Solidago altissima genotypic variation among and within populations affects
Corythucha marmorata herbivory under various drought conditions

A THESIS
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Lilhac Medina

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Timothy P. Craig

August 2018

Acknowledgements

Dedication

This thesis is dedicated to my mother who always supported my pursuit for a career in science.

Abstract

A variety of ecological and evolutionary factors contribute to the resistance of the tall goldenrod, *Solidago altissima*, to herbivory by the chrysanthemum lace bug, *Corythucha marmorata*. These factors include genetic variation due to geographic origin, genetic variation within a population, and drought stress. We established a common garden in Duluth, MN of tall goldenrod collected from several US and Japanese sites to analyze the effects of plant genotypic variation among and within geographic sites, and the effect of drought on the tall goldenrod-lace bug relationship. We found variation in lace bug performance on, and preference for, genotypes of different geographic origins and genotypes within populations. We also found that the interaction between genotype and drought changed the performance and preference of lace bugs on tall goldenrod.

Table of Contents

List of Figures

<u>Introduction</u>

Intraspecific genetic variation among plants affects their susceptibility to herbivorous insects (Wimp et al. 2010, Burkle et al. 2013). Plant species frequently extend across a large spatial scale, and adaptation to environmental heterogeneity produces genetic variation among plant populations and the herbivores found on them (Hakes and Cronin 2011, Stein et al. 2014, Deans et al. 2016). Moreover, local and temporal variation in abiotic and biotic factors can also produce genetic variation within a plant population (Turner et al. 2015, Burghardt 2016). Most studies have focused on genetic variation in susceptibility to herbivores either within plant populations (Barrios-Garcia et al. 2016, Burghardt 2016) or among populations (Utsumi et al. 2011, Grinnan et al. 2013, Turner et al. 2015), and little is known about the relative magnitude of variation at these levels.

The maintenance of genetic variation determines plant species' evolutionary potential to respond to environmental change, such as anthropogenic-induced climate change or invasion of novel environments (Reusch et al. 2005, Lanzer et al. 2017). So, it is important to consider both the genetic variation among and within populations when examining populations under different spatial and temporal conditions.

Plants interact with many herbivore species, and this interaction can strongly affect a plant population's genetic composition (Maddox and Cappuccino 1986, Maddox and Root 1987). Herbivores can strongly impact plant fitness, so plants develop tolerance and resistance strategies as defensive responses. Plant resistance to herbivores can either

be the result of genetics, environment, or a genetic and environment interaction, and will vary among individual plants (Maddox and Cappuccino 1986, Burghardt 2016).

Different intraspecific plant traits have differing effects on herbivore fitness, and this leads to herbivore preference for certain plant genotypes, such as those with more resources or with lower resistance (Hwang et al. 2008, Franks et al. 2018). Plant genotypes will differ among populations as they adapt to different abiotic and biotic factors, resulting in differences in plant-herbivore coevolution across a spatial scale (Craig et al.1999, 2007; Stein et al. 2014). For example, as plant species move into novel environments and are released from their natural enemies, they may be selected to allocate more resources to growth and reproduction and less to defense, and this may result in their loss of herbivore resistance over time (Blossey and Notzold 2010, Lind et al. 2013, Sakata et al. 2013b, Franks et al. 2018). The loss of resistance will negatively impact these populations if they are reintroduced to their natural enemies.

The interaction of plant genotype and environment frequently determines a plant's resistance to herbivory (Deans et al. 2016). Drought is a common environmental stress that can significantly impact a plant's resistance to herbivory (Helmberger et al. 2016, Shibel and Heard 2016). Drought intensity can affect the herbivory on a plant, but plant genotypes vary in their response to drought stress (Burghardt 2016). Hence, population densities of herbivores will differ among individual plants as some genotypes will be more resistant under drought stress than others (Sumerford et al. 2000, Gaylord et al. 2013, Grinnan et al. 2013). Herbivore preference for specific plant genotypes will differ under different drought conditions. Senescence feeders, specifically, would prefer

drought-stressed plants, since these herbivores can only consume soluble nitrogen which is mobilized when stressed leaves senesce (White 1993, Helmberger et al. 2016).

The tall goldenrod *Solidago altissima* (Asteraceae) is a native herbaceous perennial plant widely distributed across North America. *S. altissima* can reproduce both sexually and asexually and it is a host plant to over 100 species of insects (Maddox and Root 1990). The chrysanthemum lace bug *Corythucha marmorata* (Tingidae), is a native herbivore of *S. altissima*. *C. marmorata* is a generalist senescence feeder on several species of Asteraceae throughout North America. There are two generations of *C. marmorata* each year in its northern USA range beginning in spring when the adults emerge from diapause, to late summer when the second generation of adults overwinter in the soil (Cappuccino and Root 1992). It has multiple overlapping generations in the southern USA range and in Japan (T. Craig personal observation)

Both *S. altissima* and *C. marmorata* are invasive species in several countries, including Japan. *S. altissima* was introduced in Japan in the early 1900s. Japanese goldenrod were free from lace bug herbivory until lace bugs were also introduced in the port of Kobe in 2001. The Japanese goldenrod populations lacked defenses against lace bugs leading to high lace bug densities (Sakata et al. 2013b). However, the goldenrod populations within Japan that have experienced high herbivory have since undergone rapid evolution and developed herbivore resistance, while populations with low or no herbivory are less resistant (Sakata et al. 2014). This provides an opportunity to compare goldenrod-lace bug interactions across the native and invasive range and compare populations with different lengths of lace bug exposure.

3

I established a common garden in Duluth, MN of *S. altissima* collected from several USA and Japanese populations to examine goldenrod resistance to lace bugs. I looked at two levels of spatial variation: within-population, genotypes collected within a few hundred meters, and among-populations, genotypes collected many kilometers apart. I used *C. marmorata* performance and preference as a measure of the genotypic variation among *S. altissima.* The variation could be due to either plant resistance in response to herbivory, plant response to other abiotic or biotic factors, or an interaction of those responses (Cronin et al. 2001).

First, I tested the hypothesis that there was greater goldenrod genotypic variation in resistance to lace bug herbivory among populations than within populations. Populations farther away from each other may experience larger differences in abiotic and biotic factors (Root N. 1992, Yang et al. 2015), so I predicted that differences in plant resistance to lace bug herbivory among populations would increase with distance. I expected populations close to each other would have similar resistance to herbivory while those farther apart would have dissimilar resistance responses (Heimonen et al. 2017).

Secondly, I tested the hypothesis that plants exposed to lace bug herbivory for longer periods of time would be more resistant than those exposed for shorter time periods. Thus, populations in the USA, their native range, may have higher levels of resistance than in Japan, the introduced range, where they either have not, or only recently, been exposed to herbivory (Sakata et al. 2017). I also assumed that Duluth lace bugs would perform better on, and prefer, Duluth plant genotypes and/or genotypes from populations nearby that have similar environments. I expected lace bugs to perform

poorly and to avoid plant genotypes from the southern USA and Japanese populations that experience high lace bug densities because they are likely to have evolved stronger resistant to herbivory than northern USA populations where lace bug densities are low. However, the Japanese Hokkaido population have been free from herbivory for over a century, and so I expected Hokkaido genotypes to be highly susceptible to herbivory.

I also tested the hypotheses that drought would influence *C. marmorata* performance and preference, and that drought would interact with *S. altissima* genotypic to influence variation in insect resistance. Lace bugs perform better on drought-stressed plants (Helmberger et al. 2016), but performance may differ among plant genotypes. I expected lace bug performance and preference to be dependent on both the plant genotype and the amount of drought stress the plant is experiencing.

Finally, I hypothesized that the leaf nitrogen content of a plant would influence the performance and preference of lace bugs. Nitrogen content has been previously demonstrated to have an importance in arthropod fitness (White 1993, Cahenzli and Erhardt 2012, Massad et al. 2012). In addition, the availability of soluble nitrogen has been shown to be strongly influenced by drought (White 1993, Helmberger et al. 2016). To test this hypothesis, I measured total leaf nitrogen content among and within plant populations and under different drought conditions.

Methods

Field Site

I established a common garden in Duluth, Minnesota USA at the University of Minnesota Duluth Research and Field Studies Center. All experimental plots of goldenrod were set up under a rainout shelter for the duration of the study, to control the amount of water the plants received. The rainout shelters used in the experiments have metal and wooden frames and are covered by greenhouse grade Plexiglas with 97% light transmission.

Goldenrod Replicates

S. altissima were collected from geographic sites across the USA and Japan to represent a range of environmental conditions, length of time since being colonized by lace bugs, and current lace bug densities (Fig. 1). Genotypes at each geographic site were randomly collected by cutting rhizomes from physically connected plants, and then were propagated in Duluth, Minnesota USA at the University of Minnesota Duluth Research and Field Studies Center. Rhizomes were collected from plants that were at least 5 meters apart to assure that they were different genotypes. Plants were collected at least six years prior to the experiment and restarted from rhizomes every second year so that maternal effects were minimized.

Genotypes for experiments were replicated by cutting sections of physically connected rhizomes and planting them into 3.8 L pots in the fall and spring and grown in

the spring and summer. All replicates were watered sufficiently until the beginning of the experiments.

No-Choice and Choice Experiments

I assigned plant replicates to either a no-choice or choice experiment. The no-choice experiments measured lace bug performance and plant replicates were individually caged with 1 x 0.18 m steel poultry wire cages and Agribon™ fabric. Lace bugs were collected from non-experimental goldenrod, sorted by sex, and added to caged plants. The choice experiments measured lace bug preference for plant genotypes, and they were left uncaged to allow lace bugs to colonize them naturally.

Insect and Plant Surveys

In every survey I recorded the number of lace bug adults, nymphs and eggs on each plant to measure either or survival (no-choice experiment) or preference (choice experiment). I collected and weighed all lace bug adults individually from the no-choice experiments at the end of the season.

Plant height, flowering, and the number of leaves on each plant were also recorded. Leaves were categorized as senesced (>50% brown), senescing (>50% yellow or yellow and brown, but <50% brown), or green leaves (>50% green) to measure plant stress. The 50 % yellow criteria previously have been established as standard for determining senescence (Lim et al. 2007).

Plant Genotypic Variation Experiment Setup

To measure plant genotypic variation due to environmental heterogeneity and lace bug exposure time, one no-choice experiment and two choice experiments were conducted. Leaf samples and lace bugs adults were collected from the no-choice experiment to analyze the effect of leaf nitrogen content on lace bug performance and preference.

2017 Lace Bug No-Choice Experiment

I measured lace bug performance and lace bug and goldenrod nitrogen on ten populations: Duluth, MN; Morris, MN; Faribault, MN; Iowa-Missouri border; Pony Express, MO; Council Grove, KS; El Dorado, KS; Hokkaido Japan; Yamagata Japan; and Kyoto Japan. At the time of their collection Kyoto plants had been exposed to lace bugs for 14 years, Yamagata plants for 2 years, and Hokkaido plants had not been exposed. To maximize the genetic diversity from each population, as many genotypes as were available from within each population were used. Genotypes were not replicated and the number of genotypes from each population varied. Each pot was individually caged, and two adult lace bugs were added to every cage. Only female lace bugs were used in this experiment, since very few males were found, and females were already ovipositing eggs. After a week of oviposition, the lace bugs were removed from the cages.

I conducted surveys on June 23, 2017, July 20, 2017, and August 16, 2017. I collected green and senesced leaf samples from each plant for nitrogen analysis during

each survey. I also collected lace bug adults from each plant after the August 16 survey to measure bug weight and nitrogen.

2016 Lace Bug Choice Experiment

I examined lace bug preference for plant genotypes among and within plant populations in the summer of 2016 on nine plant populations: Hibbing, MN; Duluth, MN; Morris, MN; Faribault, MN; Iowa-Missouri border; Pony Express, MO; Council Grove, KS; El Dorado, KS; and Hokkaido Japan. I chose these populations based on previous work by Sakata et al. (2017) who showed that lace bug densities increased from north to south in Japan and the USA, however, lace bug invasion has not reached Hokkaido. Three genotypes from each site were randomly selected and 10 replicates for each genotype were made by initiating new plants from planting one rhizome in a 3.8 L pot (*n* = 270). The replicates were randomly assigned to positions in a single plot. Plants were surveyed on August 19, 2016.

