

Effects of Experimental Water Addition on Floral Nectar Traits

Mikhaela Ferguson

Mentors: Paul CaraDonna and Gwen Kirschke

Rocky Mountain Biological Laboratory

Independent Research and Rocky Mountain Ecosystems Course/REU

Abstract

Plant-pollinator interactions rely on the exchange of nectar and pollen for pollination services. Nectar traits can be influenced by changes in abiotic factors such as water availability, and such variation may in turn influence interactions with pollinators. This experiment investigated how the nectar traits in subalpine plant species responded to increased water availability compared to control conditions. We collected data on three components of floral nectar traits: nectar volume (μl), nectar sugar concentration ($\text{mg}/\mu\text{l}$), and total nectar sugar (mg) for four different wildflower species: *Delphinium nuttallianum* (Ranunculaceae), *Delphinium barbeyi* (Ranunculaceae), *Eremegone congesta* (Caryophyllaceae), and *Erigeron speciosus* (Asteraceae). *Delphinium nuttallianum*, *Delphinium barbeyi*, and *Erigeron speciosus* all showed no pattern between the control and water addition plots, suggesting these species can maintain nectar traits across variable water availability conditions. *Erigeron speciosus* showed a marginal increase in sugar content, which was not previously predicted in our hypotheses. We found that *Eremegone congesta* was the most sensitive to changes in water availability, with a significant decrease in nectar volume and sugar content due to water addition treatment.

Introduction

Nectar is a fundamental aspect of pollinator attraction and plays a key role in plant-pollinator mutualisms (Parachnowitsch et al., 2019). Nectar provides nutritional benefits to pollinators and influences the attraction of pollinators to plants. Not only do pollinators use nectar for nutrition, but plants rely on nectar foraging pollinators for the dispersal of pollen among various species (de Vega, et al., 2021). Despite the central importance of nectar as a floral reward in plant-pollinator interactions, relatively little is known about how abiotic factors influence nectar traits (Lawson & Rands, 2019).

Water availability is an aspect of ecosystems that is expected to change greatly due to global change and is especially relevant to flowering plants (Elliott et al., 2014). The effects of a changing ecosystem are already beginning to take a toll in multiple regions, such as Indonesia, where farming practices were facing change due to effects of drought (Lynch, 2018). In the Rocky Mountains specifically, drought is an expected result of continued climate change (Strzepek, et al., 2010).

Abiotic factors, such as water availability, can influence plant-pollinator interactions through the manipulation of floral attractants, such as nectar (Gallagher & Campbell, 2017). For example, Powers et al. found increased nectar concentration and reduced nectar production in *Ipomopsis aggregata* in response to low summer precipitation (2021). Waser and Price also found a decrease in nectar production with drought (2016). Furthermore, nectar dilution, whereby sugar concentrations within nectar decrease, providing less nutritional benefit to the pollinator, could increase with changes in water availability, which might negatively affect the fitness of the plant and the fitness of the pollinator by acting as an energetic constraint (Lawson & Rands, 2019). The way that water availability affects nectar traits may therefore have direct consequences for the plant-pollinator interactions. This experiment investigated how the nectar traits of subalpine plant species responded to increased water availability compared to control conditions. Through this experiment, we aimed to better our understanding of plant responses by assessing how resources are allocated to nectar in a condition of increased water availability.

Hypotheses

Our experiment aimed to address the question of how experimental water addition influences nectar traits in subalpine flowering plants. Specifically, we investigated three components of floral nectar: nectar volume (μl), nectar sugar concentration ($\text{mg}/\mu\text{l}$), and total nectar sugar (mg). Based on our question, we had four competing hypotheses. Our first hypothesis predicts that changes in water availability will have no effect on any of the nectar traits being assessed: in other words, nectar volume, concentration, and sugar content would all remain the same between control and water addition plants (Figure 1). This hypothesis operates under the reasoning that plants may work to ensure consistency in their nectar traits to remain attractive to pollinators, even when experiencing variation in water availability. Evidence consistent with this hypothesis would suggest that plants strategically allocate nectar resources as a means of quality control to maintain the mutualistic relationships needed for pollination.

