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Finding An Accurate Method to Measure Pollinator Visitation Rates

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Abstract

Pollinator visitation rates are a helpful way to monitor the health of ecosystems; however, there lacks a standardized method for obtaining these rates. The pollinator visitation rates for five plant species were collected and the relationship between the standard error of these rates and the time interval was determined. *Monarda, Echinacea purpurea, Pycnanthemum muticum, and Baptisia alba* all exhibit more accurate pollinator visitation rates as the time interval increased. *Trifolium repens* exhibited less accurate pollinator visitation rates as the time interval increased.

Introduction

Pollination ensures that ecosystems continue to grow and thrive as it allows reproduction and evolution (Potts et al. 2010). Relying on pollination as their primary method of reproduction, almost every plant species on the planet would decline in the case of declining pollination (Allen-Wardell et al. 1998). Increasing levels of pollination overall increase the sustainability of an ecosystem (Feijn and Kleijn 2017). A wide variety of factors contribute to a decline in bees and other pollinators, including habitat loss, climate change, and pesticides (Potts et al. 2010). With 70% of tropical crops and 84% of European crops in some capacity depending on pollination, the world's population would be greatly affected by a lack of pollination (Klein et al. 2007) Thus, conserving pollinating species is of utmost importance.

Some researchers have thus found interest in quantifying pollinator visitation rates for inflorescences (Tamburini et al. 2016) or individual flowers (Feijn and Kleijn 2017). These rates can be used to visualize the effects of the factors listed above. There are several papers written on this topic, however the methods used by each researchers varies. There is as yet no standardized method for obtaining accurate pollinator rates. Some research groups observed plants for 3 minutes (Tamburini et al. 2016) while others chose a longer period of 3.5 hours (Hoehn et al. 2008). There are drawbacks for both extremes; observation periods that are too short for the given plant species may produce inaccurate data while observation periods that are too long may be overall inefficient to the study (Feijn and Kleijn 2017). It should be noted that pollinator visitation rates vary greatly across species due to floral characteristics (e.g., flower shape and size, flowering duration, etc.), environmental characteristics (e.g., temperature, rainfall), and community characteristics (pollinator-to-flower ratio, coflowering species, habitat structure) (Lázaro 2013). So why study across different species? Discovering patterns in the accuracy of pollinator visitation rates may allow researchers to draw relationships between plant species with similar floral and community characteristics. The specific question I will explore in this paper is, “how does the accuracy of pollinator visitation rates change when the time interval is changed?”

Methods

For this experiment, it was important to distinguish between a pollinator visit and a pollinator simply landing on the flower. The purpose of this project was to determine the relationship between time interval and the accuracy of the pollinator visitation data; it was thus important to only count the actual pollination events. Throughout the observation periods, insects landing on the flower and quickly flying away were not counted in the data. In order to get the most relevant data, I conducted various informal observation periods prior to the formal data collection to become familiar with pollinator habits.

To answer the question of which time interval provides the most accurate pollinator visitation rates for the various species studied, I observed these species over several observation periods. Throughout the observation period, I counted the number of pollinator visits per flower; as stated in the previous paragraph, only the actual pollination events were counted towards these numbers. In this experiment, "accuracy" refers to the lowest standard error value. Standard error (SE) determines how accurately a set of data represents a population as a whole (Ejaz 2022). In relation to this experiment, the standard error determines how accurately the data from each individual observation period represents the overall data mean for that species. In this experiment, I used standard error instead of standard deviation; this is because standard deviation refers to an individual sample while standard error allows the comparing of various samples across the population (Ejaz 2022).

Figure 1. Three *Monarda* inflorescences

Table 1 summarizes the names and locations of the five flower species used in this experiment during Summer 2022, in Northeastern Ohio. These flowers each have high activity during late summer in northeast Ohio, when data collection took place. *Monarda* (Beebalm) is native to Ohio and grows everywhere in the United States (US Department of Agriculture). Each inflorescence is about 2- to 3-inches wide and blooms in mid- to late summer (National

Gardening Association). Based on personal observation (see Figure 1), each Beebalm inflorescence had about 15-20 flowers. *Monarda* rewards its various visitors – hummingbirds, butterflies, and bees – with nectar. “Nectar robbing” is a common phenomenon with this plant and was observed during this experiment. During this event, the pollinator (typically of a larger size, such as a carpenter bee) pokes a hole towards the base of the flower; the pollinator “robs” the plant by taking nectar without pollinating. Nectar robbing was not counted in this study as a flower visit.

