

Variability in the effectiveness of *Penstemon strictus* pollinators and the role that water availability plays

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Abstract:

Plant-pollinator interactions play a vital role in the functioning and stability of our ecosystems. Without pollination, angiosperms would not be able to set seed and reproduce, and most organisms would lose their primary source of nutrients and resources. Owing to the fact that pollination services are becoming increasingly threatened by anthropogenic impacts, a thorough understanding of the factors that influence the success of pollen transfer is important, relevant, and timely. In this study, we evaluate how water availability influences pollinator effectiveness. Pollinator effectiveness is defined as the amount of conspecific pollen deposited on the stigma in a single pollinator visit. Many researchers have compared and assessed pollinator effectiveness, while others have investigated how water availability influences floral and vegetative traits, but few have studied the relationship between environmental factors and conspecific pollen deposition. Using a water manipulation experiment on 80 paired *Penstemon strictus* plants growing in Crested Butte, Colorado, we were able to show that different types of pollinators vary in their behavior, handling time, and effectiveness. Our water treatment did not exert a significant effect on any of the floral or vegetative traits that we measured, so the pathway by which we predicted water availability to operate on pollinator performance was irrelevant. In our analysis of pollinator behavior, handling time and effectiveness, we therefore have not factored in water treatment or soil moisture (yet). With respect to pollinator effectiveness, we did not find a significant correlation between handling time and conspecific pollen deposition for any of the pollinators. We did, however, find a significant relationship between behavior and conspecific pollen deposition. Buzz pollination or sonication, a particular pollen foraging behavior employed by *Bombus bifarius*, yielded the greatest amount of pollen received by the stigma. Based on these findings, we suggest that behavior in conjunction with physical traits of pollinator species and genera, such as size, hairiness, and pollen-bearing structures, exert the largest influence on pollinator effectiveness.

Introduction:

Plant-pollinator interactions are fundamental to the diversity and stability of the food webs that sustain our ecosystems. Without pollination, angiosperms would not be able to set seed and reproduce, and most organisms would lose their primary source of nutrients and resources (Buchmann and Nabhan, 1996). Owing to the fact that pollination services are becoming increasingly threatened by anthropogenic impacts (Kearns et al., 1998), a thorough understanding of the factors that influence the success of pollen transfer is imperative (Sahli and Conner, 2007). Pollen transfer is largely facilitated by animals, which collectively pollinate over 85% of 352,000 flowering species (Ollerton et al., 2011; Paton et al., 2008). This study, therefore, looks at factors influencing the effectiveness of animal pollination, rather than abiotic pollination via wind or water. In the face of climate change, especially with respect to shifts in water availability, it is important to ask how pollination will be affected and what the consequences are on plant reproductive success.

Many researchers have compared and assessed pollinator effectiveness, but few have studied the relationship between environmental factors and pollinator effectiveness. Changes in water availability have the potential to influence pollination by a variety of mechanisms. Water availability can alter floral traits, which can, in turn, influence the visitation rates of pollinators (Gallagher and Campbell, 2017) or seed production (Campbell and Powers, 2015). In addition,

decreased water availability can decrease pollen production and pollen receipt of nearby flowers (Waser et al., 2016). Therefore, studies that examine the effects of water availability on ecological mutualisms need to be undertaken. A better understanding of climate change and pollination will help adequately predict and prepare for global shifts in pollination and future resource availability.

In this study, pollination by different insects was evaluated and compared by looking at the amount of conspecific pollen deposited on a stigma in a single visit. Conspecific pollen deposition leaves out any heterospecific pollen transferred to the stigma because heterospecific pollen can lead to stigma blockage of pollen (Waser and Fugate, 1986), loss of conspecific pollen (Campbell and Motten, 1985), or lower reproductive success (Morales and Traveset, 2008). Pollen transfer was evaluated using the model organism *Penstemon strictus* and its large host of *Hymenoptera* pollinators and flies. *Penstemon strictus* is a bee-syndrome perennial plant that is native to the dry subalpine meadows of the Rocky Mountains. Wide, blue-purple corollas attract frequent visitors of many different species, which vary year to year. Flowers of this plant receive a high number of visits everyday, averaging 100-200 visits a day from bumblebees alone (Thomson et al., 2000). The high number of visits that this species receives by a complex and expansive pollinator community make this an ideal study system for comparing pollinator effectiveness. In addition, its capacity to grow along an elevational gradient and survive in the high alpine make it an appropriate system to use in understanding ecological responses to shifts in water availability.

We investigated the degree to which water availability affects plants and pollinators by carrying out a water manipulation experiment on 80 paired *Penstemon strictus* plants. One individual in each of the 40 pairs was exposed to a water addition treatment of 300mL every two to three days over a period six weeks, while the other received no additional watering. We predicted that plants exposed to a water addition treatment would show increased growth, flower production, floral display, and nectar and pollen production. We also looked at pollinator identity, behavior, handling time, and conspecific pollen deposition to determine differences between pollinators. We hypothesized that the plant responses to increased water availability would have an effect on plant-pollinator interactions and facilitate an increase in conspecific pollen deposition by pollinators and ultimately higher seed set.

Methods:

Study system:

Fieldwork was conducted in a dry subalpine meadow at the Lupine Trail in Crested Butte, Colorado. *Penstemon strictus* is an upright perennial that typically blooms from July through August. The plant has characteristic purple-blue vestibular flowers, as well as broad corolla tubes and extended lips upon which insects can land (Zung et al., 2015). Flowers are held horizontally by a stiff pedicel (Castellanos et al., 2004) and new flower buds are produced at the tip of the stalk during growth. The genus *Penstemon* consists of over 275 species, which range from completely self-incompatible to self-compatible (Tepedino et al., 2006). *Penstemon strictus* is partially self-compatible, but all species on the spectrum of self-compatibility have been shown to benefit from visits and pollen transfer by animals. The pollinator community can shift year to year, but a variety of bees consistently make up the bulk of the flower visitors. The morphology of *Penstemon strictus* is of bee-syndrome character, as evidenced by its wide blue-purple

corollas, anther positioning, and slow and narrow anther dehiscence. A bird-pollinated *Penstemon* is more likely to be red, with narrower and longer corollas, and higher production and release of nectar and pollen.

