Plant-Pollinator Interactions between *Erigeron speciosus* and *Heterotheca villosa* in Virginia Basin

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REU Education Program 2024

August 14, 2024

Abstract

Plant-pollinator communities are complex networks that are essential to the promotion of biodiversity and ecosystem health. Plant pollinator networks are sensitive to environmental change, and climate change poses a severe threat to the dynamics of these networks. It is important to examine the individual dynamics within these communities to understand how they will be affected by climate change and increasing global temperatures. The focus of this study is to compare the individual mutualistic plant-pollinator relationships of *Erigeron speciosus* and *Heterotheca villosa*, two species of *Asteraceae*, the Daisy flower family, in Virginia Basin, Gothic, CO. Although *H. villosa* was more abundant than *E. speciosus*, *E. speciosus* was more attractive to pollinators. *Bombus* seemed to prefer *H. villosa*, but more sampling should be done. *E. speciosus* is likely more attractive due to its larger surface area of disc flowers. Further research should continue network sampling at Virginia Basin and investigate flower tube length and pollinator tongue length relationships and the role of flower scent and floral volatiles.

Introduction

Biodiversity patterns are determined by many factors. Species diversity across latitudinal gradients can be largely explained by heat, but the environmental factors affecting biodiversity in certain regions can be more specific and depend more on elements like temperature and precipitation (Mo et al. 2022). Particularly within narrower regions, convergent evolution plays a key role in influencing biodiversity patterns (Stayton 2015). Convergent evolution refers to the independent evolution of similar traits between different organisms caused by adaptations to similar environments, ways of life, or constraints (Stayton 2015). In plant-pollinator systems, the pollination syndrome hypothesis suggests that some flowers co-evolve with specific pollinators through convergent adaptations (Ollerton et al. 2009). Although this theory cannot be used exclusively to describe diverse floral phenotypes or predict the pollinators of most plant species, convergent evolution is still an essential component to the living world, so this theory can guide

research regarding floral diversity and attempts to understand the patterns of plant-pollinator interactions (Ollerton et al. 2009). Based on this theory, plants with similar phenotypes should be visited by similar pollinators and plants with different phenotypes should be visited by different ones. For plant species of the same family, who share common ancestors but express different phenotypes possibly due to adaptations to different pollinators, should we still expect different pollinator interactions?

My research focused on the plant-pollinator interactions of the plant-pollinator community in Virginia Basin, near the Rocky Mountain Biological Laboratory in Gothic, Colorado. Plant-pollinator interactions are essential to the health of our global ecosystem (Burkle and Alarcón, 2011) as well as humans (Hegland et al. 2008). Diverse pollinator communities are especially essential for the promotion of biodiversity and ecosystem health, as more diverse networks stimulate greater pollination, plant reproduction, and population persistence (Burkle and Alarcón, 2011). Climate change poses a serious threat to sensitive plant-pollinator communities and has the potential to disrupt them and could even result in the decoupling of mutualistic plant-pollinator interactions, which would damage such communities and their populations (Burkle and Alarcón, 2011). Small and isolated populations like that of the Virginia Basin site are particularly vulnerable to disruption from climate change (Mustajärvi et al. 2001). To better understand the evolutionary consequences posed by the threat of climate change on plant-pollinator communities like this one, we can look closer at the individual mutualistic plant-pollinator interactions within these communities.

In the Virginia Basin field site, there are many flower species that belong to the *Asteraceae* family. Many of these flowers share similar phenotypes, but the *Erigeron speciosus* differs in phenotype from most other yellow Asteraceae with its purple-colored ray flowers. Do the morphological differences between *Erigeron speciosus* and *Heterotheca villosa* result in

different plant-pollinator interactions? To assess this question, I worked at our Virginia Basin field site, where I took flower counts to determine the abundance of both *Erigeron speciosus* and *Heterotheca villosa* and made collections and observations of the pollinators interacting with these species. I performed these observations 1-2 times per week during July and August 2024 and supplemented this data with data collected from 2021 and 2022.