Echinacea purpurea (Purple Coneflower) is native to Ohio and grows nearly everywhere in mid- to eastern-United States (US Department of Agriculture). Each inflorescence (“head”) is approximately 2- to 3-inches in diameter and blooms late summer to early fall (National Gardening Association). Based on personal observation, this species contains about 150-200 flowers per inflorescence. Purple Coneflower rewards its visitors with pollen; by personal observation, the bees that visited this species often exhibited orange or yellow pollen baskets.

Figure 2. A bee with pollen baskets visiting a Purple Coneflower

Pycnanthemum muticum (Mountain Mint) is native to Ohio and grows mainly in the eastern United States (US Department of Agriculture). Each inflorescence is typically less than 1-inch in diameter and blooms in the summer (National Gardening Association). Based on personal observation, this species rewards its visitors with nectar and contains about 20-30 flowers per inflorescence.

Baptisia alba (White Indigo) is native to Ohio and grows in mid- to eastern-United States (US Department of Agriculture). Each inflorescence is less than 1-inch in diameter and blooms in late spring to early summer (National Gardening Association). Based on personal observation, White Indigo rewards its visitors with nectar and contains about 5-15 flowers per inflorescence.

Trifolium repens (White Clover) is not native to Ohio or anywhere in the United States (US Department of Agriculture). Each inflorescence is less than 1-inch in diameter and blooms between late spring and early fall. Based on personal observation, each inflorescence had about 10-15 flowers per inflorescence. White Clover has both nectar and pollen.

Common Name	Scientific Name	Location	Dates of Observations
White Indigo	*Baptisia alba*	Springfield Bog	7/29, 8/2 (2), 8/19 (2)
Beebalm	*Monarda*	Bath Nature Preserve, Springfield Bog	7/28, 7/30, 7/31, 8/16 (2)
Clustered Mountain Mint	*Pycnanthemum muticum*	Bath Nature Preserve	7/30, 8/4 (2), 8/18 (2)
Purple Coneflower	*Echinacea purpurea*	Springfield Bog	7/28, 7/29, 7/31, 8/16 (2)
White Clover	*Trifolium repens*	Firestone	8/5, 8/7 (2), 8/17 (2)

Table 1. Common Names, Scientific Names, and Locations of The Plants Used in The Study

The locations of data collection were Bath Nature Preserve, Springfield Bog Metro Park, and Firestone Metro Park-Tuscarawas Meadows Area – all located in Akron, Ohio. Table 1 summarizes the specific locations of each plant species used in the experiment. I observed each plant species during five separate observation periods over a few days, for a total of 25 observation periods.

During an observation period for data collection, I first identified the group of inflorescences that I would be observing. For Purple Coneflower, Beebalm, Mountain Mint, and White Indigo, I chose a group of less than 20 inflorescences. For White Clover, I chose an area of about 1x1m that had about 30-40 inflorescences. I walked a short distance around the location and stopped when I saw a group of inflorescences that seemed to generally have visiting pollinators. I would initially observe the group of inflorescences for about 30 seconds to one minute. If there were no pollinators visiting, I would continue and find a new group of inflorescences. If there were pollinators, I would begin my set-up for the observation period. To begin data collection, I first identified the inflorescences of observation. In order to limit the variance, I made sure to use about the same number of inflorescences for each observation period of the same plant species. I then counted the number of flowers per inflorescence. In this study, flowers are being defined as “the characteristic reproductive structure of angiosperms, a heterosporangiate strobilus, typically consisting of androecium, gynoecium, usually surrounded by a perianth and borne on an axis or receptacle, the parts showing various kinds of symmetry relationships to each other and to the inflorescence axis, also often with a distinctive merism” (Stevens 2001). In this study, one pollinator count was scored by one flower visit, not by each inflorescence visit as the pollinator could visit several flowers while remaining on the same inflorescence.