Water manipulation experiment:

To test for specific plant and pollinator responses to water availability, we performed a water manipulation experiment using a naturally occurring population of *Penstemon strictus* at the Lupine Trail in Crested Butte, CO. We marked 30 pairs of *P. strictus* on June 24, 2017 and added an additional 10 pairs to our experiment on July 15, 2017. One individual in each pair was randomly assigned a water treatment on the day that the plants were marked. A 0.042m² area around each treatment plant was watered with 250mL of water every two days from June 24, 2017 to July 15, 2017. The 250mL treatment was calculated using the area of space that would be watered (0.042m²) and the average rainfall in July (0.06747m). Average rainfall was obtained from records of rainfall at the Maxfield Meadow and is an average from the 1990s - 2009. By watering the plants with 250mL every two days, the plants received about three times the average precipitation of July. With the addition of 10 new pairs on July 15, 2017, we increased the water treatment of all treatment plants to 300mL every other day to ensure a treatment effect between individuals in each pair. This watering regime was carried out from July 15, 2017 to August 10, 2017.

To determine if the water treatment had an effect on soil moisture, measurements were taken prior to watering throughout the end of June and the month of July using a Campbell Scientific hydroprobe. We also measured the height of the tallest inflorescence of each plant before and after the watering experiment. Finally, we counted the total number of flowers of each plant as well as the peak number of flowers open at one time to see if the water treatment had an effect on plant height.

Plant resource output with respect to nectar and pollen production was also measured to determine if the water treatment had an effect on the plants. Elongated buds were tagged, bagged, and left for 48 hours to open. After 48 hours, nectar production was measured using 30 mm capillary tubes to extract the nectar of the flowers. We were unable to measure nectar concentration with a refractometer, as the refractometers did not work with the small amount and variable sugar content of the nectar. Finally, pollen production was measured by collecting and massing the undehisced anthers from flowers of *P. strictus* individuals.

Single-visit pollinator effectiveness

When *P. strictus* plants reached the elongated bud stage, they were bagged with mesh pollinator bags to prevent insects from visiting. They were later unbagged for single visit pollinator observations. One flower was randomly selected to serve as a control and immediately bagged. When a pollinator visited a flower, the species was identified and a timer was set to obtain handling time. Flowers were then tagged and bagged and left for 48 hours so that pollen tubes could germinate. Stigmas were excised with forceps and a basic fuschin gel was used to mount the stigmas to microscope slides. The number of conspecific pollen grains on the stigma was then counted under a microscope.

Many of our plants were late to bloom or did not bloom at all, so we added 19 plants to our study to use for pollinator observations. These plants were not given a water treatment and

are referred to as “wild” in our observations and statistical analysis. We measured soil moisture, plant height, flower number, floral display, and the number of inflorescences of each of these wild plants. We also used flowers from these plants to measure nectar production and pollen production.

Statistical analysis:

Plant values were analyzed using randomized block analysis of variance (ANOVA), with pair as the block, and water as the treatment. We also used R to generate boxplots of the plant traits in relation to water treatment. Pollinator effectiveness was analyzed using ANOVA with factors of insect type, behavior type, handling time, water treatment and the interaction. We similarly used R to plot the relationship between these variables. Tukey tables were used to compare relative differences between pollinator types and behavior types.

More to come...

Results:

Water manipulation:

Soil moisture measurements were taken prior to watering tagged individuals in the first 30 *P. strictus* pairs on June 24, June 27, June 30, July 3, and July 6, 2017. Measurements were also taken 5 hours after watering on July 3, 2017. On July 15, 2017, soil moisture measurements were recorded for the first 30 pairs as well as the additional 10 pairs both before watering and 5 hours after watering. Finally, soil moisture measurements were taken at the end of the month on July 30, 2017 for all 40 pairs, as well as the newly added 19 wild plants. Soil moisture measurements of the wild plants were also taken on August 1 and August 5, 2017.

To determine if the water treatment had an effect on soil moisture, July soil moisture measurements were averaged for each plant and treatment groups were compared. The soil moisture of the plants assigned to the water addition treatment was significantly higher than that of the ambient group ($F_{1, 78} = 5.46$, $P = 0.022$, Fig. 1). However, all measurements of floral attractiveness and plant resource output were not influenced by changes in water availability. For our 80 *P. strictus* plants, water availability did not have a significant effect on plant growth ($F_{1, 78} = 0.628$, $P = 0.431$, Fig. 2a), total number of flowers ($F_{1, 78} = 0.106$, $P = 0.746$, Fig. 2b), or floral display ($F_{1, 78} = 0.124$, $P = 0.726$, Fig. 2c). Treatment groups also did not differ significantly in pollen production ($F_{1, 14} = 1.341$, $P = 0.266$, Fig. 2d) and nectar production ($F_{1, 22} = 1.537$, $P = 0.228$, Fig. 2e) collected from 16 and 24 flowers respectively (Table 1). When we changed the discrete water treatment variable to a soil moisture continuum, we similarly did not see a relationship between water availability and plant growth ($F_{1, 78} = 0.692$, $P = 0.408$), flower production ($F_{1, 78} = 0.471$, $P = 0.495$), floral display ($F_{1, 78} = 0.066$, $P = 0.798$), and pollen production ($F_{1, 22} = 0.02$, $P = 0.888$) were analyzed with soil moisture as the independent variable, significant relationships were not found for any of the plant traits. However, nectar volume increased significantly with soil moisture ($F_{1, 26} = 6.472$, $P = 0.0172$, Fig. 3), but we were unable to measure concentration, which would have given us better evidence to support our prediction that nectar production increases with water availability.

