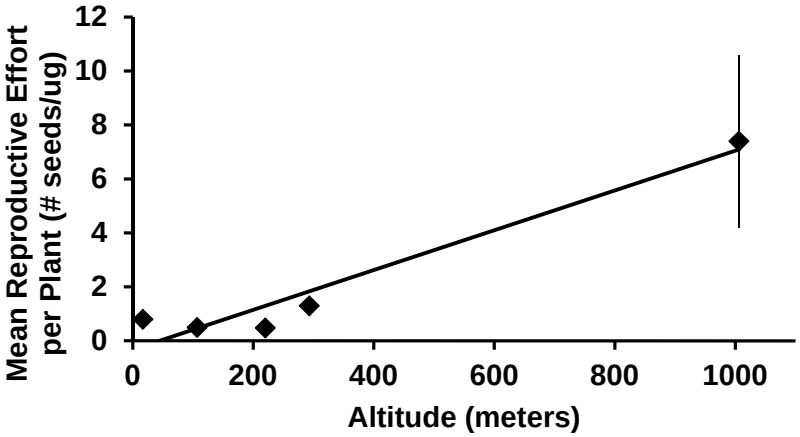


Figure 12. Reproductive effort for field plants.

Mean reproductive effort per plant for *Microstegium vimineum* for 2011 collection germinated and grown in field plots in Brown Summit, North Carolina. Vertical bars equal ± 1 SE.



APPENDIX C

SUPPLEMENTARY MATERIAL

Table C1. Germination plasticity values for experiments 1, 3, and 4.

Germination plasticity values (between warm and cool temperatures) for *Microstegium vimineum* at various altitudes from experiments 1, 3, and 4.

Altitude (meters)	October 2011	November 4, 2012	December 11, 2012
1006	-0.10127	------	------
836	------	-0.16608	-0.33392
568	------	0.00696	-0.02409
360	------	0.00696	0.23281
293	0.06895	-0.10636	-0.20614
250	------	-0.00738	-0.02387
220	0.07943	0.03125	------
107	0.0943	------	------
17	0.10263	------	------

Table C2. Germination results for individual dates and temperatures.

Altitude estimates and p-values for individual dates and temperatures for percent germination. Estimate values shown are not valid until they are multiplied by 100 and exponentiated (odds ratio). For example, the interpretation for October 2011-Warm chamber data: For every 100m increase in altitude, the odds of germination decreases by 17.3%.

Altitude Parameter	Estimate	Odds Ratio	P – value
October 2011 - Warm	-0.0019	0.827	0.0013
October 2011 – Cool	-0.0014	0.869	0.0131
November 4th - Warm	-0.0056	0.571	< 0.0001
November 4th - Cool	0.0020	1.221	0.3134
December11th - Warm	-0.0007	0.932	0.4170
December 11th - Cool	-0.0009	0.914	0.3293

Table C3. CL seed plasticity values for the October 2011 collection.

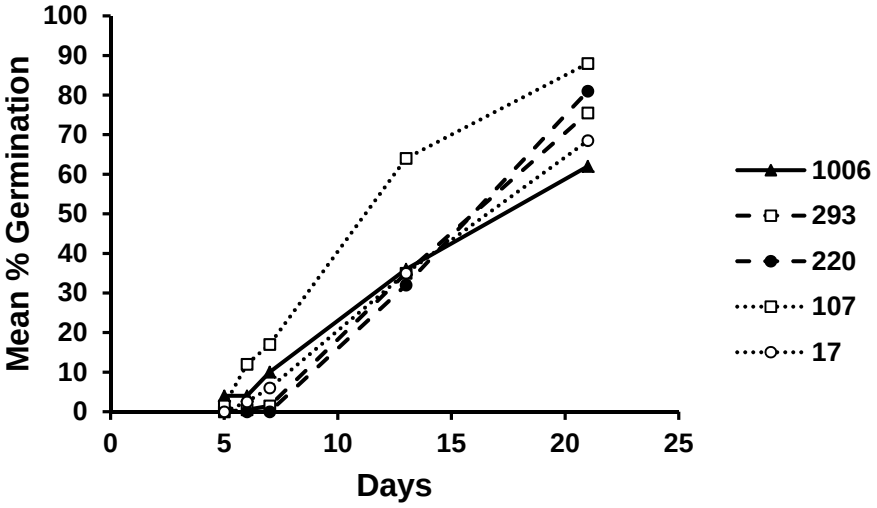
CL seed plasticity values (between warm and cool temperatures) for *Microstegium vimineum* at various altitudes. Reproductive traits were calculated for only the October 2011 collection date.

