5-2022

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Citation

Jennings, M. (2022). The Synchronization of Nocturnal Pollinator Behavior and Apple Flower Nectar Production. *Biological Sciences Undergraduate Honors Theses* Retrieved from https://scholarworks.uark.edu/biscuht/53

The Synchronization of Nocturnal Pollinator Behavior and Apple Flower Nectar Production

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Biology

By

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Spring 2022

Biological Sciences

J. William Fulbright College of Arts and Sciences

The University of Arkansas

Acknowledgements

First and foremost, I would like to thank Dr. Erica Westerman for being an exceptional mentor and dedicating her time and expertise to providing me an invaluable research experience. Her guidance has been pertinent to my academic success, and I will carry it with me throughout my future endeavors.

I would also like to thank the Westerman lab, Dr. Neelendra Joshi, and graduate student, Sushant Potdar, for welcoming me aboard this project and assisting me along the way.

Additionally, I would like to extend my gratitude to Dr. Fred Spiegel for first getting me involved in undergraduate research and remaining a source of support throughout my undergraduate career. To my honors thesis committee, Dr. Neelendra Joshi, Dr. Adam Siepielski, and Dr. Bill Levine, I am grateful for your assistance in the completion of my honors defense.

I would also like to give a special thanks to the University of Arkansas and the Honors college for funding this research through the Honors College Research Grant and a University of Arkansas Chancellor Innovation Award.

Finally, I would like to thank my family for their continued encouragement throughout this process and for always believing in me.

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Abstract

Insects perform an essential ecological service by facilitating the pollination of crops for food production worldwide. Recent declines in diurnal bee populations threaten food security and has led to growing concerns about existent pollination methods. Pollination contributions by native nocturnal insects have been documented to occur in a variety of systems, thought to be supported by the plant's attractant and reward system. If this is the case, pollination of flowers by nocturnal vectors may be influenced by circadian cycles of nectar production and insect activity. To test this hypothesis, we recorded insect abundance of nocturnal pollinators periodically throughout the night via insect light traps and by conducting transect surveys in a University of Arkansas apple orchard. We also measured nectar production of apple flowers (*Malus domestica*) over a circadian period using microcapillary tubes to determine if nectar production exhibits a circadian pattern. By testing the effect of weather and time on nectar production and insect abundance, we were then able to compare time of nectar production to time of insect abundance to determine if a synchronous relationship exists. Transect insect data were found to be significantly related to temperature and wind. Nectar production of apple flowers did not follow a circadian pattern; however, nectar was produced during the night at similar levels to diurnal nectar production. Apple flower nectar production and nocturnal insect abundance were not significantly correlated, but nectar was produced throughout the night at times when nocturnal pollinators were abundant. My findings show that apple flowers do produce nectar when nocturnal pollinators are abundant but suggest there may be an asynchronous relationship between nocturnal pollinators and apple flowers, where apple flowers produce greater amounts of nectar following high insect presence. This research provides promising insight into alternative pollination methods for the sustainment of agriculture.

Introduction

Pollination plays a vital role in the preservation of a stable ecosystem and the sustainment of life on our planet. Pollinating agents facilitate the reproduction of flowering plants through the dispersal of pollen across the natural landscape (Pardo & Borges, 2020). This improves the overall diversity of vegetation, which has cascading effects on the presence of wildlife and the availability of natural resources for human benefit (Pardo & Borges, 2020). Commercial agriculture and food production take advantage of this ecological phenomenon by using the service of insect pollinators to manage crop diversity and production (Klein et al., 2007).

Traditionally, bees have been used as the primary pollinator for food production systems worldwide. However, declines in local bee populations over the past decade threaten a collapse of agricultural markets and food security (Klein et al., 2007). To maintain equilibrium within commercial markets, cultivators are faced with the challenge of producing the same agricultural output amidst declining bee populations. As a result, the cultivation and shipment of honeybees from across the United States is used by local growers to relieve market demand. While this offers a short-term solution, it is at the expense of native pollinator populations, which appear to do a superior job at improving crop quantity and quality (Garibaldi et al., 2013; Pardo & Borges, 2020). The uncertainty for the future of pollinator-dependent crops has led researchers to seek out new solutions that can sustain the intensifying pressure of declining local bee populations.

Shifting our effort towards rebuilding and conserving the native pollinator population offers insight into more sustainable methods for crop production. Broadening our focus to include all potential pollinators revels a promising group of native pollinators that has largely been overlooked in practice, the nocturnal pollinators. Nocturnal insects, specifically moths, have been documented assisting in pollination in a variety of systems (Amorim et al., 2013; Barthelmess et al., 2006; Walton et al., 2020). Using gene flow as an indicator for pollination, nocturnal pollinators are more responsible for gene flow between populations of white campion (*Silene alba)* compared to diurnal pollinators, suggesting nocturnal pollinators are better at moving pollen across the landscape (Barthelmess et al., 2006). Another study proposed a population of nocturnal pollinators, mainly consisting of hawkmoths, contributed more to the fruit production of Inga Macaco (*Inga sessilis),* than diurnal populations (Amorim et al., 2013). To understand nocturnal pollinator-plant interactions, we must first recognize the functions required of both plants and pollinators for pollination to take place.

Pollinators form mutualistic relationships with the plants they pollinate, supported by the flower's attractant and reward system (Bengtsson et al., 2001; Burkle & Runyon, 2019; Carvalho et al., 2012). Nocturnal bees use common floral olfactory cues when searching for flower resources, indicating that nocturnal insects are likely guided by some of the same attractant cues as diurnal insects (Carvalho et al., 2012). Though not necessarily an insect pollinator, the nocturnal codling moth (*Cydia pomonella*) has evolved to use olfactory cues produced by apple trees during apple development to locate an optimal oviposition location (Bengtsson et al., 2001). Plants have also evolved to take advantage of insect behavior to support plant-pollinator interactions. For example, orchid flowers mimic the pheromones of receptive female bees to attract male pollinators to the flower and facilitate pollination (Schiestl et al., 1999). These studies help demonstrate how insect-flower interactions rely on behaviors exhibited by the flower.

In addition to a flower's attractant, the food resource offered by flowers to insects also mediates plant-pollinator interactions. Nectar is a sugary food source produced by flowers to serve as an incentive for insects to keep coming back (Nicolson, 2011). Bumble bee floral visitations to artificially manipulated field mustard (*Brassica rapa L*) are positively associated with the amount of nectar reward produced (Knauer & Schiestl, 2015). For this reason, nectar may be a resource that influences the timing of pollinator behavior. The act of insect mediated pollination requires temporal cooperation between both flowers and insects in order for pollination to be successful (Bloch et al., 2017). For example, flowers have evolved specific features to serve as advertisements for insect visitation. These traits include floral color, volatile emission, and nectar secretion. In a population of color changing rangoon creeper (*Quisqualis indica*) flowers, each floral color stage attracted different pollinators (butterflies, bees, or moths) and secreted different levels of nectar and scent emissions (Yan et al., 2016). Specific floral features may be optimized to target specific pollinators that will improve the overall reproductive fitness of the plant. To ensure the intended pollinators will receive these advertising signals, the timing of insect activity and flower advertisement need to align.

One mechanism that may facilitate the timing of plant-pollinator interactions are circadian rhythms. Disruptions to the circadian clock of Petunia negatively affected hawkmoth visitation, suggesting that the synchronous behavior of flowers and insects, guided by an internal timing mechanism, influences pollination activity (Fenske et al., 2018). These advertisements such as the release of volatiles and the production of nectar are thought to be regulated through circadian rhythms, however research assessing plant volatile and nectar production for nocturnal pollinators is limited (Fenske & Imaizumi, 2016; Garibaldi et al., 2013). Temporal control of nectar production in squash flowers was demonstrated in one study, showing consistent daily patterns of nectar secretion for squash flowers in a field and greenhouse setting (Edge et al., 2012). The attractant and reward system of scarlet sterculia (*Sterculia colorata)* also appears to maintain a circadian rhythm, with nectar production and scent production favoring nocturnal insect visitation (Prieto-Benítez et al., 2016). Each of these systems support the idea that mutualistic pollinator-plant interactions are regulated by interconnected circadian patterns, such as those of nectar production. If this is the case, nocturnal pollinator and apple flower behavior may also occur at corresponding times. However, insect and flower behavior are not the only contributing factors influencing the timing of pollination.

Weather has been observed to affect nectar secretions and insect activity abundance. Moth abundance in light traps increases on warmer nights and decreases on rainy nights (Holyoak et al., 1997). Flower visitation activity of bee populations in apple orchards is also significantly dependent on temperature, solar radiation, and wind speed, with some species more weather tolerant than others (Vicens & Bosch, 2000). If weather shapes the activity periods of pollinators, it indirectly affects the ability for pollination to occur and the kinds of pollinator that will be receptive to flower behavior. Weather also has direct effects on flower behavior, for example nectar production of onion flowers decreased relative to increasing humidity levels (Silva et al., 2004). It is likely that weather conditions affect nocturnal pollinator abundances and floral nectar production, which may work interdependently to facilitate or impede plant-pollinator interactions.