Single visit pollinator effectiveness:

We obtained pollinator effectiveness data from 124 visits to 28 of the paired treatment plants and an additional 29 visits to 13 wild plants, which we had tagged and bagged later in the season. We could not collect data from many of our paired treatment plants because 22 of the plants did not produce any flowers throughout the growing season and 13 produced three or fewer flowers. In addition, we had to discard at least 50 stigmas from our pollinator effectiveness data because the stigmas were too immature at the time of the pollinator visit. A previous study that followed the stages of flowering in *Penstemon strictus* determined that stigmas are not receptive until the style has bent more than 45 degrees. This bending can take up to 48 hours after the flower has opened to occur (Castellanos et al., 2002). Under the microscope, it was clear that these stigmas had not been fully bent and receptive to pollen when they were visited. A total of 38 control flowers from plants in the experimental groups and the wild group were collected and used for comparison.

The 146 visits used in our analysis included visits from three families of *Hymenoptera*: *Apidae*, *Halictidae*, and *Megachilidae* (Table 2). In an attempt to identify species to the lowest taxonomic level possible, we distinguished 9 different pollinator types, some at the genus level and others at the species level when possible. Visits by wasps in the *Vespoidea* family and flies in various families were not included in our analysis because we received only three and six visits by these insects, respectively. Pollinators in the *Apidae* family accounted for over two-thirds of our visits (n=108), the breakdown of which included four species of *Bombus* (*Bombus appositus* (n=19), *Bombus bifarius* (n=21), *Bombus californicus* (n=6), and *Bombus flavifrons* (n=4)) and two non-*Bombus* *Apidae* (*Anthophora terminalis* (n=38) and *Ceratina spp.* (n=7)). The number of single visits from the *Halictidae* family (n=18) and the *Megachilidae* family (n=19) was roughly equal. Within these families, taxa were identified to the genus level with *Lasioglossum spp.* and *Halictus spp.* comprising the *Halictidae* family and *Osmia spp.* comprising the *Megachilidae* family.

Pollinator effectiveness varied among the different pollinator types (Fig. 4), with *Bombus bifarius* delivering the greatest amount of pollen in a single visit ($P = ___$). *Bombus appositus*, *Bombus californicus*, and *Anthophora terminalis* delivered the second largest amount of conspecific pollen in a single visit, although deposition by these three types of pollinators was not significantly less than *Bombus bifarius*. Pollinator effectiveness by all four pollinator groups was significant when compared to pollen on the control stigmas. *Bombus flavifrons*, *Lasioglossum spp.*, *Ceratina spp.* and *Halictus spp.* delivered similar amounts of pollen but did not differ significantly from the control group.

Pollinators also differed significantly in their relative handling times (Fig. 5). *Lasioglossum spp.* and *Halictus spp.* spent significantly more time in flowers than all other pollinator groups, except *Ceratina spp.* We also found that pollinator behavior related to handling time (Fig. 6). Nectar and pollen foragers spent the most time inside the flower, while insects that foraged strictly for nectar spent the least amount of time inside the flower. *Bombus bifarius* pollen foragers, which entered upside-down, also spent very little time inside the flower. Finally, the foraging behavior exhibited by the pollinator influenced the amount of conspecific pollen that ended up on the stigma (Fig. 7). The upside-down entrance and pollen foraging behavior of *Bombus bifarius* yielded significantly higher amounts of pollen on the stigma than the nectar-only and pollen-only foraging behaviors. Pollen deposition of foragers for nectar and pollen fell in between the other behaviors, and did not differ significantly from either. Although

certain behaviors differed significantly from one another in terms of how much conspecific pollen was deposited on the stigma, handling time did not appear to produce the same outcome. For 9 pollinator identities, handling time and conspecific pollen deposition was uncorrelated (Fig. 8a,b). Furthermore, when all pollinators were grouped together, there was no significant trend for handling time and conspecific pollen deposition ($P = 0.08$).

Water availability and pollinator effectiveness: In progress...

Discussion:

Using a paired treatment experiment with 80 *Penstemon strictus* plants plus an additional 19 wild plants, we tested the hypothesis that water availability influences pollinator effectiveness by operating through changes to floral attractants and plant traits. Although we saw a difference in soil moisture resulting from our water treatment ($P = 0.022$), we found little evidence to show that water availability has an effect on floral and vegetative traits. The soil moisture gradient between treatment groups was not very large, which may have been due to the short duration of the watering experiment, the lapse periods between watering days, or the small quantity and frequent runoff of our watering treatment.

A diverse community of pollinators were identified and shown to vary significantly in the amount of conspecific pollen they deposited. In the *Apidae* family, *Bombus bifarius* appeared to be the most effective pollinator, although not significantly more effective than *Bombus appositus*, *Bombus californicus* or *Anthophora terminalis*. The latter three deposited similar amounts of pollen, all of which were significantly greater than the amount of pollen on the stigma of the controls. The small solitary bees comprising the genera *Lasioglossum*, *Halictus*, and *Ceratina* deposited similar amounts of conspecific pollen on average, making them the third most effective group. With that said, their deposition did not differ significantly from any of the other pollinators or the control group. Medium-sized pollinators in the *Osmia* genus were the least effective pollinators.