Altitude (meters)	October 2011
1006	-14.2887
293	68.7969
220	155.5681
107	76.3138
17	307.1814

Figure C1. Mean percent germination for stratified and not stratified seeds.

Mean percent germination per *Microstegium vimineum* populations sampled in 2011 for (A) stratified seeds and (B) unstratifed seeds. Stratification treatments were averaged between both warm and cool temperatures. Vertical bars equal ± 1 SE. Standard errors were so small that they are not visible in the figure. Populations are shown by altitude (meters above sea level).

A. Stratified



B. Not stratified

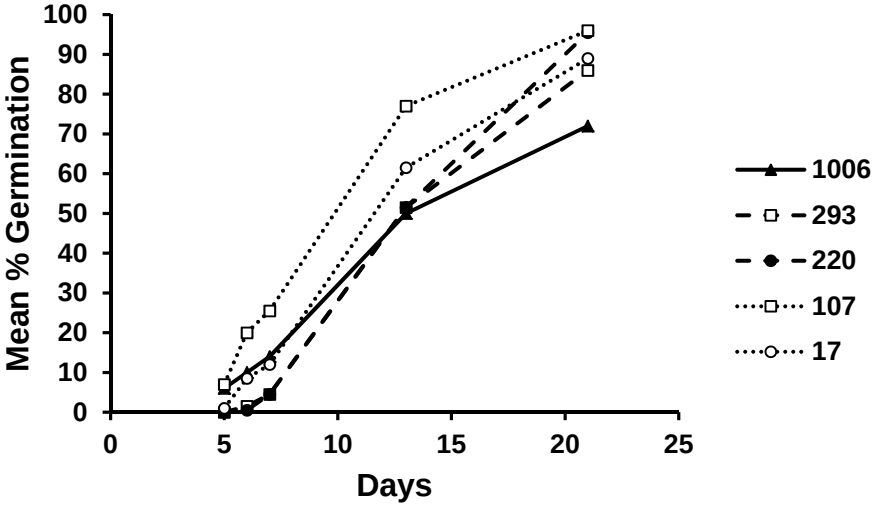


Figure C2. Mean days to flower per plant at warm and cool temperatures.

Mean days to flower per plant per population at cool and warm temperatures. Vertical bars equal ± 1 SE.

