

Consequences of Nectar Robbing in Colorado Wildflowers: Insect Variation and Nectar
Sugar Concentration

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Abstract

Nectar robbing is a process used by various insects to retrieve nectar from flowers that would otherwise be inaccessible. The community-level consequences of nectar robbing have not been widely studied, and the differences between primary and secondary robbing have been studied even less. Fitness costs to plants from nectar robbing have been documented, such as reductions in male and female components of plant reproduction, but there is room for additional experimentation. In this study, I examined the consequences of nectar robbing on insect visitation and nectar sugar concentration. This was accomplished through both manipulation and natural surveys in an attempt to observe any differences between flowers that had been robbed and those that had not. In general, I found no evidence to support that insects chose flowers based on the presence of nectar robbing in *Ipomopsis aggregata*, nor did I find evidence that sugar concentration in nectar differs depending on the size of robber holes. I found significantly more insects in robbed flowers than unrobbed flowers in *Linaria vulgaris*, but this data is not enough to prove preference. Due to this difference, further experimentation into *L. vulgaris* would help to clarify insect preference in this plant in robbed versus unrobbed flowers, and these results may provide better insight into the consequences and effects of nectar robbing.

Introduction

Many flowering plants rely on insects and birds for pollination, providing nectar as a reward for visitation (Inouye 1980). Some of these insects and birds choose to bypass legitimate nectar acquisition through nectar robbing, in which an organism makes a hole

near the base of a flower's corolla so that nectar can be extracted directly (Inouye 1980). This process inhibits interaction between the animal and pollen, preventing pollination in some cases (Inouye 1980). Nectar robbing can occur both primarily and secondarily; primary robbing involves creating the initial hole for the nectar to be removed, whereas secondary robbing involves a separate organism utilizing a previously created hole (Inouye 1980).

Nectar robbing can have negative effects on plant reproduction (Richman et al. 2016). One study showed a significant decrease in seed production between control plants and those that were primary robbed artificially, as well as significant decrease between plants that had only been primary robbed and those that had been robbed more than once (Richman et al. 2016). Repeated robbing has also been shown to effect both male and female reproductive fitness in other plants, such as *Polygala vayredae* (Castro et al. 2008). It has been suggested that nectar robbing can alter the behaviors of insect pollinators, leading to a decrease in overall fitness (Castro et al. 2008). With aspects of plant reproduction negatively tied to nectar robbing, it is possible that robbing has other community-level consequences as well.

Repeated robbing could lead to an increase in robber hole sizes, potentially allowing different insects to access the flower's nectar that would not have otherwise. Nectar robbing also affects water concentration of the flower's nectar (Pleasants 1983), which could also affect the sugar concentration of the nectar. Pleasants (1983) found that water in the nectar evaporates out of the nectar robber hole, resulting in higher nectar sugar concentration. It remains unknown whether larger nectar-robber hole sizes

associated with repeated secondary nectar robbing result in even faster rates of water evaporation and, thus, lower nectar sugar concentration.

In this study, I conducted a series of experiments to explore the effects of hole size in nectar robbed flowers on insect visitors, nectar sugar concentration, and insect visitation in other commonly robbed plants. The goal of these observations was to clarify relationships between repeated robbing and insect-plant interactions with *Ipomopsis aggregata*, *Corydalis caseana*, and *Linaria vulgaris*. First, I manipulated flowers of *I. aggregata* to simulate different degrees of nectar robbing and observed insect visitation. I predicted that there would be a different number of visitors depending on the treatment applied to the flowers due to differences in robber hole size. Second, I applied similar treatments to new *I. aggregata* and looked at the volume and concentration of nectar produced. I predicted that larger robber holes would lead to more water evaporation and therefore a lower nectar volume and a higher sugar concentration. Third, I surveyed the flowers of *C. caseana* and *L. vulgaris* to assess whether there were differences in insect visitation between naturally robbed and unrobbed flowers. I predicted that robbed flowers would have more insect visitors than unrobbed flowers.

Methods

Study species and site

Ipomopsis aggregata is a perennial wildflower in the Polemoniaceae. Long, tubular, red flowers that open up into multiple lobes characterize the plants (Weber et al. 2012). These long tubes make it a common stop for hummingbirds that have bills long enough to reach the nectar at the base of the flower (Darrow 2006)

Corydalis caseana is in the Fumariaceae, and is known for its pronounced, white spurs and bean-like fruit (Weber et al. 2012). Nectar collects in their elongated spurs, making them a target for robbing by some bumblebees (Darrow 2006).