Our ability to identify species to the lowest taxonomical level possible was facilitated by the RMBL Herbarium and special insects collection, literature on *Penstemon strictus* pollinators, and observational studies on the behaviors of the different pollinators. Functional identity was very important in matching pollinators with their taxonomical identity. For example, small solitary bees with yellow sacs on the scopae of their legs had been previously described by James Thomson as small pollen-collecting bees in the *Lasioglossum* genus (Thomson et al., 2000). Small pollen-collecting bees that lacked the external pollen-carrying sacs but still actively foraged for pollen and spent time on the anthers were later characterized as belonging to the *Halictus* genus. Medium solitary bees were matched to the *Osmia* genus due to their size, black to metallic green color, and tendency to forage for nectar (although sometimes they spent time collecting pollen as well). The final group of solitary bees made up of small nectar collectors were identified as *Ceratina*. By noting physical traits and their tendency to buzz pollen out of the anthers, we could give names to pollinators, matching them to those in Thomson's descriptive studies. *Bombus* and *Anthophora* were easy to distinguish and identify based on their distinct coloring and characteristic nectar-foraging behaviors. *Bombus bifarius*, however, did not fit into this behavioral category and instead foraged almost exclusively for pollen.

The behavior utilized by different pollinators in a single visit had significant consequences for pollen transfer. Behaviors related to both handling time and conspecific pollen

deposition. *Bombus bifarius* foraged for pollen by entering the flower upside down and sonicating on the anthers. This behavior may allow the bee to overcome the slow dehiscence schedule and narrow pubescence that prevents large amounts of pollen from removal by bees and birds (Castellanos et al., 2003). By turning upside-down and buzzing on the anthers, *Bombus bifarius* can remove copious amounts of pollen through the narrow slits in the anthers. Other bumblebees, which enter right side up and go straight to the base of the corolla where the two nectaries reside have been shown to remove smaller amounts of pollen mainly by virtue of incidentally rubbing against the anthers. Despite the similarity in handling times of all of the *Bombus* species and *Anthophora terminalis* individuals, buzz pollination by *Bombus bifarius* yielded the highest conspecific pollen transfer. This finding suggests that behavior is more crucial to pollinator effectiveness than handling time. Our results also contrast with a previous study, which showed that the upside-down pollen foraging behavior of *Apis mellifera* translated to large amounts of pollen removal but small amounts of pollen deposition (Wilson and Thomson, 1991). Wilson and Thomson posited that the pollen foragers exhibit greater amounts of in-flight grooming and consumption of pollen, both of which they categorized as contributing to pollen wastage. In our study, *Bombus bifarius* almost always visited every nearby *P. strictus* flower, so our data on pollen deposition comes from pollen transfer across very short distances. In this case, *Bombus bifarius* were not returning to the nest to drop off pollen between visits or losing pollen in flight due to long distances and grooming.

We did not find a significant relationship between handling time and conspecific pollen deposition for any of the pollinators. Many of the small solitary bees spent a significantly longer time foraging, likely because they moved around the two nectaries and four anthers, but they deposited less pollen than other foragers. Sometimes they, too, turned upside down on the front anthers and buzzed pollen out of the narrow slits, but unlike *Bombus bifarius*, their pollen-collecting foraging behavior did not translate to high conspecific pollen deposition. This finding may suggest that pollinator identity and size play a greater role in pollinator effectiveness than behavior. The sonication technique of the small solitary bees was likely weaker than that of the bumblebees, and their smaller, less hairy bodies probably restricted them from carrying as much pollen. Furthermore, as mentioned previously, *Bombus bifarius* typically did many single visits to flowers of the same inflorescence, having to move pollen only short distances without grooming between flights. Small solitary bees, on the other hand, usually spent time in only one or two flowers of the same inflorescence and then left. The *Lasioglossum*, which carried pollen sacs on the scopae of their legs may have been transporting the pollen back to their brood, as has been reported in another study (Thomson et al., 2000). Ultimately, there was wide variability in handling times, especially for the solitary bee pollen and nectar collectors. Thus, it is important to avoid generalizing too much and to consider that individual pollinators may behave in their own, unique way. Chittka and Thomson warn us against the consequences of ignoring the uniqueness of individual pollinators, devoting an entire chapter to pollinator individuality (Chittka and Thomson, 2001).

In conclusion, our study found that pollinators differed in behavior, handling time, and effectiveness. Our water treatment was not strong enough to exert an effect on the floral or vegetative traits of *P. strictus* individuals. Perhaps this species is more plastic than we realized and can adapt to water availability quite well. Future studies should attempt a more significant water availability manipulation to see if water does affect traits and then look at pollinator effectiveness in response to water availability. We are still working on adding more components

to our statistical analysis and ultimately running the statistics on water treatment and pollinator handling time and effectiveness.

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Figures and tables:

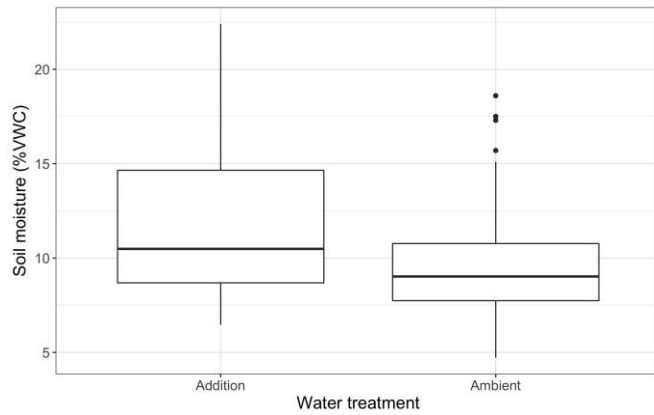
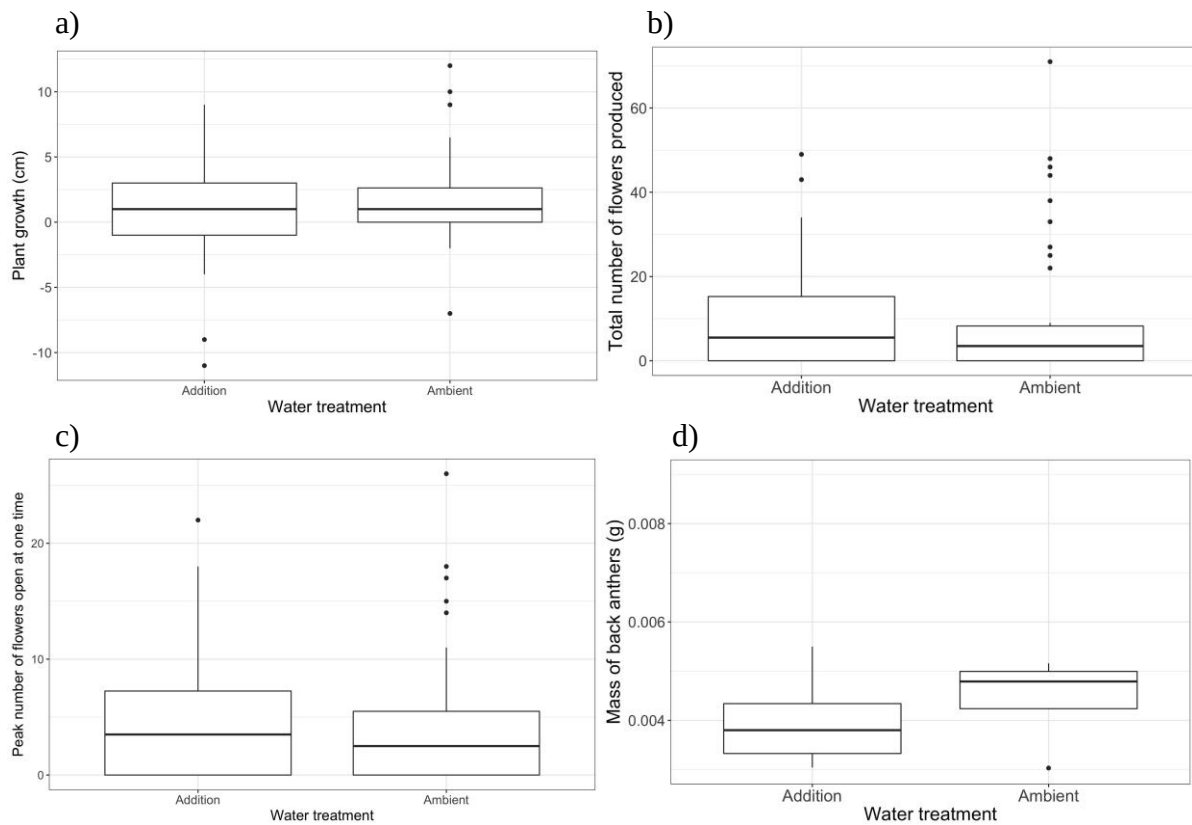


Figure 1. The effect of the water treatment on soil moisture averaged over the month of July for 80 *Penstemon strictus* plants, 40 in each water treatment group. Soil moisture was measured in percent volumetric content.



e)

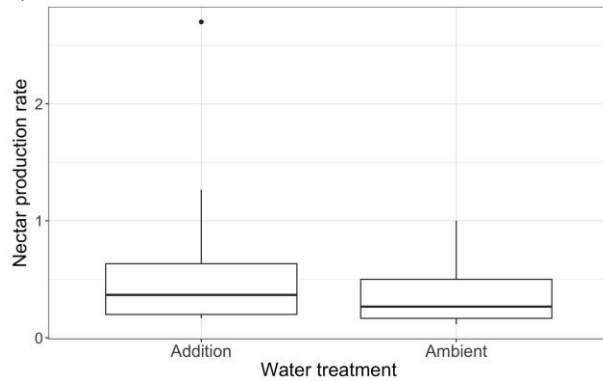


Figure 2. The effect of the water treatment on a) plant growth, b) total number of flowers, and c) floral display for 40 *Penstemon strictus* individuals in each treatment group, and the effect of water treatment on d) pollen production for 16 *P. strictus* individuals, and e) nectar production for 24 *P. strictus* individuals. No significant differences between treatment groups.

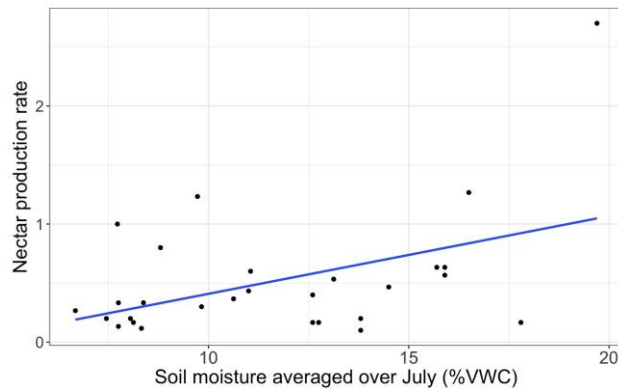


Figure 3. The relationship between soil moisture and nectar production for 28 *Penstemon strictus* individuals.

Table 1. Sample sizes of flowers used for pollen and nectar production measurements in each of the treatment groups.

Pollen and nectar collection sample sizes	Ambient	Addition	Wild	Total
Pollen	n = 4	n = 12	n = 8	n = 24
Nectar	n = 11	n = 13	n = 4	n = 28

Table 2. Sample sizes of the different pollinators that visited *Penstemon strictus* flowers of the different treatment groups as well as flowers of the control group. First grouping shows sample sizes of visitors identified by their taxonomic identifications. Second grouping shows sample sizes of pollinators in each of four behavioral categories.

