

The Effects of Flowering Phenology on Plant Fitness across a Light Quality
Gradient in the Native Mustard *Cardamine cordifolia*.

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Abstract

There are many abiotic and biotic factors that affect plant distribution. Both of these have the potential to create strong selective pressures and drive habitat specialization. Here *Cardamine cordifolia* is used to investigate the differences in flowering phenology and bittercress fitness across and among a light quality gradient. *Cardamine cordifolia* (bittercress) is a native mustard found along streams and sustainable water sources in sub-alpine regions, and in particular in the Rocky Mountains. Bittercress exhibits distinctive phenotypes at varying light qualities resulting from phenotypic plasticity and to some extent ecotypic differentiation (Whiteman et al 2012 in review). Flowering phenology for this plant was monitored to determine if a separation of flowering time within the different environments was a mechanism for a pre-zygotic isolation barrier, which would facilitate local adaptation by limiting gene flow. Furthermore, the fitness of the bittercress was assessed to gain an understanding of the degree of selective pressures faced within each environment. Due to the early snow melt and advanced life-cycles of plants only the tail end of the flowering season was captured. Even with this disadvantage a distinct trend of flowering was observed. This trend suggests that there may be a very strong isolation barrier present. It was found that the lowest light quality environment (evergreen-shade) had the highest fitness. The fitness component, seed count, was used as the closest measure to the actual fitness of the plant. Willow-shade, had the next highest fitness, and sun with the least. This study has added to our understanding of plant distributions. Given that there is much yet to be discerned. This could support the idea that the overall fitness of bittercress is not solely dependent on a higher proportion of flowers on a single stem, but is likely largely affected by pollinator behavior in addition to stem density. To determine how strong selection pressure is in aiding local adaptation, the extent of gene flow via pollination needs to be quantified.

Introduction:

Understanding the factors shaping species distributions is a fundamental problem in ecology. Numerous studies have investigated how a single abiotic or biotic factor shapes plant distributions. Common factors shaping plant distributions include light availability and quality, herbivory, and water availability. Differences in light quality among habitats have shown to have a prominent influence on local plant distributions.

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In several systems, the interaction between light quality and herbivory has played an important role in shaping plant distributions (Coley et al. 1985, Crone and Jones 1999, Dudt and Shure 1994, Greig-Smith 1983, Harper 1969, 1977, Huffaker and Kennett 1959, Louda et al 1987, Louda and Rodman 1996, Maiorana 1981, Mooney et al. 2009, Niesenbaum 1992). Previous research at the Rocky Mountain Biological Laboratory found certain plants in the sun face high herbivory and high light levels, and plants in the shade face low herbivory and low light levels (Collinge and Louda 1988, Louda 1988, Collinge and Louda 1989, Louda and Rodman 1996). In this case, specialized herbivores were more abundant in the sun than the shade (Chew 1975, 1977, Collinge and Louda 1988a, Collinge and Louda 1988b, 1989b). Thus, selection pressure from herbivores may force plants to invade less ideal light habitats, but offer enemy-free space.

Another mechanism of explaining light quality gradient distributions is variation in plant fitness. The amount of quality light a plant receives can influence the amount of energy it can invest in reproductive traits, such as flower quantity. Reproductive success is largely dependent on the life-cycle phase flowering phenology. Many out-crossing plant species rely on pollinators to vector pollen from male to female reproductive organs. Therefore, pollinators serve to facilitate the maintenance of genetic variation within populations. As such, flowering phenology can also play a critical role in creating and maintaining genotypic differentiation in a population. Variation in flowering phenology patterns within a population has the potential to limit gene flow. This potential pre-mating isolating mechanism could promote and reinforce local adaption which would in turn shape species distributions. Adding to the complexity, herbivores have been shown to reduce plant fitness (Marquis 1984, Strauss 1991). The investment in anti-herbivory defenses for survival traditionally results in a compromise of resources required for growth, thus causing a reduction in fitness. As such, when investigating species distributions it is necessary to consider the reproductive success that plant exhibits among its environments.

