

***Cardamine cordifolia*: Pollination effects on seed and fruit production.**

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Abstract

I investigated the pollination ecology of *Cardamine cordifolia*, and how pollinators affected fruit and seed production especially in relation to variation in herbivory and habitat type in 2 different sites. Site 1 was more exposed to the sunlight and site 2 was less exposed to the sunlight. I investigated the degree of self-compatibility, pollinator-visitation rates, correlation between floral traits and plant height, and fruit and seed production. I hypothesized that outcrossed pollen supplemental to flowers would increase fruit and seed set, that herbivore damage would impact the strength of pollen limitation and that longer floral traits and taller plants would be associated with increased pollinator visitation and plant reproduction. *C. cordifolia* was both self-compatible and pollinator limited, but outcrossed pollen supplemental plants produced more fruits and seeds than self-pollinated flowers. The difference in the proportion of fruit and seed set between no bag outcrossed and the open pollination treatments suggested pollen limitation of seed set. Pieridae (cabbage milky butterflies) performed more visits than any other visitors. Herbivore damage resulted in changes in how pollinators selected a plant. I found no correlation between floral traits and plant height. This study showed the complexity of a plant-animal interaction, and the benefits that each organism receives from it.

Introduction

Flowering plants are the most abundant of the seed plants with over 275,000 species. They possess a unique structure called the flower, which includes the reproductive parts of the plant (Ehrlich et al. 1976). Land plants are static; they cannot move to find food, shelter, or to seek their mates. They often rely on the mobility of other living organisms to transfer pollen and to disperse seeds (Raven and Evert, 1981). In the case of pollination, bees are common and highly efficient pollinators, but there are other visitors such as, butterflies, moths, birds, and a variety of flies, beetles, and wasps that can also accomplish pollination (Arms and Camp, 1979). Pollinators are attracted by floral traits like color, shape, odor, and fragrance that enhance the probability of pollinator visitation to flowers. Flowers presumably exhibit floral characteristics to efficiently exploit the capabilities of different animal and insect pollinators (Ray and et al. 1983), while minimizing the costs of producing floral traits and attracting floral enemies. The goal of the present study was to test the importance of pollination to the native montane crucifer, *Cardamine cordifolia* (Mustard family).

Plants and animals form a chain of mutualistic interactions, where both organisms benefit from that relationship. Plants provide food and nectar for animals, and animal pollinators promote outcrossing in plants (Krebs, 1994). However, plant-pollinator interactions are not based solely on the pairwise interaction between plants and their floral visitors. Factors like habitat, nectar robbers, herbivory, stress on plants, and seed predators can have a variety of effects on plant-pollinator interactions and on subsequent fruit and seed production in flowering plants. For example, Cariveau et al. (2004) found that fruit and seed set *Castilleja linariaefolia* (Scrophulariaceae) may be hindered, in part, by seed predation by plume moth larva and fly larvae. In this system pre-dispersal seed predators may exert stronger selection on floral traits than pollinators because of their negative effects on seed production. Such conflicts between pollination and plant damage are also common in the mustard family. For example, floral traits, such as flower color in wild radish, likely represent a compromise between pollinator attraction and plant defense (Irwin et al. 2003). In Alpine areas of Western Colorado, Louda and Rodman (1996) investigated habitat effects on *Cardamine cordifolia*. They suggested that higher insect damage to *C. cordifolia* in full sun, relative to shade, led to significantly decreased vegetative growth, reduced flowering, and decreased plant density. Moreover, if stress was applied to *C. cordifolia*, plants tended to produce fewer fruits, and herbivory increased (Louda and Collinge, 1992). The relative importance of pollination to the results of Louda and Rodman(1996) and louda and Collinge (1992) in *C cordifolia* is unknown.

The purpose of this study was to investigate the pollination ecology of *C. cordifolia*. Because little is known about its reproductive biology, it is of interest to

know how pollinators affect fruit and seed production on this plant, especially in relation to variation in herbivory and habitat type and because *C. Cordifolia* is an important wetland indicator species. In particular the following questions were addressed: (1) Is the reproduction of *C. cordifolia* limited by pollinator visitation and how that strength of pollen limitation varies with plant damage by herbivores? (2) Who are the major floral visitors? (3) Do floral and plant traits influence pollination visitation and reproduction, and are floral traits correlated? I hypothesized that outcrossed pollen supplemental to flowers would increase fruit and seed set, that herbivore damage would impact the strength of pollen limitation, and that longer floral traits and taller plants would be associated with increased pollinator visitation and plant reproduction.

