

The Effects of Exclusion Bags and Open-Top Chambers on Pollinator Activity

Carter Adamson

Independent Research & Ecosystems Course

Mentor: Dr. Amy Iler

Summer 2019

Abstract

It's common for scientists to apply experimental practices that have worked well in another study to their own studies without a complete consideration of how that manipulation may impact their system. Open-top warming chambers and pollinator exclusion bags are two such common experimental manipulations that scientists may employ with unintended side effects. This study aims to understand the potential side effects that the presence of open-top chambers (OTCs) and exclusion bags may have on the pollination of open flowers at the Rocky Mountain Biological Laboratory in Gothic, CO. Pollinator observations were conducted at three sites on two plant species (*Delphinium nuttallianum* and *Potentilla pulcherrima*). Treatment plots were manipulated either by placing an OTC around the plants or placing exclusion bags over 50-75% of the flowers per plant. Control plots were left unmanipulated during the observations. Bags did not significantly impact pollinator activity on open flowers on bagged plants compared to control plants. However, OTCs significantly reduced the pollination rates of the flowers inside compared to flowers outside the OTCs. These results suggest that, while exclusion bags are suitable for continued use in pollination studies, OTCs have significant impacts that must be considered and understood before being used in systems that rely on pollination.

Introduction

One of the most fundamental tenants of science is the experimental manipulation. Outside of observational studies, all scientific inquiry involves a researcher manipulating a variable. However, sometimes if a manipulation works well in one study, other researchers may adopt it for use in their own system without considering how the consequences and efficacy of that manipulation might differ between the studies. This study examines the unintended consequences that two common experimental manipulations may have on pollination.

Exclusion Bags

Pollination failure can result from problems in any step of the process – from the pollinator's ability to identify the flowers to the plants' spatial proximity to conspecific neighbors– and may result in reduced fruit set in the short term, and decreased biodiversity in the long term (Wilcock & Neiland 2002). In order to experimentally study the effects of pollination failure, many ecologists manipulate their systems with pollinator exclusion bags, which are small bags made of a wide array of materials, including micro-fiber and polyethylene. A mesh of small pores in the bag's surface are intended to be large enough allow air and light through to the flower, but small enough to prevent all pollinators from coming into contact with the reproductive organs of the flower. When affixed over budding flowers, these bags completely prevent pollination while altering the environmental conditions around the flower as minimally as possible.

However, the mere presence of these bags makes for a large and conspicuous physical alteration to the plant itself. The effects that these bags have on the pollination of non-bagged flowers of the same plant – whether they attract, repel, or are simply ignored by prospective pollinators – is largely untested. My first research question asked how the widely-used Del Star polyethylene bags with 0.5mm pores affect the pollination of two Colorado wildflower species, *Delphinium nuttallianum* and *Potentilla pulcherrima*, at the Rocky Mountain Biological Laboratory (RMBL). These species are part of an ongoing experimental demography project at RMBL that is testing the effects of altered pollination rates on plant population dynamics. To reduce pollination rates, the study employs pollinator exclusion bags. Having a strong understanding of the effectiveness and side-effects of this experimental manipulation is crucial for any study that aims to study pollination and reproductive success on plants that contain exclusion bags.

Open-Top Chambers

Open-top chambers (OTCs) are widely used in ecological studies to simulate the effects of climate warming in a small, localized area (Marion et al. 1997). These chambers have been shown to be

accurate and effective at providing an analogue to climate warming, but their effect on pollination is largely unknown, with one paper tentatively suggesting that they may not be ideal for studying plant-animal mutualisms such as pollination (Zhang et al. 2015). In contrast, another study found no effect of OTCs on insect pollinator visitation (Robinson & Henry 2018), pointing to the need for further research. If OTCs are to continue being used in systems where animal pollination is an essential component of plant reproduction, then a better understanding of their effects on pollinator activity must be built. As such, my second research question asked how the presence of open-top chambers affects the pollination of the same two wildflower species, *Delphinium nuttallianum* and *Potentilla pulcherrima*.

Methods

Study Sites

This study was conducted at three sites near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, from June through August of 2019. At an elevation of approximately 2900 meters above sea level, RMBL is an ideal site for pollination studies due to high diversity of perennial wildflower species and a strong community of native bees. All three sites were located within 50 meters of a county road. See Figures 6 and 7 for maps and coordinates of the study sites.

