

**The effects of *Castilleja miniata*'s parasitic relationship
with *Delphinium nuttallianum* on pollinator visitation
and pollination success**

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Abstract

Castilleja miniata is a hemiparasite capable of taking up chemical toxins from its host plant. A unique relationship is *C. miniata*'s parasitism on *Delphinium nuttallianum* (which contains alkaloids); alkaloids from *D. nuttallianum* are taken up into *C. miniata*'s nectar which could negatively affect *C. miniata*'s pollinator visitation when compared to *C. miniata* parasitizing other plants. However, *D. nuttallianum* may have an increased visitation when parasitized by *C. miniata* should the pollinators prefer the alkaloid free nectar of the close-by *D. nuttallianum* over the alkaloid-containing nectar of its host. I observed pollinator visitation to two groups of *C. miniata* (one parasitizing *D. nuttallianum* and one not) and two groups of *D. nuttallianum* (one being parasitized by *C. miniata* and one not) for a total of eighty hours and performed herbivory assessments and hand-pollinations. I also assessed seed set for all treatments to determine if pollinator visitation dictated pollination success. There were no significant differences in flowers probed/open ratios or total visitation between *C. miniata* treatments or *D. nuttallianum* treatments, although the trends were in the direction expected for *C. miniata* (decreased visitation to *C. miniata* parasitizing *D. nuttallianum*) but not for *D. nuttallianum* (decreased visitation to *D. nuttallianum* parasitized by *C. miniata*). *D. nuttallianum* seed number and seed weight did not support pollination visitation determining pollination success; both seed number per plant and seed weight per plant negatively correlated with number of visits. *C. miniata* seed data has not yet been determined.

Introduction

Many ecological studies have focused on plant hemiparasites due to the fact that hemiparasites can uptake not only nutrients and substance from their host but chemical defenses as well (Phoenix and Press, 2005). The acquisition of chemical defenses has lead to a variety of questions ranging from host chemical effects upon a hemiparasite's herbivores (Marvier, 1996, Adler and Wink 2001, Adler 2002) to pollination rates of hemiparasites (Adler and Wink 2001, Alder 2002, Marko and Stermitz 1996). *Castilleja sp.* is a parasitic plant capable of obtaining water, sugar, nitrogen, and secondary metabolites from its host (Marvier 2002). However, the chemical makeup of *Castilleja* can differ depending which host it chooses to parasitize (Adler and Wink 2000, Marko

and Stermitz 1996). For example, in a study regarding *Castilleja* parasitizing *Lupinus*, analysis showed that host alkaloids were present in the leaves and outer floral tissue of the *Castilleja* but not in the nectar (Adler and Wink 2001) whereas in another study regarding *Castilleja* and *Delphinium*, host alkaloid presence was found in all growth (stem, roots, and leaves) of *Castilleja* including the nectar (Marko and Stermitz 1996).

Interestingly, *Castilleja*'s uptake of chemicals does not necessarily mirror the location of chemicals in the host. *Delphinium* has high alkaloid concentrations in the roots and stems which could lessen its attraction to herbivores without deterring pollinators (Williams et al. 2001), but *Castilleja* parasitizing *Delphinium* contains the same alkaloids in all parts of the plant with the highest concentrations in the flowers, seeds, nectar, and leaves (Marko and Stermitz 1996). The uptake of alkaloids to all parts of the plant may serve to discourage herbivores but might also have the effect of discouraging pollinators due to the high toxicity of alkaloids (Marko and Stermitz 1996).

When *Castilleja* parasitizes *Lupinus*, the relationship has no effect on *Castilleja* pollinator preference due to the lack of alkaloids in *Castilleja* nectar (Adler 2002). However, no such study has been conducted on a parasitic relationship between *Castilleja* and *Delphinium*. Such a relationship may affect pollinator preference in a unique way; *Castilleja* parasitizing *Delphinium* could experience lower visitation than were it not parasitizing *Delphinium*, and the parasitized *Delphinium* could experience higher visitation than were it not being parasitized. The reason for these potential results is based on the following premise: Hummingbirds pollinate both *Castilleja* and *Delphinium*, but as a bright red flower, *Castilleja* is thought to be more attractive to hummingbirds than *Delphinium* (Williams et al. 2001). When *Castilleja* is parasitizing

Delphinium, initial attraction to the *Castilleja* could lead to avoidance due to alkaloid presence in the nectar (thus a decreased visitation relative to *Castilleja* parasitizing other plants). Concurrently, this attraction and rejection of *Castilleja* parasitizing *Delphinium* could result in an increased preference for the nearest alternative, i.e., the *Delphinium* host (thus an increased visitation relative to *Delphinium* not parasitized by *Castilleja*), thereby increasing the suite of pollinators that visit *Delphinium* parasitized by *Castilleja* relative to non-parasitized *Delphinium*.