Methods

Cardamine. cordifolia is a perennial plant that belongs to the Mustard family. It appears after snow melt and grows in moist and wet open sites throughout the Rocky Mountains from Colorado to Montana and New Mexico (Kershaw et al. 1998). It flourishes on old beaver dams, along cold streams, and in flowing or standing water from montane to subalpine ecosystems (Warren, 1998). The flowers of *C. cordifolia* are characterized by 4 white petals with blooms in racemes; and the leaves have a heart-kidney shape. *Cardamine. cordofilia* blooms throughout the summer from June to August and grows up to 80 cm tall. When it reaches maturity, the fruiting pods (called siliques) are less than 2 cm long (Harte, 2003).

To study *C. cordifolia*, I chose two sites. Site 1 was located near Copper Creek (38°57.360'N, 106°59.328'W) under partial shade; and Site 2 was located along the U.S. Forest service trail 401 (38°58.453'N, 106°59.447'W) close to beaver dams and more exposed to the sunlight. In each site, 40 plants were randomly selected and divided into four subgroups of ten. Plants were chosen prior to flowering. I applied the following treatments: (1) Bagged self-pollinated (The pollen of one flower was transferred to the stigma of a different flower on the same plant to test for self compatibility), (2) Bagged outcrossed (The pollen of a plant was transferred to the stigma of a different plant), (3) Unbagged outcrossed with supplemental pollen (Pollen of one plant was transferred to the stigma of a different individual) and (4) Unbagged, left for open pollination. Bags made of breided veil were used to deter pollinators from foraging on plants in the “Bagged” treatments. Bags were placed over the plants in the bud stage and were removed once plants ceased blooming.

Cardamine. cordifolia plants on the U.S. Forest Service 401 trail were more damage than plants at the copper Creek site (see results). To test if plant damage by herbivores affected plant reproduction, a third site was chosen. Site 3 (38°57.358'N, 106°59.310'W) was located 13.2 meters away from Site 1. I randomly selected 16 plants and divided them into subgroups of 4. To apply the treatments mentioned above; each subgroup was marked from 1 to 4 and 4 leaves per plant were hand damaged by making one hole in each leaf, approximately 10% leaf removal, with a hole punch. The same data collection, pollinator visits, and floral measurements described below were performed and compared with Site 2.

Hand pollination treatments were performed once the flowers opened and the stigma became receptive (flowers may be slightly protandrous, personal observation by M. Rodriguez). Every other day flowers were hand-pollinated and the following information was recorded: the number of flowers open in each treatment plant, the number of flowers hand-pollinated, and herbivore and plant damage (number of leaves damage and number of flower damage). Once plants were in full bloom, I recorded floral measurements (petal length and width) and plant height. I recorded these characters because they have been shown to influence pollinator visitation and plant fitness in other systems (Cariveau et al. 2004). Pollinator observations were made twice per week for 2 hours per site approximately in the morning and at mid-day. Pollinator visits to unbagged plants and to the whole site in general were observed. Visiting species were identified; the number of plants visited, the number of flowers probed, and the time per flower were recorded. For each observation period, I calculated the mean # of flowers opened per site, the mean of damage flowers per site, and the mean of damage leaves per site. Finally, when fruits were mature, I collected all fruits, recorded whether or not they aborted, and I counted the number of seeds in each successful fruit. To see how pollen limitation varied with plant damage by herbivores in the reproduction of *C. cordifolia*, a MANOVA analysis was performed. Multiple Variance analysis was done to explain how visitation varied per site and how leaf and flower damage was related to it. Last, Analysis of variance was performed to see the difference between the two sites among 3 variables: Percent fruit set, seeds per fruit, and the total seeds per plant.