Methods

The field site in Virginia Basin is located at 3500 m elevation, 3 kilometers northeast of the Rocky Mountain Biological Laboratory in Gothic, CO. It is comprised of 8 total transects divided into two columns of four. Each transect is 20m X 1m wide, with one meter of walking space between adjacent transects. Each transect is divided into 10 2m segments, marked and labeled with tape. I visited our field site 1-2 times per week to assist in taking flower counts, perform pollinator flower visit observations, and collect the pollinators from these visits. Because our field site is an exposed, high-elevation zone, afternoon thunderstorms and lightening posed a significant threat to our data collection. We scheduled Mondays as our regular data collection day to allow more flexibility if inclement weather affected data collection.

Each data collection day, we spent the first portion of our data collection counting and recording all flowering flower species in each segment of every transect. The flower counts for *Erigeron speciosus and Heterotheca villosa* were used for my research. After this was complete, we observed plant-pollinator interactions for all segments in a randomized order to prevent bias. We observed each segment for 3 minutes, 1.5 minutes from either side, keeping track with a stopwatch. When we observed a pollinator landing on an open flower head, we paused our stopwatch, netted the insect, placed it into a pre-labeled vial with a unique identification number (initials, insect # of the day), and recorded the insect information, ID number, and flower

information from the interaction onto our data sheet. Then, we resumed the timer and continued this process until the 3 minutes was complete and proceed through the rest of the transect segments. We did not be collect *Bombus* bees. Instead, we caught them, identified their species, recorded their respective data, and released them. Once we finished our observations for the day, we returned to the lab and deposited our full insect vials into the freezer for 12-48 hours before pinning them. Once sufficiently refrigerated, we retrieved our specimen vials and returned to the lab to pin the insects and transfer all our field data into Dr. Alarcón's excel spreadsheet for analysis.

For my research, I compared the pollinator visitation information between *Erigeron speciosus and Heterotheca villosa*. I looked for differences between the two in major taxonomic groups or differences in percentage of visits by specific species. I compared these ratios using a chi-square test.

To see if morphological traits might have influenced the differences in pollinator composition and mean visitation between these species, I used a digital caliper to measure the diameter of the capitula and their disc flower centers of 10 *E. speciosus* and *H. villosa* individuals. I performed these measurements in the meadow near Gothic. To avoid skewing the data, I measured one haphazardly selected individual from any cluster that was at least 2 m away from any other sampled cluster and avoided flowers that had begun to senesce.

Results

In 2021, flower abundance of *E. speciosus* had a mean of 14.3 per segment (SE = 1.755), with 80 total observations, and H. villosa had a mean of 27.971 (SE = 3.071), with 105 total observations (Figure 1). T-test assuming unequal variances suggests a significant difference in mean flower abundance between the two species ($t = -3.866$, $df = 160$, $p = 0.0002$). In 2022,

flower abundance of *E. speciosus* had a mean of 7.369 per segment (SE = 0.863), with 65 total observations, and *H. villosa* had a mean of 19.890 (SE = 3.748), with 73 total observations (Figure 2). T-test assuming unequal variances suggests a significant difference in mean flower abundance between the two species ($t = -3.256$, $df = 80$, $p = 0.0012$).

In 2021, mean pollinator visits per flower was 0.137 (SE = 0.028) for *E. speciosus*, with 30 total visits, and 0.105 (SE = 0.029) for *H. villosa*, with 40 total visits (Figure 3). T-test assuming equal variances suggests no significant difference in mean visitation between species ($t = 0.7697$, $df = 68$, $p = 0.444$). In 2022, mean pollinator visits per flower was 0.121 (SE = 0.023) for *E. speciosus*, with 13 total visits, and 0.048 (SE = 0.009) for *H. villosa*, with 12 total visits (Figure 4). T-test assuming unequal variances suggests a significant difference in mean visitation between species ($t = 2.961$, $df = 16$, $p = 0.009$).

