

Pollinator Community Compositions Across Four Subalpine Plant Species

Victoria DeLira

Mentor: Paul CaraDonna

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Abstract

Studies have shown that there have been pollinator declines occurring worldwide (e.g., Beismiejer et al. 2006; Potts et al. 2010; Burkle et al. 2013; Goulson et al. 2015). It is important to understand plant-pollinator interactions as thoroughly as possible, including which species are interacting with each other, to understand the possible consequences that may follow these declines. which prompts a number of problems considering their importance in plant reproduction. The primary objective of this study was to explore the variation in pollinator visitation rates and community composition across four subalpine species that bloom sequentially throughout the summer growing season. This study was conducted at the Rocky Mountain Biological Laboratory (RMBL) located at Gothic, Colorado. Timed pollinator observations of various patches of four subalpine plant species, *Delphinium nuttallianum*, *Hydrophyllum fendleri*, *Erigeron speciosus* and *Potentilla pulcherrima*, throughout their flowering periods between June and early August showed that pollinator communities are composed by visitation rates, alpha-diversity, and beta-diversity. This information will provides basic information on the type and frequency of visiting pollinators over time and across plants with their various shapes, blooming times, colors, and elevation.

The secondary objective of this study was to create an understanding of how pollinator exclusion bags affect studies in which the bags are used to simulate decreased pollination. Observations were made as the ones previously mentioned of plots that have the same four plants with some flowers covered by pollinator exclusion bags compared to plots with no bags. These plots are under the Iler-CaraDonna study looking at the effect of pollinator declines on plant reproduction. This secondary objective showed that there does not appear to be an effect of the pollinator exclusion bags on pollinator foraging behavior.

Introduction

Pollination involves the transfer of pollen from one plant's anthers to another's stigma (Meeuse, 1961). Animal pollinators are essential to this process because of the role they play as efficient and reliable pollen vectors. In fact, approximately 87.5% of flowering plant species worldwide are estimated to rely on animal pollination for at least some aspect of their reproduction (Ollerton et al. 2011). Since pollinators ultimately determine how much plants can reproduce, "pollinators are as critical as light and water" (Levin 1971; Kearns & Inouye 1998). Pollinators also benefit from their role as pollen vectors because as they transfer pollen from one plant to another, they collect nectar and pollen for their own survival and reproduction, as well as the growth and development of their offspring (Meeuse, 1961). Within this mutualistic relationship, pollinators also have a relationship where they tend to prefer plants based off their flower shapes, colors, sizes, etc. For example, there are a variety of bees that have different tongue lengths and that are smaller or larger than others, allowing them to access flowers of different depths (Faegri & van der Pijl 1979; as cited by Rosas-Guerrero et al, 2014). This effects the

Recently, there have been observations that pollinator populations have been declining in a variety of locations across the globe (e.g., Beismiejer et al. 2006; Potts et al. 2010; Burkle et al. 2013; Goulson et al. 2015). For example, Burkle et al. 2013 found that 76% of plant-pollinator interactions had disappeared in the last 120 years, many due to pollinators going locally extinct. Beismiejer et al. 2006 found that from 1980, bee richness alone decreased in Britain by 52% and in the Netherlands by approximately 67%. Pollinators have had the potential to decline quickly for a variety of reasons. There is a variety of different kinds of pollinators that are native to the Colorado Rocky Mountains, including solitary bees, bumble bees, and wasps, flies, and

hummingbirds. Pollinator declines and shifts in peak flowing times for different species as was seen this year have the potential to affect which pollinators can and are interacting with which plants. To understand what consequences will follow these declines it is important to understand basic pollination ecology, including which pollinators are interacting with which plants and when.

The primary objective of this study is to explore the variation in pollinator visitation rates and community composition across four subalpine species: *Delphinium nuttallianum*, *Hydrophyllum fendleri*, *Erigeron speciosus*, and *Potentilla pulcherrima*. Each of these plants can self-pollinate, are polymorphic, and they bloom sequentially throughout the summer growing season. It is expected that if plant species bloom at different times and exhibit different shapes and quantities of flowers, then the pollinator community composition for each will vary because different pollinators will be active at different times of the year and will have preference for different plants due to their flower structures. Learning this will provide basic, but essential information on the type and frequency of visiting pollinators, and how this also varies throughout the summer. This information is essential for understanding how these plants may respond to potential pollinator declines.

