

The Effects of Early Snow-melt on the Pollination and Seed Production of *Delphinium
nuttallianum*.

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Abstract: Plant-pollinator interactions are an integral part of many terrestrial ecosystems. However, phenological mismatches caused by global climate change have the potential to decouple plants and pollinators partnerships. Phenological mismatch can be defined as a change in life history timing that affects interactions between species, with the life stage of one or more species occurring earlier or later than recent historical norms. As the impacts of global climate change increase, so does the potential for phenological mismatch between species. This project examines accelerated snowmelt, an artifact of climate change, and its impacts on the phenology, pollination, and seed set of a subalpine plant, *Delphinium nuttallianum*. To test pollination limitation, we compared three pollination treatments; open, open-hand and exclusion. These treatments were conducted within two manipulated plots and two control plots. The manipulated plots simulated global climate change via accelerated snow-melt, and the control plots were kept consistent. We hypothesized that *D. nuttallianum* was pollen limited and that pollination and seed production would be lower in plots with accelerated snow melt. From this experiment, we observed that *D. nuttallianum* is potentially pollen limited, however accelerated snow-melt, in our case, led counterintuitively to higher seed set.

Introduction: Pollination is critical to the reproduction of thousands of species of plants (Ollerton et al. 2011) and affects a wide variety of natural and anthropogenic ecosystems. Within agricultural systems specifically, pollinators offer an important service that many plants depend on for fruit and seed set. Pollinator-plant interactions have persisted for millions of years, and have provided a foundation for humans to mimic them via hand pollination, thus increasing productivity for growers and food for millions of people world-wide. This contribution led to an estimated \$29 billion dollars in production for the US alone in 2010 (Calderone, 2012). Outside of agricultural systems, pollination contributes to increased seed set in 70% of all flowering plants (Wilson and Carril, 2016).

However, global climate change poses possible challenges for plants and pollinators in the form of mismatching phenological activity between these species. Phenology is the timing of species activity dependent on climate. Therefore, if an area is subjugated to a significant change

in climate, it could affect the interactions of species, including reproductive aspects. . In low elevation environments, climate change may result in longer growing seasons, earlier migrations, and earlier reproduction in many species of animals and plants (Inouye et al. 1999). Flowers may bloom earlier due to accelerated snow melt, pollinators may fail to track this change in timing, leading to a decrease in pollination services and seed production (Schweiger et al. 2008). Gezon et al. (2016) experimentally removed snow to determine the potential effects of global climate change on plant phenology and how that affects pollinator-plant interaction. They found that the flowering of *Claytonia lanceolata* occurred approximately 10 days earlier within snow-removal plots as compared to their control plots. With the snow melt manipulations, there was also higher pollinator visitation rates, increased frost damage to plants and lower reproduction rates (regardless of pollen limitation potential.) However, in the early snow melt manipulation, plants that survived the frost damage showed higher reproductive success (Gezon et al. 2016).

Flowering plants display reproductive success through two phases; pre-emergent and post-emergent success. Pre-emergence can be defined as the amount of seeds that are able to germinate within their immediate environment, while post-emergence can be defined as the amount of seeds that successfully germinate and produce offspring (Wiens et al. 1987). For plants that require biotic pollination, the potential for shifts in earlier phenological activity due to climate change may cause flowering plants to decrease reproductive success rates within these two phases. In addition, potential shifts in the behavior of the insects that pollinate these plants could also lead to decreased seed production. For example, populations of insects that originate in the north have been shifting to genetically resemble insects that originate in the south (Bradshaw and Holzapfel, 2006). These combined changes may provide the potential for mismatch between flower emergence and pollinator activity leading to a decrease in successful pollination and seed production in flowering plants.

