

Impact of Decreased Flower Attractiveness on Pollinator Visitation Rates and Pollinator Community Composition

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Abstract

Plant-pollinator interactions are extremely familiar to many different ecosystems all over the world. Many floral species and pollinators have developed intricate and essential adaptations to benefit one another. Aspects of plant reproduction require pollinator visits and many pollinators rely on floral species for food sources. Several studies have focused on how they serve one another and form a part of complex ecological networks. However, there is a need in community-level ecology to understand how disturbances within such networks can create ripple effects throughout the entire system. Therefore, our experimental design aims to provide information on pollinator adaptation to small-scale disturbances within an already-established plant community. To test this, we manipulated floral attractiveness in three different subalpine meadows of the Gunnison National Forest. At each transect, a floral species was sprayed with quinine and actively sampled pollinators who landed on all open flowers. We identified the type of pollinator caught and which floral species it landed on. This data was used to determine how pollinator total visits and pollinator community composition differed in the control and the manipulation throughout the summer season. Due to the application of quinine creating a disturbance to the pollinators' food source, we saw a decrease in overall pollinator visits and a slight change in our insect ID groups. A decrease in the overall pollinator visits to the area could be attributed to certain species leaving for other floral communities. A change within insect group IDs can also be attributed to an increased need for competition for the flowers not containing quinine and their inability to adapt to the disturbance. This research question does not aim to explain all the ripple effects of a disturbance within a system. However, it is essential to continue testing the strength of these networks to understand how other more complex biological disturbances can potentially impact them.

Introduction

It is no secret that plant-pollinator interactions are a foundation for successful ecosystems. It is estimated that nearly 90% of flowering plant species depend on animal visitors for some aspect of their reproduction (Ollerton et al., 2011). We also know that over 200,000 animal species rely on floral resources for food (Inouye & Ogilvie, 2017), making them mutualistic interactions where both parties rely on one another to reproduce and survive successfully. These networks are highly interconnected, and understanding their response to future disturbances is highly beneficial in preserving these communities.

Climate change is one of the biggest anthropogenic disturbance factors imposed on ecosystems today. Therefore, recent studies have analyzed plant-pollinator interactions in this context to try

and understand how warming temperatures will affect these networks. Relevant studies have shown that increasing average temperatures have a varied effect on the physiology of flowering plants. Some characteristics that vary are the decision to flower and quantity of flower production, flower size, floral scent, pollen production, and plant height (Scaven and Rafferty, 2013). These characteristics of floral physiology play a large part in pollinator activity, determining whether they forage on those flowers or look for others. Therefore, the responses of flowering plants to increasing temperatures will likely affect floral visitors, and ultimately the benefits may or may not accrue (Scaven and Rafferty, 2013). While there is limited knowledge on the physiological response of common pollinators to climate change, how they are affected has been predicted based on what is known from thermal ecology. Pollinator foraging behaviors, body size at maturity, and life span are all characteristics that were found to be most likely affected due to warming temperatures (Scaven and Rafferty, 2013). These physiological changes may be varied, but they have the potential to alter the plant-pollinator networks as a whole.

Understanding how pollinators forage is also essential when analyzing their response to environmental disturbances. Pollinators' foraging behaviors can shift in response to the taste and smell of flowers. For instance, bees can learn to find flowers based on the quality or abundance of nectar available, and also learn how to avoid visiting flowers with nectar containing toxic secondary compounds (Stevenson et. al., 2016).

At a community level, how pollinators experience interspecific competition with other pollinators, despite the occasional interference while accessing flowers, has rarely been studied (Goulson, 1999). We have observed bumblebees avoiding flowers previously visited by other *Bombus* species as a sign of decreased reward (Goulson, 1999). Other studies across various ecosystems indicate interspecific competition can lead to changes in ecological specialization over short time scales through behavioral or morphological plasticity (Brosi and Briggs, 2013). Two key drivers of plasticity are the range of available floral resources and interactions with other pollinators for those resources (Brosi, 2016). When certain disturbances occur in plant communities, it is beneficial to understand how different groups of pollinators shift their foraging behaviors and how competition between pollinator species changes. There are many mechanisms involved in pollinator foraging, but having flexible preferences enables insects to adapt their strategy according to the changing availability of rewards in different floral species (Goulson, 1999).