In 2021, there were 46 observed pollinator visits to *E. speciosus* and 78 observed visits to *H. villosa*. 50% of visits to *E. speciosus* were from *Bombus* and 50% were from other pollinators (big solitary bee, *Bombyliidae*, hemipteran, moth, other fly, small black solitary bee, syrphid, wasp; Figure 5). In contrast, 73% of visits to *H. villosa* were from *Bombus* and 27% were from other pollinators. Chi-square test of independence suggests a significant difference in pollinator composition between these species (Chi square = 6.73, $df = 1$, $p < 0.05$). In 2022, there were 15 observed pollinator visits to *E. speciosus* and 24 observed visits to *H. villosa*. There were no visits by *Bombus* to *E. speciosus* in 2022, with 100% of the visitors being other pollinators (Figure 6). However, 21% of visits to *H. villosa* were *Bombus* and 79% were other pollinators. Chi-square test of independence suggested no significant difference in pollinator composition between these species for this year (Chi-square = 3.58, $df = 1$, $p > 0.05$).

For my capitula measurements, the average diameter of the capitula of 10 *E. speciosus* was 33.383 mm (SE = 1.608) compared to 25.381 mm (SE = 1.522) for *H. villosa* (Figure 7). T-test assuming equal variances suggests a significant difference in their mean diameters ($t=3.614$, $df= 18$, $p=0.002$). The average of the disc flowers of the 10 *E. speciosus* was 10.645 mm (SE = 0.621) compared to 5.353 mm (SE = 0.170) for *H. villosa* (Figure 8). T-test assuming unequal variances also suggests a significant difference in the mean diameters of their disc flowers ($t= 8.217$, $df=10$, $p<0.001$).

Due to an unusually late flowering season and poor weather conditions in August, I was unable to collect sufficient flower abundance and visitation data for the 2024 season at Virginia Basin, and thus primarily report those data from 2021 and 2022.

Discussion

The results indicate that mean floral abundance between *E. speciosus and H. villosa* was significantly different in both 2021 and 2022, with *H. villosa* showing significantly greater mean abundance than *E. speciosus* in both years. Despite seeing greater abundance in *H. villosa*, our results supported a significant trend of greater mean pollinator visits per flower to *E. speciosus* in 2022. In 2021, the difference in mean visits per flower between the two species was not significant, but we saw a similar trend, *with E. speciosus* receiving a slightly higher number of mean visits per flower than *H. villosa*. Regarding pollinator visit composition, we saw a significant difference between the two species in 2021, with *H. villosa* receiving a higher proportion of *Bombus* visitors over other pollinator types than *E. speciosus*. While this trend was still present in 2022, with *H. villosa* receiving a greater portion of *Bombus* visitors than *E. speciosus*, which received 0 observed *Bombus* visits that year, the difference was not significant. This is likely due to 2022's small sample size, as there was a significant drop-off in overall

pollinator visits, specifically *Bombus* visits, compared to other years. This likely has to do with climate variance between years. Timing of snowmelt affects the flowering of these sub-alpine species communities significantly, as earlier melt out dates leave flowers more vulnerable to frost damage later in the summer (Inouye 2008). *E. speciosus* has a significant negative relationship between the first date of bare ground and the abundance of flowering the following summer (Inouye 2008). In fact, if snow melts out before May 19, there is a high chance of frost damage the next summer (Inouye 2008). The first date of bare ground in 2021 occurred on May 7, which could explain the decline in abundance of *E. speciosus* and *H. villosa* from 2021 to 2022 and, in turn, 2022's decline in pollinators, because pollinators are sensitive to the size and density of plant populations (Mustajärvi et al. 2001). However, flower production of whole populations is very sensitive and can vary significantly due to other changes in local climate such as annual variations in temperature and rainfall (Alarcón et al. 2008).