2017 Lace Bug Choice Experiment

I examined lace bug preference for goldenrod genotypes in the summer of 2017 on eight plant populations: Duluth, MN; Morris, MN; Faribault, MN; Iowa-Missouri border; Pony Express, MO; Council Grove, KS; El Dorado, KS; and Saga, Japan. Not enough Hibbing, MN and Hokkaido Japan replicates grew during this season and so these sites were excluded from this experiment. Saga is a site where plants had been exposed to lace bug herbivory for only two years prior to their collection (Tokuda pers. comm.).

9

Four genotypes from each of the sites were selected and 4 replicates for each genotype were made from rhizomes. The replicates were randomly assigned to positions in one plot ($n = 128$). Three surveys were conducted on June 22, 2017, July 19, 2017, and August 15, 2017.

Drought and Within Population Genotypic Variation Experiment Setup

To examine how lace bug performance and preference on plant genotypes is influenced by drought, four plots were made. Two plots measured performance and preference of lace bugs on different plant genotype by drought treatments. Two plots measured the effect of drought on leaf and lace bug nitrogen content.

Drought Treatments

Water availability to plants was controlled by keeping the soil moisture level in the following ranges: low drought treatment ≥8% soil moisture, moderate drought treatment 5-7.9% soil moisture, and high drought treatment 1-4.9%.

I measured soil moisture in each pot three times a week using a handheld digital soil moisture meter (OMEGA Engineering, Stamford, CT, USA), and watered plants based on the drought treatment designated for that replicate and the current soil moisture level. For low drought plants, 50 mL of water was added if the soil moisture was 8-10%, 100 mL was added if the moisture was 5-7.9%, and 150mL was added if it was <5%. For moderate drought plants 50 mL of water was added if the soil moisture was 5-7.9%, and 100mL was added if it was <5%. For high drought plants 50 mL was added if moisture

was 1-4.9%, and 100 mL was added if <1%. If soil moisture was above the designated range, the plant was not watered and if the plant was too stressed, i.e. drooping, stem was senescing, additional water was added.

2016 Lace Bug No-Choice and Choice Experiment

I measured the effect of plant genotype and drought treatment on lace bug performance and preference on 10 genotypes from the Carlos Avery population. Each genotype had six replicates in each of the three drought treatments. The two treatments in this experiment were a no-choice treatment ($n = 90$) and a choice treatment ($n = 90$). Each plot consisted of nine replicates of each of the ten genotypes, with three replicates in each drought treatment (Fig. 2). Plants were randomly assigned into plots; however, both the no-choice and choice treatments had an identical layout (Fig. 3). One survey was conducted on August 20, 2016.

2016 Drought Nitrogen Experiments

I measured nitrogen concentration in lace bugs and goldenrod under three drought conditions. I created 36 replicates from rhizomes from each of two goldenrod genotypes from the Carlos Avery site (Fig. 4). The replicates were randomly assigned into either a no-lace-bug control or a lace bug treatment. The no-lace bug treatment ($n = 36$) was placed in a single cage to prevent herbivory (Fig. 5a). The lace bug treatment ($n = 36$) had individually caged replicates with a female and male lace bug added to each cage (Fig. 5b).

11

Goldenrod leaves were collected for nitrogen sampling three times: on August 10, 2016, September 4, 2016, and September 22, 2016. After leaves were collected, the plant sampled was discarded so that no replicate was sampled twice to prevent damage-induced defense responses from the plants.

Lace bug adults were collected for nitrogen sampling on September 22. Two replicates in each plant genotype by drought treatment were sampled for nitrogen during each of the three surveys and 1-2g of wet weight leaves were collected for each plant.

Nitrogen Analysis

Leaf and lace bug samples collected for nitrogen analysis were dried at 65°C for 2-3 days. 8-10 mg of each leaf sample was weighed out and run through a Thermo Scientific FLASH EA 1112 Elemental Analyzer to determine total nitrogen concentration. 5-15 lace bugs were assorted into composites to form 1mg of sample to run through the analyzer.

Statistical Analysis

I used R version 3.3.1 to analyze all the data.

Plants that grew multiple stems in one pot were considered one replicate, and the data collected for all stems in a pot were combined. Preliminary analysis determined that plant height, number of leaf nodes, and the number of offshoots from the main stem did not significantly influence the goldenrod-lace bug interaction, and so these traits were excluded from further analysis. We used the proportion of senesced, senescing, and green

12

leaves and plant flowering as traits in the analysis. Although the proportion of green leaves is inversely proportional to senescing and senesced leaves, I analyzed all three categories since lace bugs are senescence feeders, and there is likely a distinction in the amount of resources available to lace bugs among these leaf categories.

Lace bug abundance was determined by dividing the total number of lace bugs on each plant replicate by the stem height.

Boxplots, Bartlett tests, and qq plots were used to check assumptions for the statistical models. Lace bug abundance for each life stage was fourth-root transformed to homogenize variances. Tukey's Honest Significant Difference tests were used when ANOVA models showed a significant difference result.

2017 Plant Genotypic Variation: Lace Bug No-Choice Experiment

A one-way ANOVA was used to analyze differences in lace bug survival and adult weight and nitrogen concentration among plant genotypes in the 2017 no-choice experiment.

A repeated-measures ANOVA was used to analyze variation in the leaf nitrogen concentration with plant population and date surveyed as factors in the 2017 no-choice plot experiment. One-way ANOVAs were also used to analyze leaf nitrogen among plant populations for each survey date individually.

2016 and 2017 Plant Genotypic Variation: Lace Bug Choice Experiments

Nested ANOVAs were used to analyze variation in the abundance of lace bugs among plant genotypes in the 2016 and 2017 choice plots, with genotypes nested within plant populations. One-way ANOVAs were also used to determine which groups were significantly different via Tukey's HSD test.

Correlations and simple linear regression were used to determine the relationship between plant traits and lace bug performance and preference. ANCOVA models were used to include among and within population genotypes as a factor in the regression models.

2016 Drought and Within Population Genotypic Variation: Lace Bug No-Choice and Choice Experiments

Two-Factor ANOVAs were used to analyze variation the abundance of lace bugs among plant genotypes and drought treatments in the no-choice and choice plots. Two-Factor ANOVAs were also used to analyze the weight of lace bug adults in the no-choice plot. If there was no significant interaction between plant genotype and drought, the interaction term was removed, and the Two-Factor ANOVA was rerun.

Correlations and simple linear regression were used to determine the relationship among plant traits, soil moisture, and lace bug performance and preference. ANCOVA models were used to include plant genotypes as a factor in the regression models.

2016 Drought Nitrogen Experiments

Two-Factor ANOVAs analyzed variation in the leaf nitrogen concentration among plant genotypes and drought treatments for each survey date in the no-lace bug and lace bug nitrogen plots.

Results

Plant Genotypic Variation Experiment

2017 Lace Bug No-Choice Experiment

Lace bug nymph survival significantly varied among plant populations in July 2017 (One-Way ANOVA, $F_{8,63} = 3.29$, $P < 0.0033$; Fig. 6a), but not in August. Adult survival (One-Way ANOVA, $F_{8,56} = 2.63$, $P < 0.016$; Fig. 6b) varied among populations only in the August 2017 survey. The Tukey's HSD test revealed that there were significantly more nymphs on El Dorado genotypes than on other plant populations, but the test did not detect any significant differences among populations in adult numbers. There was no significant variation in lace bug egg numbers among populations throughout the sampling season.

Egg abundance was positively related to the proportion of senescing leaves in June ($y = 1 + 1.07x$, $F_{1,76} = 4.17$, $P < 0.044$, $r^2 = 0.05$). In August, egg abundance was negatively associated with the proportion of green leaves ($y = 1.44 - 0.66x$, $F_{1,63} = 7.34$, $P < 0.0087$, $r^2 = 0.10$), while positively associated with senesced leaves ($y = 0.78+0.66x$, $F_{1,63} = 7.35$, $P < 0.0086$, $r^2 = 0.10$). There were no other significant relationships found between lace bugs and the proportion of senesced, senescing and green leaves in the no-choice treatment.

Lace bug adults weighed more on Morris, MN; Pony Express, MO; Yamagata, Japan; and Kyoto Japan than the other plant populations (One-Way ANOVA $F_{8,252} = 11.65$, $P < 0.0001$; Fig. 7).

Duluth, MN; Morris, MN; and Kyoto Japan were the only populations to have

flowering replicates in the 2017 no-choice treatment.

2016 and 2017 Lace Bug Choice Experiment

The abundance of eggs ($F_{18,189} = 2.62$, $P < 0.0006$; Fig. 8a) and nymphs ($F_{18,190} = 2.08$, $P < 0.008$; Fig. 8b) varied significantly among genotypes within plant populations in the 2016 experiment when analyzed with a nested ANOVA. Plant origin did not affect either the number of lace bug eggs nor nymph abundance. Lace bug adults showed no preference for genotypes either among or within populations.

There was no difference in the number of lace bugs among or within populations in the 2017 experiment in June or July. In August 2017, the number of eggs ($F_{24,93}=1.8$, $P < 0.02$; Fig. 9a) and adults ($F_{24,93} = 1.66$, $P < 0.05$; Fig. 9b) varied significantly within population genotypes, but not among populations. Nymph abundance did not vary among or within populations in any sample period.

The abundance of eggs ($y = 0.92\text{-}0.65x$, $F_{1,213} = 6.46$, $P < 0.012$, $r^2 = 0.05$) and nymphs ($y = 0.67\text{-}0.49x$, $F_{1,213} = 5.65$, $P < 0.019$, $r^2 = 0.04$) on a plant decreased as the proportion of green leaves increased in August 2017. There were no other significant relationships found between lace bugs and the proportion of senesced, senescing and green leaves in the 2016 or 2017 choice plots.

The abundance of nymphs was significantly affected by the interaction of among-population genotypes and green leaf proportion ($F_{7,109} = 3.26$, $P < 0.0035$), and the interaction of within-population genotypes and senesced leaf proportion ($F_{7,109} =3.75$, $P <$

0.0011) in July. The ANCOVAs showed the factors and covariates not to be significant individually, and there were no other significant interactions in the 2016 and 2017 choice experiments.

Hibbing, MN; Duluth, MN; and Morris, MN were the only populations to have flowering replicates in the 2016 choice treatment. Duluth, MN; Morris, MN; and Pony Express, MO had flowering replicates in the 2017 choice plot.

2017 Leaf and Lace Bug Nitrogen Content Analysis

Egg abundance was negatively associated with green leaf nitrogen in the August survey ($y = 1.36-0.062x$, $F_{1,56} = 11.96$, $P < 0.001$, $r^2 = 0.18$). There was no relationship between green leaf nitrogen content and nymph and adult survival.

The ANCOVAs showed that an interaction of plant population and green leaf nitrogen affected egg abundance in July ($F_{8,52} = 2.306$, $P < 0.0338$), but there were no other significant results.

Total green leaf nitrogen concentration varied significantly among populations ($F_{8,48} = 2.45$, $P < 0.026$), survey dates ($F_{2,96} = 9.6$, $P < 0.0001$) and there was a significant interaction among plant populations and survey dates ($F_{16,96} = 2.5$ $P < 0.0031$) according to a repeated-measures ANOVA. One-Way ANOVAs for each survey showed green leaf nitrogen varied among plant populations in the June ($F_{8,68} = 5.59$, $P < 0.0001$) and July ($F_{8,62} = 2.84$, $P < 0.0094$) surveys (Fig. 10). The Hokkaido Japan plant genotypes had higher green leaf nitrogen content than the other populations in June, and the Kyoto Japan genotypes had higher nitrogen content in July.