To further test the breadths of plasticity in plant-pollinator interactions, our manipulation of floral attractiveness can show whether these dynamic interactions are capable of shifting under different conditions. For example, bees have been shown to reject flowers with unpleasant tastes, but the response of other common pollinators has yet to be studied. Other studies have shown that the removal of generalist plants from a landscape can dramatically reduce visitation rates and redistribute visitors to the remaining plant community (Bain et. al, 2022). For this

experimental design, we will be investigating how the application of quinine (a bitter compound) to one flower species impacts the number and changes of pollinator visits to the whole floral community. Our first aim is to determine if pollinators who frequent those areas adapt to the disturbance by staying or leaving. This will be measured by assessing if there is a change in total pollinator visits to the area. Our second aim is to quantify how the average visits differ between different insect groups. While this experimental design does not analyze plant-pollinator interactions in relation to climate change, the disturbance to their food source, which we will be manipulating, can model changes in the foraging behaviors of the pollinator community as an adaptation to small-scale disturbances.

We asked how the spraying of quinine on flowering plants influences the number of pollinator visits to the area and the composition of pollinators in the area. Specifically, we asked (1) whether total visits to the manipulated area are similar or different to the control area; and (2) whether those who remained in the treatment consist of the same species or vary. Based on previous experimental designs, I hypothesize that pollinator visits will decrease overall. I also hypothesize that some insect groups will be affected more than others with the presence of quinine. To be more specific and based on what is known about their foraging behaviors, bumblebees will be the most impacted and decrease their visits to the manipulated areas. Due to their ability to fly longer distances, they can flexibly adapt their flight range to local environmental conditions (Herascu R., 2017). Altogether, this work provides an experimental test to improve our empirical understanding of the consequences of disturbances within a community.

Materials and Methods

Study Sites

We worked in three field sites near Rocky Mountain Biological Laboratory (38°95'53"N, 106°98'685"W, 2898 m above sea level), in the Gunnison National Forest of western Colorado, United States called Gothic Town (GT) (38°56'53"N 106°59'27"W, 2878 m above sea level), Avery Picnic (AP) (38°58'49"N 106°59'57"W, 2980 m above sea level), and Rustler's Gulch (RG) (38°59'24"N 107°00'43"W, 2988 m above sea level). They were subalpine meadows that shared comparable densities of pollinators and flower species. All three field sites had four 20m x 1 m transects labeled P1, P3, P5, and P7. Each transect was then divided into ten 2m x 1m segments labeled S1 through S10. We used data collected in the summer months of June through August 2023 and 2024.

