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A field study of Mimulus alatus and its visitors

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Abstract

The purpose of this field study was to determine the flower characteristics of *Mimulus alatus* and observe the behaviors of visitors to the plant. Members of *M. alatus* were observed in their natural habitat to ascertain the flowering period for the plants and the lifespan of a single bloom; nectar and pollen levels were also periodically measured. Behavioral patterns of visitors to the flowers were recorded and the species identified. Statistical analysis was performed to deduce the relationships between time of day and nectar and pollen levels, as well as visitor behavior. The blooming period occurred from early July to mid-August and lifespan of open flowers was found to span roughly 24 hours. Nectar standing crop and pollen levels decreased monotonically throughout the day and were depleted by 11 AM. Visitors of the genus *Bombus* occupied the early morning hours and non-*Bombus* visitors increased in prevalence later in the day; visitation rates were generally higher for *Bombus* species than other groups.

Overview

Flowering plants rely on several methods for pollination to occur, utilizing wind or water, or most commonly, pollinators. In return for food in the form of pollen, nectar, or fruit, these visitors transfer pollen between flowers to fertilize them (Mitchell et al. 2009). This complex relationship between the flowering plant and its visitors is critical for each party’s reproduction or foraging. To understand this relationship in our plant of interest, *Mimulus alatus*, it’s first necessary to know the basic characteristics and interactions of visitors with *M. alatus*.

Several factors determine visitation: flower shape, blooming season and period (time of day when flowers open), competition, and weather conditions. Flower shape and display dictates what visitors enter the flower for food (Christopher et al. 2021), while the blooming season restricts potential visitors to those that are active during that time of year. Furthermore, the time of day during which flowers are open or potential visitors are active must by necessity overlap for visitors to be potential pollinators. Competition between pollinator species and other flower species drawing attention away from *M. alatus* can alter visitation rates (Flanagan et al. 2011). Changes in climate or weather patterns could also affect a visitor’s ability to interact with flowers (Bertin and Sholes, 1993). The combination of these factors selects for specific visitors to cooperate with each plant species.

The Flowers – *Joshua Stefan*

Introduction

In this chapter, we focus on the physical characteristics of *M. alatus* (sharp-wing monkeyflower) plants and the flowers they produce. Very little published literature is available about *M. alatus* apart from information on its habitat, where it might be found in the United States and its basic appearance (Valerie 1993). The sharp-wing monkeyflower is best found in wooded areas with damp soil consisting of high amounts of clay, silt and sand (**Figure 1-1**). Often compared to its cousin, *Mimulus ringens* (square-stem monkeyflower), our plant is distinguished by its leaves with toothed edges and a longer petiole than *M. ringens*, along with a shorter pedicel connecting the flowers to the stem. Aside from these basic characteristics, the only other publication regarding *M. alatus* indicates that it is capable of hybridizing with its aforementioned cousin when both species inhabit the same territory (Windler et al. 1976).

Figure 1-1. A patch of *M. alatus* at Brecksville Reservation. Most of the green vegetation seen on either side of the 1 m wide streambed is *M. alatus*.

A baseline field study such as this will provide invaluable information for future research into *M. alatus*. Simply understanding the length of time during which these plants produce blooms and how long an individual flower lasts will help in planning for further studies.

Additionally, information as simple as the number of flowers present on a single stem might prove an indicator for the degree of self-fertilization occurring in this species, as it does for *M. ringens* (Christopher et al. 2021). Understanding the basic behavior of the flowers and the resources provided by the plant can also help mold future pollinator and visitor studies for *M. alatus*. Figuring out whether pollen and nectar levels decrease monotonically throughout the day can guide researchers to what species of pollinators might be expected to be visiting flowers at a particular time of day.

This study will attempt to determine the blooming season for the sharp-wing monkeyflower as well as the life cycle of a single flower from bud to the time its petals fall off. Furthermore, we will observe when nectar is produced and whether its levels (standing crop) drop steadily throughout the day or if it is replenished at some point. Another point of interest will be how much nectar is produced in a flower before visitation and whether the number of flowers on a plant affects nectar levels within each flower. This will identify whether each stem makes a set amount of nectar that is distributed to the flowers proportionately or if the stem produces enough nectar to fill each flower individually. Additionally, we will explore whether stigma closure and pollen levels correlate to nectar standing crop and can be used to infer nectar volumes within flowers. This will also determine whether visitors to flowers are transferring pollen and contributing to pollination instead of simply feeding themselves. Finally, the effect of sunlight on nectar production will be explored to find whether the light setting can be used to predict nectar levels.

Methods

Casual observation of several sites where larger populations of *M. alatus* might be found revealed that only Brecksville Reservation (41°18'11.2"N 81°36'37.1"W) contained a sample

size deemed large enough to study. The population was located in a wooded valley along a small streambed consisting mostly of small pools of standing water, except after a large rainfall when some flow was present in the stream. The flower population was divided into nine patches based mostly on geographical boundaries, such as long bends in the streambed or large fallen trees blocking direct access from an adjacent patch. Patches contained an estimated range of 50 to 400 individual *M. alatus* plants, with each plant arising from its own stem. The typical distance between patches was an estimated 40 meters, with some as close as 15 meters apart and a few separated by as much as 70 meters. Observation of the *M. alatus* population ran from 7/28, 2020 when plants were seen to bear flower buds in large quantities, until 8/22, when very few plants were producing new buds.

Data was collected at varying times of the day to obtain a spectrum of values. Collection of flower characteristics occurred at opportunistic intervals throughout the day and varied in what observations were noted. Sometimes only one variable such as stigma closure was looked at, while other sampling periods looked at multiple categories of interest. Old flowers (defined by wilting or discoloration of petals, or missing stamen) were ignored for the purpose of maintaining data only for active flowers. Anywhere from 20 to 50 flowers would be sampled during a single 20-40 minute walk through all of the patches. Fresh flowers were picked off a plant haphazardly and if the flower did not display obvious signs of aging, then it

Figure 1-2. A bud and flower that has dropped its petals on *M. alatus*. Neither was counted among normal samples.

was counted as a sample (**Figure 1-2**). Once a flower was chosen from a plant, information gathered included whether the flower was shaded or in sun at that moment in time, whether the stigma was open or closed, if there was any discoloration of the stamens and how much pollen was present to the naked eye (scored as full, moderate, or empty). The total number of flowers on the plant off which a flower was chosen was also noted, although buds (defined as flowers whose petals were not yet unfolded and exposing the interior components) were not included in the tally. Additionally, the amount of nectar in a flower (standing crop) was measured using capillary tubes (length = 30 mm, volume = 5 uL).

If a plant or set of flowers needed to be isolated to obtain results that wouldn't be affected by animal or insect behavior, a microweave net (hole size = 1 mm) was placed over the plant/flowers and secured at the base and above the plant's uppermost leaves. This was largely done to collect baseline values for nectar production in flowers. It also improved confidence when counting the number of flowers made per plant because animals couldn't remove flowers before counting and if branches or flowers did snap off of the stem, the flowers would still be trapped in the net and accurately counted. Additionally, discovery of how long a flower lasted on a plant without interference (from bud with a visible white nub inside to petals falling off) was achieved through use of the nets. To prevent plants from being damaged by the weight of the nets, stakes were often used as supports. A range of 5-20 plants would be bagged for observation for 24-72 hours at a time. Bagging was used successfully five times throughout the field study. For plants that were to be observed for multiple days but where insects or animals were not a concern, colored tags wrapped around the stem were used for identification purposes. Plants chosen for closer observation were often trimmed of all open and aged flowers so that data points were collected only for new flowers.

Samples were gathered from over 450 flowers during six visits to the field site (standing crop of nectar was not measured on one visit). Total sampling time per day was around three hours, broken up during several times of day, ranging from 6 AM to 4 PM. Additionally, five separate visits were made to the field site to check on over 110 newly opened flowers on plants isolated by microweave nets. All sampling of new flowers was done between 7 AM and 12 PM in one 45 minute walkthrough per day.

Statistical analysis was performed using JMP Pro 15 software. A χ^2 test was used to analyze the relationship between pollen levels and stigma closure. Analysis of nectar standing crop among pollen content and stigma closure groups was achieved via ANOVA, as were comparisons of nectar levels in new flowers by time of day and among plants with a certain number of flowers. A t-test was performed to analyze nectar standing crop among sunlit and shaded flowers, nectar production in unvisited flowers among pollen content and stigma closure, and nectar levels in unvisited flowers among shaded versus sunlit flowers.

Results

Prior to collecting most of the data, seven to ten plants were isolated using microweave nets on three separate occasions to determine when flowers opened, and consequently fell off, to ascertain the best times for data collection. It was determined that flower buds swollen in the afternoon opened between sunset that evening and 5:30 AM the next day. The progression from new green bud to open flower took roughly 72 hours to complete from the time the new bud could be visually identified by the tiny nub of white petals inside its sepals. Old flowers would fall off by 10 AM the morning after which they had first opened (unpublished data).

There is a general decrease in nectar levels as the day progresses until volumes are depleted around 11 AM (**Figure 1-3**). The first day has roughly 2.5x the average nectar at any

given time over the next closest day. Pollen contents also decrease as the day wears on, with anthers emptying near 11 AM (**Figure 1-4**). The majority of stigmas close by 10 AM (**Figure 1-5**).

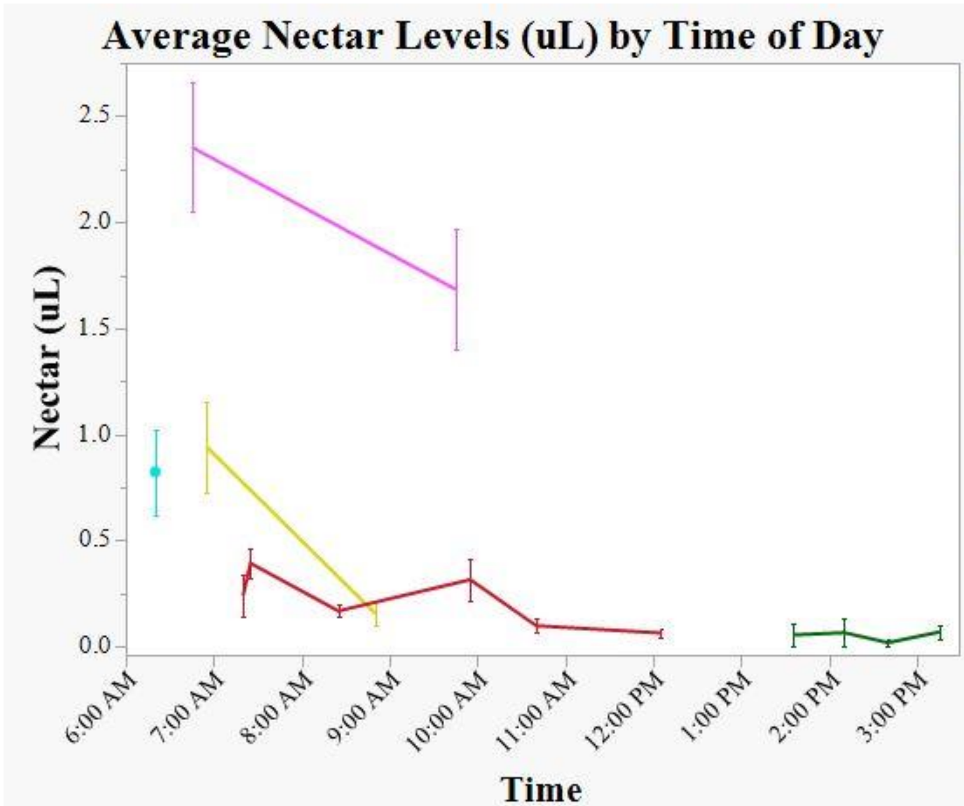

Figure 1-3. Average standing crop of nectar (uL) at various times of the day. N ≈ 18 flowers sampled per data point. Data points are coded by date: pink = 7/28, yellow = 7/29, cyan = 7/30, red = 8/10, dark green = 8/21. Nectar measurements were not taken on 8/6, so it is not shown on the graph. Largest volumes of nectar are seen early in the day with standing crop depleted by 11 AM.

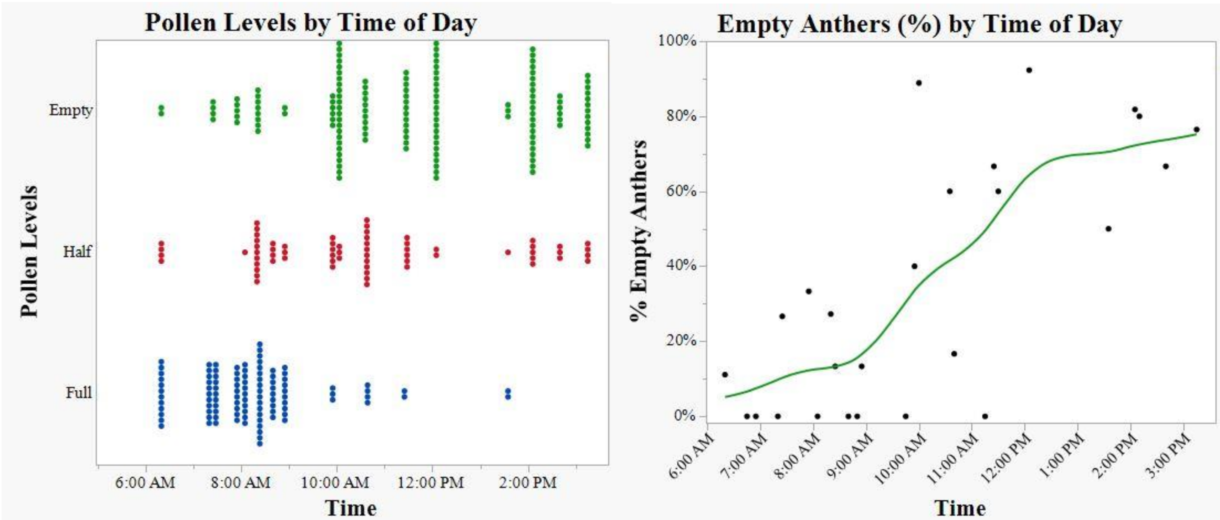

Figure 1-4. Pollen levels throughout the day across five days of observation. Total N = 312 flowers. Highest levels of pollen seen early in the day with the majority of flowers being emptied by 11 AM.