Total lace bug nitrogen did not vary significantly among plant populations.

Drought and Within-Population Genotypic Variation Experiment

2016 Drought No-Choice Experiment

The number of lace bug adults, nymphs and eggs did not differ among plant genotypes or drought in a Two-Factor ANOVA with the interaction included. When the Two-Factor ANOVA was rerun without the interaction term, adult survival significantly differed among drought treatments ($F_{2,63} = 3.14$, $P < 0.05$) but not among genotypes. The Tukey HSD revealed adult survival was higher at high drought treatments than at low drought treatments.

Lace bug adult survival was negatively associated with the proportion of green leaves ($y = 0.51\text{-}0.29x$, $F_{1,73} = 4.91$, $P < 0.03$, $r^2 = 0.06$), but positively associated with senesced leaves ($y = 0.23\text{+}0.28x$, $F_{1,73} = 4.37$, $P < 0.04$, $r^2 = 0.06$). There was no other significant relationship found between lace bugs and the proportion of senesced, senescing and green leaves in the no-choice treatment.

Plant genotype ($F_{8,237} = 14.24$, $P < 0.0001$), drought treatment ($F_{2,237} = 9.43$, $P < 0.0001$) and the interaction of genotype and drought ($F_{13,237} = 3.4$, $P < 0.0001$) significantly affected lace bug adult weight (Fig. 11). Overall, lace bugs were significantly smaller on genotype G, but within each genotype the lace bug size varied among the three drought treatments.

Lace bug adult weight was negatively associated with the proportion of green leaves ($y = 0.19\text{-}0.035x$, $F_{1,259} = 4.025$, $P < 0.046$, $r^2 = 0.02$) and senescing ($y = 0.18\text{-}$

$0.37x$, $F_{1,247} = 18.95$, $P < 0.0001$, $r^2 = 0.07$), but positively associated with senesced leaves ($y = 0.16+0.043x$, $F_{1,259} = 7.33$, $P < 0.0073$, $r^2 = 0.02$). There was no significant relationship found between lace bugs and percent soil moisture.

The interaction of genotype and soil moisture (ANCOVA $F_{8,243} = 6.74$, $P < 0.0001$), significantly affected lace bug weight. Genotype also interacted with the proportion of green (ANCOVA $F_{8,243} = 3.84$, $P < 0.0003$), senescing (ANCOVA $F_{7,232} = 3.97$, $P < 0.0004$), and senesced leaves (ANCOVA $F_{8,243} = 3.8$, $P < 0.0003$), to influence lace bug weights.

The number of flowering plants differed among genotypes but not among drought treatments in the no-choice treatment. Within the flowering genotypes, drought showed an effect on the number of flowering with many genotypes only flowering in low or moderate drought. Genotype, drought treatment, and the interaction of genotype and drought did not significantly affect plant flowering in the choice plot.

The only plants that flowered in the lace bug nitrogen treatment were in the low-drought treatment. There was no difference in flowering among genotypes and there was no interaction of genotype and drought. Similarly, there was no variation in flowering in the lace bug free nitrogen plot on any level.

2016 Drought Choice Experiment

The number of nymphs varied significantly among plant genotypes (Two-Factor ANOVA $F_{9,71} = 2.007$, $P < 0.051$), but not drought treatment when the interaction term was removed. Lace bug adults did not show a preference for plant genotype or drought

treatments. However, the interaction of plant genotype and drought treatment had a significant impact on lace bug adult preference ($F_{18,53} = 1.77$, $P < 0.053$ Two-Factor ANOVA; Fig. 12). Oviposition preference was not affected by genotype or drought.

There was no significant relationship between lace bug preference and the proportion of senesced, senescing and green leaves using simple regression models.

The interaction of drought treatment and proportion of senescing leaves affected egg abundance (ANCOVA $F_{2,77} = 3.75$, $P < 0.028$). The interaction of plant genotype and proportion of green (ANCOVA $F_{9,63} = 2.31$, $P < 0.026$) and senesced leaves (ANCOVA $F_{9,63} = 2.034$, $P < 0.049$) affected adult abundance. The factors and covariates were not significant individually, and there were no other significant interactions in the choice experiment.

Genotype, drought treatment, and the interaction of genotype and drought did not affect plant flowering in the choice plot.

2016 Drought Nitrogen Analysis

In the lace bug-free treatment, green leaf nitrogen (Two-Factor ANOVA $F_{2,20} = 4.1$, $P < 0.032$, Fig. 13) and senescing leaf nitrogen (Two-Factor ANOVA $F_{2,13} = 4.66$, $P < 0.03$, Fig. 14) increased with drought. In the lace bug treatment, green leaf nitrogen varied among plant genotypes in the September 4th survey (Two-Factor ANOVA $F_{1,10} = 15.6$, $P < 0.0027$, Fig. 14), but not among drought treatments.

The nitrogen concentration of lace bug adults did not differ among plant genotypes or drought treatments, but it was significantly lower in females than males (One-Way ANOVA $F_{1,9} = 13.6$, $P < 0.005$).

The only plants that flowered in the lace bug nitrogen treatment were in the low-drought treatment. There was no difference in flowering among genotypes and there was no interaction of genotype and drought. Similarly, there was no variation in flowering in the lace bug free nitrogen plot on any level.

Discussion

Plant genotypic variation among and within populations, and its interaction with environmental variation, affected lace bug performance and preference. Plant genotypes varied in lace bug resistance, and how drought altered this resistance varied among genotypes. The following hypotheses, or a combination of them, could explain these patterns. First, genotypic differences in goldenrod resistance could be the result of plant adaptation to geographic and temporal environmental heterogeneity (Hakes and Cronin 2011). Second, plants could have evolved genotypic differences in resistance due to their history of herbivory by lace bugs (Sakata 2014). Third, plants adapt to stress through the leaf senescence and reallocation of nitrogen to other areas of the plant (White 1993). This could influence lace bug performance and preference because lace bugs are senescence feeders and rely on the availability of soluble nitrogen (Helmberger et al. 2016). Fourth, the availability of soluble leaf nitrogen may vary among and within plant populations, which could influence lace bug performance and preference. The overall resistance of a plant could be the result of the interaction of all these factors. The mechanisms underlying this variation in resistance could be physical or chemical defenses, nitrogen availability, or some combination of the two.

Geographic and Temporal Environment Adaptation

Spatiotemporal heterogeneity may result in strong variation in resistance among plant populations. Geographic variation in the herbivore community (Sakata et al. 2017), and environmental factors other than growing season length, such as drought frequency,

influence a population's resistance to herbivory (Johnson and Agrawal 2005, Heimonen et al. 2015). Plant defenses are costly, so plants may invest resources into other mechanisms if abiotic factors are a stronger impact on plant growth and reproduction (Blossey and Notzold 2010, Dostalek et al. 2016, Shibel and Heard 2016, Franks et al. 2018).

Strong genotypic variation in plant resistance was seen in lace bug performance and preference within all plant populations. Genotypic variation among populations affected the survival of lace bugs by the end of the sampling season (Fig 6). Except for Morris, MN, the local lace bugs weighed more on populations from the southern USA and from southern Japan (Fig 7), and these plant populations are from areas with a hotter climate and higher lace bug densities than Duluth, MN.

Climate adaptation could also explain the differences in flowering among plant populations. I found that only a few goldenrod populations from the north and south populations invested in sexual reproduction under the conditions in this study. Hibbing, MN; Duluth, MN; and Morris, MN populations are adapted to the colder climate and shorter season in Minnesota, and so it is not surprising that they flowered. However, Pony Express, MO and Kyoto Japan are adapted to hotter climates and a longer growing season, and so they should continue to invest in asexual production rather than switching to sexual reproduction at a time when this would be adaptive in Duluth.

Within-population plant genotypic variation, but not among-population genotypic variation, influenced lace bug egg and nymph abundance and lace bug adult preference (Fig. 8 and 9). This indicates that local environmental and temporal variation selects for

24

variation in resistance of the same magnitude or larger than that found at much larger

spatial scales. Variation among genotypes within populations may obscure the overall

trends seen among populations in previous studies that did not measure within plant

population genetic variation (Grinnan et al. 2013, Sakata et al. 2014, Burghardt 2016). I

examined only a few genotypes within each population, and perhaps including a larger

number of genotypes would show a clearer trend among populations. The results of this

study and previous studies (Craig et al. 2007, Craig and Itami 2011, Utsumi et al. 2011,

Sakata et al. 2014) may differ because lace bug performance and preference were

measured under different conditions. Sakata et al. (2014, 2017) used a reciprocal

transplant design, and only focused genetic variation among populations, but this study

was limited to one location and one population of lace bugs. To explain the differences in

these results, future studies should be conducted at multiple sites, and include within and

among site plant genotypic variation.

Plant Adaption to Lace Bug Exposure

A plant population's history of herbivory can influence resistance to herbivory

(Sakata et al. 2014). This hypothesis is consistent with some, but not all the variation in

performance and preference observed in this study. Traits of local populations of host

plants and herbivores are hypothesized to be more coevolved with each other than those

of plants and herbivores from different populations (Thompson 2005). Local herbivores

are often better adapted to local plant populations than to foreign populations, but the

reverse also occurs with host plants being more resistant to local herbivores than foreign

herbivores (Kawecki and Ebert 2004, Craig and Itami 2011). However, I did not support these hypotheses as Duluth lace bug populations showed no consistent pattern of better performance on, or stronger preference for, the local or foreign host plant populations (Fig. 7-9). Instead, there was large variation in lace bug performance and preference among local Duluth plant genotypes.

The history of exposure and intensity of lace bug herbivory did not have a consistent impact on a plant population's resistance to lace bugs. Sakata et al. (2014) found that the length of exposure time to lace bug herbivory was positively associated with lace bug resistance in Japan. They found that plant populations free from lace bug herbivory for over 100 years were initially highly susceptible to herbivory, but rapidly evolved resistance that, in some cases, exceed that in the native USA range. Sakata et al. (2017) also found that the intensity of lace bug herbivory was higher in more southern latitudes with warmer temperatures and longer growing seasons. Thus, I predicted that the Kyoto plant population, with a longer exposure to lace bug herbivory than the Yamagata population, would be more resistant. I also predicted that all USA and Japanese plant populations would be more resistant to lace bugs than the Hokkaido population, which have not recently been exposed to herbivory. However, the results of this study did not consistently support any of these patterns (Fig. 7-9), possibly because the plant populations were grown under the same conditions in this common garden study, rather than the diverse conditions to which they have adapted.

Drought Influence on Lace Bug Performance and Preference

Plant responses to water stress influenced lace bug performance and preference, but the pattern of the response was influenced by plant genotype. Lace bugs are senescence feeders (Helmberger et al. 2016), and so they are more likely to prefer senescing over healthy plants and perform better on them (White 1993). I found that lace bug performance and preference increased with the proportion of senescing and senesced leaves, but that there was also an interaction of leaf senescence with plant genotype. The effect of increasing leaf senescence on lace bug performance and preference varied among and within populations. This implies that different plant genotypes with the same amount of leaf senescence will have different plant qualities for lace bugs. The phenotypic plasticity of plant resistance within a population could have evolved as a response to spatiotemporal heterogeneity.

Drought can increase plant senescence, and the availability of soluble nitrogen (White 1993). I found that genotype, drought, and the interaction of genotype and drought all significantly affected lace bug adult weight (Fig. 11), however drought was the only factor that affected lace bug survival. Overall, there was higher lace bug survival on high-drought stressed plants over low-drought plants, most likely because high-drought plants are more senesced, and therefore have more soluble nitrogen available to the lace bug (White 1993, Helmberger et al. 2016). Resistance to lace bugs as indicated by lace bug weight resulted from the interaction of drought treatment with plant genotype on weight. This was further supported by the pattern of lace bug preference. Lace bug adults did not prefer any genotype or drought treatment, but the interaction of genotype and drought influenced lace bug choice (Fig. 12). This interaction could be due to

27

differences in plant response to drought among genotypes. Some genotypes may be more resistance to leaf senescence in response to drought, or some genotypes may have an induced chemical defense against herbivory that is only induced on plants experiencing water stress (Grinnan et al. 2013).