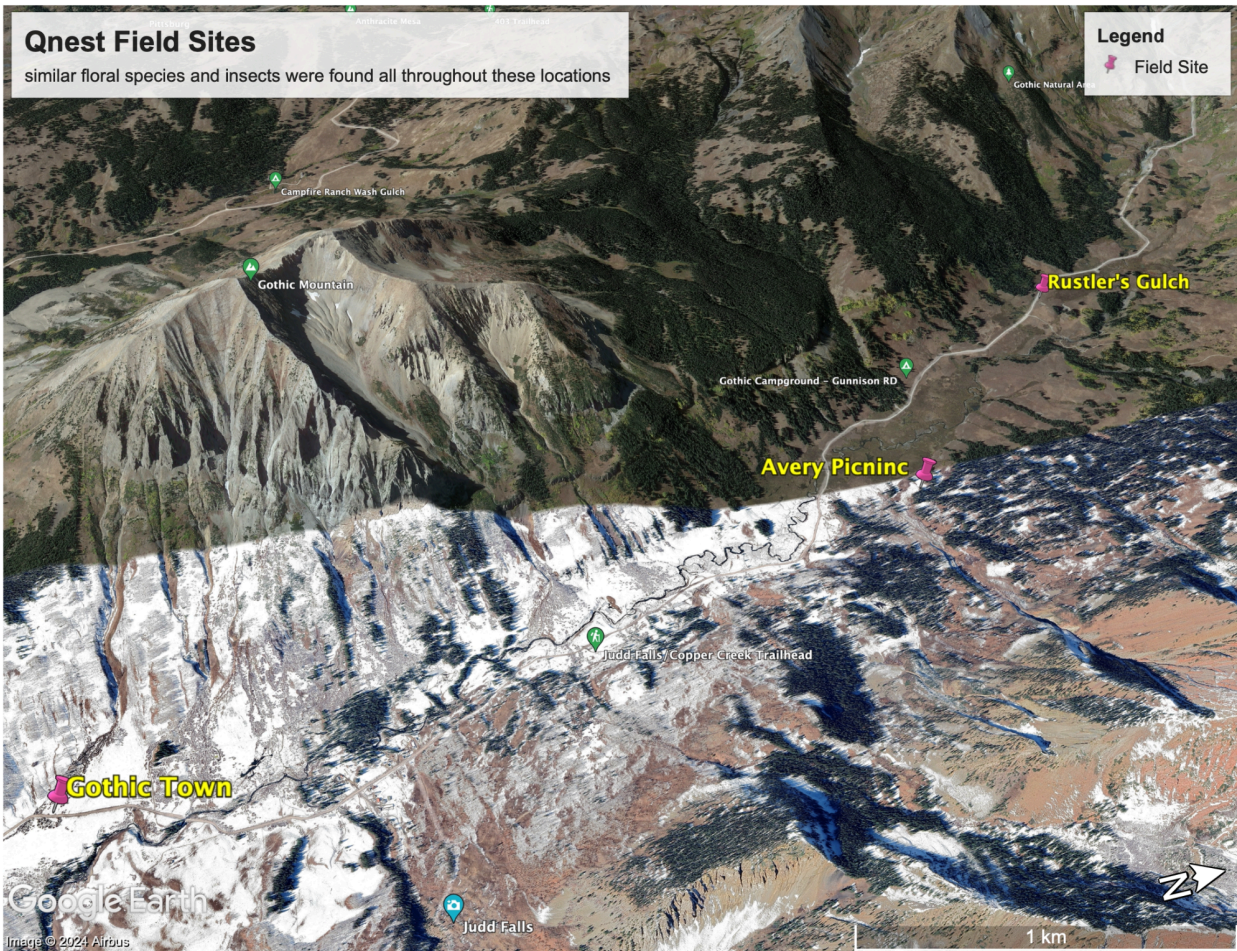


Figure 1. Map showing field sites used in this study. These sites were labeled with their corresponding coordinate points. Each field site had similar floral species and insects and experienced similar weather patterns.

Treatment: Control and Manipulation

The three field sites served as both our control and manipulated areas. On the control days, a floral survey was conducted. The floral surveys included counts of each flower species within each 2m x 1m segment and were identified to species level. The flower surveys only included plants that were flowering (i.e. those with their reproductive organs visible and accessible to pollinators). Our field team then prepared for pollinator sampling by prepping glass vials with a few drops of ethyl acetate to euthanize the captured insect. Each observer visited an equal number of segments between all four transects, randomly assigned to prevent potential sampling bias. To obtain the number of visits and identity of pollinators at each field site, we sampled all pollinators that landed on the reproductive organs of the flowers present within the transects. Insects collected fell into the following gross ID categories: solitary bee, Megachile (leafcutter bee), Syrphidae, Bombyliidae, other fly, Wasp, Moth, Beetle, Hemipteran, Bombus, and

unknown (if the insect was not identifiable). Some insects excluded were ants, grasshoppers, and spiders. Bumblebees were also caught but released after being identified.

The total active sampling time was 120 person minutes. Each segment was observed for three minutes total, one minute and thirty seconds on each side to prevent missing any pollinators from landing on hidden flowers. The timer was paused with each catch and then resumed. Each pollinator caught was then placed into a vial with a written description of the vial number, field site, date, and flower species it landed on. During this process, we also recorded “misses” when an insect we intended to capture escaped or got lost. Sampling was also conducted during different rounds, allowing for replication and a larger sample size each day. The number of rounds sampled each day was also determined by weather conditions, but they were kept equal for a given control and manipulation pair day. Finally, we conducted our control sampling at a specific site on one day, followed by our manipulation treatment on the subsequent day. This approach ensured consistency in weather conditions, floral species availability, and insect abundance across both sampling periods.

