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## Using AI to examine nocturnal moth pollination

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**Using AI to examine nocturnal moth pollination of flowering apple trees**

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

By

Conor Moriarty

Spring 2022

Biology

J. William Fulbright College of Arts and Sciences

**The University of Arkansas**

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## Abstract

The importance of insect-mediated agriculture cannot be understated especially in the wake of pollinator population declines. Most research of insect-mediated pollination is focused on diurnal pollinators like the honeybee (*Apis mellifera*). Deepening our understanding of how other pollinators, such as nocturnal pollinators, can benefit agriculture and ecosystems will be very important as honeybee populations decline. I explored nocturnal moth interactions with flowering apple flowers during their pollination season to better understand how these nocturnal pollinators interact with the plants. To accomplish this, I used a University of Arkansas farm area with a dedicated apple orchard. I gathered my raw data in Spring 2021 by setting up night vision cameras to record apple trees that had many flowering flowers over a period of 8 nights. I used the computer program DeepMeerkat, a motion detection AI, to process the video footage to discern the quantity, size and the family of the moths that visited the flowers. Depending on the time of day, I found that moth visitation was impacted by size, visit time to flower, amount of daylight, and length of visit. There were no significant differences between time of day and number of flowers visited. This research improves our understanding of plant-pollinator interactions and apple production, which is one of the most harvested fruits in the world.

## Introduction

Animal pollinators are essential in reproduction for many plants (Kearns et al 1998). This pollination process is generally mutualistic between plant and pollinator, as the plant provides resources such as nectar for the pollinator and in turn, the pollinators transfer pollen from stamens to stigmas (Kevan and Baker 1983). Around 85% of angiosperms are dependent on animal pollination, and this service is largely carried out by insects (Potts et al. 2010). Due to the importance of this relationship, observed declining rates of pollinators is alarming for the biodiversity of ecosystems (Biesmeijer et al 2006). Due to these declines, awareness has been raised for pollinators other than honeybees (*Apis mellifera*) (Brittain et al. 2013). Within agricultural systems, honeybees are considered the main pollinator of crops, but there is evidence that moths can also positively impact agriculture (Brittain et al. 2013). More importantly, it is vital to conserve diversity of pollinators because they can buffer the impacts of environmental changes on plant pollination (Brittain et al. 2013).

Lepidoptera have shown to be valuable pollinators. Between butterflies and moths, they show diurnal and nocturnal habits and can transport pollen short and long distances (Pettersson 1991). Nocturnal pollination may represent a significant percentage of plant-pollinator interactions (Devoto et al. 2011), with moths being the majority of night pollinators. In studies done by Clinebell et al 2004, less than 10% of the moths in the study had more than 50 pollen grains attached to their bodies. While this may not seem like a lot, certain plant species can be effectively pollinated by small amounts of pollen (Clinebell et al. 2004). This is true especially when these pollinators are frequent flower visitors (Clinebell et al. 2004).

In a study done by Dar et al (2006), benefits of nocturnal pollinators in conjunction with diurnal pollination were shown. When flowers were exposed to both types of pollinators, the fruit set increased. Because the plants were more open to a complimentary pollination system, it

promoted a wider group of pollination visitors. This system also acts as a fail-safe in areas where diurnal or nocturnal organisms are not as abundant (Dar et al. 2006). Moths also take part in extensive food webs and provide food for different types of birds and mammals like bats (Svensson et al. 1999), shrews (Buckner 1969) and bears (White et al. 1998). Moths in these communities not only affect plant reproduction but also can greatly affect organisms higher up the food web. It is important to understand the role of moths in these systems for conservation of moths as a whole and the plants and animals that depend on them for pollination and food sources (Devoto 2010).