The purpose of my research was to assess whether *E. speciosus* and *H. villosa* have different pollinator interactions due to their morphological differences. The results indicated that *E. speciosus* and *H. villosa* did differ in their pollinator visit compositions, as *H. villosa* attracted a higher proportion of *Bombus* in both 2021 and 2022, although 2022's results were insignificant. Additionally, although only 2022's data showed significant results, we saw a trend of *E. speciosus* showing a greater mean number of visits across both years, despite seeing a significantly greater mean floral abundance in *H. villosa* for both years. Pollinators tend to prefer large populations of plants because they offer more rewards over small populations (Gascon 2021), but our results showed the opposite, as the species with higher abundance, *H. villosa*, received fewer visits on average than the less abundant species, *E. speciosus.* One factor that could explain this difference is in the average amount of reward available per individual for each of these species. More sparse plant populations have been found to attract a higher mean number

of visits per plant than larger populations, possibly because sparce plant populations often have larger inflorescences (Mustajärvi et al. 2001). This concept was supported by the flower measurements of these species. I found that the disc flower centers of *E. speciosus'* capitula were nearly 2 times as large on average as those of *H. villosa*. With larger disc flower centers, *E. speciosus* is likely more attractive due to its larger display and greater amount of available reward.

As for the trend found in 2021 of *Bombus* preferring *H. villosa* over *E. speciosus*, many factors are likely responsible for this. One factor that I did not investigate was flower tube length. Because bumblebees have longer tongues, they likely prefer longer tubed flowers. This could possibly indicate that *H. villosa* have longer tubed flowers than *E. speciosus*. Future research should take this factor into account and examine it further. Additionally, bumblebees are larger than most insects and thus also require greater amounts of nectar. *E. speciosus* may be less attractive to bumblebees because of its attractiveness, as it receives more visits from other pollinators, leaving less nectar for the bumblebees. Future research could also examine scent as a factor in the differences in attractiveness between these two species. This could be done by comparing their floral fragrance and volatile profile patterns.

For 2024, there was a late blooming season that delayed flowering of *E. speciosus* and *H. villosa* to late July and early August. Consistent unsafe weather conditions through the first two weeks of August prevented me from collecting any abundance or network data on these species for 2024. Future research should continue network sampling in Virginia Basin. It is important to monitor changes in sensitive pollinator communities like this one, as rapid environmental change and population declines in plants and pollinators put the diversity and stability of these communities at serious risk (Burkle and Alarcón, 2011).

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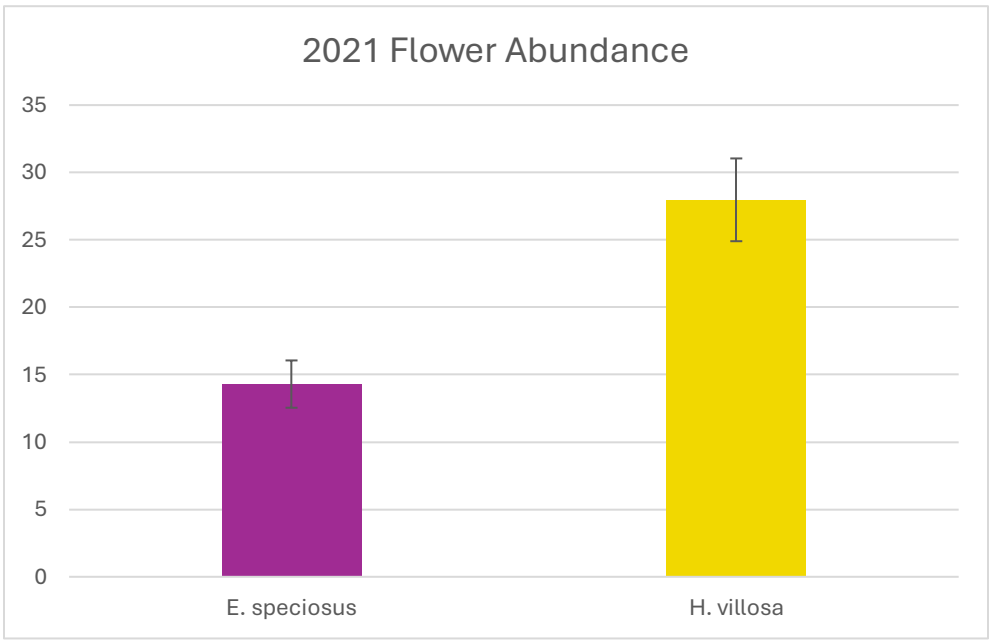

