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Spatial Variation and Tradeoffs in Species Interactions

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SPATIAL VARIATION AND TRADEOFFS IN SPECIES INTERACTIONS

A Thesis Presented

by

HOLLY BERNARDO

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
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Organismic and Evolutionary Biology

DEDICATION

To Alvan Toews (1928-2009)

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I first want to thank my husband for his help, understanding, shoulder and love.

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ABSTRACT

SPATIAL VARIATION AND TRADEOFFS IN SPECIES INTERACTIONS

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The geographic mosaic theory of coevolution predicts that spatial differences in species interactions result in a patchwork of evolutionary hot and cold spots across a landscape. We used horsenettle (*Solanum carolinense* L.), a perennial weed with a diverse insect community found in old fields and meadows, to examine local adaptation and resource-mediated selection. The goals of this study were to (1) determine the potential for a selection mosaic by identifying local adaptation through trait-interaction matching with herbivores, pollinators and plant competitors, and (2) determine the potential for indirect selection through resource allocation tradeoffs. The potential for local adaptation was determined by measuring interactions in four populations across Massachusetts and relating those findings to plant traits measured on offspring grown from those populations in a ‘common garden’ greenhouse experiment. Allocation tradeoffs between growth, herbivore resistance, and floral traits were also assessed in the common garden experiment. We found high herbivore damage in the field associated with decreased root:shoot ratios in greenhouse-grown plants, which may indicate an herbivore-mediated effect on life-history through selection for a more annual strategy. We found high levels of pollination associated with small flowers and floral displays, indicating that high pollination may relax selection via

decreased competition for pollinators. By examining allocation tradeoffs we found evidence of two distinct reproductive strategies in this perennial plant. Negative correlations between reproductive traits and both growth and defense suggest that individuals either favor current growth and reproduction over defense, or invest in current survival and defense while delaying reproduction. Overall, this study sheds light on how selection changes over space and time, which are of many of the fascinating traits we find in plants and animals today.

Keywords: Selection mosaic, resource allocation tradeoff, herbivory, pollination, competition, horsenettle, *Solanum*

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CHAPTER 1

SPATIAL VARIATION AND TRADEOFFS IN SPECIES INTERACTIONS

Introduction

The geographic mosaic theory of coevolution predicts that spatial differences in species interactions result in a patchwork of evolutionary hot and cold spots across a landscape (Thompson 1999, Gomulkiewicz et al. 2000). Within hot spots, species interactions have a high impact on fitness and there is selection on traits that mediate interactions (Gomulkiewicz et al. 2000). Within cold spots, the same interactions have little impact on fitness, either because the one species is less abundant or because other interactions create stronger pressures, and selection on traits mediating the interaction is relaxed (Gomulkiewicz et al. 2000). If trait remixing between populations is low, then possible evolutionary mosaics can be identified by finding variable local adaptation, or a good ‘fit’ between trait expression and interaction intensity in some locations and a bad or no ‘fit’ at others (Gomez et al. 2009a). Evidence of local adaptation suggests that a population is experiencing a selective episode and a geographic mosaic across the population is possible. Evidence of local maladaptation, i. e., a bad ‘fit’ or no relationship between traits and interactions, suggests that a population is at the beginning of a selective episode or that the population is a cold spot for the interactions of interest.

Plant-insect interactions have long been used to examine the role of coevolution in shaping natural selection on traits mediating species interactions (Berenbaum 1983, Leebens-Mack et al. 1998, Thompson 1999, Rudgers and Strauss 2004, Berenbaum and Zangerl 2006). Specialized plant-herbivore systems are especially useful because herbivore populations and pressures often vary across a landscape, and plant defenses can be costly

(Baldwin et al. 1990, Bergelson and Purrington 1996, Strauss et al. 2002) and traits often respond strongly to selection (Berenbaum 1983, Agrawal 2005, Berenbaum and Zangerl 2006). For example, over twenty years of research on wild parsnip (*Pastinaca sativa*) and the parsnip webworm (*Depressaria pastinacella*) has provided much evidence that plant chemical defenses and insect detoxification mechanisms have coevolved (Berenbaum 1983, Berenbaum and Zangerl 2006, Zangerl et al. 2008). A recent introduction of the webworm to New Zealand found selection regimes consistent with a geographic mosaic. Defenses in wild parsnip had diminished in the insect's absence, and Zangerl et al. (2008) found substantial increases in selection on defense chemicals and the greatest decrease in fitness near the epicenter of the outbreak. If greater herbivore pressure generally results in decreased fitness and increased positive selection for defense traits, then plants from sites with high herbivory should be under selection for increased defense levels, whereas plants from sites with low herbivory should experience relaxed selection for defenses. Differences in historical selection pressures could be evident in the trait values of first generation plants grown in a common garden environment, such as greenhouse, where environmental variation in trait values is eliminated. Increased defenses could also contribute to population-level differences in other traits. Plants that invest more energy in defenses may have less to allocate to other functions (Agrawal et al. 1999, Strauss et al. 1999), and it has been shown that plants with high herbivore resistance often produce fewer and smaller flowers (Strauss et al. 1999). If selection for increased defenses resulted in reduced allocation to reproductive traits, then greenhouse-grown offspring from populations with high herbivory may have fewer or smaller flowers.

Pollinators, like herbivores, select for plant traits in many systems. For example, pollinators exert selective pressure on floral attractive traits, like shape (Conner et al. 1996, Galen and Cuba 2001), flower stalk height (Gomez 2008, Gomez et al. 2009b), and number of flowers (Conner et al. 1996). Evidence that plants and pollinators coevolve has been well documented in a few specialized systems, such as the classic yucca and yucca moth example (Leebens-Mack et al. 1998). Current research has also found geographic mosaics of selection on floral traits via pollinators (Gomez et al. 2009a, Gomez et al. 2009b). Gomez et al. (2009a) documented a selection mosaic on *Erysimum mediohispanicum* in which guilds of generalist pollinators selected for floral traits differently across a landscape and in the presence of various herbivores. Thus, even in systems with generalist pollinators one could expect trait-interaction matching between greenhouse-grown offspring and populations with differing levels of pollination. We predict that sites with the lowest pollinator activity would have plants with the largest and most showy flowers, as competition for pollinator services is likely intense. Conversely, sites with the most pollinator activity would have plants with the smallest flowers, as selection on attractiveness may have relaxed due to decreased competition for pollinators. Relaxed selection can occur when a strong selective force is removed (Lahti et al. 2009), such as competition. Relaxed selection on plant defenses has been documented when a plant species invades an area with few herbivores (Blair and Wolfe 2004), but to our knowledge has not been demonstrated in response to saturated pollination services (Lahti et al. 2009).

Along with plant-insect interactions, plant-plant competition can exert selective pressure on plant traits (Jones et al. 2006). The intensity of competition varies spatially since it can be regulated by local abiotic conditions (Liancourt and Tielborger 2009). Competition

is thought to be most extreme in favorable environments, and decrease in importance when environments are harsher and more species are excluded (Sambatti and Rice 2006, Liancourt and Tielborger 2009). Favorable environments allow many species to establish and compete for resources, and often select for increased plant height, growth or investment in survival (Liancourt and Tielborger 2009). Therefore, we predict that plants in favorable sites with the strongest competition will have offspring that grow faster and larger, and invest more in the current seasons growth (shoots) than survival (roots) in the greenhouse.