Using *Castilleja miniata* and *Delphinium nuttallianum*, I propose to study the effects *Castilleja*'s parasitic relationship with *Delphinium* has on the pollinator visitation and pollination success of both plants. Should parasitizing *D. nuttallianum* negatively affect *C. miniata*'s pollination visitation and/or pollination success, one would expect evolutionary pressure on *Castilleja* not to choose *Delphinium* as a host plant. However, parasitizing *Delphinium* may reduce herbivory rates for *Castilleja* due to alkaloid presence. Concurrently, this study may suggest the parasitism to be advantageous for *D. nuttallianum* by increasing pollinator visitation (thus potentially raising *D. nuttallianum* pollination success rates).

The questions this study seeks to answer are 1) Does a parasitic relationship between *C. miniata* and *D. nuttallianum* change pollinator visitation rates to *C. miniata* and/or *D. nuttallianum* versus when the two species are separate from each other? 2) Do pollinator visitation rates mirror pollination success in both *C. miniata* and *D. nuttallianum*? 3) Does a parasitic relationship between *C. miniata* and *D. nuttallianum* affect herbivory of *C. miniata*?

Materials and Methods

This experiment was conducted near the Rocky Mountain Biological Laboratory in Gothic, Colorado at an elevation of 2930 meters. My study sites are off Copper Creek trail and comprise of a mixed community of individual *C. miniata*, *Castilleja miniata* parasitizing *Delphinium nuttallianum*, *D. nuttallianum* being parasitized by *C. miniata*, and individual *D. nuttallianum*. I used the following criteria as a guide for placing the study plants into respective groups:

- Individual *C. miniata*: must be at least six inches away from nearest *D. nuttallianum*, must show promise of flowering this season (have at least one bud with red bracts), and preferably not have all flowers in full bloom at the time of being chosen
- Individual *D. nuttallianum*: must be at least six inches away from nearest *C. miniata*, must have buds, and preferably not have all flowers in full bloom at the time of being chosen
- C. miniata* parasitizing *D. nuttallianum*: must be in immediate proximity of *D. nuttallianum* such that leaves of both species are in contact, must show promise of flowering this season (have at least one bud with red bracts), and preferably not have all flowers in full bloom at the time of being chosen
- D. nuttallianum* parasitized by *C. miniata*: must be in immediate proximity of *C. miniata* such that leaves of both species are in contact, must have buds, and preferably not have all flowers in full bloom at the time of being chosen

At the site I flagged and marked the following numbers of each group: thirty individual *C. miniata*, thirty *C. miniata* parasitizing *D. nuttallianum*, thirty *D. nuttallianum* being parasitized by *C. miniata* (the same *D. nuttallianum* involved with the above mentioned parasitic *C. miniata*), and thirty individual *D. nuttallianum* in such a way that all groups were intermixed and no one area of the site contained exclusively one group. Fifteen random plants from each group were hand pollinated every three days. I assessed each plant for herbivory damage (# of stalks eaten) at the onset of the study and will do so each subsequent two weeks thereafter. I used a one-tailed t-test to judge

whether the parasitism affects herbivory rates of *C. miniata* with the independent variable as whether the plant is parasitizing or not and the response variable as the percentage of stalks eaten. *D. nuttallianum* herbivory was scored as a '0' or '1' (0 = stalk not eaten, 1 = stalk eaten) and was calculated as a mean percent in each treatment.

To examine if *C. miniata* parasitizing *D. nuttallianum* changes pollinator visitation rates to *C. miniata* and/or *D. nuttallianum*, I conducted observation periods at the site with a hand-held tape recorder, totaling eighty hours of observation time. The observation periods occurred anywhere between 8am and 8 pm on any given day (morning, early afternoon, and late afternoon times emphasized due to high pollinator activity) and consisted of observing hummingbird visits (as well as other pollinators) to any marked plant from an inconspicuous location via eye and binoculars. During all observations I recorded the species of visitor, plant number, number of visits to each plant, number of flowers probed per visit, and number of flowers open on each plant.

To answer the question “Do pollinator visitation rates to *C. miniata* and *D. nuttallianum* differ when *C. miniata* is parasitizing *D. nuttallianum* as opposed to when both species are separate?” I ran one-tailed t-tests examining the effect of parasitism on pollinator visitation. The independent variable is whether *C. miniata* and *D. nuttallianum* are involved with each other in a parasitic relationship or not. The response variable is per plant visitation rates to either *C. miniata* or *D. nuttallianum*. I also ran a χ^2 test to see whether total visits (number of visits/treatment) of *C. miniata* parasitizing *D. nuttallianum* differed from total visits of *C. miniata* parasitizing other plants. A χ^2 test was also performed between *D. nuttallianum* treatments for total visitation.

To examine if pollinator visitation rates mirror pollination success in both *C. miniata* and *D. nuttallianum*, I gathered, counted, and weighed seeds. Total seed number and total seed weight per individual plant was determined by counting all the seeds an individual plant yields and weighing the seeds collectively. I performed two regressions testing effects of pollinator visitation on pollination success: one regression testing the effects of pollinator visitation on seed number (total seed number/individual plant as the response variable) and a second regression testing the effects of pollinator visitation on seed weight (total seed weight/individual plant as the response variable). In both regressions the independent variable is number of pollinator visits to respective plants. The statistics were calculated with Excel version 2003 and JMP version 5.1.