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Figures

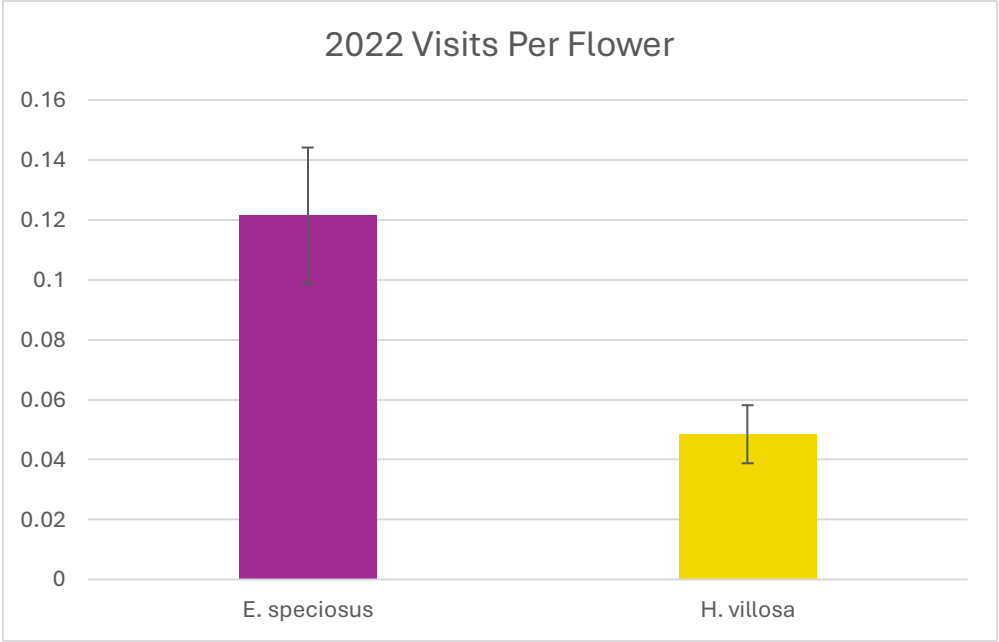

Figure 1. Mean (+/- SE) number of flowers per segment for *Erigeron speciosus* and *Heterotheca villosa* in 2021.