In addition to studies of nocturnal pollinators in nature, nocturnal pollinators have also been observed in an agricultural setting. Nocturnal pollination of lowbush blueberry results in significant fruit set and comparable fruit weight to that of diurnal pollinators (Cutler et al., 2012). A recent study found that nocturnal pollinators in a Northwest Arkansas orchard participate in the pollination of apple flowers and provide similar levels of apple production as their diurnal counterparts (Robertson et al., 2021). Though nocturnal pollinators have been recognized in nature for their role in pollination, the cause and function of nocturnal pollination for crops remain underdeveloped (Wonderlin et al., 2019). If we can better understand the role of nocturnal pollinators in these plant-pollinator interactions, we can determine how they can be used to improve fruit production on a global scale.

The goal of my study is to establish if a synchronous relationship exists between nocturnal pollinators and apple flower behavior by comparing the activity patterns of nocturnal pollinators to the circadian rhythms, if existent, of nectar production in apple flowers. Additionally, I assessed the effect of weather on insect abundances and nectar production to determine if abiotic factors influence timing of pollination. I hypothesized the activity times of pollinators would align with nectar production, suggesting that apple flowers may take advantage of all pollinators to increase the chance of pollination occurring. The results of this study can provide agriculturalists with new ways to enhance the effectiveness of native pollinators.

Methods

Study Site

We selected a University of Arkansas (UARK) apple orchard at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, Arkansas for the location of our study (36°06’05”N, 94°10’00”W). The 1.2-acre orchard consists of ten rows of approximately 222 apple trees of different varieties, including Enterprise, Goldrush, AR 127, and AR 124. As part of the research space for UARK’s Horticulture department, this space is typically used to study fruit production and sustainable agriculture. It is located in a semi-rural environment surrounded by several other field sites. The orchard’s secluded setting and surrounding vegetation provides a suitable habitat for moths and other various insect species. Additionally, the lack of light pollution in the field during the night ensured the light traps used for our study would not be competing with any other light sources.

Study Animal

In an effort to involve all potential nocturnal pollinators, the study system encompassed all species of moth (*lepidoptera*) present in the orchard throughout the circadian period. The exact species of moth observed and captured were not recorded, however the moths we encountered were categorized by size, consisting of small, medium, and large. Based on this information and the known species of moths found in Arkansas, the likely moths inhabiting the field include Sphingidae, Noctuidae, and Geometridae.

Insect Sample Collection

Data collection was conducted in the spring of 2021, occurring over a two-week field season between April 10th and April 23rd, when apple flowers were in bloom and pollinators were active. Insect sampling took place over 7 nights of the two-week field season. To identify the abundance of pollinators and when they were present in the orchard, we collected two types of insect data: transect surveys and insect light traps. In order to monitor the presence of moths throughout the night, transect sampling began just before dusk and continued until dawn with a total of four sampling times at 6:00PM, 10:00PM, 2:00AM, and 6:00AM. We performed five-minute transect surveys at each sampling time by counting the number of moths encountered along a transect in the orchard and recording the number in a Rite in the Rain notebook. We also noted the size of each moth, categorized by small, medium, and large.

In addition to the five-minute surveys, we positioned three pairs of quarter acre DynaTrap insect traps in different locations throughout the orchard to get a more thorough account of the pollinators present. Each pair of traps were connected to a rechargeable battery that was enclosed in a waterproof tarp to ensure the traps would stay on throughout the sampling period. Trap collection occurred from 6:00PM to 6:00AM, with 3 sampling periods from 6:00PM to 10:00PM, 10:00PM to 2:00AM, and 2:00AM to 6:00AM. Per sampling period, only a single pair of traps were turned on, so that every four hours a new orchard location would be sampled for insects. The insects caught in the light traps during each four-hour interval were then relocated to zip lock bags and maintained in a -30 C freezer. I counted the number of insects collected in each bag to quantify the number of insects in the orchard at particular times and transferred the data to a spreadsheet.

Nectar Sample Collection

Nectar data was also collected to determine whether nectar production of apple flowers was circadian and produced at night. A total of 779 nectar samples from 32 sampling times were collected in Drummond Calibrated Micropipettes over 7 circadian days. A circadian day was a 24-hour sampling period from 6:00AM to 6:00AM and was divided into 5 sampling times: 6:00AM, 11:00AM, 4:00PM, 9:00PM, and 2:00AM. Approximately every five hours, we sampled nectar from five randomly selected in bloom trees. Five flowers were then selected for nectar sampling from each tree, resulting in a total sample of 25 microcapillary tubes per sampling time. Flowers were chosen on the basis of maturity and whether or not they appeared to be in bloom. Nectar was collected by inserting the microcapillary tube into the base of each flower where nectar is present. To measure the amount of nectar in each sampling tube, I created a scale bar by marking the length of the microcapillary tube on a piece of paper and dividing it into ten equal segments. Using the scale bar as a reference, I measured the volume of nectar in each microcapillary tube under a dissecting microscope.

Weather Data

To account for any effects of weather on our data, we documented temperature and wind during the sampling times of nectar collection. For insect collection, the weather conditions were recorded using Wunderground's weather archives, which includes data for temperature and wind. The data from Wunderground is based on weather conditions at Northwest Arkansas Regional Airport in Bentonville, AR. (https://www.wunderground.com/weather/us/ar/fayetteville)

Statistical Analysis

I analyzed insect, weather, and nectar data in Rstudio Version 1.4.1103. The counts of insect activity (transect and trap data) and nectar abundance served as my dependent variables, while time of day and weather acted as my independent variables. To evaluate the effect of time, temperature, and wind on insect abundances, I conducted two generalized linear models, one for transect data, and another for trap data. To assess the effect of time, temperature, and wind on nectar production, I conducted another generalized linear model. Two calculations for nectar were included, one based on the summation of nectar per tree and another on the average of nectar per tree. I also performed two linear regressions to determine if there was a correlation between insect activity (transect data and trap data) and nectar abundance. For this analysis, the 25 nectar samples per time point were first averaged by flower and then by tree to obtain one nectar value per sampling point. Insect data and nectar data had different sample sizes, for this reason, 10 nectar values and 2 transect insect values were excluded from the data set, resulting in a total sample size of 22 for transect insects and nectar. The sample size for the linear regression of trap insects and nectar was 18 and excluded 14 nectar values and one insect value from the data set.

Ethics Statement

Due to the nature of the study and using traps as the most efficient means of determining moth activity throughout the night, moths were affected by the study; however, we aimed to minimize our interactions with them as to not disturb their natural behavior. All moths that were caught were euthanized by freezing to preserve for future analysis.

Results

Summary

We surveyed 29 insects from transect walks and captured 36 insects via light traps, for a total observation of 65 nocturnal insects across 7 nights (Table 8). Due to inclement weather, 4 transect insect sampling times of the original 28 were canceled, resulting in 6 sampling points for 6:00PM, 7 sampling points for 10:00PM, 6 sampling points for 2:00AM, and 5 sampling points for 6:00AM (Table 5). Of the original 21 sampling times for trap insects, 2 were canceled, resulting in 7 sampling points for 10:00PM, 6 sampling points for 2:00AM, and 6 sampling points for 6:00AM (Table 6). We also collected a total of 779 nectar samples from 156 apple trees over 7 circadian periods (Table 9). Due to inclement weather, 10 nectar sampling times of the original 42 were cancelled within the 7 circadian periods. This resulted in 10 sampling points for 6:00AM, 5 sampling points for 11:00AM, 5 sampling points for 4:00PM, 6 sampling points for 9:00PM, and 6 sampling points for 2:00 AM (Table 7). We surveyed a total of 48 trees at timepoints 1 and 6 (6:00AM), 30 trees at timepoint 2 (11:00AM), 19 trees at timepoint 3 (4:00PM), 30 trees at timepoint 4 (9:00PM), and 29 trees at time point 5 (2:00AM).

Insect Data: Weather and Time

Transect insect data was found to be significantly affected by temperature and wind. Insect abundance increased with temperatures above 12° C (p-value = 0.004, T = 3.315, and N = 24) (Table 1, Figure 2). Insect abundances increased below wind gusts of 3 mph and decreased with greater wind speeds (p-value = 0.037, T = 2.274, N = 24) (Table 1, Figure 3). There was also a significant interaction between temperature and wind for transect insect data (p-value = 0.010, T = -2.94, and N = 24) (Table 1). Additionally, transect insect abundance had a significant relationship with the interaction term of time and temperature (p-value = 0.041, T = -2.21, N = 24) (Table 1). Trap insect abundance was not significantly influenced by time of day, temperature, or wind (Table 2).