Linaria vulgaris is in the Plantaginaceae, and is non-native to Colorado (Weber et al. 2012). The yellow flowers have wide openings, with nectar collecting near the base of the flower (Darrow 2006).

All three plant species are nectar robbed by the bumble bees *Bombus occidentalis* and *Bombus mixtus*. Both species can act as primary and secondary nectar robbers. In addition, the plant species can be secondary robbed by other bumble bee species as well as other insects foraging for nectar, such as flies and wasps.

I collected data around the Gothic Townsite in Gothic, CO (elevation 9,485 ft) and near Keblar's Pass (elevation 10,007 ft).

Insect visitation based on robber hole size in Ipomopsis aggregata

For the first experiment, I selected 60 *I. aggregata* plants near Ore House cabin. I grouped the plants into triplets based on their proximity to one another, and marked each plant with a string tag to differentiate between group number and which treatment was applied to the flowers. I used three different treatments, one for each plant in a triplet, consisting of a control treatment, primary robbing treatment, and secondary robbing treatment. For the control plants, I brushed my hands along the stem of the flower to maintain consistency between other treatments, but otherwise did not manipulate the flowers. For the primary robbing treatment, I used pointed forceps to make a hole that went through the entire flower, near the base of each open flower's corolla. This made

two holes that resembled the two incisions made by natural robbers when they first rob a flower. For the secondary robbing treatment, I repeated the primary procedure but connected the two holes with the forceps, making one larger hole to represent multiple robber visits. For both the primary and secondary robbing treatments, I manipulated all open flowers on the plant and used a 10 μ L glass tube to remove any nectar in the flowers to simulate robbing.

After treating flowers on a plant, I selected up to three flowers to destructively sample by removing them from the plant and using my fingers to open them up and look for insects inside. I also checked for insects around the sepals. I destructively sampled no more than half of the available open flowers for each plant to avoid interfering with plant fitness. For each day of sampling, I recorded the number of open flowers on each plant, the number of insects visible on the plant before manipulation, and the number of insects in each sampled flower and took note of which insects were present.

I sampled these plants on 10 separate occasions between June 27, 2017 and July 21, 2017.

Effects of robber hole size on sugar concentration in nectar

In the second experiment, I used the same manipulation procedures as in the first experiment to treat *I. aggregata* flowers in the meadow behind the Gothic Research Center in Gothic, CO. For each plant I selected, I treated between six and nine flowers, dividing them up between the three treatments. I grouped flowers based on which reproductive phase they were in so that I could consistently record nectar data. I marked each treated flower's base with colored paint pens, using different colors to represent

different treatments. I did not remove any nectar from the primary and secondary flowers so that it could build up. After treating the flowers, I put pliable aluminum cages over them to prevent nectar removal by hummingbirds. I let each of the plants sit for 48 to 72 hours to allow nectar to accumulate. I recorded the date and times for treatments to maintain consistency between sampling. The number of plants I treated varied between 9 and 20, depending on how many plants had at least six open flowers.

When it was time to collect nectar, I removed the flowers and squeezed the top while holding a 10 μ L glass tube against the base to collect any nectar inside. If there was nectar, I measured how much of the tube it filled up in millimeters using Mituyo digital calipers. I later converted millimeters into microliters using the length of the tube (41.00 mm). I used the nectar from all flowers of a particular treatment to take my measurement. I then put the nectar on the prism assembly of a Leica Brix50 refractometer to measure the percentage of sugar in the nectar.

*Insect visitation in *Corydalis caseana* and *Linaria vulgaris**

For the final experiment, I surveyed *C. caseana* and *L. vulgaris* in two separate plots, the first being in Keblar's Pass and the second near Ruby Lounge in Gothic, CO. For *C. caseana*, we sampled 200 individual stalks, looking at a flower that had been robbed and another that had not on each stalk, so 400 total flowers. We destructively sampled flowers by removing them from the stem and opening them up to observe any insects inside. We caught insects in the flowers and stored them in plastic vials with a 90% ethanol solution for later identification. For flowers with robber holes, we measured

and recorded the length and width of the holes in millimeters using Mituyo digital calipers. We also recorded the number of robber holes observed on each robbed flower.