Results

Plants at site 1 (Willey Site) produced significantly, more successful fruits than plants at site 2 (401 trail) (ANOVA: $F_{1,7}=7.14$, $P=0.03$), with 74.5% higher fruit set (Fig.1 and Table 1 on Appendix). In comparing pollen limitation between the four treatments in both sites, I observed that unbagged outcrossed plants that received supplemental pollen had a significant higher total number of seeds per plant (ANOVA: $F_{3,79}=4.56$, $P=0.005$), % fruit per (ANOVA: $F_{3,79}=2.94$, $P=0.03$), and seed per fruit (ANOVA: $F_{3,79}=4.84$, $p=0.003$) compared with plants under bagged self –pollinated, bagged outcrossed, and open pollination treatments (Fig.2 on Appendix). These differences in fruit and seed production in the supplemental pollen treatment and the other 3 treatments showed that they were statistical significant different (MANOVA: $F_{3,73}=0.35$, $p=0.0001$, milk's $\lambda=0.0098$). Natural levels of flower and leaf damage had effects on visitation at both sites, meaning that there was a significant relationship between the number of damaged flowers and visitation components (ANOVA: $F_{1,7}=8.12$, $p=0.0001$) and damaged leaves and visitation components (ANOVA: $F_{1,7}=54.48$, $p=0.0001$) in site 1 and site 2. To avoid Type I error on the above analysis, a MANOVA analysis was done using the same 2 variables of damage by visitor and site. The analysis in Site 2 (MANOVA; $F_{2,53}=28.35$, Wilk's $\lambda=0.0001$) showed a significant difference. Site 1 also showed a statistical significant difference (MANOVA: $F_{2,542}=2.53$, Wilk's $\lambda=0.0001$); suggesting that visitors selected plants based on leaf and flower damage in both sites.

Site 1 and Site 2 varied in components of pollinator visitation (MANOVA; $F=0.86$, $p=0.02$), including the proportion number of flowers probed, mean time per flower, and mean time per plant (Fig.3 a-b at Appendix), but there was not a significant

difference on visitor species in relation to both sites (MANOVA: $F=0.86$, $p=0.16$).

Pieridae (Cabbage white butterflies) performed more visits per plant in Site 1 (ANOVA: $F=4.23$, $p=0.04$) and probed more flowers (ANOVA; $F=2.88$, $p=0.001$) than any other visitor. (Fig.3 c-d and Table 2 at Appendix).

In terms of floral measurements, there was a significant difference in floral measurements between sites (MANOVA: $F=0.28$, $p=0.0001$) but not among treatments ($p=0.31$). Plants in Site 1 had larger floral displays signs than plants in Site 2. There was no correlation between the floral measurements (length and width) and plant height in Site 1 and Site 2. The analysis of variance showed a significant difference between mean petal length (ANOVA; $F_{1,72}=26.76$, $p=0.0001$) and mean petal width (ANOVA: $F_{1,72}=5.46$, $p=0.02$) within both sites (Fig.4 a-d at Appendix). On the other hand, analysis of variance between means on petal length and width by treatments (Fig. 4 c-d at Appendix) showed no significant difference in petal length (ANOVA: $F_{3,72}=1.76$, $p=0.16$) and petal width (ANOVA: $F_{3,73}=1.02$, $p=0.38$). By performing a multivariate correlation, I found that Site 2 had a positive but weak correlation in petal length and width, Site 2 showed a positive and stronger correlation, but they were not significant. However, there was a significant difference in plant height by site (ANOVA: $F_{1,239}=110.58$, $p=0.0001$); showing that plants in Site 2 were taller with wider petals, and plants in Site 1 were smaller with longer petals (Fig.4e at Appendix).

Discussion

My results are consistent with the first two predictions of my hypothesis that outcross supplemental pollen to flowers increased seed and fruit set and that herbivore damage led to pollen limitation. The study provided evidence that herbivore damage

resulted in changes in the way pollinator selected plants and how indirectly affected pollen limitation in *C. cordifolia* plants. The results agreed with the study made by Louda and Rodman (1996) that higher insect damage led to reduced flowering and thus to less fruit and seed set. Plants outcrossed with supplemental pollen including those under bagged outcrossed and no bag outcrossed treatments in both sites, represented higher fruit and seed set with 66.88% of the total successful fruits and 69.70% of the total seed production. Compared with the other two treatments, plants self-pollinated in Site 1 had the least fruit and seed set than self-pollinated plants in Site 2, where control plants had the lower fruit and seed production. The results are related to the fact that Site 1 had more number of flowers open, higher proportion of flowers probed and thus, more visits per plant. Hand pollinated and outcrossed with supplemental pollen plants received more pollen than control plants, therefore, there was an increased in fruit and seed set, giving as a result that *C. cordifolia* is both self-compatible and pollinator limited since self-pollinated plants produced fruits and seeds but in lower levels, as well as, control plants. I concluded that pollinators do not carry enough pollen so plants could not produce a higher proportion of fruit and seed set.