Pollinator taxonomic ID	Ambient	Addition	Wild	Total
<i>Bombus appositus</i>	n = 3	n = 12	n = 4	n = 19
<i>Bombus bifarius</i>	n = 7	n = 14	n = 2	n = 23
<i>Bombus californicus</i>	n = 0	n = 0	n = 6	n = 6
<i>Bombus flavifrons</i>	n = 0	n = 4	n = 0	n = 4
<i>Anthophora terminalis</i>	n = 22	n = 15	n = 18	n = 55
<i>Ceratina spp.</i>	n = 4	n = 1	n = 2	n = 7
<i>Halictus spp.</i>	n = 6	n = 5	n = 2	n = 13
<i>Lasioglossum spp.</i>	n = 13	n = 5	n = 3	n = 21
<i>Osmia spp.</i>	n = 3	n = 6	n = 8	n = 17
<i>Pseudomasaris vespoides</i>	n = 0	n = 0	n = 2	n = 2
Flies	n = 1	n = 3	n = 2	n = 6
Control	n = 13	n = 10	n = 15	n = 38

Pollinator functional ID	Ambient	Addition	Wild	Total
Nectar forager	n = 37	N = 41	n = 22	n = 100
Pollen forager	n = 10	n = 5	n = 3	n = 18
Nectar and pollen forager	n = 5	n = 5	n = 4	n = 14
Pollen forager via tilt	n = 7	n = 14	n = 2	n = 23
Control	n = 13	n = 10	n = 15	n = 38

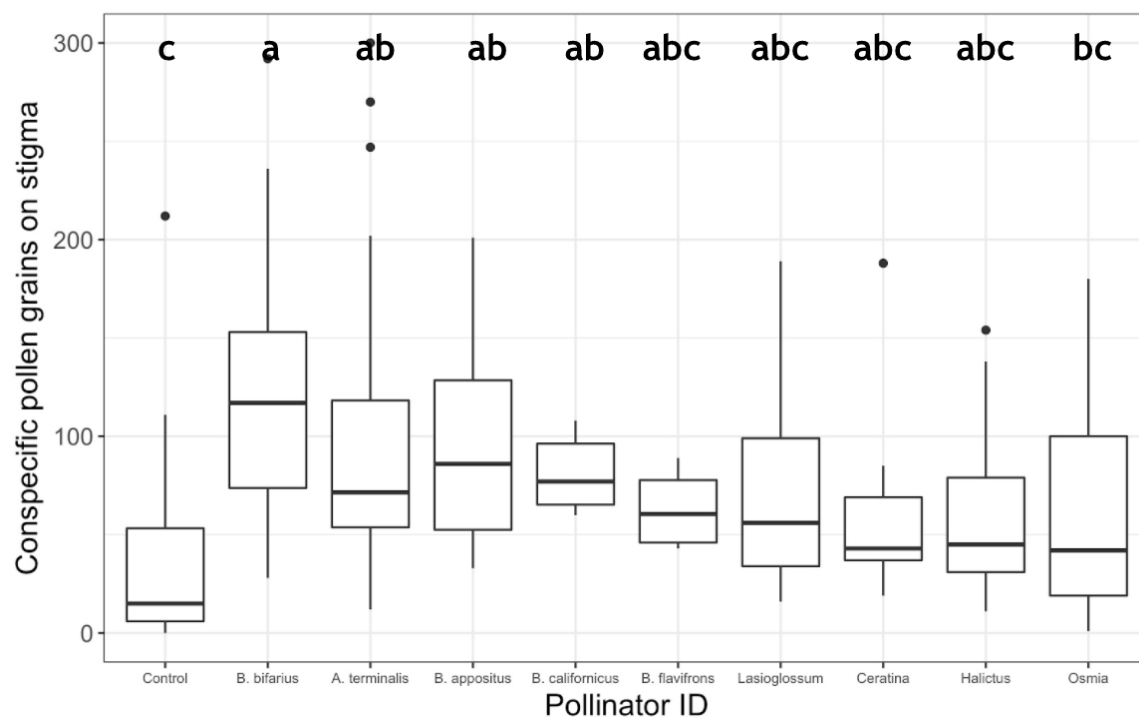


Figure 4. Comparison of the effectiveness of 9 different pollinator groups. Letters represent significance codes.

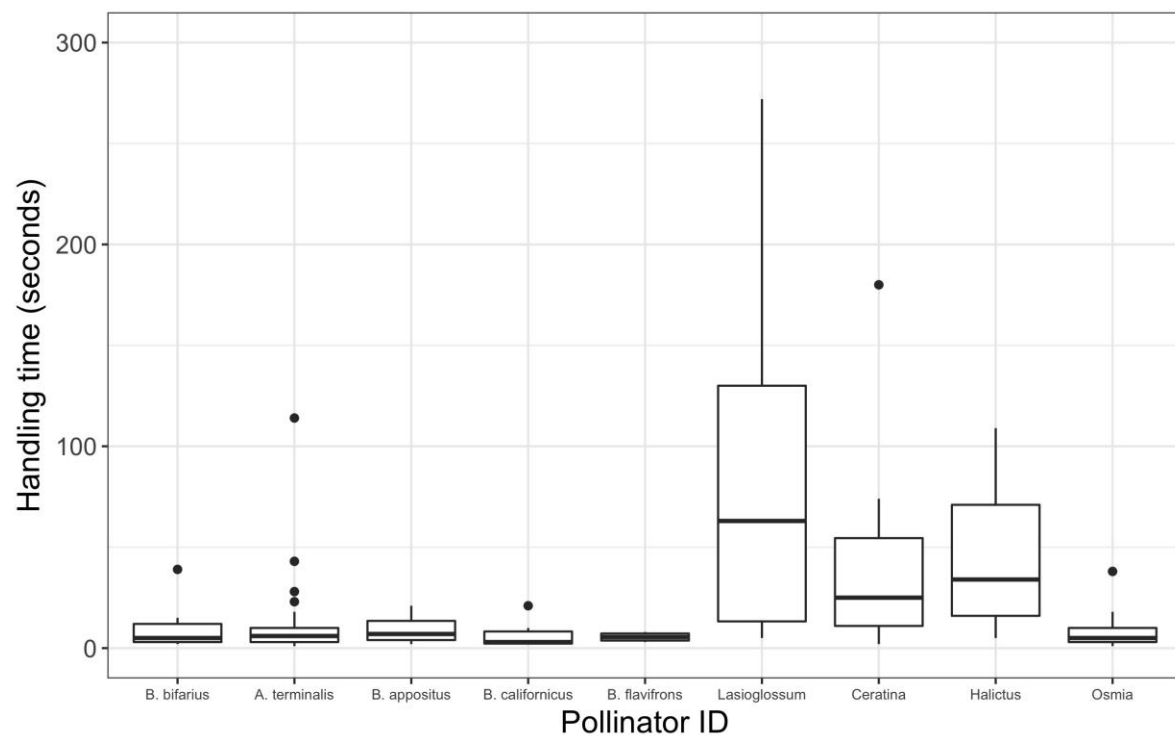


Figure 5. Comparisons of the amount of time different pollinator groups spent inside the flowers foraging.