Nectar Data: Weather and Time

Nectar production was not influenced by time, temperature, or wind (Table 3, 4). However, nectar was produced during the night at similar or greater levels to diurnal nectar production (Figure 7, 10). Nectar production in apple flowers was not circadian, as consistent amounts were produced during the day and night (Figure 7, 10).

Nectar and Insect Data

Nectar production and transect insect abundance were not correlated (p-value = 0.71, T = - 0.383, and N = 22). Additionally, trap insect abundance was not influenced by nectar production (p-value = 0.66, T = -0.449, N = 18).

Discussion

We found transect insect abundance was significantly affected by temperature and wind, with highest insect presence occurring at warmer temperatures and lower wind speeds. There was not an effect of time found for insect abundance, however we observed and collected a total of 65 insects in the orchard at night. Nectar production was not influenced by time, temperature, or wind, but was produced consistently both diurnally and nocturnally. Additionally, insect abundance and nectar production were not correlated. Given the lack of temporal control for insect abundance and nectar production, our findings suggest there is not a synchronous relationship between nocturnal pollinators and apple flower behavior.

Apple flowers were not only found to produce nectar at night, but levels of nectar collected during the night were equal to diurnal amounts. This finding expands upon the results of a preliminary experiment that recognized the involvement of nocturnal pollinators in apple production (Robertson et al., 2021). The presence of nectar at night may attract nocturnal pollinators to apple flowers and reinforce diurnal pollination. If apple flowers play a role in facilitating nocturnal pollination events via resource advertisement, it implies that the previously reported significant contribution of nocturnal pollination was not random or coincidental.

In the context of the current body of literature surrounding nocturnal pollination, this study appears to be the first of its kind to document nectar production occurring at night for fruit crops. Previous studies have acknowledged the involvement of nocturnal pollinators in fruit production, but do not provide a clear mechanism for facilitating nocturnal pollinator visitation. For example, significant levels of fruit set were obtained from blueberry crops that were exposed to nocturnal pollination, but flower behavior was not observed (Cutler et al., 2012). Other studies have considered the role of nectar production in supporting pollinator interactions with fruit crops but did not document nectar production over a circadian period. For example, diurnal changes in rate of nectar production were measured for two Sumatran wild bananas, however nocturnal nectar production was not reviewed (Itino et al., 1991). In conjunction with existing studies, my results are significant because it provides evidence to suggest producing nectar at night has been evolutionarily selected for in apple flowers, perhaps because nocturnal pollinators benefit their reproductive success.

One outcome of the study that was unexpected, was the lack of a significant circadian pattern to nectar production. Compared to previous studies that found temporal control of nectar production in flowers, nectar production of apple flowers remained consistent throughout the circadian period with overall low averages of nectar per flower (Edge et al., 2012; Prieto-Benítez et al., 2016; Silva et al., 2004). In contrast to our results, squash flowers revealed consistent changes in nectar volume throughout the day under greenhouse conditions, however in the presence of active pollinators, nectar production was greatly reduced and highly variable (Edge et al., 2012). This is consistent with our findings of overall low nectar averages for apple flowers. Nectar production in Scarlet Sterculia (*S. Colorata)* was also very low, however unlike our results, there was a significant difference in nectar volume between time shifts (Prieto-Benítez et al., 2016). Likewise, onion flowers exhibited significant differences in the amount of nectar produced at different time points on a 24-hour cycle (Silva et al., 2004). Although, the lack of circadian control for apple flower nectar production is inconsistent with previous research, our results showing decreased amounts of nectar with high variability are comparable to nectar production in other crops and flowers under natural conditions.

Temporal regulation of nectar rewards has been suggested as a method used by flowers for conserving energy and maximizing reproductive fitness (Lovett-Doust & Lovett-Doust, 1988). While the production of nectar can facilitate plant-pollinator interactions that result in pollination, it can also come at the expense of seed yield and condition (Pyke, 1991). Nectar production was found to lower seed quality and quantity in Christmas Bells (*Blandfordia nobilis*) (Pyke, 1991). With this in mind, apple flowers may produce lower amounts of nectar consistently to target a variety of pollinators without exerting all their energy.

Additionally, the lack of circadian rhythm for nectar production could suggest there is less competition among apple flowers and other plants to attract insect visitors. Asynchronous timing of nectar production is one suggested strategy that plants may use in habitats with increased competition because it provides each species with individual pollination opportunities (Bentley & Elias, 1983).

The significant influence of temperature and wind on transect insect abundance aligns with the results of other studies that found insect activity increased on warmer nights and decreased on windy nights (Holyoak et al., 1997; Vicens & Bosch, 2000). Our sampling of transect insects had a wide temperature range of 4° to 23° C with highest insect abundances occurring at 20° C (Figure 2). Decreased insect abundances were generally observed at lower temperatures, with a few exceptions that may be explained by the interaction of temperature and wind. This phenomenon suggests nocturnal pollination may be more likely to occur on warmer nights compared to cooler nights due to the pollinators having a lower tolerance for cold temperatures.

Extreme cold weather can have lethal effects on insect survival caused by protein damage and cellular strain (Chidawanyika & Terblanche, 2011). In response, insects have developed behavioral and physiological strategies to withstand cold weather (Chidawanyika & Terblanche, 2011). For example, noctuid moths can perform behavioral thermoregulation by choosing microhabitats that provide protection from the cold (Heinrich, 1987). Although the temperatures never reached levels of freezing, lower temperatures may influence nocturnal pollinator behavior, causing them to seek out shelter as opposed to preforming basic behaviors such as feeding, mating, and pollinating flowers.

The negative effect of wind on insect abundance may result from changes in flight behavior. This has been demonstrated in Hymenoptera that experienced reduced flight capacity due to higher wind velocities (Marchand & Mcneil, 2000). Abundance patterns of flying insects have also been shown to decrease in cold and windy weather conditions, resembling our results (Grüebler et al., 2008). Successful pollination requires that insects efficiently travel distances between flowers so that pollen can be dispersed across the landscape. If windy weather reduces nocturnal pollinator flight, this will also affect pollination. The present work highlights differences in insect activity as a result of weather condition, which in turn may impact pollination occurrence.

Insect abundance was not significantly correlated with nectar abundance and individually neither had a significant relationship to time. This result differs from other findings that appear to show a synchronous relationship between insect visitation and the circadian rhythm of flower behavior (Edge et al., 2012; Fenske & Imaizumi, 2016; Prieto-Benítez et al., 2016). Although there is not a significant correlation in our study, it is worth noting that the highest amounts of nectar were produced around 2:00AM to 6:00AM, while higher amounts of insects occurred earlier around 10:00 PM (Figure 13). This may suggest that insect presence encourages nectar production. One explanation may be that as insects remove nectar from apple flowers, flowers produce more nectar in response, leading to higher amounts later in the night. The effect of removal-enhanced nectar replenishment has been demonstrated in animal pollinated plant species, however whether this is guided by insect presence is unclear (Luo et al., 2014). The lack of a significant relationship between nectar, insects, and time fails to confirm this trend, so future research is necessary. It is also important to recognize that data was only collected for one season and doesn't offer a complete understanding of this system. Nectar production and insect abundance may vary annually, so additional data is needed to strengthen current findings.

Although a synchronous relationship was not established between insect and nectar behavior, the results of our study demonstrate a consistent pattern of apple flower nectar production that occurs diurnally and nocturnally. This is significant for our understanding of nocturnal pollination in fruit crops because it implies that apple flowers have evolved to produce nectar nocturnally, perhaps on account of nocturnal pollinators. The characterization of this relationship can be used to improve natural pollination methods for crop production.

Conclusion

Although additional investigation is necessary to confirm a consistent nectar production pattern over time, my findings clearly indicate that nectar is produced during the night by apple flowers. This provides insight into the mechanism of nocturnal mediated apple flower pollination, as it appears to operate using similar attraction and reward strategies as that of diurnal pollination. According to these findings, weather conditions may dictate insect abundance to a greater extent than nectar production. Future studies exploring insect visitation of specific nocturnal species and associated nectar production under consistent weather conditions may alter our understanding of insect and apple flower temporal cooperation. Further investigations into apple flower behavior can be made by examining volatile emission or nectar quality over time. This study highlights the importance of investigating novel plant-pollinator interactions to determine if nocturnal pollination is facilitated by nectar production in other systems. Our results contribute to current endeavors to determine the involvement of nocturnal pollinators to successful pollination and utilize their service in crop production.

