

The effects of phenology on *Delphinium nuttallianum*.

Student: Amber Datta

Mentors: Rebecca Irwin and Zak Gezon

Independent Research
Summer 2011

Abstract

The phenology of plants in the alpine environment of the Rocky Mountains is heavily dependent on snowmelt time (Inouye and McGuire 1991, Thomson 2010). In this study we have manipulated the timing of snowmelt in order to change the phenology of *Delphinium nuttallianum* in the subalpine environment of the Rocky Mountains; treatments included snow addition, snow removal and control. The objective of this study was to examine the effects of phenological change on *D. nuttallianum* in terms of peak flower abundance, species interactions, floral morphology, and pollen receipt. The date of peak flowering was significantly earlier in snow removal plots, resulting in altered floral diameter and petal length. Changes in floral display may alter plant-pollinator interactions and could thereby affect the reproductive success of the plant (Waser and Price 1991). Ninety percent of angiosperms rely on such plant-pollinator interactions, suggesting that changes in snowmelt may have significant impacts on many plant species (Engel and Irwin 2003). The potential for future changes in snow duration and melt time in the Rocky Mountains due to dust events makes this study particularly relevant to the future of *D. nuttallianum* and other wildflower species in this area (Painter *et. al.* 2007).

Introduction

Phenology is the study and documentation of the timing of natural history events. The first flowers of spring, the ripening of fruit, the emergence of bees from their nests, and the changing colors of fall leaves are all matters of phenology (Chuine 2010, Forrest and Miller-Rushing 2010). Historically these biological events have been important for both celebration, as in the Japanese cherry blossom festival, and survival, as when farmers choose the proper time to plant their crops and thereby provide food (Aono and Kazoi 2007). Studies of phenology have focused on both agriculture and ecological systems (Forrest and Miller-Rushing 2010). The phenology of plants in these systems is cued by environmental conditions that occur during or prior to flowering; many species are thus suspected to be quite sensitive to changes in climate (Forrest *et. al.* 2010). In the Rocky Mountains, climate change and dust events have begun to affect snowmelt, a phenological cue that significantly affects plant growth (Painter *et. al.* 2007, Inouye and McGuire 1991). In this study we have manipulated the timing of snowmelt in order to examine the effects of phenology on *Delphinium nuttallianum*, a species sensitive to this particular factor (Inouye and McGuire 1991).

Previous studies have found that early snowmelt causes *D. nuttallianum* to flower earlier and also decreases the peak number of flowers produced (Miller-Rushing and Inouye 2009). An earlier study conducted by Inouye and McGuire (1991) also noted a decrease in peak flower amounts after early snowmelt, and concluded that earlier snowmelt may have both direct and indirect negative effects on the seed production of animal-pollinated plants in subalpine meadows of the Rocky Mountains. The timing of snowmelt is also suspected to affect pollinator interactions with *D. nuttallianum* and other animal-pollinated plants because of the change in floral abundance (Inouye and McGuire 1991). In order to further explore the effects of phenology on pollinator interactions with *D. nuttallianum* we have examined the affects of manipulated snowmelt on the timing and abundance of peak flowering, the morphology of the flowers, the height of the plants, and the amount of pollen received by plants in different snowmelt treatments.

Floral morphology and plant height are suspected to affect reproductive success. In *Ipomopsis aggregata*, taller plants attract more pollinators (Engel and Irwin 2003). Seed set in *Ipomopsis* also increases with plant height (Mitchell 1994). Pollinator interactions are also affected by the appeal of a floral display, as demonstrated by studies involving *Ipomopsis aggregata* and *Raphanus raphanistrum*, where larger flowers received more visits due to increased appeal to pollinators (Campbell 1996, Connor and Rush 1996). To the best of our knowledge there have not yet been any studies on the effects of *D. nuttallianum* morphology on plant-pollinator interactions. In this study, measurements of petal width, petal length, nectar spur length, and diameter were included in order to detect whether phenology can affect pollinator interactions by changing floral morphology.