Our second hypothesis is that experimentally adding water would result in the plants producing overall more nectar that is more dilute (lower sugar concentration) but with the same sugar content (mg) as control plants (Figure 1). Evidence for this hypothesis would suggest that plants respond to increased water availability by allocating the water directly to floral nectar.

Our third hypothesis predicts that plants will produce nectar with more sugar and have a higher nectar sugar concentration than the control, but with no change in nectar volume from the control (Figure 1). Evidence for this hypothesis would suggest that plants are allocating resources mobilized by the addition of water to add more sugar, but not toward producing more nectar overall.

Our fourth hypothesis predicts that the plant would respond to increased water availability by producing more nectar, in addition to more sugar within (Figure 1). In this case, the increased water availability would allow for the mobilization of nutrients, allowing the plant to allocate more resources to sugar production. This would result in a higher nectar volume than the control, a higher sugar concentration than the control, and a higher sugar content than the control. Evidence for this hypothesis would suggest that water is a limit for nectar quality.

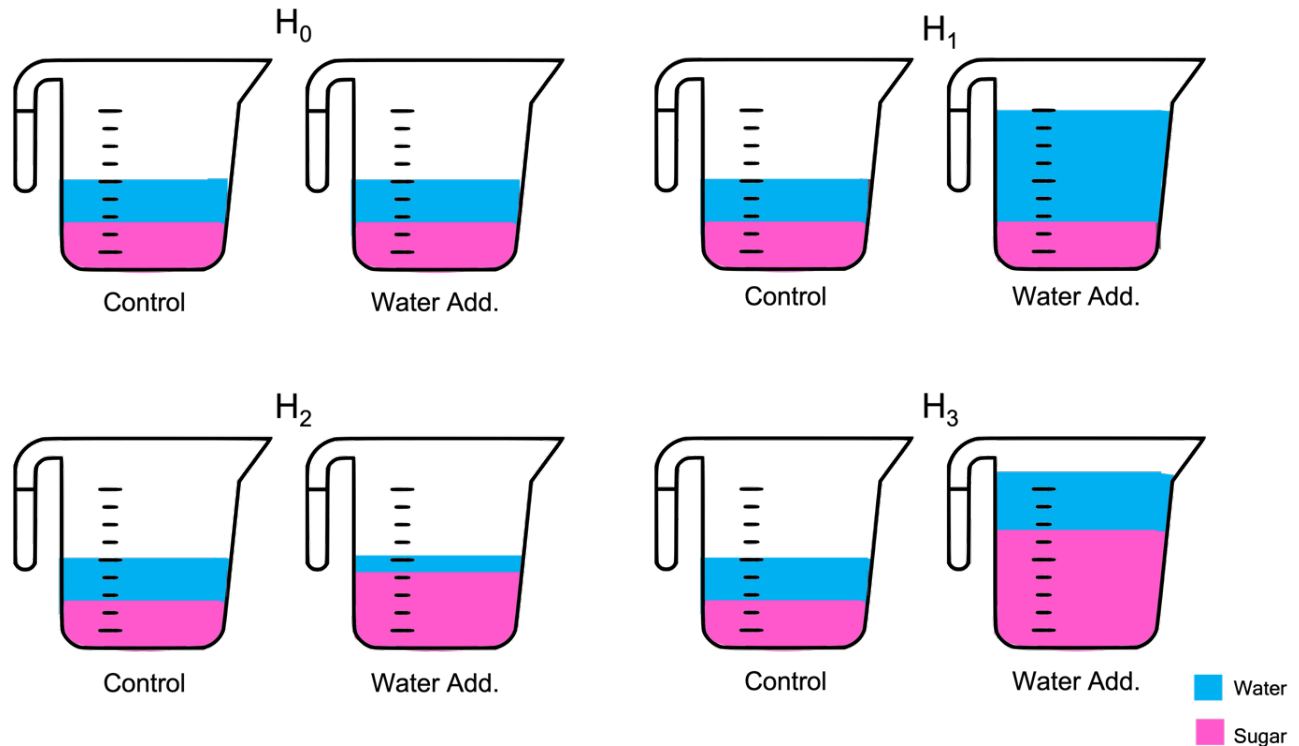


Figure 1. Hypotheses and predictions of nectar traits for control and water addition plots.