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Figures and Tables

Table 1. Generalized Linear Model for Transect Insects and Weather (N=24)

	Estimate	Std. Error	t-value	p-value
Time	0.417	0.373	1.120	0.279
Temperature	2.97	0.896	3.315	0.004*
Wind	1.78	0.781	2.274	0.037*
Time:Temp	-0.105	0.048	-2.218	0.041*
Time:Wind	-0.055	0.059	-0.944	0.359
Temp:Wind	-0.396	0.135	-2.940	0.010*
Time:Temp:Wind	0.016	0.008	2.058	0.056

Table 2. Generalized Linear Model for Trap Insects and Weather (N=19)

	Estimate	Std. Error	t-value	p-value
Time	-0.45	0.67	-0.67	0.52
Temperature	0.46	0.94	0.49	0.64
Wind	0.88	1.79	0.49	0.64
Time:Temp	0.05	0.05	1.02	0.33
Time:Wind	0.09	0.13	0.67	0.52
Temp:Wind	-0.06	0.13	-0.47	0.65
Time:Temp:Wind	-0.01	0.01	-0.91	0.38

Table 3. Generalized Linear Model of Nectar Average per tree, Weather, and Time (N=156)

	Estimate	Std. Error	t-value	p-value
Time	9.32e-03	7.90e-03	1.18	0.240
Temperature	4.36e-04	1.11e-02	0.039	0.969
Wind	5.92e-03	1.52e-02	0.39	0.697
Time:Temp	-7.93e-04	7.77e-04	-1.02	0.309
Time:Wind	-6.14e-04	1.096e-03	-0.56	0.576
Temp:Wind	-8.60e-05	1.53e-03	-0.06	0.955
Time:Temp:Wind	2.11e-05	1.03e-04	0.21	0.838

Table 4. Generalized Linear Model for Nectar Sum per tree, Weather, and Time (N=156)

	Estimate	Std. Error	t-value	p-value
Time	0.047	0.040	1.183	0.239
Temperature	0.002	0.056	0.039	0.969
Wind	0.030	0.076	0.392	0.696
Time:Temp	-0.004	0.004	-1.020	0.309
Time:Wind	-0.003	0.005	-0.563	0.575
Temp:Wind	-0.000	0.008	-0.056	0.955
Time:Temp:Wind	0.000	0.001	0.205	0.838

Table 5. Sample Size of Transect Insect Sampling Times

Sampling Time	Sample Size
6:00 PM	6
10:00 PM	7
2:00 AM	6
6:00 AM	5
Total	24

Table 6. Sample Size of Trap Insect Sampling Times

Sampling Time	Sample Size
10:00PM	7
2:00AM	6
6:00AM	6
Total	19

Table 7. Sample Size of Nectar Sampling Times

Sampling Time	Sample Size
6:00 AM	10
11:00 AM	5
4:00 PM	5
9:00 PM	6
2:00 AM	6
Total	32

Table 8. Insect Sample Size per Sampling Time

Sampling Time	Insects
6:00 PM	7
10:00 PM	38
2:00 AM	16
6:00 AM	4
Total	65

Table 9. Nectar Sample Size per Sampling Time

Sampling Time	Nectar Samples
6:00 AM	240
11:00 PM	125
4:00 PM	120
9:00 PM	150
2:00 AM	144
Total	779

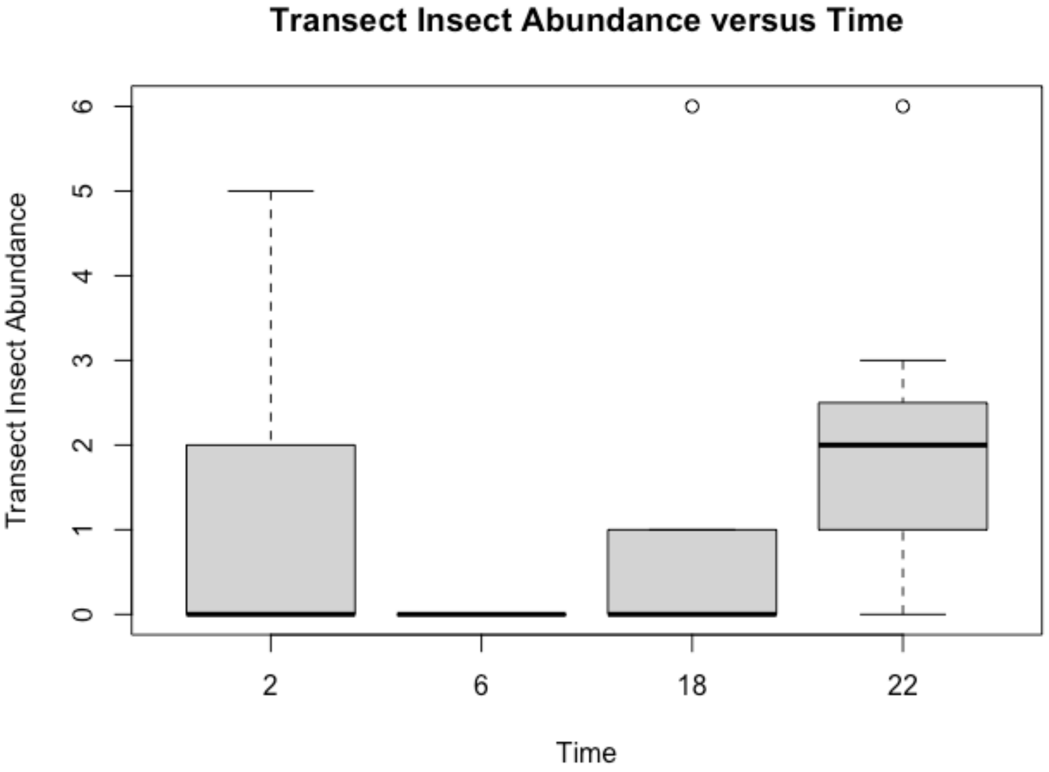

Figure 1: No effect of survey time on transect insect abundance, see glm results in Table 1 (N=24 surveys; with N=6 for Time 2, N=5 for Time 6, N=6 for Time 18, and N=7 for Time 22).

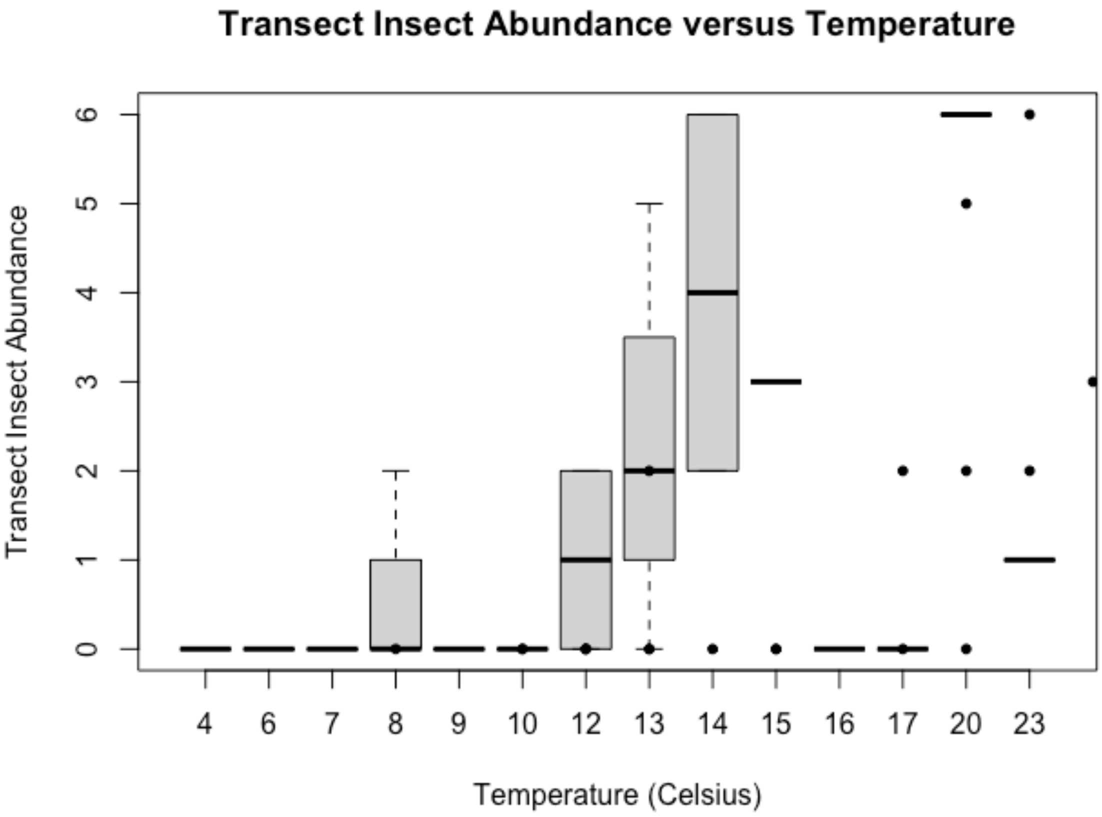

Figure 2: There was an effect of temperature on transect insect abundance, see glm results in table 1 (N=24).

