

Impacts of early snow removal and frost on the
reproductive success of *Delphinium nuttallianum*

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ABSTRACT

Anthropogenic climate change is causing increases in global average temperature, changes in seasonality, and altered precipitation patterns which are linked to upslope range shifts, population declines, and phenological changes. This study examines the impacts of climate induced changes in snowmelt date and episodic frost on the reproductive success of a subalpine forb species, *Delphinium nuttallianum*, in the Rocky Mountains of south-central Colorado, USA. *D. nuttallianum* plants were exposed to early or natural snowmelt, reduced or natural frost, and natural pollination and supplemental pollen treatments and reproductive success was measured in order to explore the effects of climate change on plant reproductive fitness. While plant density within a plot and pollinator visitation rate was significantly higher in early snowmelt treatments, there was no significant difference between any of the climate change related treatments in reproductive fitness, indicating that snow melt, frost exposure, and supplemental pollination did not significantly impact the reproductive success of *D. nuttallianum* in 2016. However, this may partially be due to dry conditions which caused many plants to abort a substantial portion of flowers and fruits, diluting the effects of climate-related variables. These data indicate that while plants that bloom earlier might be at an advantage for attracting pollinators, further research is needed to elucidate the effects of climate change on the reproductive fitness of *Delphinium nuttallianum* and other subalpine forbs.

INTRODUCTION

Global average temperatures are rising as a result of anthropogenic carbon forcing. Since 1880, global temperatures have increased by 0.85° C, with some areas experiencing more than 1° C of warming (IPCC 2013). On a local scale, the effects of this overall temperature increase are highly variable, differentially impacting precipitation, seasonality and short-term weather events, in addition to temperature change due to differences in topography, hydrology, and regional climate (Inouye 2000, Parmesan and Yohe 2003, Parmesan *et al.* 2013, CaraDonna *et al.* 2014). The effects of intensifying climate change on temperature, precipitation and seasonality patterns has been linked to upslope and latitudinal range shifts, changes in abundance, and phenological shifts (McLaughlin *et al.* 2002, Parmesan and Yohe 2003, Battisti *et al.* 2005, Wilson *et al.* 2005, Walther *et al.* 2005, Kelly and Goulden 2008, Kearney *et al.* 2010, Yang and Rudolf 2010, VanDer Wal *et al.* 2012, CaraDonna *et al.* 2014, Gezon *et al.* 2016). Additionally, species may react differently to climate change, resulting in the disruption of species interactions and community dynamics (Dunne *et al.* 2003, Hegland *et al.* 2009, Gliman *et al.* 2012, Forrest 2015).

In montane regions, climate change has manifested itself through increasing summer temperatures and earlier snowmelt. In the Rocky Mountains of Colorado, average summer air temperature has increased by approximately 0.4° C per decade and the date of spring snowmelt has advanced by an average of 3.5 days per decade since the 1970's (CaraDonna *et al.* 2014). As plant phenology is sensitive to temperature, precipitation and timing of snow melt (Høye *et al.* 2007, Lambert *et al.* 2010, CaraDonna *et al.* 2014), these changes have the potential to greatly impact the reproductive success of alpine forbs.

Changes in plant phenology can impact reproductive success by altering the abiotic conditions experienced by a plant (Gezon *et al.* 2016). In the context of earlier

emergence and advanced flowering phenology caused by increasing temperatures and earlier snow melt (Dunne *et al.* 2003, CaraDonna *et al.* 2014), susceptibility to frost and access to adequate water resources are of primary concern when considering the effects of climate change on plant reproductive success. Earlier emergence puts plants at increased risk of damage due to episodic frost events (Molgaard and Christensen 1997, Inouye 2008). This can have a disproportionate impact on reproductive success, as reproductive tissues are very vulnerable to frost damage (Inouye 2000, Agrawal *et al.* 2004). Early snow melt also leads to drier summer soils, reducing summer water availability, especially during dry years (Dunne *et al.* 2003, Iler *et al.* 2013). This in turn may reduce reproductive success by causing water stress, though this effect is variable in depending on the plant species (de Valpine and Harte 2001, Gezon *et al.* 2016).