Moths are far more abundant than butterflies in the order *Lepidoptera*. Butterflies amount to around 20,000 species out of the 180,000 in this order (New 2004). Butterflies are generally seen with a positive image by the public and thus deemed worthy of conservation efforts. Moths on the other hand, are typically viewed as pests and not viewed as favorably as butterflies are (New 1997). While they have not been studied as extensively as butterflies, moths have important ecological roles. Plants that benefit from diurnal pollinators like bees and butterflies may require the nocturnal pollination that moths provide to obtain full reproductive success in their environment.

To put this into further perspective, 70% of the crops that are used directly by human consumption are dependent on pollination and over 1500 crops require pollination as well (Klein et al. 2007). Simply by the numbers, pollination is a key service to biodiversity as a whole and maintaining that the human population maintains stable food production. However, with increasing food production and land use, pollinators have been negatively affected (Wagner 2020). Land-use changes, climate change, invasive species, and diseases have adversely affected pollinator populations (Potts et al. 2016). Pesticides have been shown to display negative effects such as birth defects, disorders, endocrine disruption and even death upon wild and managed

pollinator populations alike. In addition, many pesticides labeled as “reduced risk” by the United States EPA are in fact toxic to non-targeted insects and disruptive to pest control programs in fruit trees suggesting a broader need for pesticide reform overall (Biddinger et al. 2013).

The Food and Agriculture Organization of the U.N estimate that most of the food supply for 146 countries are pollinated by bees but the rest are pollinated by wasps, flies and moths who have important contributions (FAOSTAT 2019). Many plants owe their lives to these pollinators. The important link between pollinators and plants has never been more important and it is clear to see why both must be consciously conserved to increase food security and biodiversity. The apple plant is one of the most important fruit crops on the planet. Like many other plants, apple trees depend on insect mediated pollination to propagate their fruit which we then can consume. Without these crucial services from insects the security of our fruit markets come into serious question (Aizen et al. 2009). To continue agricultural production, pollination is essential. In 2016, the global production of apples reached 85 million tons which equaled to a value of 45 billion dollars (FAOSTAT 2019). The apple's contribution to the global economy is evident by its revenue and insect pollinators have been shown to increase the quality of fruit sets (Garratt et al. 2014).

Colony Collapse Disorder is one such reason for honeybee decline. It has affected over 30 states in the United States with colonies seeing bee losses as high as 100% death. There is the possibility of up to an estimated 90 billion dollars. The North American diet consists of  $\frac{1}{3}$  of pollinated foods such as fruits, vegetables, seeds and nuts. It goes without saying how detrimental it would be without pollination contributions. Because honeybee populations are becoming more threatened due to Colony Collapse Disorder (CCD), it is more important than ever to understand how secondary pollinators such as the moth can contribute to food security and biodiversity.

In a study done by Robertson et al. (2021), it was found that nocturnal pollinators significantly contributed to apple pollination. They found that nocturnal pollinators may be as efficient in pollinating apples as diurnal pollinators. Importantly, they found evidence of nocturnal pollination of apples but were unable to determine which nocturnal insects were responsible. My project will be to determine the frequency of moth visits and apple flowers during pollination season as well as to assess the moth size and family that visits most often. To do this, I will be using the motion detection program DeepMeerkat to analyze night video footage taken during apple tree pollination season (Weinstein 2018).

## **Methods**

Study Species: Since the video recordings were filmed in infrared, I classified moths to family using primarily shape and size. Moths were classified into *Arctiidae*, *Geometridae*, *Noctuidae*, *Saturniidae*, and *Sphingidae* families. We expected to see large amounts of *Noctuidae* due to this family being the most common in the United States and many of its species being nocturnal. In a recent study done by Robertson et al. on nocturnal pollinators contributing to apple production in the same orchard, he found that moths from the family *Noctuidae* were most observed at night. The two most common being *Mythimna unipuncta* Haworth and *Peridroma saucia* Hübner both from family *Noctuidae* (Robertson, Dowling, Wiedenmann, Joshi, and Westerman 2021).