Many predictions regarding local adaptation assume that plants have limited resources, and resource-mediated selection is thought to be a major factor in the creation of selection mosaics across a landscape (Bergelson and Purrington 1996, Gomulkiewicz et al. 2000, Strauss et al. 2002). Plant resource allocation has been described as “a triangle of competing functions: growth, reproduction and defence” (Obeso 2002). Essentially, energy allocated to one function, like growth, cannot be used for other functions, like reproduction or defense. Thus, plants cannot be perfectly adapted to all conditions and, assuming trait variation has a genetic basis, selection will on traits that most contribute to fitness in a specific environment (Obeso 2002, Hautier et al. 2009). Therefore, identifying allocation tradeoffs is another way to identify possible selection on traits. Resource-mediated costs are generally identified by negative correlations between traits associated with different functions (Obeso 2002, Andersson 2006, Jones et al. 2006, but see Knops et al. 2007). In this study, we predict negative correlations between defense and growth, size and reproductive traits. This prediction is based on the premise that resources put into defense are unavailable for other functions. One could also predict negative associations between growth or size traits and reproductive traits. However, positive relationships between these traits are often found

(e.g. Gomez 2008, Hautier et al. 2009), and are generally interpreted as indicating that bigger plants have more resources and are therefore able to make more flowers (Obeso 2002). Thus, relationships between growth or size and reproduction could be interpreted in several ways. A negative correlation between reproduction and growth or size would indicate the possibility of conflicting selection between these functions. For example, perennial plants that invest more in root growth in one year may store those extra resources for future sexual reproduction at the expense of current reproduction. A positive correlation would indicate that selection could act on growth or size and reproduction in a similar manner.

Solanum carolinense L. (horsenettle; Solanaceae) is an herbaceous, perennial plant native to the southeastern U.S. and found throughout the eastern U.S. in old fields, meadows and agricultural fields (USDA 2010). It reproduces clonally through lateral roots and sexually through seeds. Horsenettle is self-incompatible (Elle 1998, Mena-Ali et al. 2008) and is only effectively pollinated by bumblebees that release pollen during buzz pollination (Mena-Ali et al. 2008). Horsenettle harbors a diverse, well documented herbivore community, with 32 species reported to regularly feed on the plant (Wise 2007b). Effects of multiple herbivores on survival and reproduction have been documented in this and closely related *Solanum* species (Wise and Sacchi 1996, Wise and Weinberg 2002, Viswanathan et al. 2007, Viswanathan et al. 2008), and many plant traits, including trichomes, thorns and chemical alkaloids, have been hypothesized to act as herbivore deterrents (Wise 2007a). Many plant traits associated with survival and reproduction have been shown to have a heritable component in this species (Elle 1998).

Horsenettle is a serious pest in pastures and agricultural fields. Its toxic fruit and leaves sicken livestock (Gorrell et al. 1981, Cipollini and Levey 1997). It can reduce yield

through competitive effects with crop species (Hackett et al. 1987, Frank 1990). It is an alternate host to many Solanaceous insect pests, exacerbating its negative impact on related crops through increased damage or disease (Gorrell et al. 1981, Nichols et al. 1992, Wise 2007a, b). Its extensive root system and clonal growth make it difficult to control (Prostko et al. 1994). Understanding the selective pressures on, and tradeoffs between, plant traits in horsenettle's natural habitat may provide insight into controlling this species' growth and reproduction in pastures and fields.

This study had two goals. The first was to determine the potential for a selection mosaic in horsenettle by identifying local adaptation through trait-interaction matching. Specifically, we asked whether sites differ in biotic characteristics, including herbivory, pollinator activity, and plant community, that could affect selection on plant traits, and whether those differences predicted population-level trait differences of horsenettle grown in a common garden. The second goal was to determine whether resource allocation tradeoffs exist between defense, reproduction, growth or size traits in greenhouse-grown plants.

Methods

Species Interactions

To describe the selective environment of each horsenettle population, insect herbivory, bumblebee pollination and plant community composition were measured at four sites during the summer of 2009 (Table 1).

Herbivory. A summary of damage types, defining characteristics, and method of measurement are in Table 2 (based on Wise 2007a). Leaf and floral herbivory were identified to species based on descriptions in Wise (2007a and 2007b). Leaf herbivore damage was surveyed once between Aug 5-7 on the 5 youngest fully expanded leaves of 30 haphazardly

chosen ramets at least 2 m apart in each population (Wise et al. 2008). Wise et al. (2008) found two waves of intense herbivore damage occur in a season. Our survey dates were at the beginning of the second wave in order to identify as much of the first and second set of herbivores as possible. Five of 7 damage types were easily identified; the 2 unknown types were only found rarely, with < 2% leaf area removed at each population. Two metrics were calculated to represent leaf herbivory; leaf herbivore diversity as the number of damage types per plant, and the extent of leaf damage as the number of leaves damaged out of 5 surveyed. Unidentified damage was included in these metrics. Florivory included damage to buds, petals and anthers. Sixty buds and 40 flowers on up to 40 ramets were haphazardly surveyed for damage at each population. Only bud damage had uniquely identifying characteristics that could be assigned to a species.

Pollination. Pollination was estimated by observing bumblebee visitation in 15-minute intervals for a total of 6 hours over 2 days at each site. Observations took place between Aug 4-16 on warm, sunny days between 10am and 2pm. Two to 6 flowers were chosen on 1-4 ramets for each 15-minute observation. For each bee, the total number of visits, defined as direct contact with a flower's reproductive parts, was recorded. The total visits per flower per minute was calculated as a metric representing pollination.

Plant Community. The dominant flora at each site was measured using three 1 m x 1 m plots randomly placed along three 10 m transects at each site for a total of 9 plots surveyed per site between July 23-Aug 6. A total of 16 taxa (*Ailanthus altissima*, *Parthenocissus quinquefolia*, *Impatiens capensis*, *Daucus carota*, *Rosa multiflora*, *Trifolium* sp., *Gallium* sp., *Asclepias* sp., *Mentha* sp., *Oxalis* sp., *Solidago* sp., *Vitis* sp., *Rubus* sp., *Plantago* sp., several grasses, and 1 unidentified composite) in addition to horsetail were found. The 3 to

5 species that comprised the majority (about 80%) of each plot were measured either by counting the number of stems present or by estimating the percent cover within each plot. Horsenettle density was measured by counting the number of stems per plot, and its flowering density by noting how many stems had open flowers out of the total.

Plant Traits

To describe trait expression in each population, traits estimating herbivore defense, pollinator attraction, sexual reproduction, growth, above- and belowground biomass, and size were measured on horsenettle grown in a greenhouse at the University of Massachusetts during the winter of 2008-09.