Interspecific interactions like those between *D. nuttallianum* and its pollinators can affect reproductive success in flowering plants. If a plant receives too little pollen it can fail to meet its reproductive potential, a phenomenon known as ‘pollinator limitation.’ Pollen receipt is found to increase with increased pollinator visitation rates (Engel and Irwin 2003, Thomson 2010). Interactions between a plant and its nectar robbers and herbivores are also likely to limit a plant’s reproductive success. Nectar robbers are species that manage to receive the nectar reward offered by plants without pollinating the flowers. Nectar robbed plants suffer lowered reproductive success due to damaged flower structure or to a related increase in pollinator limitation (Irwin and Brody 1999). If a plant is consumed by herbivores it is no longer capable of reproduction. Pollinator, nectar robber, and herbivore interactions may be affected by phenology and therefore could have an impact on plant reproduction when snowmelt time is shifted. It is already suspected that phenology affects reproductive success of *D. nuttallianum* via influencing its interactions with pollinators, but the effects of phenology on reproduction due to interactions with nectar robbers and herbivores have not been explored (Miller-Rushing *et. al.* 2010, Forest and Thomson 2011, Thomson 2010). The interactions of pollinators, nectar robbers and herbivores with *D. nuttallianum* at the Rocky Mountain Biological Laboratory have thus been included in this study. Pollinators of *D. nuttallianum* observed in this area include Broad-tailed hummingbirds (*Selasphorus platycircus*) and several species of *Bombus* including *nevadensis*, *californicus*, *appositus*, and *flavifrons* (Burkle *et. al.* 2007). The main nectar-robber of *D. nuttallianum* is *Bombus occidentalis* while its main herbivores are large rodents (Waser and Price 1981, Dr. Mary Price, personal communication).

The objective of this study was to examine the effects of phenological change on *D. nuttallianum* in terms of peak flowering time, peak flower abundance, species interactions, floral morphology, plant height, and pollen receipt. Determining the effects of snowmelt on this species has implications for the plant itself and for the ecological community to which it belongs. Our *a priori* predictions include the following: 1) the time of peak flowering will shift with snow manipulations so that plants in the removal plots bloom first, followed plants in the disturbance control plots, and lastly plants in the addition plots, 2) pollinator and nectar robber visitation rates are expected to increase as the growing season progresses and these species emerge from their nests, and 3) pollen receipt will therefore increase as the growing season progresses and pollinator visits increase (Engel and Irwin 2003, Forrest and Thomson 2011, Inouye 1991).

Methods

The duration of this study was approximately seven weeks, stretching from 26 June 2011 to 12 August 2011. A subalpine meadow on the 401 Trail in Gunnison County was used as the study site; the site was roughly 280 m North of the Judd Falls parking lot (38°57' 58.612"N 106°59'25.168"W). On May 16th 2011 we haphazardly set up 15, 2 x 2 m plots and randomly assigned each plot to one of three snow manipulation treatments: snow removal, snow addition, and control. Snow was shoveled off of the “removal” plots on 23 May 2011; at the time of snow removal the mean snow depth in the meadow was 123cm, the snow was removed until only 5cm remained. Four days after the snow removal an average of 93% of the ground within each plot was bare. Snow was added to the “addition” plots in order to maintain a depth of 1 m for as long as possible. In order to ensure that observed differences between treatments was due to changes in phenology rather than to other disturbances incurred while performing the snow removal treatments, the control plots were subjected to the same disturbances experienced by the snow removal plots with the exception of snow being removed. Specifically, we walked around inside the control plots when the snow reached a depth of about 5 cm, as was as necessary in the snow removal plots.

Within each plot, six *D. nuttallianum* plants were numbered and labeled. The labels consisted of a loop of string stuck together with blue tape and wrapped loosely around each plant's base, so as not to affect the growth of the plant.

During the study we collected data on pollinator visitation, nectar-robbing, herbivory, flower morphology, phenology and plant height. Observations were conducted every three days throughout the course of the growing season of *D. nuttallianum*. We began each field day with pollinator observations. Pollinator observations consisted of watching each plot for 20 minutes, collecting data on the species of pollinators that visited, the number and type of plants they visited, the number of flowers they visited, and the length of time spent on each flower. We recorded the plant ID of all marked *D. nuttallianum* plants visited by pollinators during the observations. The length of time spent on each flower was intended to give some indication of the amount of pollen deposited on the stigma. Longer pollinator visits per flower typically result in more pollen removal from anthers and more pollen deposited on stigmas (Castellanos *et. al.* 2003).