Figure 3. A group of Purple Coneflower inflorescences for observation

I noted the UV index, temperature, and general notes about the weather (windy, cloudy, sunny.) The values for both UV index and temperature were obtained from the Apple Weather app (The Weather Channel). In order to limit the independent variables due to weather, I did not make observations on days that it was raining or overcast. I chose days that were between 21.1^{o}C (70^{o}F) and 32.2^{o}C (90^{o}F). I noted characteristics about the flowers I was observing, such if the flowers were in direct sunlight, if there were surrounding flowers, etc., however no distinct patterns were observed, so this information was not used in analyses. All observation periods were begun between 10:00 a.m. and 12:00 p.m. During each observation period, I stood about 1-2 meters from the flowers being observed. I used a timer app on my phone for 30 minutes with a sound made each one minute interval. When a pollinator visited, I would write the letter corresponding to the species on my data collection sheet. Each letter indicated one flower visit. After each one minute interval, I would move to a new line on the data collection sheet. Table 2 summarizes the pollinator species represented in this experiment.

Common Name	Scientific Name	Plant Species Visited
Honeybee	*Apis*	*Trifolium repens, Baptisia alba, Monarda, Echinacea purpurea, Pycnanthemum muticum*
Bumblebee	*Bombus*	*Trifolium repens, Baptisia alba, Monarda, Echinacea purpurea*
Moth	*Lepidoptera*	*Monarda*
Wasp	*Vespa*	*Pycnanthemum muticum*

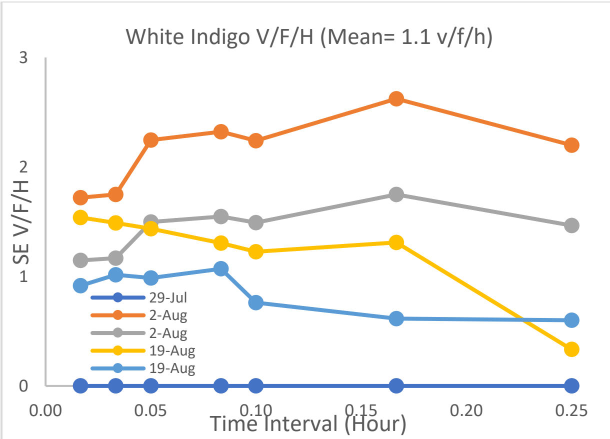
Table 2. Common Names, Scientific Names, and Plants Visited of the Pollinators Studied

Data was analyzed using Microsoft Excel. Data from each observation period was entered onto its own Excel sheet. This original data included the number of pollinators, the number of flowers visited, and the number of inflorescences visited; however, only the number of flowers visited was used. The data was then grouped into several different time intervals to evaluate how the length of an observation period affects the SE– 1 minute (observation periods), 2 minutes, 3 minutes, 5 minutes, 6 minutes, 10 minutes, and 15 minutes. The mean and SE of the flower visits by pollinators was configured for each time interval. The SE of the mean number of flowers visited was divided by the total number of flowers for that observation period (number of observed inflorescences x number of flowers per observed inflorescence) and multiplied by 60 (60 minutes in one hour) in order to provide the results in units of visits/flower/hour. By converting all data to the same units, I was able to compare data across different species. I used this method across the data from all the observation periods.

Results

Figures 4, 6, 8, 10, and 12 show the mean pollinator visits per flower per hour (v/f/h) across all five observation periods of the respective species. In each of these figures the standard error of v/f/h is plotted against the time interval. Each line represents a distinct observation period with the date indicated in the legend. Each data point represents the SE of v/f/h for that specific time interval and observation date.

Figures 5, 7, 9, 11, and 13 shows the general trend between time interval and pollinator visitation. In each of these figures, the mean SE v/f/h is plotted against the time interval. Each data point represents the mean SE v/f/h for that specific time interval across all five observation periods of same species. A regression line has been added to the figure to clearly indicate the trend that the data follows (slope) and how closely the data follows this trend (R^2).