Methods

Study Site & Study Species

Our experiment took place near RMBL in subalpine plant communities, where plant-pollinator systems have been thoroughly studied, including research done by CaraDonna and Waser (2020) looking at temporal flexibility in plant-pollinator networks. The plots for all four species were located near Vera Falls (Figure 3). At our study site, we had 6 control and 6 watering plots for *Delphinium nuttallianum* (Ranunculaceae), *Erigeron speciosus* (Asteraceae), and *Eremegone congesta* (Caryophyllaceae) paired to ensure similar environmental conditions, taking into consideration vegetation cover, incline of the hill, and amount of sun or shade. There were 10 pairs of plots for *Delphinium barbeyi* paired based on size, space, and location. Because *Delphinium barbeyi* is a larger plant, plots were centered around a particular study plant, as only one could fit within the parameters. Plots were 1m², located uphill from the East River at an elevation of 2,900m.

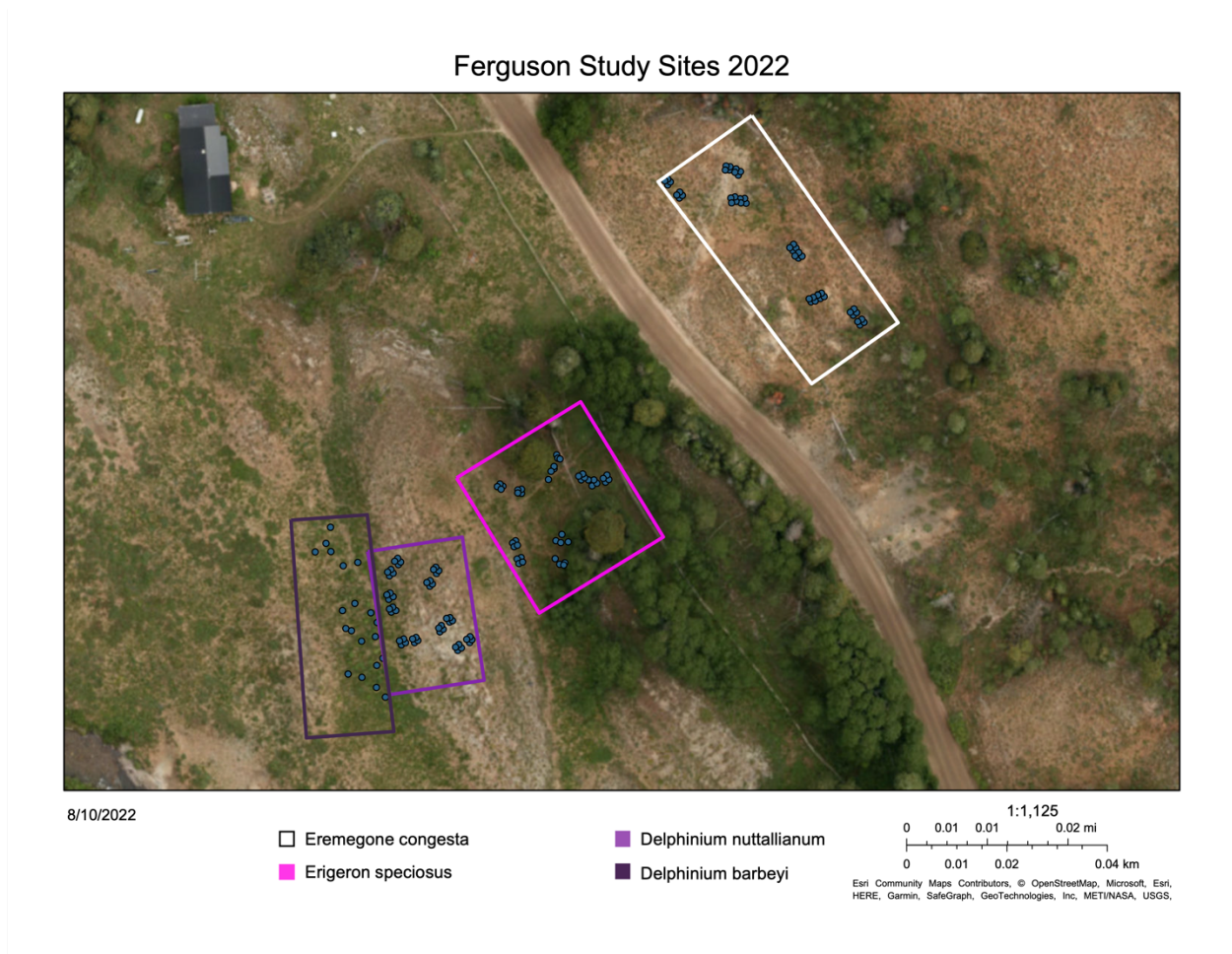


Figure 3. Map of experimental water addition and control sites for all study species.

Watering Treatment

Plots receiving the watering treatment were watered 3 times per week. Each 1m² plot received 4 liters of water, as outlined in a previous RMBL experiment within a comparable ecosystem, following previous research in meadows at the study site by Faust & Iler (2022). The water used for experimental water addition was collected from rivers near our study plots (East River and Copper Creek) and put through a filter into a SprayMate backpack with a battery-powered nozzle. The watering pack allowed for 8L of water to be transported from the river to the plot

site at a time, enough for two treatment plots. Plots were watered in the late afternoon to minimize evaporation.

Quantification of Nectar traits