For my research project, I tested the effects of increased snow-melt on the pollination and seed production of *Delphinium nuttallianum*. This was conducted using a snowmelt manipulation experiment, designed to accelerate snowmelt and flowering timing while keeping naturally available pollinators from the surrounding area relatively unaffected. *Delphinium nuttallianum* is a wildflower in the family Ranunculaceae that is prevalent in the western United States in ecosystems such as Mesic Meadows. In the Colorado Rocky Mountains, *D. nuttallianum* blooms

in early spring (late May-mid June) and may be an important food resource for newly emerging bumble bee (*Bombus spp.*) queens during early colony establishment (Goulson 2003). These bumblebees may also be important pollinators of this plant. However, with different suites of abiotic cues caused by global climate change, the potential for mismatches in phenology may affect the reproductive success of the plant. I hypothesized that *D. nuttallianum* will have reduced seed production within the early snowmelt manipulated plots compared to control plots, because of the possibility of pollinators, such as bumblebees, being fewer in number earlier in the season, as well as the potential for the flowers they pollinate to emerge earlier.

Methods:

Site Locations

I conducted experiments to test pollen limitation in the seed production of *D. nuttallianum* at the Rocky Mountain Biological Laboratory in Gothic, Colorado (38.98°N, -107°W). Experiments were conducted over the course of 9 weeks from June 12th to August 7th at two sites in Washington Gulch (Figure 1), named “Pfieler” (Figure 2) and “Meridian” (Figure 3). Within each site, we established two 10 x 14 meter experimental plots. These plots were spaced between 30-100 meters apart in order to keep a buffer between the manipulated and control plots, without allowing large environmental variation between plots. As perennial mesic meadows, these sites shared similar plant composition, moisture levels, aspect and slope, in order to minimize the bias within our comparisons across plots, as well as sites.

Snowmelt Manipulations

At each site, we accelerated snowmelt timing in one plot whilst the others served as an unmanipulated control. Snow melt was accelerated using black 50% shade cloth, which was laid over the snow-covered plots 4 weeks before the estimated natural snowmelt date, and served to absorb and retain more of the sun’s heat. This caused local snowmelt and subsequent plant phenology to be advanced by one to three weeks (Steltzer et al. 2009). In control plots, snow melt occurred under natural conditions.

Pollinator Observations

In order to understand possible shifts in pollinator-plant interactions due to accelerated snow melt, we conducted floral visitation observations. Through this nine week interval, we conducted observations and recorded visitation rates when a pollinator came into contact with the reproductive parts (stigma or anthers) of any blooming plant within the paired plots. This was done by using 10 meter by 1 meter sampling transects within the control and manipulated plots. We conducted timed observations of three minutes, split between two intervals of one minute and thirty seconds. The observations were held on each side of 2 meter segments within the transects of each plot. These segments were randomized and the observer would wait for a pollinator to touch the reproductive parts of the blooming plant. Once an insect was spotted on the flowers' reproductive parts, the individual stopped their timer, insects were netted and destructively sampled in order to identify to species in the lab. If a bumblebee (*Bombus spp.*) or butterfly landed in the segments during the observation, they were captured, marked and then released. These taxa were and placed into a large centrifuge tube to be identified using an identification key. Bumble bees would be chilled in a cooler bag with ice packs for approximately 10 minutes, identified to species, marked with paint pens and released, so as to account for recaptures. In addition, we assessed plant diversity between the control and the manipulated plots to understand the plant diversity and abundance and how that may influence pollinator-plant interactions between the plots. We also tracked the effects of early snowmelt on bloom timing of all flowering species by censusing individuals in four 1x1m quadrats within transects and recording number of individuals within the following phenophases: bud initiation, anthesis start, anthesis maximum, flower senescence, and ovule expansion. We also counted all fully blooming flowers within the transects to have a census to compare pollination activity with flower availability.

Pollen Limitation Experiments

Delphinium nuttallianum is often pollinated by bumble bee queens, hummingbirds and solitary bees (Bosch and Washer, 1999). Like other flowering plants, the anthers of *Delphinium* dehisce before their stamen, making it a viable test species. For this portion of the experiment, I determined the dependency of *Delphinium nuttallianum* on pollinators for successful seed production. To do this, I performed pollen limitation experiments at the two snow melt locations listed above; Meridian and Pfeiler by comparing seed counts between treatments in these plots.