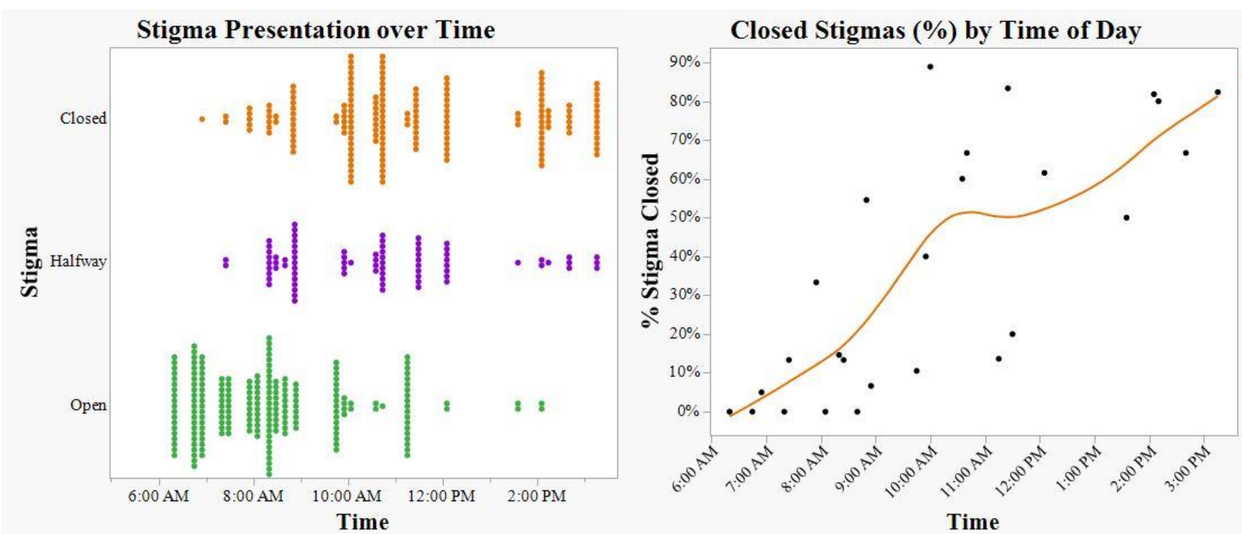

Figure 1-5. Presentation of stigmas throughout the day across six days of observation. Total N = 462 flowers. Stigmas are open earliest in the morning and begin closing on a majority of plants at 10 AM.

We wanted to determine whether stigma presentation and pollen amount could be used to infer nectar volume for a flower. Stigma presentation and pollen levels correlate strongly with nectar standing crop. Flowers with an open stigma contain an average of 1.07 ± 0.09 uL of nectar

(mean ± SE), while half-closed stigmas present an average of 0.14 ± 0.15 uL and flowers with fully closed stigmas an average of 0.16 ± 0.12 uL (**Figure 1-6**). Standing crop levels differed significantly among stigma presentation groups (P<0.0001). Flowers scored as being full of pollen had a mean of 0.41 ± 0.06 uL of nectar per flower, while half-filled flowers presented a mean of 0.10 ± 0.08 uL and flowers without pollen showed a mean of 0.11 ± 0.05 uL (**Figure 1-7**). Comparison of mean nectar levels among pollen score types were statistically significant (P=0.0007).

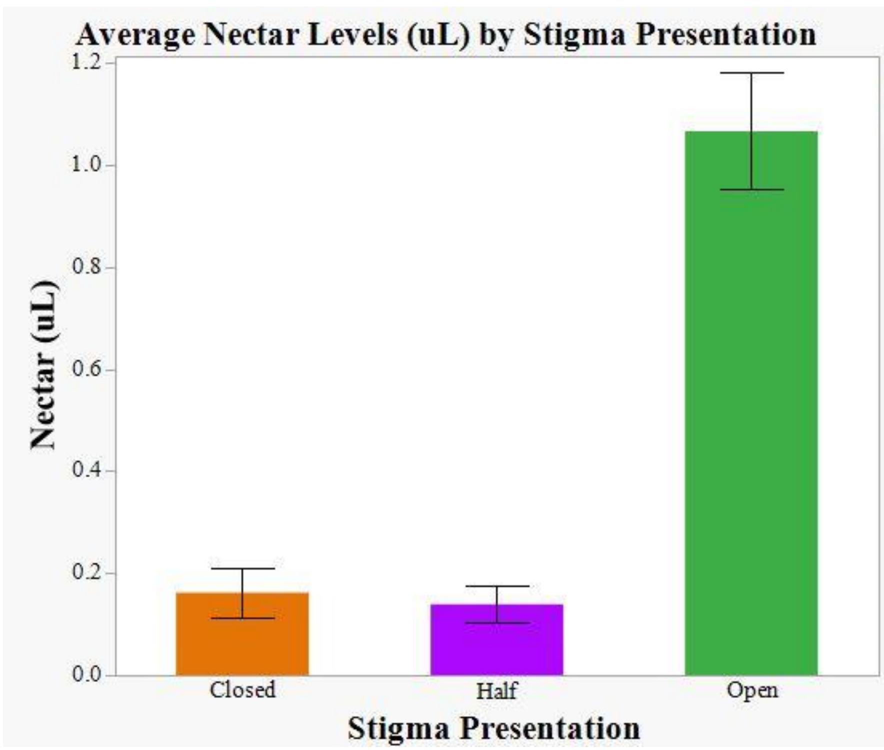

Figure 1-6. Comparison of mean standing crop of nectar (uL) versus stigma presentation. Flowers with an open stigma contained higher levels of nectar than those with half or fully closed stigmas. Standing crops differed significantly among stigma closure categories (F(2,219)= 26.4, P<0.0001). Error bars represent one standard error.

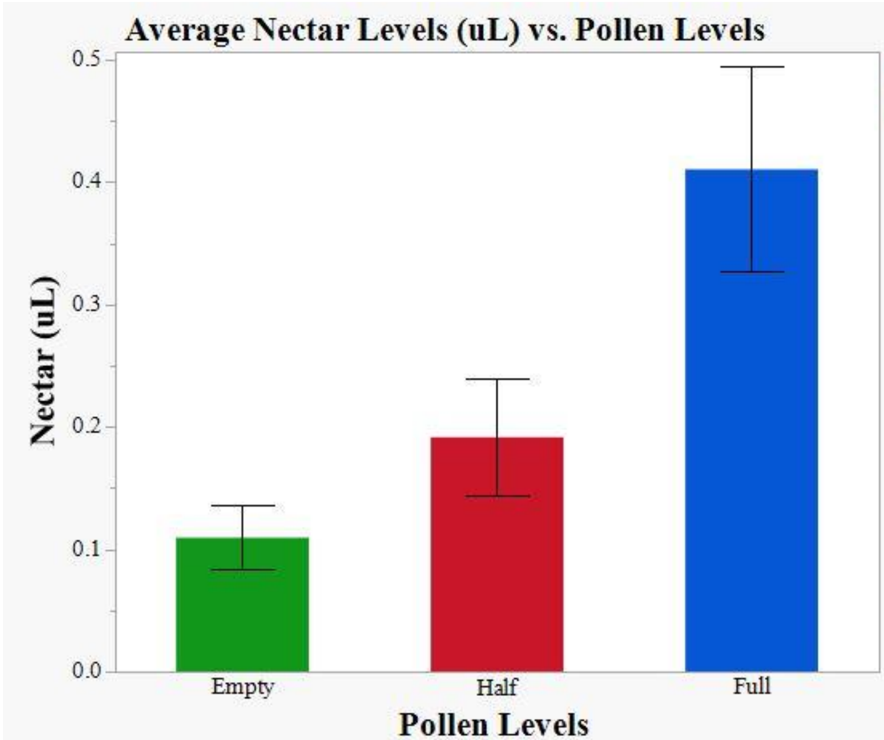

Figure 1-7. Comparison of mean standing crop of nectar (uL) versus pollen levels. Flowers with full pollen contained greater amounts of nectar than flowers with half-filled anthers, which had higher nectar content than flowers lacking pollen. Standing crops differed significantly among pollen content levels ($F(2,139)= 7.7$, $P=0.0007$). Error bars represent one standard error.

Stigma presentation and pollen levels are not randomly associated ($P<0.0001$). They mirror each other in that when stigmas are open, pollen levels are high, and when stigmas close, pollen levels are decreased (**Figure 1-8**). Stigmas that are half-closed indicate that pollen levels are at about half of their full capacity.

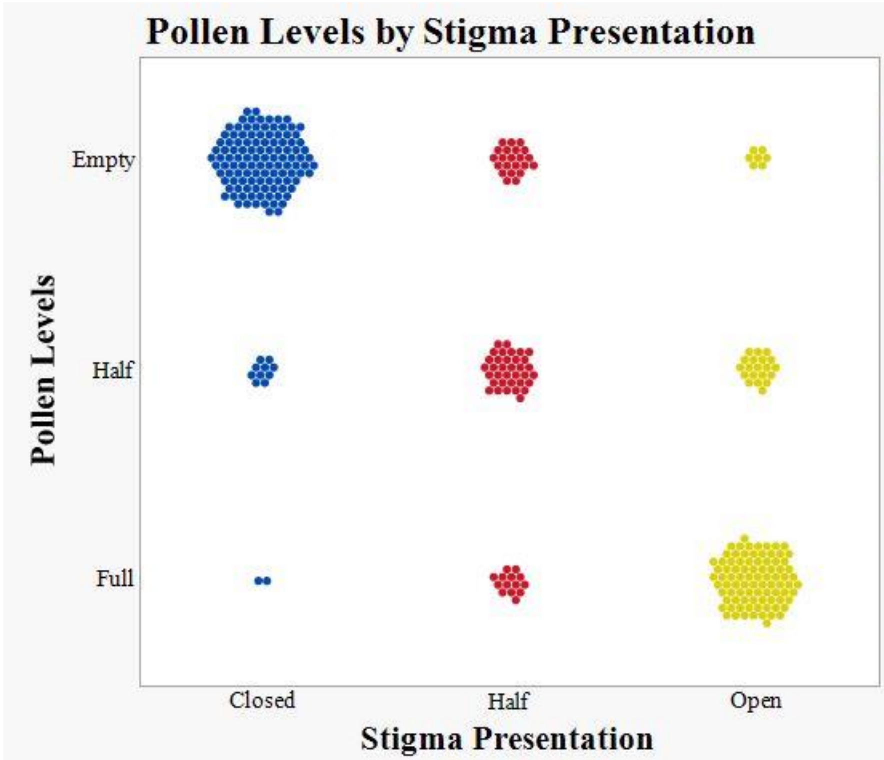

Figure 1-8. Comparison of pollen levels based on stigma presentation. A likelihood ratio test found that pollen contents and stigma closure were not randomly associated (χ^2 = 262, df = 4, P<0.0001).

Another point of interest was to determine whether sunlight had any effect on nectar standing crop. There is a significant difference in the amount of nectar found in shaded flowers versus sunlit flowers (P=0.0002). Shaded flowers contain an average of 0.78 ± 0.08 uL of nectar in comparison to sunlit flowers which averaged 0.15 ± 0.14 uL in nectar (**Figure 1-9**). The average time of day when flowers were found in shade was 9:35 AM ± 7 minutes, while flowers found in sunlight averaged 10:40 AM ± 13 minutes. The average time of day when flowers were found in shade versus sunlight differed significantly (P<0.0001).