Results

Pollinator Visitation

C. miniata visitation data were divided into two time intervals dictated by *D. nuttallianum*'s flowering stage (early *C. miniata*; June 21-July 3) and fruiting stage (late *C. miniata*; July 6-July 24). Average per plant visitation for early *C. miniata* did not differ significantly between *C. miniata* parasitizing other plants and *C. miniata* parasitizing *D. nuttallianum* (Fig. 1, $t = 1.51$, $df = 52$, $P = 0.0688$) nor did it differ between late *C. miniata* treatments (Fig. 2, $t = 0.715$, $df = 56$, $P = 0.239$). However, the trends were in the general direction hypothesized; *C. miniata* parasitizing *D. nuttallianum* tended to have lower per plant visitation than *C. miniata* parasitizing other plants. Per plant visitation was marginally higher for non-parasitized *D. nuttallianum* than

parasitized *D. nuttallianum* (Fig. 3, $t = 1.39$, $df = 58$, $P = 0.0855$); the trend was not in the direction hypothesized.

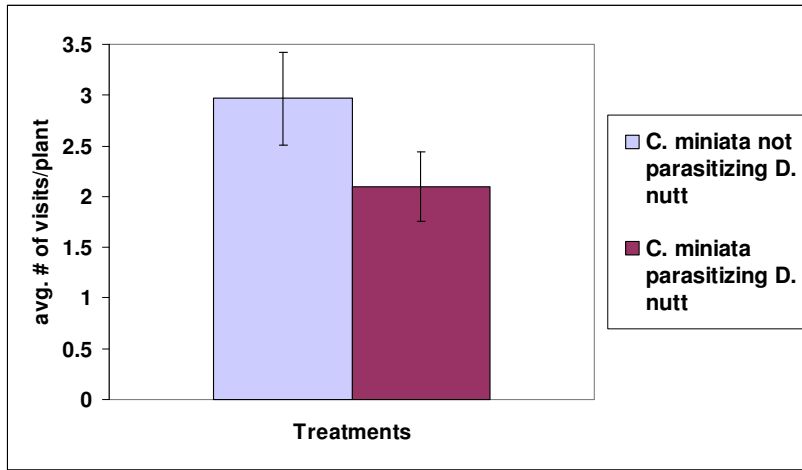


Fig. 1: Average Total Visits/Plant of *C. miniata* treatments during the time period when *D. nuttallianum* treatments are in bloom (early *C. miniata*) (Mean $\pm$ 1 SE)

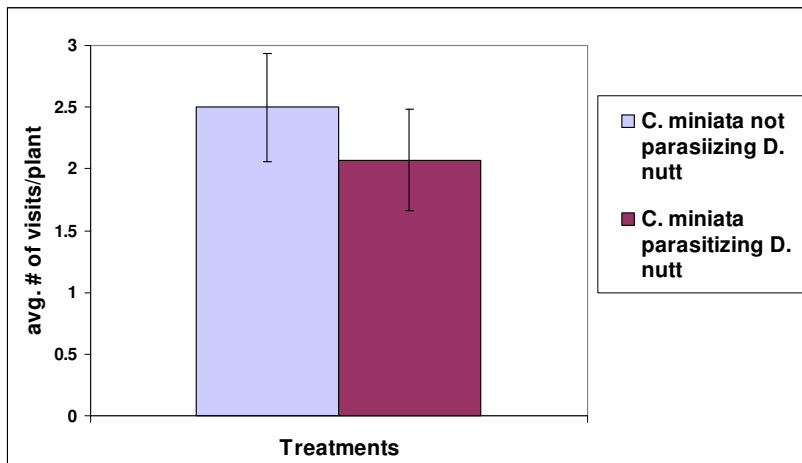


Fig. 2: Average Total Visits/Plant of *C. miniata* treatments during the time period when *D. nuttallianum* treatments are in fruit (late *C. miniata*) (Mean $\pm$ 1 SE)

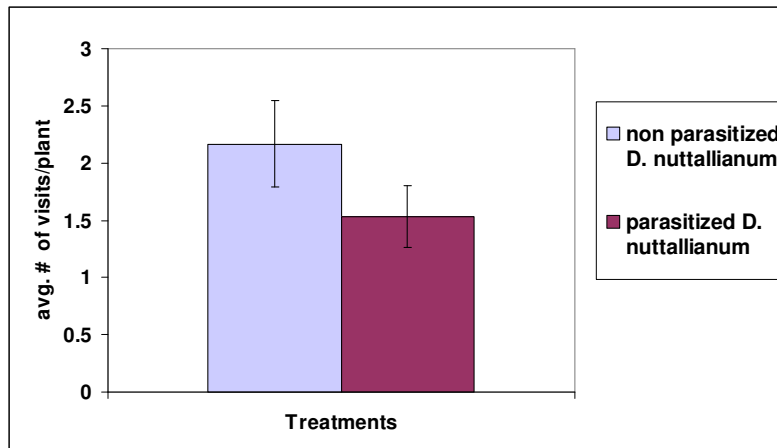


Fig 3: Average Total Visits/Plant of *D. nuttallianum* treatments (Mean $\pm$ 1 SE)

None of the differences between treatments were significant in a one-tailed t-test for average ratio of flowers probed/flowers open (Table 1).