Floral phenology and abundance surveys were taken by counting the number of open flowers on marked *D. nuttallianum*. Flowers were considered open if their petals were unfurled enough for potential pollinators to visit and if they had not begun to dehisce petals or dry up (Inouye 1991). Floral abundance was calculated in terms of the average number of open flowers per plant per plot. Peak floral abundance was determined as the date with the highest mean number of flowers per plant.

After pollinator observations and surveys were conducted, nectar robbing was quantified by counting the number of flowers per plant that were robbed and the number of holes made in the nectar spur on each flower. The number of flowers open was counted, allowing nectar robbing to be quantified as a percent of the total number of open flowers (Burkel *et. al.* 2007). Herbivory

was measured at the same time as nectar robbing and was quantified by recording the number of plants that had been browsed; if a plant was browsed we noted the extent and location of the damage done.

Floral morphology was quantified by measuring, in mm, three flowers per plant using digital calipers. For each flower we measured the petal length of either the left or right petal, starting the measurement where the petal separated from the corolla base. The petal width was measured across the broadest part of either the left or right petal. The floral diameter was measured across the face of the flower, and the length of the nectar spur was measured starting at the base of the corolla. Each plant's height was measured in cm at the time of first flowering. The mean petal width, petal length, diameter, spur length and plant height was calculated for each plot, and subsequently for each snow manipulation treatment. After the flowers wilted, we carefully collected the stigmas off the ends of the fruit using forceps, thereby minimizing any disturbance to the development of these fruit.

The amount of pollen deposited on each stigma was quantified by placing collected stigmas onto a microscope slide with basic fuchsin gel (Kearns and Inouye 1993). The amount of *D. nuttallianum* pollen and the amount of heterospecific pollen was counted on each slide using a microscope with 20x magnification.

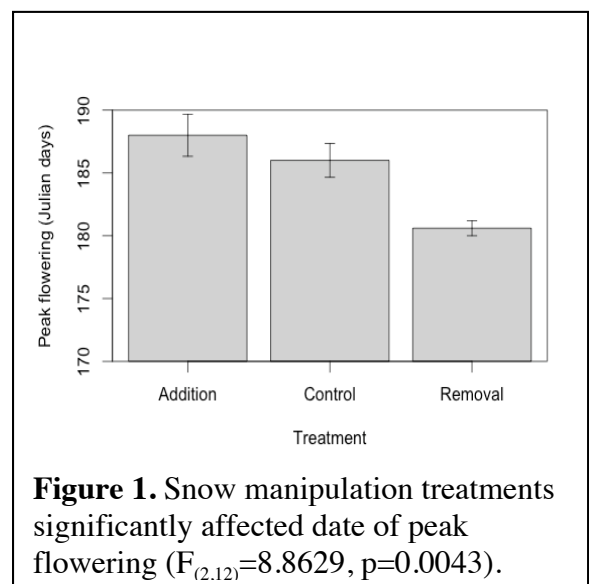
In individual tests, ANOVA was used to test whether the following variables varied significantly with snowmelt treatments: herbivory, nectar robbing, pollen receipt, petal length, petal width, nectar spur length, plant height, and floral diameter. The unit of replication was the plot, giving a total of 15 samples; in most plots more than one individual plant survived, so the mean of all plants within each plot was used for the ANOVAs. Significant results were investigated further using Tukey post-hoc multiple comparisons.