### Study Sites:

This study took place at the Milo J. Shult Agricultural Research Extension Center and the apple orchards of the University of Arkansas (36.101, -94.166) in Fayetteville, Arkansas. The plot of land that served as the study site was a rectangular shape roughly 30 by 11 apple trees of mixed



variety. In the surrounding areas there are other fields used by the University as well as a moderately busy road, approximately hundred meters from the edge of the orchard.

### Experimental Design:

I assisted in setting up 5 Ghost Stop cameras with infrared capability at night over 7 nights during a 14-day observation period from April 9, 2021-April 23, 2021, to determine peak moth visitation time and visitation rates. The specific sampling dates were 4/9/21, 4/11/21, 4/13/21, 4/17/21, 4/19/21, 4/21/21, 4/23/21. Cameras were set to record from 5:45pm to 5:45am on each sampling day and were checked 4 times each night at 5:45pm, 9:45pm, 1:45am, and 5:45am. If the cameras stopped recording for any reason or the batteries died, they would be turned back on or set to charge. If adverse weather such as rain or frost occurred, the affected shift times would be skipped. The cameras were set up on tripods in boxes that protected from environmental factors such as wind and rain. They were about 2 meters off the ground focused on apple flower clusters presumed to be attractive to pollinators (flower clusters containing multiple fresh or newly opened flowers). Over the 7 nights of sampling, the 5 cameras recorded approximately 350 hours of video footage which could be used for data processing and analyzing.

### Data Processing:

To process the video footage, I used the motion detection AI program DeepMeerkat which specializes in video-based biodiversity surveys (Weinstein 2018). DeepMeerkat can be trained to recognize moth movement to cut down on time spent processing videos manually. I used the parameters 0.0001 for minimum object size (% of frame) and 0.99 for tensor flow threshold for moth detection in DeepMeerkat. This is also necessary for the program to avoid extraneous

movement from non-moth sources. When the program successfully detects moth movement, the AI indicated which are the moth positive video frames, then I could look through the videos to find the specific moment of moth activity in each of the indicated frames. Once moths were found in the videos, their behavior was recorded. I created and used two ethograms (Table 1 and 2) to record the behaviors and data. Date, time, video number, and which camera were recorded as well as approximate moth size (small, medium, large), visit time to flowers, how many flowers were visited, whether other moths or insects were present at the visitation, and whether it was light or dark outside. For the statistical analysis, time was sorted into 2-hour bins. 6:00pm – 8:00pm, 8:00pm – 10:00pm, 10:00pm – Midnight, Midnight – 2:00am, 2:00am – 4:00am, 4:00am – 6:00am to see if moth behavior differed by the time of day.

### Statistical analysis:

After counting and sorting through the number of different sizes, and differentiating family identification, and scoring behaviors in the video footage I used RStudio to run different statistical analyses. I ran Kruskal-Wallis tests to compare time of night to length of flower visit and number of flowers visited. Shapiro-Wilk normality tests were run on both tests to test for a normal distribution. Chi-square tests of Independence were run on time of night and size, visit time, amount of light. Size and visit time to flower were also tested with the Chi-square test.

### **Results**

I watched 309 videos and observed 30 total moths and 69 insects in all the videos. I saw 2 moths in the 6:00pm – 8:00pm time, 17 from 8:00pm – 10:00pm, 4 from 10:00pm – Midnight, 7 from Midnight – 2:00am, 0 from 2:00am – 4:00am, 0 from 4:00am – 6:00am across the observation dates. The 8:00pm -10:00pm time slot had the greatest number of moths and 2:00am

– 4:00am and 4:00am – 6:00am had the least amount (Figure 1). On 4/11/21, I observed 6 moths: 2 from 6:00pm – 8:00pm, 2 from 8:00pm – 10:00pm, 2 from 12:00am – 2:00am. On 4/12/2021, I observed 2 moths, both from 12:00am – 2:00am. On 4/13/21, I observed 13 of the 30 moths found in this study, all observed from 8:00pm – 10:00pm. 9 of these 13 were viewed from the same camera in a 30-minute period. On 4/14/21, I observed 5 moths: 1 from 8:00pm – 10:00pm and 4 from 10:00pm – 12:00am. On 4/15/21, I observed 3 moths from 12:00am – 2:00am. On 4/22/21, I observed 1 moth from 8:00pm – 10:00pm.