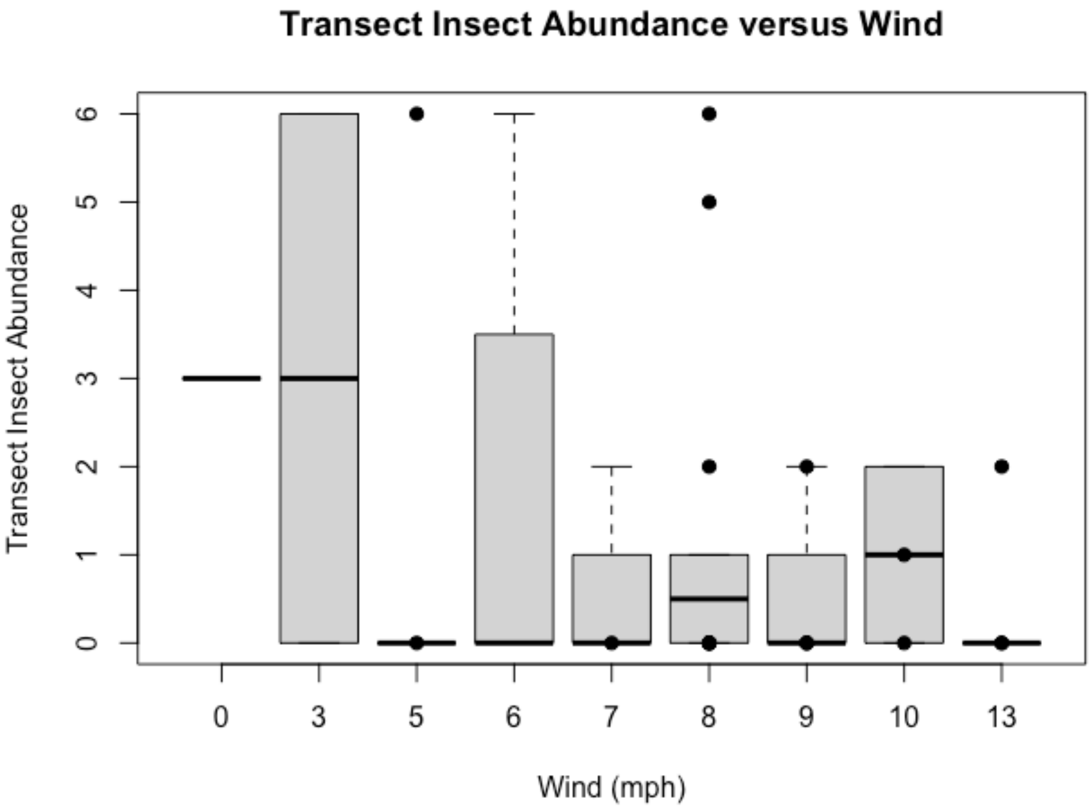

Figure 3: There was an effect of wind on transect insect abundance, see glm results in table 1 (N=24).

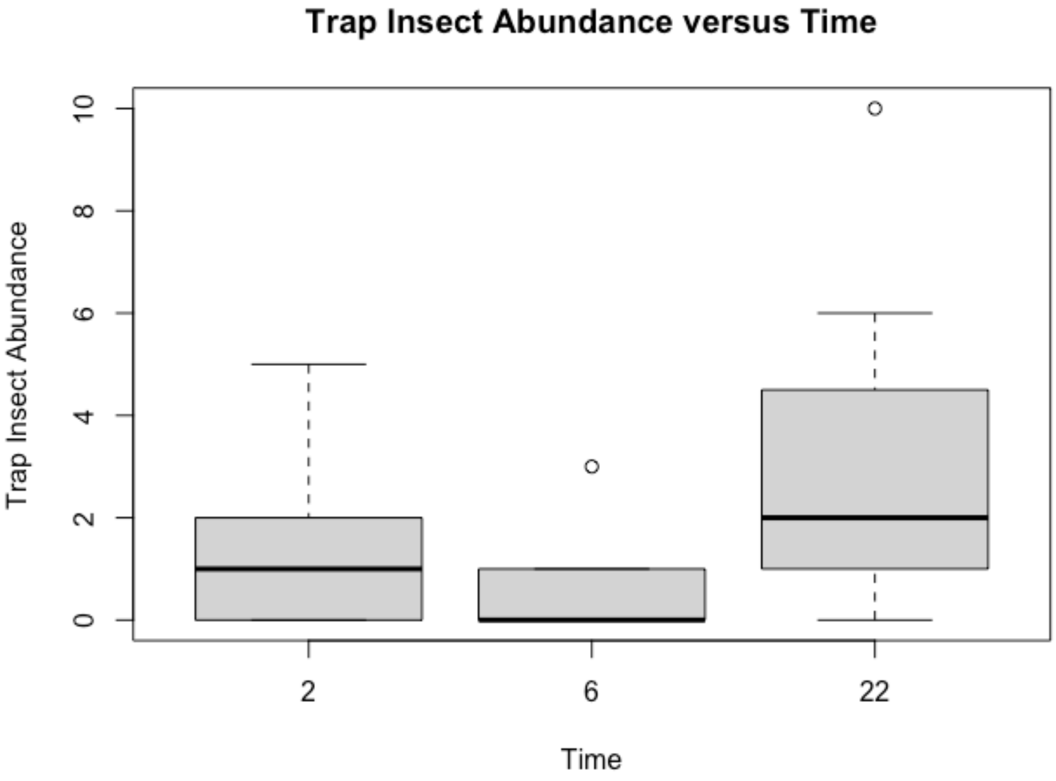

Figure 4: No effect of time on trap insect abundance, results of glm in table 2. Time 22 accounts for insects collected from 6:00PM to 10:00PM, Time 2 accounts for insects collected from 10:00PM to 2:00AM, and Time 6 accounts for insects collected from 2:00AM to 6:00 AM. (N=19 surveys; with N=6 for Time 2, N=6 for Time 6, N=7 for Time 22).

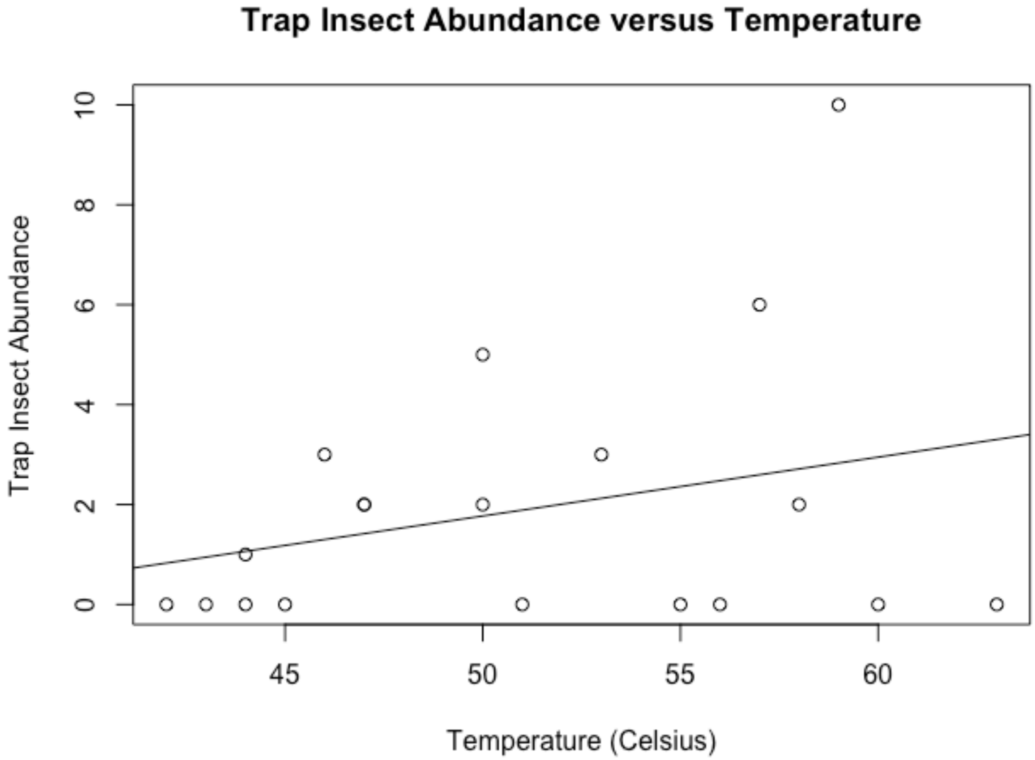

Figure 5: No effect of temperature on trap insect abundance, see glm results in table 2 (N=19).