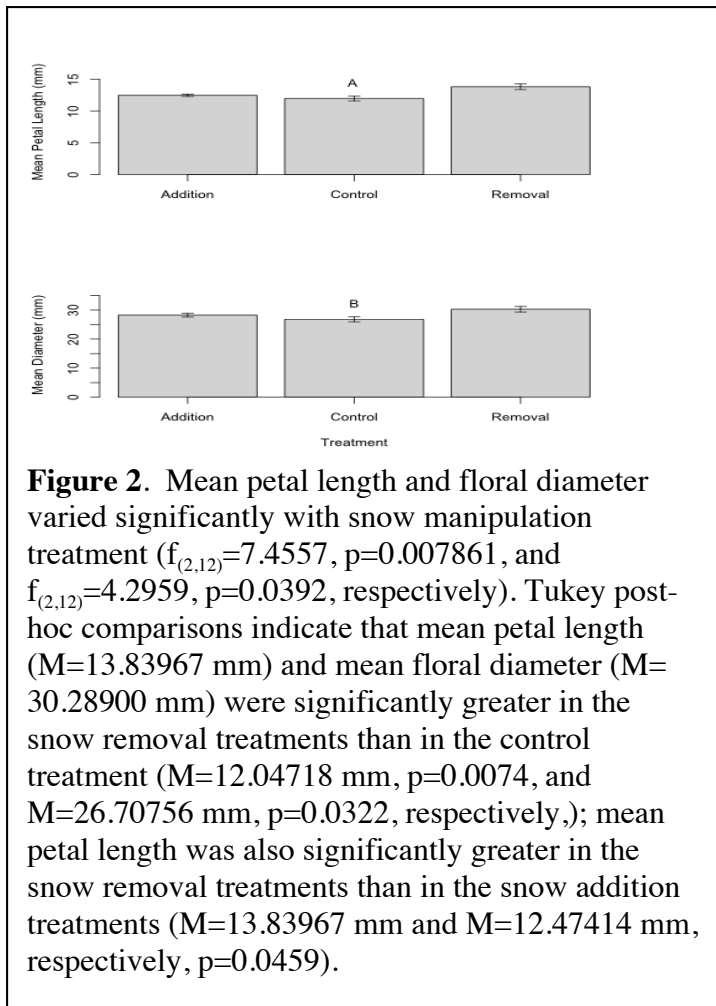
Results

Phenology

Snow manipulation treatments had a significant effect on the date of peak floral abundance (Figure 1).

Tukey post-hoc comparisons indicate that the snow removal treatments reached peak floral abundance at a significantly earlier date ($M = 180.6$) than in the control treatment ($M = 186.0$, $p = 0.0292$), as well as the snow addition treatments ($M = 188.0$, $p = 0.0041$). There was no significant difference in the date of peak flowering between the snow addition treatment and the disturbance control plots ($p = 0.5321$). At the time of peak flowering the mean number of flowers per plant did not vary significantly with treatment (Figure S1-A). Additionally, the mean total number of flowers produced per plant did not vary significantly with treatment (Figure S1-B).





Species interactions

Nectar robbing, quantified as percent total flowers robbed per plot, did not vary significantly with treatment ($F_{(2,12)}=0.0866$, $p=0.9176$). Herbivory, quantified as percent of plants consumed or damaged was not found to vary significantly with treatment ($F_{(2,12)}=0.28$, $p=0.7606$). Pollen receipt did not vary significantly with treatment (Figure S2). We were unable to compare pollinator visitation across treatments because pollinators were never observed to visit marked plants in the plots.

Morphology

The snow manipulations had a significant effect on some of the morphological features of *D. nuttallianum*. Average petal length and mean floral diameter varied significantly with treatment (Figure 2, A&B). The average petal width, nectar spur length, and plant height did not vary significantly with treatment. (Figure S3).

Discussion

Trends towards earlier snowmelt have been occurring throughout western North America in recent decades (Stewart *et al.* 2005). At the Rocky Mountain Biological Laboratory this change towards earlier snowmelt time has not yet occurred but predictions for the southwestern Rocky Mountain area suggest that this may change in the near future (Inouye *et al.* 2000 and Painter 2007). These changes in snowmelt time may affect plant and pollinator species in different ways; in this study the manipulation of snowmelt affected the morphology of *D. nuttallianum*.

The variation in floral morphology between treatments has implications for the effects of changes in snowmelt time on pollinator interactions. According to studies of *Mimulus*, and *Polemonium viscosum*, among many others, floral display affects interactions with pollinators, often by making a given species more or less attractive to certain species of pollinators (Galen 1989, Schemske 1999). The theory of plant/pollinator coevolution states that “size, arrangement, and shape of flowers have evolved to improve the efficiency of pollination or the number of pollen grains delivered to the stigma surface during a pollinator visit” (Galen 1989). Although such studies have not been done on *D. nuttallianum*, it seems likely that its floral display has

evolved to appeal to its pollinators; if this interaction is consistent with other types of plant-pollinator co-evolution it seems likely that pollinators will be attracted to flowers with larger diameters. Thus, this plant-pollinator interaction may therefore be affected by shifts in the phenology of the plant.