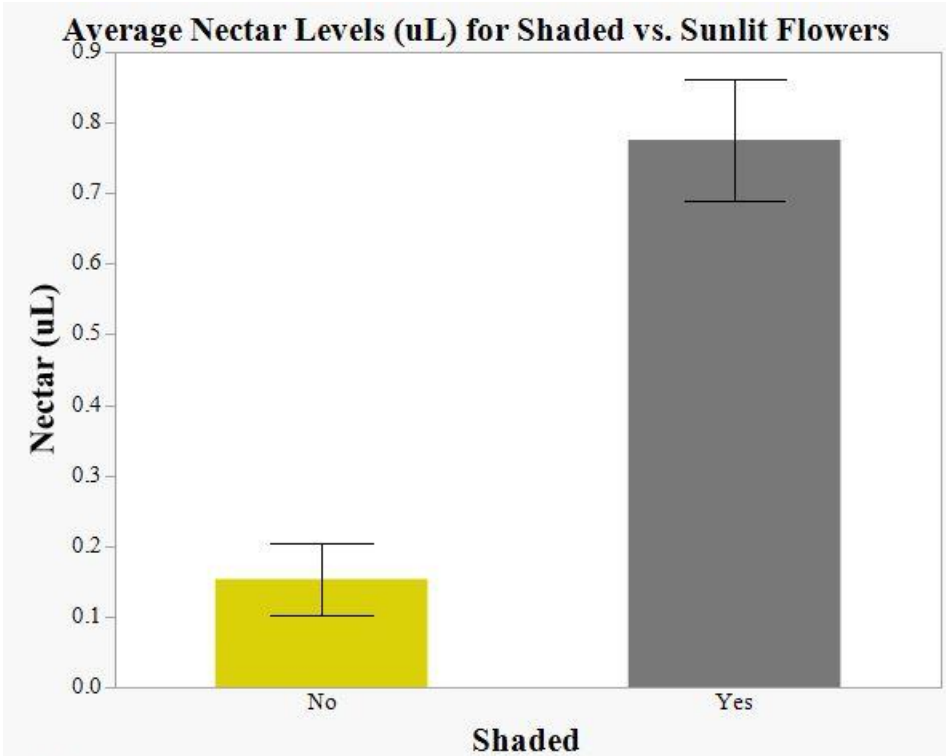

Figure 1-9. Comparison of average standing crop of nectar (uL) for flowers in shade versus sunlit flowers. Flowers on shaded plants contained greater amounts of nectar than sunlit flowers. A two-tail t-test confirmed that the two groups were statistically significant (P=0.0002). Error bars represent one standard error.

New Flowers

Additional measurements of flower characteristics were collected from new flowers bagged to prevent pollinator visitation ('bagged' and 'new' will be used interchangeably to describe flowers in this section). On 8/19, three separate plants each produced one new flower that had a closed stigma and empty anthers.

New flowers vary in nectar amounts (uL) based on the time of day when collection is performed (**Figure 1-10**). Levels generally increase as the day progresses. The average amount of nectar present in a new flower is 1.97 $\pm$ 0.10 uL.

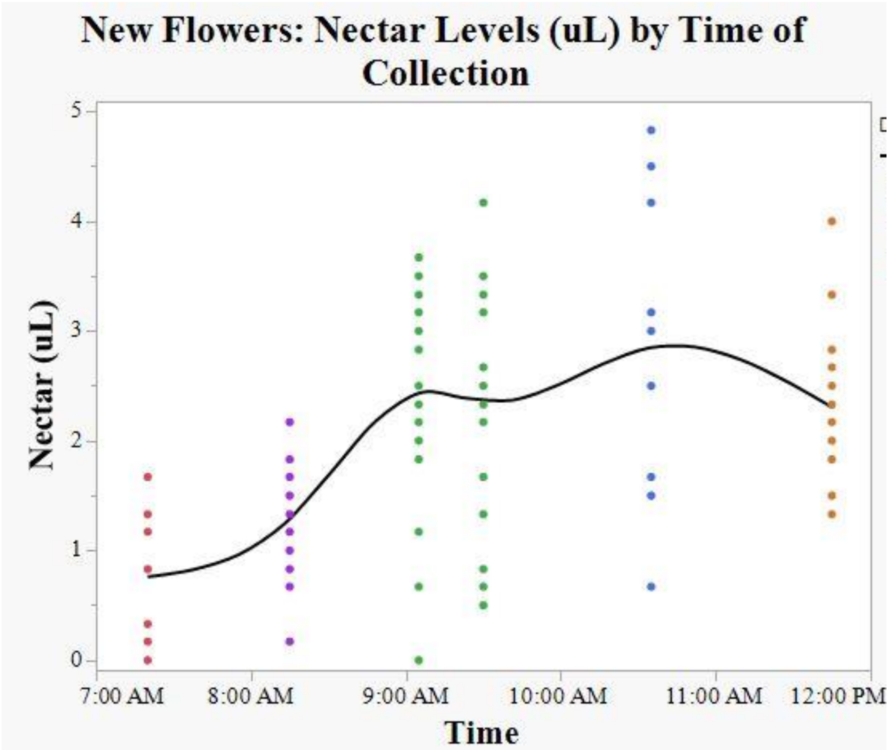

Figure 1-10. Nectar amounts (uL) measured in new flowers by time of day when collections were made. Data points are coded by date: blue = 8/8, red = 8/10, green = 8/11, purple = 8/19, orange = 8/22. Nectar levels trend up significantly from the morning throughout the day ($F(1,111)= 25.7$, $P<0.0001$).

One question we wanted to answer was whether the number of flowers on a plant is related to the nectar levels in each flower. Although an increasing number of flowers on a plant tends to be associated with higher amounts of nectar in each flower, analysis of pairwise differences did not yield any significant results (**Figure 1-11**). Plants with only one flower contain an average of 1.60 ± 0.20 uL of nectar per flower, while flowers on plants with two flowers contain an average of 2.01 ± 0.17 uL of nectar. Flowers on plants with three flowers contain an average of 2.29 ± 0.17 uL of nectar and plants with four flowers contain a nearly identical mean of 2.29 ± 0.50 uL of nectar per flower. An average of 2.53 ± 0.44 uL of nectar is present in flowers on plants with five flowers. Flowers on plants with six flowers contain a mean of 1.28 ± 0.31 uL of nectar. Stigma closure and pollen content comparisons to nectar levels also

did not yield statistically significant results (**Figure 1-12** and **Figure 1-13**). Flowers with full anthers contained an average of 2.06 ± 0.10 uL of nectar while empty ones averaged 1.39 ± 0.59 uL. Similarly, flowers with open stigmas contained a mean of 2.06 ± 0.10 uL of nectar, whereas flowers with closed stigmas held only 1.39 ± 0.59 uL of nectar.

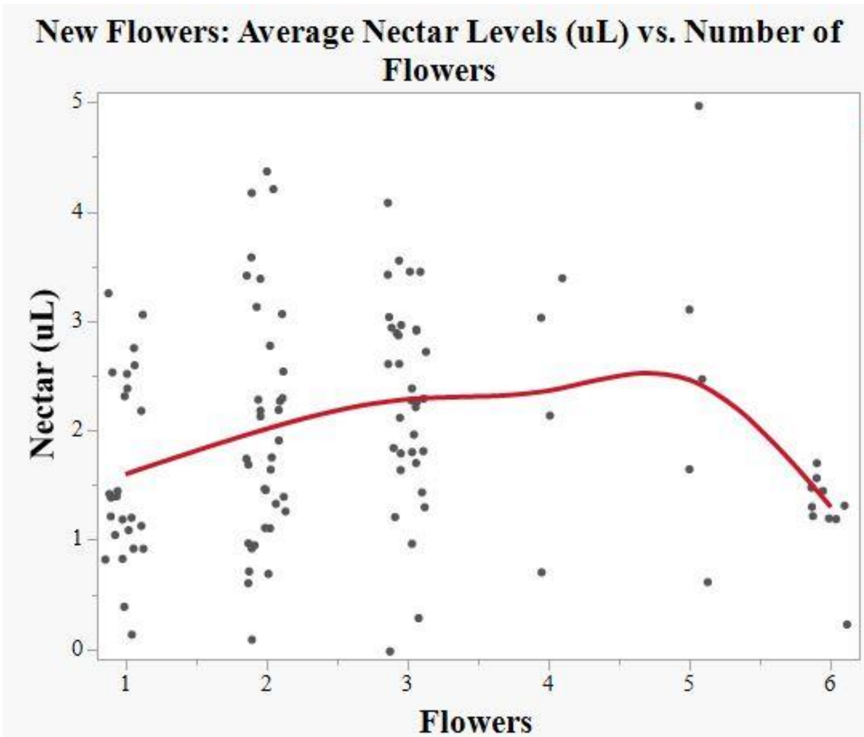

Figure 1-11. Mean nectar levels (uL) versus the number of flowers present on the plant. Generally, the more flowers present on a plant, the more nectar contained in each flower. Analysis by ANOVA gave significant results (F(5,107)= 2.8, P=0.0210), but no significant pairwise differences emerged.

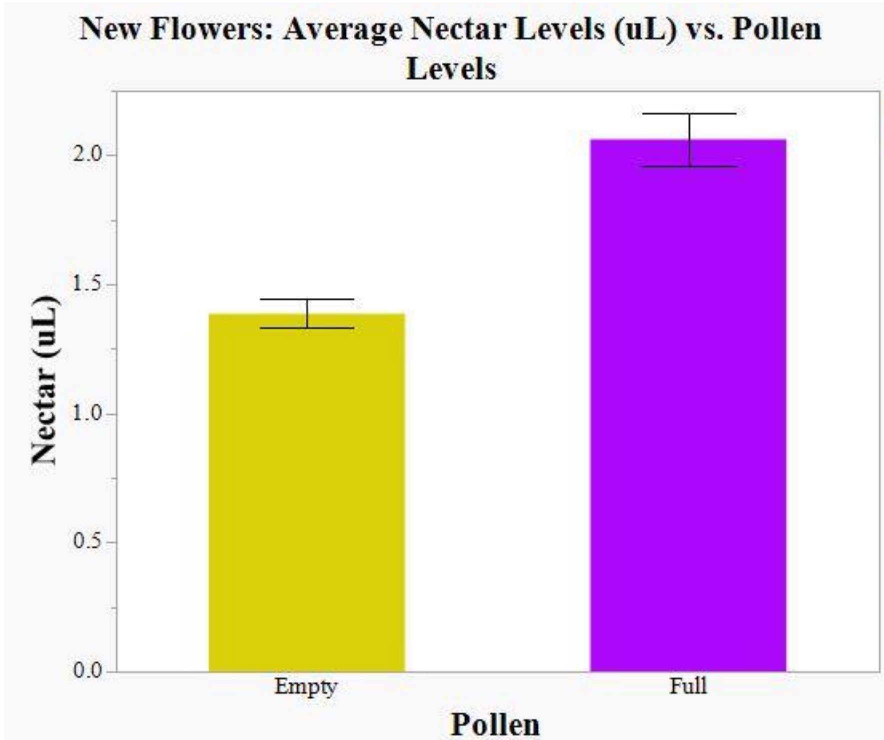

Figure 1-12. Nectar levels (uL) in new flowers compared to pollen levels. Flowers containing full anthers held more nectar on average than flowers without pollen. No significant results were found between the two groups (P=0.26). Error bars represent one standard error.

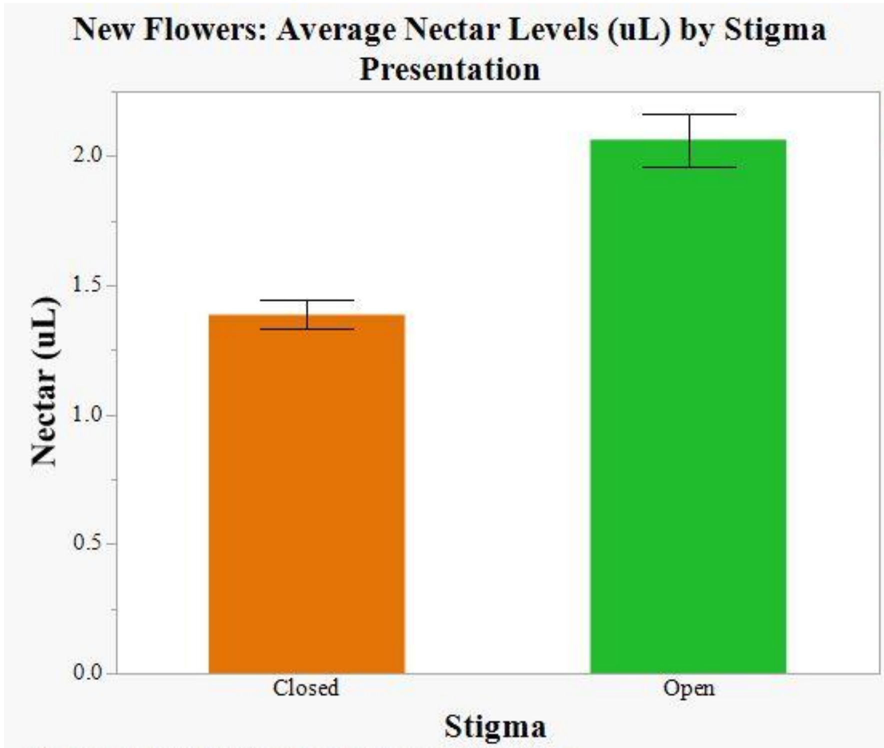

Figure 1-13. Mean nectar levels (uL) by stigma presentation. Flowers with open stigmas contained higher amounts of nectar on average than flowers with closed stigmas. Analysis of the two groups did not reveal any significant results (P=0.26). Error bars represent one standard error.

Whether or not sunlight affected production of nectar was another point we wanted to determine. Nectar levels in new flowers are unaffected by the light setting; comparison by a t-test yields no significant difference between shaded and sunlit flowers (**Figure 1-14**). Shaded flowers contained an average of 1.95 $\pm$ 0.11 uL of nectar whereas flowers in sunlight held a mean of 2.08 $\pm$ 0.24 uL. The average time of day when flowers were found in shade was 9:16 AM $\pm$ 8 minutes, while flowers found in sunlight averaged 10:02 AM $\pm$ 18 minutes. The average time of day when flowers were found in shade versus sunlight differed significantly (P=0.0248).