Additionally, plant reproductive fitness in non-wind pollinated species is highly dependent on pollinators. Phenological changes have the potential to negatively impact plant-pollinator interactions by causing phenological mismatches (Parmesan and Yohe 2003, Burkle *et al.* 2013, Forrest 2015) or by increasing competition for pollination resources due increased co-flowering (CaraDonna *et al.* 2014). While the flowering phenology of many plant species are strongly influenced by snowmelt date (Dunne *et al.* 2003, CaraDonna *et al.* 2014), it seems less important for determining insect pollinator emergence (Forrest and Thomson 2011), suggesting the potential for a loss of plant fitness caused by temporal asynchrony (Kudo and Ida 2013, Kudo 2014). However, other recent studies have found little evidence of temporal mismatch (Rafferty and Ives 2011, Iler *et al.* 2013, Gezon *et al.* 2016). In addition to potential asynchrony, shifts in plant phenology may also impact reproductive success by increasing interspecific competition for pollination resources, increasing deposition of non-conspecific pollen and changing the way that pollinators interact with the plant community (Waser 1978, Pleasants 1980, Ghazoul 2006, CaraDonna *et al.* 2014).

Plant phenological changes in response to climate change are linked to different variables in different species (Dunne *et al.* 2003). Flower phenology has advanced more rapidly in early flowering species compared with those which flower later in the season (Dunne *et al.* 2003, CaraDonna *et al.* 2014), indicating that reproductive effects of early snow melt and frost sensitivity may differ between early and late flowering forb species. Additionally, phenology changes in different forb species are tied to different variables (Dunne *et al.* 2003, CaraDonna *et al.* 2014), suggesting that species may also exhibit variation in climate mediated reproductive fitness. Gezon *et al.* (2016) found that in *Claytonia lanceolata*, the first wildflower to bloom in the spring, individuals exposed to early snow removal had higher visitation and reproduction when undamaged, but were also more likely to have reproductive tissues damaged by frost. However, plants which flower later in the growing season may display different responses to changing climate variables.

In this study, I expand upon the investigation of Gezon *et al.* (2016) by exploring the factors impacting reproductive success in another subalpine wildflower, *Delphinium nuttallianum*. First, pollinator observations were used to determine the effects of snowmelt and frost treatment on pollinator visitation rate. Second, I used seed set as a metric for reproductive fitness to explore the impacts of snow and frost treatment on plant reproductive success. Third, I compared plants given supplemental pollen with those in natural pollen treatment to investigate the effects of pollen limitation on plants exposed to different abiotic conditions.

MATERIALS AND METHODS

This study was conducted in a subalpine meadow north of Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA at an elevation of approximately 3000m. In April 2016, 40 2x2m plots were created in two parallel transects (Fig. 1). Each of the 40 plots was randomly assigned to either early snow removal or natural snow melt. Within each of the two snow treatments, 10 plots were protected from frost and 10 were exposed to natural frost. In the early snow removal plots, snow was manually removed down to a depth of 5cm once overall snow depth reached 75 cm (per Gezon *et al.* 2016). Early snow removal resulted in snow melt 12-14 days sooner than in natural snow melt plots. Frost protected plots were insulated from below freezing temperatures by the placement of an Easy Gardener® cloth tarp the evening before a frost event was predicted to occur, while the natural frost plots remained uncovered.

Delphinium nuttallianum Pritz. (Ranunculaceae) is a montane herbaceous perennial which flowers between early June and early July (CaraDonna and Bain 2015). *D. nuttallianum* is protandrous, with male reproductive parts maturing first, followed by females within any given flower. *D. nuttallianum* phenology has advanced since the 1970's with date of first flowering, peak flowering, and final flowering all moving forward (CaraDonna *et al.* 2014). *D. nuttallianum* reproductive tissues are sensitive to frost damage, while leaves are not (CaraDonna and Bain 2015).