We randomly assigned three experimental treatments to 15 individual plants (5 plants per treatment) and tracked the seed production of a single flower per plant labelled with a jeweler's tag. In each snowmelt and control plot, I selected five groups of three individuals that were spaced no more than 50 cm apart, and randomly assigned the three individuals in each group one of three treatments. Plants were selected in areas outside of pollinator observation transects, but within snowmelt and control plots. To isolate the effects of insect pollinators on seed production, I first excluded pollinators to all experimental individuals by covering racemes of virgin flowers (including the tagged flower) in mesh bags made of organza fabric, approximately 15 cm x 15 cm in size, gently secured to peduncles with soft wire. To prevent the weight of the mesh bags from damaging plants, bags were also be attached to supports made of stiff wire, inserted into the ground at least 5 cm away from the base of the plant. In five of the experimental individuals, I left these mesh bags for the entire duration of the study, and measured seed production when individuals received no insect pollinators ('pollinator exclusion' treatment). In another five individuals, I removed the mesh bags for a period of 7 days during peak blooming, to allow natural insect pollination to occur, and then reattached mesh bags ('natural pollination' treatment). During the 7-day pollination period, I also hand-pollinated flowers of the remaining 5 individuals twice on the first and fifth day, and I also allowed natural insect pollination to occur ('Open-Hand-pollination' treatment). This hand pollination treatment simulates zero pollen limitation, and provided a reference for the maximum reproductive output when pollen is not limiting. Within our experimental process however, due to environmental factors, we were not able to conduct Open-Hand pollination at our 'Pfiler' site, limiting our potential to draw effective conclusions for this treatment. In addition, I measured plant height and number of flowers per plant as covariates to help account for variability in seed production due to differences in individual size. Once the flowers produced seed, I counted the number of seeds produced. I then compared the reproductive success using a linear mixed effect model analyzed using the R statistical software.

Results:

We counted >600 seeds and linked seed production to pollen availability and accelerated snow melt. The exclusion treatments had a mean of 2.3 developed seeds, the open treatments had a mean of 5.45 developed seeds and the open-hand treatments had a mean of 7 developed seeds

(Table 1). The standard deviation of the developed seeds were 3.0128. For this experiment, it was seen that *Delphinium nuttallianum* produced more developed seeds at the Pfieler site and in the manipulated plots (Table 2, Table 3). Leading us to the consensus that with earlier emergence and no pollen limitation, *Delphinium nuttallianum* has a larger developed seed production than when it is pollen limited and emerges later.

Discussion:

Global climate change is an ongoing phenomenon that has the potential to cause phonological mismatch between certain plants and pollinators. One of the implementations of global climate change is accelerated snow-melt. To understand the association between *Delphinium nuttallianum* seed production and accelerated snow melt, we used a field experiment testing pollination limitation treatments on individual plants amongst controlled and manipulated plots.

We found that there is a positive correlation between hand pollination and accelerated snow melt between the sites that were used. Also, we found that the exclusion treatment producing lesser amounts of seed set in comparison to the other pollination treatments leads us to conclude that this species is dependent on pollinators.

Putting this result into context of our experimental sites and procedures may account for variable results if this study were to be duplicated. One of the more prevalent ecological issues found within our experimentation process was water availability between different elevations. Meridian, being at a higher elevation provided to have less water availability, making it difficult to hand pollinate the *Delphinium nuttallianum* before the plant withered. Pfieler on the other hand being at a lower elevation provided for relatively successful hand pollination because of the availability of water. Within these two sites, the pollen limitation may have also cause an alternation in the overall experiment. Open pollination for example, had less pollen limitation potential within the manipulated plots due to the accelerated availability of flower species to be pollinated. Also, being subjugated to a field study, the treatment plants were exposed to the potential of herbivory, anthropocentric destruction and other biotic and abiotic factors that may have influenced developed and underdeveloped seed production between both sites.