Leaf Nitrogen Content

Differences in lace bug performance and preference for plants could be due to an increase in soluble nitrogen (White 1993, Cahenzli and Erhardt 2012, Helmberger et al. 2016). Leaf nitrogen was generally higher in high-drought treatment plants than in low- or moderate-drought plants, but this trend was only seen in the absence of lace bugs (Fig. 13 and 14). In the presence of lace bugs, leaf nitrogen did not differ among drought treatments. This could be because lace bugs were consuming the available soluble leaf nitrogen until total leaf nitrogen concentration was equalized among drought treatments. Alternatively, herbivory could induce the plants to change their resource allocation rate. Furthermore, lace bug herbivory impacted goldenrod fitness. Moderate and high-drought stressed plants only flowered in the absence of lace bugs, and there was less total leaf nitrogen in the lace bug treated plants than in the lace bug-free plants (Fig 13, Fig 14).

Of the two genotypes sampled for nitrogen, lace bugs weighed less on the high-drought J genotype and did not survive on the high-drought S genotypes. Additionally, female lace bugs had less total nitrogen than males, suggesting that females may have invested this missing nitrogen in egg production. These results are consistent with the hypothesis that these genotypes changed their leaf nitrogen allocation rate in response to

drought in a way that influenced lace bug performance and preference. If drought reduced soluble nitrogen availability via decreasing the allocation rate, this could decrease lace bug performance. Conversely, if drought increased the rate of withdrawal of soluble nitrogen from the leaf, then this could reduce the time when a leaf was suitable for lace bug herbivory. Further studies on how the rate of nitrogen removal influences lace bug fitness are needed to test these hypotheses.

Plant populations with higher total nitrogen content could have more soluble nitrogen, which could increase lace bug performance and preference. Lace bug performance on USA populations, such as Faribault and Iowa-Missouri, are consistent with this hypothesis as their total green leaf nitrogen levels were constantly lower throughout the sampling season (Fig. 10) and lace bugs were smaller on those genotypes (Fig 7). Moreover, lace bugs performed well on Yamagata and Kyoto population genotypes, which had high total green leaf nitrogen content.

Lace bugs could also be influenced by variation in the pattern of nitrogen allocation within plants among populations. The southernmost USA populations, Pony Express and El Dorado, had a very different seasonal strategy of green leaf nitrogen allocation than the other USA populations. Green leaf nitrogen decreased in these plant populations midseason (Fig 10). Different habitats will favor different phenology of growth, and allocation of resources to different plant parts such as leaves, rhizomes and flowers. Since lace bugs access nitrogen when it is soluble and being transported among plant tissues, then nitrogen allocation should increase lace bug herbivory. The change in green leaf nitrogen midseason could indicate that there is more soluble nitrogen available

for lace bug consumption as plants move nitrogen out of their leaves. Lace bugs are indeed larger on the Pony Express, MO genotypes than the other USA populations (Fig. 7), except for Morris, MN which is a prairie subspecies of goldenrod (Semple 2006?). This indicates that subspecies of *S. altissima* may influence lace bug performance. Lace bugs on the El Dorado, KS genotypes, however, weighed the same as the rest of the USA populations.

Environmental factors may influence the evolution of goldenrod population more than lace bug herbivory. Lace bugs should have better performance on, and higher preference for the Japanese Hokkaido population since it had the highest green leaf nitrogen concentration of all the populations sampled and had the largest decrease in nitrogen midseason (Fig 10). However, there was high variation in lace bug weight and survival on this population (Fig. 6 and 7). The relatively poor performance of lace bugs on the Hokkaido population is surprising since it had not been exposed to lace bugs for over a century, and therefore it would be expected to have low resistance to lace bugs (Sakata et al. 2014). Lace bug weights were also low on Duluth plant genotypes, which is a population that experiences low lace bug herbivory, and therefore would not be expected to have evolved strong resistance (Sakata et al. in press). It is not surprising that Hokkaido and Duluth plant populations have similar resistance since they both are adapted to similar cold climates and encounter low or absent lace bug densities. However, it was unexpected for these populations to include high lace-bug resistance genotypes without high lace bug herbivory.

Conclusion

Genotypic variation among and within populations of tall goldenrod affected lace bug performance and preference. Contrary to expectations, variation in plant resistance to lace bugs was larger within plant populations than among plant populations. Moreover, a longer history of lace bug herbivory did not increase a goldenrod population's resistance as predicted. The large variation within and among plant populations in lace bug resistance suggests that high genetic variation evolved in response to both large and fine-scaled environmental heterogeneity. Previous studies primarily measured genotypic variation in plant resistance to herbivores either within local populations, or only at larger geographic scales, and this may give a limited understanding of the evolutionary genetics of plant-herbivore interactions.

I also found that drought stress interacted with plant genotype and resulted in an increase or decrease of plant resistance to lace bugs. Plant resistance due to plant genotype and drought stressed was associated with leaf nitrogen availability, but further research is needed to understand what factors limit its availability to lace bugs.

While common garden studies, like the one I conducted, are critical for revealing the presence of genotypic plant variation in resistance to herbivory, they provide an incomplete understanding of coevolution of plant-insect interactions. Plant resistance to herbivory is a coevolved plant genotype x insect genotype x environment interaction (Thompson 2005). Thus, additional studies that include variation in insect genotypes are also necessary to understand the coevolution of lace bugs and goldenrods.
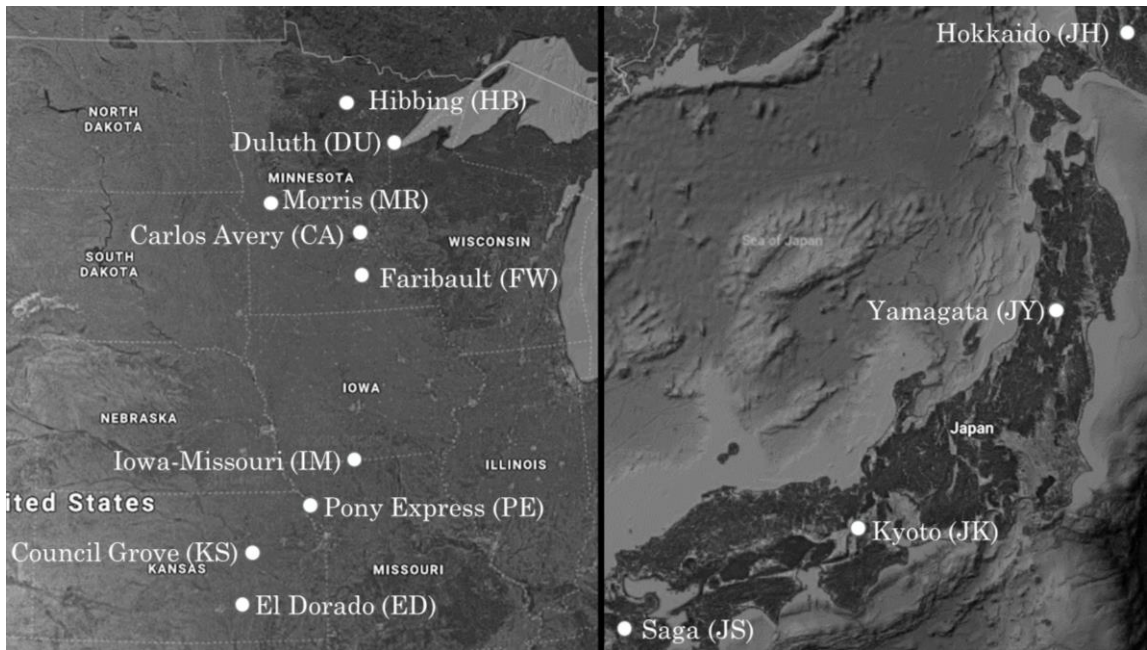
31