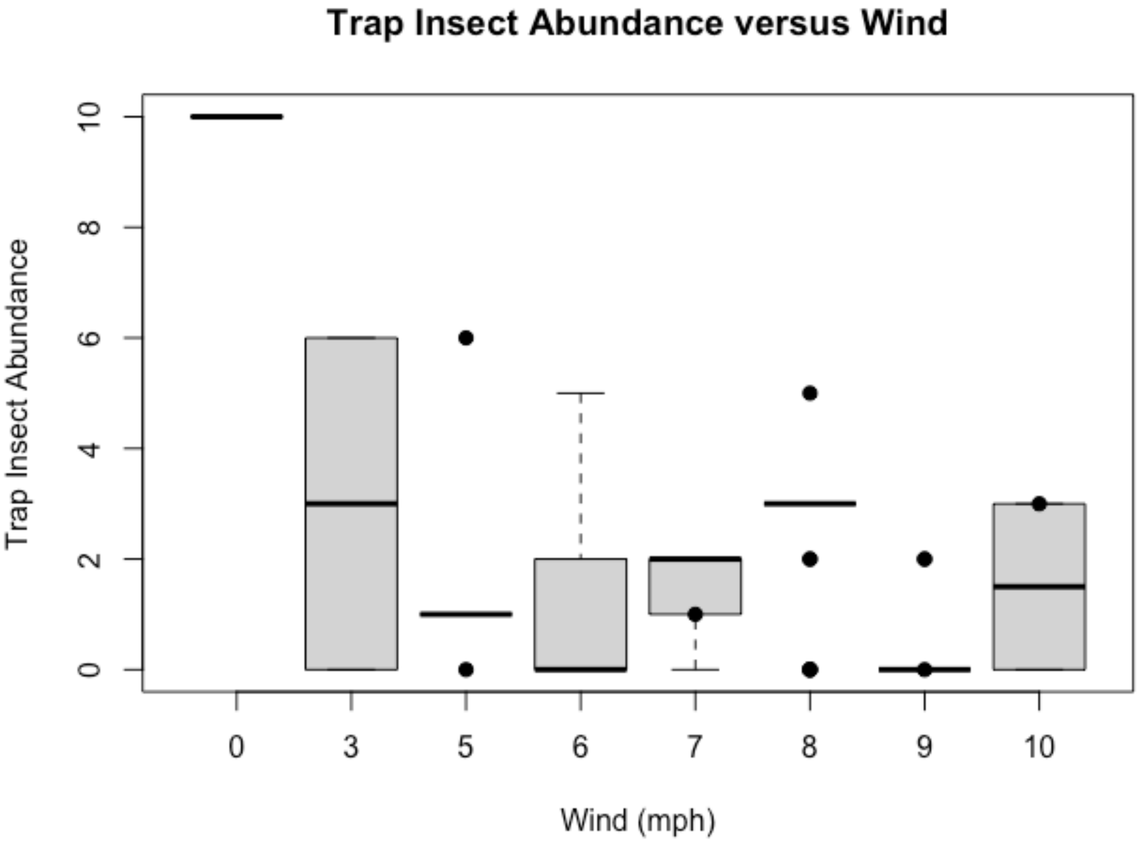

Figure 6: No effect of wind on trap insect abundance, see glm results in table 2 (N=19).

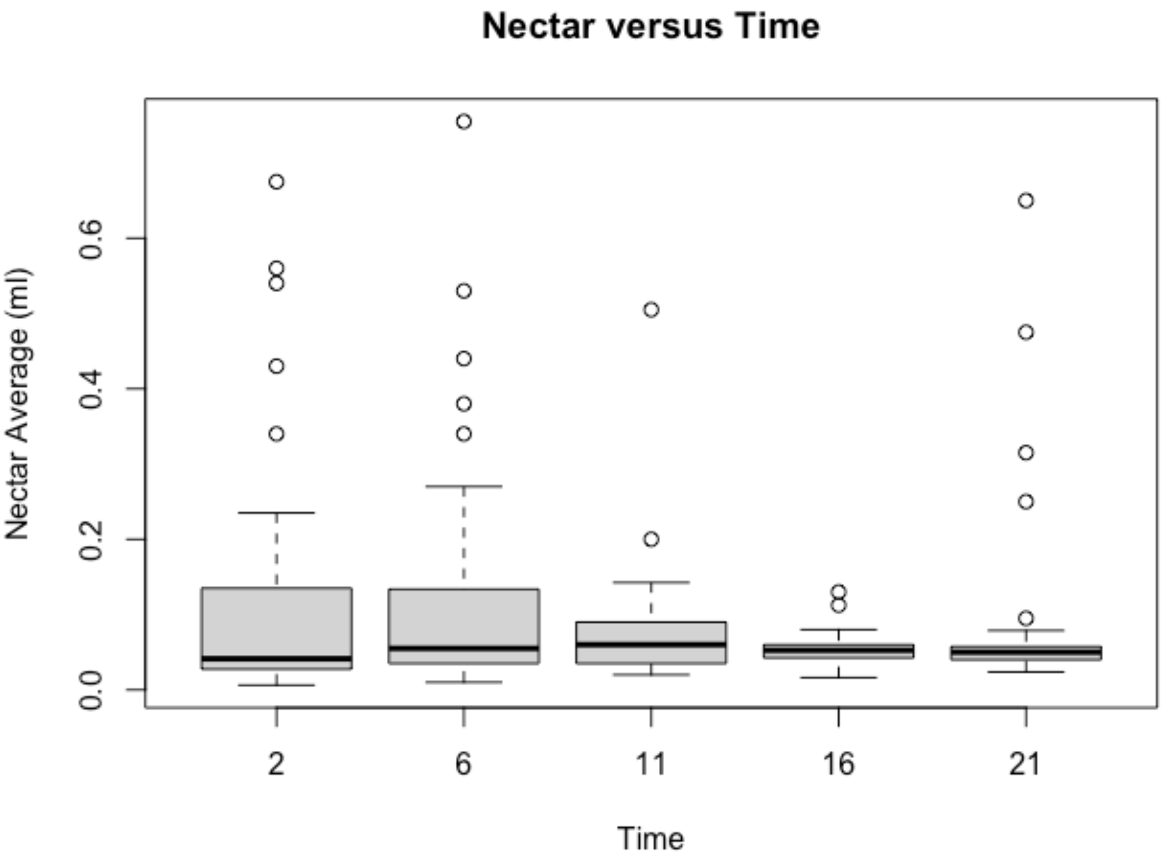

Figure 7: No effect of time on nectar average per flower per tree, see glm results in table 3 (N=156; with N=29 for Time 2, N=48 for Time 6, N=25 for Time 11, N=24 for Time 16, and N=30 for Time 21).

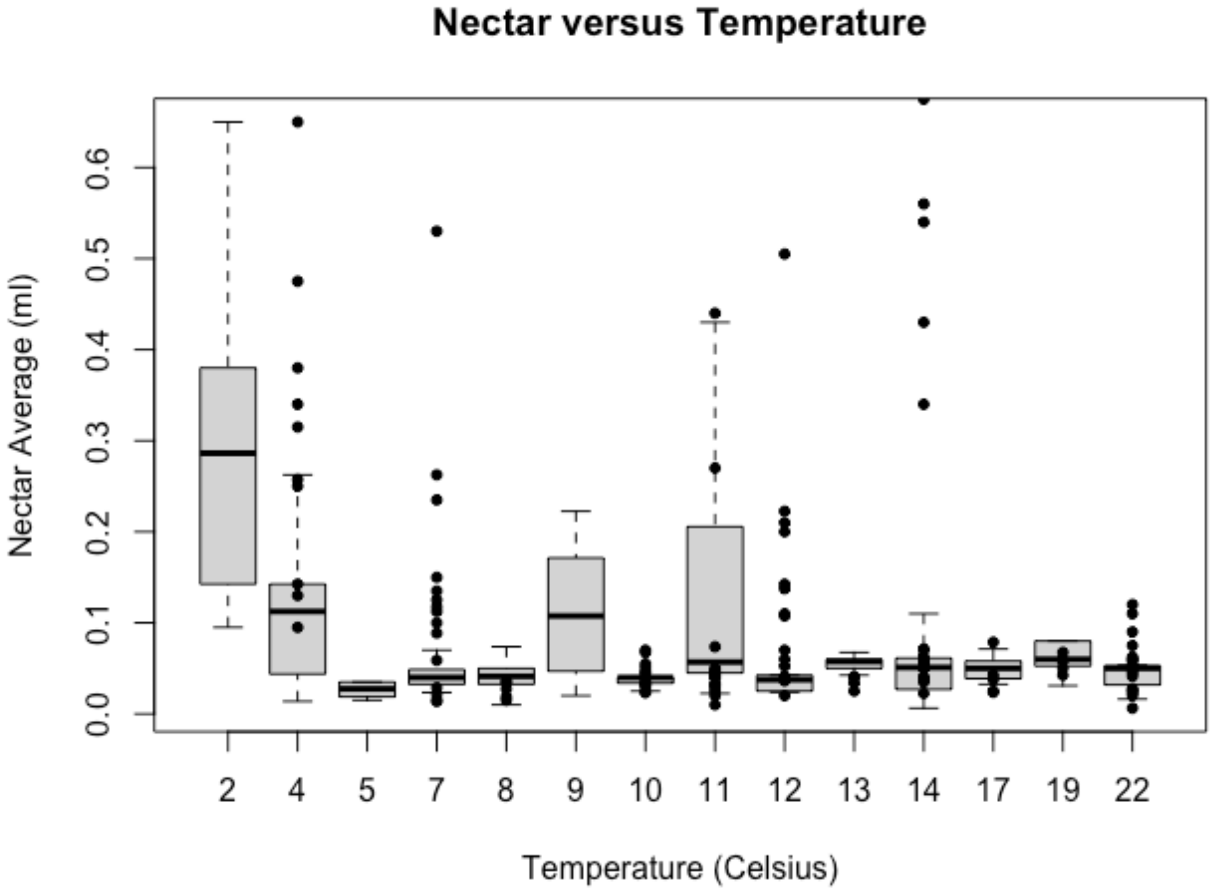

Figure 8: No effect of temperature on nectar average per flower per tree, see glm results in table 3 (N=156).