Additionally, in studies about pollination biology, pollinator exclusion bags are commonly used to cover flowers to simulate a decrease in pollination. While there are different options of which pollination bags to use, little to no research has been conducted on how the use of these pollination bags might affect the behavior of pollinators to other, nearby unbagged plants. The commonly used bags are mesh bags that, while they have large enough holes to allow air flow, prohibit pollinators such as bees from accessing the flowers to pollinate and collect nectar and pollen. The optimal foraging theory, which describes how an animal might choose to

spend time and energy when looking for food, might suggest that pollinators may avoid areas where they may recognize a physical barrier to their food resources. Alternatively, pollinators may not be deterred by the bags and instead might regularly visit a flower patch with bagged flowers. With a lack of research in this area, it is unknown if, besides physically impeding plant-pollinator interactions, there might be a deliberate choice on the pollinators part to visit areas under study that are using pollination bags. With this second part of the study, we are interested in learning if the bags effect pollinator foraging. We expect that if pollinator exclusion bags are used within a patch of flowering plants, then the rate at which pollinators visit those plots will decrease because they will be discouraged by the unnatural barrier keeping them from maximizing their pollination rates within a given area. This part of the experiment is important because of the insight it will offer regarding a piece of equipment that is commonly used in pollination biology. If the results suggest that there is an effect of these bags on pollinator foraging, further experiments might be necessary to determine if there is a better option to simulate reduced pollination; if no effect is implied, pollinator biologists may be able to continue to use these bags.

Methods

For this observational experiment, we chose *D. nuttallianum*, *H. fendleri*, *E. speciosus*, and *P. pulcherrima* because they are within the larger Iler+CaraDonna demography project. These species were selected because they can all self-fertilize, are polymorphic, and they each have staggered blooming times, allowing us to observe the present pollinators between June and August of 2019.

Each species was observed as they begin to bloom until they begin to senesce. *D. nuttallianum* was observed from June 20 to July 12, *H. fendleri* was observed between July 9 and

July 27, *P. pulcherrima* was observed between July 15 and August 14, and *E. speciosus* was observed between August 2 and August 14. As much time as possible between 9:00 and 16:00, the focus species is observed in increments of 5 minutes. Within each patch, the flowers being observed are ones that are open and accessible for pollination, and each of these are counted. The date and start time in a 24-hour format are recorded and conditions including temperature at the beginning of the observation period, and an estimated percent cloud cover before each individual observation, are recorded.

During the 5-minute observation period, any pollinators that make contact with the reproductive parts of the flowers were counted and identified to the lowest taxa and each flower it visits is counted as a visit from that pollinator. Each pollinator was also sorted into one of the following function groups: bumble bees, large solitary bees, medium solitary bees, small solitary bees, flower flies, other flies, butterflies, and hummingbirds.

After we concluded pollinator observations for each plant, we found the alpha-diversity of the pollinators visiting each plant. After we collected data for each species, we found the beta-diversity for each plant. This will also look at the differences in the specific taxa that are coming to visit as well as the functional groups. Furthermore, we calculated the visitation rates of the pollinators for each plant. This was put on a timeline showing how pollinator visitation rates changed throughout the June-August observation period. The alpha- and beta-diversity will be found using R, running the Simpson diversity function for the alpha-diversity, and running Whitaker's dissimilarity and Bray-Curtis dissimilarity as a way to look at beta-diversity, with a focus on Bray-Curtis dissimilarity. A series of ANOVA tests were run to compare every plant in pairs to learn if the pollinator communities that visit them are significantly different.

We also did pollinator observations for each of these plants within the plots set up for the Iler+CaraDonna demography experiment. The same 5 minutes observation structure was followed, but here, the goal was to learn if there is an effect by the pollination bags on pollinator activity. We counted the number of bags within the plots in addition to the number of open flowers, and the plots adjacent to these with pollinator exclusion bags were noted as such. Similar to the first part of the experiment, we calculated pollinator visitation rates, but this time, ANOVA test were run to compare the visitation rates between patches of flowers adjacent to plots with bags and with bags.