Study Species

Both *Delphinium nuttallianum* and *Potentilla pulcherrima* are iteroparous perennial wildflowers that have been well-studied at RMBL. *Delphinium* flowers exhibit bilateral symmetry and are mainly pollinated by bumble bee queens and broad-tailed hummingbirds (Waser & Price 1981). *Potentilla* flowers exhibit radial symmetry and are pollinated by a diversity of flies and bees (CaraDonna et al. 2017). Additionally, *Potentilla* is capable of autogamous self-pollination (Burkle & Irwin 2010). The species were chosen based on their involvement in the experimental demography project, as well as their differing flowering phenology; *Delphinium* blooms earlier than *Potentilla*, facilitating data

collection throughout the season. *Delphinium* data were collected over the course of 33 days from June 21 to July 24, and *Potentilla* data were collected over the course of 30 days from July 10 to August 9.

Experimental Design

In order to determine the effects of these manipulations of pollinator activity, the response variables for these experiments were the number of pollinator visitors, the number of pollinator visits, pollinator visitation rate (number of pollinator visits per flower), and pollen deposition (number of pollen grains per stigma). Pollen deposition was only measured for the second research question to avoid any potential consequences of stigma collection on plant reproduction from plants within the experimental demography project.

To test the first research question, I compared pollinator visitation rate between open and bagged treatments of both target species. This was accomplished by visiting existing sites in use for the Iler lab's investigation of the effects of pollinator declines on plant populations, which includes five different plant species and four levels of pollination. The plots at these sites were 1m by 10m, with eight treatments per plot arranged according to a randomized block design. The only subplots among these that fell under the scope of my investigation were those that used the reduced pollination and control treatments at the *D. nuttallianum* and *P. pulcherrima* sites. In the reduced pollination treatment, approximately 50% of the flowers per plant were covered by pollinator exclusion bags. The control plants were unmanipulated, resulting in a higher average number of flowers in the control treatment than the bagged treatment. Due to this, response variables like the number of pollinator visitors and visits were not suitable – visitation rate was determined by dividing the number of visits by the number of flowers. These visitation data were gathered through five-minute visual observations of 1m x 1m portions of these plots, recording the number of pollinator visits and the number of flowers. These observations were repeated many times for each plot over the flowering period of each plant species, such that over one hundred replicate data points were gathered for each treatment and each species.

To test the second research question, I compared pollination between control plots and plots with OTCs at another Iler lab site that is shared with Drs. Kailen Mooney and Will Petry, following the same pollinator observation protocol outlined above. Unlike the bags, the presence of chambers did not reduce the average number of available flowers in the plots, meaning that in addition to visitation rate, the number of visiting pollinators and the total number of visits were also able to be measured as response variables. Halfway through the data collection period, on-site conflicts forced me to switch from collecting data in fourteen plots with seven chambers each, to only seven chambers total. On every day that pollinator observations were conducted, flowers inside and adjacent to the chambers (within a 2m radius) were observed. For the OTC research question, pollen deposition was also included as a response variable, as measured by the number of pollen grains per stigma. Stigmas were collected by using forceps to remove stigmas from two flowers of each species in each chamber and the same number from outside the chambers. This totaled fourteen flowers of each species in the chambered treatment, and fourteen in the non-chambered treatment. The stigmas were squashed on microscope slides with fuchsin dye so that conspecific pollen grains could be counted under a compound microscope. I made a reference slide of each species' pollen to facilitate the identification of conspecific pollen grains.

Analysis

To determine whether pollinator visitation rates differed between bagged and unbagged plants, I attempted to use a generalized linear mixed effects model with visitation rate as a binomial response variable, and date as a random intercept term to account for repeated samples across dates. However, this model was very overdispersed, so I averaged visitation rates across all observation sessions for each day of the study, within each treatment, to avoid pseudoreplication from repeated observations of the same plots. I then compared mean visitation rates between treatments with a Welch two-sample t-test.

To determine whether the number of pollinator visitors differed between plants within and without chambers, I attempted to use a generalized linear mixed effects model with the number of visitors as a binomial response variable, and date as a random intercept term to account for repeated samples across dates. However, this model failed to converge. I therefore averaged the number of visitors across all observation sessions for each day of the study, within each treatment, to avoid pseudoreplication from repeated observations of the same plots. I then compared mean number of visitors between treatments with a Welch two-sample t-test.