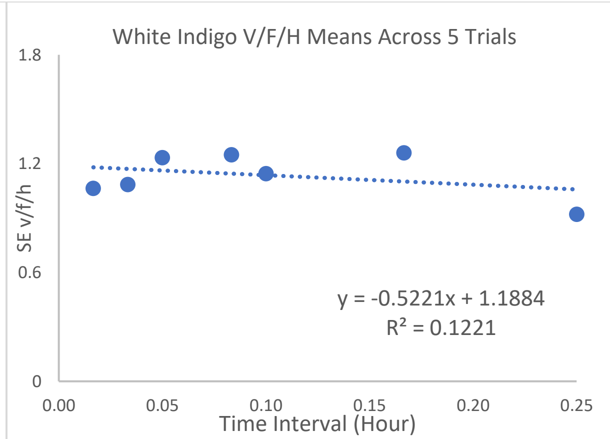
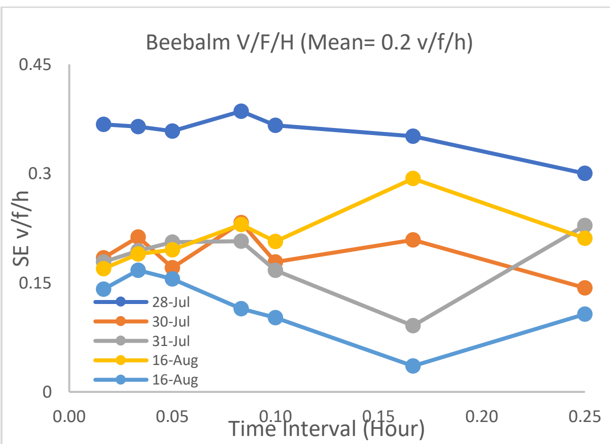


Figure 4. Comparing White Indigo Pollinator Visits Per Flower Across Different Time Intervals

Figure 5. Average White Indigo Pollinator Visits Per Flower Across 5 Trials



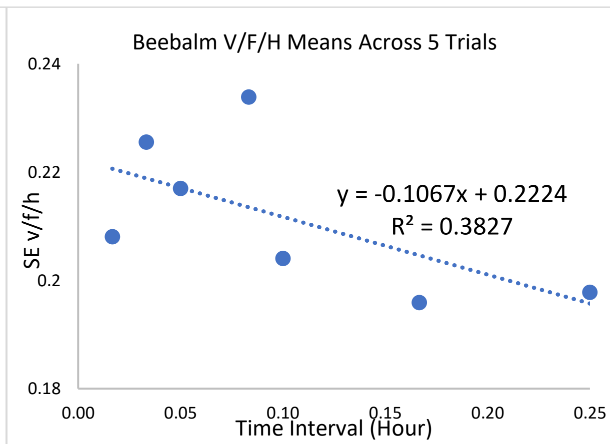
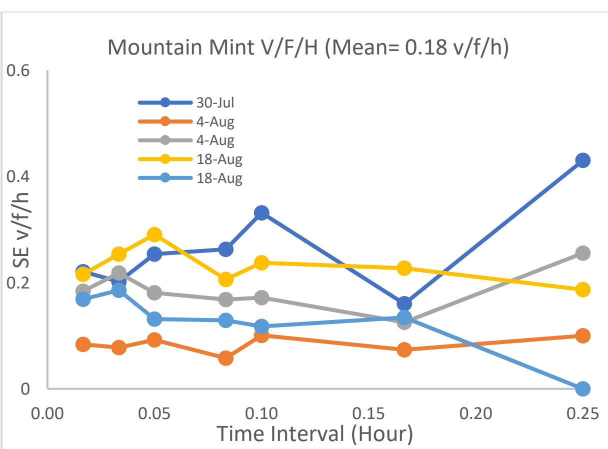


Figure 6. Comparing Beebalm Pollinator Visits Per Flower Across Different Time Intervals