Results

Visitation rates for *D. nuttallianum*, *H. fendleri*, *P. pulcherrima*, and *E. speciosus* all vary. We observed the lowest visitation rate for *D. nuttallianum*, which had an average visitation rate of 0.016 visits per flower. *D. nuttallianum* was the first of the four plants to bloom in the 2019 summer season followed by *H. fendleri* which had the second highest average visitation rate at 0.035 visits per flower. *P. potentilla* was the third plant to bloom and had the highest average visitation rate at 0.044 visits per flower. The final plant to bloom was *E. speciosus*, which followed *D. nuttallianum*'s average visitation rate as the second lowest average visitation rate at 0.037 visits per flower. From the ANOVA test run on R, $F = 3.211$, and $p = 0.033$. The visitation rates overall across all four species saw a general increasing trend through the summer season, with a slope of 0.00037, $R^2 = 0.025$, $p = 0.16$.

The Simpson diversity function was used on R to find the Alpha-diversity of each species. The diversities for each plant were compared to each other using an ANOVA test where $F = 3.341$, and $p = 0.0289$. The average diversity for *D. nuttallianum* was 0.24, for *H. fendleri* was 0.56, for *P. pulcherrima* was 0.38, and for *E. speciosus* was 0.53.

The Beta-diversity for each plant species was also found. We looked at Whitaker's Dissimilarity and Bray-Curtis Dissimilarity. The Whitaker's Dissimilarity model gave a beta-diversity for *D. nuttallianum* of 0.8, 0.75 for *H. fendleri*, 0.66 for *P. pulcherrima*, and 0.44 for *E. speciosus*. The Bray-Curtis Dissimilarity gave a beta-diversity for *D. nuttallianum* of 0.91, 0.84 for *H. fendleri*, 0.77 for *P. pulcherrima*, and 0.74 for *E. speciosus*.

We compared visitation rates for plots in the Iler+CaraDonna project to compare visitation rates in plots with bagged plants to plots adjacent to bagged plants. We calculated the average visitation rates for each species by treatment, whether there were bagged flowers in the plot or if the plot had no bagged flowers but was adjacent to a plot with bagged flowers. *D. nuttallianum* had an average of 0.022 pollinator visits per flower per 5-minute observation period for plots with bagged plants and a 0.014 rate for patches with no bags; *H. fendleri* had a visitation rate of 0.031 for bagged plots and 0.00 for unbagged plots; *P. pulcherrima* had a rate of 0.00 for bagged plots and 0.037 for unbagged plots; and *E. speciosus* had a rate of 0.038 for bagged plots 0.029 for unbagged plots. Comparing the visitation rates for the two treatments overall, we found that $F = 0.007$, and $p = 0.934$.

Discussion

Pollinator visitation rates varied for each species. We observed that *D. nuttallianum* had the lowest visitation rates compared to the later three plant species. We also observed that *D. nuttallianum* had the lowest Simpson diversity, or the lowest overall diversity of the pollinator species and function groups (small, medium, or large bees, flies, ants, etc) compared to the other three plants that were coming to pollinate these flowers. Despite these lower visitation rates and the low Simpson diversity, we did observe that *D. nuttallianum* had the highest Bray-Curtis diversity compared to the rest of the plant species.

H. fendleri, the second plant to bloom in the season, had higher visitation rates than *D. nuttallianum*, and had the second highest visitation rate range, but its average visitation rate made it the third highest. *H. fendleri* had the highest Simpson diversity but had the smallest Bray-Curtis diversity. While *H. fendleri* had more variety overall in pollinators, it saw less diversity in which pollinators were visiting from day to day and between each visit.

P. pulcharrima, the third plant to boom in the season. *P. pulcharrima* had the highest visitation rates of all the plants. It also had the largest range of Simpson diversity, but overall it had a lower Simpson diversity than *H. fendleri*. For the Bray-Curtis diversity, *P. pulcharrima* had the value as *E. speciosus*; these two had a beta-diversity just higher than *H. fendleri*, but both were well below *D. nuttallianum*'s beta diversity.

E. speciosus, the final plant to bloom, had visitation rates just higher than *D. nuttallianum* but remained lower than *H. fendleri* and *P. pulcharrima*, although its average visitation rate was just higher than *H. fendleri*'s. *E. speciosus* had a higher Simpson diversity than *D. nuttallianum* but had a lower and more narrow diversity than *H. fendleri* and *P. pulcharrima*.