To determine whether the number of pollinator visits differed between plants within and without chambers, I used a generalized linear effects model with the number of visits as a binomial response variable, and date as a random intercept term to account for repeated samples across dates.

To determine whether pollinator visitation rates differed between plants within and without chambers, I used a generalized linear effects model with visitation rate as a binomial response variable, and date as a random intercept term to account for repeated samples across dates.

To determine whether pollen deposition differed between plants within and without chambers, I used a generalized linear effects model with the number of pollen grains per stigma as a binomial response variable, and plot as a random intercept term to account for stigmas being gathered from multiple plants per treatment.

Results

Exclusion Bags

The presence of exclusion bags did not have a significant effect on the average visitation rate to *Delphinium nuttallianum* (Welch two-sample t-test; $t = -0.20035$, $n = 23$ days, $p = 0.8434$) or *Potentilla pulcherrima* (Welch two-sample t-test; $t = -0.13931$, $n = 14$ days, $p = 0.8915$). The maximum mean visitation rate was .0015 visits/flower higher in non-bagged treatment for *D. nuttallianum*, and the

median mean visitation rate was .032 higher in non-bagged treatments for *P. pulcherrima* (Figure 1), but these differences were not statistically significant. We fail to reject the null hypothesis that exclusion bags have no effect on pollination of nearby flowers.

Open-Top Chambers

The presence of open-top chambers significantly reduced the mean number of visitors per observation session per day on *D. nuttallianum* (Welch two-sample t-test; $n = 16$, $p = 0.01287$), with the no-chamber treatment having a mean of 0.377 visitors compared to a mean of 0.040 visitors for the chamber treatment (Figure 3). The presence of open-top chambers also significantly reduced the mean number of visitors per observation session per day on *P. pulcherrima* (Welch two-sample t-test; $n = 12$, $p = 0.01299$), with the no-chamber treatment having a mean of 0.421 visitors compared to a mean of 0.051 visitors for the chamber treatment.

For the number of visits, a linear mixed effects model was used with treatment and plant species as fixed effects and date of the observation session as a random intercept. The best model for the number of visits included an additive effect of species but no species by treatment interaction ($\Delta AIC = 1.7156$). The model showed that the chambers significantly reduced the number of visits per observation session and that species had no effect on the number of visits (Table 1; Figure 4).

For visitation rate, a linear mixed effects model was used with treatment and plant species as fixed effects. The best model included the species by treatment interaction ($\Delta AIC = 394.7156$), although this interaction was not significant, indicating that the effect of treatment does not depend on species (Table 2). The model showed that the chambers significantly reduced the visitation rate and that visitation rates did not differ between species (Table 2; Figure 2). For both species, the daily visitation rate per flower is significantly higher in the control treatments, with a median of zero for the OTC treatments (Figure 2).

For pollen deposition, a linear mixed effects model was used with treatment and species as fixed effects. The best model for pollen deposition included the species by treatment interaction ($\Delta AIC = 22.71$). The model showed that the chambers significantly reduced the amount of pollen grains found on stigmas, and that this effect was stronger for *Delphinium* than for *Potentilla* (Table 3; Figure 5)

Discussion

This study investigated the effects of two common experimental manipulations and found evidence that pollinator exclusion bags have no effect on pollination of open flowers, while OTCs reduce pollination. These results indicate that pollinator exclusion bags are not reducing pollination more than intended and support their continued use in pollination studies. However, they also indicate that OTCs are not suitable for studies where animal pollination is a key component. Further research should be conducted to determine the mechanism by which the OTCs are discouraging pollinators so that researchers can modify their use of OTCs to impact pollinators as little as possible.

Exclusion Bags

Exclusion bags had no effect on the pollination of flowers. This result confirms that the conspicuous physical presence of the bags does not deter or encourage pollinators in any way, which supports the continued widespread use of the bags. However, it is worth noting the small sample size for these results, especially for *P. pulcherrima*. Though it is not statistically significant, a trend towards lower visitation rates in the bagged treatments is clearly observable (Fig 1). It is possible that, had more data been gathered, this trend may have grown, and I would have had more statistical power to detect a difference between the treatments. This would mean that the presence of bags *does* alter pollinator behavior in some small way, which would line up well with qualitative observations of pollinators landing on the bags and attempting to reach the flowers within, which may reduce the efficiency of their foraging.