Table 1: Average number of flowers probed/flower open ratios

Treatment	Avg. flower probed/open $\pm$ S.E.	t-value	df	p-value
Early <i>C. miniata</i> parasitizing other plants	0.65 $\pm$ 0.097	-0.770	57	0.222
Early <i>C. miniata</i> parasitizing <i>D. nuttallianum</i>	0.54 $\pm$ 0.101			
Late <i>C. miniata</i> parasitizing other plants	0.33 $\pm$ 0.055	1.30	56	0.0996
Late <i>C. miniata</i> parasitizing <i>D. nuttallianum</i>	0.23 $\pm$ 0.055			
<i>D. nuttallianum</i> not parasitized	0.58 $\pm$ 0.071	0.98	58	0.165
<i>D. nuttallianum</i> parasitized	0.50 $\pm$ 0.069			

Hummingbird visitation did not differ between non-parasitized *D. nuttallianum* and parasitized *D. nuttallianum* (3.08% of total visitation and 4.35% of total visitation, respectively). The remaining visits to both *D. nuttallianum* groups were from *Bombus*.

Non-parasitized *D. nuttallianum* had 3.70 flowers per plant $\pm$ 0.259, and parasitized *D. nuttallianum* had 3.05 ± 0.329 (p-value of 0.042 analyzed with a two-tailed t-test).

Total visitation to each group resulted in marginally significant differences between treatments (Chi-Square analysis); *C. miniata* parasitizing other plants received more visits than *C. miniata* parasitizing *D. nuttallianum* in both early (Fig. 4, $\chi^2 = 3.56$, df = 1, P = 0.0590) and late treatments (Fig. 5, $\chi^2 = 2.15$, df = 1, P = 0.143). Non-parasitized *D. nuttallianum* received more visits than parasitized *D. nuttallianum* (Fig. 6, $\chi^2 = 3.26$, df = 1, P = 0.0706).

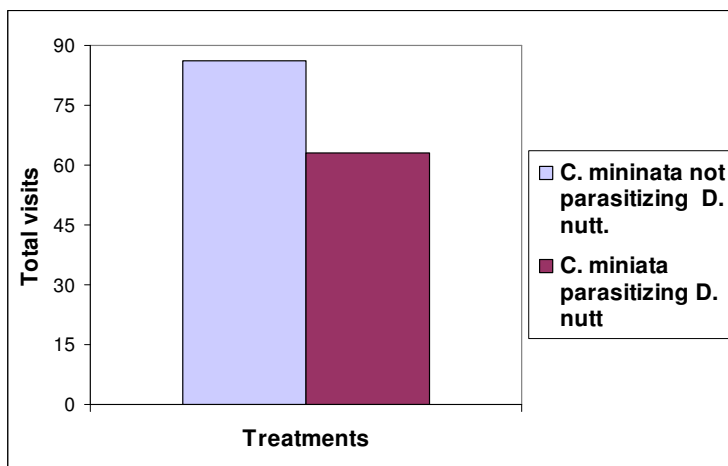


Fig. 4: Total visitation to *C. miniata* treatments during the time period when *D. nuttallianum* treatments are in bloom (early *C. miniata*)

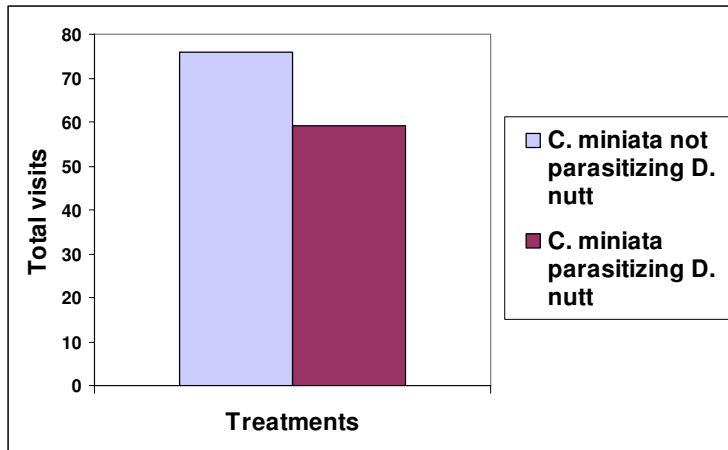


Fig. 5: Total visitation to Late *C. miniata* treatments during the time period when *D. nuttallianum* treatments are in fruit (late *C. miniata*)