In this study a native mustard, *Cardamine cordifolia* (bittercress), common name heart-leaf bittercress, is used to investigate plant distributions. Bittercress is commonly found along mountain streams and water sources in the Rocky Mountains. It exhibits heart-shaped leaves, four-petaled flowers on a dense raceme, and dense growth clusters interconnected by underground rhizomes (Harrington 1954; Chew 1977; Louda and Rodman 1983). Bittercress is capable of clonal reproduction and sexual reproduction (Source). Plants require pollinators to deliver pollen, but are capable of being selfed (R. Irwin, personal communication).

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Bittercress serves as an excellent model organism to explore light and herbivory gradient driven plant distributions. Bittercress is found in a variety of micro-habitats, each of which exhibits distinctive phenotypic traits. Over ten years has been spent characterizing the herbivory differentiation along a light quality gradient. A series of experiments were conducted to test the hypothesis that herbivory pushes bittercress into ecotonal and dense shade. Louda did find that herbivory was a major driver of bittercress spatial distribution, but surprisingly did not find evidence of ecotypic differentiation (cite Louda). In 2012, Humphrey et al. (in review) conducted a common garden reciprocal transplant experiment of rhizomes from sun-exposed and evergreen-shaded source habitats. Humphrey et al. (in review) found the effects of the light environment depended on the source population for a variety of vegetative plant traits, as well as differential resistance to herbivores (Figure 6). The common garden experiment supports that hypothesis that ecotypic differentiation is present between light quality habitats; further making bittercress an excellent system to understand the factors contributing to local adaptation.

Still unknown are the precise phenology patterns of bittercress among these light quality habitats. Past studies have seen that before the fourth week after snow melt, plants in sun-exposed habitats are phenologically more advanced than ecotonal willow-shaded habitats, but after the fourth week the willow-shaded plants catch up to the sun-exposed plants (Collinge and Louda 1989). However, no studies were conducted on plants from evergreen-shade, which is deeper and relatively permanent shade. If the period of flowering between these habitats does not overlap, or overlap only slightly, the differences in flowering phenology may act as a barrier to gene flow, via pollinators, between the different light quality gradients. This barrier would facilitate habitat specialization and ecotypic differentiation between evergreen shade and open sun habitats; supported by Humphrey et al.'s preliminary evidence.

When comparing plant distributions, I hypothesize bittercress will be phenologically more advanced as light quality increases across this environmental gradient. I also predict that bittercress may employ multiple strategies between habitats to maintain a relatively constant fitness along the light quality gradient as this native mustard trades-off between various biotic and abiotic stressors; mainly high herbivory verses low light quality.

The main objectives of this study were to: (1) Monitor bittercress flowering phenology within and across a light quality gradient, (2) Assess bittercress fitness within and across a light quality gradient, and integrate this information to (3) Estimate the potential role pollinators play in mediating gene flow

among plants within and across a light quality gradient based on flowering phenology and plant fitness differences among habitats. Understanding flowering phenology and fitness differences between these habitats is essential for determining the role of gene flow in affecting local adaptation.

Methods:

Three differential light quality gradients of bittercress were chosen to study; open-sun (<5% canopy coverage), evergreen-shade (>80% evergreen canopy coverage), and willow ecotonal (> 5% but <80% deciduous canopy coverage). A densiometer was used to determine the percent of canopy cover for each patch.

Phenology and Fitness along a Light Quality Gradient

Along the 401 trail two sun, two evergreen shade, and two ecotonal willow locations were established to observe flowering phenology and assess plant fitness. Within each of these six field sites, patches of clusters of *Cardamine cordifolia* (we assume stems are clonal within a cluster) were designated. In each patch, five focal plants were marked with flagging tape. These focal plants were used to measure flowering phenology traits and fitness components of bittercress. In total three-hundred and fifteen plants were monitored (Figure 1). Conditions at each plot area was quantified by percentage canopy coverage, PAR (photosynthetic active radiation), and marked with a GPS unit (Figure 1).