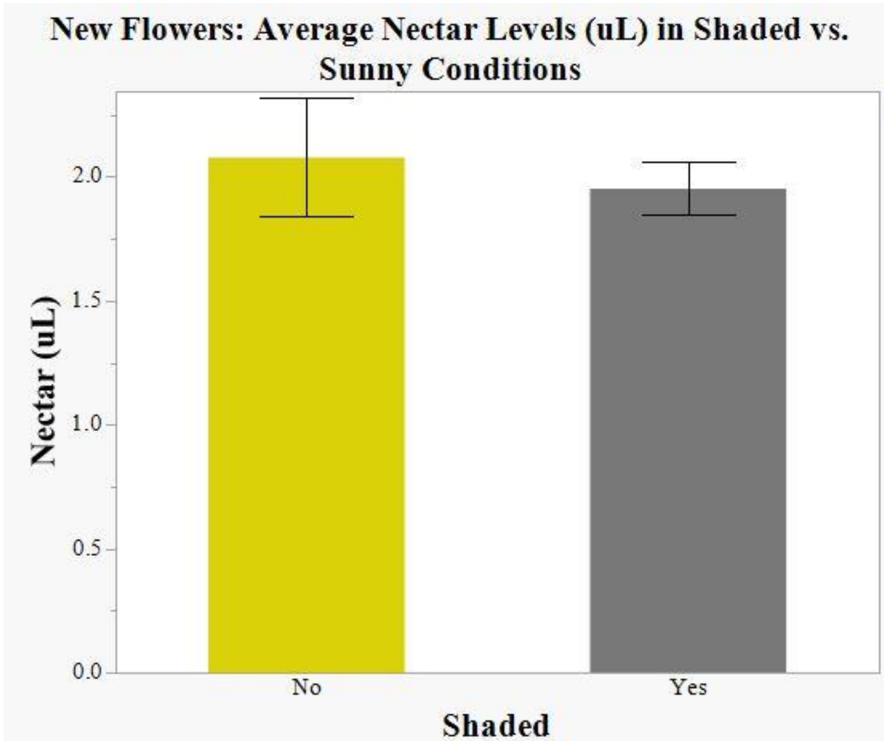

Figure 1-14. Comparison of mean nectar levels (uL) in flowers from shaded versus sunlit plants. Flowers from shaded plants and sunlit plants contained roughly equal amounts of nectar. No significant difference was found between the two groups (P=0.6276). Error bars represent one standard error.

As the season progressed, production of new flowers per plant decreased (**Figure 1-15**). 8/08 averaged 3.67 ± 0.45 flowers/plant while 8/10 had a mean of 4.43 ± 0.51 flowers/plant. 8/11 showed 2.51 ± 0.21 flowers/plant, whereas 8/19 yielded 2.40 ± 0.23 flowers/plant and 8/22 had a mean of 2.24 ± 0.29 flowers/plant. Flowers per plant declined significantly over the flowering season, with plants on 8/10 holding significantly more flowers than any of the later dates (P=0.0064, 0.0038, 0.0028, for comparisons between 8/10 and each later date, respectively). Conversely, there was no obvious trend in nectar production (uL) from early to late in the blooming season. Although there were significant differences in nectar levels between certain sampling periods, there wasn't a steady decrease in the concentrations (**Figure 1-16**). 8/8 averaged 2.89 ± 0.28 uL in nectar and fell to 0.79 ± 0.31 uL on 8/10, before climbing back up to

a mean of 2.41 ± 0.13 uL on 8/11. 8/19 averaged 1.27 ± 0.14 uL of nectar and 8/22 averaged 2.30 ± 0.18 uL.

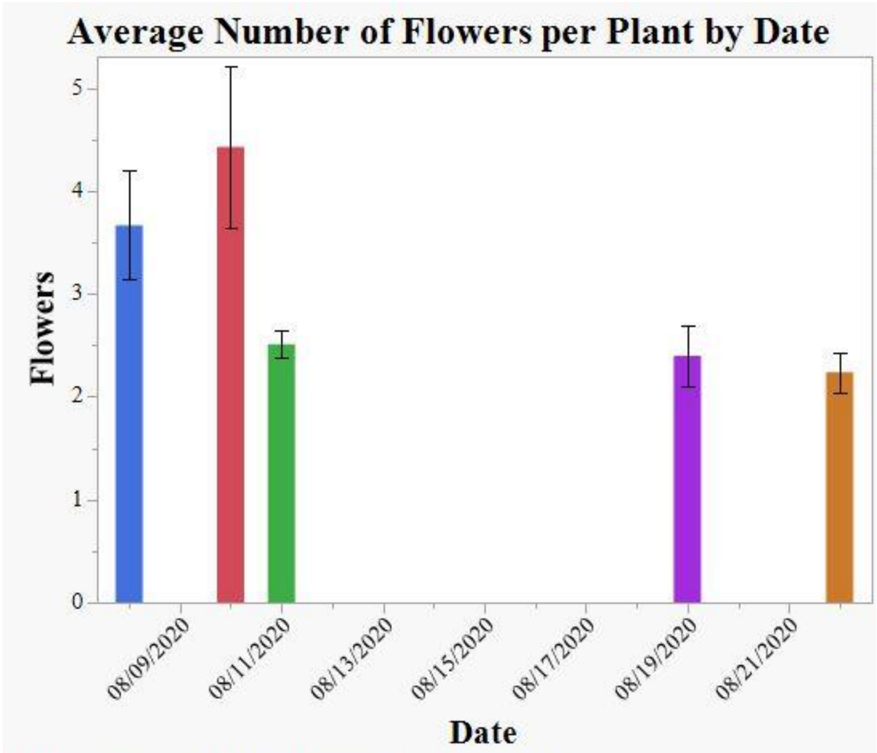

Figure 1-15. Comparison of mean number of flowers per isolated plant by date. There is significant decrease in flowers produced per plant as the days move later into the year ($F(4,108)= 5.2$, $P=0.0007$). Error bars represent one standard error.

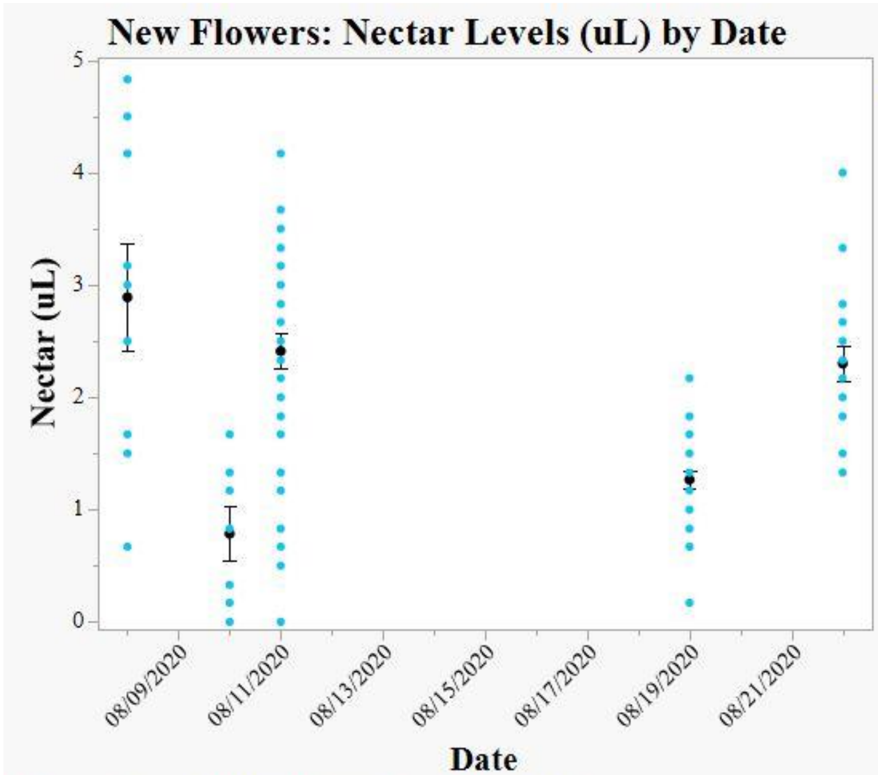

Figure 1-16. Comparison of nectar levels (uL) by date of collection. Points in black indicate mean $\pm$ SE. No obvious trend is seen in nectar production from early to later in the year, although results differed significantly among groups ($F(4,108)= 16.5$, $P<0.0001$). A Tukey's analysis showed that Days 1, 3 and 5 were significantly different from Days 2 and 4 (P= Day 1→2 < 0.0001 , 1→4 < 0.0001 , 3→2 < 0.0001 , 3→4 < 0.0001 , 5→2 = 0.0005, 5→4 = 0.0001).

Discussion

We wanted to determine whether nectar standing crop dropped steadily throughout the day, or if the plant replenished nectar in the flowers. We found that nectar standing crop decreases over time in a monotonic fashion (**Figure 1-3**). On the first day of sampling, nectar levels were highly elevated (roughly 2x) compared to subsequent days; it may be that flowers were waterlogged from that morning's rainfall, artificially increasing nectar volume. Due to lack of proper sunlight, sucrose concentration values were unable to be obtained by use of a refractometer to confirm the presence of excessive water.

We also wanted to decipher whether sigma closure or pollen content could be used to infer nectar standing crop. It was discovered that stigma closure and pollen content follow a strong trend over time with a majority of stigmas closed and anthers empty by 11 AM (**Figure 1-4** and **Figure 1-5**). Pollen content and stigma closure are also strongly related to each other, indicating that visitors are not simply collecting pollen but leaving some behind as well (**Figure 1-8**). This has important implications when observing pollination and visitation of *M. alatus*. An individual flower's nectar content can be inferred by looking at stigma closure; as the nectar levels decrease, stigmas will progress from an open presentation to a closed presentation, with discernable "half-open" steps between the two extremes (**Figure 1-6**). Pollen levels follow a similar trend. Visitors to the flowers drink the nectar and take some of the pollen with them, so that anthers are emptied over the day in conjunction with nectar standing crop (**Figure 1-7**).

Another point of interest to conclude was whether the light setting a flower was found in could predict the amount of nectar in the flower. Intriguingly, whether a flower is found in shade or sunlight is indicative of the amount of nectar that can be expected (**Figure 1-9**). However, because the times at which flowers are found in sun versus shade are significantly different, it is likely that the nectar levels are more indicative of the trend in nectar volumes over time than any effect the sun might have on nectar production. Because the population of flowers that we studied was located in a valley, many of the plants were not hit by direct sunlight until the sun crested the ridge and surrounding forest canopy, which occurred later in the day. The idea that the difference in nectar levels among sunlit versus shaded flowers is more tied to time than actual sunlight is confirmed when observing unvisited flowers, where nectar levels are essentially equal for flowers both in the sun and shade, even with taking time into account (**Figure 1-14**).

We desired to ascertain whether nectar was produced in fixed amounts or if each flower was filled individually. The conclusion reached from observing unvisited flowers is that nectar production is not tied to the number of flowers produced on a plant (**Figure 1-11**). This shows that plants fill up each flower present on the stem, rather than producing a set volume of nectar and distributing it equally to each flower. It's difficult to determine when members of *M. alatus* produce their nectar. In unaffected flowers, nectar levels trend upwards throughout the morning (**Figure 1-10**). Production seems to begin in the late evening sometime, as a visit to the field at 8 PM indicated that buds judged to be ready to open the following morning contained just under 1 uL of nectar on average. Whether nectar production would continue throughout the day in the absence of visitors remains to be explored. Towards the end of the flowering season, nectar produced per flower remains unaffected (**Figure 1-16**), even as the number of flowers present on a plant decrease (**Figure 1-15**). A fixed amount of nectar produced would have led to higher nectar volumes per flower later in the season as flower production dropped, which is not what we observed. This further supports our hypothesis that regardless of the number of flowers present on a stem, each one will be individually filled rather than obtaining a percentage of a fixed volume of nectar produced by the plant.

There are points of interest that should be explored further. For a reason we are unable to discern, stems stripped of old flowers and bagged to prevent intrusion from visitors infrequently produce "dud" flowers. These duds contain no pollen and have closed stigmas, despite containing nectar. Although unlikely, it may be that a visitor was trapped with the buds in the bagging process and was able to visit the new flowers before we could observe them, leaving empty shells behind. However, this would not fully explain why there is still nectar present, nor why only one flower on the stem was a dud and the rest were 'normal'. Another question to

delve into would be to see when nectar production drops off. It should be determined whether flowers continue to produce nectar until its first visitor, a set time of the day, or simply until the flower is filled to maximum capacity. If it is the last option, what the maximum capacity for nectar in a flower is would be a natural question to also answer. Further data should be collected on nectar standing crop throughout the day to better pin down the trend and demonstrate how much variation there truly is among days. It should be determined whether stigma on *M. alatus* close in response to pollen transfer or if simply being touched without the stimulation of pollen is enough to induce closure; for simplicity, it was assumed for the purposes of this study that closure was in response to pollen-laden visitors brushing some of their load onto the stigma. However, it may be that both touch and pollen can initiate stigma closure, in which case finding if there is a difference in response time – as is present in *Mimulus aurantiacus* (Fetscher and Kohn 1999) – between the two scenarios would be beneficial. A final experiment would be to count how many dead flowers remain attached to their sepals after 10 AM. Because of the protected nature of the habitat, it was fairly common to see wilted and dead flowers remaining on plants well into the afternoon. This could affect how visitors interact with *M. alatus* later in the day.