An average of 2.37 flowers were visited per moth visitation. The average flower visitation time was 98 seconds. The average moth size was small. At the time of moth visitation, no other moth or insects were observed. Out of the 30 moth observations, 29 were at night and 1 was during daytime.

There was an effect of time of day on flower visitation time, with moths spending the most time visiting flowers between 10:00pm and 12:00am ( $N=30$ ,  $\chi^2=8.275$ ,  $p = 0.0407$ , Figure 2). The Shapiro-Wilk normality test had a p-value of 0.0001. For the Chi-square tests, there was an effect of time of day on size, with 26 out of the 30 moths seen, classified as small ( $N=30$ ,  $\chi^2=85.503$   $p=9.839e-8$ ). There was an effect of time of day on amount of light during visitation, only 1 moth was seen during daytime, and it was during the 6:00pm-8:00pm time bin when dusk was turning to night, ( $N=30$ ,  $\chi^2=50.414$ ,  $p=3.403e-8$ ). I did not find an effect of size on flower visitation time ( $N=30$ ,  $\chi^2=67.261$ ,  $p=0.0079$ ), nor did I find an effect of time of day on number of flowers visited per moth ( $N=30$ ,  $\chi^2=2.635$ ,  $p=0.6209$ , Figure 3), Shapiro-Wilk normality test had a p-value of 0.0256.

## Discussion

The initial goal of this study was to determine the relationship between time of day and moth pollination. Given that previous studies have indicated the importance of moth pollination in the wake of primary pollinator declines (Biesmeijer et al 2006), it is important to gain a greater understanding of when these nocturnal pollinators prefer visit flowers. Of the 5 variables tested, 3 showed significant differences: an effect of time of day on flower visitation time, effect of time of day on size, effect of time of day on amount of light during visitation.

Our data showed a significant difference between time of day and flower visitation which was one of the main relationships we wanted to investigate. The time bins showed that out of the 30 moths, 17 of them were seen in the 8:00-10:00pm time bin possibility indicating the stage of night that these moths are most active. However, the significance shown for this time bin could be considered circumstantial because 14 of the 17 moths were recorded all in the same night and may not be representative of true moth distributions. It is possible that these moths were influenced by the amount of food reward available. Heinrich (1975) conducted an experiment on native bees in Maine and their flower pollination. He found pollinators would discriminate between two similar blooming flowers if one was perceived to be more common or provided greater food reward. Bees forage where they can make the most profit, which is not only limited to greatest amount of food return. This can include the number of pollinators already present at a certain site or how well the pollinator can manipulate the flower (Heinrich 1975). The behaviors shown by these native bees could provide one possible explanation why one camera recorded large amounts activity in a small-time frame of 30 minutes,, indicating that the flowers in front of this particular camera contain a large food reward.

A study done by Goodwin et al. (2011) on honeybees showed that honeybees have shorter visitation times than we found for moths. They found that honeybees took an average of

about 11 seconds to leave a flower, visit a second flower and leave it, averaging 1 flower visit every 3.23 second. This contrasts with our moth data which shows a much higher average time spent visiting, an average of 2.37 flowers for 98 seconds per flower visitation. A 2-year study done by Adlerz (1965) displayed that the honeybees longest single visit was 29 seconds with a mean visitation time of 5.7 seconds. In the second year, the longest visit was 60 seconds with mean visitation times of 8 seconds.