Figures

Figure 1. Locations of *S. altissima* populations. Several genotypes were collected from within each population across the USA and Japan (Imagery ©2018 Google, TerraMetrics, NOAA)
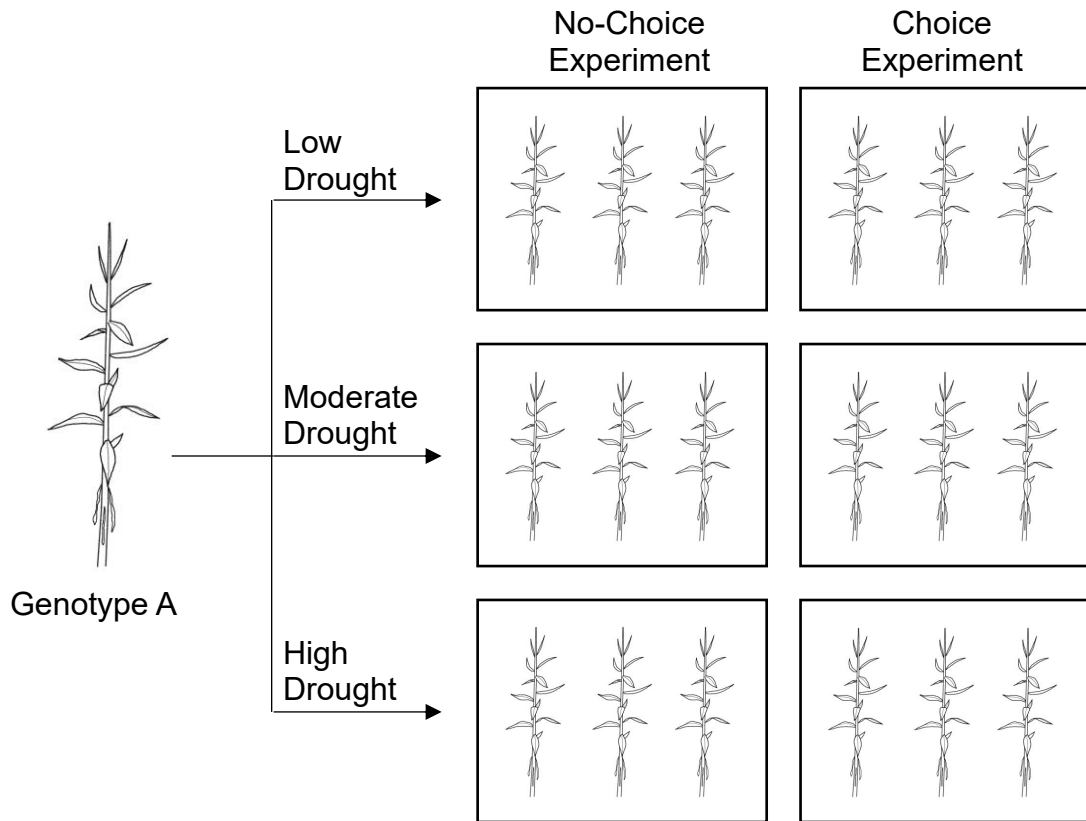
Figure 2. Division of plant genotypes into drought treatments for the 2016 lace bug no-choice and choice drought experiments. Ten plant genotypes from the Carlos Avery, MN population were used. For each genotype, six replicates were put into each drought treatment, and three replicates of each drought treatment were assorted into the no-choice and choice plots.
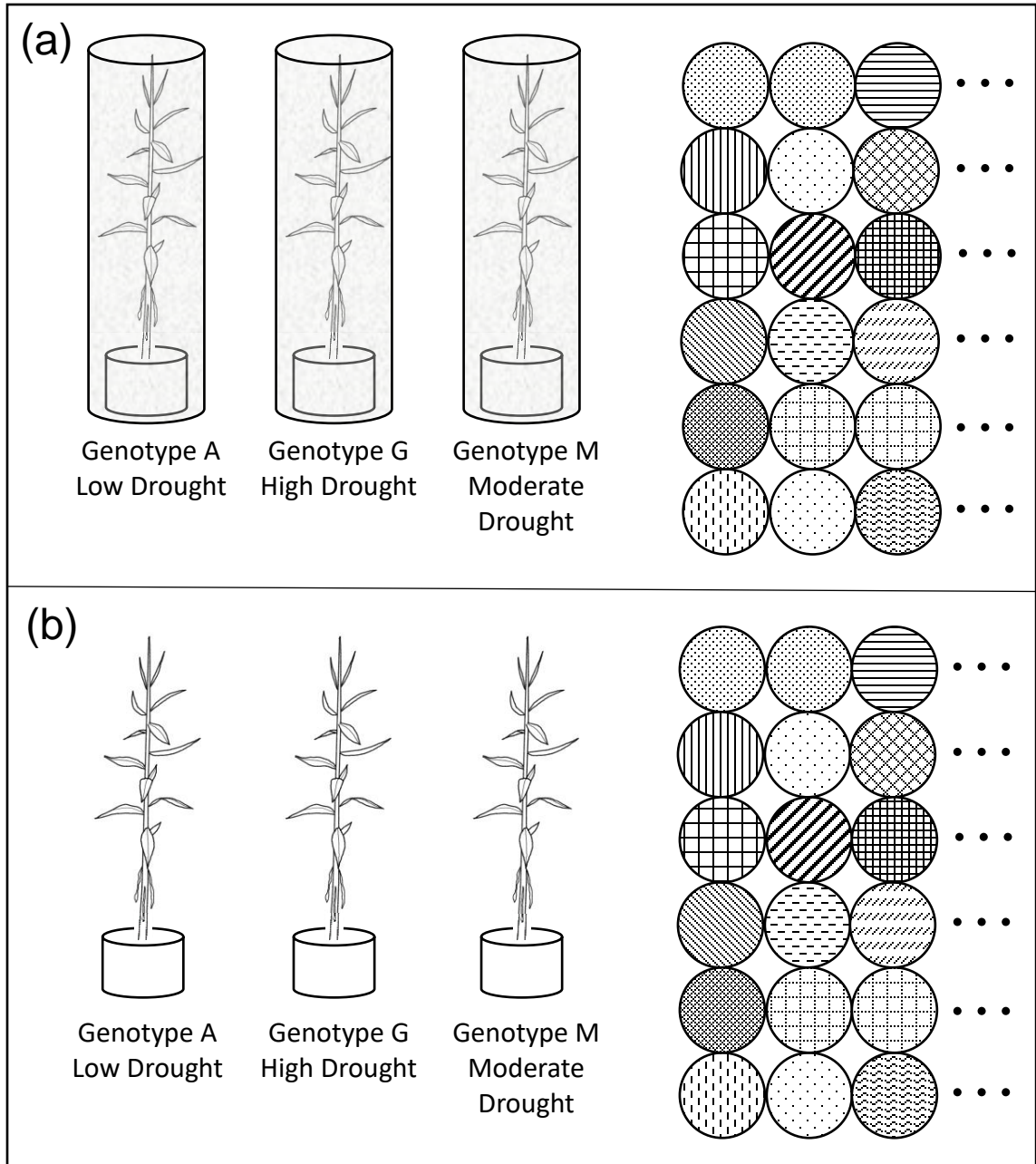
Figure 3. Experimental design of the 2016 lace bug no-choice and choice drought experiments. Ten Carlos Avery genotypes were used and replicates for each genotype were treated with either low, moderate, or high drought. All genotype x drought replicates were randomly assorted into two plots, however, the assortment of the two plots were identical to each other. a) The no-choice experiment had individually caged plants, and a female and male lace bug was added to each cage and removed after one week. b) The choice experiment had uncaged plants to allow natural lace bug colonization.
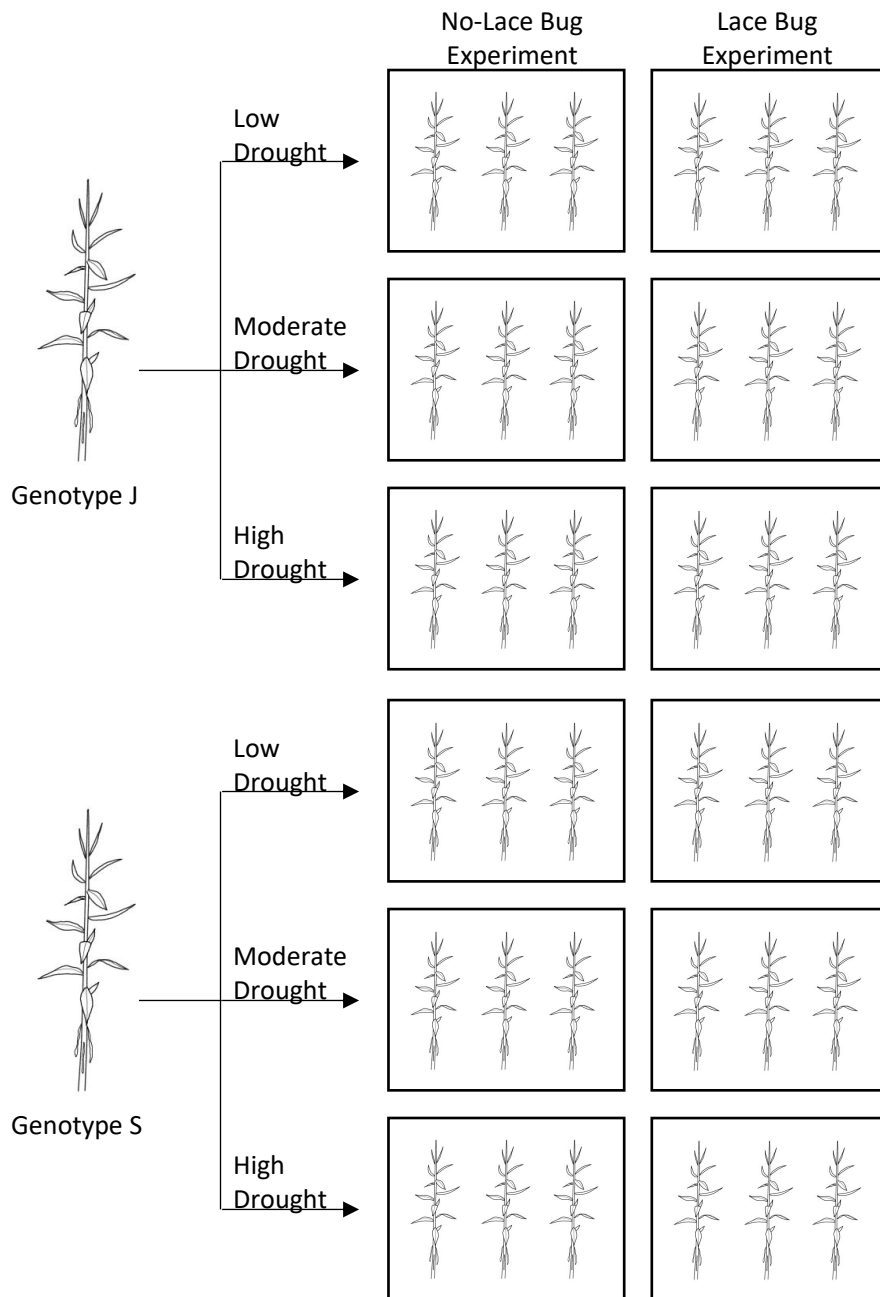
Figure 4. Division of plant genotypes into drought treatments for the 2016 drought nitrogen experiments. Two plant genotypes from the Carlos Avery, MN population were used. For each genotype, six replicates were put into each drought treatment, and three replicates of each drought treatment were assorted into the no-lace bug and lace bug plots.
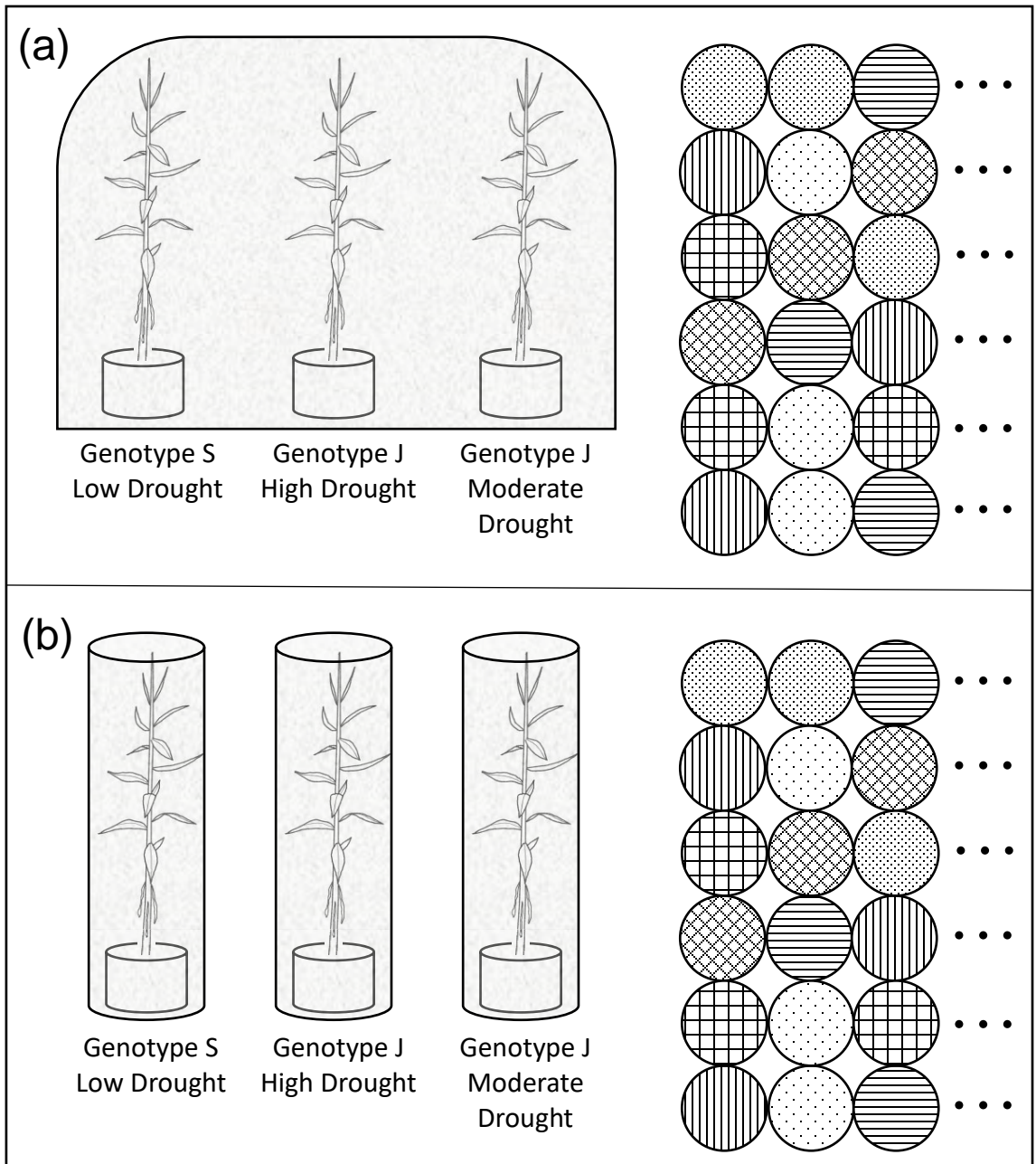
Figure 5. Experimental design of the 2016 drought nitrogen experiments. Two Carlos Avery genotypes were used and replicates for each genotype were treated with either low, moderate, or high drought. All genotype x drought replicates were randomly assorted into two plots, however, the assortment of the two plots were identical to each other. a) The no-lace bug experiment had the entire plot caged to prevent herbivory. b) The lace bug experiment had individually caged plants, and a female and male lace bug was added to each cage and removed after one week.
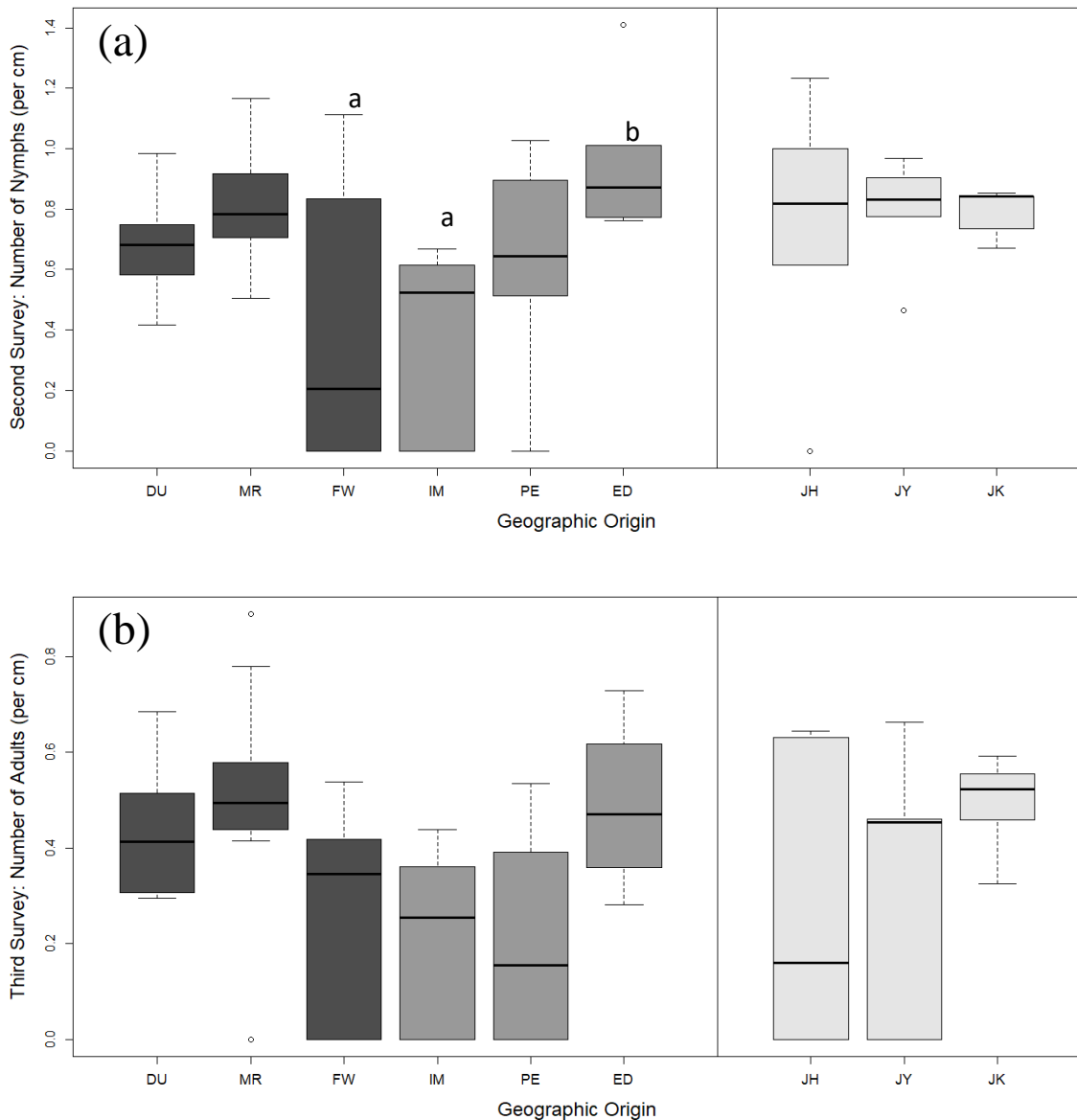
Figure 6. *C. marmorata* nymph and adult survival among *S. altissima* populations in the 2017 genotypic variation no-choice experiment. a) There was higher nymph survival on the El Dorado, KS (ED) plant genotypes than on Faribault, MN (FW) and Iowa-Missouri border genotypes during the second survey in July. b) There was higher adult survival during the third survey in August. The Tukey HSD could not detect which plant populations were significantly different
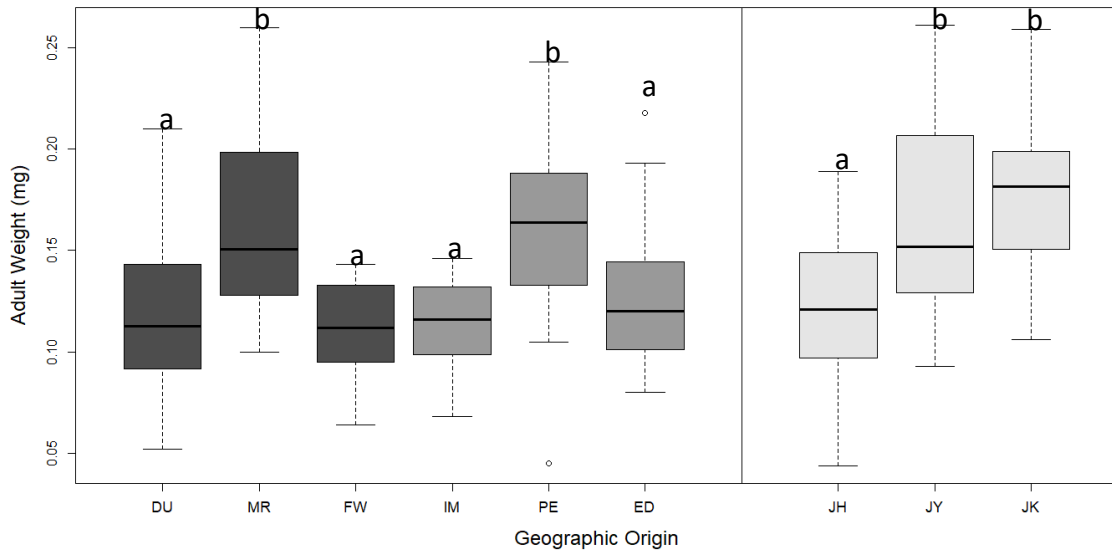
Figure 7. *C. marmorata* adult weight among *S. altissima* populations in the 2017 genotypic variation no-choice experiment. Adults were larger on Morris, MN (MR), Pony Express, MO (PE), Yamagata Japan (JY), and Kyoto Japan (JK) genotypes.
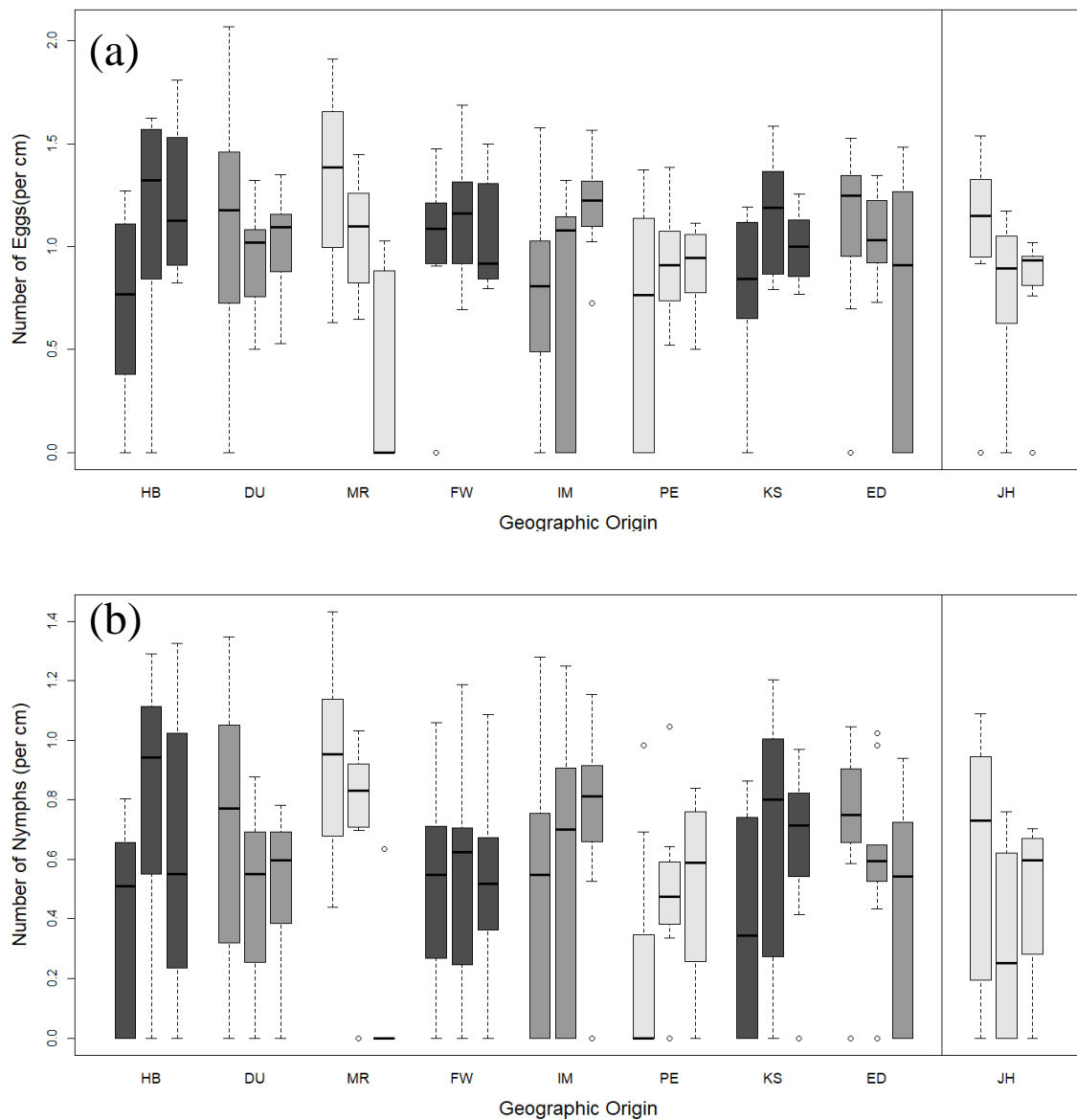
Figure 8. *C. marmorata* egg and nymph abundance among and within *S. altissima* populations in the 2016 genotypic variation choice experiment. Three genotypes from each of 9 populations were used. Differences in a) egg and b) nymph numbers were found within populations but not among populations.
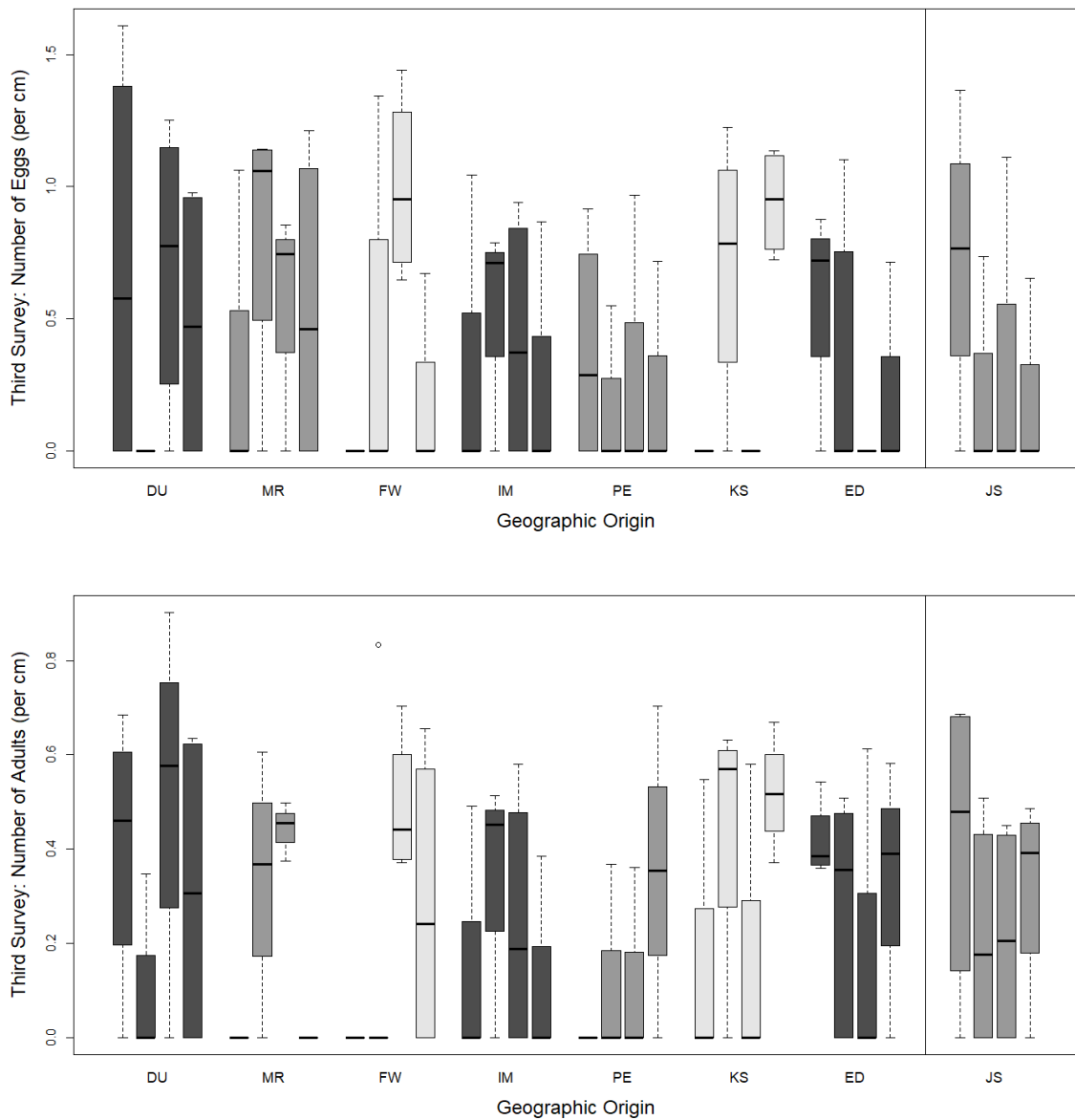
39