The Visitors – *Nathan Stefan*

Introduction

A particular species of monkeyflower—*Mimulus alatus* (sharp-wing monkeyflower)—was the focus of this study. A native to eastern North America, the plant is an herbaceous eudicot perennial that grows near streambeds and creeks, moist woodlands, and ditches. Unfortunately, the relationship between *M. alatus* and its visitors is poorly understood since little data has been published on *M. alatus* to date; however, another monkeyflower in the same genus—*Mimulus*

ringens—may provide clues to what visitors interact with *M. alatus*. Both *Mimulus* species are found in generally similar habitats and have similar flowers in terms of size, shape, and color (Gleason and Cronquist, 1991). It's reasonable that the same species of visitors may frequent both; what's more, the principal pollinators for *M. ringens* were discovered to be *Bombus* species, primarily *B. fervidus*, *B. vagans*, and *B. impatiens* (but dependent on the site location, as some species are more common in specific areas of the U.S.) (Flanagan et al., 2009; Mitchell et al., 2004). Combined, this hints that *Bombus* species may also be a potential visitor (and pollinator) of *M. alatus*, though other visitors cannot be ruled out.

In investigating the relationship between *M. alatus* and its visitors, we concentrated on identifying the primary visitors to *M. alatus* and elucidating their behavior when interacting with flowers. Anecdotal evidence from similar *Mimulus* species indicated that *Bombus* species may be primary visitors (and pollinators), but investigation was needed; thus, our study addressed several questions: (1) Who visits *M. alatus*, and does overall visitation behavior vary over time, by day, or with weather conditions? (2) Do *Bombus* visitors differ in foraging behavior from non-*Bombus* visitors, such as in visitation rates or movement distance between flowers? (3) Which visitors are engaged in pollen collection and carrying?

Methods

A population of *Mimulus alatus* plants located in the Cuyahoga Valley National Park in Brecksville, Ohio, U.S.A. (41°18'11.2"N 81°36'37.0"W) was studied. *M. alatus* flowers are pale blue, with petals resembling a monkey's face, and bloom between early July and late August (Gleason and Cronquist, 1991); our study spanned this time-period during summer 2020. The site consisted of nine patches of plants strung out along a shaded streambed, most containing roughly 50-100 plants each, and several containing ~500-1000 plants (**Figures 2-1, 2-2**). Most of

the patches were organized into smaller clusters of plants spaced about a meter or two apart. The rest were one continuous group, with no discernable division. The typical distance between patches was around 40 meters; a couple were as close together as 15 meters or as far as 70 meters apart. Individual *M. alatus* plants almost never occurred in between these patches. Each patch was tagged and given an identification number for data collection. Other vegetation present in the habitat was mainly composed of mosses, ferns, and weeds clustered around the streambanks. The presence of other flowering plants was almost non-existent, except for isolated patches of tiny purple flowers (unidentified); visitors to *M. alatus* ignored these flowers until the very end of the season, when *M. alatus* flowers were dying off in earnest.

Figure 2-1: Shows the typical habitat in which *M. alatus* is found, a shaded streambed or creek with clusters of plants growing adjacent to the water or on the bank. This photo shows patch four in the foreground, divided into two smaller groups on either side of the streambed.

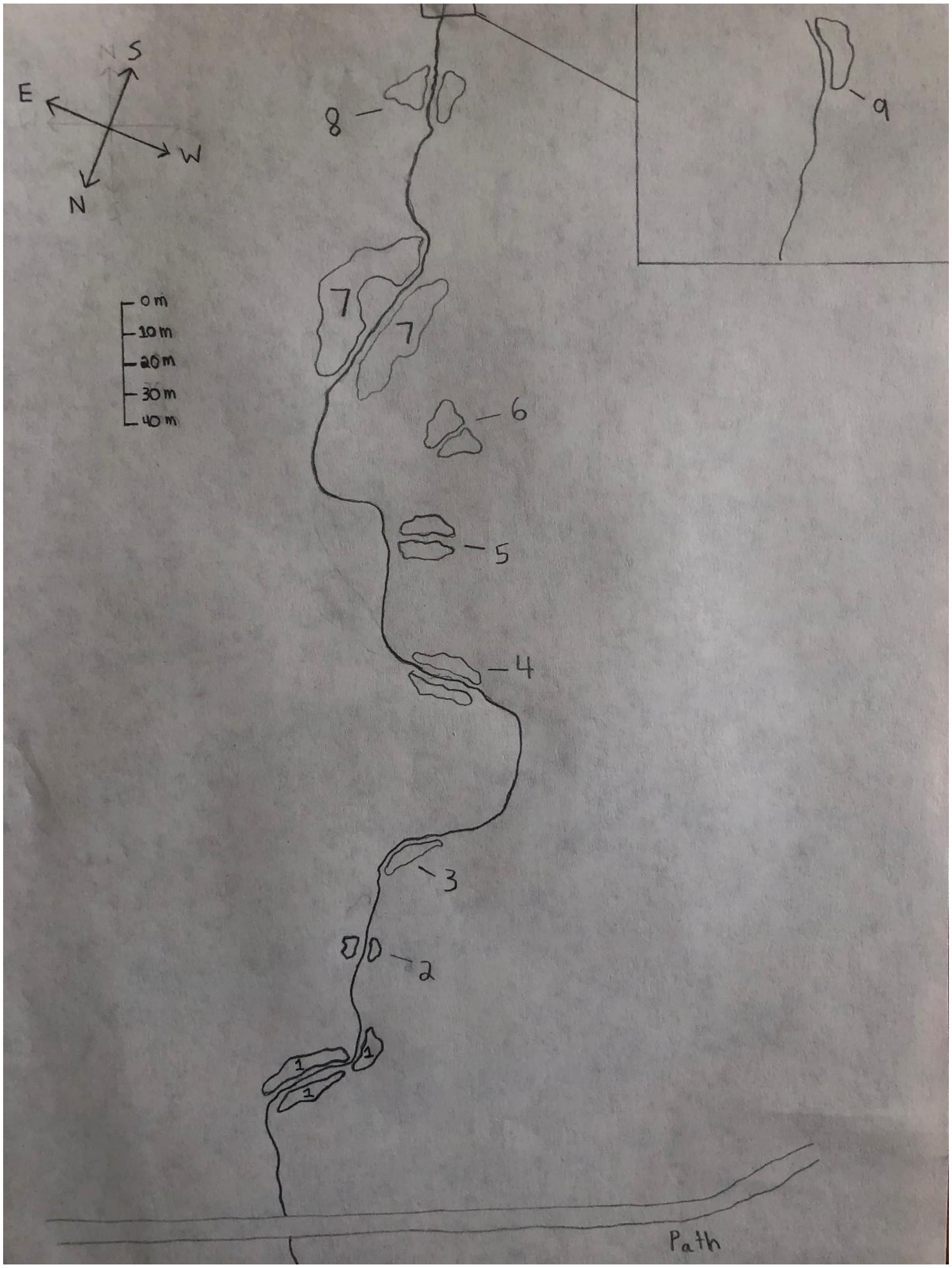

Figure 2-2: Shows a rough sketch of the location of the nine patches that were studied. The stream ran directly through most of the patches, as plants tended to grow on either side of the water rather than in drier areas further away (such as patch six). The sketch was not drawn to scale.

Upon arriving at the site each day, the weather and plant conditions (wet or damaged flowers, leaves showing signs of predation) were assessed and recorded for later use in interpreting collected data. Five days were spent in collecting visitor data; the first three days each consisted of about five hours of observation, plus two hours each during the last two days. In total, 59 observation periods were conducted at various times of day between two observers over the five-day span (**Table 2-2**). Before collecting visitor data, we selected a patch to observe and established an observation area in which a group of flowers could be observed at the same time, without missing any visits. Estimates of how many plants resided in a typical area within our direct line of sight (not the entire patch) were calculated only once for each patch. Each plant estimate was multiplied by the mean flowers per plant for each day (determined after collected flower data was analyzed) to find the total flowers per observation area (for each patch). Most patches contained about 40 plants within a typical observation area, the largest area containing 75 plants and the smallest only 15 plants. The patch number and time were then noted, and the observation period begun.

Each observation period consisted of 15 minutes. Any visitor that entered the observation area and interacted with *M. alatus plants* and flowers was identified and recorded as accurately as possible (to species if able). The presence or absence of pollen bodies on the visitor were also noted. When the visitor actively entered a flower by thrusting its head inside, it was considered to have visited the flower, regardless of whether it collected any food or not. This rule was established because it would have been difficult to ascertain whether the visitor had collected any pollen or nectar, especially if it were already covered in pollen to begin with. Any visitor that was observed carrying pollen (in baskets or on the forehead) was recorded as having transferred pollen inside every flower it visited.

Each visitor was tracked to observe total flowers that it visited in succession in the established area of observation before leaving. Total visits in a 15-minute period were divided by the total estimated flowers in the observation area to yield visits per flower per 15-minute period (v/f/15 min). When able, the distance between two consecutive flower visits was recorded in cm; rough visual estimations were used to calculate the distance. This data was used to determine the mean movement distance between visits to observe how visitor behavior varied over time and date. Once the visitor left the observation area, new visitors were tracked and recorded. If two or more visitors arrived at the same time, they were tracked simultaneously as best as possible. Visitor specimens were periodically collected to identify observed species. When the flowering season for *M. alatus* wound to a close, data collection was completed, and the data compiled and analyzed using Excel and JMP Pro 15. Most statistics were done using an ANOVA to compare groups over time of day and across days. T-tail tests were used to determine where differences occurred amongst groups.

Results

We observed visitors on five separate days, between 06:00 and 17:00. A total of fifty-nine 15-minute observation periods were conducted over the five days, with observations mainly being done in the morning the first three days and the afternoon the last two. Over the course of the study, dawn occurred around 06:20. The first day (7/28) was cool and sunny but leaves and flowers were covered in water droplets as it had rained heavily the day before. The following two days (7/29, 7/30), conditions had dried up considerably and leaves and flowers were not waterlogged by droplets. The fourth day (8/06) was also dry and clear, despite the rain a couple nights before. The final day of observation (8/21) saw clear and warm conditions without precipitation.

At least 10 types of visitors were observed over the five days, consisting mostly of bees. Visitors were divided into two visitor groups, '*Bombus*' and 'Non-*Bombus*'. *Bombus* species comprised 52.1% of all visitors (**Table 2-1**). All other types of visitors were grouped as Non-*Bombus*; the vast majority were sweat and carpenter bees. Anecdotally, peak visitation times were around 09:00 in the morning and 14:30 in the afternoon during the periods of observation we observed over the course of the study (**Figure 2-3**).

Abbreviation	Classification	N Observed	N w/ Pollen
BOMIMP	*Bombus impatiens*	76 (24.9%)	44
BOMVAG	*Bombus vagans*	50 (16.4%)	30
BOM	Unknown *Bombus*	33 (10.8%)	5
Total:		159 (52.1%)	79
HAL	*Halictidae* (sweat bees)	61 (20.0%)	13
CER	*Ceratina* (small carpenter bees)	34 (11.1%)	5
VESALA	*Vespula alascensis* (Yellowjacket)	25 (8.2%)	0
ANTTER	*Anthophora terminalis* (digger bee)	13 (4.3%)	3
PAPTRO	*Papilio Troilus* (spicebush swallowtail)	6 (2.0%)	0
COC	*Coccinellidae* (Lady Beetle)	2 (0.7%)	0
MOT	Moth (species unknown)	2 (0.7%)	0
UNK	Unknown Visitor	2 (0.7%)	0
POAZAB	*Poanes zabulon* (zabulon skipper)	1 (0.3%)	0
Total:		146 (47.9%)	21
Overall:		305	100

Table 2-1: shows each visitor observed over the course of 59 observation periods. Genus and species names were given when known; otherwise, the type was used (e.g. Moth). Each visitor

type was given an abbreviation to simplify discussion. The convention used was the first three letters each of the genus and species (if known) of the visitor (e.g. BONIMP). If neither was known, short form of the type was used. Unknown *Bombus* visitors were simply labelled BOM, as their species was unknown. The total N observed for each visitor was tallied and percentages of total N determined. Most common visitors consisted of *Bombus* species and sweat bees, while various butterflies and moths were least common. Finally, the total N w/ pollen for each visitor type was found.

Observation Days	6:00	7:00	8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00
7/28/2020		1	3	6	4	1					
7/29/2020		3	4	3	2						2
7/30/2020	2	7	2	5							
8/6/2020									4	4	
8/21/2020								3	3		

Table 2-2: Shows the observation times (green boxes) of every day in which observations were made. Five days of observation were performed, ranging between 06:00 and 17:00. Numbers inside green boxes indicate the number of observation periods performed each hour. In total, 59 observation periods were done, with 43 of those periods occurring before noon and 16 in the afternoon. Very little data was collected within the 11:00-13:00 time-interval.

Observation periods were heavily clustered during early morning hours during the first three days of observation, and in the afternoon the last two days (**Table 2-2**). Morning periods were done during peak blooming season, while afternoon periods were performed towards the end of the season; data may reflect these trends. The volume of periods in the morning compared to the afternoon were also substantially higher.

Date	7/28/2020	7/29/2020	7/30/2020	8/6/2020	8/21/2020
Mean Flowers Per Plant	1.82	2.11	2.04	2.32	2.59

Table 2-3: Shows the mean flowers per plant on the five days that observation periods were conducted. Overall mean flowers per plant is 2.18.

To address whether visitation behavior varied in visitation rate over time and amongst species, we needed to normalize our data. Patches contained wide ranges of plants, making visitation partially dependent on the number of flowers present in an area; in addition, plants displayed different numbers of flowers each day (from variation in the bloom/flower death ratio). A method was devised to ascertain the true visitation rates by first determining the mean flowers on a plant across all patches, for each day of observation (**Table 2-3**).