Figure 7. Average Beebalm Pollinator Visits Per Flower Across 5 Trials



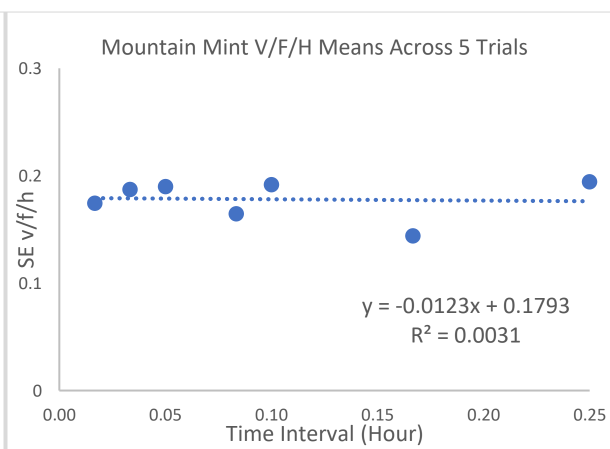
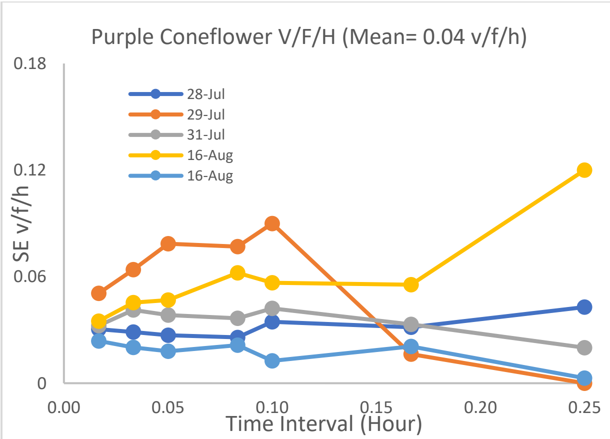


Figure 8. Comparing Mountain Mint Pollinator Visits Per Flower Across Different Time Intervals

Figure 9. Average Mountain Mint Pollinator Visits Per Flower Across 5 Trials



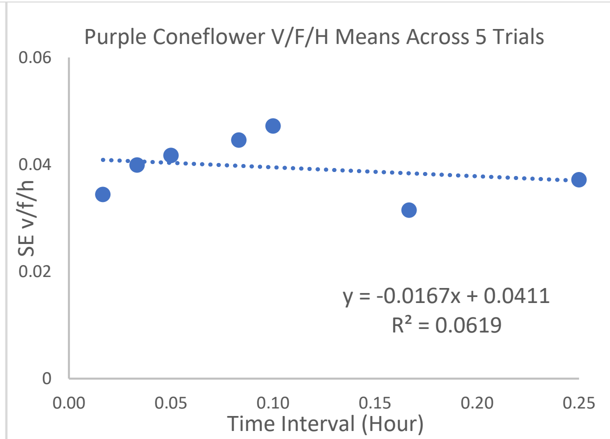
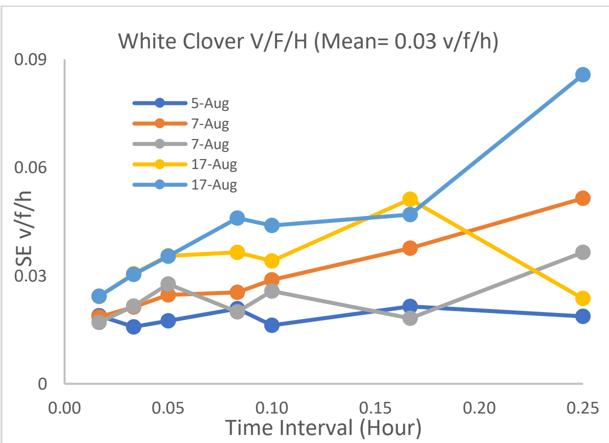


Figure 10. Comparing Purple Coneflower Pollinator Visits Per Flower Across Different Time Intervals
Figure 11. Average Purple Coneflower Pollinator Visits Per Flower Across 5 Trials