Figure 9. *C. marmorata* egg abundance and adult preference among and within *S. altissima* populations in the 2017 genotypic variation choice experiment. Four genotypes from each of 8 populations were used. Differences in a) egg abundance and b) adult preference were found within populations but not among populations in the third survey in August.
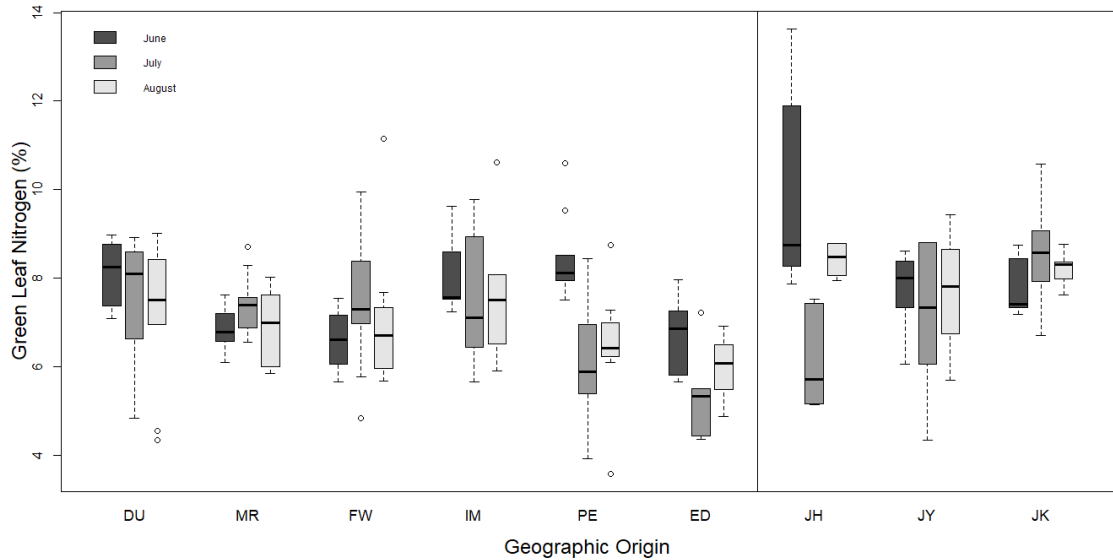
Figure 10. Total green leaf nitrogen content among *S. altissima* populations in the 2017 genotypic variation no-choice experiment. Nitrogen concentration changed significantly among survey dates, and Hokkaido Japan (JH) and Kyoto Japan (JK) had the highest nitrogen concentration in the first and second survey, respectively.
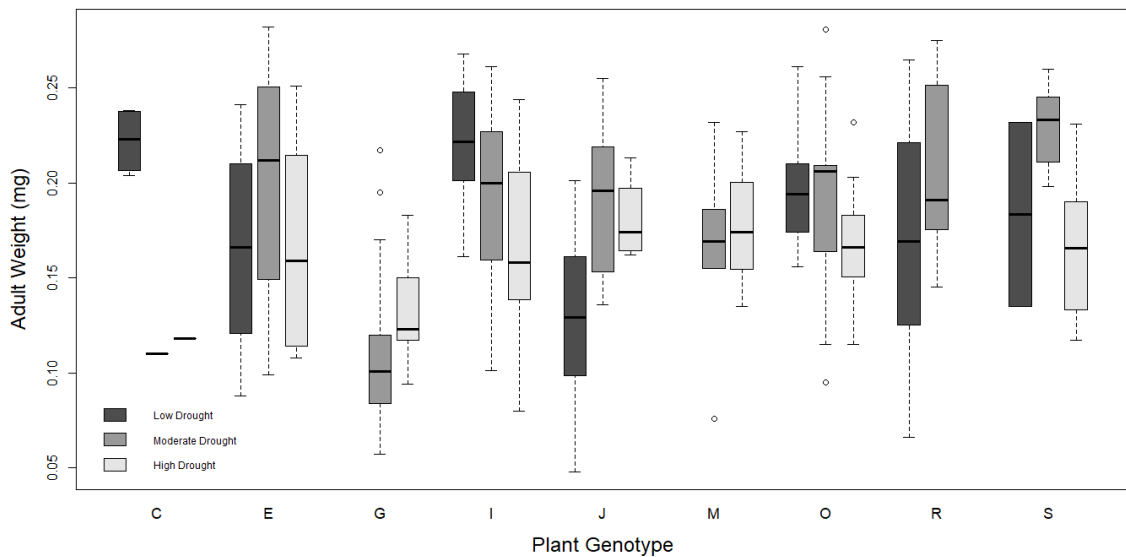