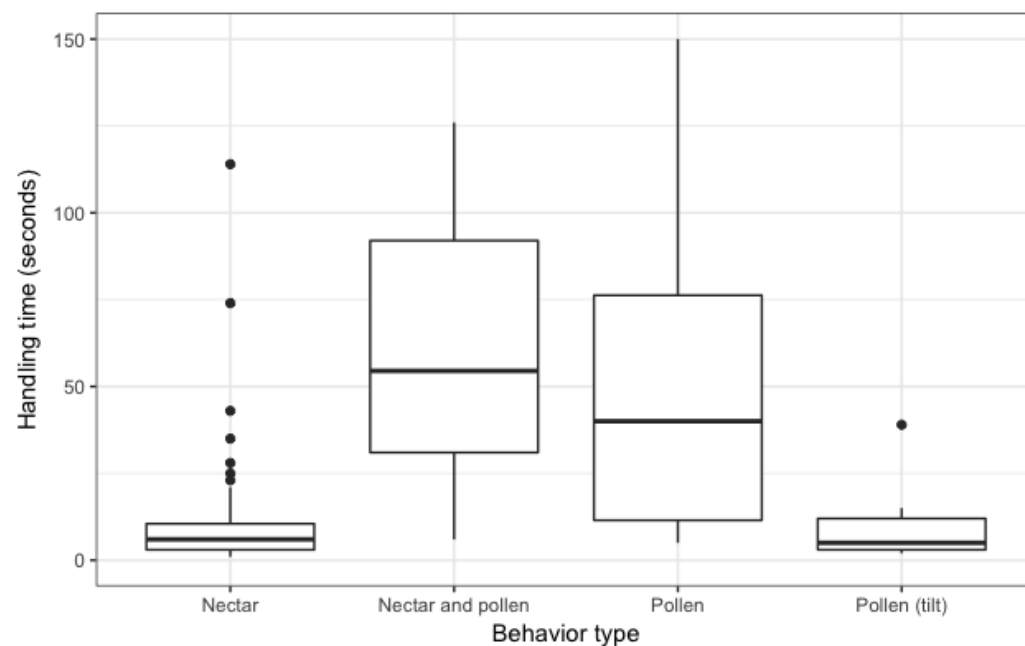


Figure 6. Relationship between pollinator foraging behavior and handling time. Pollen foraging by “tilt” refers to the foraging behavior of *Bombus bifarius*, which involves entering the flower upside down and buzzing on the anthers. Letters represent significance.

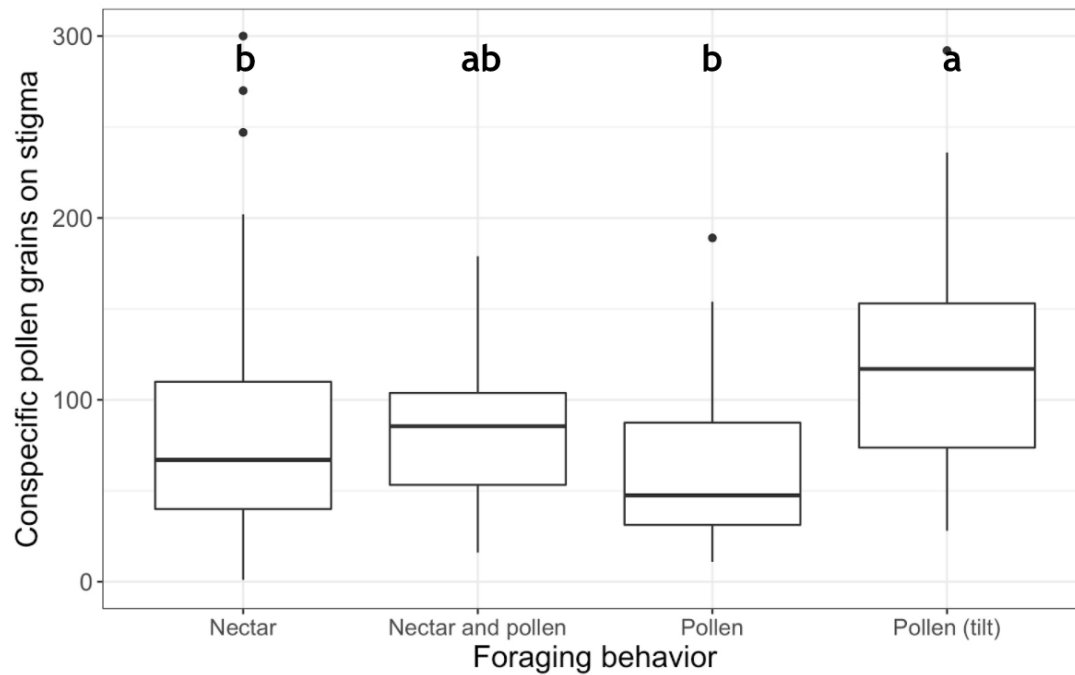


Figure 7. Relationship between pollinator foraging behavior and the amount of conspecific pollen deposited on the stigma. Pollen foraging by “tilt” refers to the foraging behavior of *Bombus bifarius*, which involves entering the flower upside down and buzzing on the anthers. Letters represent significance.