Higher visitation rates to flowers on plants without bags could occur due to pollinators selecting areas of higher flower density for foraging, given the reduced number of flowers available in the bagged treatment. Some research suggests that increasing floral density has a positive impact on visitation rate (Grindeland et al. 2005). However, other papers suggest that floral density does not impact visitation rate at all (Schmitt 1983), and still others suggest a negative relationship (Mustajärvi et al. 2001). Future studies should repeat this examination of the effects of bags on pollination, but with much larger sample sizes so that if such a pattern indeed exists, it can be better detected with increased statistical power.

In the ongoing experimental demography study that motivated this research question, bagging is used to reduce pollination. Therefore, even in the case that bags do reduce pollination of open flowers and this study did not have the statistical power to detect it, the use of bags is still justified within the context of the study.

Open-Top Chambers

The presence of OTCs significantly reduces pollinator visitation rates, the number of visits, the number of visitors, and pollen deposition for the flowers inside (Figure 2). These results indicate that researchers should not use OTCs in studies where pollination is a critical component, which corroborates the tentative suggestion of Zhang et al. (2015) that OTCs are not suited for studies of plant-insect interactions. Although this pattern is quite clear, what is less clear is why the chambers have this effect. I have three hypotheses as to why this may be. First, one salient difference between exclusion bags and OTCs is that exclusion bags do not alter environmental conditions within, whereas OTCs are specifically designed to alter air temperature and may have additional effects on abiotic factors such as soil temperature and moisture (Dabros et al. 2010). It is possible that this alteration of environmental conditions is what discourages prospective pollinators. Second, it's possible that pollinators may choose not to fly up and into the chambers to reach flowers if there is a large abundance of flowers immediately outside the chamber. I observed that often when pollinators began foraging near OTCs, they chose to

pollinate the flowers around the OTC rather than enter it. And third, the OTC may impose a vertical barrier that is simply too high for the bees to forage past. Previous studies have shown that many pollinator groups, including bees and butterflies, have a preferred height range that they are not inclined to leave while foraging, which is referred to as foraging height constancy (Faulkner 1975; Levin & Watkins 1984). Additionally, they tend to forage low to the ground, starting with a plant's bottom-most flowers (Haynes & Mesler 1984). Future studies should attempt to determine which, if any, of these three hypotheses may explain why visitation was so low inside the OTCs. Such a study could involve treatments with dummy chambers that do not affect environmental conditions to address the first hypothesis, treatments with varying densities and abundances of flowers around the chambers to address the second, and treatments with shorter chambers, or chambers with holes at foraging height for pollinators to pass through, to address the third. If the reason why OTCs reduce visitation rates can be determined, then researchers can try to design OTCs to have less of an effect on visitation rates while still warming air temperatures (e.g. by designing shorter OTCs if the third hypothesis holds true).

It's worth noting that for pollen deposition, the results were much stronger for *Delphinium* than *Potentilla*. This may be due to carelessness when emasculating *Potentilla* before collecting the stigmas, or perhaps due to *Potentilla*'s capacity for self-pollination.

Acknowledgements

Thanks to Dr. Amy Iler for her immensely helpful assistance and support in every aspect of this study, Drs. Nick Waser and Mary Price for their comments and the use of laboratory equipment, and to the Rocky Mountain Biological Laboratory for a generous scholarship that helped to make this study possible.