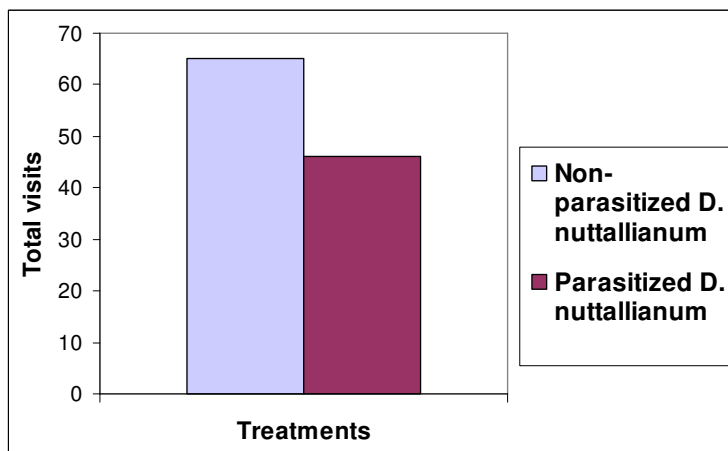


Fig. 6: Total visitation to *D. nuttallianum* treatments

Herbivory

Herbivory analysis for *C. miniata* showed no significant difference in the percent stalks eaten in each treatment (*C. miniata* parasitizing other plants had mean 43.4% stalks consumed $\pm$ 5.12% and *C. miniata* parasitizing *D. nuttallianum* had mean 34.7% stalks consumed $\pm$ 5.98% (one-tailed t-test; $t = 1.11$, $df = 58$, $P = 0.137$). A noticeable portion of *D. nuttallianum* stalks in each treatment was snapped at the base by an

unknown animal. The proportion of stalks snapped in each treatment did not differ; non-parasitized *D. nuttallianum* had 30% destruction and parasitized *D. nuttallianum* had 36.7% destruction ($\chi^2 = 0.672$). Herbivory of *D. nuttallianum* did not differ between treatments ($\chi^2 = 0.333$) and accounted for less than 6% of seed set loss in both treatments.

Hand-pollination

In a one-tailed t-test analysis of non hand-pollinated treatments, non-parasitized *D. nuttallianum* had a significantly higher chance of a flower setting fruit (83.4%) than parasitized *D. nuttallianum* (64.5%; $t = 2.07$, $df = 37$, $P = 0.0228$). However, in the hand-pollinated treatments, non-parasitized *D. nuttallianum* did not differ from parasitized *D. nuttallianum* in percent chance of a flower setting fruit (90.5% and 83.3%, respectively, $t = 0.747$, $df = 23$, $P = 0.479$). When hand-pollinated treatments were analyzed in a one-tailed t-test, non-parasitized *D. nuttallianum* had more seeds per plant than parasitized *D. nuttallianum* ($t = 2.45$, $df = 18$, $P = 0.0148$), but total seed count per plant did not differ significantly between the non hand-pollinated treatments of *D. nuttallianum* ($t = 0.293$, $df = 26$, $P = 0.386$) or between hand-pollinated and non hand-pollinated *D. nuttallianum* not parasitized treatments ($t = -0.0224$, $df = 21$, $P = 0.491$). Total seed weight/plant followed the same pattern between the *D. nuttallianum* groups previously described (Table 2); for hand-pollinated treatments, non-parasitized *D. nuttallianum* had higher seed weight per plant than parasitized *D. nuttallianum* ($t = 2.20$, $df = 18$, $P = 0.0248$). Total seed weight per plant did not differ significantly between the non hand-pollinated treatments of *D. nuttallianum* ($t = 0.448$, $df = 21$, $P = 0.329$) or between hand-pollinated and non hand-

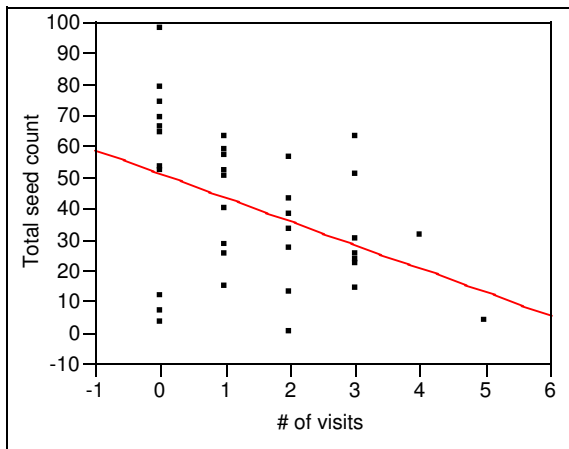
pollinated *D. nuttallianum* not parasitized treatments ($t = 0.664$, $df = 26$, $P = 0.257$). *C. miniata* seed count and seed weight have not yet been analyzed.