Plant Propagation and Study Design. Fruit were collected in fall 2008 from the four sites used to measure species interactions. Three to 5 ripe fruit were taken from as many ramets as possible while keeping collections spaced a minimum of 2 m apart to increase the chances that each ramet was a genetically distinct individual (Wise et al. 2008). Seeds were removed from the fruit and soaked in a 5% HCl solution for 30-40 minutes to increase the germination rate. Seeds were left to air-dry overnight, then refrigerated (4° C) for a minimum of 6 weeks before being planted on December 14, in 4 cm x 5 cm x 5.5 cm propagation trays in Fafard Grow Mix # 2 (Conrad Fafard, Inc., P.O. Box 790 Agawam, MA 01001) at 24-27° C (16:8 D:N cycle).

Traits were measured on three offspring per maternal plant for a total of 240 plants (3 seeds/plant x 20 plants/population x 4 populations). Plants were arranged in three spatial blocks, each containing one individual per maternal plant. The bench location of each plant within a block was randomized every two weeks. Plants emerged between Dec 28-Jan 29. Due to the long period of emergence, plants were transplanted in groups upon reaching the 4-

5 leaf stage, with a new group every 4-5 days to control for phenological differences. Seedlings at the 4-5 leaf stage were transplanted into round 473 ml pots with 2 g slow release fertilizer (Osmocote Classic 14-14-14; The Scotts Company, Marysville, OH); for the first two groups fertilizer was added 1 week and 2 days after transplant, respectively, but this delay did not affect growth or final size (data not shown). All subsequent trait measurements, fertilizer applications, transplanting and harvesting were done at a uniform number of days post transplant to control for phenological differences. Plants were transplanted to 7.57 L pots 30 days post transplant and harvested 80 days post transplant, for a total growing time (emergence to harvest) of about 110 days per plant. Liquid fertilizer (500 mL of Technigro 17-5-24; Technigro, Burleigh BC., QLD, AUS) was applied at 55 and 70 days post transplant. Pesticides were applied four times to control whiteflies and thrips. On March 6, 16, and 27 1.3 mL/L of Azatin XL (OHP INC., Mainland, PA) and 15.65 mL/L of M-Pede (Dow AgroScience, LLC., Indianapolis, IN) were applied, and on April 6 1.3 mL/L of Avid 0.15 EC (Syngenta, Greensboro, NC) and 1.3 mL/L of Coserve SC (Dow AgroScience, LLC., Indianapolis, IN) were applied.

Trait Measurements. Trichome density, thorn density, and thorn size were measured as defensive traits. Although horsenettle also produces chemical defenses (Walls et al. 2005), exploring these were beyond the scope of this study. Trichome density was estimated under a dissecting microscope as the average number of trichomes in 3 haphazardly placed 1 mm x 5 mm rectangles on 2 cm of stem obtained at harvest from just above the soil line. Leaf thorn density was measured as the number of thorns on a leaf divided by leaf area (estimated as midvein length times width at the bottom lobe), on the 3 youngest fully expanded leaves at 55 days post transplant. Thorn size was measured as length with digital calipers on 3

randomly chosen thorns removed from the stem 3-5 internodes below the apical meristem shortly before harvesting.

Reproductive traits included flower size, size of reproductive structures, total number of flowers (hermaphrodite and male combined) produced per plant, and proportion of plants that flowered. Flower size was measured by corolla diameter, and length and width of 1 randomly chosen petal on each of the first 3 flowers produced. All 3 measures and flower area estimates calculated from them were highly correlated (all $r > 0.67$, $p < 0.0001$); corolla diameter was chosen to represent flower size in statistical analyses. Anther size was estimated by the volume of 1 randomly chosen anther (treated as a cylinder, $V = \pi r^2 h$) in each of the first 3 flowers. Style length was measured in the first 3 flowers from the base of the petals to the tip of the stigma. Investment in sexual reproduction was estimated as the total number of flowers produced until harvest and proportion of plants that flowered.

Aboveground growth rate and the root:shoot ratio were measured as indicators of competitive ability and investment in current versus future growth for this perennial species. Aboveground growth rate was calculated by dividing the difference in height between the time of transplantation to the 473 mL pot and the time of harvest by the number of days between the 2 measures (80), and is reported as growth in mm per day. Height is often associated with competitive ability in plants (Purrington 2000, Jones et al. 2006). The root:shoot ratio was calculated from the dry weight of aboveground and belowground plant material after harvest. Measuring the root:shoot ratio in pot grown plants may artificially deflate the value, as plants can continue to grow aboveground but are restricted below. The short growth period of our study should not have allowed for extreme skew in these values, but these results should be interpreted with caution. A higher root:shoot ratio was interpreted

as a higher investment in survival to future seasons (since this plant overwinters as roots), compared to investment in the current season's growth and reproduction.

Overall size was estimated for both the entire plant and for leaves specifically. Plant size was measured at the time of harvest by height, stem diameter, number of branches, number of internodes, and the dry weights of root and shoot biomass. Leaf size was estimated on the 3 youngest fully expanded leaves at 55 days post transplant by the length of the midvein, from the beginning of the petiole to the tip of the leaf, and the width of the top and bottom lobes. Estimates of leaf area calculated from these measures were highly correlated with midvein length (all $r > 0.79$, $p < 0.0001$); midvein length was chosen to represent leaf size in statistical analyses.

Statistical Analysis

All statistical analyses were conducted using the Analyst program in SAS 9.2.

Question 1: Species Interactions. First, we asked whether leaf herbivore diversity, the extent of leaf damage, and the 3 florivory estimates (petal, bud and anther damage) varied across sites. Herbivore diversity was log transformed to improve normality and analyzed with one-way ANOVA with site as a fixed factor (as in all analyses) and a Tukey's HSD test. Leaf damage and the florivory measures were nonnormal even after transformations, and were analyzed using individual Kruskal-Wallis' and Tukey's HSD tests. Second, the total number of pollinator visits per flower per minute could not be made normal with transformations and was analyzed with Kruskal-Wallis and Tukey's HSD tests. Last, to compare plant communities we analyzed only the top 2 most frequent and ubiquitous taxa found across all sites, grasses and goldenrod. Site differences in grass percent cover were tested using ANOVA and Tukey's HSD tests. The number of goldenrod stems per plot could

not be made normal and was analyzed with Kruskal Wallis and Tukey's HSD tests. Lastly, the variation in total, flowering and non-flowering horsenettle densities across sites, with $\log(x+1)$ transformations, was compared using ANOVAs and Tukey's HSD tests.

Question 1: Plant Traits. Two MANOVAs were performed to test for overall site differences between defenses (trichome density, thorn density, thorn size) and floral traits (corolla diameter, anther volume, style length). Thorn density, trichome density and anther volume were log transformed to improve normality; all other variables were normal without transformations. Univariate ANOVAs were performed after significant MANOVAs to determine which traits differed between sites. Next, number of flowers was log transformed to improve normality, and an ANOVA was performed to determine differences between sites in allocation to sexual reproduction. A Chi Square Goodness-of-Fit test was calculated by hand to test for differences in the proportion of plants that flowered across sites using the average proportion as the expected value. Two ANOVAs with Tukey's HSD tests were performed to test whether growth rate and the root:shoot ratio differed between sites. A MANOVA was performed using all 7 plant and leaf size traits to determine if plant size differed across sites. This was significant and subsequent ANOVAs and Tukey's HSD tests were performed to determine which traits differed significantly across sites.