Ollerton et al. (2008) studied bird pollinators and found that the type of plant visited influenced visitation duration. They observed *Phylloscopus canariensis*, the Canary chiffchaff, and *Parus caeruleus*, the blue tit, visiting the flower *Canarina* typically for only 1 to 3 seconds. However, when visiting *Isoplexis* and *Lotus* flowers, they would stay up to 120 seconds to feed. The bird pollinators showed much lower visitation rates compared to the moths in our study. The *Canarina* were visited once every 5.5 hours, *Isoplexis* once every 16 hours, and *Lotus* once every 7 days (Ollerton et al. 2008). It would be beneficial to build off this study to see if moths also adjust their visitation time based on the plant they are visiting.

While our data showed that time of night had no significant effect on the number of flowers visited, the moths visited about 2 flowers per flower cluster visitation. This result corresponds to the results of Robertson et al. (2021), indicating that these moths could be pollinating during their visits, and supports the finding that nocturnal pollinators contribute to apple pollination. In addition to this, a study on the pollination of *Silene Alba* (*Caryophyllaceae*), found that the seed sets from moth visitation were twice that of bees (Young, 2002). However, flower visitation does not always guarantee pollination (Park et al. 2016) which displays the need for further studies on this topic.

While the quality of the cameras used during filming were not good enough to classify the moths any further than family, we can say that the moths identified were most likely from the

family *Noctuidiae*. Pollinating moths are generally distinguished into two broad categories: large sphinx moths and small moths of *Noctuidiae* (Bawa 1990). Our results showed a positive correlation size with time of day. 26 out of the 30 moths were small and the other 4 medium size. The small size of the moths helped classify them into the family *Noctuidiae*. These findings are consistent with Robertson et al. (2021). They were unable to determine which nocturnal insects were responsible, but found that the armyworm (*Mythimna unipuncta*), Haworth (Lepidoptera: *Noctuidiae*) and the variegated cutworm (*Peridroma saucia*) Hubner (Lepidoptera: *Noctuidiae*) were the most observed insects visiting apple flowers at night during their study.

While there were hardly any moths seen in the daylight this does not necessarily mean they were not active during the daytime. Our cameras were only set up to record from 6:00pm to 6:00am the next morning. There was no video footage during the daytime where it is possible moth activity could have occurred. In addition to the limitations of moth viewing, there were only 5 cameras set up directed at trees that we had decided beforehand that would be the trees pollinators would most likely visit based on the visual appearance of the budding flowers. This means we only have a limited sample and could have missed large amounts of moth visitation due to the restrictions of our study. There is a possibility of false replication of moth counting since every moth that entered the video frame was considered a new moth. It is possible that moths could have been counted multiple times. As stated before, due to the camera quality it was difficult to classify the moths. They were all about the same size and I could not make out any defining features to accurately differentiate them.

## **Conclusions**

In this study, I examined the effect of time of night on visit frequency and duration of moths to apple flowers to better understand nocturnal moth pollination. I found differences between time

of night on flower visitation duration but did not with time of night on number of flowers visited. It is important to understand the role of moths in these systems for conservation of moths as a whole and the plants and animals that depend on them for pollination and sources of food. A possible future direction is to expand upon the parameters of this study and have more cameras to more accurately account for the number of moths active visiting apple flowers.