We measured three nectar traits: nectar volume (μl), nectar sugar concentration ($\text{mg}/\mu\text{l}$), and total nectar sugar (mg). Plants were sampled twice per week to quantify these nectar traits. Flowers were bagged 12-24 hours prior to nectar sampling to ensure the pollinators did not deplete nectar from study flowers, which replenish their nectar supply once per day. A potential limitation of our methodology is that nectar replenishment could've been impacted in a way we could not measure (Luo et al, 2014). Nectar was sampled from flowers using Drummond microcapillary tubes. The volume of the microcapillary tube used depended on the species being sampled, with a range of 1–5 μl , based on previous research (Kirschke & CaraDonna unpublished data). Once the nectar was in the tube, a ruler was used to determine how much of the tube was filled with nectar. This measurement of the proportion of the microcapillary tube taken up by nectar, along with the total volume of the tube, was used to calculate the volume of nectar sampled. The nectar was then dispensed onto a handheld sugar refractometer and read for sugar concentration. The sugar concentration was multiplied by the nectar volume to obtain total nectar sugar content (mg).

For the flower species that could not be directly sampled due to the structure of their nectaries (*Erigeron speciosus*, and *Eremegone congesta*), the flowers were centrifuged to extract the nectar. For *Erigeron speciosus*, the ray peddles were removed before the flower was centrifuged. For *Eremegone congesta*, which does not have ray peddles, the flower was placed into the

centrifuge tube intact. All of the flowers on one stalk of *Eremegone congesta* were centrifuged together, and the volume was divided by the number of flowers on the stalk. From there, the process is the same as for the other flowers, treating the small centrifuge tube as a large nectary. Statistical analysis was run using a linear mixed effects models with experimental water addition treatment as predictor variables, nectar traits as response variables, date as an interaction term, and plot as a random intercept term. All statistical tests were performed using R statistical software (v4.2.0, R Core Team 2022).