Table 2: Avg. total seed count and seed weight of non-parasitized and parasitized *D. nuttallianum* (hand-pollinated = hp and non hand-pollinated = non-hp)

Treatment	Avg. total seed count/plant $\pm$ S.E.	Avg. total seed weight/plant (mg) $\pm$ S.E.
Non-parasitized HP	37.67 $\pm$ 7.14	25.1 $\pm$ 5.16
Non-parasitized NON-HP	37.43 $\pm$ 7.05	30.9 $\pm$ 6.43
Parasitized HP	19.1 $\pm$ 2.86	13.8 $\pm$ 2.72
Parasitized NON-HP	34.64 $\pm$ 6.37	27.4 $\pm$ 4.53

Visitation effects on seed set

Regressions of visitor number effects on *D. nuttallianum* seed count and seed weight yielded significant negative correlations (Fig. 7, Fig. 8); as visitation to *D. nuttallianum* increased, both the number of total seeds per plant and total seed weight per plant decreased. I will run regressions testing effects of pollinator visitation on *C. miniata* seed weight and seed count when the data have been analyzed.

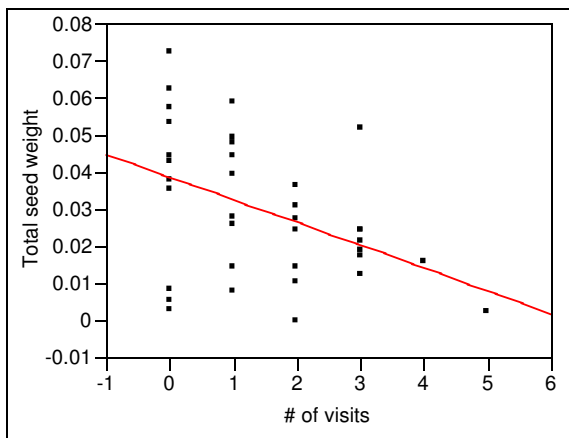


Linear Fit

Total seed count = $51.2 - 7.65 (\text{\# of visits})$

$R^2 = .179$ $P = .0101$

Fig. 7: Bivariate fit of *D. nuttallianum* total seed number by total no. of visits to each plant



Linear Fit

Total seed weight = $0.0387 - 0.00613 (\text{\# of visits})$

$R^2 = .186$ $P = .0087$

Fig. 8: Bivariate fit of *D. nuttallianum* total seed weight (grams) by total no. of visits to each plant

Discussion

In this study, *C. miniata* parasitizing *D. nuttallianum* tended to receive fewer visits than *C. miniata* parasitizing other plants, whereas non-parasitized *D. nuttallianum* tended to receive more visits than parasitized *D. nuttallianum*. There was no evident change in pollinator pool to parasitized *D. nuttallianum* versus non-parasitized *D. nuttallianum*, nor was there any significant herbivory in both *D. nuttallianum* treatments (whereas both *C. miniata* treatments experienced considerable herbivory although in roughly equal amounts). I found no negative effects of parasitism on seed count and seed weight of parasitized *D. nuttallianum* when compared to seed count and seed weight of non-parasitized *D. nuttallianum*. However, parasitized *D. nuttallianum* had less chance of a flower setting fruit than non-parasitized *D. nuttallianum*. This difference is most likely determined by visit limitation, not parasitism. *D. nuttallianum* seed count and seed weight per plant had a strong tendency to decrease as visitation to the plant increased. *C. miniata* seed count and seed weight has not yet been determined, but it will be interesting to see if seed set of *C. miniata* parasitizing *D. nuttallianum* mirrors pollinator visitation; seed set may be higher than expected due to low insect herbivory (higher alkaloid concentration in *C. miniata* parasitizing *D. nuttallianum*).

Pollinator Visitation

Although significant difference was not found in per plant visitation or total visitation for early and late *C. miniata*, the trends were in the direction that supported my hypothesis; *C. miniata* parasitizing *D. nuttallianum* received fewer visits than *C. miniata* parasitizing other plants. The reason for lower visitation to *C. miniata* parasitizing *D.*

nuttallianum may be due to the presence of nectar alkaloids as suspected. *D. nuttallianum* per plant visitation and total visitation trends did not support my hypothesis in that non-parasitized *D. nuttallianum* received higher visitation than parasitized *D. nuttallianum*. Pollinators may have preferred non-parasitized *D. nuttallianum* due to the higher average number of flowers per plant and/or because non-parasitized *D. nuttallianum* was more exposed and easy for *Bombus* (the majority of observed *D. nuttallianum* pollinators) to access whereas the parasitized *D. nuttallianum* was often less accessible due to the taller *C. miniata* growth physically surrounding parasitized *D. nuttallianum*.