Phenology

To determine if the flowering phenology differed on a light quality gradient, reproductive traits were measured at regular intervals on focal plants. The following traits were recorded for each inflorescence; number of open flowers, phase of floral development of flowers (Delvin and Stephenson 1985), number of aborted buds, number of aborted outgrowths, number of aborted siliques, number of potential siliques, number of developing siliques, and length of each developing silique.

Fitness

To determine if fitness differed on a light quality gradient, the fitness component seed count was as a representation of the reproductive success of bittercress. At the end of the season, defined as when siliques were first observed to be dehiscent, the inflorescences of all focal plants were collected, the length and width of each silique was measured, and number of viable seeds for each silique was

counted. In the case of siliques that had already dehisced, or had induced dehiscence upon collection, the seed count was determined by the indentation left in the silique.

In addition to assessing fitness differences I sought to determine if length or girth of a silique was an accurate indicator of number of seeds for that silique. To assess if a relationship may exist between seed count and silique length or width for bittercress the measured length and girth of a silique was compared to the observed seed count.

Results:

Phenology and Fitness along a Light Quality Gradient

Statistical Analysis for Figures 2 and 3 were performed using R. Statistical Analysis for Figures 4 and 5 were conducted with JMP 10.

Phenology

Quantification of variation in flowering phenology was done by comparing observations of focal plants among and within each environment. The total number of flowers present in each environment was tested at three different time points corresponding to the approximate dates June 29, 2012; July 05, 2012 and July 13, 2012 (Figure 2). To account for an un-equal sample size from each light quality gradient the proportion of stems flowering was used to assess variation in flowering phenology. Furthermore, based on the Shapiro-Wilk normality test these proportions were over-dispersed. A generally accepted way to reduce the effects of over dispersion on hypothesis testing is to compare the observed F-statistic of an ANOVA against an empirical distribution of F-statistics generated by randomly assigning factor levels to response variables. An ANOVA (light environment as sole factor with three levels) was compared against an empirical distribution of F-statistics by randomly assigning the factor levels (shade, sun, willow) to response variables (10,000 replicates). For each time point a p-value of 0.233 and 0.007 was obtained respectively. The first time point measured showed no statistically significant difference, whereas the second time point showed willow plants to have a higher proportion of flowers than sun or shade plants.

Fitness

Statistical comparisons of variation in fitness were done by comparing the total number of seeds produced by each plant among different environments (Figure 3). The number of seeds did not follow a

normal distribution but after being square root-transformed, it approximated a normal distribution (Shapiro-Wilk normality test). The square-root of number of seeds per plant vs. habitat type showed a statistically significant difference among habitats ($F=6.03$, $p=0.0075$).

Dissecting fitness variation one step further, the amount of seeds produced from each silique was taken into account. A ANOVA was conducted comparing average number of seeds per plant per fruit by habitat type and yielded a statistically significant result (Figure 4) ($F = 9.2792$, $p = 0.0002$). The mean number of seeds per plant per fruit was significantly higher in the shade (mean = 5.30111), than in the sun or willow environments, but the sun and willow habitats were not significantly different from one another (mean = 3.23428, 3.44606 respectively).

Linear regressions were performed to determine the relationship, if any, between plant fitness (number of seeds), and silique length or girth. A bivariate model was used to compare seed count by length or girth for each environment (Figure 5). When fitness was compared to length the Rsquared values derived were X,Y, and Z in the shade, willow, and sun habitats respectively. When fitness was compared to girth the Rsquared values were X,Y, and Z in the shade, willow and sun habitats respectively.