Our experimental design relied on manipulating the attractiveness of floral species in our plots by using quinine. We used a 10 mM quinine solution to alter the taste of the flower species in our sample sites. This method ensures a disturbance to the pollinator community’s food source. Because our manipulation occurs the day after the control at the same site, we do not conduct a floral survey, assuming the floral community remains constant. In turn, an individual floral species is previously selected to be manipulated and sprayed with quinine. The selected species has two main considerations: (1) the one that showed the most pollinator interactions and (2) practicality. We use flower guards when spraying the flowers to avoid the solution getting onto non-focal flowers in the plot. Each flower of the selected species is sprayed within all transects, all segments, and within a 1m buffer zone. The plot is then sampled the same as the control. Quinine deteriorates with sun exposure so respraying was done when necessary.

Data Analysis

Because we care about how overall pollinator visits and composition differ between each control and manipulation day pair, we grouped total visits by date, site, and round. Some manipulated days had fewer rounds than their control paired days due to different circumstances, so we had to group by rounds to only use data from days where they equaled each other. For our insect composition data analysis, we included “gross ID”. The pollinator composition was determined by separating all pollinators caught into 11 insect ID groups: solitary bee, Megachile (leafcutter), Syrphidae, Bombyliidae, other fly, Wasp, Moth, Beetle, and Hemipteran, Bombus, unknown. After testing for normality (Shapiro-Wilk normality test), a paired t-test was conducted to determine any significance in the differences between the total pollinator visits. We also tested for equal variances (F-test). The same data sets were analyzed to obtain the average total visits

for each gross ID group per treatment. A paired t-test was then used to identify any statistical significance between differences in average total visits. All statistical analysis was done using R-studio version 2024.04.2.

Results

By the end of data collection, we obtained a sample size of 22 paired observations of control and manipulation treatments. This was used to determine a pattern in total pollinator visits throughout the sampling period. This data set showed that there was an overall decrease in the amount of total visits due to the quinine treatment. For our second sample size, we grouped insects into 11 different gross ID groups: beetle, Bombus, Bombyllidae, butterfly, hemipteran, moth, other flies, solitary bee, Syrphidae, wasps, and unknown. Overall, there was no statistical difference in average visits between control and manipulation for each gross ID group, except bumblebees (*bombus* spp.).

Our first aim was to determine if the application of quinine to a flower species in the community would impact the overall pollinator visits. Some values between our paired observations showed decreases in total visits, such as 225 total visits observed in the control on 7/11/24 and 164 visits the following day with the manipulation treatment. Other paired values showed increases in total visits, such as 101 total visits in the control on 7/18/2024 and 138 total visits the following day in the manipulation treatment. While there was some variability, we found that on average there were more pollinator visits in control treatments than in manipulation treatments. Our control had an average of 117 visits and our manipulation had 94 visits. After analyzing all of our data sets, we determined a statistical significance in the decrease in total pollinator visits between control and manipulation treatments ($p = 0.03318$, paired t-test, $N = 22$).