I carried out the same procedure with the *L. vulgaris* flowers, but sampling 100 separate plants and 200 flowers.

Data analysis

I conducted all data analyses using R software program version 3.4.1 (R Core Team 2017). To determine if there was an association between the number of insect visitors on the plants based on the treatment, I averaged the number of insects over the ten separate days of sampling for each treatment (Control, Primary, Secondary) and ran an ANOVA with treatment as the independent variable and mean number of insects as the dependent variable. I also ran a linear regression with the number of open flowers as an independent variable and the number of insects seen on the plant as a dependent variable.

For nectar volume and concentration, I ran ANOVAs for nectar volume against treatment and nectar concentration against treatment. For sugar concentration and nectar volume, I ran a linear regression.

We did not find any insects in either robbed or unrobbed flowers for *Corydalis caseana*, so I performed no statistical analyses for this data set.

To determine if there was an association between insect visitation and robber holes in *Linaria vulgaris*, I ran an ANOVA with treatment (Control, Robbed) as the independent variable and the number of visitors per flower as the dependent variable.

Results

Insect visitation in Ipomopsis aggregata

Mean insect visitors per treatment did not vary over the period of observation ($P > 0.05$; Fig. 1). The mean number of insect visitors across all treatments was 0.30 insects per plant per day. There was a significant difference in the number of insect visitors based on the number of open flowers, with more insects on plants with more open flowers, but with a low R^2 value ($R^2 = 0.08$, $P < 0.001$; Fig. 2).

Nectar concentration in Ipomopsis aggregata

There was a significant correlation between volume of nectar and sugar concentration when excluding treatment from the analysis, with a negative trend but low R^2 value ($R^2 = 0.07$, $P < 0.01$; Fig. 3). There was a significant difference in the mean nectar volume ($P < 0.05$; Fig. 4) between the three treatments (Control, Primary, Secondary), with control flowers having more nectar than flowers in the primary and secondary robbing treatments. It is important to note that I observed this difference even though I did not remove nectar from the flowers after making robbing holes. Nonetheless, there was no significant difference between the mean sugar concentrations among the treatments ($P > 0.05$; Fig. 5).

Insect visitation in Linaria vulgaris

There was a significant difference between the number of insects observed in robbed and unrobbed flowers, with more insects observed in the robbed flowers ($P < 0.05$; Fig. 6).

Discussion

*Insect visitors in *Ipomopsis**

In *Ipomopsis aggregata*, there was no significant difference in the number of insect visitors, regardless of treatment. There are several factors that could have influenced this finding. It is possible that insects use the number of flowers to choose which plants to visit as opposed to whether or not those flowers are robbed. I found a significant positive correlation between the number of insects and the number of open flowers, with more insects observed on plants with more open flowers. Since many insects rely on visual cues when choosing flowers to visit (Kevan et al. 1983), the number of open and therefore visible flowers may influence their decision-making. Further experimentation with a larger sample size and more days of observation would be necessary to better inform this speculation.

Sugar concentration

Since it is known that water evaporates from flowers that have been nectar robbed (Pleasants 1983), it follows that sugar concentration may increase relative to the remaining nectar. I found that the nectar volume was significantly higher in untreated flowers than in treated, or robbed, flowers. These data support the hypothesis of water evaporation occurring from robbed flowers; it should be noted, however, that there were several variables that could not be accounted for through the experimental design. Many flowers were wilted or had already fallen from the plant when I went to sample them, preventing nectar collection. This could be due to the changing weather, where the climate would go from hot and dry to cool and wet. Heavy rainfall may have also had a

role in dislodging flowers from their sepals. These factors may have influenced the amount of nectar available for sampling based on the treatments. While there was significance between treatment and volume, there was no significance between treatment and sugar concentration. Again, heavy rainfall may have played a part in diluting the nectar of some flowers, skewing the data. In future experiments, it would be helpful to sample earlier in the summer so that the weather would be more consistent. Weather ports might also help with this consistency. For sugar volume and nectar concentration, I found a significant negative correlation between the two, with sugar concentration decreasing as the nectar volume increased. More data are needed to better explore this relationship.