Seven times during the *D. nuttallianum* flowering period the total number of *D. nuttallianum* plants in each plot and the number of flowers on ten plants was counted as a part of a larger floral resource census. Based on this information, the average and maximum number of *D. nuttallianum* plants in each plot was calculated, as well as the average number of flowers per plant within each plot over the course of the flowering period. Pollinator observations conducted 2-3 times per week throughout the flowering season in order to determine pollinator visitation rates in plots with different environmental treatments. During pollinator observations, each plot was observed for 10 minutes in a random order. During this time, all pollinator visitations were observed and the length of time on each flower visited, total time in the plot, and identification was recorded. If a pollinator could not be identified on the wing it was collected for later identification in the lab. Bees in the genera *Bombus* and *Osmia* individuals were not collected in order to avoid damaging important pollinator populations.

Within each plot, eight *D. nuttallianum* individuals were marked with colored tape and given an identifying number. Four individuals were randomly designated as part of the supplemental pollen treatment, while the other four comprise the natural pollination treatment. Supplemental pollen plants were hand pollinated using pollen from conspecifics collected at least 10m away from the plot. Only flowers with receptive stigmas, indicated by their light purple color, were hand pollinated. Several times per week, all flowers on each tagged plant were recorded and all receptive flowers on supplemental pollen plants hand pollinated.

Once flowers senesced and fruits fully developed, all marked plants were collected and taken to the lab in order to determine seed set. For each plant, the number of developed fruits, number of aborted fruits, the number of viable seeds, and the number of aborted seeds were recorded. These data in turn were used to calculate total seed set, proportion of successful seed set, total fruit set, and proportion successful fruit set (per Gezon *et al.* 2016), allowing for the quantification of

reproductive success. Each measure of reproductive success was compared between different treatments for each species with ANOVAs (Chambers *et al.* 1992) and mixed models (Skaug and Fournier 2006) using R statistical software (R Core Team 2016).

RESULTS

The maximum number of individual *Delphinium nuttallianum* plants flowering within a plot was weakly correlated with snowmelt treatment ($df=1$, $f=3.26$, $p=0.08$), with early snowmelt plots averaging 27.2 ± 4.61 plants per plot and natural snowmelt plots averaging 18.0 ± 2.71 plants per plot (Fig. 2). There was no relationship between frost treatment and maximum plants per plot ($df=1$, $f=0.009$, $p=0.92$).

Pollinator visitation was also highly influenced by snowmelt treatment. Both total time on flowers ($df=1$, $f=14.73$, $p=0.0002$) and total flower visitations ($df=1$, $f=30.62$, $p<0.0001$) were significantly impacted by snowmelt treatment. Pollinators spent an average of 22.05 ± 3.77 seconds on flowers and visited an average of 3.97 ± 0.39 flowers per plot in early snowmelt plots, while pollinators spent only 7.14 ± 1.27 seconds on flowers and visited an average of 1.53 ± 0.22 flowers per plot in natural snowmelt plots (Fig. 3a and b).

In spite of differences in pollinator visitation, pollination treatment did not have a significant impact on *Delphinium nuttallianum* reproductive fitness. There was no significant difference in total fruits ($df=1$, $f=0.11$, $p=0.74$), total seeds ($df=1$, $f=1.29$, $p=0.26$), proportion successful fruits ($df=1$, $f=0.74$, $p=0.39$), and proportion successful seeds ($df=1$, $f=3.26$, $p=0.073$). For all metrics of reproductive fitness, fitness was slightly higher in the natural pollination treatment than in the supplemental pollen treatment (Figure 4a-d), but these differences were minor, and were not present when random effects were included in our mixed modeling.