While visitation trends of late *C. miniata* were in the direction supportive of my hypothesis, the tendency for *C. miniata* parasitizing *D. nuttallianum* to have lower visitation than *C. miniata* parasitizing other plants decreased as the summer progressed. This could be explained by possible alkaloid concentration changes in certain parts of *D. nuttallianum* (roots and stems) due to certain growth developments (fruiting). For example, alkaloid concentration in the roots and shoot tips of *Conium maculatum* (poison hemlock) was maximal in seedlings and sharply decreased or disappeared in mature plants. Alkaloid analysis of mature *Conium maculatum* found high alkaloid concentration in the fruits, suggesting that they are supplied continuously with alkaloids from root and shoot tips (Vetter, 2004) which could resemble *D. nuttallianum*'s maturation. If this is the case, *D. nuttallianum* alkaloid movement (triggered by *D. nuttallianum*'s switch from flower to fruit) could result in lowering *C. miniata* nectar alkaloid concentration later in the season due to the fact that *C. miniata* later in the season may not be able to access *D. nuttallianum* alkaloids as readily as *C. miniata* early in the

season. In addition, lower alkaloid concentration in *C. miniata* later in the season could have affected herbivory between *C. miniata* treatments; herbivores may have fewer qualms about eating *C. miniata* parasitizing *D. nuttallianum* later in the season (opposed to eating *C. miniata* parasitizing other plants) if the alkaloid content decreased later in the season.

For *D. nuttallianum* visitation, parasitized *D. nuttallianum* received the same suite of visitors as non-parasitized *D. nuttallianum*, and hummingbirds only accounted for less than 5% of the visitation to both treatments; my hypothesis of *C. miniata* attracting more pollinators to parasitized *D. nuttallianum* was not supported. The low hummingbird visitation to the parasitized *D. nuttallianum* treatment suggests that a hummingbird encountering *C. miniata* containing alkaloids in the nectar may prefer to go to another *C. miniata* (or other plant species) rather than parasitized *D. nuttallianum* despite parasitized *D. nuttallianum*'s close proximity to *C. miniata*.

Hand-pollination

Although parasitized *D. nuttallianum* may not have gained any benefits from its host in visitation, *C. miniata* had no negative effects on seed set of parasitized *D. nuttallianum* with the possible exception of hand-pollinated parasitized *D. nuttallianum*. Aside from hand-pollinated parasitized *D. nuttallianum*, all other *D. nuttallianum* groups (hand-pollinated and not) were very similar in seed weight and seed count between parasitized and non-parasitized treatments. Parasitized *D. nuttallianum* may have similar seed set to non-parasitized *D. nuttallianum* due to potential resource movement in *D. nuttallianum*. If *D. nuttallianum* may be capable of moving alkaloids to areas that are

less accessible to *C. miniata*, it may also be capable of moving important resources as well (such as nitrogen and water) (Preston, 1998), thereby lessening any negative effects *C. miniata* might have on seed set. I could not account the low seed count and seed weight of hand-pollinated parasitized *D. nuttallianum* to parasitic effects as the non-hand pollinated *D. nuttallianum* showed no significant difference between treatments for seed set, nor could I account the low seed set to damaging hand-pollination techniques as non-parasitized hand-pollinated *D. nuttallianum* showed no effects of the flowers being damaged during hand-pollination. However, approximately 33% of the *D. nuttallianum* were destroyed in each group (thought to result from identity tag removal by a rodent), therefore sample size was low (making it difficult to discern why hand-pollinated parasitized *D. nuttallianum* had unusually low seed set).

Hand-pollination analysis also did not support negative effects due to *C. miniata* parasitism in the percent *D. nuttallianum* flowers that fruited. There was no significant difference in the chance of a flower fruiting between the hand-pollinated treatments (*D. nuttallianum*), but there was significant difference in fruit set between the non hand-pollinated *D. nuttallianum* treatments. This leads me to believe that visitation, not parasitism, determine fruit set.

Visitation effects on seed set

Perhaps the most interesting results of my study, *D. nuttallianum*'s significant decrease in seed weight and seed count as visitation increased could be explained by one of two possible factors; either the decrease resulted from damage to the reproductive organs or it resulted from resource allocation. The reasoning behind the first factor is the

more a *D. nuttallianum* flower is visited, the higher the risk of damage to the female reproductive organs (over-visitation of the flower), thus a decreased seed set. For example, increased pollen-eating ant and honey bee visitation has been cited in several papers as damaging to seed set of various plants (Bhattacharya 2004, do Carmo et al. 2004). However, this explanation is not as strongly suspected as the second due to the fact that *Bombus* were the primary observed pollinators of *D. nuttallianum* and are not known to cause damage during visitation (Bhattacharya, 2004). The second possible explanation results from lack of resources for seed set; the plant may have over-compensated in the flower production stage. In a study on *Wurmbea dioica* (early Nancy), flower size was negatively correlated to seed set in that the larger the flower, the lower the seed set, implying a trade off of reproductive success with pollinator attraction (Vaughton, 1998). Something similar may occur with *D. nuttallianum*; a large flower or large number of flowers on a plant might receive many visitors but could potentially be draining the resources needed for seed set. Future research could investigate *D. nuttallianum* flower size to further test the resource allocation hypothesis.