There appeared to be a trend in pollinator visitation rates. Visitation rates tended to increase from day 170 to approximately day 200, and they tended to decrease afterward through the end of the observation period. However, there was not a significant R^2 to confirm that this trend was significant.

The observations made in the first part of this observational study contribute to our understanding of the effect pollinator declines might have on pollination. It gives us a broader understanding of how pollinator communities are composed. These results illustrate how there are many ways pollination patterns may vary. *D. nuttallianum* has a lower pollinator diversity

and lower visitation rates, so if its pollinators are declining, it might be lacking in enough pollinators for its reproductive fitness. It also has higher diversity from day to day, which might be helpful because if only certain of its pollinators decline, it might ensure having something come and pollinate on a consistent basis spread out through its reproductive life. Looking at a plant like *H. fendleri*, with higher overall diversity and lower day to day diversity, it might be more secure with a higher number of pollinator species and groups, but it might not be secure if it depends on the same ones each day. These variations all contribute to our understanding of how pollinator declines might affect pollinator communities which might affect the reproductive fitness of plants based on their pollinator communities.

The second part of the observational experiment showed the differences in pollinator visits for each species for plots that had most flowers bagged with pollinator exclusion bags and plots that were adjacent to these with no flowers bagged. Through this part of the experiment, we found no significant difference between the visitation rates to plots with bags and without bags. This is important because it suggests that using pollinator exclusion bags does not affect on the study since it appears to not be altering pollinator foraging behavior, and they can continue to be used in pollination experiments.

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Figures and Tables

Figure 1: Pollinator Observation Sites

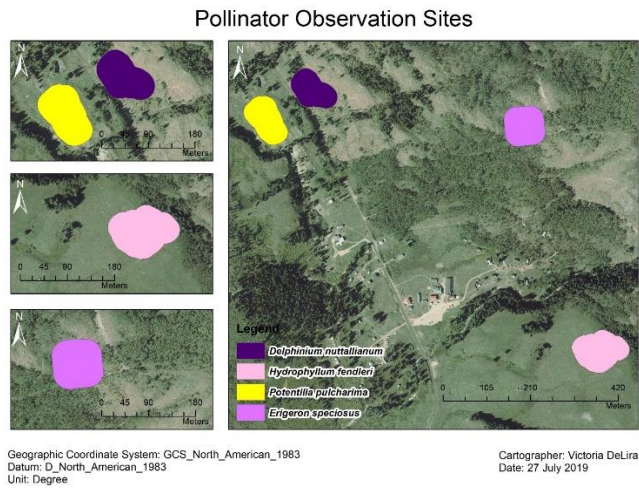


Figure 1 shows the locations of each site for pollinator observations for each target plant species.

Figure 2: Pollinator Visitation Rates per Plant

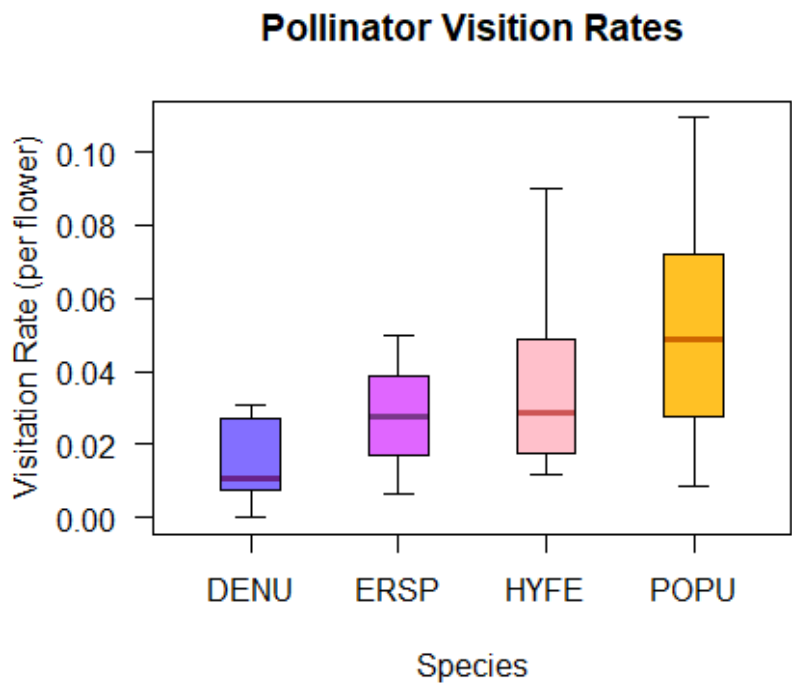


Figure 2 shows the visitation rates as visits per flower for the four plant species.