Plants in Site 2 were more damaged (see fig.5) and had lower fruit and seed production. The study provided evidence that herbivore damage resulted in changes in the way pollinator selected plants and how indirectly affected pollen limitation in *C. cordifolia* plants. The results agreed with the study made by Louda and Rodman (1996) that higher insect damage led to reduced flowering and thus to less fruit and seed set. For example, plants in Site 2 had more damaged leaves and had less fruit and seed set. Plants on Site 1 had less damaged leaves and had a higher proportion of fruit and seed set.

Brassicacea species plants are often pollinated by lepidopteran species, which includes butterflies and moths. There was a total of 82 visits in both sites (see table 2), which 72.82% corresponded to Pieridae (milky butterflies). MANOVA ($F=0.86$, $p=0.02$) analysis gave a statistical significant difference in visitation between Site 1 and Site 2. From the total pollinator visits, 96.34% corresponded to site 1 and only 3.66% to Site 2. Therefore the major visitor was Pieridae, but other like, flies, solitary bees, and bumble bees visited the plants, but not at high levels. With the above information, it is observed that the reduced pollinator visits affected the fruit and seed production in Site 2.

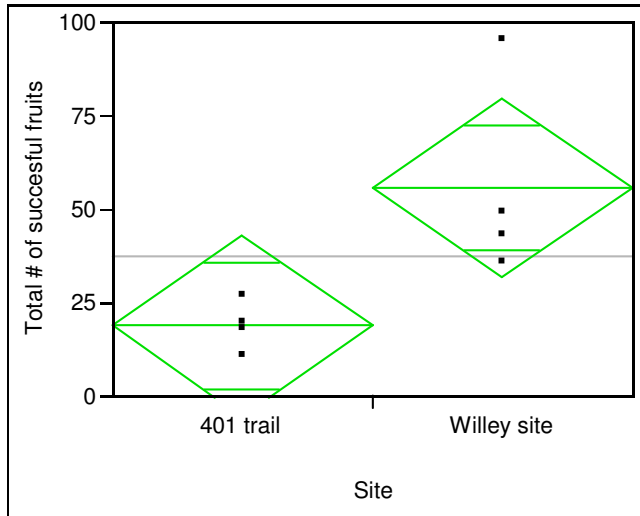
According to MANOVA ($F=0.28$, $p=0.0001$), there was a significant difference of floral displacements in variation to habitat type. Plants in Site 1 were smaller and plants in site 2 were taller, ANOVA ($F=110.58$, $p=0.0001$) showed that plant height in both sites was different. I predicted that longer floral traits and taller plants would be associated with pollination visitation and plant production, but a $p\text{-value}=0.0001$ demonstrated that the prediction was not accurate. Multivariate correlation found no strong correlation between floral traits. I observed that plants in Site 1 were smaller with longer petals, and plants in Site 2 were taller with wider petals, therefore floral traits and taller plants were not associated with an increase of pollinator visitation and plant reproduction. Plant reproduction is not solely based in pollinator visitation, it also depends on the quantity of pollen transferred, and the way it is transferred, whether via pollinators, or by self-pollination. Finally, weather is an important factor that affects the availability of pollen and has negative effects on pollinator visits. During the time of the study process, there was rain, clouds, and wind; such factors affected my observations, my hand pollination treatments, and the data taken.

Appendix

Fruit and seed production

Total # of successful fruits by Site

Fig 1. Graph shows that Willey Site has a higher number of successful fruit set, than 401 Site (ANOVA: $F=13.21$, $p=0.01$)



Differences in fruit an seed set by site and treatment

Site	Treatment	# successful fruits	# of aborted fruits	Total seeds
Site 1	Bag Self	36	78	110
Site 1	Bag Outcross	49	31	214
Site 1	No bag outcross	95	34	497
Site 1	Open	43	43	175
total		223	186	996
Site2	Bag Self	18	52	70
Site 2	Bag Out	20	37	92
Site 2	No bag Out	27	22	131
Site2	Open	11	27	51
total		76	138	344

Table 1. Site 1 had higher fruit and seed production than site 2. No bag outcross and bag outcross had the higher fruit and seed set.