Results

As predicted, the different species responded to water addition treatment in different ways. The *Delphinium* species had similar results to one another, while the remaining species responded in ways not predicted in our hypotheses. *Delphinium nuttallianum* maintained its nectar traits for volume, concentration, and sugar content when compared to the control with little variation and outliers (Figure 2). *Delphinium barbeyi* responded similarly to *Delphinium nuttallianum*, with both species aligning with our null hypothesis (Figure 2.1). *Eremegone congesta* showed no significant change for sugar concentration, but there was a significant decrease in nectar volume and sugar content (Table 1, Figure 2.2). *Erigeron speciosus* showed no significant change in nectar volume or concentration between control and water addition plots, but there was a marginal increase in sugar content for the water addition plots (Table 1, Figure 2.3).

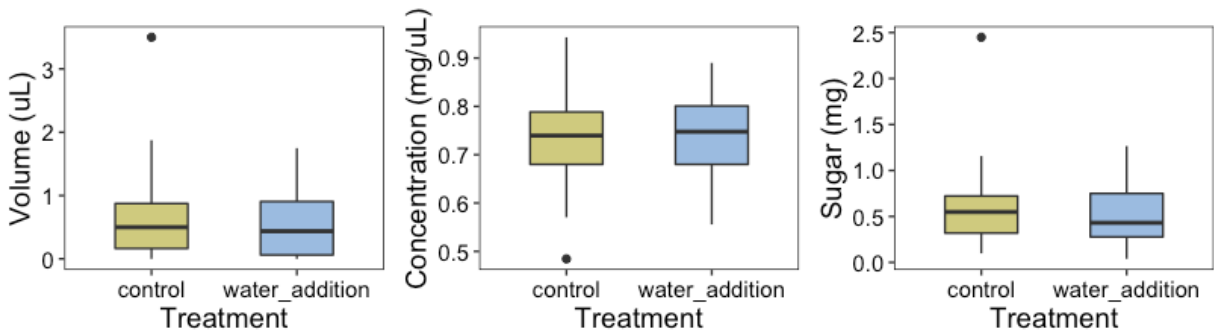


Figure 2. Means of control and water addition of nectar traits (volume, concentration, and sugar content) for *Delphinium nuttallianum*.

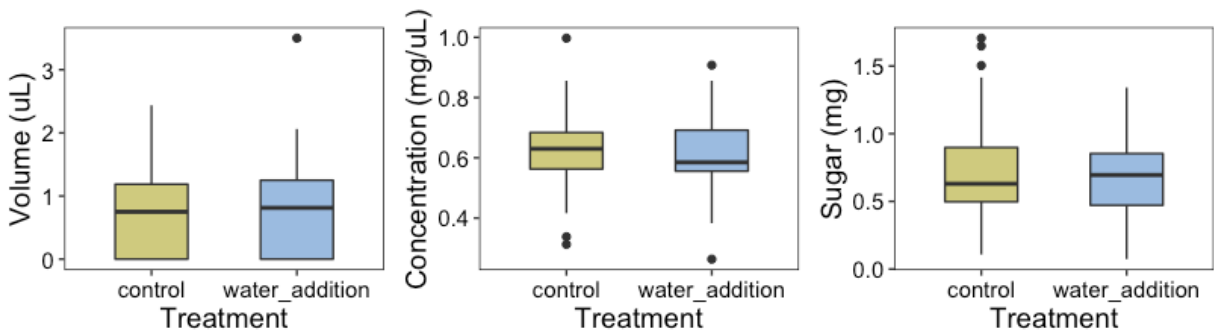


Figure 2.1. Means of control and water addition of nectar traits (volume, concentration, and sugar content) for *Delphinium barbeyi*.