*Insect visitors in *Corydalis caseana* and *Linaria vulgaris**

Though often targeted by nectar robbers (Darrow 2006), we did not find any insects within the 400 *Corydalis caseana* flowers we surveyed, and therefore cannot make any claims about the relationship between insect visitors and nectar robbing. The morning we surveyed the flowers came after a night of heavy rain, so it is possible that there was an effect on insect visitation, but multiple surveys would be necessary to support this speculation.

For the 200 *Linaria vulgaris* flowers I sampled, there were significantly more insects in the robbed flowers than there were in the unrobbed flowers. This finding could support my prediction that the presence of robber holes, at least in *Linaria vulgaris*, allows certain insects to access the inside of the flower more easily than without robber holes. It is possible that there only happened to be more insects in the robbed flowers, and

sampling on multiple days would provide better support for these claims beyond the significance of this experiment.

Figures

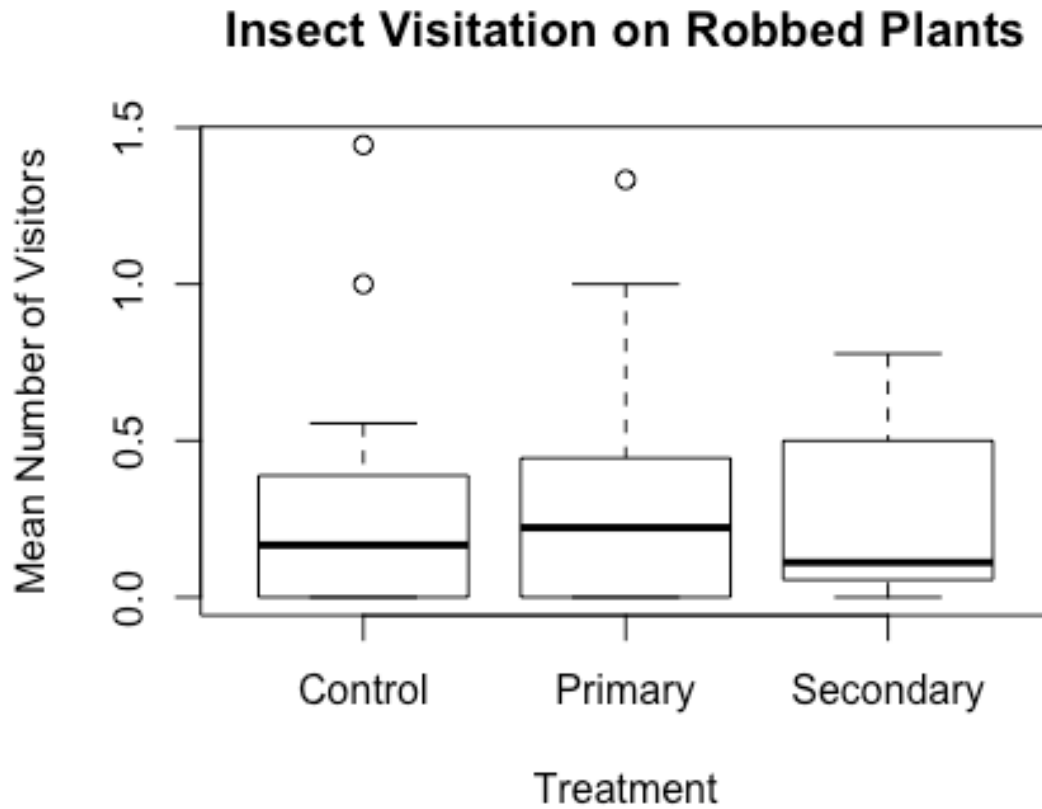


Figure 1. Mean number of insect visitors per treatment over ten separate days of sampling. Hollow circles are outliers, and thick bars represent the means for each treatment.