Table 1: Average Visitation Rates per Plant

Plant	DENU	HYFE	POPU	ERSP
Average Rate	0.016	0.035	0.044	0.037

Table 1 shows the average visitation rates for each plant, as they bloom sequentially throughout the summer season. Each rate is the average visits per flower.

Figure 3: Pollinator Visitation Rates by Day of Year

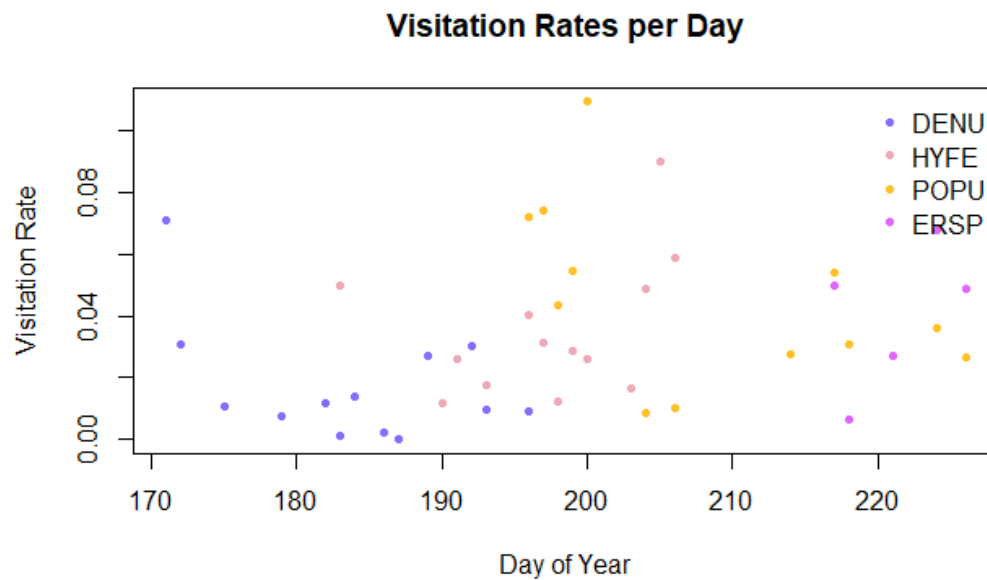


Figure 3 shows the visitation rates to each plant species per day of year.

Figure 4: Alpha Diversity

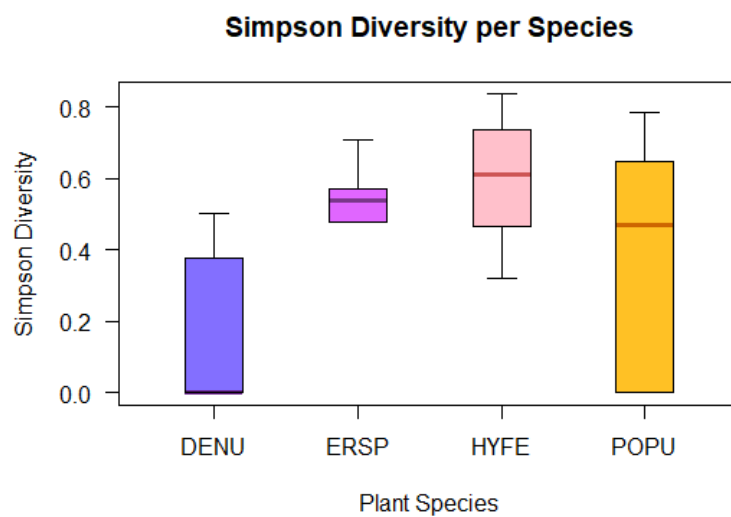


Figure 4 uses Simpson diversity to illustrate the alpha-diversity of the pollinators visiting each plant species.