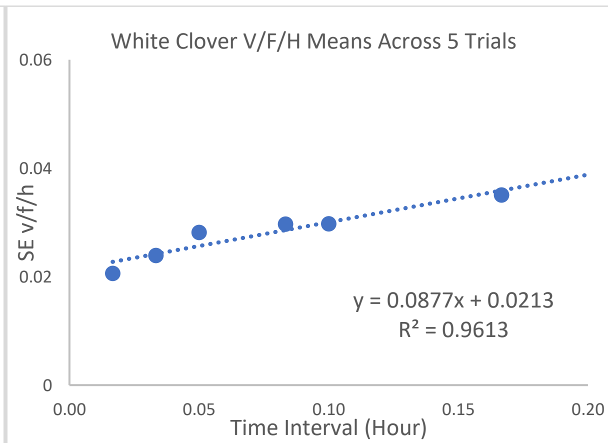


Figure 12. Comparing White Clover Pollinator Visits Per Flower Across Different Time Intervals
Figure 13. Average White Clover Pollinator Visits Per Flower Across 5 Trials

The standard error of the pollinator visitation rate and the mean visitation rate varied greatly among plant species (Table 3). The least variation across time intervals occurred for White Clover (SE= 0.03), even though it had one of the highest visitation rates. On the other hand, the largest SE (1.10) occurred for White Indigo, which had the highest visitation rate (2.17). The slopes from the regression line of SE v/f/h plotted against time interval indicate their relationship . A negative slope indicates that the SE v/f/h decreased as the time interval increased, or the data became more accurate as the time interval was increased. Likewise, a positive slope indicates that the SE v/f/h increased as the time interval was increased; in other words, the data became less accurate as the time interval was increased. The R^2 values indicate how closely this line fits the values; significant findings are indicated with an asterisk. The only significant relationship was that found in White Clover, which had an R^2 value of 0.9613 (Table 3). Among the five species, White Clover had the lowest SE v/f/h and it's mean v/f/h was neither the highest nor the lowest value. White Clover had the only positive slope when time interval was plotted against the SE v/f/h, as in Figure 5.

Plant Species	Mean SE v/f/h	Mean v/f/h	Slope of Time Interval vs SE	R^2 Value
White Indigo	1.10	2.1736	-0.5221	0.1221
Beebalm	0.20	1.3665	-0.10607	0.3827
Mountain Mint	0.18	0.6866	-0.123	0.0031
Purple Coneflower	0.04	0.2316	-0.0167	0.0619
White Clover	0.03	0.9520	0.0877	0.9613*

Table 3. Summary of Pollinator Visitation Rates Across 5 Species (*Indicates Significant Regression With 1 and 5 df)

Discussion

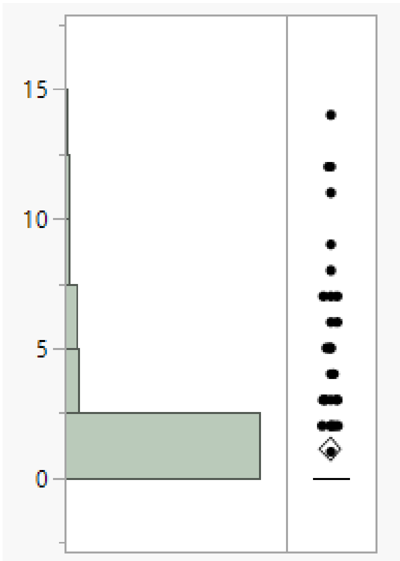
Overall, the accuracy of the data tended to increase as the time interval of the observations increased, although these patterns were not significant. This was the case for four of the five species I studied: *Monarda, E. purpurea, P. muticum, and B. alba.* This shows that for these species, the longer observation periods (15 minutes) tended to provide more accurate pollinator visitation rates than shorter observation periods (1 minute), although none of these relationships was significant. For *Monarda,* the standard error decreased by 87% between the highest and lowest data points. For *E. purpurea*, there was a 67% decrease. For *P. muticum*, there was an 87% decrease. For *B. alba*, there was a 74% decrease.

Surprisingly, the accuracy of the *T. repens* data decreased as the time interval of the observations increased. For this species, the standard error had a 48% increase. As indicated in Table 3, this was a statistically significant finding. It is important to consider the reasoning behind this finding for *T. repens* as opposed to the rest of the plant species studied. It is possible that this difference is due to a flaw in the experimental design. During the observation periods, inflorescences were chosen for observation if there were already some pollinators visiting; this serves as a potential source of bias. This essentially ensures that the first few minutes have more visitors than the rest of the observation period and increase the overall variation when visits are rare. In order to adjust for this design flaw in future experiments, the inflorescences to be observed should be selected at random and not based on pollinators.