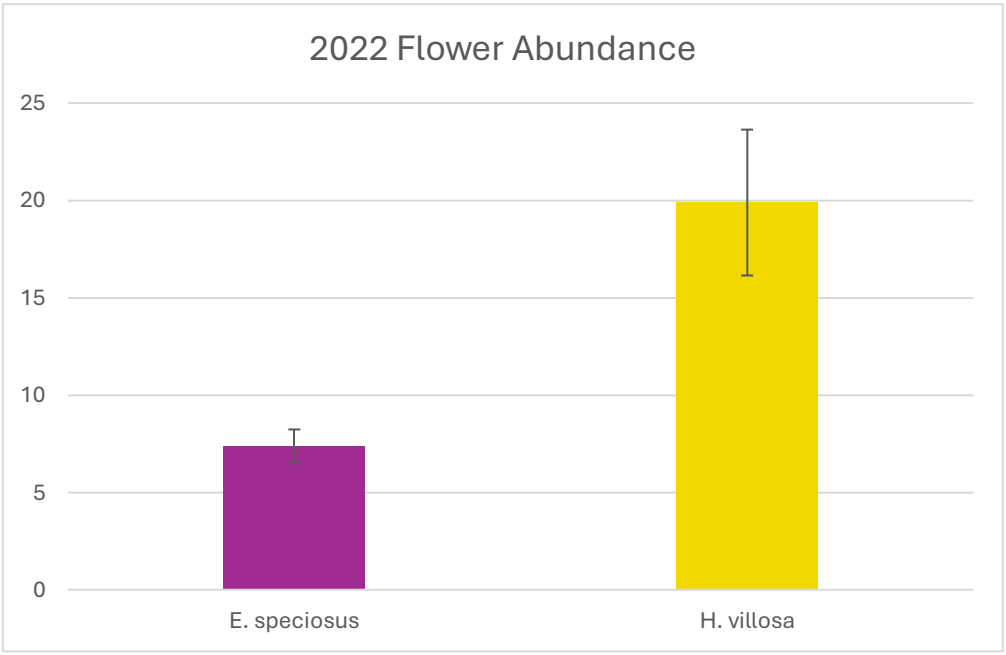

Figure 2. Mean (+/- SE) number of flowers per segment for *Erigeron speciosus* and *Heterotheca villosa* in 2022.

Figure 3. Mean (+/- SE) number of pollinator visits per flower for *Erigeron speciosus* and *Heterotheca villosa* in 2021.

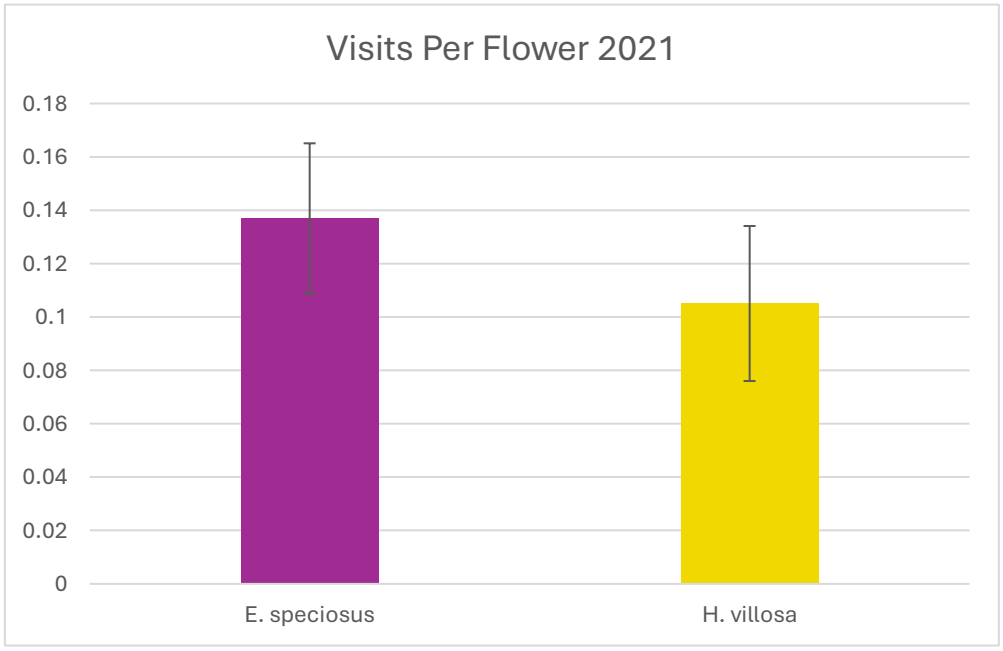

Figure 4. Mean (+/- SE) number of pollinator visits per flower for *Erigeron speciosus* and *Heterotheca villosa* in 2022.