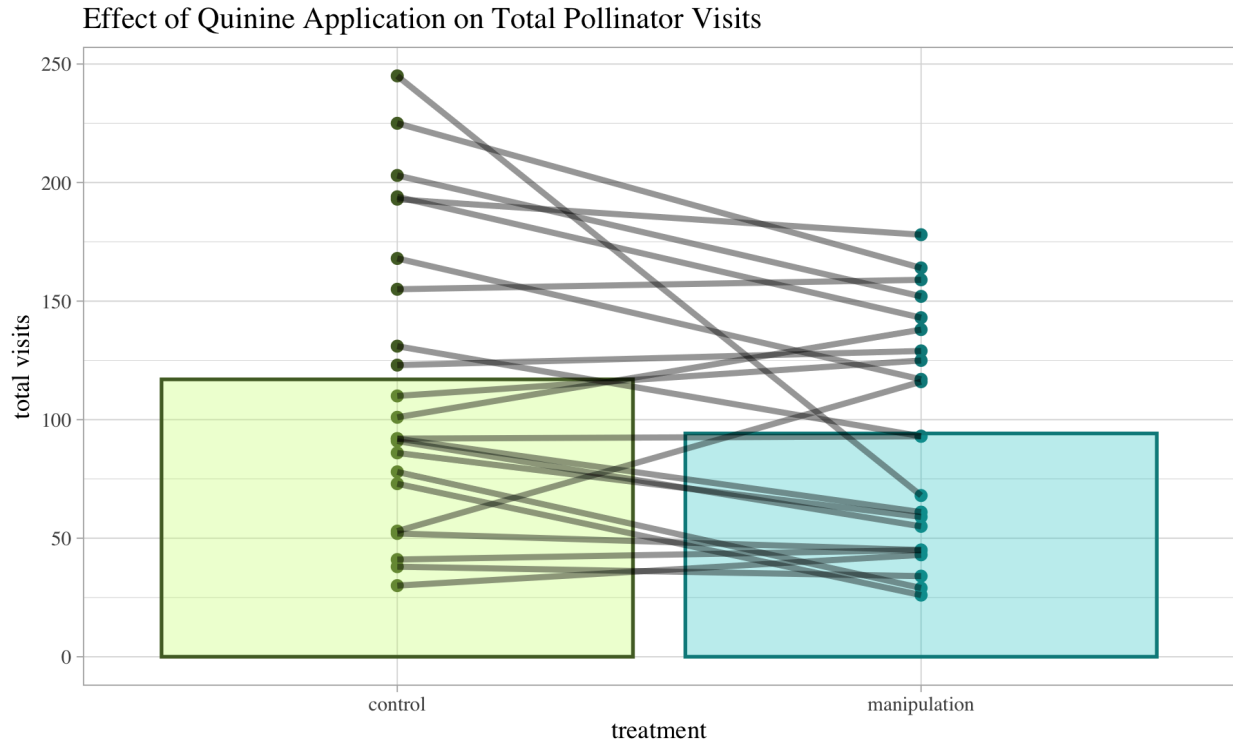


Figure 2. There was an overall total decrease in pollinator visits. Total visits were grouped by date sampled, equal number of rounds, and treatment. Each control value has a corresponding manipulation value which can be traced with a semi-transparent line showcasing the change in total visits. The bar graphs show the average total visits for each treatment group.

Since we did determine a change in total pollinator visits between both treatment groups with the presence of quinine, our second aim was to see how the treatment impacted the average total visits within the insect community. After analyzing our entire data set, we only saw a strong statistical significance in the decrease in average total visits of bumblebees ($p = 0.00591$, paired t-test, $N = 19$). There was an average of 7.84 total bumblebee visits in our control and an average of 4.11 total bumblebee visits in our manipulation. We ran a t-test for the rest of the gross ID groups to ensure there was no statistical significance between any of the observed differences; we saw none. Our most abundant gross ID group was “other fly”, with an average of 53.70 visits between both treatment groups. Our second most abundant gross ID group was “syrphidae” followed by “solitary bee”. Due to the significant decrease in bumblebee average visits, we can say that the presence of quinine led to a shift in the insect community.