The effects of snowmelt and frost treatment on reproductive fitness were not significant for any measure of reproductive success. Using a negative binomial mixed model, we found the number of total fruits, total seeds and total seeds per fruit, respectively, were unrelated to snow treatment ($z=0.21$, $p=0.83$; $z=-0.73$, $p=0.47$; and $z=-0.42$, $p=0.67$), frost treatment ($z=1.41$, $p=0.16$; $z=-1.02$, $p=0.31$; and $z=-0.43$, $p=0.67$) or snow plus frost treatment ($z=-1.18$, $p=0.24$; $z=0.25$, $p=0.80$; and $z=0.83$, $p=0.41$), (Fig. 5a and b). Similarly, using a quasibinomial mixed model for proportional data, the proportion of fruits successful and the proportion of seeds successful, respectively, were also not significantly correlated with snow treatment ($t=-0.16$, $p=0.877$ and $t=-0.60$, $p=0.55$), frost treatment ($t=-0.04$, $p=0.97$ and $t=-0.41$, $p=0.68$) and snow plus frost treatment ($t=0.04$, $p=0.97$ and $t=0.44$, $p=0.67$). Modeling relating pollination to snow and frost treatments similarly showed no significance for any measure of reproductive fitness.

DISCUSSION

These data show that within our experimental plots, climate change related treatments had a complicated effect on the fitness of *Delphinium nuttallianum*. Early snowmelt was loosely related to *D. nuttallianum* density within a given plot, and was strongly correlated with pollinator visitation rates. The precise factors which led to higher plant density are not clear, but this trend may be partially explained by greater

light or water availability in early snowmelt plots, or by a compression in the growing season, leading to higher number of co-flowering individuals (Dunne *et al.* 2003). However, these variables were not specifically measured. Higher pollination visitation rates in early snowmelt plots are probably partially explained by the higher conspecific density, which has been linked to pollinator approach rate in other studies (Kunin 1997, Bosch and Waser 1999). Early snowmelt plots also likely had higher visitation rates due to less competition for pollination resources earlier in the growing season (Iler *et al.* 2013, Gezon *et al.* 2016).

Despite differences in density and pollinator visitation rates between the different snowmelt treatments, we did not see any evidence that pollination impacted reproductive fitness in *Delphinium nuttallianum*. Early snowmelt plots did not have higher reproductive fitness in spite of having higher conspecific density and pollinator visitation. In addition, there was not difference in fecundity between supplemental hand pollination and natural pollination treatments. The fact that plants that were given supplemental pollen did not have higher reproductive fitness suggests that *D. nuttallianum* plants were not pollen limited for reproduction. Instead, abiotic conditions are likely the limiting factor controlling the plants reproductive success.

While abiotic conditions are likely the limiting factor determining seed set and fruit set in *Delphinium nuttallianum*, snowmelt and frost treatment do not appear to have had a significant role in determining reproductive fitness. We found that that neither treatment was predictive of fecundity, indicating that snowmelt and frost did not play an important role in determining the reproductive success of *D. nuttallianum* during the 2016 growing season. The lack of a differences between frost treatments may be due to the fact that while frost events occurred periodically during the growing season of *D. nuttallianum*, temperatures cooler than -8°C are necessary to cause damage in *D. nuttallianum* (CaraDonna and Bain 2015). Temperatures below this threshold are uncommon during the flowering period of *D. nuttallianum*, reducing the potential for differentiation between frost treatments. Additionally, we suspect that dry conditions during the flowering period and the time of fruit development in June and July caused plants to become water stressed, leading them to abort a number of fruits and seeds and washing out any difference in fitness which might have existed between different snowmelt, frost, and pollination treatments explored in this study. The effects of climate change related variables has been previously demonstrate to show significant interannual variation. (Dunne *et al.* 2003, Inouye 2008, Gezon *et al.* 2016). As such, a continued study over the course of several growing seasons are needed to effectively assess the likely impacts of climate change *Delphinium nuttallianum* and other subalpine forb species.