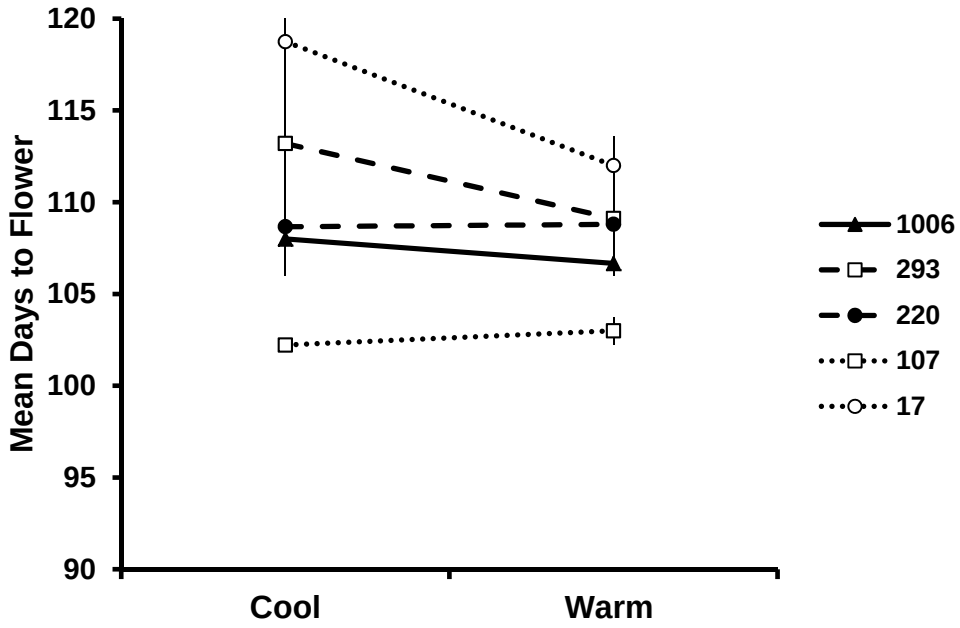
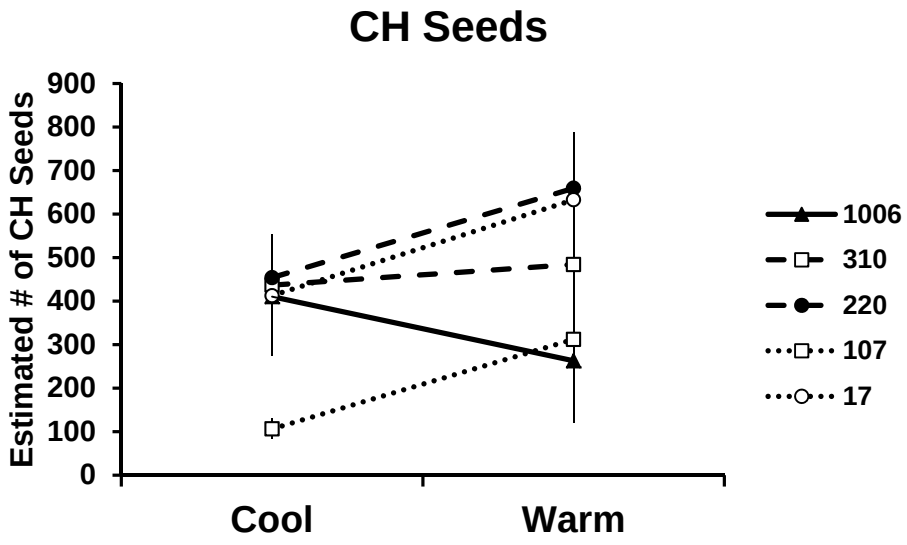


Figure C3. Estimated number of CH and CL seeds at warm and cool temperatures.

Estimated number of *M. vimineum* seeds produced per population at cool (C) and warm (W) temperatures. Parent plants were grown from seeds collected in 2011 and germinated in W and C temperature growth chambers on February 24, 2012. Seeds were separated into (A) CH seeds and (B) CL seeds. Vertical bars equal ± 1 SE.

A.



B.