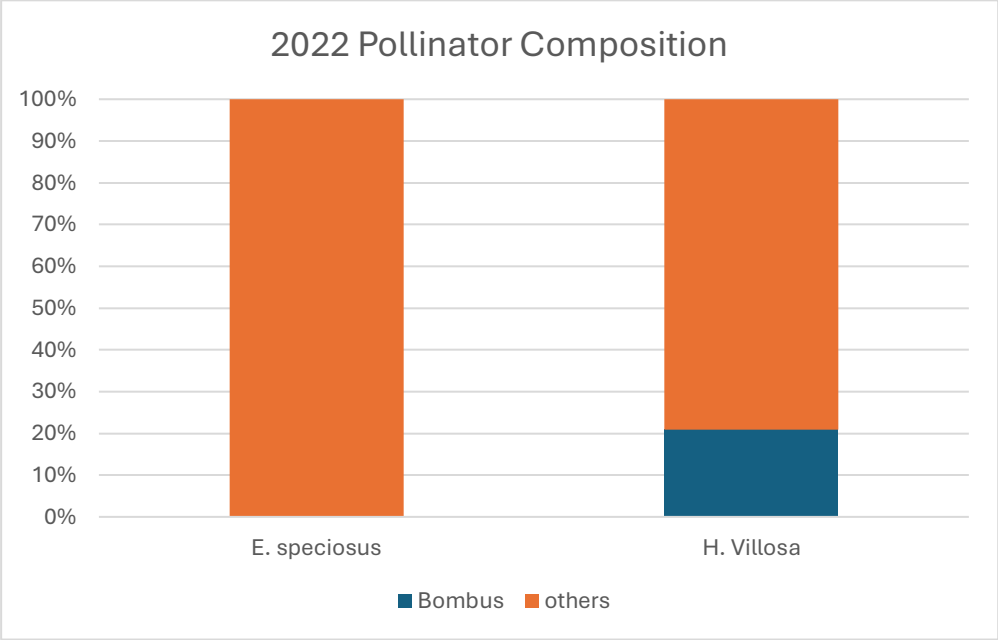

Figure 5. Composition of pollinator visits to *Erigeron speciosus* and *Heterotheca villosa* in 2021.

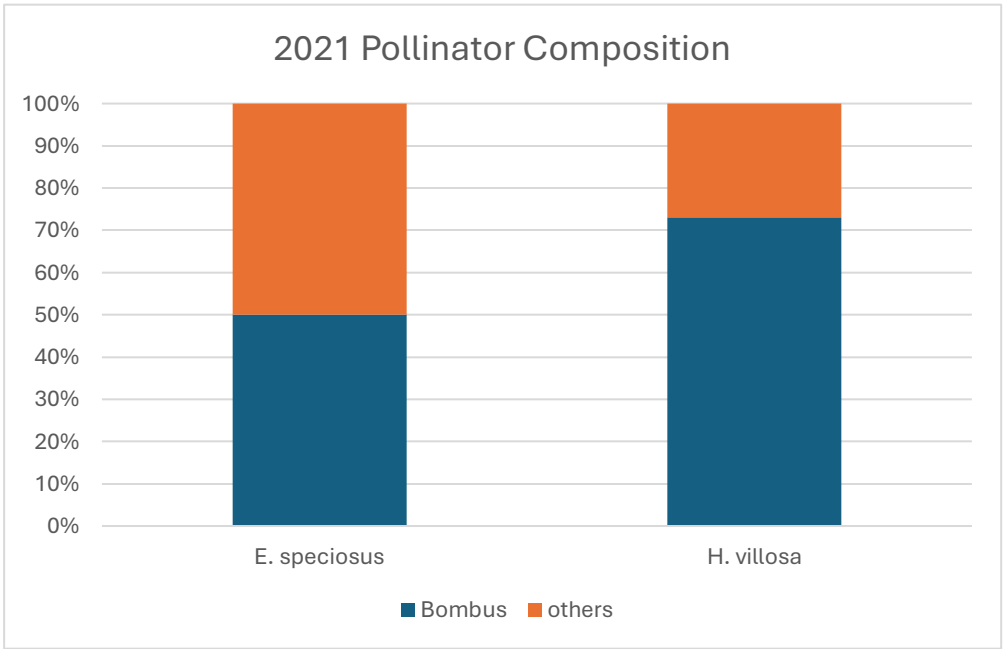

Figure 6. Composition of pollinator visits to *Erigeron speciosus* and *Heterotheca villosa* in 2022.