Conclusions

I can not yet determine whether *C. miniata* benefits from its relationship with *D. nuttallianum*, but from the results that have been analyzed, it appears that *D. nuttallianum* seed set remains unaffected by *C. miniata* parasitism (with possible exception to hand-pollinated parasitized *D. nuttallianum*) and that it was strongly affected by visitation (increased visitation correlated with decreased seed set). An interesting question regarding parasitized *D. nuttallianum* is whether *C. miniata* is purposely not harming its

host's reproductive success (thereby ensuring more future hosts for *C. miniata* to parasitize) or whether *D. nuttallianum* is thwarting *C. miniata*'s attempts to drain resources (thereby putting pressure on *C. miniata* not to parasitize *D. nuttallianum*). Analysis of *C. miniata* seed set will help answer this question; if *C. miniata* parasitizing *D. nuttallianum* has lower seed set than *C. miniata* parasitizing other plants, it would suggest that *D. nuttallianum* is blocking *C. miniata*'s attempts to pirate resources from *D. nuttallianum* rather than *C. miniata* purposely harming its own reproductive success at the benefit of its host. However, if *C. miniata* seed set shows no difference between both treatments, it may suggest that *C. miniata* parasitizing *D. nuttallianum* is purposely draining its host in a conservative manner. The main question *C. miniata* seed set will answer is 'does parasitizing *D. nuttallianum* benefit *C. miniata*'s reproductive success?'

However, *C. miniata* hand-pollination results might support a pollinator limitation hypothesis between the two treatments; that is, that seed set is dependent upon the number of visits and not any advantages that the host plant might provide. If pollinator visitation is the deciding factor for *C. miniata* seed set, then *C. miniata* would not benefit by choosing *D. nuttallianum* as a host plant; *C. miniata* parasitizing *D. nuttallianum* received less visits than *C. miniata* parasitizing other plants. An interesting procedure for future studies would be to perform the Dragendorff Reagent test which can determine the concentration of alkaloids in nectar of *C. miniata* parasitizing *D. nuttallianum*. *C. miniata* parasitizing *D. nuttallianum* may vary in nectar alkaloid concentration according to *C. miniata* size, *D. nuttallianum* size, and/or time of the season (flowering *D. nuttallianum* versus fruiting *D. nuttallianum*). Pinpointing variation in nectar alkaloid

concentration within and between *C. miniata* may help examine the link between nectar alkaloid concentration and pollinator visitation to *C. miniata*.

Another unexpected result that needs further investigation is the negative correlation between *D. nuttallianum* seed set and pollinator visitation. Determining this relationship would need to include such considerations as flower number on plant, flower size, amount of pollen produced, ratio of anthers to stigmas, and plant height. Looking at each of these factors individually would help determine what is most attractive to visitors and what limits *D. nuttallianum* seed set.

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Literature Cited

- Adler, L. S. 2002. Host effects on herbivory and pollination in a hemiparasitic plant. *Ecology* **83**:2700-2710.
- Adler, L. S. and Wink, M. 2001. Transfer of quinolizidine alkaloids from hosts to hemiparasites in two *Castilleja-Lupinus* associations: analysis of floral and vegetative tissues. *Biochemical Systematics and Ecology* **29**: 551-561
- Bhattacharya, A. 2004. Flower visitors and fruitset of *Anacardium occidentale*. *Annales Botanici Fennici* **41**: 385-392
- do Carmo, R. M. and Franceschinelli, E.V. and da Silveira, F.A. 2004. Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* **36**: 371-376
- Irwin, R. E. 2000. Hummingbird avoidance of nectar-robbed plants: spatial location or

- visual cues. *Oikos* **91**: 499-506.
- Marko, M. D. and Stermitz, F. R. 1997. Transfer of alkaloids from *Delphinium* to *Castilleja* via root parasitism. Norditerpenoid alkaloid analysis by electrospray mass spectrometry. *Biochemical Systematics and Ecology* **25**:279-285.
- Marvier, M. A. 1996. Parasitic plant-host interactions: plant performance and indirect effects on parasite-feeding herbivores. *Ecology* **77**: 1398-1409.
- Phoenix, G. K. and Press, M. C. 2005. Linking physiological traits to impacts on plant community structure and function; the role of root hemiparasitic Orobanchaceae (ex-Scrophulariaceae). *Journal of Ecology* **93**: 67-78.
- Preston, K. A. 1998. The effects of developmental stage and source leaf position on integration and sectorial patterns of carbohydrate movement in an annual plant, *Perilla frutescens* (Lamiaceae). *American Journal of Botany* **85**: 1695-1703
- Vaughton, G. and Ramsey, M. 1998. Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* **115**: 93-101
- Vetter, J. 2004. Poison hemlock (*Conium maculatum* L.) review. *Food and Chemical Toxicology* **42**, Issue **9**: 1373-1382.
- Williams, C. F. and Ruvinsky, J. and Scott, P.E., and Hews, D.K. 2001. Pollination, breeding system, and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species. *American Journal of Botany* **88**: 1623-1633.