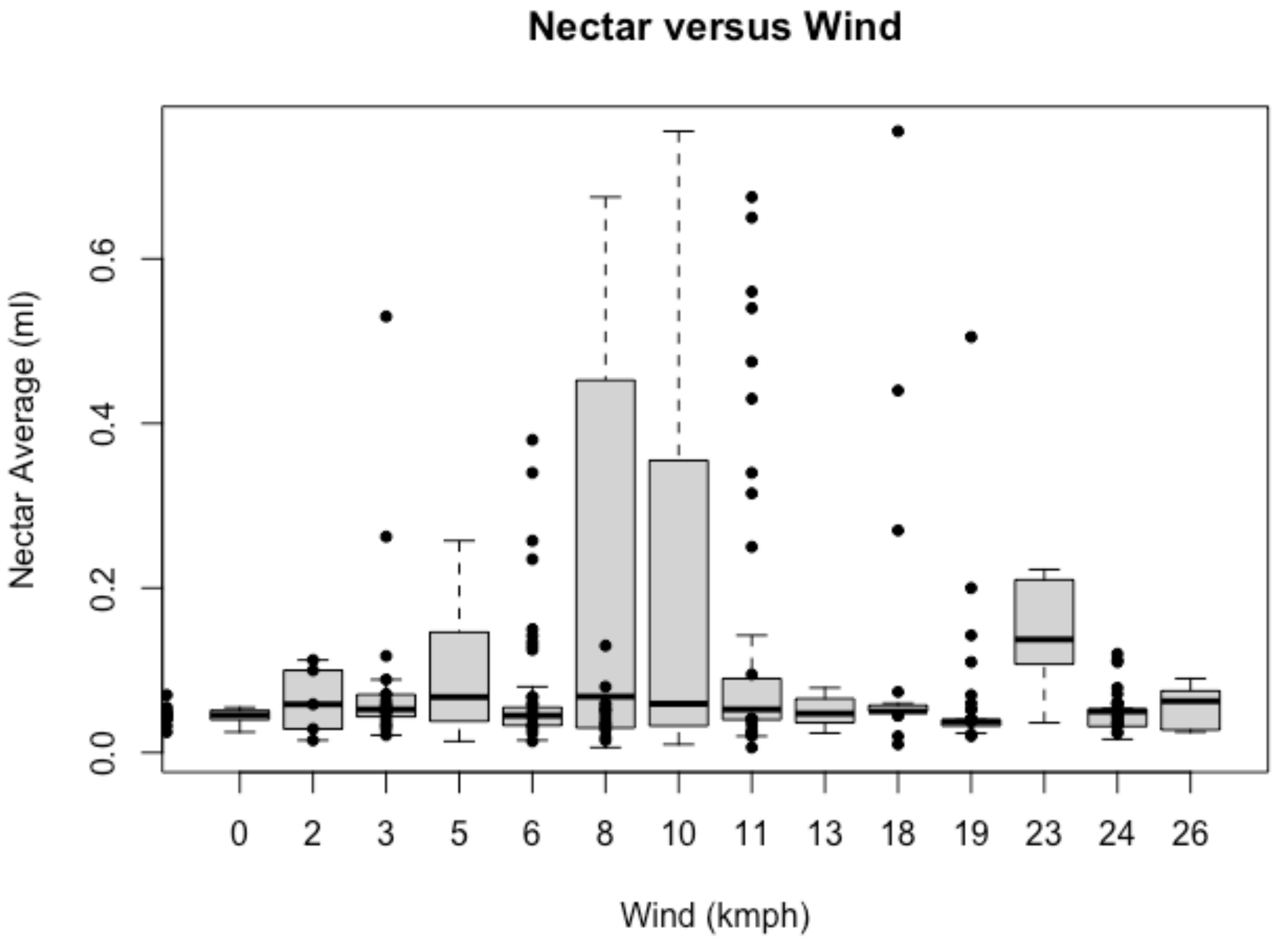

Figure 9: No effect of Wind on nectar average per flower per tree, see glm results in table 3 (N=156).

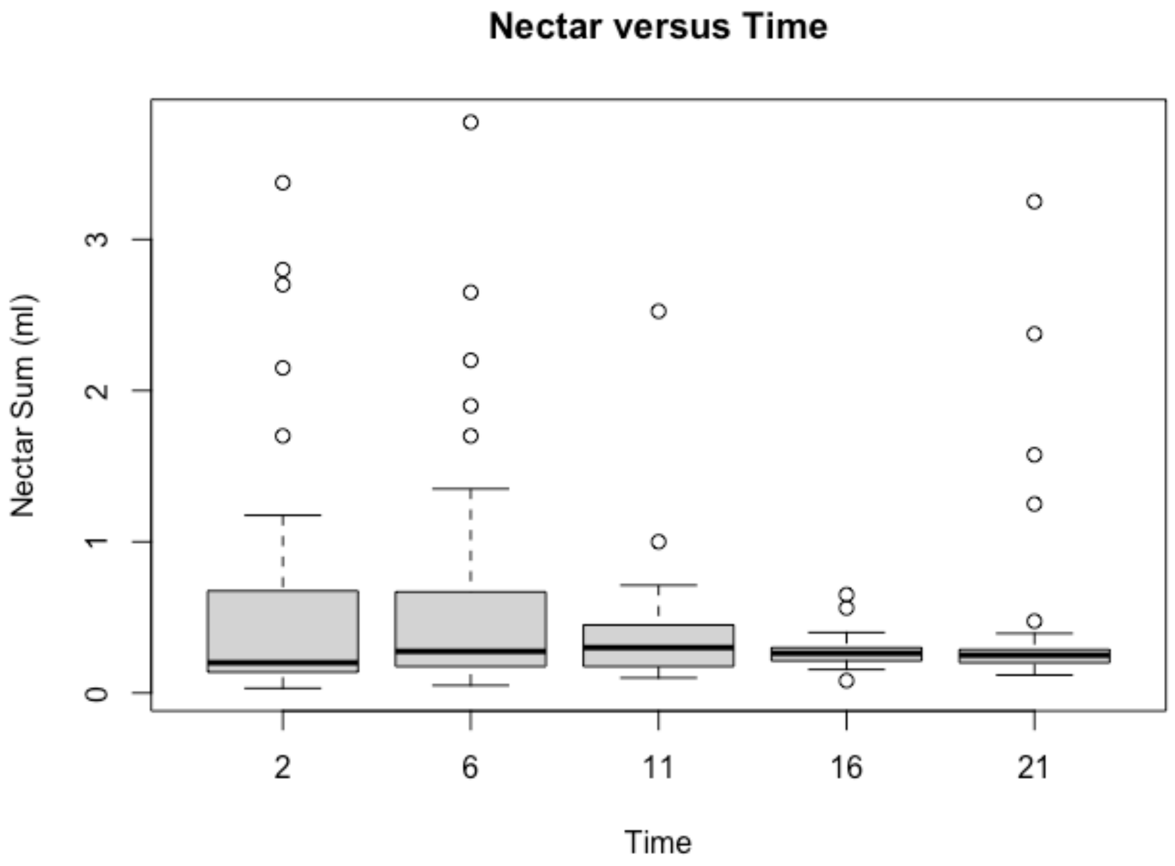

Figure 10: No effect of time on the summation of nectar per tree, see glm results in table 4 (N=156; with N=29 for Time 2, N=48 for Time 6, N=25 for Time 11, N=24 for Time 16, and N=30 for Time 21).