Discussion:

Phenology

After the first time-point (A), the proportions of stems flowering in willow and evergreen-shade light quality categories had higher means and were outside of the standard error bars of the sun light quality category (although $P>0.05$). After the second time point (B) I found a proportionally larger number of flowers in the willow habitat than in the sun or shade. Even at time point A, there was a relatively low overall proportion of stems flowering. Perhaps if the study has started earlier in the season there would have been a larger proportion flowering and a distinctive trend could be observed. It was clear there was a decrease in the proportions of stems flowering over the time points, suggesting in addition to the increase in post-flower outgrowths, the flowering trend observed was not an artifact of buds waiting to bloom, but was in fact the tail end of the flowering life cycle stage for the season among all the environments. Due to the early snow melt and advanced cycles of all plants in the basin we were unable to record the entire flowering duration of all the environment types. Therefore the lack of significance found may not be because there is not difference, but is based on the timing of the

observations. Unfortunately the extent of the overlap in flowering phenology between environments could not be properly assessed this season.

Fitness

The stems of *C. cordifolia* in the shade exhibited the highest observed level of fitness (number of seeds), in comparison to those in the willow, or sun environments. As discussed previously, one would assume there would be a cost and benefit to living in any environment. In the case of shade bittercress the benefit they gain from contending with low light quality is increased fitness. It seems paradoxical then that bittercress would be distributed in the sun where it has the lowest fitness and highest herbivory associated with it. In the past Louda conducted an experiment that showed a decrease in herbivory, via use of insecticides, resulted in an increase of fitness; suggesting that herbivory may be a major factor regulating fitness (cite). Another explanation for the observed decrease in fitness may be due to the extremely dry conditions faced by plants this summer. Perhaps the sun plants were more water stressed than in previous years, thus negatively affecting their fitness. Based on global climate change and David Inouye's snow melt data, the snow melt is happening on average three days earlier this year (cite). This could mean in future years on average bittercress will be faced with environmental conditions similar to this season, meaning that unless the sun environment better adapts to the sun; their decreased fitness will result in an even more reduced distribution among the sun.

The length of siliques is a relatively strong indicator of the fitness. It seems like common sense that silique length would be correlated with the number of seeds in a silique, because a) a longer pod provides more room for seed surface area, and b) why would a plant invest energy for growth if there is no benefit to it. Then it seems logical that silique girth would also be correlated with the number of seeds in a silique for the same reason, yet this is not the case. One explanation is that that since the range of the girth was so small (0.5- 2.3 mm), the distribution could not be accurately assessed. Another cause for this lack of significance could be rooted in the level of maturity of the siliques. Even in an immature pod, the number of would-be healthy seeds could be counted. Perhaps given more time to mature these siliques would widen and then more accurately reflect the amount of seeds contained within.

This study has added to our understanding of plant distributions. Given that there is much yet to be discerned. For example, it is interesting to note that the statistically significant higher proportion of stems flowering in the willow environment at time point B did not result in a parallel increase in overall

fitness. This could support the idea that the overall fitness of bittercress is not solely dependent on a higher proportion of flowers on a single stem, but is likely largely affected by pollinator behavior in addition to stem density. To determine how strong selection pressure is in aiding local adaption, the extent of gene flow via pollination needs to be quantified.

Appendix of Figures:

Figure 1: GPS Map of Sites

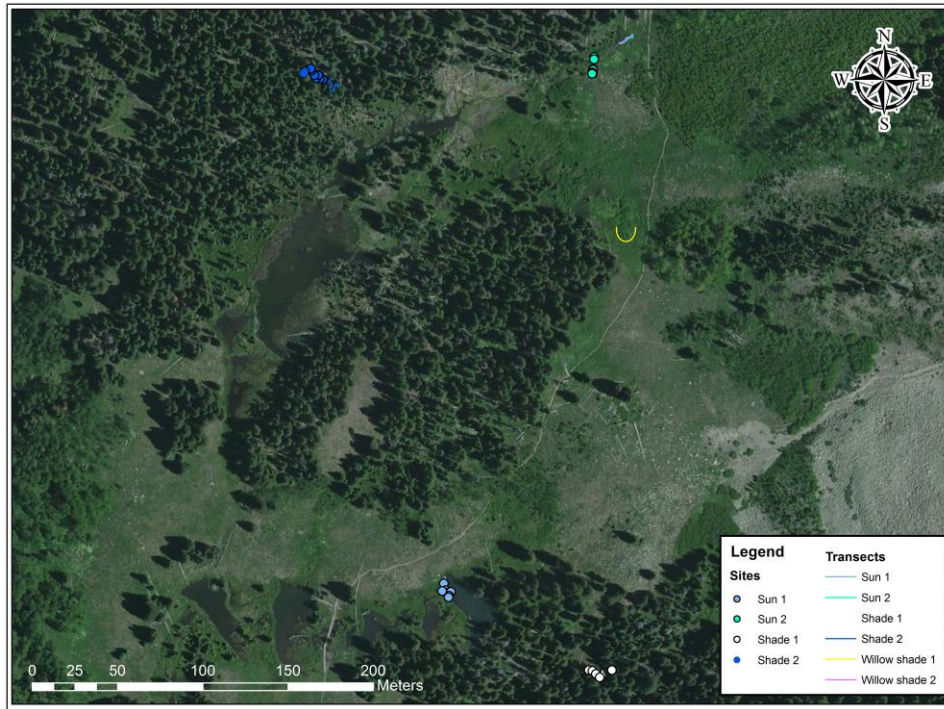


Figure 2: Hierarchy of Study Design

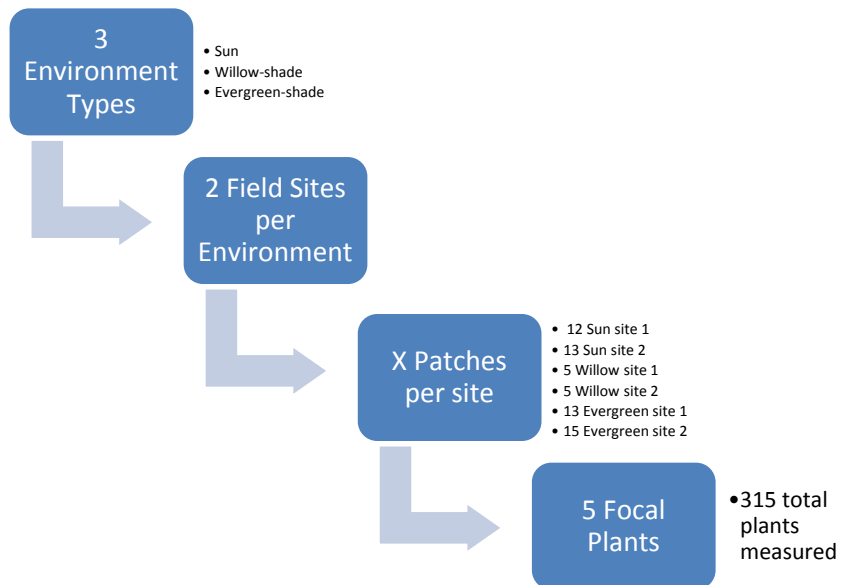


Figure 3: Flowering Phenology by Date

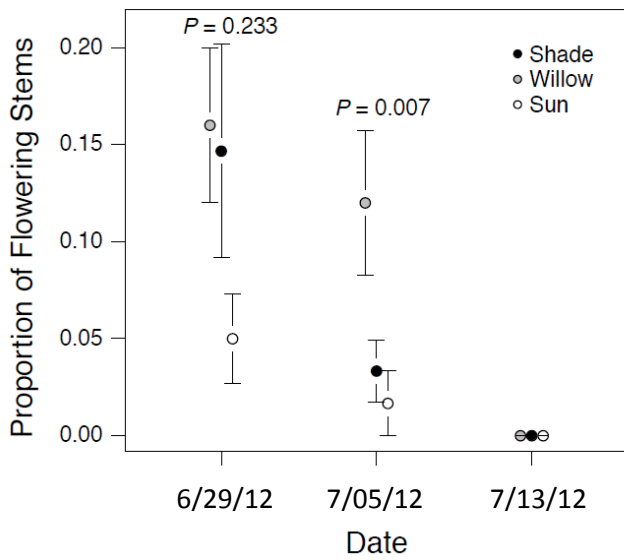


Figure 4: Plant Fitness by Environment

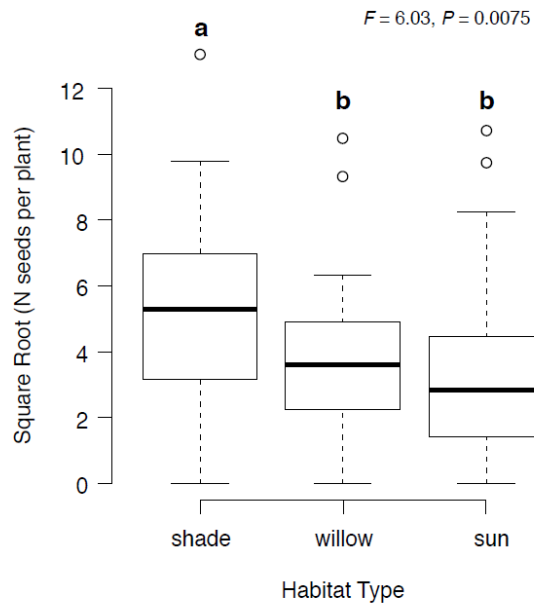


Figure 5: Comparison of Average Number of Seeds per Silique by Plant across Environments