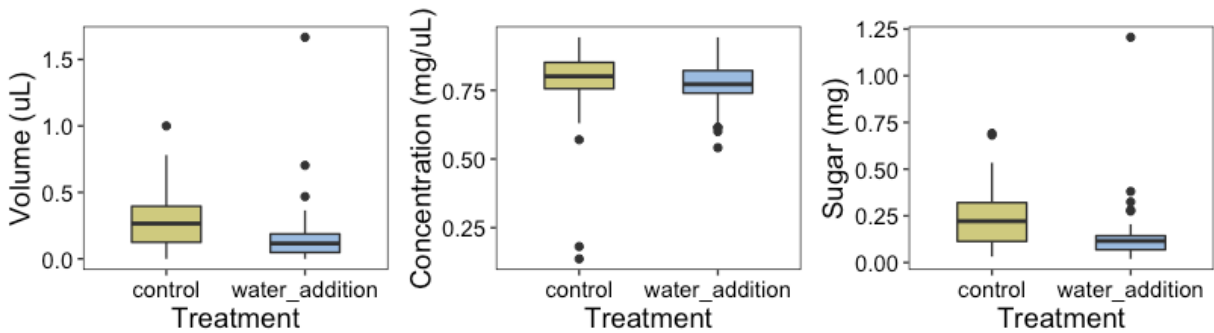


Figure 2.2. Means of control and water addition of nectar traits (volume, concentration, and sugar content) for *Eremegone congesta*.

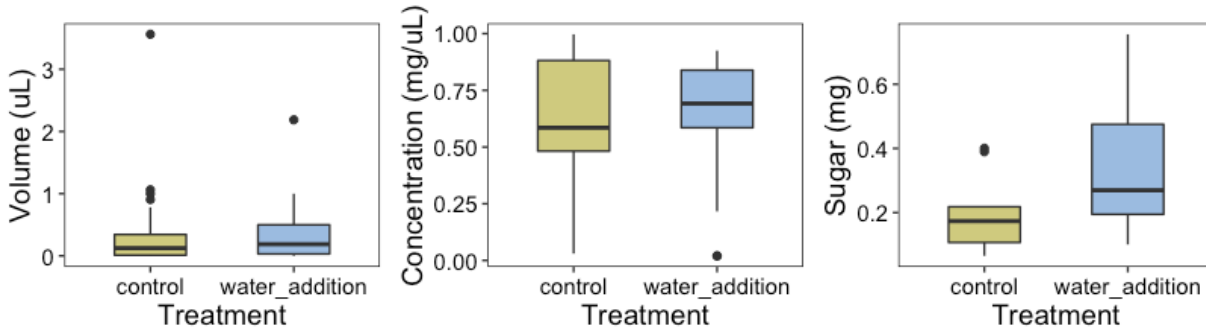


Figure 2.3. Means of control and water addition of nectar traits (volume, concentration, and sugar content) for *Erigeron speciosus*.

Table 1. Values from statistical analysis of mixed effects linear model and means of nectar traits for control and water addition plots.

Species Code	Nectar Trait	Mean (water addition)	Mean (control)	F Statistic	P Value
DEBA	Volume (μL)	0.789	0.741	0.386	0.535
	Concentration ($\text{mg}/\mu\text{L}$)	0.609	0.617	0.165	0.686
	Sugar Content (mg)	0.678	0.692	0.087	0.769
DENU	Volume (μL)	0.513	0.609	1.957	0.164
	Concentration ($\text{mg}/\mu\text{L}$)	0.739	0.732	0.204	0.653
	Sugar Content (mg)	0.531	0.573	0.826	0.366
ERCO	Volume (μL)	0.163	0.299	9.683	0.002
	Concentration ($\text{mg}/\mu\text{L}$)	0.767	0.775	0.106	0.746
	Sugar Content (mg)	0.152	0.246	7.011	0.01
ERSP	Volume (μL)	0.339	0.398	0.131	0.719
	Concentration ($\text{mg}/\mu\text{L}$)	0.653	0.592	0.222	0.641
	Sugar Content (mg)	0.328	0.193	3.355	0.079

Discussion

Results Summary/Implications

The different species responded in different ways to increased water availability, which may predict how plants allocate resources in various conditions. Experimental addition of 12 liters of water per week appeared to have little effect on nectar traits for 2 of our 4 study species. This finding suggests that, at least for these species, water may not be a primary limitation on the quality and quantity of nectar production. One species responded in a way not indicated by our four hypotheses, with decreases in nectar production in a condition of increased water availability. The different responses of our study species show the varied consequences communities face in a state of global change.