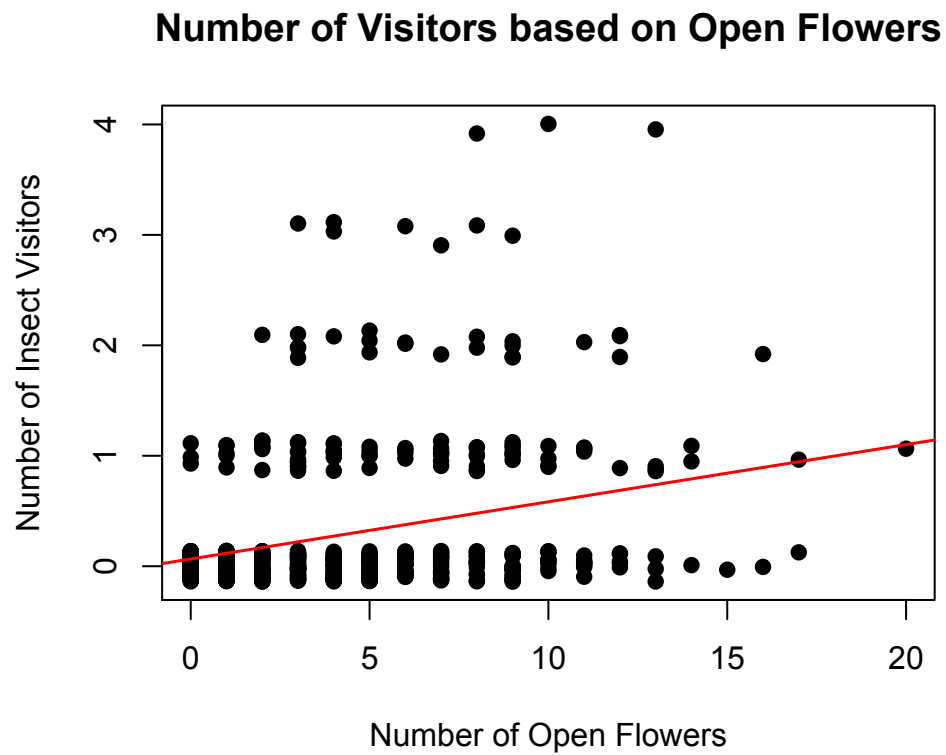


Figure 2. The number of insect visitors based on the number of open flowers available on a plant. All values are integers between 0 and 4, separated for visibility. Significant, positive trend line ($P < 0.001$) with low R^2 value ($R^2 = 0.08$).

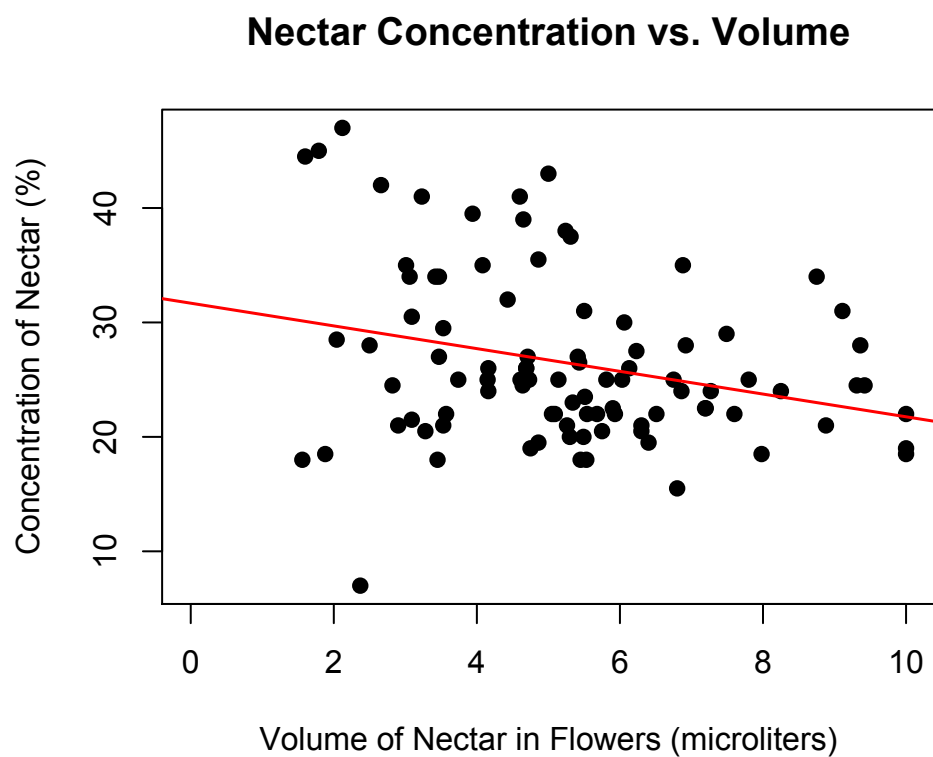


Figure 3. Concentration of sugar in nectar (%) based on the volume of nectar collected (μL). Black dots represent individual data points. Significant negative trend line ($P < 0.01$) with low R^2 value ($R^2 = 0.08$).