APENDIX 1

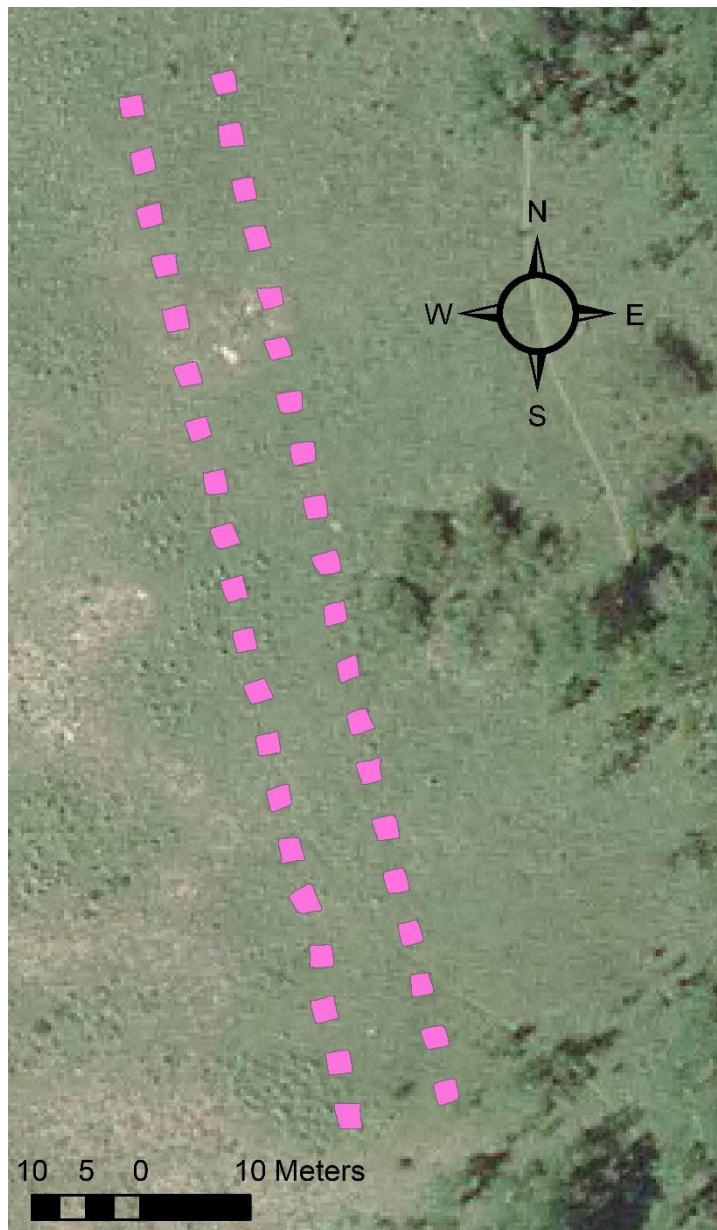


Figure 1: Map of study site, located at ~ 3000m in Gothic, Colorado, USA. Each box represents one 2x2m plot.