Over the course of this study 45 hours were spent (five hours each observation day) in the field looking for pollinator interactions with *D. nuttallianum*. It is interesting that despite the amount of time devoted to these observations, pollinators were never seen to land on the labeled plants. We noticed a few pollinators visiting *D. nuttallianum* outside of the plots, but even these observations were few and far between. It is possible that our observation periods were not long enough, or that they were not spread evenly enough throughout the day. However, this year has had a rather unusual growing season due to the late snowmelt, which occurred near the beginning of June. Anecdotally, other researchers also noted low pollinator (bumble bee) abundance during the flowering period of *D. nuttallianum*. It is therefore possible that pollinators emerged late in the season, as they are known to be sensitive to local environmental conditions (Forrest and Thomson 2011).

Pollen receipt was also recorded in this study as an indication of pollinator interactions with *D. nuttallianum*, as the pollen received by the plants increases with frequency of pollinator visits (Williams 2007, Engel and Irwin 2003). Pollen receipt in this case was relatively low compared to amounts found in other studies and did not vary significantly between the treatments, which seems contradictory to the changes in morphology (Bosch and Waser 1999, Bosch and Waser 2001). This lack of variance could indicate several things. Firstly, pollen may have been knocked off of the stigmas during the transition from the field to the lab, or possibly even before stigmas were collected. This seems possible, but somewhat unlikely as we followed a methodology common to other successful studies. Secondly, it may be that although the changes in floral morphology were statistically significant, they are not biologically significant. It is possible that pollinators in this case were not particularly sensitive to the variation in floral display. A third reason for this lack of variance in pollen receipt may again have to do with the somewhat unusual phenology caused by this year's late snowmelt. There may be several other explanations for this result; further investigations over multiple years will be necessary to determine the cause of this result and to see if it is repeated over time.

The low rates of nectar robbing this year may also be due to effects of late snowmelt on *Bombus occidentalis*. The frequency of interactions between nectar robbers and plants is in general known to have a tendency to vary from year to year (Rebecca Irwin, pers. comm.). In order to determine the effects of phenology on nectar-robbing this study would likely need to be repeated over several years.

The findings of this study suggest that changes in snowmelt time have an affect on the peak flowering time and morphology of *D. nuttallianum*. These changes may alter plant-pollinator interactions and could thereby affect the reproductive success of the plant (Waser and Price 1991). Ninety percent of angiosperms rely on such plant-pollinator interactions, suggesting that changes in snowmelt may have significant impacts on many plant species (Engel and Irwin 2003). The potential for future changes in snow duration and melt time in the Rocky Mountains due to dust events makes this study particularly relevant to the future of *D. nuttallianum* and

other wildflower species in this area (Painter *et. al.* 2007). Co-flowering communities, the overlap time between plants and their pollinators, and the frequency of frost damage are among several factors expected to be affected if such shifts in snowmelt do occur (Inouye and McGuire 1991, Miller-Rushing and Inouye 2009, Forrest *et. al.* 2010, Forrest 2011). The impacts of anthropogenic activities like agriculture in areas such as the Colorado Plateau may therefore be expected to cause significant changes in alpine ecological systems around the world (Steltzer *et. al.* 2009, Warren 1984).