Figure 2

Total Seeds per plant by treatment

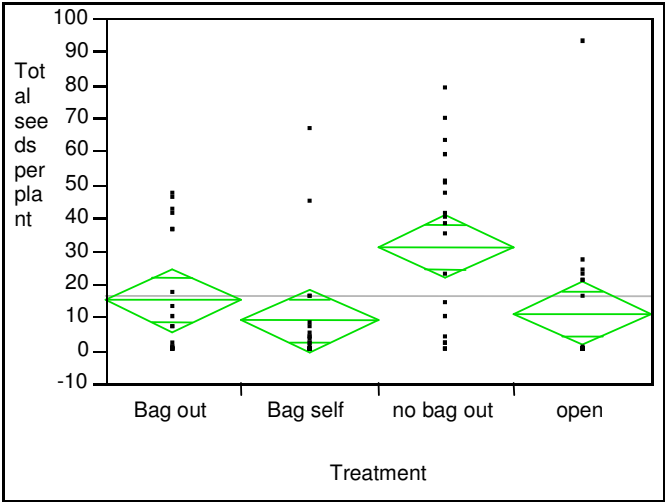


Fig 2a. Plants on no bag outcrossed treatment, had 0 plant compared with the other three treatments. (ANOVA: $F=4.56$, $p=0.005$).

% fruit per set by treatment

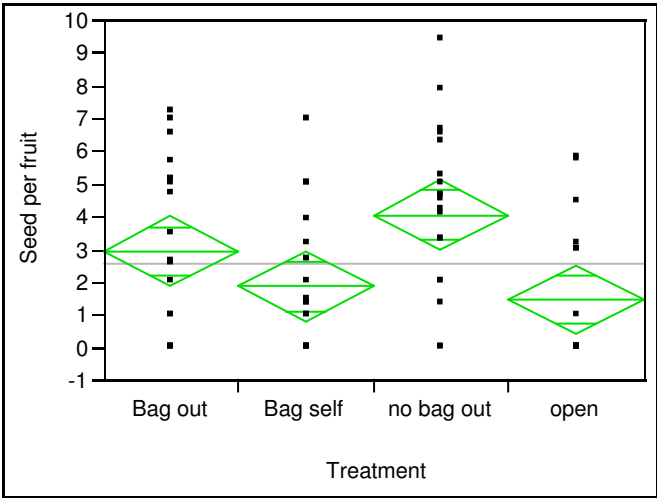


Fig 2b. No bag outcrossed treatment shows a higher % fruit per set than plants in the other treatments (ANOVA: $F=3.04$, $p=0.04$).

Seed per fruit by treatment

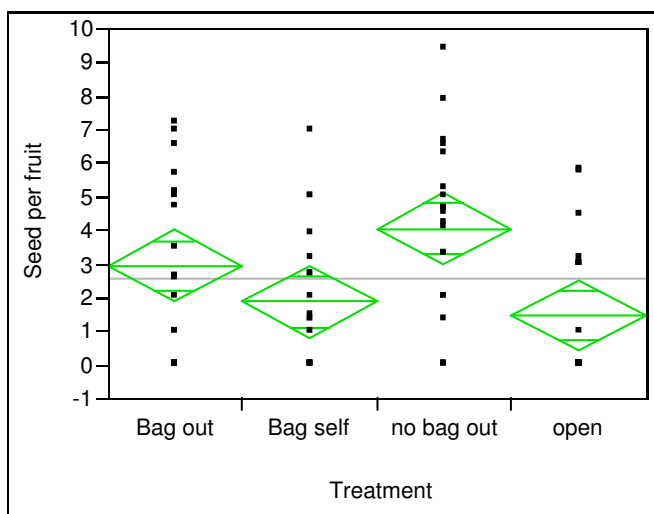


Fig 2c. Seed per fruit was higher on no bag outcrossed treatment followed by bag outcrossed (ANOVA: $F=4.84$, $p=0.00039$)

Figure 3.

Number of visits per plant by site

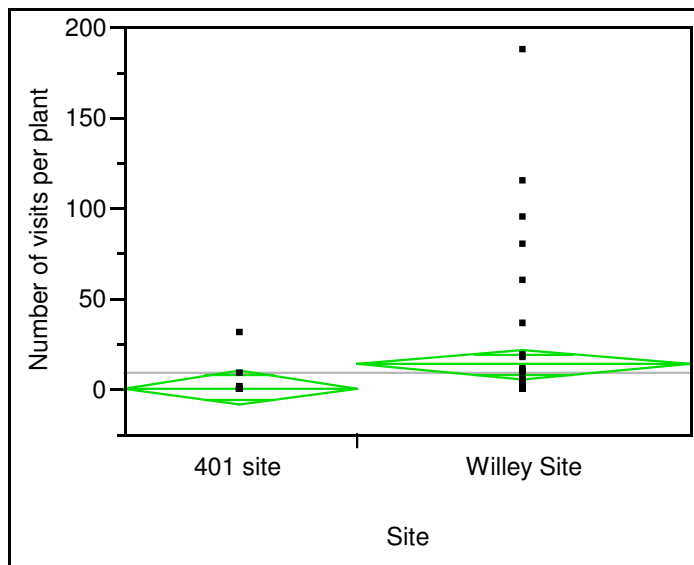


Fig 3a. Willey site received more visits, compared with the 401 site (ANOVA: $F=4.25$, $p=0.04$).