Question 2: Tradeoffs. Correlations were performed to identify the direction and magnitude of potential tradeoffs. Data from all sites were combined because all correlations at the population level were consistent with correlations found with the data from all populations, and they transformed as previously described. To determine if there was a resource allocation tradeoff between defense traits and allocation to reproduction, 3 defense traits (trichome density, thorn density, and thorn size) were correlated with 2 floral traits

(corolla diameter and total flower number). To determine if there was a tradeoff between defense and growth, the 3 defense traits were correlated with growth rate and the root:shoot ratio. To determine if there was a tradeoff between defense and size, a PCA on Z-score transformed plant and leaf size measurements was performed to produce one composite variable representing plant size. The first PC accounted for 47% of the total variation, and all variables loaded positively between 0.20 and 0.49. This principle component was then correlated with all 3 defense traits. Last, to determine if there was a tradeoff between reproduction and growth or size, both reproductive traits were correlated with the growth measurements and the PCA scores for size.

Results

Question 1: Species Interactions

Herbivory. Leaf herbivore diversity and the extent of leaf damage varied across sites (diversity: $F_{3,116} = 64.45$, $p < 0.0001$, damage: Chi Square = 65.39, $df = 3$, $p < 0.0001$; Figure 1A). The HR site had significantly higher herbivore diversity and leaf damage than all other sites. Orchard Hill had significantly more petal damage than the other sites (Chi square = 16.35, $df = 3$, $p = 0.001$), which had almost none (OH = 3.21%, HR = 1.38%, LY = 0.54%, GC = 0.18%). Anther damage also differed significantly across sites (Chi Square = 8.89, $df = 3$, $p = 0.0318$), with significantly more damage at HR than any other site (HR = 27.75%, GC = 19.00%, OH = 15.93%, LY = 14.02%). The proportion of buds damaged also differed across sites (Chi square = 8.79, $df = 3$, $p = 0.0322$), but no differences were found between individual sites in the Tukey's HSD test (GC = 0.11, HR = 0.11, OH = 0.08, LY = 0.02).

Pollination. The number of visits per flower per minute was significantly different across sites (Chi Square = 9.74, df = 3, p = 0.0208). The number of visits was highest at OH, intermediate at LY and lowest at GC and HR (Figure 1B).

Plant Community. The HR and GC sites had significantly higher percent grass cover than the OH and LY sites ($F_{3,32} = 10.37$, $p < 0.0001$; Figure 2A). Conversely, the LY and OH sites had more goldenrod stems per plot than HR and GC (Chi Square = 10.99, df = 3, $p = 0.0118$; Figure 2B). The OH and LY sites also had higher horsenettle densities than the HR and GC sites ($F_{3,32} = 9.99$, $p < 0.0001$; Figure 1C). This difference in total density was due to an increase in flowering stems only (flowering: $F_{3,32} = 7.71$, $p = 0.0005$, non-flowering: $F_{3,32} = 1.14$, $p = 0.348$; Figure 1C).

Question 1: Plant traits

Defenses. In the greenhouse, defense traits differed across plants from different sites in the MANOVA (Wilks' Lambda = 0.629, $F_{9,340.87} = 7.95$, $p < 0.0001$) and univariate analyses for all traits ($F_{11,142} > 3.2$, $p < 0.0005$). However, the prediction that defenses would be the highest in offspring from sites with the most herbivory (HR and OH) was not supported. Trichome density was highest in plants from GC and OH, thorn density was highest in plants from GC and LY, and thorn size was largest in plants from OH (Figure 3A). Expression of all defense traits was consistently, but not statistically, lowest in plants from HR, the site with the highest herbivory (Figure 3A). Defense traits differed by block (Wilks' Lambda = 0.859, $F_{6,280} = 7.95$, $p = 0.0016$) but there was no significant block by site interaction.

Reproduction. No evidence was found to support the prediction that low levels of pollination increased competition for pollinator services and selected for larger or more

flowers. While floral traits differed across sites in the MANOVA (Wilks' Lambda = 0.514, $F_{9,270.3} = 9.44$, $p < 0.0001$) and univariate analyses (Corolla Diameter: $F_{11,113} = 4.71$, $p < 0.0001$; Style Length: $F_{11,113} = 2.86$, $p = 0.0024$; Anther Volume: $F_{11,113} = 2.18$, $p = 0.0202$), neither HR nor GC, the sites with the fewest pollinator visits per flower, had consistently large floral structures (Figure 3B). Plants from LY had significantly smaller corolla diameters, and plants from GC had the longest styles but the smaller anthers. Also, the total number of flowers produced did not vary across sites ($p = 0.29$) However, evidence of relaxed selection with increased pollination was found in LY, having high pollination and the smallest corollas and medium to short styles (Figures 1B & 3B). We also found a strong trend, though not significantly different ($\text{Chi}^2 = .14$, $df = 3$, $p > 0.05$), of decreased flower production at the population level in the offspring from HR. Only 50% of the total plants from HR produced at least one flower, whereas between 80-95% of plants from the other sites produced flowers.

Growth and Size. Neither growth nor size followed the patterns predicted with respect to potential competition. If we assume that sites with high horsenettle density (LY and OH) are the most favorable sites for horsenettle, then those sites should be experiencing high competition, and plants from those sites should be the largest and fastest growing.

Aboveground growth rate did not differ significantly between sites ($F_{11,150} = 1.44$, $p = 0.1589$). Size was significantly different across sites using MANOVA (Wilks' Lambda = 0.476, $F_{21,316.41} = 4.45$, $p < 0.0001$), and individual ANOVAs found differences in leaf midvein length ($F_{11,116} = 2.54$, $p = 0.006$), root biomass ($F_{11,116} = 3.27$, $p = 0.0006$), and shoot biomass ($F_{11,116} = 2.95$, $p = 0.0018$). However, plants grown from LY and OH were not consistently the largest in any measure. Plants from HR and OH had the longest leaves and

greatest shoot biomass, but plants from OH also had the greatest root biomass (Figure 3C). Similarly, the prediction that plants from sites with high competition would have increased investment in current growth was not supported. Plants from HR had significantly lower root:shoot ratios than any other site ($F_{11,116} = 3.99$, $p < 0.0001$), but no other among site differences were found (Figure 4).

Question 2: Tradeoffs

Assuming resources are limited in this plant, tradeoffs between potentially costly traits should be observed. First, several negative correlations with defense and other functions were found, as predicted. Two of the three defense traits were significantly negatively correlated with the PCA scores for plant size, and one negative association was found between thorn density and corolla diameter (Table 3). However, no evidence of a tradeoff was found between any defense traits and aboveground growth rate. Finally, a positive relationship was found between thorn and trichome densities and the root:shoot ratio, indicating that plants that invest highly in defending aboveground tissue also invest in future survival over current aboveground growth (Table 3).