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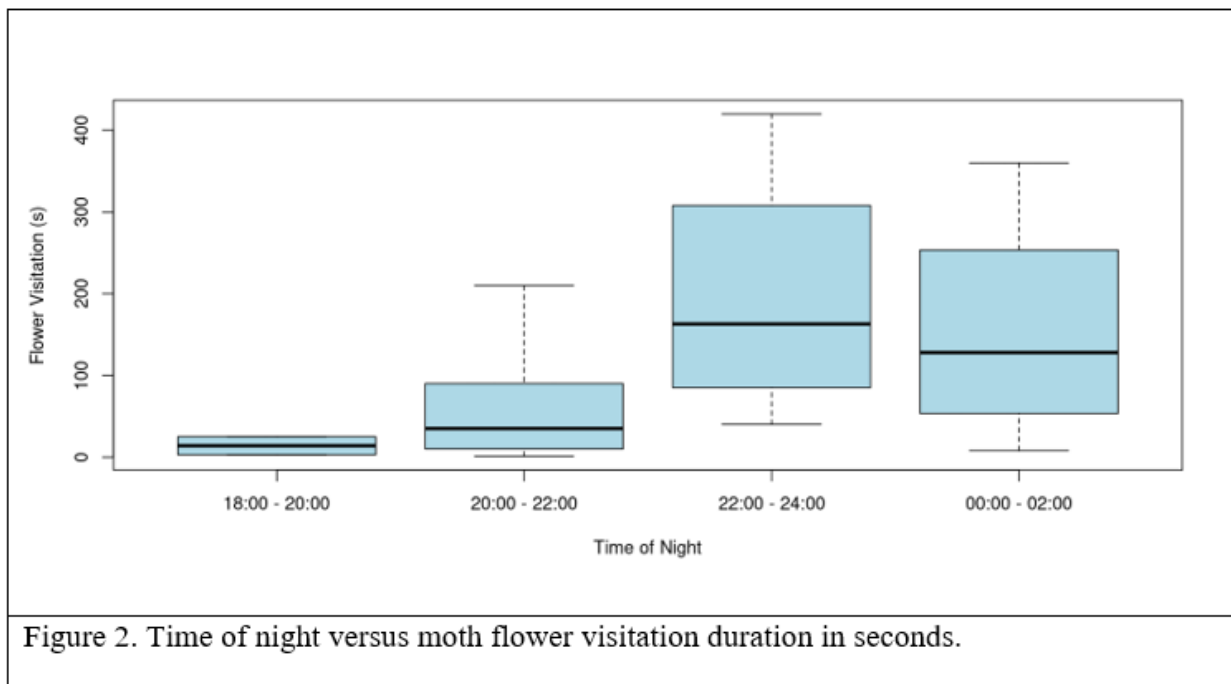
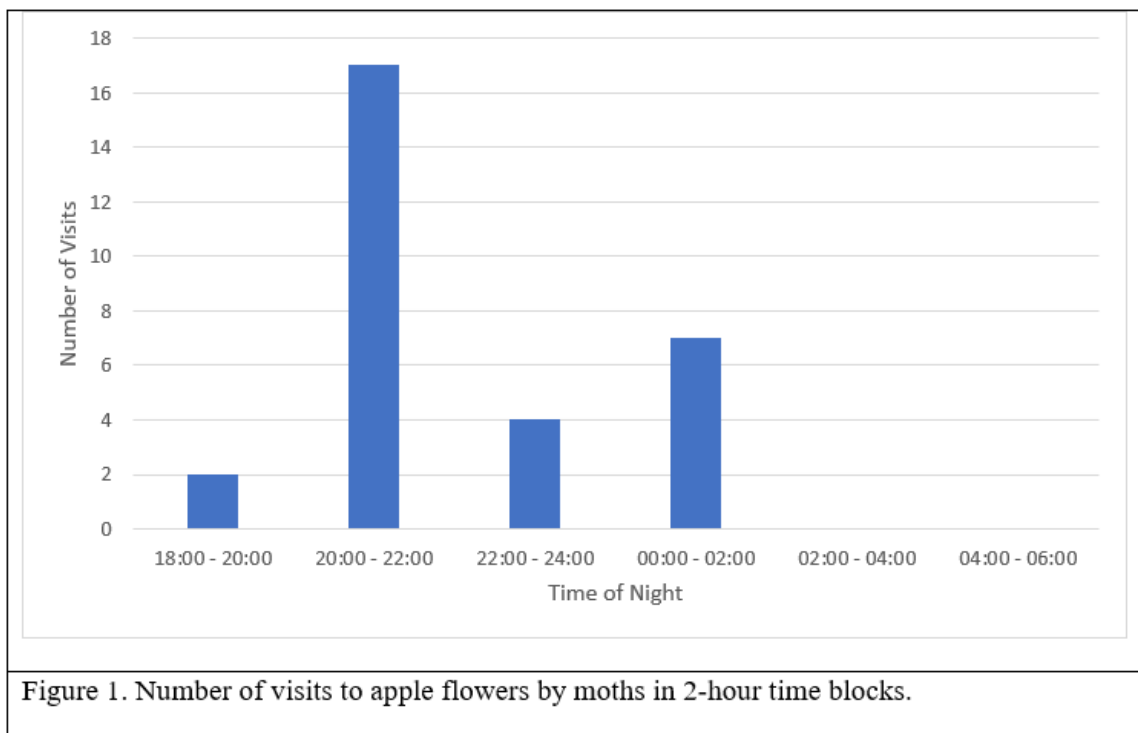
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**Figures**