Proportion of flowers probed by site

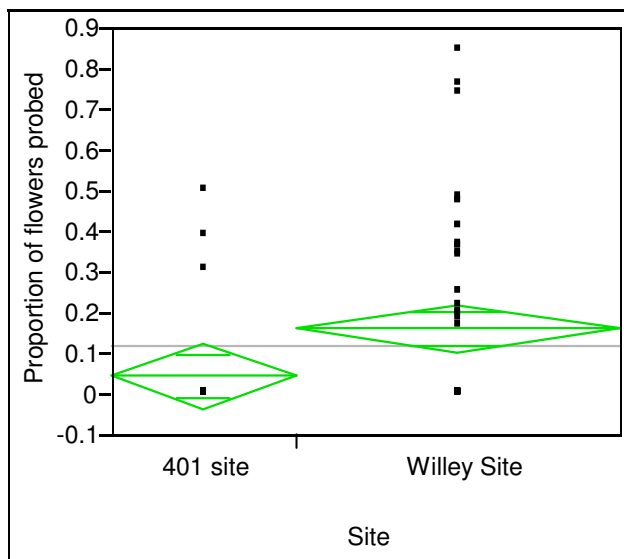


Fig 3b. More flowers were probed in Willey site in relation to 401 site (ANOVA: $F=5.73$, $p=0.01$).

Number of visits per plant by visitor

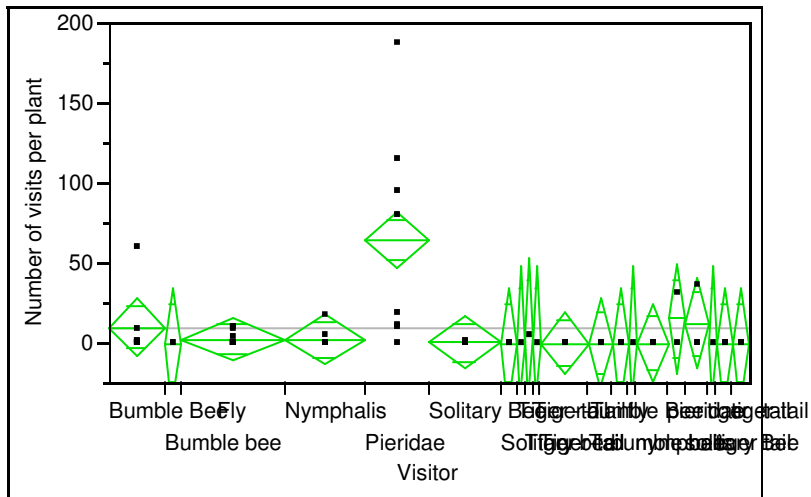


Fig 3c. Pieridae make more visits per plant than any other visitor (ANOVA: $F=2.52$, $p=0.003$).

Proportion of flowers probed by visitor

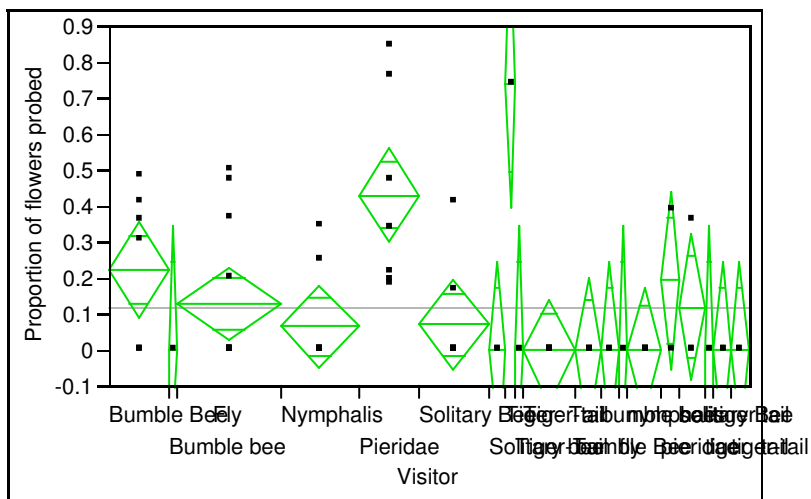


Fig 3d. Pieridae made more visits, but it also probed more flowers than any other visitor.