Literature Cited

- Burkle, L.A. & Irwin, R.E. (2010). Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. *Journal of Ecology*, 98(3): 705-717.
- CaraDonna, P.J., Petry, W.K., Brennan, R.M., Cunningham, J.L., Bronstein, J.L., Waser, N.M., & Sanders, N.J. (2017). Interaction rewiring and the rapid turnover of plant-pollinator networks. *Ecology Letters*, 20(3): 385-394.
- Dabros, A., Fyles, J.W., & Strachan, I.B. (2010). Effects of open-top chambers on physical properties of air and soil at post-disturbance sites in northwestern Quebec. *Plant and Soil*, 333(1): 203-218.
- Faulkner, G.J. (1975). Honeybee behaviour as affected by plant height and flower color in brussels sprouts. *Journal of Apicultural Research*, 15(1): 15-18.
- Grindeland, J.M., Sletvold, M., & Ims, R.A. (2005). Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Functional Ecology*, 19(3): 383-390.
- Haynes, J. & Mesler, M. (1984). Pollen foraging by bumblebees: Foraging patterns and efficiency on *Lupinus polyphyllus*. *Oecologia*, 61(2): 249-253.
- Levin, D.A. & Watkins, L. (1984). Assortative mating in *Phlox*. *Heredity*, 53(3): 595-602.
- Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Levesque, E., Molau, U., Molgaard, P., Parsons, A.N., Svoboda, J., & Virginia, R.A. (1997). Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, 3(1): 20-32.
- Mustajärvi, K., Siikamäki, P., Rytkönen, S., & Lammi, A. (2001). Consequences of plant population size and density for plant-pollinator interactions and plant performance. *Journal of Ecology*, 89(1): 80-87.
- Robinson, S.V.J. & Henry, G.H.R. (2018). High Arctic plants show independent responses to pollination and experimental warming. *Botany*, 96(6): 385-396.

Schmitt, J. (1983). Flowering plant density and pollinator visitation in *Senecio*. *Oecologia* 60(1): 97-102.

Waser, N.M. & Price, M.V. (1981). Pollinator choice and stabilizing selection for flower color in

Delphinium nelsonii. *Evolution*, 35(2): 376-390.

Wilcock, C. & Neiland, R. (2002). Pollination failure in plants: why it happens and when it matters.

Trends in Plant Science, 7(6): 270-277.

Zhang, C., Wang, L., Yang, Y., & Duan, W. (2015). Flower evolution of alpine forbs in open top chambers

(OTCs) from the Qinghai-Tibet Plateau. *Scientific Reports*, 5: 10254.

Tables & Figures

Table 1. Results of the mixed linear effects model for the number of visits in the OTC experiment.

	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-1.9773	0.3702	-5.341	< 0.0001
Treatment	2.0759	0.4184	4.962	< 0.0001
Plant Species	-0.1692	0.4439	-0.381	0.703

Table 2. Results of the mixed linear effects model for visitation rate in the OTC experiment.

	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-5.4844	0.4492	-12.210	< 0.0001
Treatment	2.5356	0.4264	5.946	< 0.0001
Plant Species	0.8493	0.6772	1.254	0.210
Treatment x Species	-0.8600	0.6584	-1.306	0.191

Table 3. Results of the mixed linear effects model for pollen deposition in the OTC experiment.

	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	1.8969	0.1604	11.830	< 0.0001
Treatment	1.2840	0.2639	4.866	< 0.0001
Plant Species	0.7452	0.1499	4.972	< 0.0001
Treatment x Species	-1.1413	0.2255	-5.062	< 0.0001

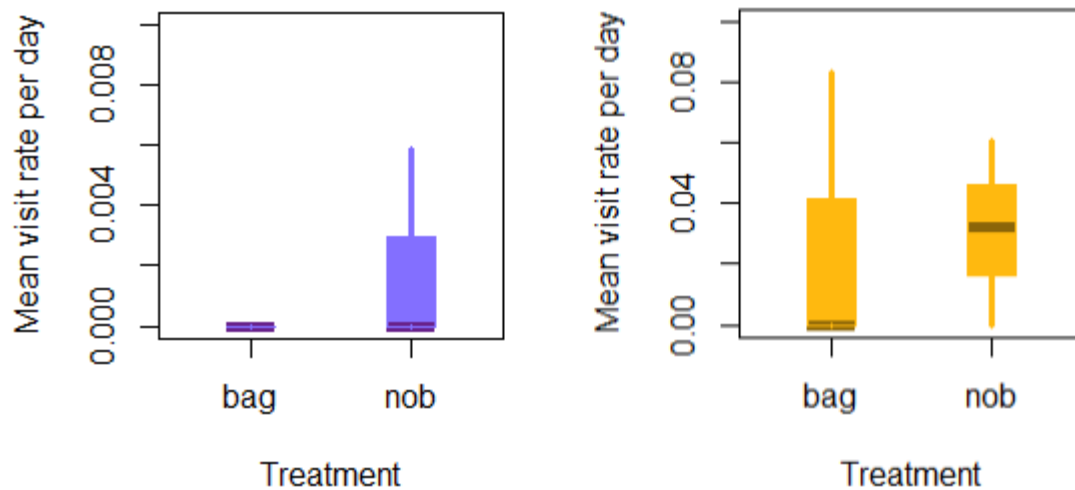


Figure 1. Treatment vs. mean visitation rate per day for *D. nuttallianum* (blue) and *P. pulcherrima* (yellow) in the exclusion bag experiment. “bag” refers to the bagged treatments, whereas “nob” refers to the control treatments.