Literature Cited

- Aono, Yasuyuki and Keiko Kazui. 2007. Phenological data series of cherry tree flowering in Kyoto, Japan, and its application to the reconstruction of springtime temperatures since the 9th century. *International Journal of Climatology*. 28:905-914.
- Burkle, Laura A., Rebecca E. Irwin, and Daniel A. Newman. 2007. Predicting the effects of Nectar Robbing on Plant Reproduction: Implications of Pollen Limitation and Plant Mating System. *American Journal of Botany*. 94: 1935-1943.
- Castellanos, Maria Clara, Paul Wilson, and James D. Thomson. Pollen Transfer by Hummingbirds and Bumblebees, and the Divergence of Pollination Modes in *Penstemon*. *Evolution*. 57:2742-2752.
- Chuine, Isabelle. 2010. Why does phenology drive species distribution?. *Philosophical Transactions of the Royal Society*. 365:3149-3160.
- Engel, E. Cayenne, and Rebecca E. Irwin. 2003. Linking Pollinator Visitation Rate and Pollen Receipt. *American Journal of Botany*. 90: 1612-1618.
- Forrest, Jessica, and Abraham J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society*. 365:3101-3112.
- Forrest, Jessica, David W. Inouye, and James D. Thomson. 2010. Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns?. *Ecology*. 91: 431-440.
- Forrest, Jessica R. K., and James D. Thomson. In-press. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Society of America*.
- Galen, Candace, and Maureen Stanton. 1989. Bumble Bee Pollination and Floral Morphology: Factors Influencing Pollen Dispersal in the Alpine Sky Pilot, *Polemonium vicosum* (Polemoniaceae). *American Journal of Botany*. 76:419-426.
- Inouye, David W., and A. David McGuire. 1991. Effects of Snowpack on Timing and Abundance of Flowering in *Delphinium Nelsonii* (Ranunculaceae): Implications for Climate Change. *American Journal of Botany*. 78:997-1001.
- Inouye, David W., Billy Barr, Kenneth B. Armitage, and Brian D. Inouye. 2000. Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences*. 97:1630-1633.
- Inouye, David W. 22 June 2011. Personal communication.
- Irwin, Rebecca E., and Alison K. Brody. 1999. Nectar-robbing Bumble Bees Reduce the Fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology*. 80: 1703-1712.
- Kearns, C.A., Inouye, D. W. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, CO.
- Miller-Rushing, Abraham, and David Inouye. 2009. Variation in the Impact of Climate Change on Flowering Phenology and Abundance: An Examination of Two Pairs of Closely Related Wildflower Species. *American Journal of Botany*. 96: 1821-1829.
- Miller-Rushing, Abraham J., Toke T. Hoyer, David W. Inouye, and Eric Post. 2010. The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society*. 365: 3177-3186.
- Painter, Thomas H., Andrew P. Barrett, Christopher C. Landry, Jason C. Neff, Maureen P. Cassidy, Corey R. Lawrence, Kathleen E. McBride, and G. Lang Farmer. 2007. Impact of

disturbed desert soils on duration of mountain snow cover. Geophysical Research Letters. 34.

Price, Mary V. 23 June 2011. Personal communication.

Schemske, Douglas W. and H. D. Bradshaw, Jr. Pollinator preference and the evolution of Floral Traits in Monkeyflowers (*Mimulus*). Proceedings of the National Academy of Sciences of the United States of America. 96: 11910-11915.

Thomson, James D. 2010. Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. Philosophical Transactions of the Royal Society. 385: 3187-3199.

Warren, Stephen G. 1984. Impurities in Snow: Effects on Albedo and Snowmelt. Annals of Glaciology. 5. (Review).

Waser, Nickolas, and Mary V. Price. 1981. Pollinator Choice and Stabilizing Selection for Flower Color in *Delphinium Nelsonii*. Evolution. 35: 376-390.

Williams, Charles F. 2007. Effects of Floral Display Size and Biparental Inbreeding on Outcrossing rates in *Delphinium Barbeyi* (Ranunculaceae). American Journal of Botany. 94: 1996-1705.