There are many factors possibly contributing to the differences in pollinator visitation rates amongst the studied species. As discussed in the introduction, pollinator visitation rates can be affected by floral, community, and environmental characteristics. Each plant species and location studied in this experiment had a variety of factors that set them apart from the others. Some plant species were located in a small garden beside the University of Akron Field Station, while others were located in a vast field dense with plants. Although the temperature range and weather conditions during which observations are made can be controlled, it is impossible to control for every single independent variable when working in nature.

An important factor to consider the overall efficiency of observation periods. In my experiment, it took about 5 minutes to set up for each observation site. Set-up included determining the temperature and UV index, noting the weather and flower conditions, and counting the inflorescences and flowers. Including the 5-minute set-up time, the first minute-long observation period actually took six minutes. Likewise, a 15 minute observation actually takes 20 minutes, a 30 minute observation actually takes 35 minutes, and so on. This efficiency would need to be considered, especially in experiments that are using equipment or sample sizes that require a longer time to set up. Depending on how the precision changes with the time interval, it may be preferential to use the shorter time intervals in order to increase the sample size. Overall, the set-up process could be inefficient to the experiment as a whole if the accuracy of the data doesn't change across different time intervals.

It's been established that both short and long intervals may have benefits and restraints to obtaining pollinator visitation rates. It should be considered that short and long time intervals might require different statistical approaches. I used White Indigo (highest mean pollinator visitation rate) to compare the distribution of the species' one-minute interval (Figure 14) and 30-minute interval (Figure 15). The one-minute intervals are very swayed towards zero, while the 30-minute intervals are closer to a bell-shaped curve. In this case, shorter intervals don't follow a normal distribution and thus require non-parametric statistics to analyze the data. On the other hand, the longer intervals are closer to a normal distribution and thus can use parametric statistics to analyze the data.



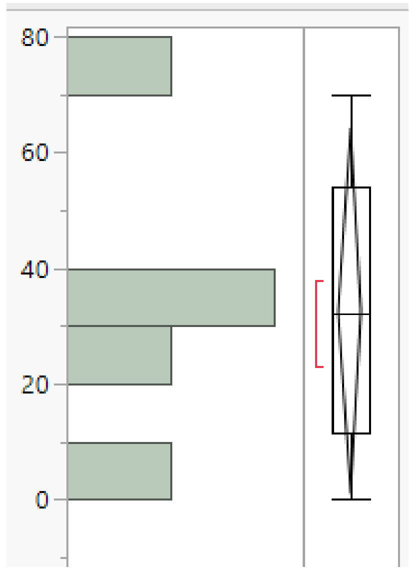


Figure 14. Distribution of White Indigo Data from One-Minute Time Intervals
Figure 15. Distribution of White Indigo Data from 30-Minute Time Intervals

Further research would be needed in order to gain a better understanding of the patterns of the accuracy of pollinator visitation rates as the time interval changes, as well as the potential causes of these patterns. Since the longer observation periods tended to be more accurate in these trials, it would be helpful to use even longer observation periods in future studies. This would allow further insight to the relationship between time interval and accuracy of the data; the accuracy could continue to increase with time interval or there could possibly be an upper limit. Further research on both extremes – very long and very short observation periods – may allow for the discovery of an ideal range to make pollinator visitation observations. If the proper materials were available, such as reliable stop-motion cameras, this would allow observation periods outside of what is reasonable for a human to manually record. In addition to longer observation periods, more observation periods overall would help in the future. Additional plant species could be included in future studies; by studying a variety of plant species with varying floral characteristics, researchers may be able to come to conclusions about the most accurate time intervals for different species with similar floral characteristics. Plants with very low pollinator visit rates may be beneficial to study as they are likely to show different patterns than those with very high pollinator visitation rates; the plants with low rates would likely have more variation. More in-depth knowledge on the differences between the species of plants and pollinators would be helpful for future research, as I suspect some of these differences may play a key role in the differences in pollinator visitation rates.

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