Our understanding of the implications and consequences of global climate change on plant-pollinator interaction is growing. However, there is still potential for more learning in the future for *Delphinium nuttallianum*, as well as other species of plants that pollinators are dependent on. Continued studies are essential for us to comprehend these complex systems within plant communities and have the potential to contribute to our knowledge of and preparation for global climate change.

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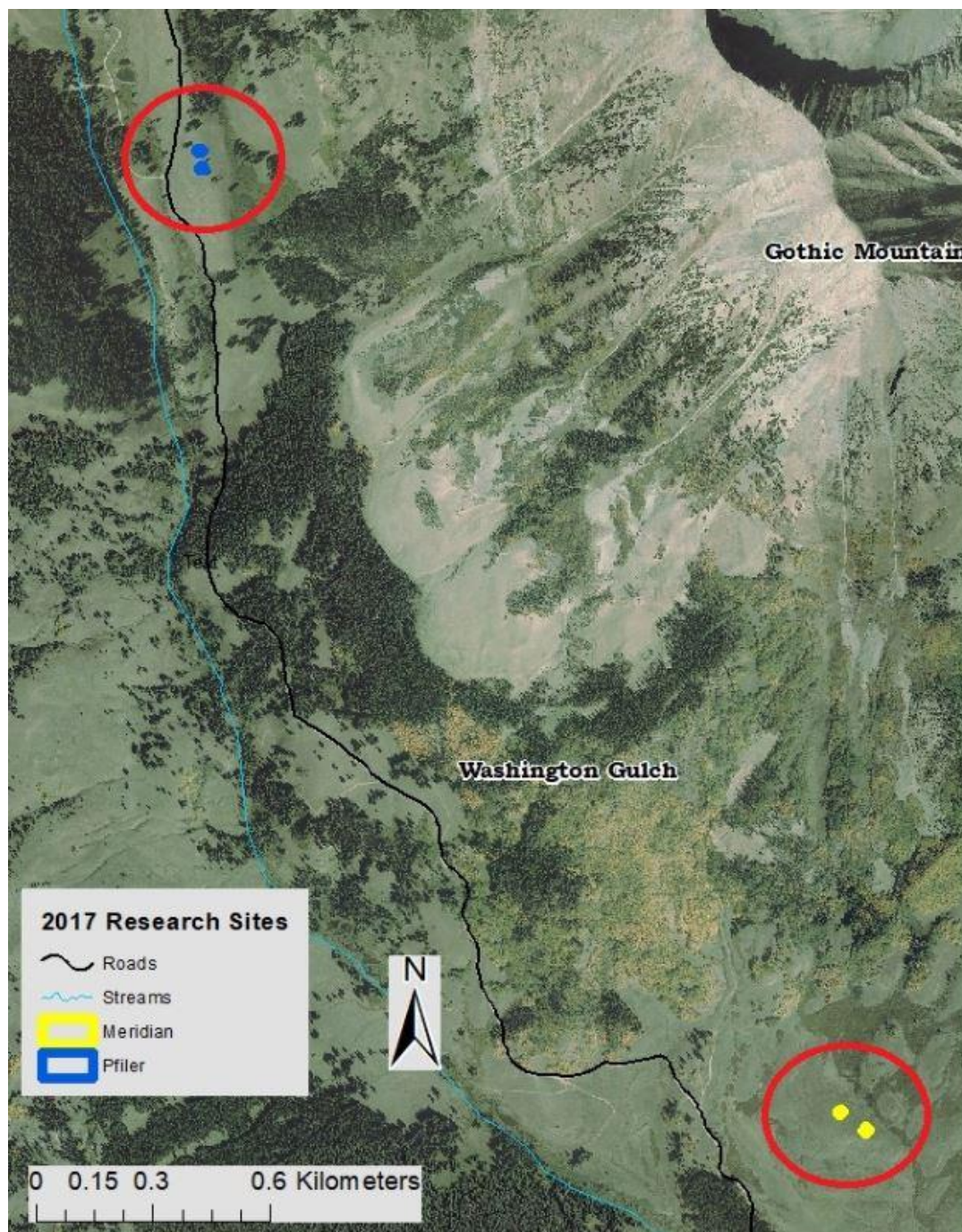
Tables and Figures:

Figure 1: Vicinity map of experimental sites (Meridian and Pfiler) generated via ArcMap programming.