Eremegone congesta had a decrease in volume and sugar content for the water addition plots. We hypothesize that 12L of water per week was enough to overwhelm the plant with too many resources, causing its nectaries to become stressed and not produce as much nectar, which is a response that was not previously predicted in our hypotheses. This is because the concentration of the nectar remained the same between the control and treatment, suggesting the plant is producing the same amount of nectar, but a lower volume in the increased water availability condition. *Eremegone congesta* was the most sensitive to water availability change out of the species studied. *Eremegone congesta* generally prefers drier areas with more rocky soil (Ackerfield, 2015). Results from *Eremegone congesta* suggest that too much water may not be beneficial for nectar production.

Erigeron speciosus had results consistent with our first hypotheses for nectar volume and concentration. However, *Erigeron speciosus* did show a marginal increase in sugar content, which is a result not previously predicted (Table 1) (Figure 2.3).

Both *Delphinium* species responded similarly to increased water availability despite difference in habitat preference. *Delphinium nuttallianum*, like *Eremegone congesta*, prefer drier conditions with rocky soil. Despite this preference for dry habitat conditions, *Delphinium nuttallianum* was more resistant to changes in water availability than *Eremegone congesta*. *Delphinium barbeyi* also showed no significant change between control and water addition plots for all nectar traits assessed. *Delphinium barbeyi* had a high incidence of florivory, making some of its flowers unable to be sampled (see Appendix). *Delphinium* species appear to be less sensitive to changes in water availability, which may make them more attractive to pollinators due to a consistency in their nectar traits that cannot be promised by more sensitive species. *Delphinium barbeyi* also has a large number of flowers per plant, making them popular for a variety of pollinator species (Williams et al, 2001).

Fifth hypothesis

Analysis of the response of *Erigeron speciosus* led us to develop a fifth hypothesis, specifically for plants that thrive in drier environments. Our fifth hypothesis predicts that if plants prefer drier conditions, then increased water availability may result in reduced nectar production. This could be due to destruction of nectary structures or an excess of water resources.

Future Research

The various species studied in this experiment responded differently to the same water addition treatment, presumably due to habitat preferences and resistance to changes in water availability.

Future research would benefit from assessing a larger variety of species across a spectrum of habitat preferences. It would also be beneficial to compare plant families at a larger scale than this experiment to see if there is a phylogenetic relationship to resistance or sensitivity to changes in water availability. A repetition of this experiment could also benefit from multiple water addition treatments, as opposed to one. Creating a gradient of water availability with intervals could assist in more specifically identifying the level of water availability to initiate change by species.

This experiment considered nectar as an aspect of plant-pollinator interactions; however, we aren't sure how water availability influences the relationship directly. It would be interesting to do pollinator observations to see if there is a difference in visitation between control and water addition plots. Additionally, it would be useful in determining the role that nectar traits play in plant-pollinator interactions at the microclimate level. In this case, there would need to be accommodations to ensure the plots are not observed while flowers are bagged.

Utilizing measurements of water use efficiency at the end of the experiment could also aid in understanding the allocation of additional water availability by species. Depending on habitat preference, water use efficiency could increase or decrease. Certain species have evolved either low or high water use efficiency mechanisms to increase their fitness within their environment (Campitelli et al, 2016). These adaptations could impact how plants respond to consequences of global change.

Appendix: Florivory in the nectaries of *Delphinium barbeyi*

When we were sampling *Delphinium barbeyi*, there was noticed a high incidence of florivory in within the nectaries. Specifically, we observed that some nectaries were browned and had a stunted shape (Figure 4). Other flowers had larvae within the nectary (Figure 4). To further

investigate this observation, we sampled nectar standing crop and took note of the incidence of florivory. For some plants, florivory damage was as high as 100% for the flowers sampled (Table 2). Most of the florivores were insects in the larval stage. A majority of the nectaries with bugs or evidence of larval florivory were no longer producing nectar. This could be because the infestation destroyed the nectary structure, or the plant could be choosing to no longer allocate nectar resources to flowers with florivory present. It would be interesting to investigate the prevalence of florivory in this species across time and space, how florivory contributes to the nectar traits of the remaining flowers on the plant, and how nectar traits compare between florivorized and unflorivorized plants.