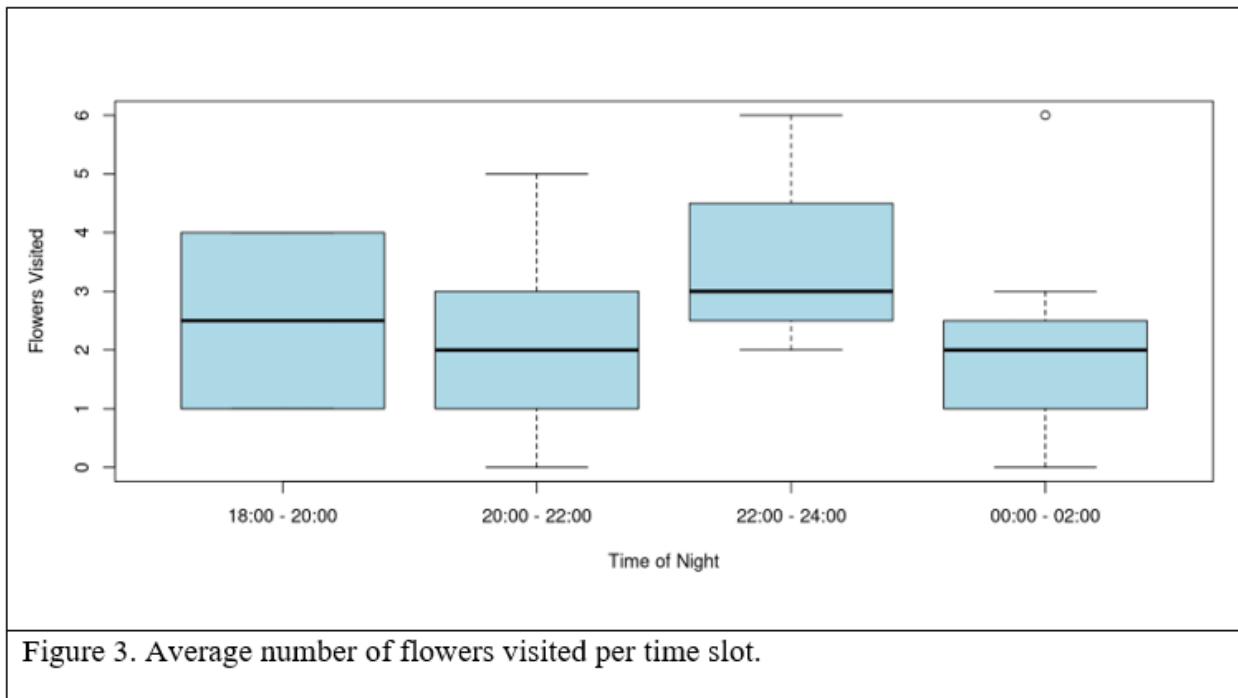


Figure 3. Average number of flowers visited per time slot.