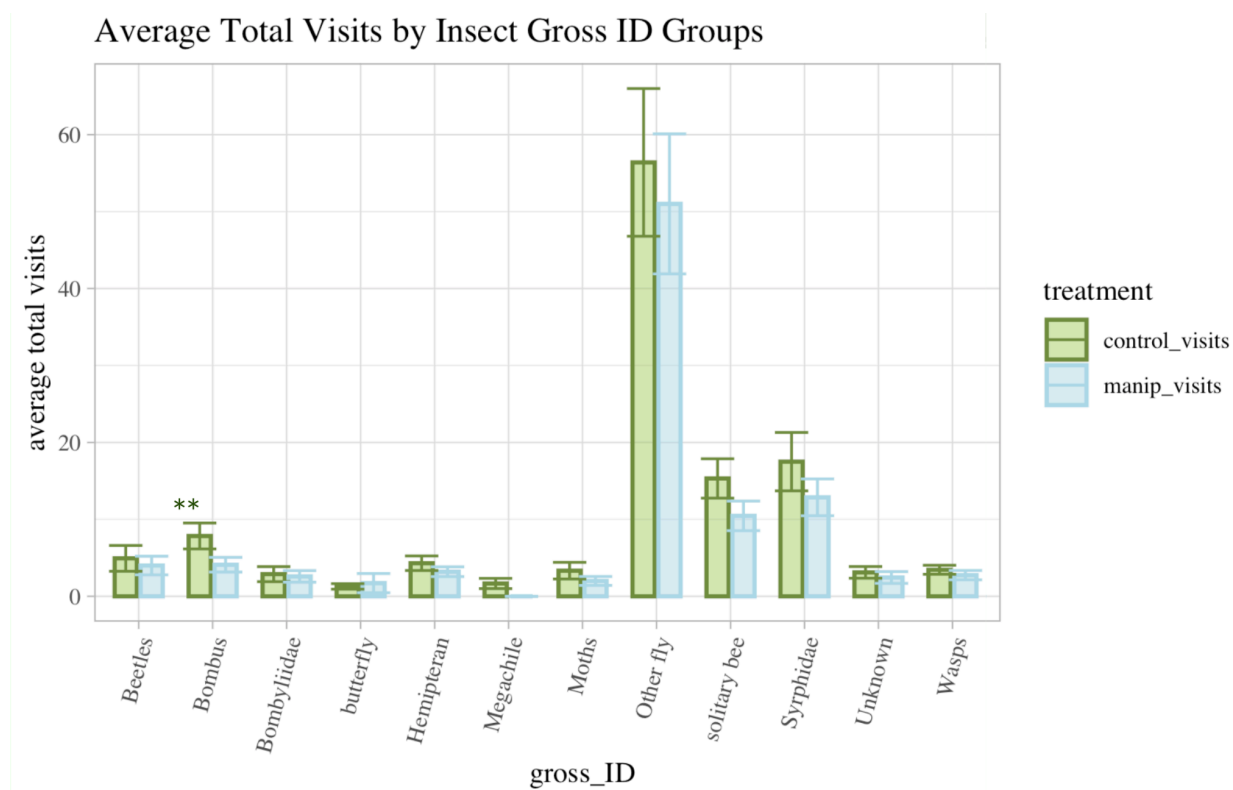


Figure 3. We analyzed how the application of quinine impacted the average total visits by insect gross ID groups. We categorized our insects caught into 11 different gross ID groups. The most number of visits were from the “Other fly” group. The most significant difference was identified in the “Bombus” group with a p-value less than 0.001. This strong significance is signaled by the two asterisks above the bar graphs. Paired t-tests were also done for all other gross ID groups.

Discussion

Our results demonstrate how pollinators in identical communities react to a small disturbance. Our experimental design used quinine to reduce flower attractiveness and therefore impact the food source in a community. There were two possible reactions to this disturbance: (1) the pollinators left the area or (2) the pollinators remained in the area but shifted foraging preferences to flowers unaffected by the quinine. While we saw a reduction in the total pollinator visits to the area, it is hard to determine which of the two scenarios can be the true response. This decline can be due to many pollinators choosing to forage elsewhere, but it can also be that they

stayed but left due to increasing competition for the available resources. Nonetheless, this reaction is in line with what other studies found about how pollinators detect certain chemicals.