Figure 4. Photos of florivory in *Delphinium barbeyi*. Furthest left photo depicts a healthy nectary capable of sampling. The center image depicts a damaged nectary due to previous bug infestation. Furthest right image depicts a larvae found within a nectary.

Table 2. Percent florivory damage for standing crop sampling in *Delphinium barbeyi* (see Appendix)

Plant Number	Sampling Round	Percent Florivory
1	1	66.7
	2	71.4
	3	33.3
	4	42.9
2	1	76.9
	2	50
	3	10
	4	75
3	1	42.9
	2	28.6
	3	50
	4	10
4	1	73.3
	2	100
	3	62.5
	4	12.5
5	2	50
	3	41.7

References

- Ackerfield, J. (2015). *Flora of Colorado*. Brit.
- Campitelli, B. E., Des Marais, D. L., & Juenger, T. E. (2016). Ecological interactions and the fitness effect of water-use efficiency: Competition and drought alter the impact of natural MPK12 alleles in *Arabidopsis*. *Ecology letters*, 19(4), 424-434.
- CaraDonna, P. J., & Waser, N. M. (2020). Temporal flexibility in the structure of plant–pollinator interaction networks. *Oikos*, 129(9), 1369-1380.
- de Vega, C., Álvarez-Pérez, S., Albaladejo, R. G., Steenhuisen, S. L., Lachance, M. A., Johnson, S. D., & Herrera, C. M. (2021). The role of plant–pollinator interactions in structuring nectar microbial communities. *Journal of Ecology*, 109(9), 3379-3395.
- Elliott, J., Deryng, D., Müller, C., Frieler, K., Konzmann, M., Gerten, D., ... & Wisser, D. (2014). Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proceedings of the National Academy of Sciences*, 111(9), 3239-3244.
- Faust, M. N., & Iler, A. M. (2022). Pollinator-mediated reproductive consequences of altered co-flowering under climate change conditions depend on abiotic context. *Climate Change Ecology*, 3, 100043.
- Gallagher, M. K., & Campbell, D. R. (2017). Shifts in water availability mediate plant–pollinator interactions. *New Phytologist*, 215(2), 792-802.
- Lawson, D. A., & Rands, S. A. (2019). The effects of rainfall on plant–pollinator interactions. *Arthropod-Plant Interactions*, 13(4), 561-569.

Luo, E. Y., Ogilvie, J. E., & Thomson, J. D. (2014). Stimulation of flower nectar replenishment by removal: a survey of eleven animal-pollinated plant species. *Journal of Pollination Ecology*, 12, 52-62.

Lynch, M. (2018, June 19). *In Indonesia, villagers find innovative ways to adapt to climate change*. Conservation International. Retrieved June 30, 2022, from <https://www.conservation.org/blog/in-indonesia-villagers-find-innovative-ways-to-adapt-to-climate-change>

Parachnowitsch, A. L., Manson, J. S., & Sletvold, N. (2019). Evolutionary ecology of nectar. *Annals of Botany*, 123(2), 247-261.

Powers, J. M., Briggs, H. M., Dickson, R. G., Li, X., & Campbell, D. R. (2021). Earlier snow melt and reduced summer precipitation alter floral traits important to pollination. *Global Change Biology*, 28(1), 323-339.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Strzepek, K., Yohe, G., Neumann, J., & Boehlert, B. (2010). Characterizing changes in drought risk for the United States from climate change. *Environmental Research Letters*, 5(4), 044012.

Waser, N. M., & Price, M. V. (2016). Drought, pollen and nectar availability, and pollination success. *Ecology*, 97(6), 1400-1409.

Williams, C. F., Ruvinsky, J., Scott, P. E., & Hews, D. K. (2001). Pollination, breeding system, and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species. *American Journal of Botany*, 88(9), 1623-1633.