There was also evidence for a tradeoff between reproduction and allocation to survival. Both flower size and total number of flowers were negatively correlated with the root:shoot ratio (Table 3), suggesting that plants have strategies of investing either in current sexual reproduction or in survival for future reproduction. Conversely, a positive relationship was found between plant size and both reproductive measures and between aboveground growth rate and flower size (Table 3). This is consistent with the prediction that larger plants have more resources overall and can thus allocate more to sexual reproduction.

Discussion

Local Adaptation

We hypothesized that if horsenettle populations experienced a selection mosaic, we would find trait values in a common garden that corresponded to interaction levels in the population of origin. Since this study found significant differences between sites in herbivore intensity, pollination and community composition, specific predictions about local adaptation to each of these interactions can be assessed. First, we predicted that if herbivores were a significant selective agent on plant traits, we would see increased defenses in greenhouse grown offspring from populations with high damage levels, but not in plants from populations with low damage levels. Our data show no significant evidence of local adaptation of defenses to herbivory, since mechanical defenses were not higher in plants from sites with high herbivory (HR and OH; Figures 1A and 3A). In fact, plants from the site with the highest herbivory (HR) consistently had the lowest expression of all defense traits, indicating that herbivory was highest at the site with the least defended plants. This pattern could occur if historically low defense levels have led to increased population growth or recruitment of herbivores to this site. If herbivore pressure strongly affects fitness, and if these defenses reduce damage, this site could become an evolutionary hot spot in the future. Other work in horsenettle and a closely related species showed that herbivore levels can reduce plant growth, reproduction and induce the production of chemical defenses (Wise 2003, Walls et al. 2005, Viswanathan et al. 2007, Wise 2007a, Viswanathan et al. 2008), and Wise (2003) found seven herbivores that selected for resistance in horsenettle. Overall, these results do not support the existence of a current selection mosaic on horsenettle's mechanical

defenses caused by herbivore pressure, but they do show the potential for a hot spot to develop.

Second, we predicted that resource allocation to increased defenses might contribute to selection for energy conservation in other traits, such as decreased floral size and number. Although not statistically significant, there is a trend supporting this prediction. The HR site had the most leaf herbivory and anther damage, and the plants grown from the HR site qualitatively produced the fewest flowers and had the smallest percentage of plants that flowered. This suggests that even though we did not find higher mechanical defense levels in plants from the high-herbivory sites there may be other defense traits, such as chemical compounds that we did not consider, that could trade off with reproduction. Walls et al. (2005) found a significant cost to aboveground growth from inducible chemical defenses in horsenettle under certain circumstances. Also, Baldwin et al. (1990) found that simulated herbivore damage only decreased seed set in *Nicotiana sylvestris* when the induction of chemical defenses was allowed to occur; regardless of constitutive defense levels. If a similar phenomenon occurred with the induction of chemical defenses in horsenettle, then our data would support the prediction that the costs of producing defenses reduced reproductive output.

Pollinators may also exert selection on plant traits. We predicted that low pollinator activity would increase competition for pollinator services and result in selection for increased floral size and floral displays, while high pollinator activity might relax selection on floral traits and result in smaller flowers and floral displays. The GC and HR sites had significantly less pollinator activity than the LY and OH sites, and the LY site had smaller corollas than any other site (Figures 1B & 3B). If this finding represents relaxed selection

due to abundant pollination it may be due to the benefits of saving resources, since smaller flowers may receive adequate pollination when pollinators are abundant but cost the plant less to produce (Lahti et al. 2009). However, no trend was found for increased floral size in plants from GC and HR (Figure 3B), meaning we did not find evidence that competition for pollinators selected for increased floral size. Our field data revealed another suggestive pattern regarding flowering strategy and pollination. Sites with the most pollination also had the highest flowering horsenettle density, but all sites had equal densities of non-flowering stems (Figure 1C). This suggests that in populations with increased pollination, horsenettle produced more flowering stems, not more flowers per stem, to take advantage of those services. However, the same trend would be seen if pollinators prefer sites with more flowering stems. Our greenhouse experiment did not allow confirmation that this pattern had a genetic basis because plants were grown from seed and only produced one main stem.

Lastly, we predicted that strong competition would result in selection for larger, faster growing plants that invest heavily in aboveground tissue. We found that sites with the lowest horsenettle densities (least intraspecific competition) also had high grass densities (GC and HR), and that sites with the highest horsenettle densities (most intraspecific competition) also had high goldenrod densities (LY and OH). Plants from the LY and OH sites did not grow faster, become larger or have lower root:shoot ratios than plants from GC and HR. Thus, we found no evidence that intraspecific competition selected for these traits in horsenettle. These trait differences could be due to plasticity in response to crowding (Maliakal et al. 1999, Dorn et al. 2000), rather than due to genetic differences as a result of selection. Several other species in the *Solanum* genus exhibit plastic responses in trait expression (Diggle 1994, Miller and Diggle 2003). For example, when *Solanum hirtum* were allowed to set fruit they

produced more male-only flowers than genetically identical plants that were not allowed to set fruit, indicating that this species changes its sexual expression in response to the energy demands of fruit production (Diggle 1994). We noted in the field that plants growing within dense vegetation of any kind (common at GC, LY and HR) were tall and had few branches, while plants growing on patch edges (most plants at OH) were short and had many branches (H. Bernardo, pers. obs.). However, greenhouse-grown plants from OH also did not differ in size, growth rate, or the root:shoot ratio compared to plants from other sites, suggesting that plant density did not drive genetic differences at our sites and that differences observed in the field are a plastic response to the environment.

While there was no trend supporting a role of competition in the evolution of the root:shoot ratio, there is evidence that herbivory might influence the root:shoot ratio in horsenettle. Plants from the HR site, which had the highest herbivory, had significantly lower root:shoot ratios than plants from other sites. This finding might be evidence that herbivores select for a more annual resource allocation strategy. Many environmental factors have been shown to influence selection on semelparous versus iteroparous reproductive strategies in plants (e.g. Evans et al. 2005, Hautier et al. 2009). However, empirical evidence regarding the role herbivory plays in the evolution of plant reproductive life-history strategies has only been found recently. Miller et al. (2008) found that tree cholla (*Opuntia imbricata*) with more flowers had higher chances of damage by a specialized cactus bug (*Narnia pallidicornis*). High damage decreased flowering success and favored plants with fewer flowers and with a more iteroparous (perennial) life-history strategy. Our data suggest that a similar herbivore-mediated effect on reproduction and life-history strategy might occur in horsenettle, although in the opposite direction.