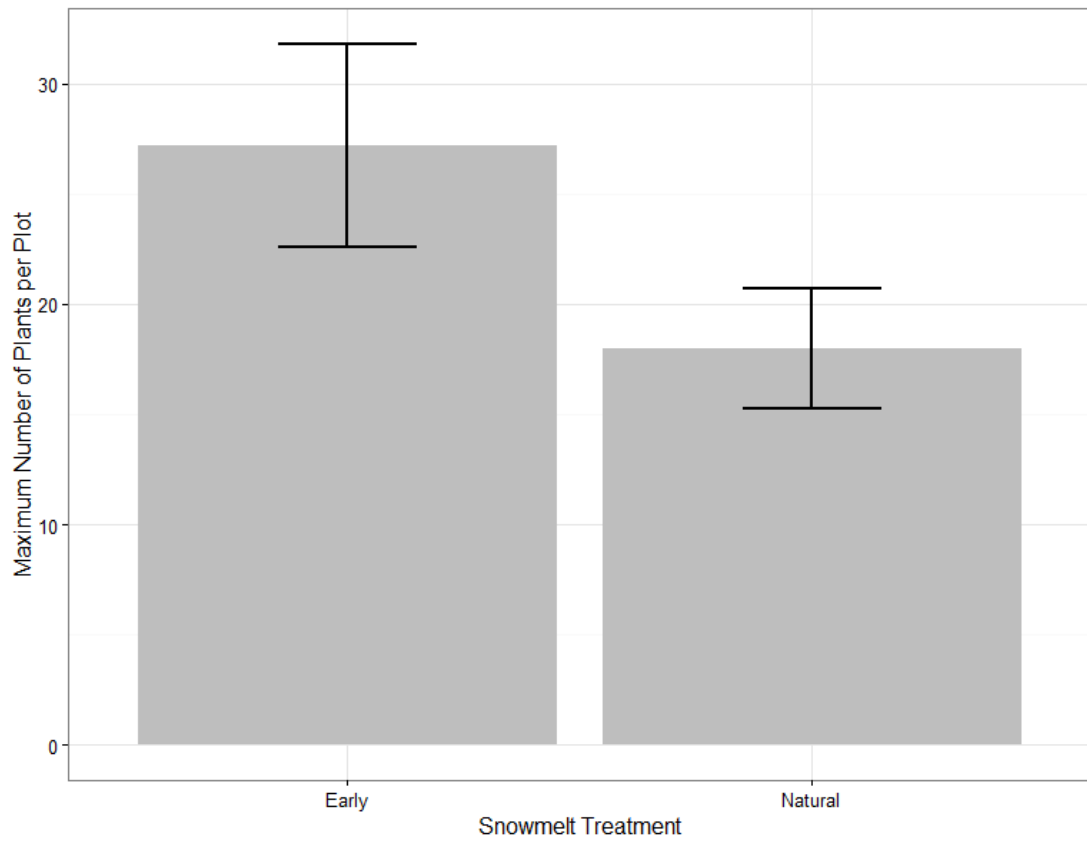


Figure 2: Mean ($\pm$ SE) maximum number of *Delphinium nuttallianum* plants per plot in natural and early snowmelt treatments.

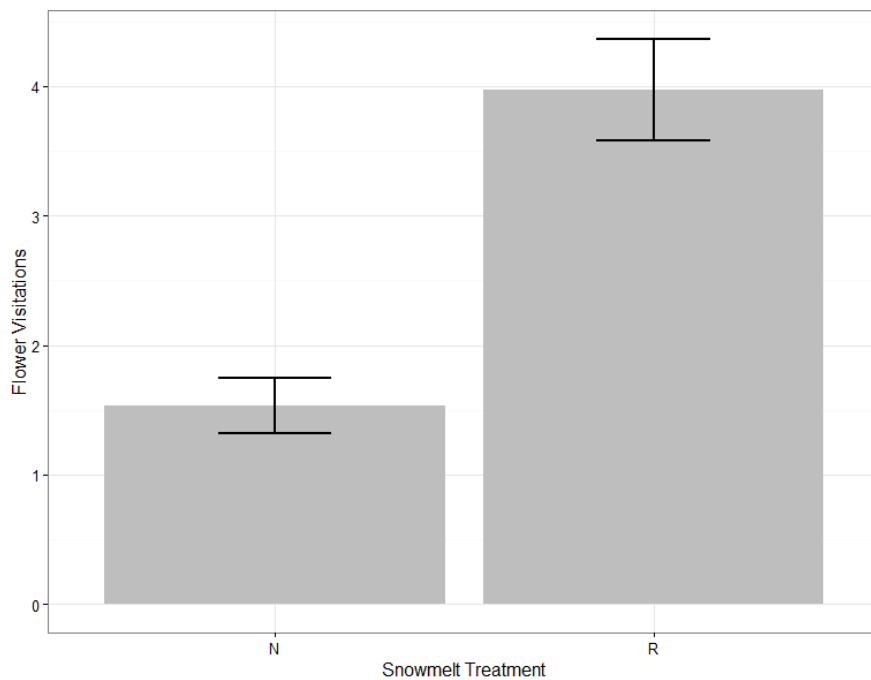


Figure 3a: Mean ($\pm$ SE) number of *Delphinium nuttallianum* flowers visited by pollinators per plot in natural and early snowmelt treatments.