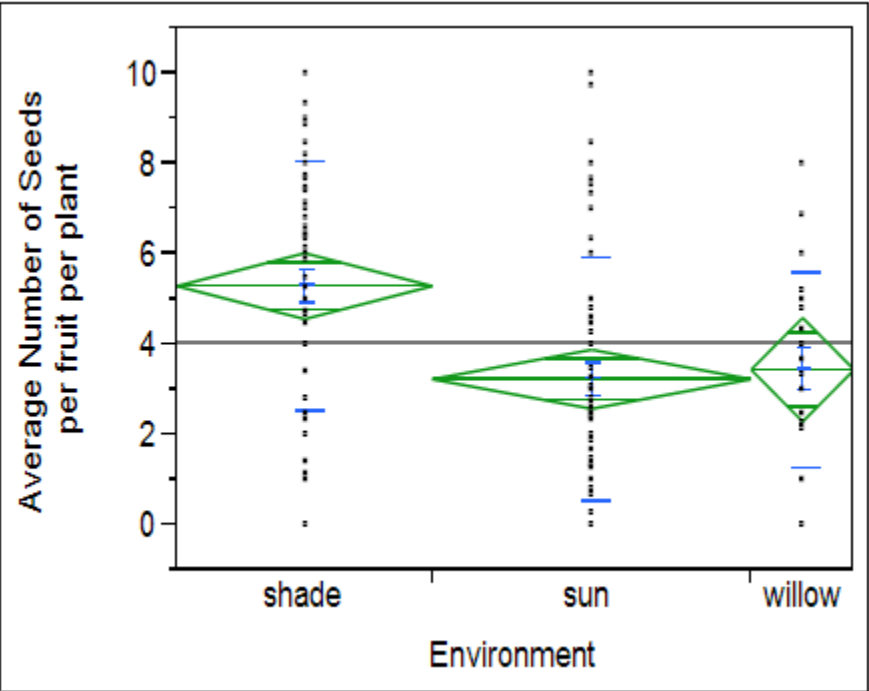


Figure 6: Correlation of Silique Length and Width by Environment

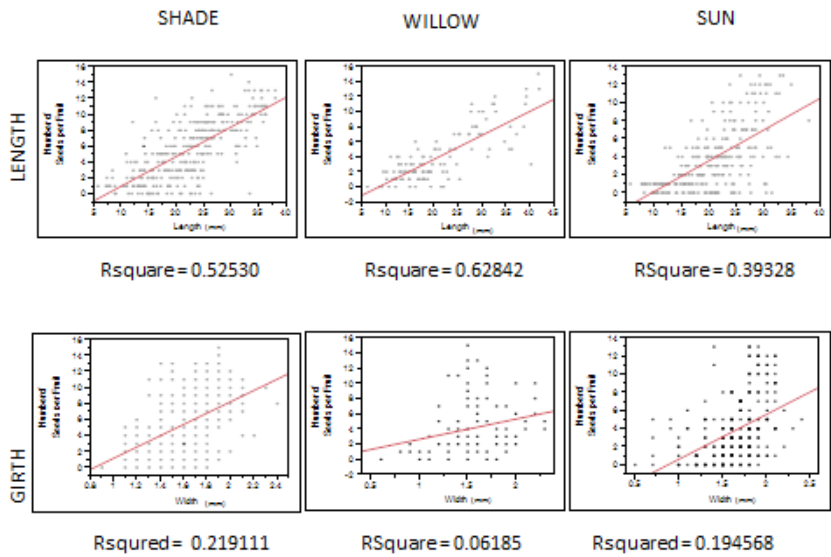