Table 2

Major Visitors

Site	Major visitor	Total # of visits
Site 1		
1	Pieridae	63
2	Solitary Bee	4
3	Bumble Bee	4
4	Fly	5
5	Tiger-tail shawoll	1
6	Nymphalis	2
Site 2		
1	Pieridae	2
2	Solitary Bee	0
3	Bumble Bee	0
4	Fly	1
5	Tiger-tail	0
6	Nymphalis	0

Table 2. Pieridae performed more visits than any other visitor. Only in site one there were 79 visits out 82. Site 2 only received 3 visits out of 82.

Figure 4: Floral Measurments

Mean Petal Length (mm) by Site

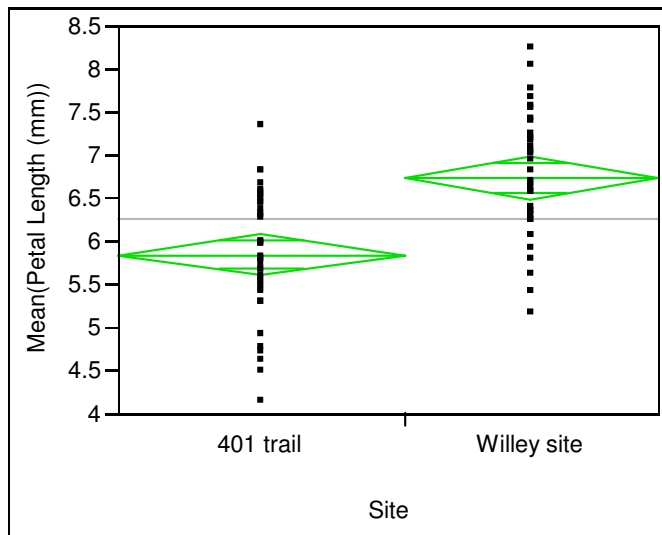


Fig 4a. Flowers in Willey site contained larger petals than 401 site (ANOVA: $F=26.76$, $p=0.0001$).

Mean Petal Width (mm) by site

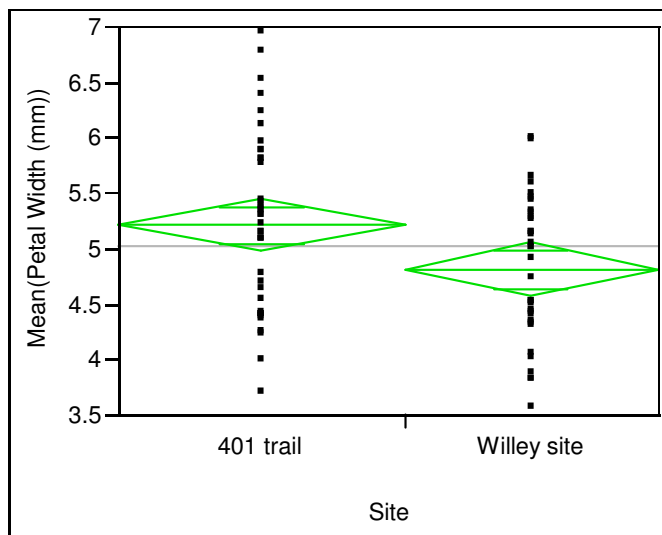


Fig 4b. the 401 site has wider petals than Willey site (ANOVA: $F=5.46$, $n=0.02$).

Mean Petal Length (mm) by Treatment

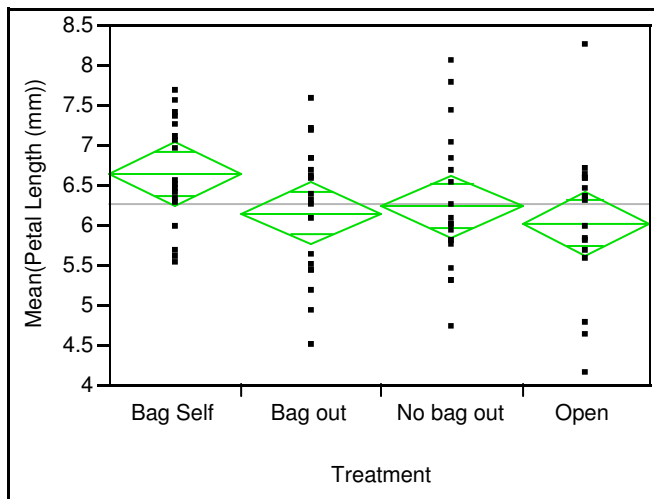


Fig 4c. There is not a significant difference in petal length between treatments (ANOVA: $F=1.76$).