## Supplemental Tables

Date	TimeCategory	Time	VideoCapturedFrom	Size	NearCamera	FlowerVisitation	VisitTimetoFlowerSeconds	VisitMultipleFlowers	OtherMothsorInsectsPresentatVisitation?	LightorDarkOutside
4/11/2021	18:00 - 20:00	19:25:50	20210411_182144	S	Far	<5	3	1		0 Light
4/11/2021	18:00 - 20:00	19:37:05	20210411_193704	S	Far	>20	25	4		0 Dark
4/11/2021	20:00 - 22:00	21:12:37	20210411_211236	S	Far	>10	5	2		0 Dark
4/11/2021	20:00 - 22:00	21:24:50	20210411_211236	S	Medium	>20	25	2		0 Dark
4/11/2021	00:00 - 02:00	1:08:10	20210411_232208	M	Medium	>100	360	2		0 Dark
4/11/2021	00:00 - 02:00	1:16:20	20210411_232208	M	Medium	>100	128	1		0 Dark
4/12/2021	00:00 - 02:00	1:20:28	20210412_012027	M	Medium	>100	270	3		0 Dark
4/12/2021	00:00 - 02:00	1:55:32	20210412_012027	M	Medium	>100	237	1		0 Dark
4/13/2021	20:00 - 22:00	20:27:28	20210413_195613	S	Far	>100	80	1		0 Dark
4/13/2021	20:00 - 22:00	20:35:28	20210413_195613	S	Far	>50	78	5		0 Dark
4/13/2021	20:00 - 22:00	20:39:39	20210413_195613	S	Far	>50	70	3		0 Dark
4/13/2021	20:00 - 22:00	20:35:31	20210413_195613	S	Far	>100	110	5		0 Dark
4/13/2021	20:00 - 22:00	20:39:31	20210413_195613	S	Far	>100	160	2		0 Dark
4/13/2021	20:00 - 22:00	20:46:35	20210413_195613	S	Medium	>10	12	1		0 Dark
4/13/2021	20:00 - 22:00	20:52:47	20210413_195613	S	Medium	>5	7	1		0 Dark
4/13/2021	20:00 - 22:00	20:55:00	20210413_195613	S	Medium	>20	35	4		0 Dark
4/13/2021	20:00 - 22:00	20:56:00	20210413_195613	S	Far	>5	10	1		0 Dark
4/13/2021	20:00 - 22:00	21:27:51	20210413_211926	S	Medium	>100	210	5		0 Dark
4/13/2021	20:00 - 22:00	21:44:43	20210413_211926	S	Medium	>50	90	3		0 Dark
4/13/2021	20:00 - 22:00	21:11:11	"video with moth"	S	Medium	<5	5	0		0 Dark
4/13/2021	20:00 - 22:00	20:23:01	"video with moth 2"	S	Medium	<5	1	0		0 Dark
4/14/2021	20:00 - 22:00	20:12:33	20210414_200727	S	Medium	>100	105	2		0 Dark
4/14/2021	22:00 - 24:00	22:12:48	"video with moth 3"	S	Medium	>100	196	3		0 Dark
4/14/2021	22:00 - 24:00	22:12:50	20210414_221003	S	Medium	>20	40	3		0 Dark
4/14/2021	22:00 - 24:00	22:23:20	20210414_222003	S	Far	>100	420	6		0 Dark
4/14/2021	22:00 - 24:00	23:11:16	20210414_231003	S	Medium	>100	130	2		0 Dark
4/15/2021	00:00 - 02:00	1:14:07	20210415_011003	S	Medium	>5	8	0		0 Dark
4/15/2021	00:00 - 02:00	1:27:45	20210415_012003	S	Medium	>50	53	2		0 Dark
4/15/2021	00:00 - 02:00	1:27:50	20210415_012003	S	Medium	>50	54	6		0 Dark
4/22/2021	20:00 - 22:00	21:10:31	20210422_201202	S	Medium	>10	14	0		0 Dark

Table 1. Individual statistics for each moth visitation to apple flowers.



Table 2. Master list of all camera footage.

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