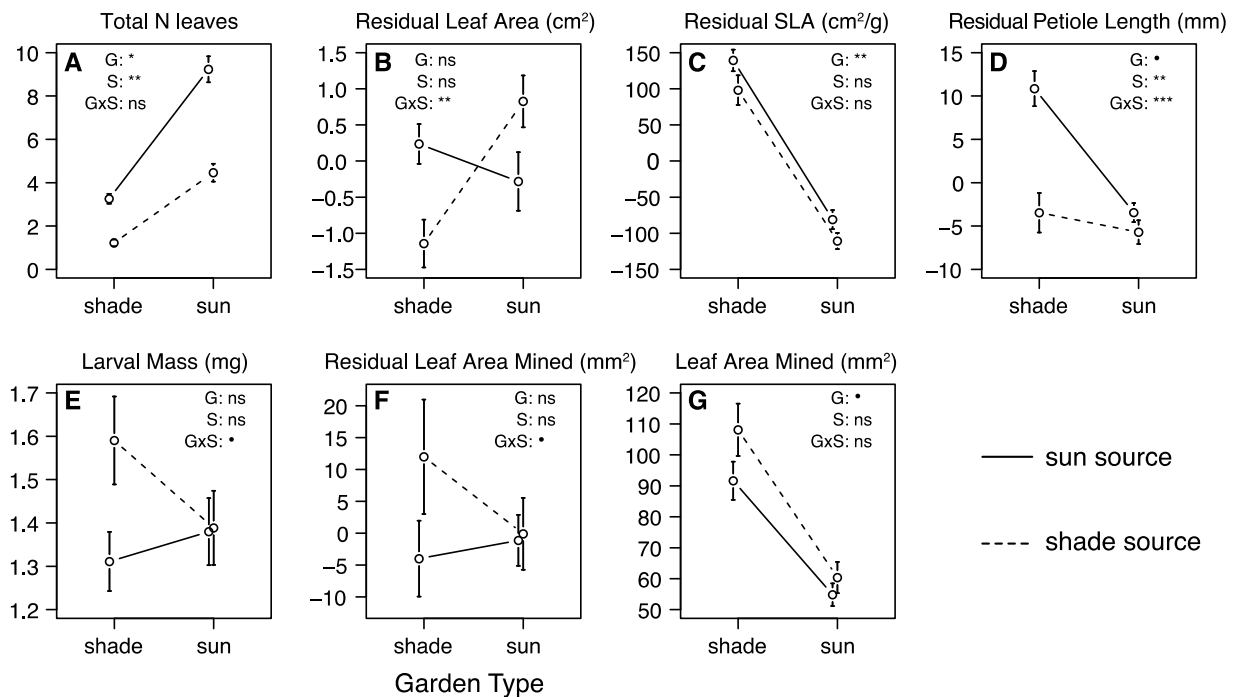


Figure 7: Humphrey al. et. 2012 Source x Garden Experiment



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