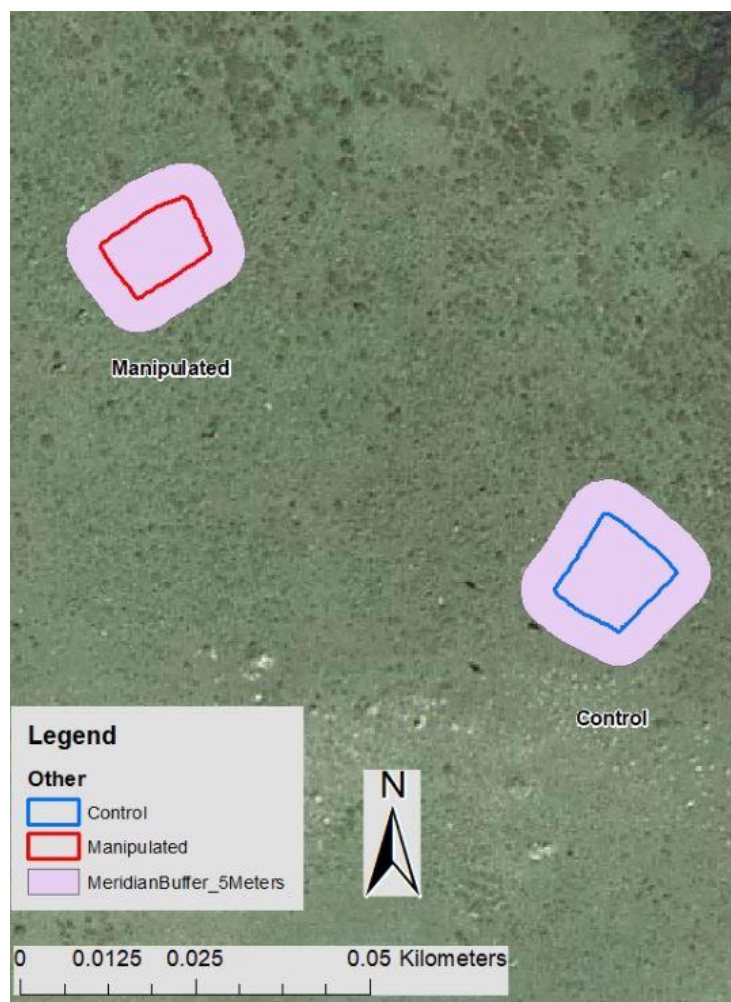


Figure 2: Meridian experimental site containing one control plot and one manipulated plot with a 5 meter buffer. Generated via ArcMap programming.