Figure 5: Beta-Diversity per Plant

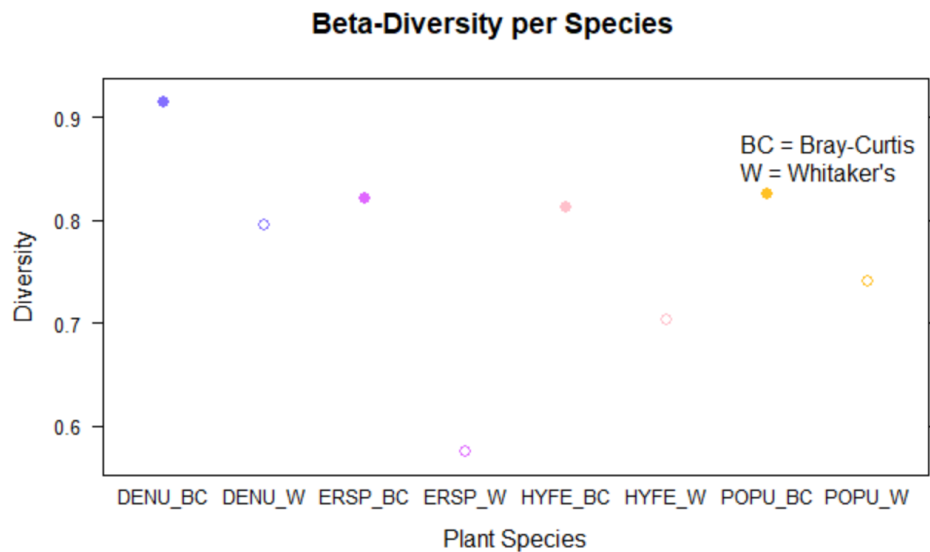


Figure 5 illustrates the differences in beta diversity for each plant between Whitaker's dissimilarity and Bray-Curtis dissimilarity.

Table 2: Beta-Diversity per Plant

Plant	DENU	HYFE	POPU	ERSP
Whitaker's Dissimilarity	0.80	0.70	0.74	0.57
Bray-Curtis Dissimilarity	0.91	0.81	0.82	0.82

Table 2 shows the beta-diversity for each plant, considering both the Whitaker and Bray-Curtis models for dissimilarity. Whitaker's dissimilarity takes into account the presence and absence of pollinators, and Bray-Curtis dissimilarity includes visitation numbers by the pollinators.

Figure 6: Bags vs No Bags Visitation Rates

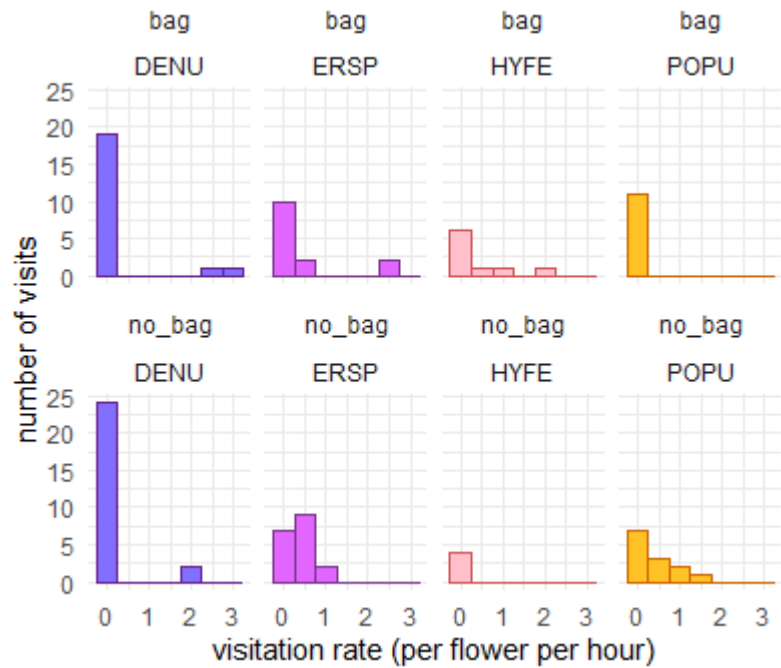


Figure 6 shows the visitation rates of pollinators to flower patches by hour for each different plant species.

Table 3: Average Visitation Rates by Treatment

Plant	DENU	HYFE	POPU	ERSP
Bag	0.022	0.031	0.000	0.038
No Bag	0.014	0.000	0.037	0.029

Table 3 shows the average visitation rates for each plant type per flower per 5 minutes, distinguishing between plots with pollinator exclusion bags and pots without.