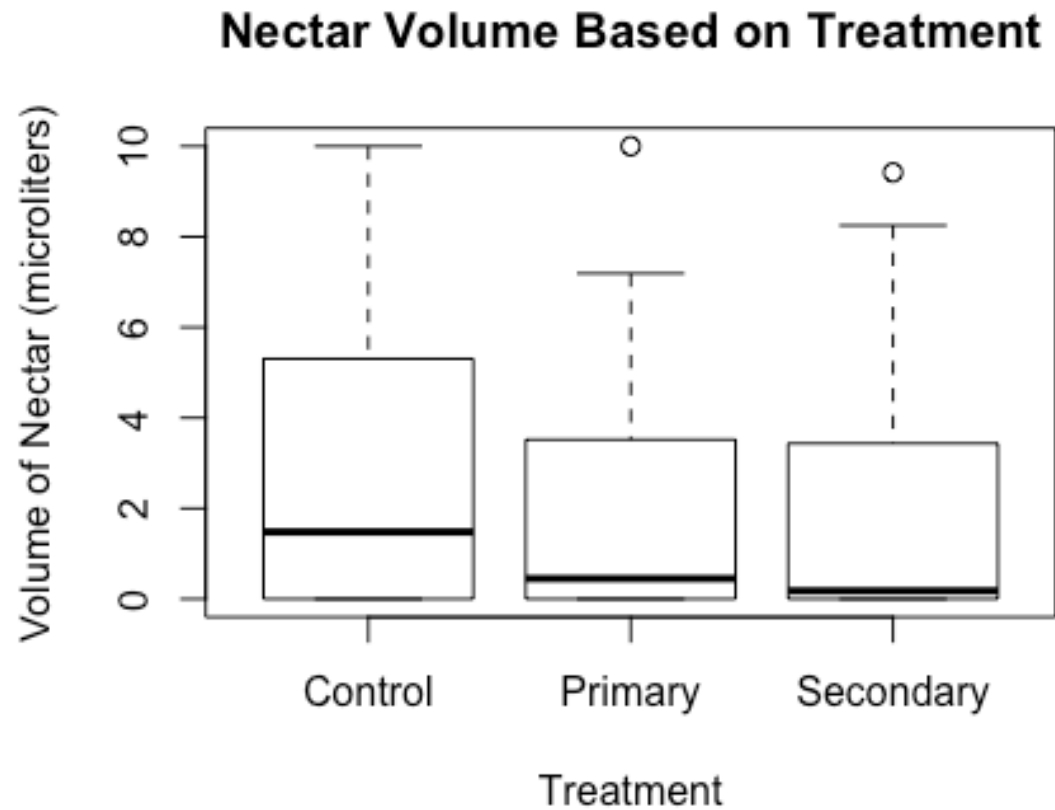


Figure 4. Volume of nectar (μL) collected from flowers based on treatment. Hollow circles represent outliers, and thick bars represent mean values. There is a significant difference between the mean of the control treatment and the means of the primary and secondary treatments.