Tradeoffs

Since resource-mediated selection can be a major component creating selection mosaics (Gomulkiewicz et al. 2000, Strauss et al. 2002), identifying allocation tradeoffs between plant functions could provide insights into selection pressures on plant traits. First, we predicted that defenses would be negatively associated with all other plant functions, as defenses are often costly to produce and only have benefits in the presence of antagonistic species. We found evidence for tradeoffs between defense and plant size, but not aboveground growth rate or reproduction (Table 3). Plant size was negatively correlated with trichome and thorn densities, but positively correlated with thorn size. This suggests that it is more costly for the plant to produce more thorns than larger thorns. One negative correlation between corolla diameter and thorn density was found, but no significant correlations between defenses and total flowers were found. This is at best weak evidence of a tradeoff between defense and reproduction, since total flowers is a stronger predictor of fitness potential than flower size (Obeso 2002). Second, we predicted that size and growth will be positively correlated with reproduction because larger plants can invest more in sexual reproduction (Gomez 2008, Hautier et al. 2009). This was also supported; size and aboveground growth rate were positively associated with reproduction (Table 3).

In this study, we interpret the root:shoot ratio as a measure of interannual growth strategies. A high root:shoot ratio indicates a high investment in survival and future growth, as this plant overwinters as roots and vegetatively spreads through creeping roots (Prostko et al. 1994), and a low root:shoot ratio indicates a high investment in the current season's growth. The root:shoot ratio was positively correlated with two of the three defense traits, indicating that plants that produced more defenses also invested more in root compared to

shoot biomass. The root:shoot ratio was negatively correlated with both measures of reproduction, indicating that plants that reproduced more allocated less to roots. These results suggest that there may be two distinct strategies employed by this species. Overall, plants that invested more in future survival reproduced less currently, a tradeoff that has been found in other plant species (Vilela et al. 2008, Hautier et al. 2009), and defended their vegetative tissue more. This suggests that this species reproduces sexually when conditions are currently favorable and invests more in survival and vegetative spread when conditions are poor. This pattern of differential allocation to reproduction based on environmental conditions has been documented in other perennial plants (Hautier et al. 2009 and citations within), and is thought to be the basis of the evolution of perennial life-history strategies (Stearns 1989, Bergelson and Purrington 1996, Miller et al. 2008, Vilela et al. 2008).

Conclusions

Species interactions can act as selective forces in many different ways under different circumstances, and these variable interactions are the basis of evolutionary and coevolutionary dynamics in meta-populations. This study demonstrates the importance of considering multiple interactions in a community context when examining how variability in ecological interactions can create mosaics in evolutionary pressures. For example, we found that plants with low root:shoot ratios also had high reproduction and low defenses, suggesting a strategy of favoring current reproduction over future survival. Other individuals with higher root:shoot ratios had higher defenses and lower current reproduction, suggesting a strategy of investing in survival and waiting to reproduce. This dichotomy is consistent with general theories on the evolution of perennial life-history strategies in plants (Obeso 2002, Miller et al. 2008), but we would not have uncovered it had we not examined multiple

traits known to mediate each type of interaction. Understanding how selection changes due to changing interactions is especially important when studying agricultural weeds because the selection pressures between the field and the natural habitat may be very different. Overall, this study sheds light on how selection changes over space and time, which is the basis for adaptation and the basis of many of the fascinating traits we find in plants and animals today.

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APPENDIX A

TABLES

Table 1. Description and location of each site.

Site (abbreviation)	Location	Latitude / Longitude	Description
Greenfield Community College (GC)	Greenfield, MA	42°35'48.54"N 72°37'25.31"W	small meadow surrounded by mostly urbanized land
Holyoke Range (HR)	South Hadley, MA	42°17'4.09"N 72°35'51.81"W	small meadow abutting a forested mountain
Leyden Wildlife Management Area (LY)	Greenfield, MA	42°39'17.48"N 72°35'55.00"W	large open meadow surrounded by large tracts of meadow and forest mostly growing along the edges of
Orchard Hill (OH)	Amherst, MA	42°23'37.12"N 72°31'16.05"W	trails running through a medium sized, patchy meadow surrounded by patches of forest and buildings

Table 2. Herbivore damage characteristics and method of measurement

Species	Characteristics	Measurement Method
Leaf Herbivory		
Eggplant Lace Bug, <i>Gargaphia solani</i> (Tingidae)	small, yellow to brown dying spots	number of spots per leaf
Eggplant Leafminer, <i>Tildenia inconspicuenta</i> (Gelechiidae)	miner trails in leaves	number of trails per leaf
False Colorado Potato Beetle, <i>Leptinotarsa juncta</i> (Chrysomelidae)	round, chewed leaf edges	percent leaf area removed
Eggplant Tortoise Beetle, <i>Gratiana pallidula</i> (Chrysomelidae)	large interior holes on leaves	percent leaf area removed
Eggplant Flea Beetle, <i>Epitrix fuscata</i> (Chrysomelidae)	numerous, very small holes in a leaf	grid with 25 4 mm x 4 mm squares placed on the top right of each leaf, number of squares out of the total that contained at least one hole
Unknown #1	small, brown chewed areas	percent leaf area removed
Unknown #2	chewing damage not of any other agent	percent leaf area removed
Floral Herbivory		
Potato Bud Weevil, <i>Anthonomus nigrinus</i> (Curculionidae)	small hole chewed into flower bud base	proportion buds with holes out of total surveyed
Petal Damage	holes in petals	percent area removed
Anther Damage	brown chew marks	percent surface damaged or removed

Table 3. Pearson Product-Moment correlation coefficients. Bold indicates significant values ($p \leq 0.05$), and italics indicate marginal significance ($p = 0.07$). Size PC refers PCA scores obtained from combining all size measurements. Growth rate is the aboveground growth rate in mm per day. Root:Shoot refers to the ratio of root dry biomass to shoot dry biomass.

	Size PC	Growth Rate	Root:Shoot	Total Flowers	Corolla Diameter
Trichome Density	-0.35	-0.02	0.40	-0.06	-0.06
Thorn Density	-0.17	-0.05	0.18	0.08	-0.21
Thorn Size	0.26	0.12	-0.11	0.13	0.03
Total Flowers	0.21	0.11	<i>-0.18</i>	---	---
Corolla Diameter	0.21	0.21	-0.33	-0.02	---

APPENDIX B

FIGURES

Figure 1. Site variation in: A) The average herbivore diversity and extent of damage per plant; B) The average number of pollinator visits per flower per minute; C) Total horsenettle density and total density broken into flowering and non flowering stems. Bars represent standard error. Different letters indicate statistically significant differences in the same response across sites.