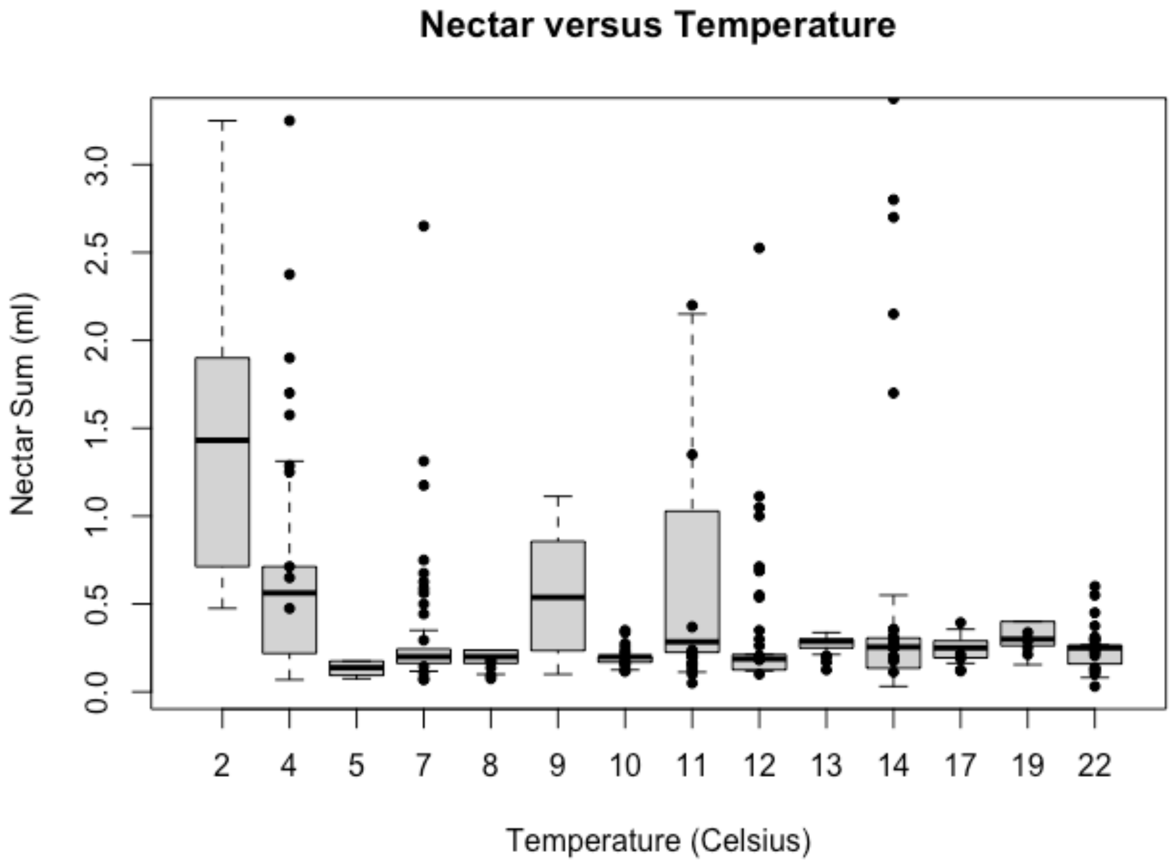

Figure 11: No effect of temperature on the summation of nectar per tree, see glm results in table 4 (N=156).

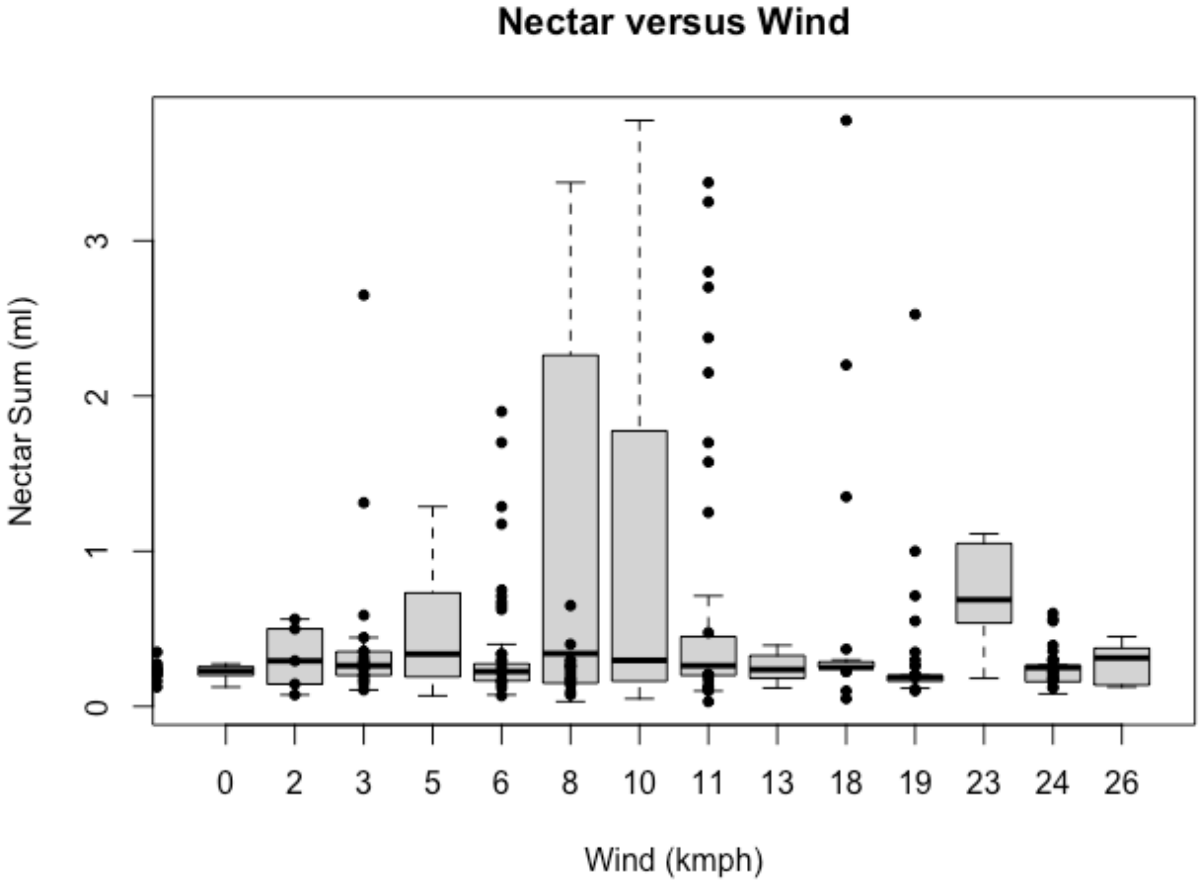

Figure 12: No effect of wind on the summation of nectar per tree, see glm results in table 4 (N=156).

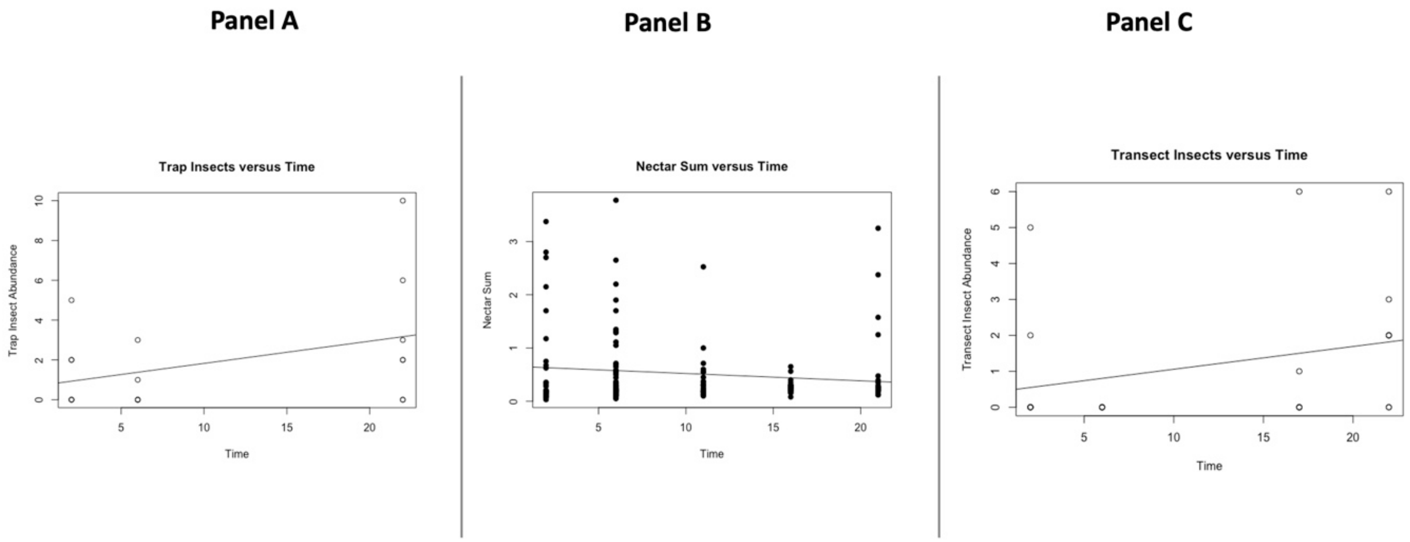

Figure 13: Insect and nectar abundances are not correlated in a circadian fashion. A) Trap insect abundance over time, N= 19; B) Nectar sum of 5 trees per sampling point over time, N=156; C) Transect insect abundance over time, N=22