Mean Petal Width (mm) by Treatment

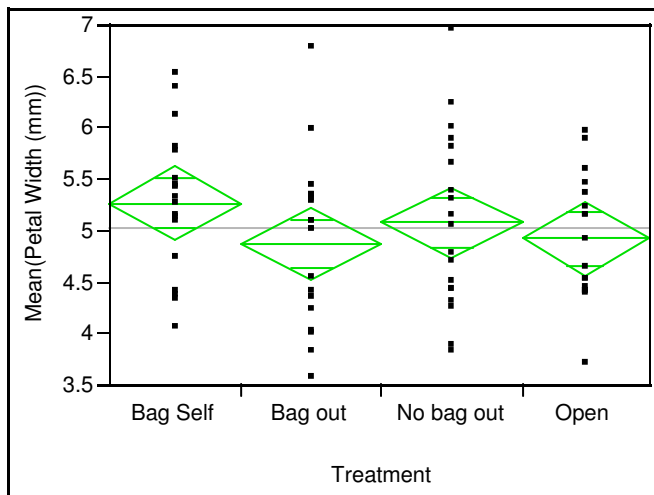


Fig 4d. There was not a significant difference in petal width in any treatment (ANOVA: $F= 1.02$, $p= 0.38$).

Plant Height (mm) by Site

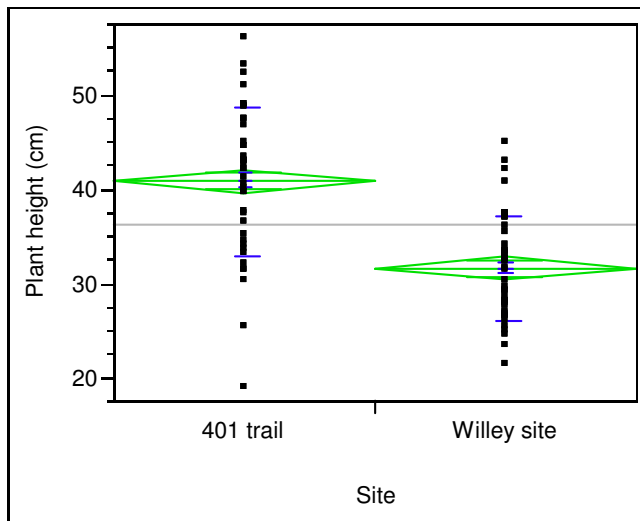


Fig 4e. There was a significant difference in plant height in both sites. Plants in 401 were taller than plants in the Willey site (ANOVA:

Comparison of Leaf damage in both sites

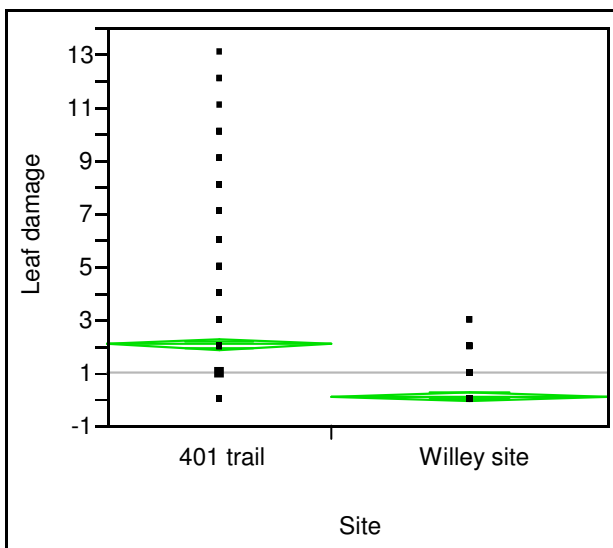


Fig 5. Shows that 401 trail had more leaf damage in comparison to Willey site (ANOVA: $F = 217.38$, $p = 0.0001$).

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