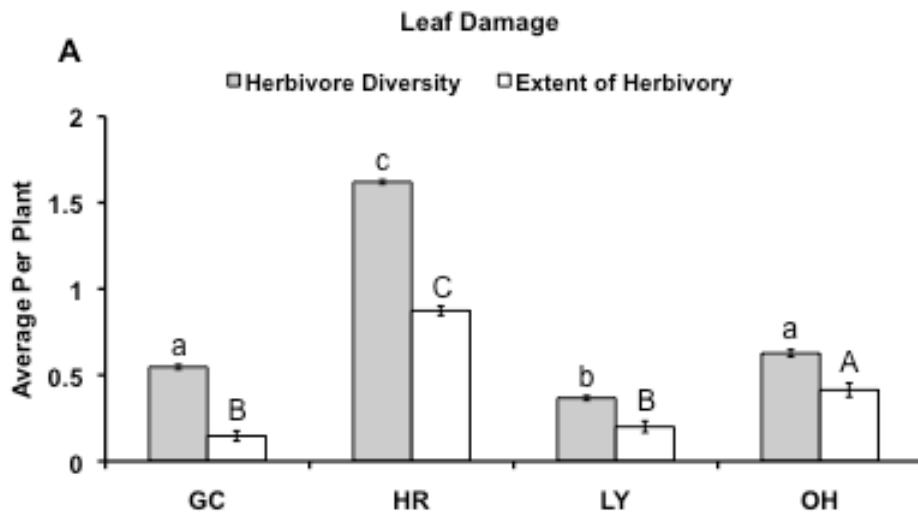


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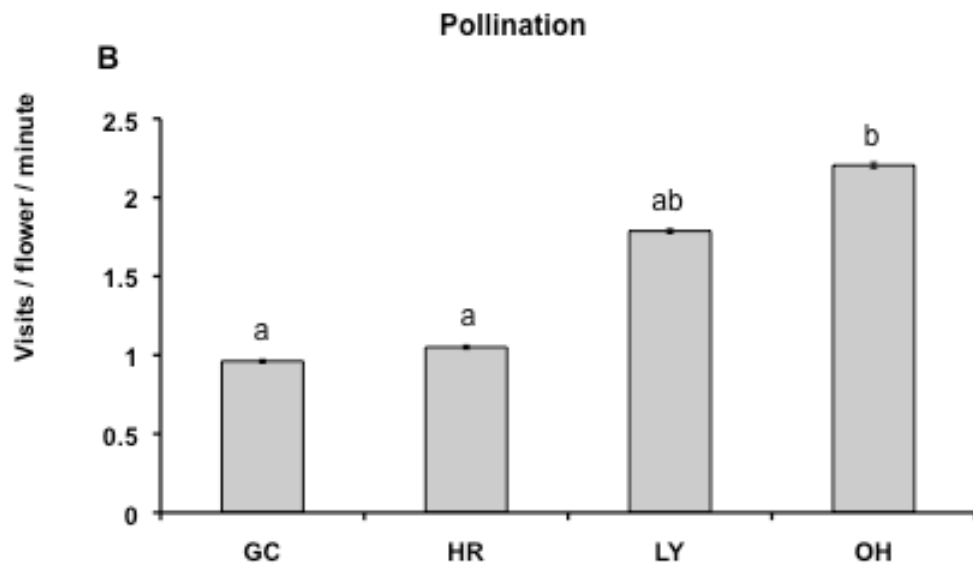


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Horsenettle Densities

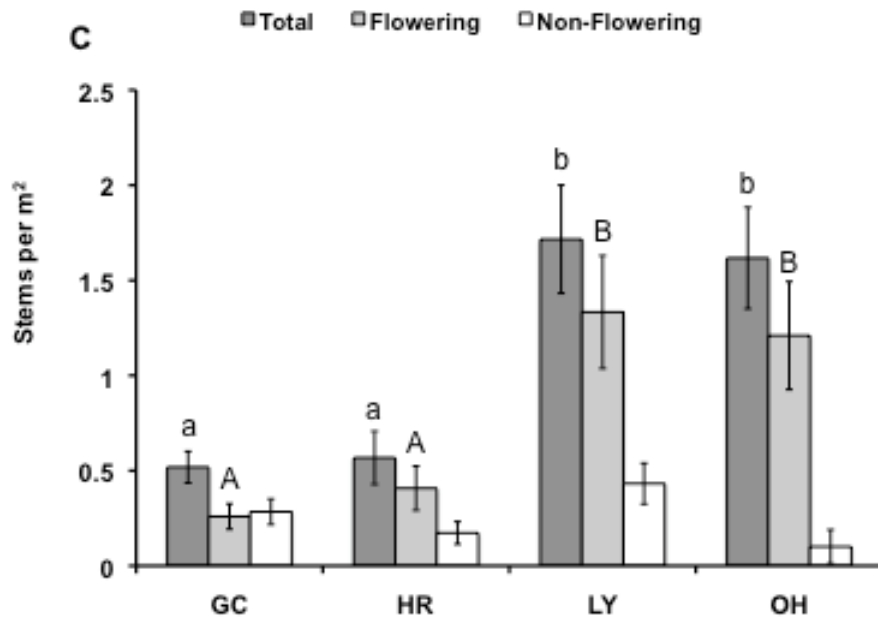


Figure 2. Site variation in: A) The average percent grass cover per plot; B) The average number of goldenrod stems per plot. Bars represent standard error. Different letters indicate statistically significant differences across sites.

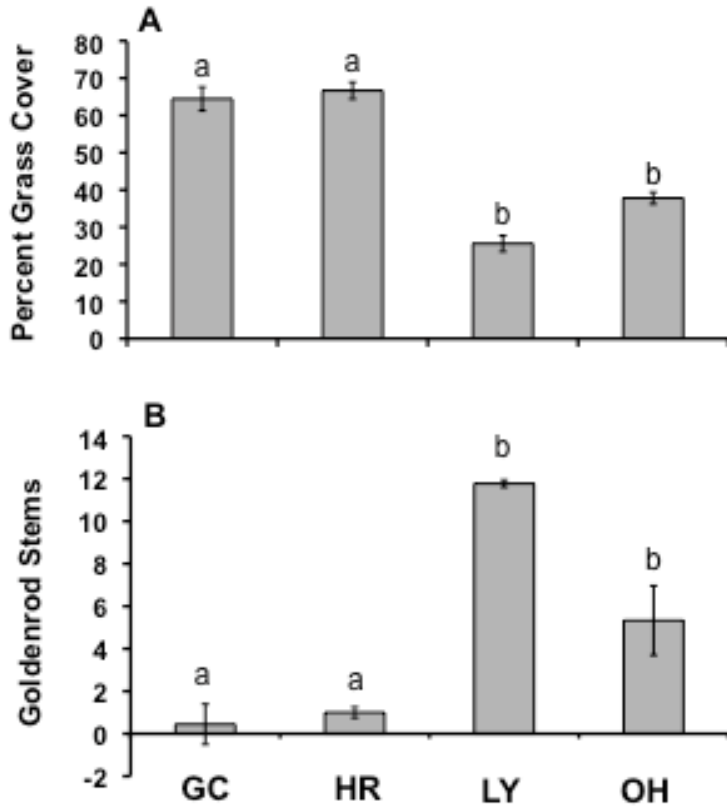


Figure 3. Site variation in: A) Trichome density and thorn density in number per mm², and thorn size in mm; B) Corolla diameter and style length in mm, and anther volume in mm²; C) Significant size traits, shoot and root biomass in g, and midvein length in mm. Bars represent standard error. Different letters indicate statistically significant differences in the same response across sites.