Figure 11. *C. marmorata* adult weight among *S. altissima* genotypes and three drought treatments in the 2016 drought no-choice experiment. Nine genotypes from Carlos Avery were used. *C. marmorata* weight among genotype, drought, and the interaction of genotype and drought was significantly different.
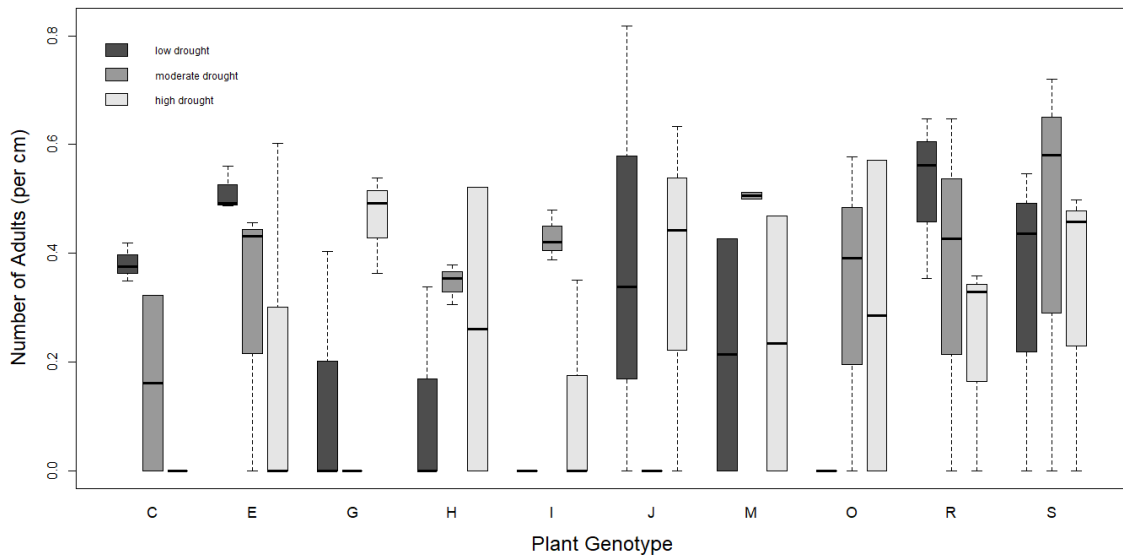
Figure 12. *C. marmorata* adult preference among *S. altissima* genotypes and three drought treatments in the 2016 drought choice experiment. Ten genotypes from Carlos Avery were used. *C. marmorata* had no preference for genotype or drought treatment, but the interaction of genotype and drought influenced lace bug preference.
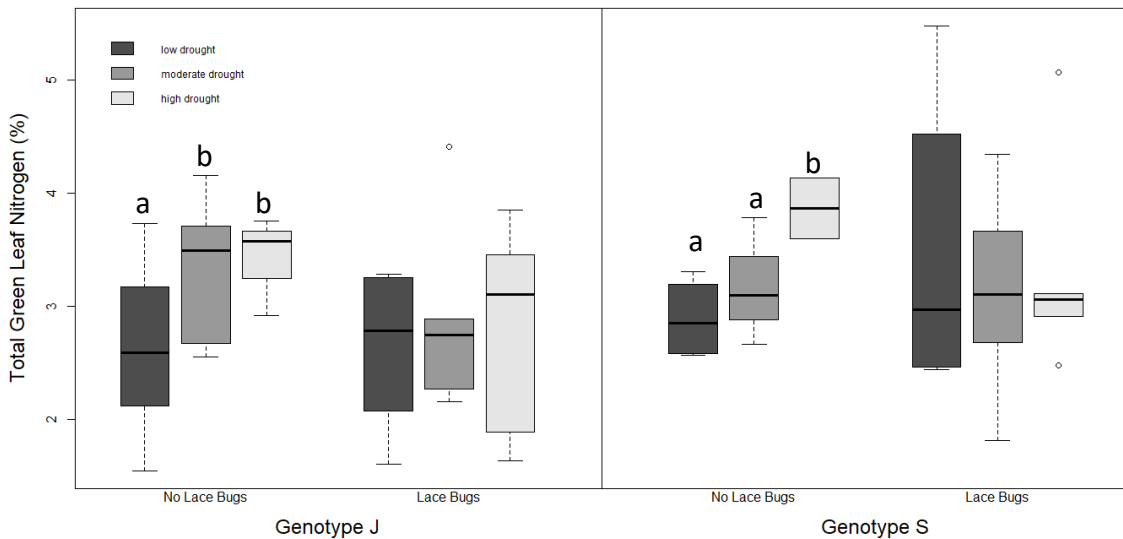