	Mean Plant Totals in Observation Area by Patch								
Patch	1	2	3	4	5	6	7	8	9
Mean Plants	35	35	25	40	30	50	75	40	15

Table 2-4: Shows the mean number of plants in a typical observation area for each patch. Mean values hovered around 35 flowers, with 75 and 15 being the maximum and minimum.

It was impractical to count the plants before every period to estimate of flower totals. Instead, the number of plants in a typical observation area for each patch were calculated once and used for all subsequent observation periods (**Table 2-4**). Plus, since many of the patches could only be observed from one location (either due to inaccessibility, or because the patch contained only one cluster of plants), the mean number of plants in a typical observation area within a patch was assumed to be fairly constant. Estimations were made for mean total plants present in an observation area for each patch and were assumed to be representative of the patch regardless of date or time (**Table 2-4**).

Mean Flower Total in Observation Area by Patch and Date					
Patch	7/28/2020	7/29/2020	7/30/2020	8/6/2020	8/21/2020
1	64	74	71	81	91
2	64	74	71	81	91
3	46	53	51	58	65
4	73	84	82	93	104
5	55	63	61	70	78
6	91	106	102	116	130
7	137	158	153	174	194
8	73	84	82	93	104
9	27	32	31	35	39

Table 2-5: Shows the mean flower totals of an observation area in each patch, by date. Totals were rounded to the nearest whole number. Flower totals steadily increased by date and peaked on 8/21.

Mean flowers per plant by date (**Table 2-3**) were multiplied by mean plants per observation area in a patch (**Table 2-4**) to yield the mean flower totals of a typical observation area in each patch by date (**Table 2-5**). This value helped show the normalized visitation behavior of visitors, adjusted to compensate for date and by patch. The total visits made by a visitor during a 15-minute period were then divided by the total estimated flowers in the observation area (**Table 2-**5) to yield visits per flower per 15-minute period (v/f/15 min).

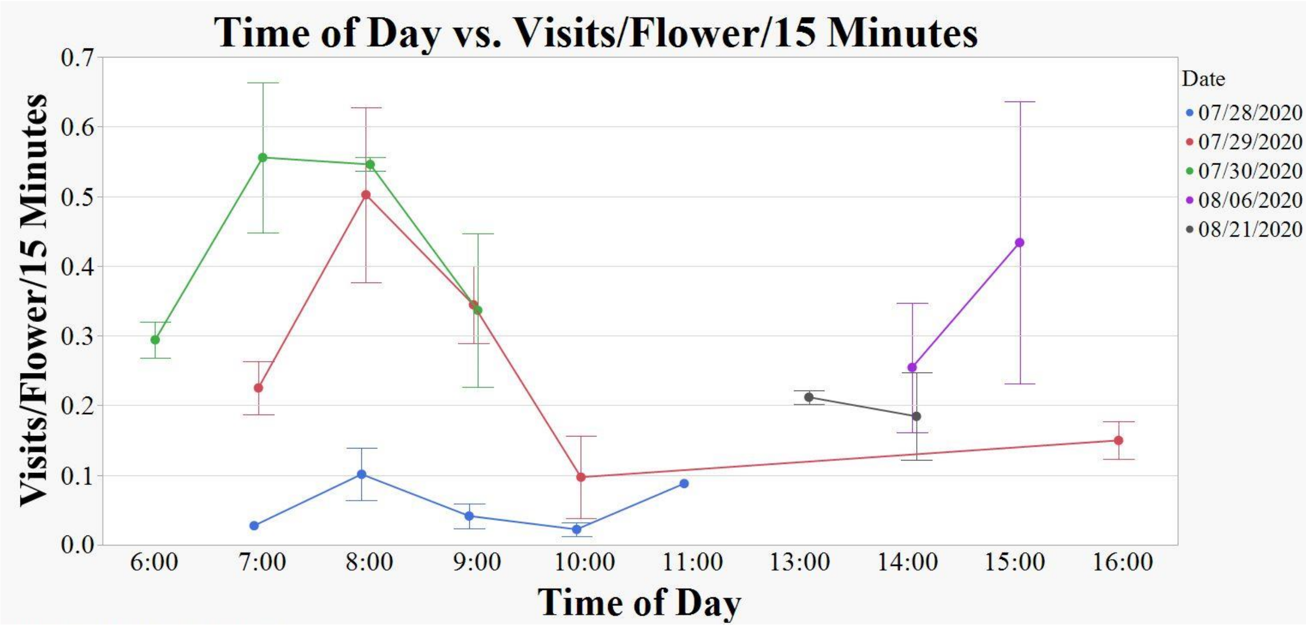

Figure 2-3: Shows the mean visits per flower present in the observation area (over 15-minute periods), over time (v/f/15 min); mean visits per flower were grouped by hour and color coded by date. Differences in mean visits per flower were not significant over time for each of the five days ($F(4,10)=1.7$, $p=0.2129$; $F(4,9)=3.2$, $p=0.0703$; $F(3,12)=1.1$, $p=0.3699$; $F(1,6)=0.6$, $p=0.4525$; $F(1,4)=0.2$, $p=0.6868$). Mean visits per flower differed significantly across the five days ($F(4,54)=8.3$, $p<0.0001$). Analysis of pair-wise differences determined that 7/28 differed significantly from 7/29, 7/30, and 8/06 ($p=0.0127$; $p<0.0001$; $p=0.0132$). Error bars represent one standard error.

To determine whether foraging behavior varied with regard to visitors visiting different numbers of flowers over time and by day, visits per flower in an observation area (over a 15-minute period) were calculated. The overall mean visits per flower in a period on each day were 0.05, 0.30, 0.45, 0.34, and 0.20 respectively (**Figure 2-3**). 7/28 clearly had the lowest mean, despite observations occurring around peak visitation times. In fact, both days directly following 7/28 experienced significantly higher mean visits per flower, despite having similar observation times. Time of day differences in mean visits/flower/15 minutes weren’t significant for any day but were significant across the five days. The most likely explanation for these statistics was the

weather on each day, with flowers on 7/28 being saturated with water and deterring visitors from staying long.

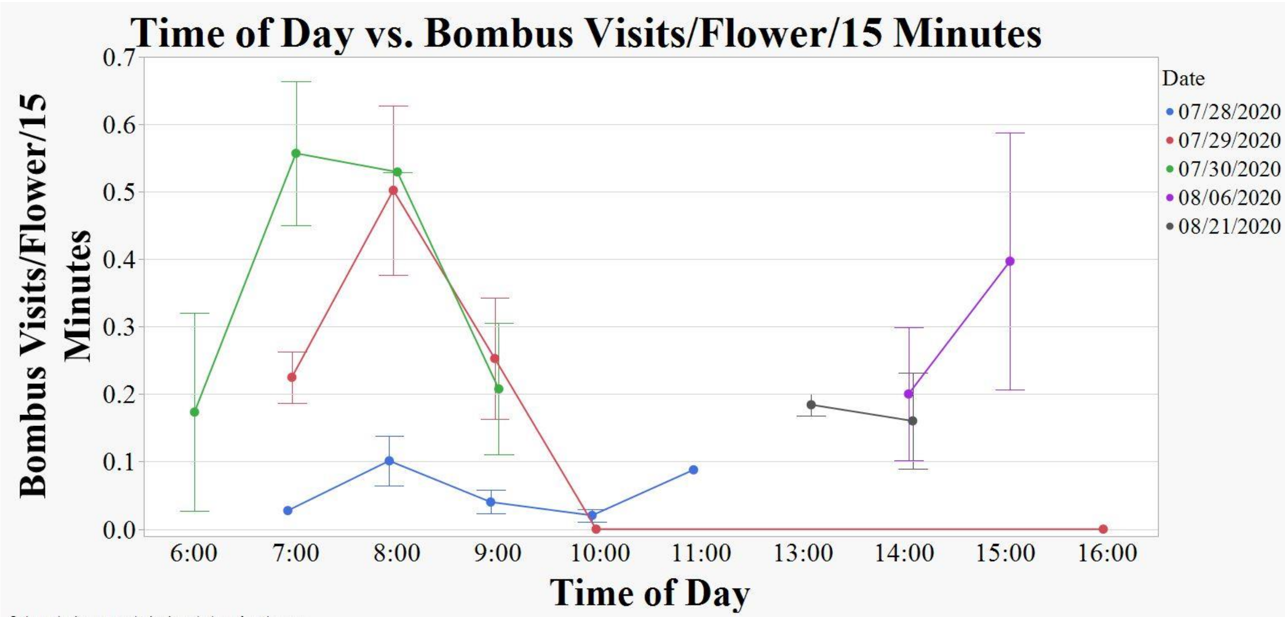

Figure 2-4: Shows the mean *Bombus* visits per flower present in the observation area (over 15-minute periods), over time (v/f/15 min); mean visits per flower were grouped by hour and color coded by date. Differences in mean *Bombus* visits per flower were significant over time for 7/29 and 8/06, but not the other three days ($F(4,10)=1.9$, $p=0.1901$; $F(4,9)=4.6$, $p=0.0263$; $F(3,12)=2.8$, $p=0.0868$; $F(1,6)=0.8$, $p=0.0317$; $F(1,4)=0.1$, $p=0.7599$). Mean *Bombus* visits per flower differed significantly across the five days ($F(4,54)=5.1$, $p=0.0016$). Analysis of pair-wise differences determined that 7/28 differed significantly from 7/30 ($p=0.0005$). Error bars represent one standard error.

The overall mean *Bombus* visits per flower in a period on each day were 0.05, 0.25, 0.40, 0.30, and 0.17 respectively (**Figure 2-4**). 7/28 again had the lowest mean, and as before, both days directly following 7/28 experienced significantly higher mean visits per flower. Contrasting with overall mean visits per flower, time of day differences in mean *Bombus* visits/flower/15 minutes were significant for 7/29 and 8/06, as well as across the five days. This signals that a change in visitation behavior (in terms of the number of flowers visited) occurred in *Bombus*

visitors over time of day. Again, weather was the likely factor causing differences in mean *Bombus* visits per flower across days due to the wet conditions on 7/28.

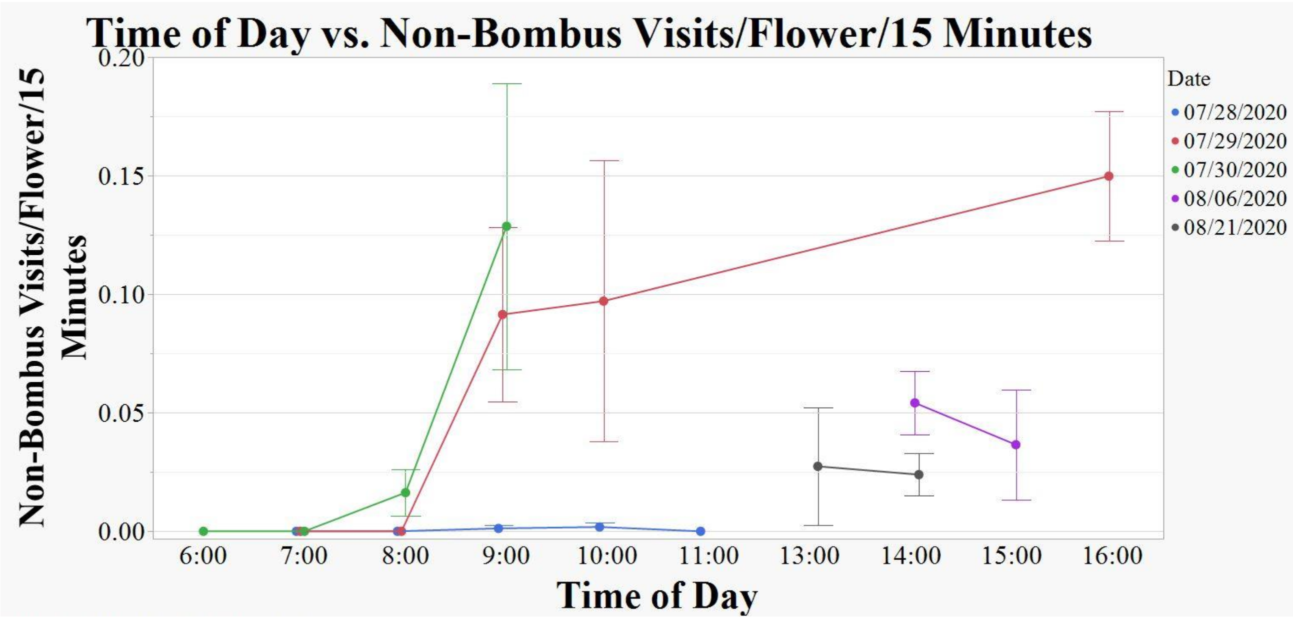

Figure 2-5: Shows the mean non-*Bombus* visits per flower present in the observation area (over 15-minute periods), over time (v/f/15 min); mean visits per flower were grouped by hour and color coded by date. Differences in mean non-*Bombus* visits per flower were significant over time for 7/29 and 8/06 ($F(4,10)=0.2$, $p=0.9112$; $F(4,9)=6.3$, $p=0.0105$; $F(3,12)=3.0$, $p=0.0727$; $F(1,6)=0.4$, $p=0.0147$; $F(1,4)=0.02$, $p=0.9009$). Mean non-*Bombus* visits per flower didn't differ significantly across the four days analyzed ($F(4,54)=1.6$, $p=0.1755$). Error bars represent one standard error.