After seeing that there was indeed a decrease in total visits, it was intriguing to see how that shift translated into the different gross ID groups. By taking the average total visits of all insect groups, we saw changes in visits between control and experimental treatments. The difference between bumblebee visits after the manipulation was statistically significant. This was a strong statistical significance, which meant there was strong evidence that the decrease in average bumble bee visits between control and manipulation days was due to the application of quinine. These findings are supported by previous studies where quinine has been used as an aversive compound in nectar-based behavioral assays (Chittka et al., 2003). Other studies have used quinine to determine if bees had a behavioral response to differences in pollen chemical composition (Muth et al., 2016) and nectar containing toxic secondary compounds (Stevenson et al., 2017). Most of these studies were done on honeybees and bumblebees and showed that they learned to reject flowers that contained quinine-laced pollen. If some were to taste the quinine-laced pollen, they were most likely to switch flower preferences (Muth et al., 2016). Foraging behaviors among bumblebees are also one of the most studied among pollinators. Bumblebees are one of several pollinators that exhibit flower constancy, which is described as a learned preference for a specific flower that has previously provided a reward (Goulson, 1999). They are also known to abandon this foraging behavior when their preferred flower is scarce (Chittka et al., 2003) and rapidly replace these learned preferences with new ones when the flower rewards cease (Goulson, 1999). By applying quinine to the center of a specific flower species we disrupted the main reward for most bumblebees. Therefore, their response to this disruption is justified and expected based on their known foraging behaviors.

Another interesting finding was the “other fly” group, which was the most abundant pollinator throughout the communities we sampled. While their response to quinine was not statistically significant, it shows that they react differently to quinine than bumblebees. When we were sampling, they were the slowest to react to the quinine. We also saw them landing and staying on the manipulated flower the most out of all the other groups. Despite these observations, their reaction to ecological disturbances or specific foraging behaviors is well understudied. The high abundance we saw is, however, supported by relevant studies. We classified the insects caught under this gross ID group to be part of the Diptera order, but non-syrphid Diptera. 71 families in the Diptera order are floral visitors to at least 555 flower species (Larson, et al., 2001). More notably, studies conducted in the United Kingdom analyzing farmland pollination networks discovered that non-syrphid Diptera comprised 67% of all flower-visitor abundance (Orford et al., 2015). Due to observed wild pollinator declines in various ecosystems, mostly observed in bee populations, these alternative pollinator taxa can provide some insurance against bee losses (Orford et al., 2015). Therefore, future exploration of their foraging behaviors can be valuable in understanding the role of non-syrphid Diptera as pollinators.

Limitations and Weaknesses

Our findings greatly depended on the pollinator sampling we conducted on each treatment day. To minimize error, we recorded all missed insects to the best of our abilities and only sampled them when we were actively observing the area. However, one aspect we could not control was the weather. Pollinators usually forage under ideal environmental conditions, which include sunny and dry days. However, summers in our field station experienced several days of rain and strong winds. There were days our sampling was cut short due to incoming thunderstorms. We also continued sampling despite strong winds which could have resulted in higher missed insects. Weather also posed an issue when comparing paired control and manipulation days, and can be a reason for drastic declines in total visits with the manipulation treatment. Another consideration for future studies is the concentration of quinine. While the quinine proved to be effective for the majority of the sampling period, early manipulation treatments used a quinine solution with a lower concentration.

Future Directions and Importance

As we previously stated, most plant-pollinator interaction studies focus on bees, hoverflies, and butterflies. We know much about their foraging behaviors, preferences, and importance. Therefore, with the continued use of quinine, future studies could analyze how specific insect pollinator groups change their floral preferences as a response to the disturbance. There could also be more in-depth studies on how non-syrphid Diptera change their foraging preference and behavior. On the same note, another summer conducting this exact experiment could provide more evidence. This could either support the findings of this study and give a stronger statistical significance in the decrease of pollinator visits, or show the lack of it. Nonetheless, continuing to use quinine as a small-scale disturbance can give valuable insight into plant-pollinator networks. The use of quinine is not meant to replicate any real environmental disturbance. However, this should not disregard the value of its use in experimental designs. Understanding how wild communities function and respond to disturbances can be difficult due to the many abiotic and biotic conditions they experience. We know much about individual components of certain communities, but knowing what happens when we poke at a system in a controlled way and how far those effects ripple through is still a valuable area of study. Given how understudied many pollinator groups still are and the large unknowns of climate change on these systems, improving our understanding of plant-pollinator communities is necessary.

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