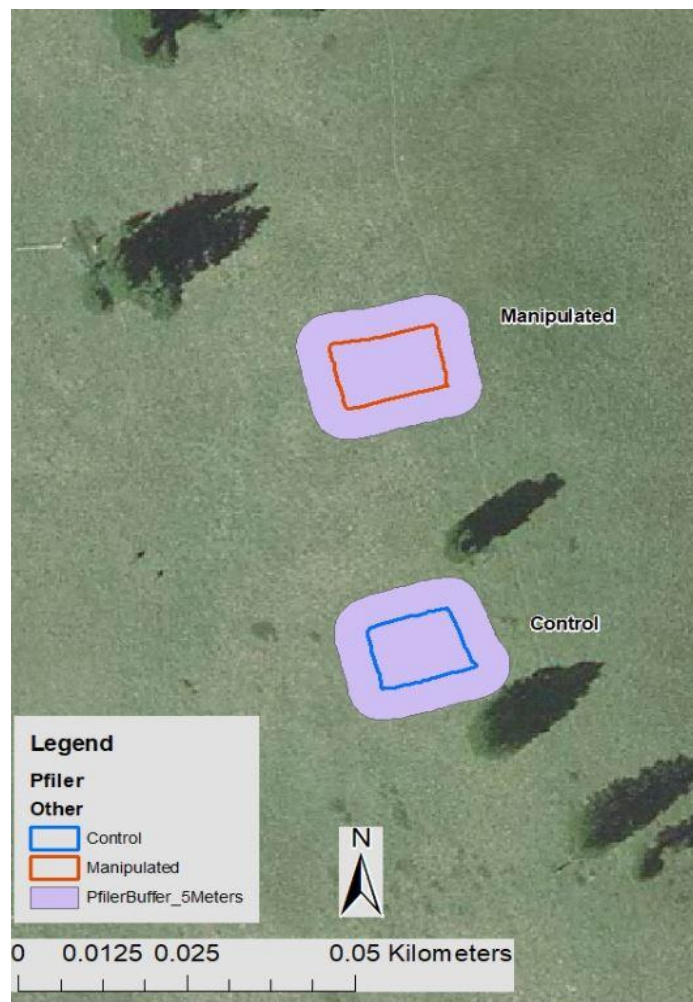


Figure 3: Pfiler experimental site containing one control plot and one manipulated plot with a 5 meter buffer. Generated via ArcMap programming.

Table 1: Simple statistics on developed seeds between plant treatment, plot and site generated via R studio programming.

	Plant.Treatment	Plot	Site	N	mean	sd	se
1	Exclusion	Control	Meridian	5	2.0	3.4641016	1.549193
2	Exclusion	Control	Pfieler	5	0.4	0.8944272	0.400000
3	Exclusion	Manipulated	Meridian	5	0.0	0.0000000	0.000000
4	Exclusion	Manipulated	Pfieler	5	6.8	13.0843418	5.851496
5	Open	Control	Meridian	5	0.0	0.0000000	0.000000
6	Open	Control	Pfieler	5	6.4	7.1624018	3.203123
7	Open	Manipulated	Meridian	5	2.6	3.7148351	1.661325
8	Open	Manipulated	Pfieler	5	12.8	14.0249777	6.272161
9	Open-Hand	Control	Pfieler	5	6.4	10.0647901	4.501111
10	Open-Hand	Manipulated	Meridian	5	6.4	10.0647901	4.501111
11	Open-Hand	Manipulated	Pfieler	5	15.2	12.6372465	5.651548