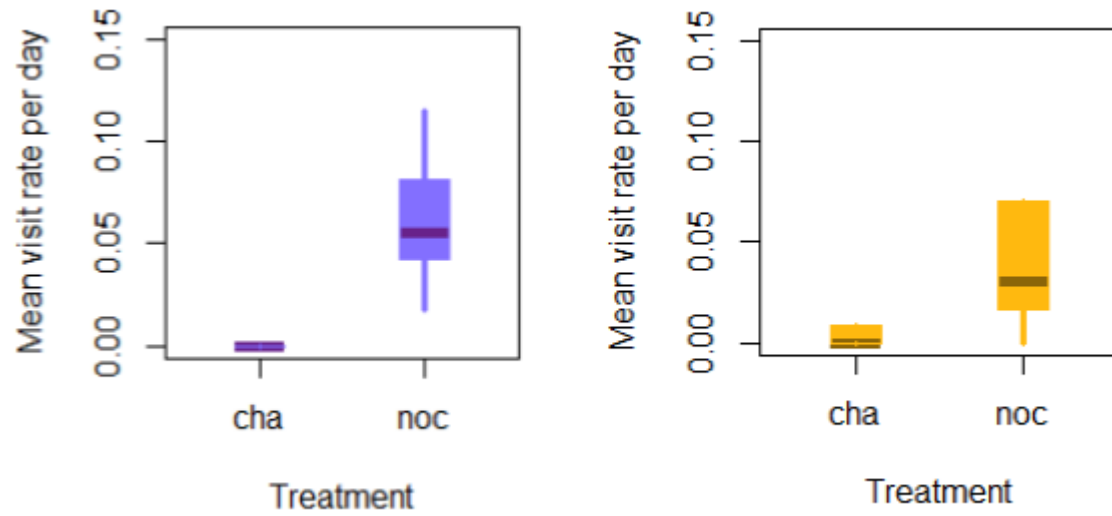


Figure 2. Treatment vs. mean visitation rate per day for *D. nuttallianum* (blue) and *P. pulcherrima* (yellow) in the OTC experiment. “cha” refers to the OTC treatments, whereas “noc” refers to the control treatments.

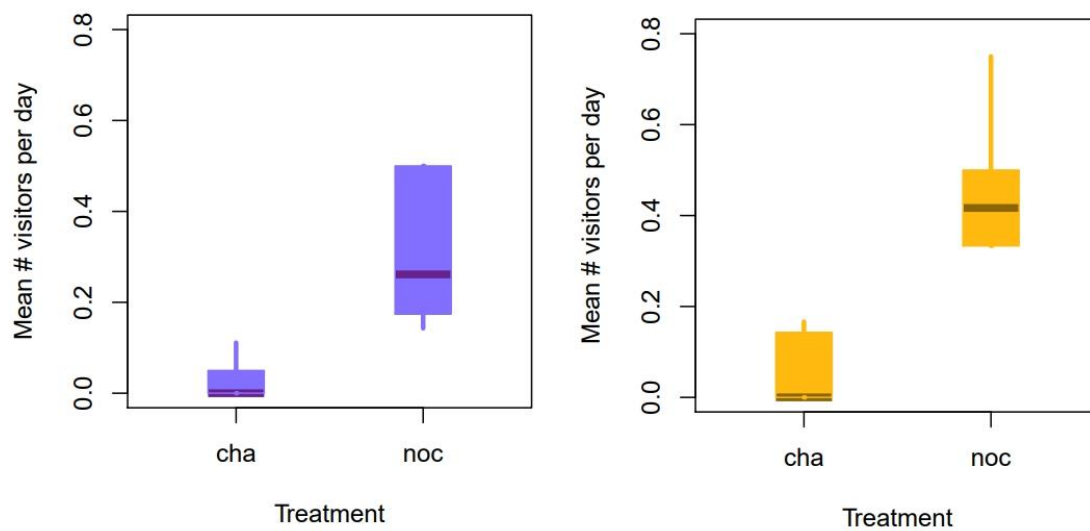


Figure 3. Treatment vs. number of visitors per observation session per day for *D. nuttallianum* (blue) and *P. pulcherrima* (yellow) in the OTC experiment.