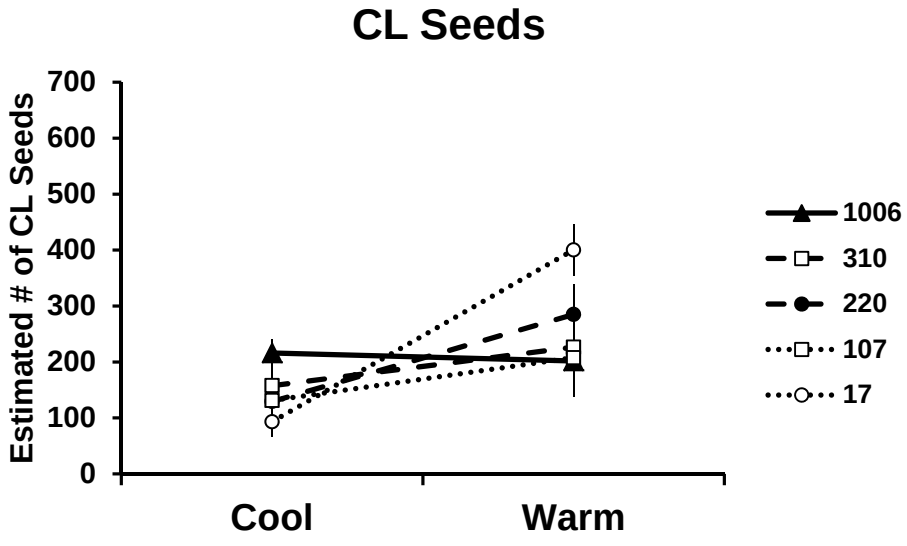
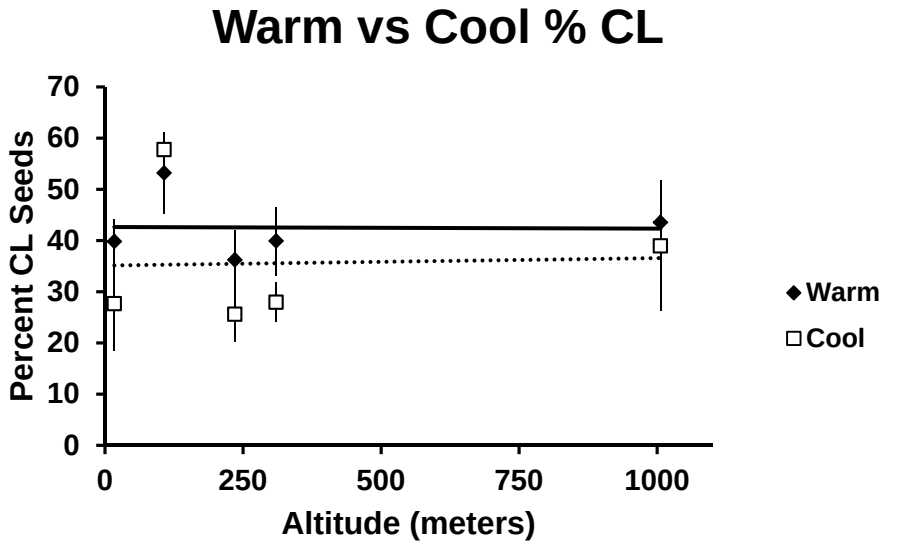


Figure C4. Percent CL seeds produced at warm and cool temperatures.

Percent *Microstegium vimineum* CL seeds produced at warm and cool temperatures. Seeds produced came from the parent 2011 seed collection. (A) All populations are shown and (B) the Raleigh population is removed. Vertical bars equal ± 1 SE.

A.



B.

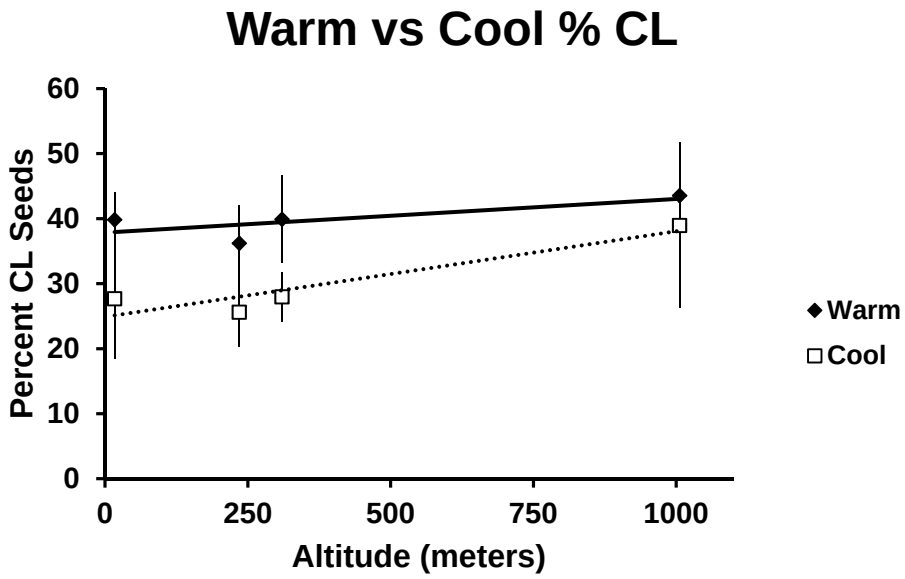


Figure C5. Reproductive effort at warm and cool temperatures.

Mean reproductive effort in micrograms for *Microstegium vimineum.* Seeds were sampled in 2011 and germinated/grown in warm and cool temperature growth chambers on February 24, 2012. Vertical bars equal ± 1 SE.