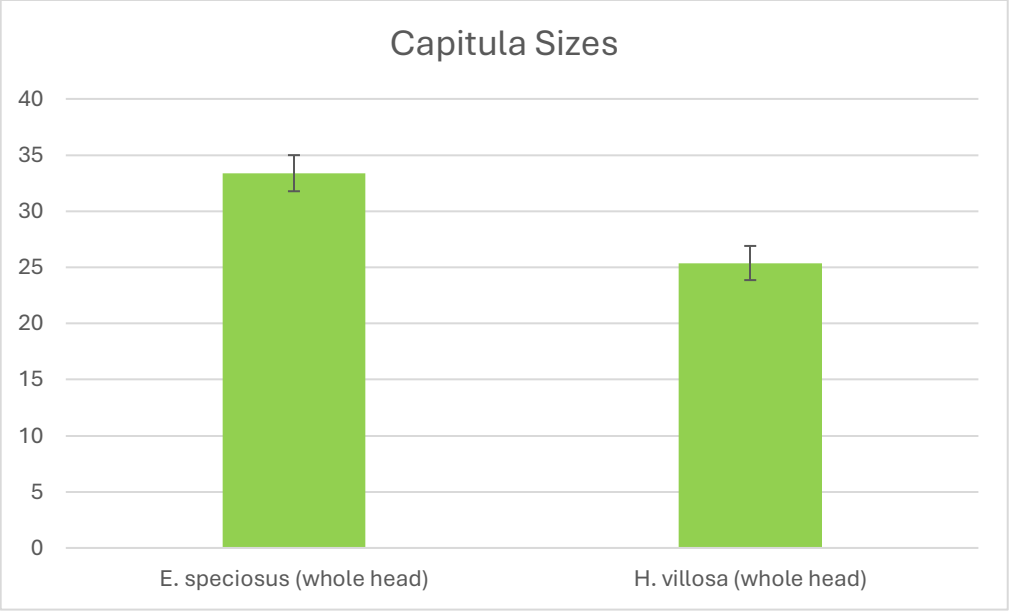

Figure 7. Figure 4. Mean (+/- SE) diameters of *Erigeron speciosus* and *Heterotheca villosa* capitula.

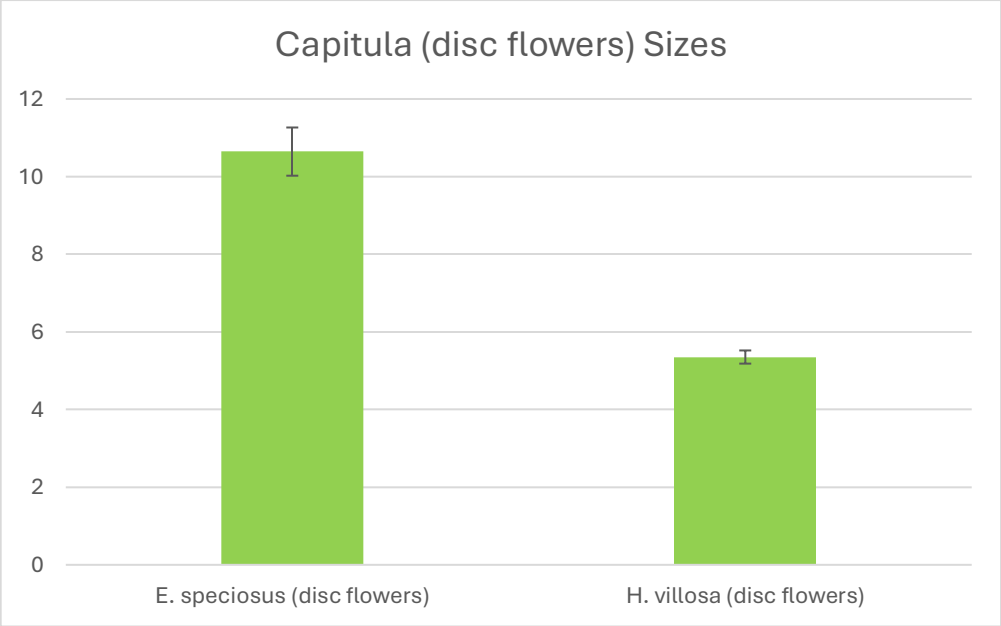

Figure 8. Figure 4. Mean (+/- SE) diameters of *Erigeron speciosus* and *Heterotheca villosa* disc flowers.