The overall mean non-*Bombus* visits per flower in a period on each day were 0.001, 0.05, 0.04, 0.05, and 0.03 respectively (**Figure 2-5**). On 7/28, practically no non-*Bombus* species visited, yielding a mean of nearly 0. Again, mean visits per flower spiked for the following days, in a similar pattern to previous data presented. Time of day differences in mean visits/flower/15 minutes were again significant for 7/29 and 8/06, just like *Bombus* species. Interestingly, on 7/29 *Bombus* visits per flower were observed to drop from the morning to the afternoon (**Figure 2-4**), while non-*Bombus* species did the exact opposite (**Figure 2-5**). Our field observations were that

Bombus visitation in general decreased from the morning to afternoon, while non-*Bombus* visitation was almost nonexistent in the morning and spiked as the day wore on. Differences in mean non-*Bombus* visits per flower across days were again probably due to weather.

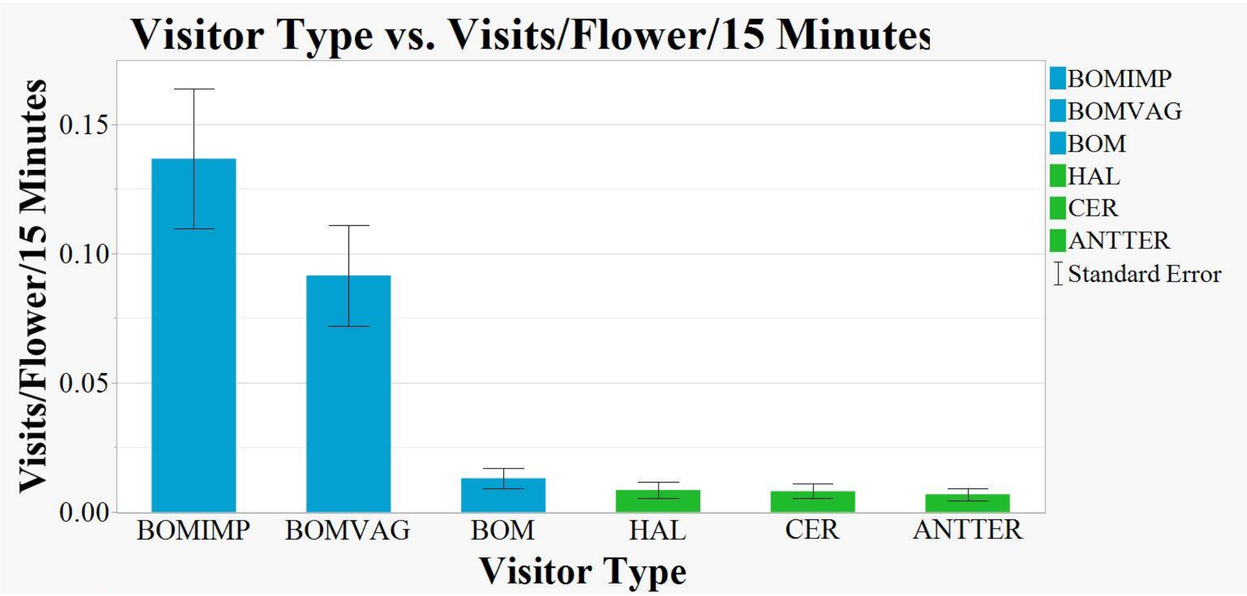

Figure 2-6: Shows the mean visits per flower in an observation area (over 15-minute periods), by visitor type (v/f/15 min). Only types that were observed at least five times and were considered possible pollinators were graphed, meaning COC, MOT, PAPTRO, POAZAB, VESALA, and UNK categories were excluded. Types were color coded into two categories, *Bombus* (blue) and non-*Bombus* (green). Differences in mean visits per flower were significant amongst visitor types, as well as between *Bombus* and non-*Bombus* groups (F(5,348)=16.3, p<0.0001; F(1,352)=37.2, p<0.0001). Analysis of pair-wise differences determined that BOMIMP and BOMVAG differed significantly from every other type (sequentially from left to right), except from each other (p<0.0001; p<0.0001; p<0.0001; p<0.0001; p=0.0011; p=0.0004; p=0.0004; p=0.0003). Error bars represent one standard error.

Bombus species had much higher visitation rates (0.08 v/f/15 min) than the non-*Bombus* group (0.008 v/f/15 min) (**Figure 2-6**). Visitation rates were significantly different amongst visitor types and between *Bombus* and non-*Bombus* groups (**Figure 2-6**). The astoundingly low visitation rates of the two most populous non-*Bombus* visitor types (HAL and CER) is

interesting, considering both were such common visitors; while both visitation rates may be artificially low due to difficulty in tracking them from flower to flower, it's still clear that they chose to visit in short bursts and leave soon thereafter, as opposed to longer forays for *Bombus* visitors. Our observations were that non-*Bombus* visitors were also much more likely to enter an observation area and rest on plants for short periods (without interacting with flowers), then fly off; *Bombus* visitors that entered an area almost never rested on a plant and would spend much longer in the area.

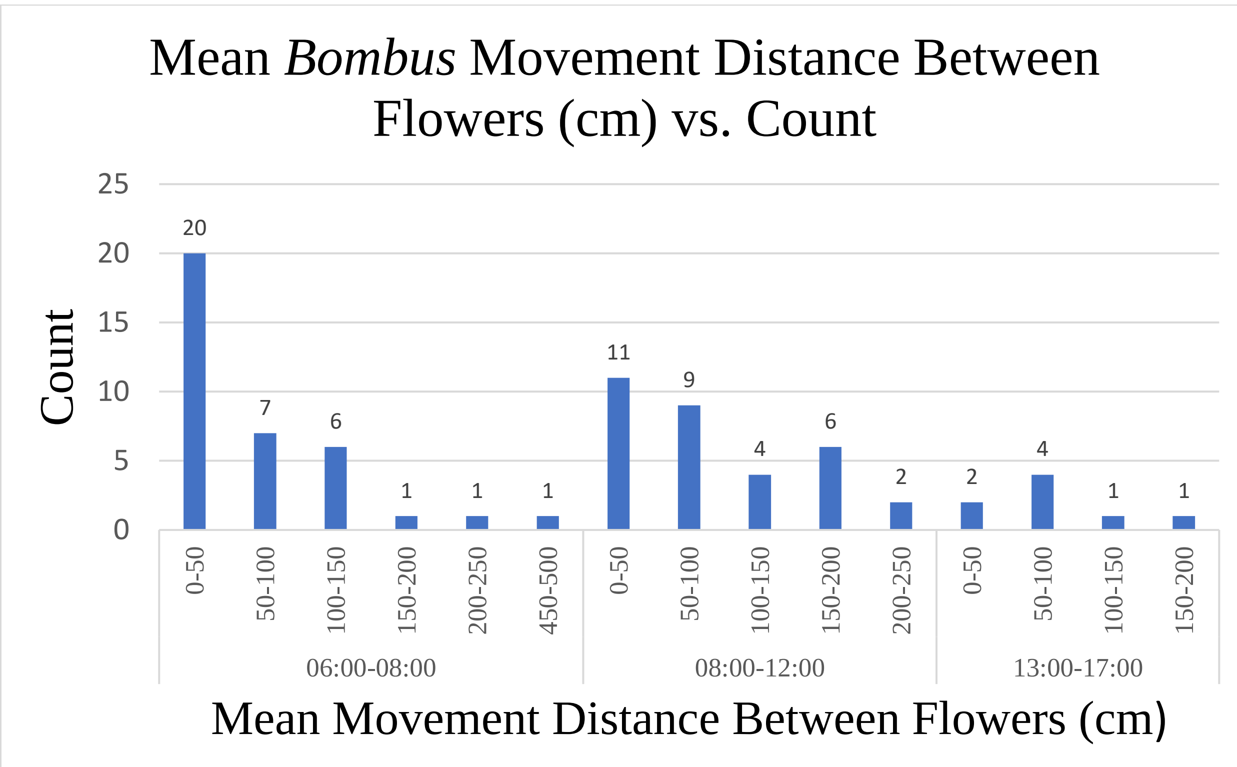

Figure 2-7: Shows the mean *Bombus* movement distance (cm) travelled between each flower visited by a visitor in a 15-minute period over time. Periods were grouped by range and three time-intervals: 06:00-08:00, 08:00-12:00, and 13:00-17:00. Just 76 mean movements were graphed, as many visitors only visited one flower (and thus, were not included). The overall mean movement distance between flowers for the three time-intervals were 74.6 ± 14.3 cm (N = 36), 89.0 ± 10.7 cm (N = 32), and 77.8 ± 14.8 cm (N = 8).

In conjunction with mean visits per flower, verifying visitor behavior between flowers was also helpful for answering the question of what overall visitor behavior was like over time and date. We focused on the mean movement distance between flowers for every visitor over the five days of observation (**Figures 2-7, 2-8**). A breakdown was found of the general trend in mean movement distance during specific time-intervals, regardless of the day. Mean *Bombus* movement distance roughly resembled one half of a bell curve in distribution in each interval, with frequency decreasing as mean movement distance lengthened (cm) (**Figure 2-7**). The number of mean movements also decreased over time, due in part to the fact that fewer observation periods were done in the afternoon, but possibly also due to lower *Bombus* visitation in the afternoon. Each time-interval also yielded similar overall mean movement distances between flowers, an indication that *Bombus* species don't change their foraging behavior between plants over time of day. When analyzed across the five days, differences in mean movement distance between flowers were again insignificant ($F(4,71)=0.3$, $p=0.8822$).

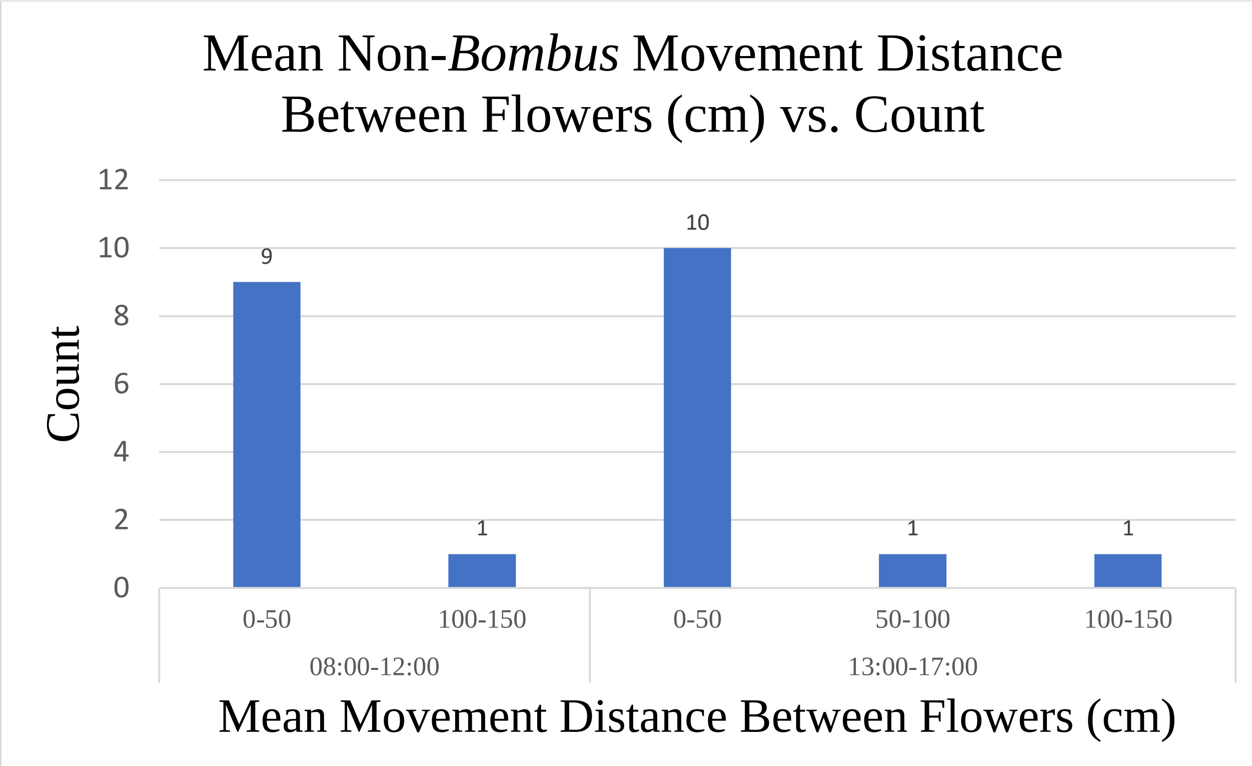

Figure 2-8: Shows the mean non-*Bombus* movement distance (cm) travelled between each flower visited by a visitor in a 15-minute period over time. Periods were grouped by range and two time-intervals: 08:00-12:00, and 13:00-17:00. Only 22 mean movements were graphed. The overall mean movement distance between flowers for the two time-intervals were 36.7 ± 12.8 cm (N = 10) and 25.9 ± 8.4 cm (N = 12).

Mean non-*Bombus* movement distance roughly resembled one half of a bell curve in distribution in each interval, with frequency decreasing as mean movement distance lengthened (cm) (**Figure 2-8**). Interestingly, not a single non-*Bombus* species was recorded having visited two flowers in succession between 06:00-08:00. This is more evidence that non-*Bombus* species wait to visit *M. alatus* until later in the morning (or even afternoon). In contrast to *Bombus* visitors, the number of mean movements increased over time, despite the fact that less observation periods were done in the afternoon than morning. The overall mean movement distance decreased somewhat from the first interval to the second but remained within standard

error. When analyzed across the five days, differences in mean movement distance between flowers were insignificant (F(3,18)=1.1, p=0.3670).