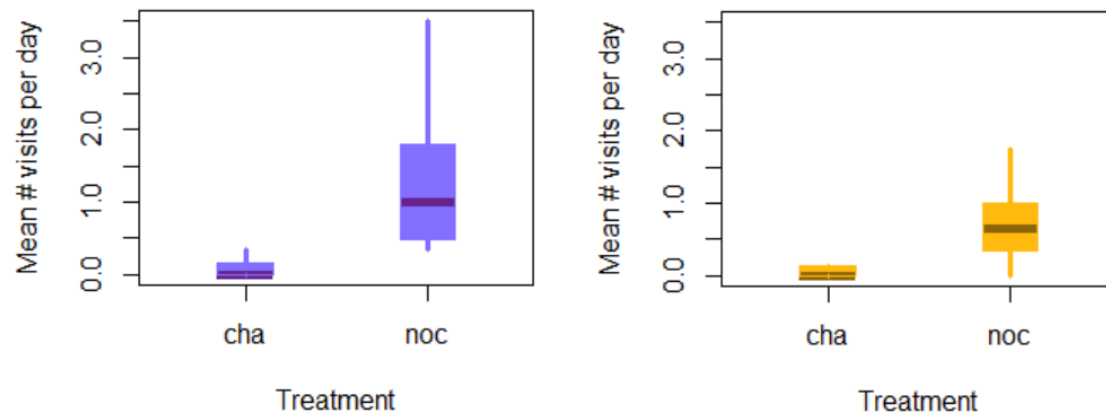


Figure 4. Treatment vs. number of visits per observation session per day for *D. nuttallianum* (blue) and *P. pulcherrima* (yellow) in the OTC experiment.

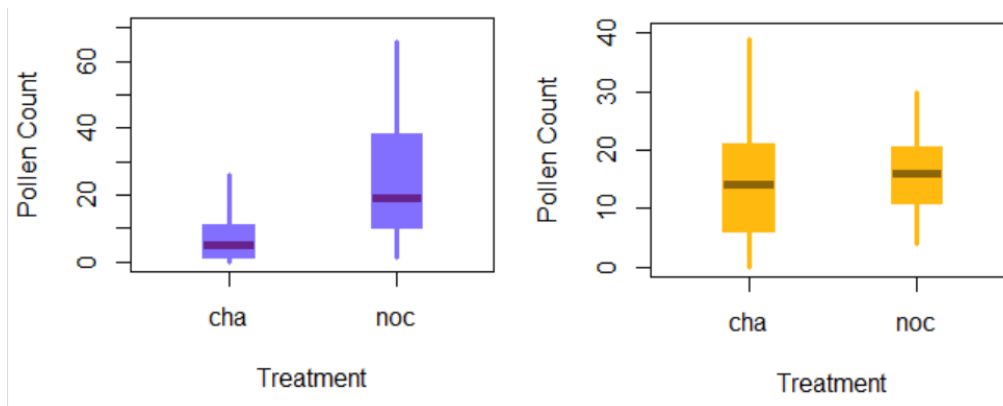


Figure 5. Treatment vs. pollen grains per stigma for *D. nuttallianum* (blue) and *P. pulcherrima* (yellow) in the OTC experiment.



Figure 6. Study sites for the exclusion bag experiment. The *D. nuttallianum* plots are blue, the *P. pulcherrima* plots are yellow, and the bagged treatments are indicated by pink circles. *D. nuttallianum* site centerpoint: 106°59'30.138"W, 38°57'45.677"N. *P. pulcherrima* site centerpoint: 106°59'34.332"W, 38°57'43.752" N.



Figure 7. Study site for the OTC experiment after on-site conflicts reduced the number of plots and chambers available. The plots are grey and the chambers are indicated by pink circles. Centerpoint: 107°0'53.806"W, 38°59'39.449"N.