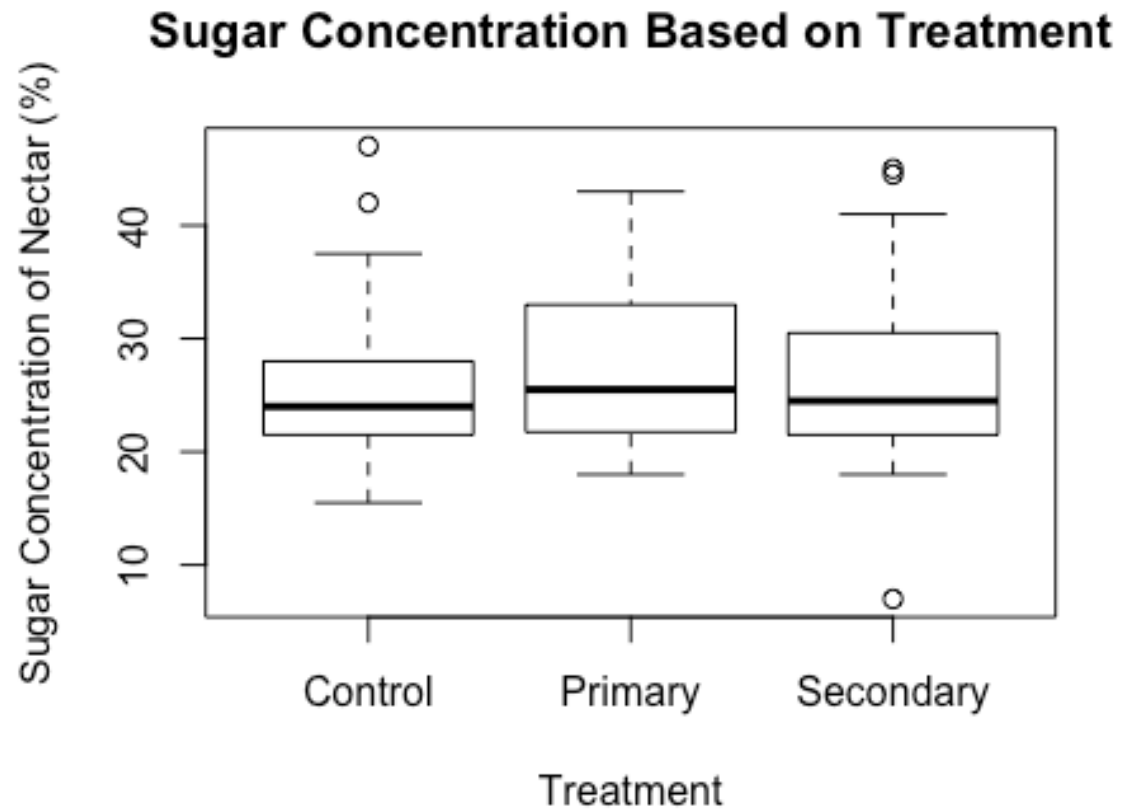


Figure 5. Sugar concentration of nectar (%) based on treatment. Hollow circles represent outliers, and thick bars represent mean values.

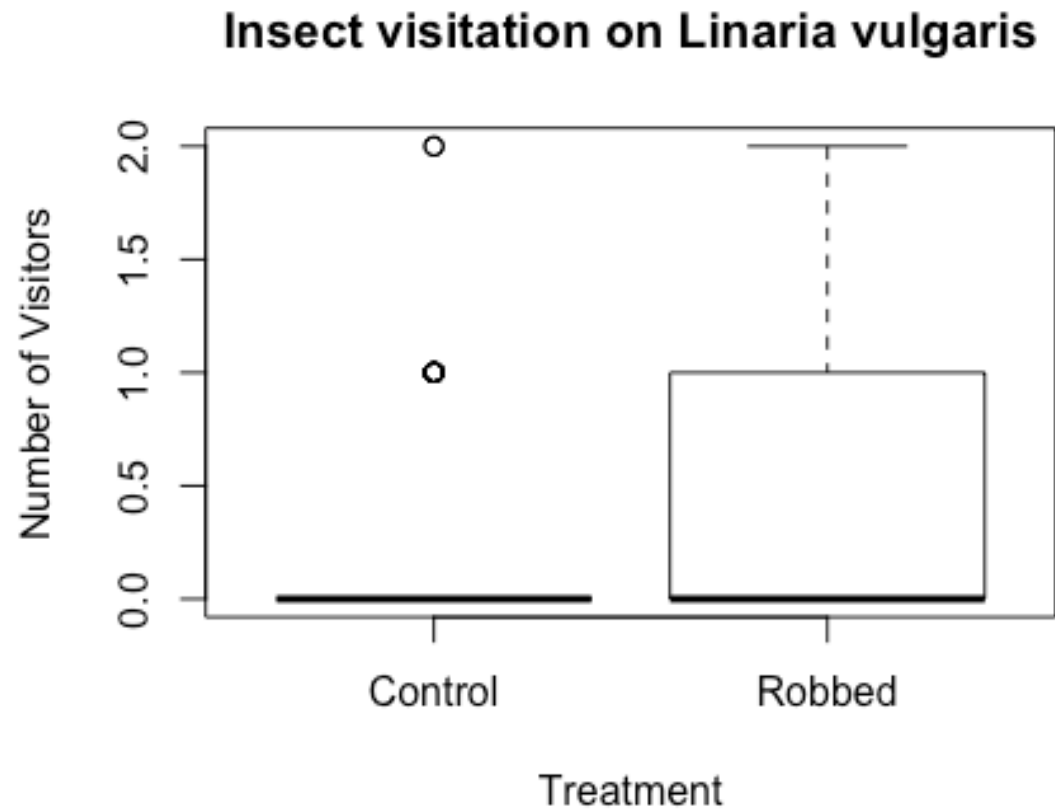


Figure 6. Number of insect visitors based on presence of robber holes in *Linaria vulgaris*. Hollow circles represent outliers, and thick bars represent mean values. There is a significant difference between the means of the treatments.