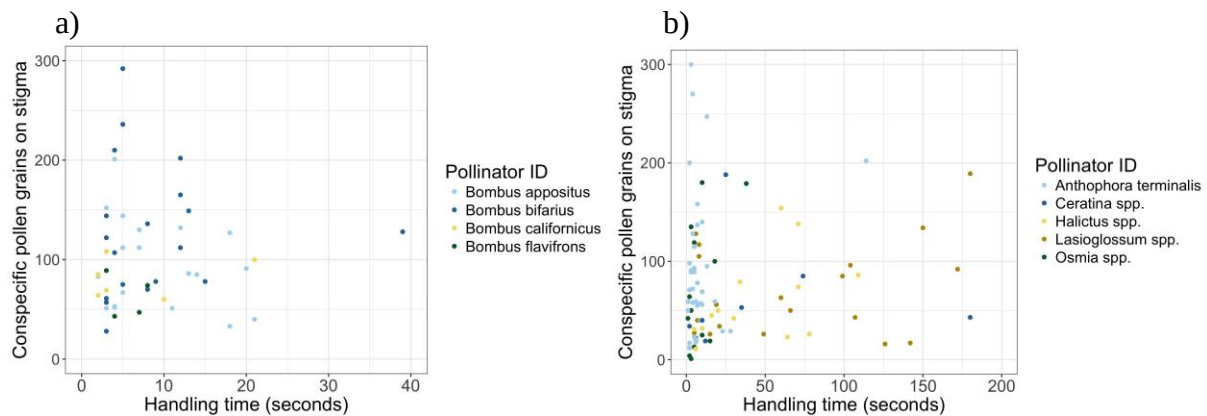
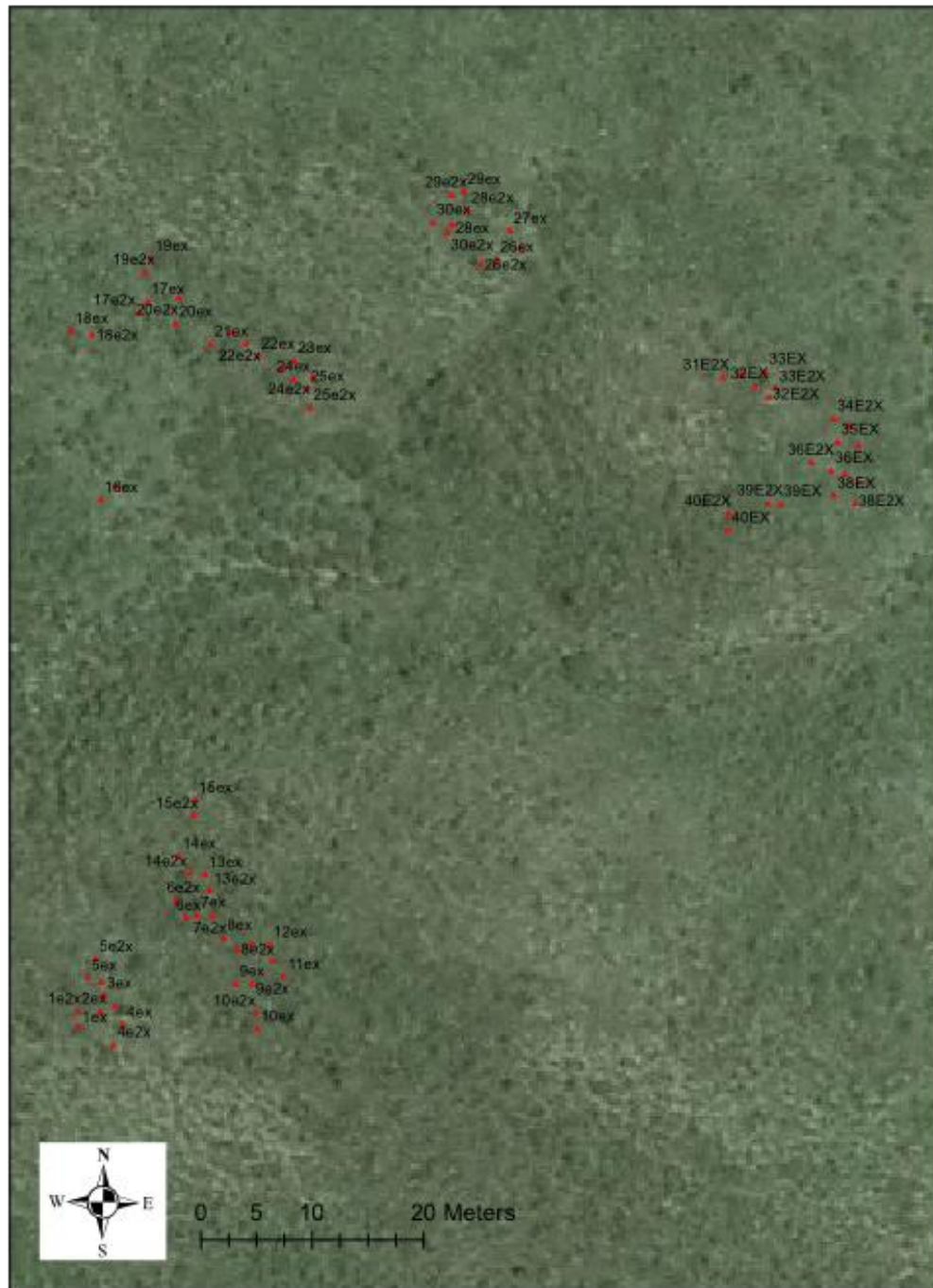


Figure 8. Relationship between handling time and conspecific pollen deposition for a) *Bombus* species and b) non-*Bombus* species of pollinators.

Maps of study site:

Vicinity map of *Penstemon strictus* studied at the
Lupine trail in Crested Butte, CO



Map of Ashley Morse's research on *Penstemon strictus*
2017