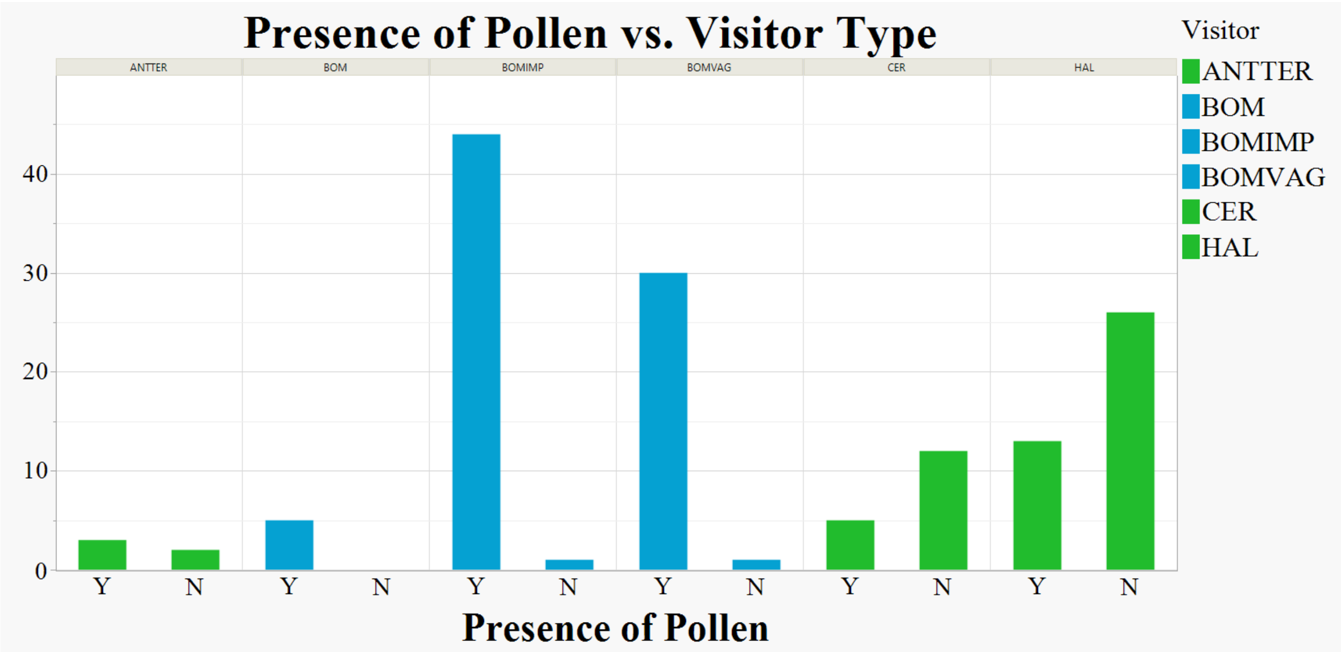

Figure 2-9: Shows the presence of pollen (either on the head or in pollen baskets) by visitor type, divided into 'Y' and 'N' categories. If pollen couldn't be confirmed present (Y) or absent (N), that visit wasn't included. The VESALA, PAPTRO, COC, MOT, UNK, and POAZABW types were excluded; they either weren't deemed likely pollinators, or no visits were recorded in which pollen was present. Visitor types were listed in alphabetical order as listed in the legend. *Bombus* species were clearly the most frequent pollen carriers, followed by sweat bees (HAL).

In our field observations, *Bombus* species were seen carrying pollen almost every time a bee visited, even in very early morning before visitors had done much visitation. While no distinction was made between pollen baskets and pollen on the head, our personal observations were that the majority of *Bombus* bees who carried pollen did so using both baskets and their head. We also noticed that *Bombus* species possessed bristly hair ideal for brushing across pollen to cause adhesion for collection during visits. Non-*Bombus* visitors, like sweat bees or carpenter bees, contained smaller hairs than *Bombus* species, making it more difficult to ascertain whether pollen was present or not. This may have caused visitor totals recorded as carrying no pollen to

be artificially high, and those with pollen to be low. Anecdotally, many less carried any pollen in baskets compared to *Bombus* species; it was more common to observe pollen on the head instead.

Overall, data in **Figure 2-9** indicates that the primary pollinators are likely *Bombus* species, with sweat bees (HAL), carpenter bees (CER), and digger bees (ANTTER) all possible secondary pollinators. Almost half of all *Bombus* visitors carried pollen (49.7%), compared to only 19.4% of all selected non-*Bombus* visitors. This is further backed by the evidence that *Bombus* species are also the principal pollinators for *M. ringens* (Flanagan et al., 2009; Mitchell et al., 2004).

Discussion

In answer to our first research question of who visits *M.* alatus, we found that the primary visitors were predominantly *Bombus* species, constituting over half of all recorded visitors (**Table 2-1**). Surprisingly, sweat and carpenter bees were also common, making up another third of all visitors (31.1%). With regards to overall visitation behavior, mean visits per flower over a period were not found to be statistically significant over time of day; thus, visitation rates remained steady over the time periods that we observed (**Figure 2-3**). However, very few observation periods were done in the afternoon, and we never observed for more than 4-5 hours at a time, making it impossible to compare from the morning to afternoon within a particular day. Visitation rate did differ significantly between days (between 7/28 and 7/29, 7/30, and 8/06, visitation rate ranged from 0.05 to 0.34 v/f/15 min) (**Figure 2-3**). Thus, visitation rates fluctuate by day, but remain steady during each day over time. We postulate that mean v/f/15 min were much lower on 7/28 compared to every other day because of the heavy rain that took place the

night previous, wetting the flowers and discouraging visitors from visiting, as pollen and nectar were waterlogged.

How did *Bombus* visitors compare to non-*Bombus* visitors in visitation rates? *Bombus* visitation rates varied significantly with time of day on 7/29 and 8/06 (**Figure 2-4**). Our personal observations were that *Bombus* activity was high throughout the morning, and that activity was slightly less in the afternoon compared to the morning. When analyzed between days, a difference in visitation rate was found between 7/28 and 7/30 (**Figure 2-4**). Daily visitation rates ranged from 0.05 v/f/15 min on 7/28 to 0.40 v/f/15 min on 7/30. Again, we believe that visitation was low on 7/28 due to wet conditions.

Non-*Bombus* visitation rates varied significantly with time of day on 7/29 and 8/06 as well (**Figure 2-5**). Our field observations were that non-*Bombus* activity was very low in the morning until around 09:00. It's important to note that despite most observation periods occurring in the morning, not a single non-Bombus visitor was ever recorded visiting more than one flower before 08:00 (**Figure 2-5**). From there, activity rapidly increased into the afternoon; in fact, the sheer number of sweat and carpenter bees that traversed the observation areas was so large (and their movements so fast) that they were difficult to track and record. Thus, small visitors were likely under-reported, especially in the afternoon. Conversely, Non-*Bombus* visitation rates didn't vary significantly between days, despite rates ranging from 0.001 v/f/15 min (7/28) to 0.05 v/f/15 min (7/29 and 8/06) (**Figure 2-5**). It's possible that the lack of observation periods in the afternoon combined with difficulty in tracking non-*Bombus* visitors masked visitation trends over time of day and across days.

Directly addressing how *Bombus* visitation compared to non-*Bombus* visitation, mean visitation rates were also determined for each visitor type of interest (possible pollinators who

visited more than five times). *Bombus* species had much higher overall visitation rates across the five days (0.08 v/f/15 min) compared to the non-*Bombus* group (0.008 v/f/15 min) (**Figure 2-6**). Visitation rates were significantly different amongst visitor types and between *Bombus* and non-*Bombus* groups. As mentioned previously, *Bombus* species were observed to visit for longer periods (and more flowers) than non-*Bombus* species, which would visit in short bursts; **Figure 2-6** indirectly illustrates this behavior, with non-Bombus species exhibiting such low visitation rates compared to *Bombus* species.

The second aspect of overall visitor behavior we studied was the mean movement distance between visits. When graphed within ranges of time across the day, intervals for both *Bombus* and non-*Bombus* mean movement distance distributions resembled half-bell curves, with short distances heavily favored and frequency rapidly decreasing with increased distance (**Figures 2-7, 2-8**). This is consistent with the fact that patches usually consisted of groups of tightly clustered plants in close proximity to each other. Generally, the overall mean movement distances for each interval were remarkably similar within groups (**Figures 2-7, 2-8**). In addition, no differences in movement distances were found between days for either group. Together, this indicates that while visitation rates may change over time of day (and by day), visitors don't alter movement behavior between flowers.

Over the course of observations, visitors were observed to visit flowers in patterns of sorts, such as visiting every flower on a plant before moving on to an adjacent plant and travelling in straight lines down an entire row of plants, as if maximizing foraging over the smallest movement distance (personal observations). Furthermore, we cannot recall a single instance in which a visitor ever visited the same flower twice, even after hitting dozens of

flowers in the same area. This indicates that the visitor was able to recognize previously visited flowers, either by sight or through other methods.

Our last research question focused on what visitors collected and carried pollen, to make an educated guess as to the primary pollinators of *M. alatus*. Preliminary evidence from *M. ringens* indicated that *Bombus* species would be the most likely pollinators, and our data agreed with this supposition (**Figure 2-9**) (Flanagan et al., 2009; Mitchell et al., 2004). Half of all *Bombus* visitors carried pollen, compared to only a fifth of selected non-*Bombus* visitors. Coupled with much higher visitation rates, *Bombus* species would likely be more effective pollinators than other species, though further study is needed to determine the validity of this claim.

In retrospect, several aspects of the study could have been altered to improve data acquisition and findings. Only five days of observation were done, mostly in the morning hours, and almost no observations were performed between 11:00-13:00. Performing more over the entire length of the day (and for additional days) would have provided a better indication of temporal patterns. We discovered that determining the mean visits by a visitor was tricky because the number of flowers present in a patch were not constant over time. Devising a more accurate and precise method for measuring visitation rates would be prudent. Also, measuring the movement distances of visitors needs to be standardized and recorded in a manner more efficient than estimating by sight; one method might be to videotape visitors and measure the exact distances travelled between flowers. When determining pollen presence on visitors, it was sometimes hard to definitively state whether a visitor carried any pollen, short of catching and examining it. We also never distinguished between pollen on the head vs. pollen baskets.

Revising our methods of testing pollen presence should allow more accurate measurements useful for studies pertaining to pollination and plant reproduction.

Overall, our findings open new possibilities and questions that need to be addressed to gain a clearer picture of the visitors of *M. alatus* and their interactions. For example, while we determined the main visitors of *M. alatus* and offered circumstantial evidence for possible pollinators, further study must be done to put the matter to rest. During observations, we also noticed that certain visitors travelled between flowers in ordered sequences. It would be beneficial to elucidate whether patterns of visitation exist for primary pollinators, as this may determine how pollination and fertilization of flowers occurs amongst and between patches.

Final Thoughts

Thus far, we've discussed the characteristics of just visitors or plants, but haven't delved into the overlap between the two topics. This discussion seeks to clarify those points. Flower resources, such as nectar and pollen, do not diminish independent of outside influence (**Figure 1-10**). Nectar standing crop (**Figure 1-3**) and pollen levels (**Figure 1-4**) are depleted over the course of the day by visitor activity, and virtually non-existent by 11 AM. Most of the resources are collected by *Bombus* species, as visitation is high during the morning hours (**Figure 2-4**); non-*Bombus* species basically never visited before 8 AM, and only sparsely throughout the morning (**Figure 2-5**). A reasonable assumption is that *Bombus* species are the primary collectors of pollen and nectar, and non-*Bombus* species arrive later to scavenge for remaining resources. As such, *Bombus* species are the suspected primary pollinators of *M. alatus*. This is backed by the fact that *Bombus* species had the highest prevalence of pollen bodies out of any visitor type (**Figure 2-9**), and by the evidence that *Bombus* is also the primary pollinator of *M. ringens* (Flanagan et al., 2009; Mitchell et al., 2004). Further study would need to be done to

record the transfer of pollen to stigmas of other flowers by *Bombus*, confirming their role as pollinators.

Visitor behavior changed over the course of the field study, with visitors almost exclusively probing *M. alatus* flowers early in the blooming season; when flowers began dying off late in the blooming season, we observed that visitors diverted their attentions towards co-existing flowering species nearby (personal observations). Weather patterns also dictated visitor behavior and flower resource levels over time and by day. For example, nectar standing crop was 2.5x higher on 7/28 compared to any other day (**Figure 1-3**), and visitation rates were significantly lower (4x less than any other day) (**Figure 2-3**). On 7/27, it had rained heavily, saturating the nectar, and discouraging visitors from foraging as long as flowers remained waterlogged. The following two days (7/29 and 7/30), nectar standing crop was lower and visitor activity spiked. We postulate that the drier conditions (and flowers) were more conducive for visitation.

While the main visitors of *M. alatus* have been identified and *Bombus* species suspected of being primary pollinators, further investigation of the pollination of *M. alatus* would yield insight into the pollination patterns that govern *M. alatus* reproduction. Worth noting is the fact that early in the study, multiple flowers were found to contain no nectar, despite being closed the day prior to taking measurements early the next morning (roughly 6 AM) (unpublished data). This presents the possibility that 'nectar robbers' may frequent *M. alatus* during the night. Night-time monitoring may resolve the issue and determine whether such a 'robber' exists, or whether this phenomenon is due to other unforeseen factors (such as plant disease).

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