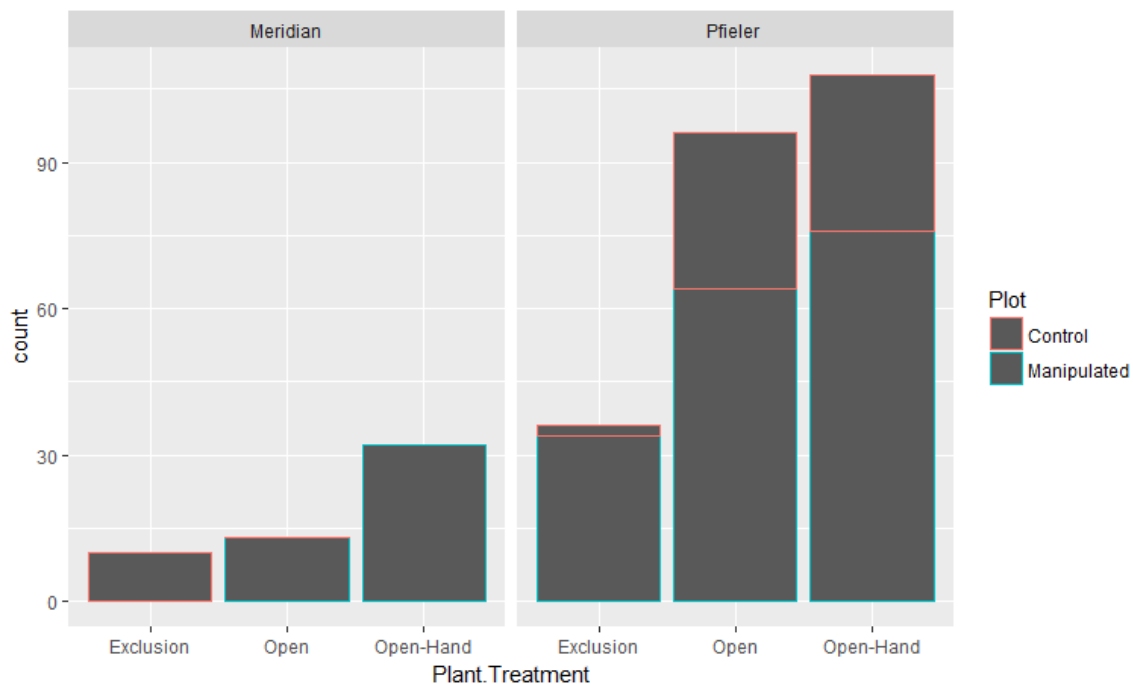


Figure 4: Comparison stacked bar graph displaying developed seed count between pollination treatments, experimental sites and controlled or manipulated plots. Generated via R Studio Programming.

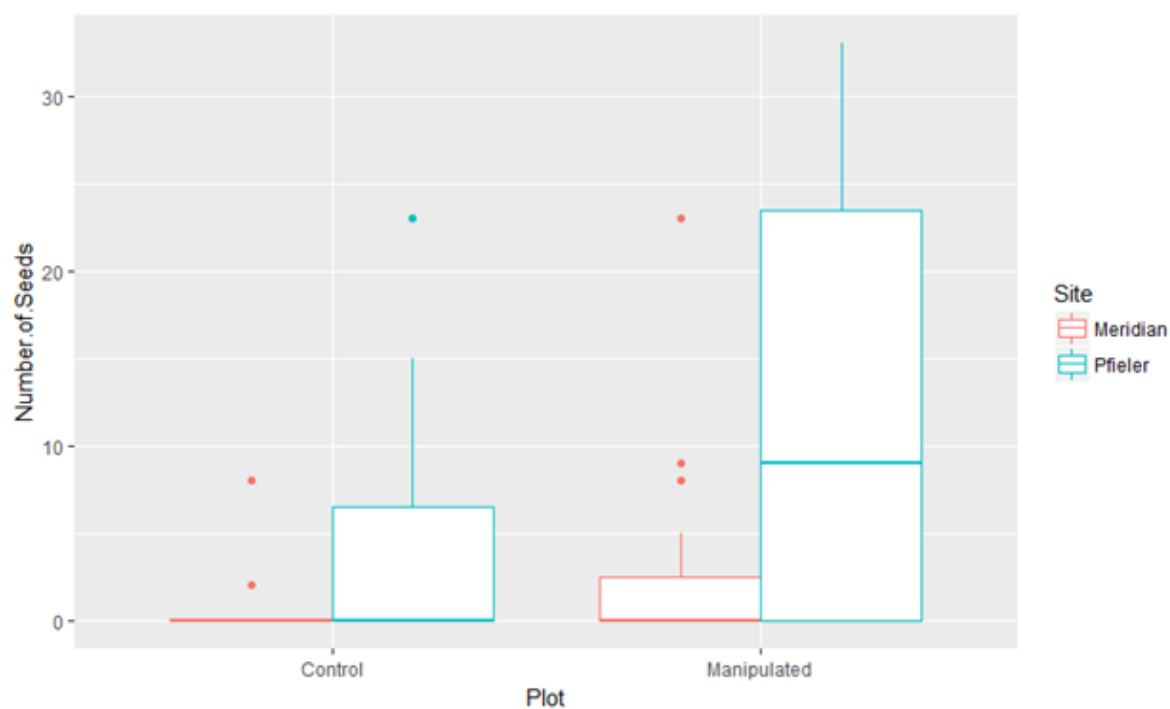


Figure 5: Comparison box plot displaying developed seed count between controlled or manipulated plot and experimental sites. Combined developed seed count for each site. Generated via R Studio Programming.