Figure 13. Total green leaf nitrogen concentration among three drought treatments in the absence and presence *C. marmorata* for the 2016 Drought nitrogen experiment. Two *S. altissima* genotypes from the Carlos Avery population were used. Nitrogen increases with drought in the absence of *C. marmorata*. No variation among treatments or genotype was found in the lace bug plots.
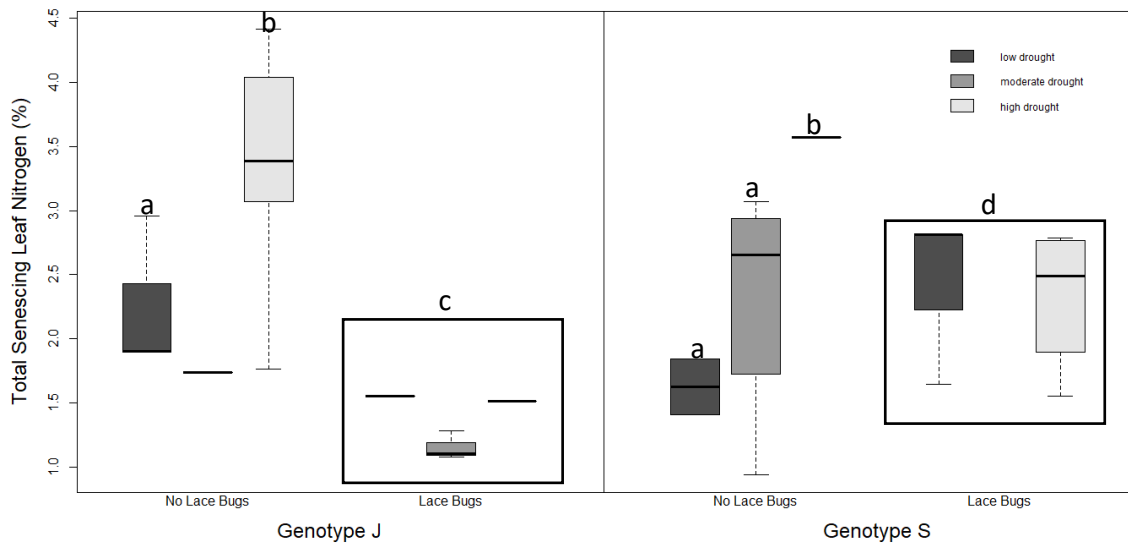
Figure 14. Total senescing leaf nitrogen concentration among three drought treatments in the absence and presence *C. marmorata* for the 2016 Drought nitrogen experiment. Two *S. altissima* genotypes from the Carlos Avery population were used. Nitrogen increased with drought in the absence of *C. marmorata* marked with "a" and "b". There were genotypic differences in nitrogen in the presence of lace bugs, boxed and marked with "c" and "d".

Literature Cited

Barrios-Garcia, M. N., M. A. Rodriguez-Cabal, J. A. Rudgers, and G. M. Crutsinger. 2016. Soil fertilization does not alter plant architectural effects on arthropod communities. Journal of Plant Ecology 10:800–807.

Blossey, B., and R. Notzold. 2010. Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants : A Hypothesis Author (s): Bernd Blossey and Rolf Notzold Source : Journal of Ecology , Vol . 83 , No . 5 (Oct ., 1995), pp . 887-889 Published by : British Ecological Society. Journal of Ecology 83:887–889.

Burghardt, K. T. 2016. Nutrient supply alters goldenrod's induced response to herbivory. Functional Ecology 30:1769–1778.

Burkle, L. a., L. Souza, M. a. Genung, and G. M. Crutsinger. 2013. Plant genotype, nutrients, and G × E interactions structure floral visitor communities. Ecosphere 4:art113.

Cahenzli, F., and A. Erhardt. 2012. Host plant defence in the larval stage affects feeding behaviour in adult butterflies. Animal Behaviour 84:995–1000.

Cappuccino, N., and R. B. Root. 1992. The significance of host patch edges to the colonization and development of Corythucha marmorata (Hemiptera: Tingidae). Ecological Entomology 17:109–113.

Craig, T., Abrahamson, W., Itami, J., & Horner, J. (1999). Oviposition Preference and Offspring Performance of Eurosta solidaginis on Genotypes of Solidago altissima. Oikos, 86(1), 119-128.

Craig, T. P., and J. K. Itami. 2011. Divergence of Eurosta Solidaginis in Response To Host Plant Variation and Natural Enemies. Evolution 65:802–817.

Craig, T. P., J. K. Itami, and J. V. Craig. 2007. Host plant genotype influences survival of hybrids between Eurosta solidaginis host races. Evolution 61:2607–2613.

Craig, T. P., J. K. Itami, T. Ohgushi, Y. Ando, and S. Utsumi. 2011. Bridges and barriers to host shifts resulting from host plant genotypic variation. Journal of Plant Interactions 6:141–145.

Cronin, J. T., W. G. Abrahamson, and T. P. Craig. 2001. Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. Oikos 93:312–320.

Deans, C. A., S. T. Behmer, J. Fiene, and G. A. Sword. 2016. Spatio-Temporal, Genotypic, and Environmental Effects on Plant Soluble Protein and Digestible Carbohydrate Content: Implications for Insect Herbivores with Cotton as an Exemplar. Journal of Chemical Ecology 42:1151–1163.

Dostalek, T., M. B. Rokaya, P. Marsik, J. Rezek, J. Skuhrovec, R. Pavela, and Z. Munzbergova. 2016. Trade-off among different anti-herbivore defence strategies along an altitudinal gradient. AoB PLANTS 8.

Franks, S. J., N. Genovese, M. Stockdale, J. J. Weber, B. H. Ansaldi, and E. van Wilgenburg. 2018. The Effects of Artificial Selection for Rapid Cycling in *Brassica rapa* on Herbivore Preference and Performance. International Journal of Plant Sciences 179:000–000.

Gaylord, M. L., T. E. Kolb, W. T. Pockman, J. A. Plaut, E. A. Yepez, A. K. Macalady, R.

E. Pangle, and N. G. Mcdowell. 2013. Drought predisposes piñon-juniper woodlands to insect attacks and mortality. New Phytologist 198:567–578.

Grinnan, R., T. E. Carter, and M. T. J. Johnson. 2013. Effects of drought, temperature, herbivory, and genotype on plant-insect interactions in soybean (Glycine max). Arthropod-Plant Interactions 7:201–215.

Hakes, A. S., and J. T. Cronin. 2011. Environmental heterogeneity and spatiotemporal variability in plant defense traits. Oikos 120:452–462.

Heimonen, K., A. Valtonen, S. Kontunen-Soppela, S. Keski-Saari, M. Rousi, E. Oksanen, and H. Roininen. 2015. Colonization of a host tree by herbivorous insects under a changing climate. Oikos 124:1013–1022.

Heimonen, K., A. Valtonen, S. Kontunen-Soppela, S. Keski-Saari, M. Rousi, E. Oksanen, and H. Roininen. 2017. Susceptibility of silver birch (Betula pendula) to herbivorous insects is associated with the size and phenology of birch–implications for climate warming. Scandinavian Journal of Forest Research 32:95–104.

Helmberger, M. S., T. P. Craig, and J. K. Itami. 2016. Effects of drought stress on oviposition preference and offspring performance of the lace bug Corythucha marmorata on its goldenrod host, Solidago altissima. Entomologia Experimentalis et Applicata 160:1–10.

Hwang, S. Y., C. H. Liu, and T. C. Shen. 2008. Effects of plant nutrient availability and host plant species on the performance of two Pieris butterflies (Lepidoptera: Pieridae). Biochemical Systematics and Ecology 36:505–513.

Johnson, M. T. J., and A. a. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (Oenothera biennis). Ecology 86:874–885.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.

Lanzer, N. B., T. D. Lee, M. J. Ducey, and S. E. Eisenhaure. 2017. Sapling white pine (Pinus strobus L.) exhibits growth response following selective release from competition with glossy buckthorn (Frangula alnus P. Mill) and associated vegetation. Forest Ecology and Management 404:280–288.

Lind, E. M., E. Borer, E. Seabloom, P. Adler, J. D. Bakker, D. M. Blumenthal, M. Crawley, K. Davies, J. Firn, D. S. Gruner, W. Stanley Harpole, Y. Hautier, H. Hillebrand, J. Knops, B. Melbourne, B. Mortensen, A. C. Risch, M. Schuetz, C. Stevens, and P. D. Wragg. 2013. Life-history constraints in grassland plant species: A growth-defence trade-off is the norm. Ecology Letters 16:513–521.

Maddox, G. D., and N. Cappuccino. 1986. Genetic Determination of Plant Susceptibility to an Herbivorous Insect Depends on Environmental Context. Evolution 40:863–866.

Maddox, G. D., and R. B. Root. 1987. Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, Solidago altissima: genetic variation and heritability. Oecologia 72:8–14.

Maddox, G. D., and R. B. Root. 1990. Struture of the Encounter between Goldenrod (Solidago altissima) and its Diverse Insect Fauna. Ecological Society of America 71:2115–2124.

Massad, T. J., L. A. Dyer, and G. Vega C. 2012. Costs of Defense and a Test of the Carbon-Nutrient Balance and Growth-Differentiation Balance Hypotheses for Two Co-Occurring Classes of Plant Defense. PLoS ONE 7.

Reusch, T. B. H., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences of the United States of America 102:2826–2831.

Root N., R. B. . C. 1992. Patterns in population change and the organization of the insect community associated with goldenrod. Ecological Monographs 62:393–420.

Sakata, Y., J. Itami, Y. Isagi, and T. Ohgushi. 2015. Multiple and mass introductions from limited origins: genetic diversity and structure of Solidago altissima in the native and invaded range. Journal of Plant Research 128:909–921.

Sakata, Y., S. Kaneko, A. Hayano, M. Inoue-Murayama, T. Ohgushi, and Y. Isagi. 2013a. Isolation and characterization of microsatellite loci in the invasive herb Solidago altissima (Asteraceae). Applications in plant sciences 1:1200313.

Sakata, Y., T. Ohgushi, and Y. Isagi. 2013b. Geographic variations in phenotypic traits of the exotic herb Solidago altissima and abundance of recent established exotic herbivorous insects. Journal of Plant Interactions 8:216–218.

Sakata, Y., M. Yamasaki, Y. Isagi, and T. Ohgushi. 2014. An exotic herbivorous insect drives the evolution of resistance in the exotic perennial herb Solidago altissima. Ecology 95:2569–2578.

Sakata, Y., M. Yamasaki, and T. Ohgushi. 2016. Urban landscape and forest vegetation regulate the range expansion of an exotic lace bug Corythucha marmorata (Hemiptera: Tingidae). Entomological Science 19:315–318.

Shibel, Z., and S. B. Heard. 2016. Synergistic and additive effects of drought stress and simulated herbivory on two goldenrods, Solidago altissima and S. gigantea. Botany 94:635–642.

Stein, A., K. Gerstner, and H. Kreft. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecology Letters 17:866–880.

Sumerford, D. V, W. G. Abrahamson, and a E. Weis. 2000. The effects of drought on the Solidago altissima-Eurosta solidaginis-natural enemy complex: population dynamics, local extirpations, and measures of selection intensity on gall size. Oecologia 122:240–248.

Thompson, J. N. 2005. Coevolution: The Geographic Mosaic of Coevolutionary Arms Races. Current Biology 15:R992–R994.

Turner, K. G., H. Fréville, and L. H. Rieseberg. 2015. Adaptive plasticity and niche expansion in an invasive thistle. Ecology and Evolution 5:3183–3197.

Utsumi, S., Y. Ando, T. P. Craig, and T. Ohgushi. 2011. Plant genotypic diversity increases population size of a herbivorous insect. Proceedings of the Royal Society B: Biological Sciences 278:3108–3115.

White, T. C. . 1993. The Inadequate Environment: Nitrogen and the Abundance of Animals. Springer-Verlag Berlin Heidelberg.

Wimp, G. M., G. D. Martinsen, K. D. Floate, R. K. Bangert, and G. Whitham. 2010. Plant Genetic Determinants of Arthropod Community Structure and Diversity.

Society for the Study of Evolution. 59:61–69.

Yang, Z., X. Liu, M. Zhou, D. Ai, G. Wang, Y. Wang, C. Chu, and J. T. Lundholm. 2015. The effect of environmental heterogeneity on species richness depends on community position along the environmental gradient. Scientific reports 5:15723.