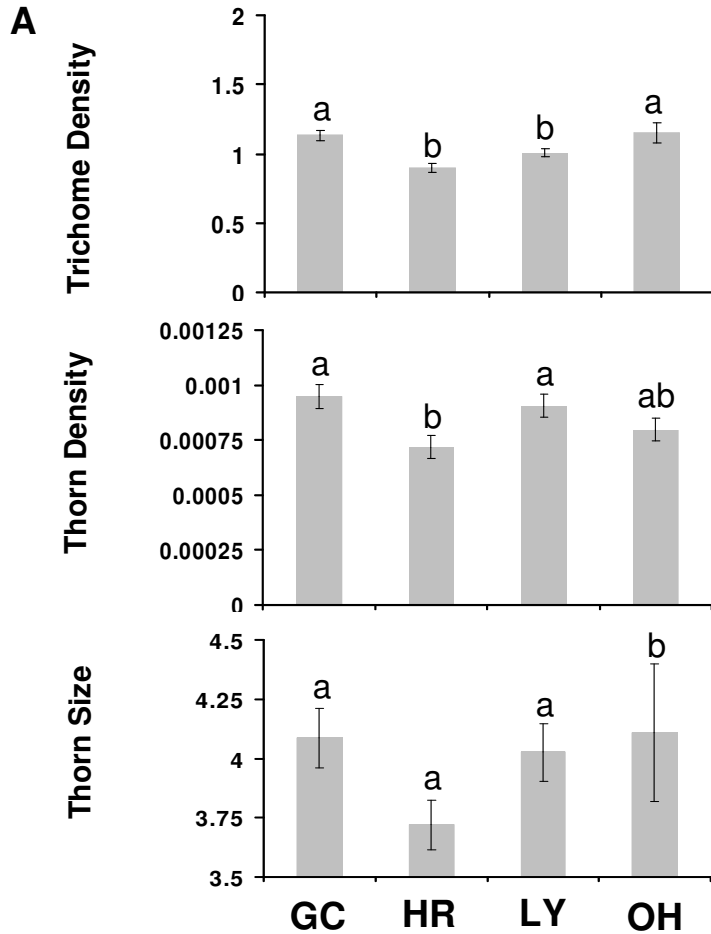


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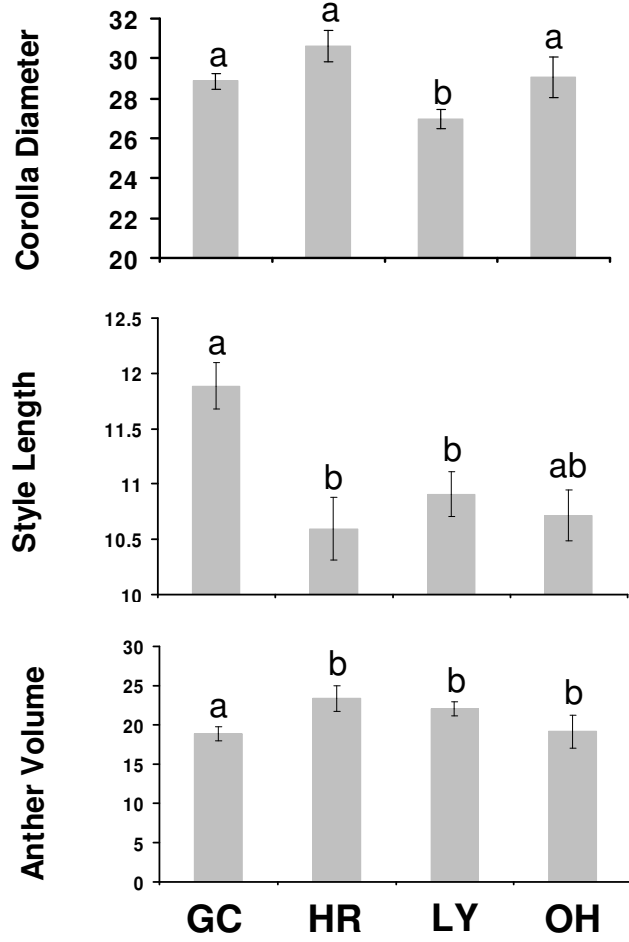


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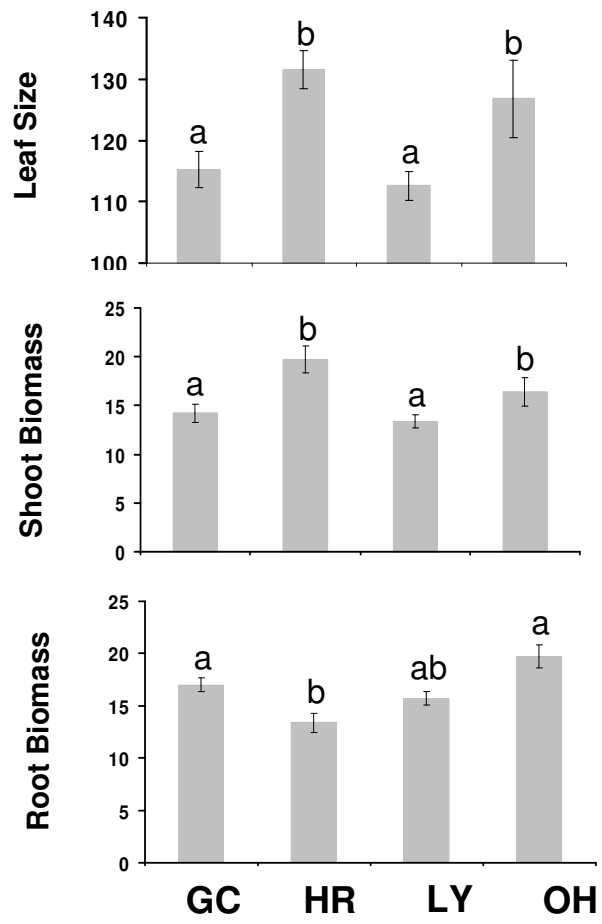
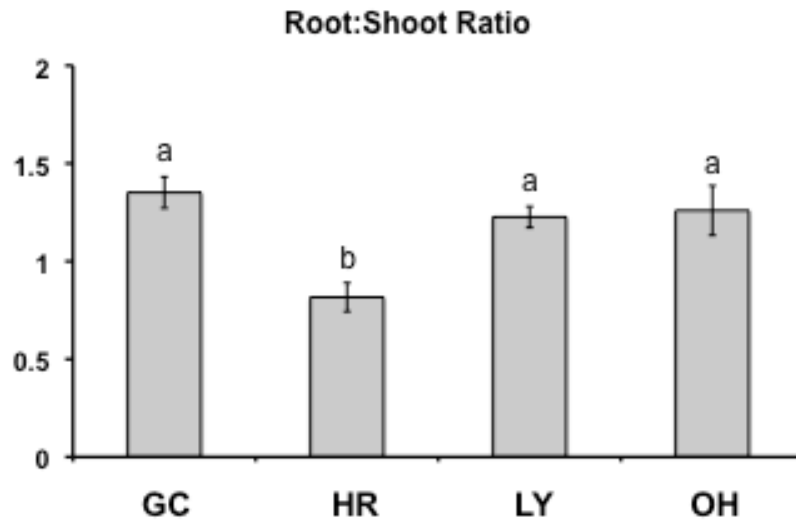


Figure 4. Site variation in the root to shoot ratio. Bars represent standard error. Different letters indicate statistically significant differences across sites.



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