2017 Gothic Townsite

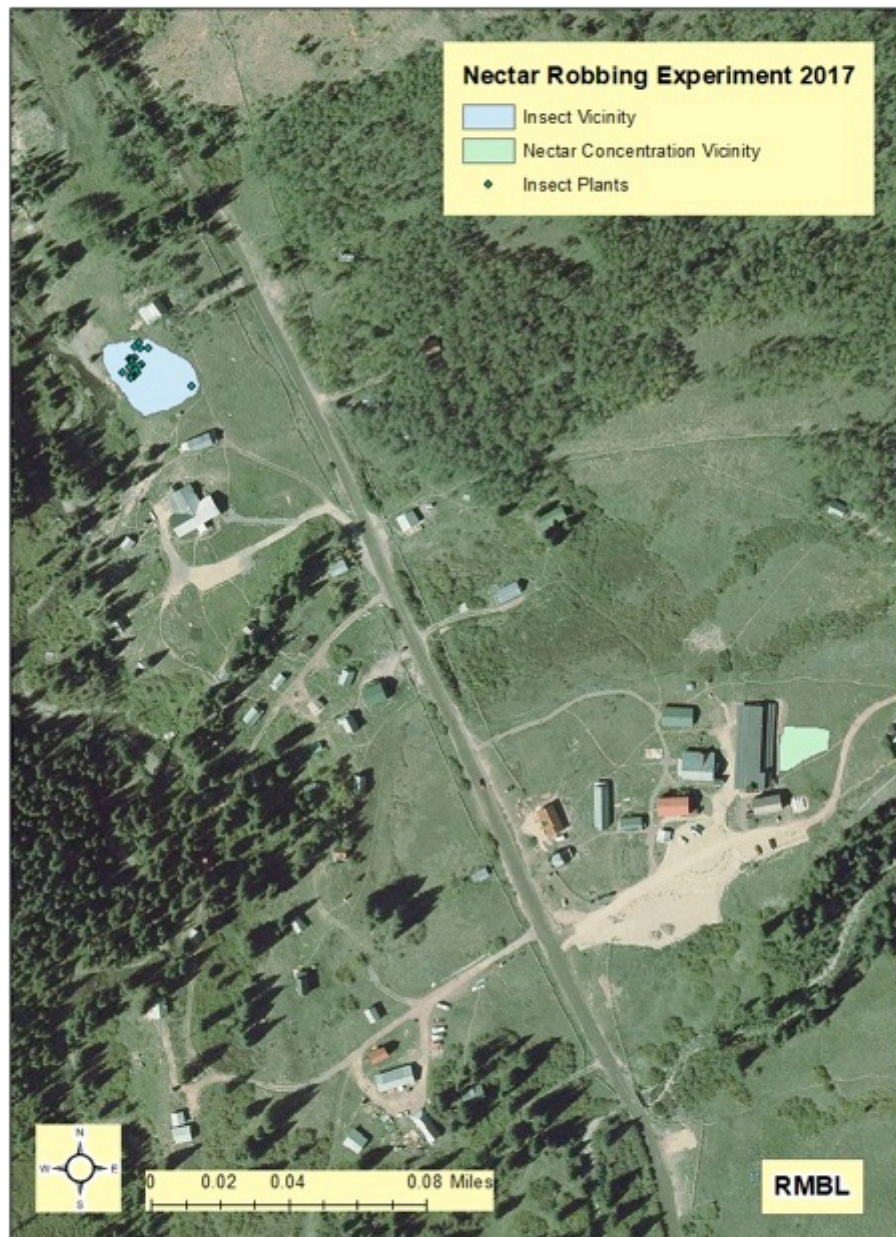


Figure 7. Map of Gothic Townsite showing locations of nectar robbing experiments on *I. aggregata*.

Literature Cited

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