Supplemental Figures

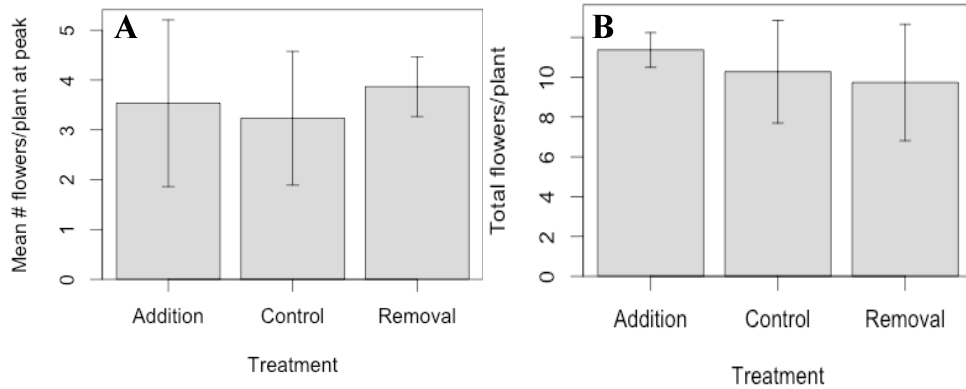


Figure S1. Mean number of flowers per plant (A) and total number of flowers produced per plant (B) did not vary significantly with treatment ($F_{(2,12)}=0.2184$, $p=0.807$ and $F_{(2,12)}=0.1303$, $p=0.8791$, respectively).

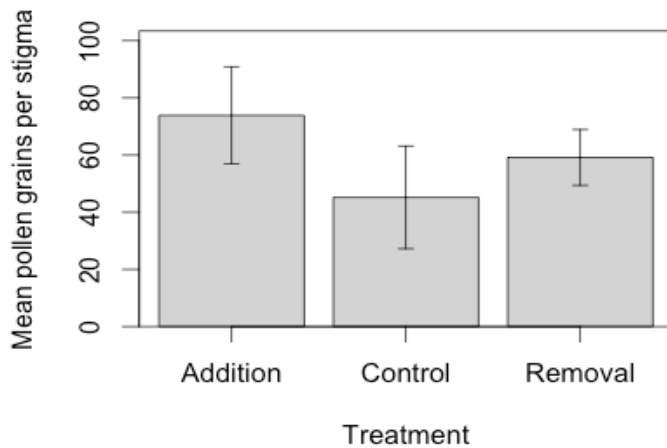


Figure S2. The mean number of pollen grains per stigma did not vary significantly with snow manipulation treatment ($F_{(2,12)}=0.6412$, $p=0.5438$).

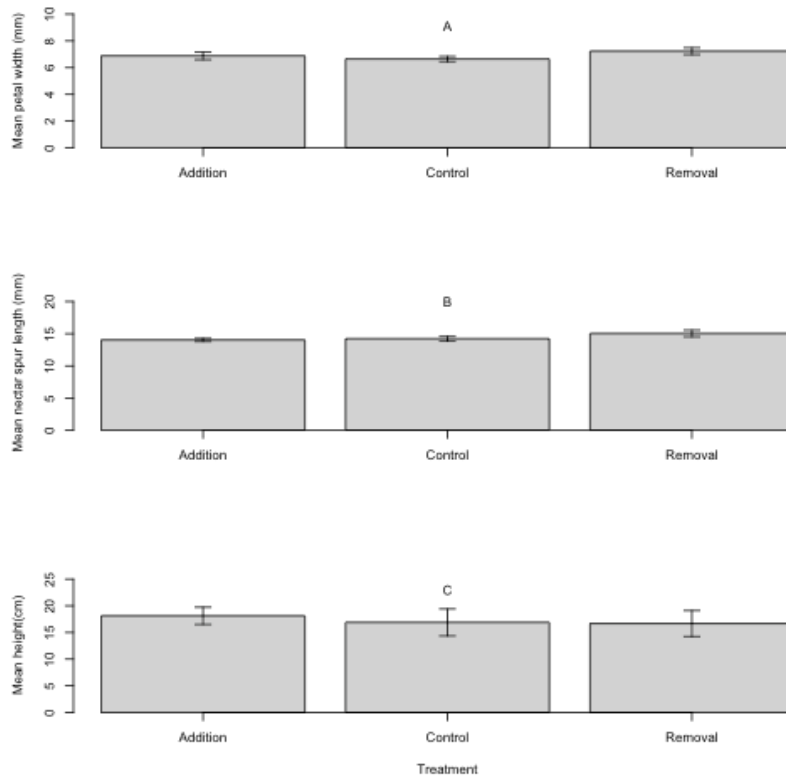


Figure S3. Means of petal width (A), nectar spur length (B), and plant height (B) did not vary significantly with snow manipulation treatment ($F_{(2,12)}=1.3796$, $p=0.2889$; $F_{(2,12)}= 1.7862$, $p=0.2094$; $F_{(2,12)}=0.1219$, $p=0.8863$, respectively).