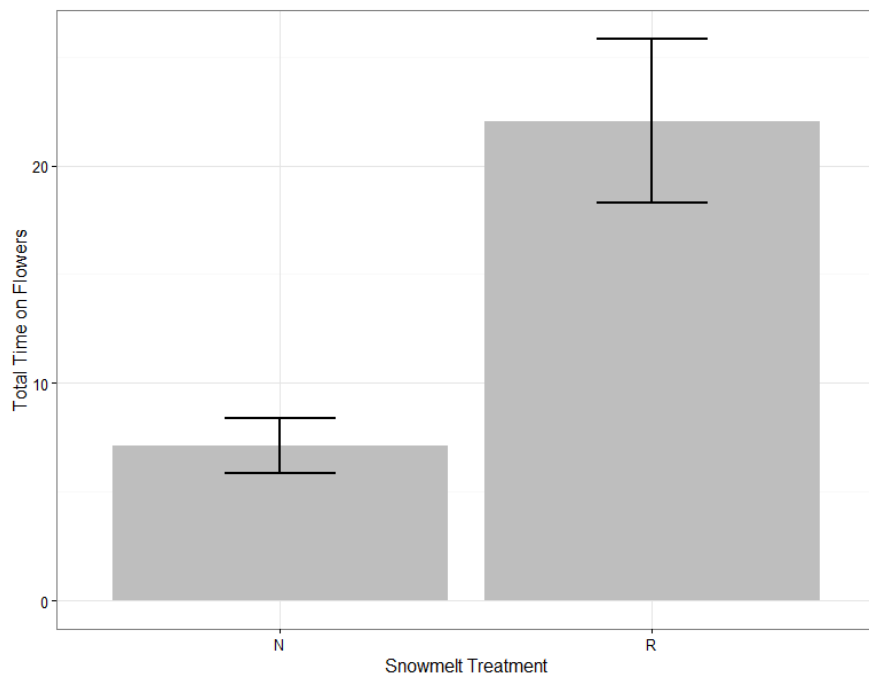


Figure 3b: Mean ($\pm$ SE) total time spent on *Delphinium nuttallianum* flowers by pollinators per plot in natural and early snowmelt treatments.

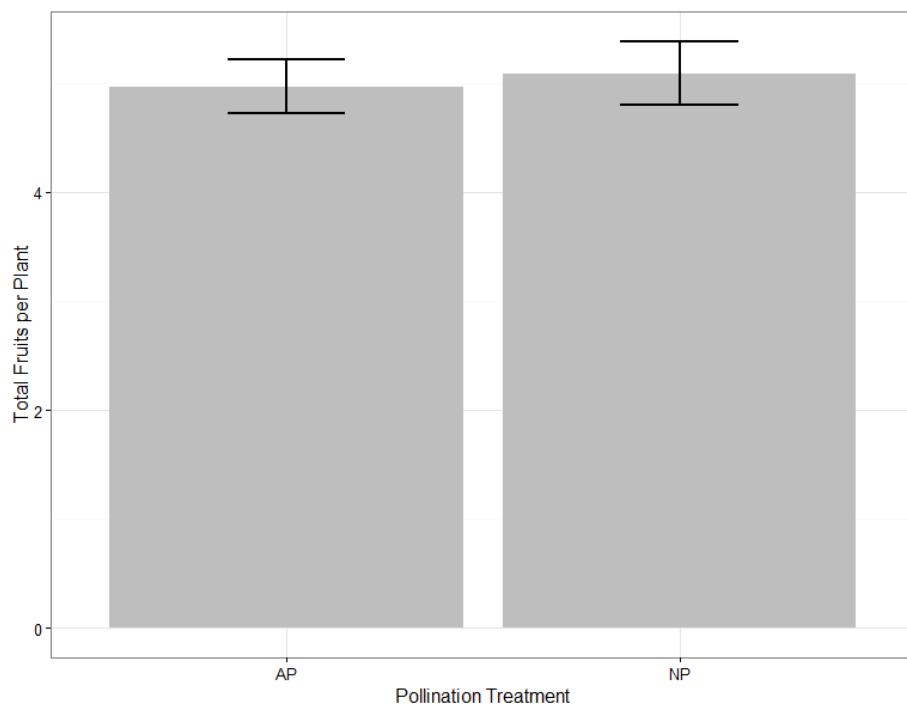


Figure 4a: Mean ($\pm$ SE) total number of *Delphinium nuttallianum* fruits per plant in natural and supplemental pollination treatments.

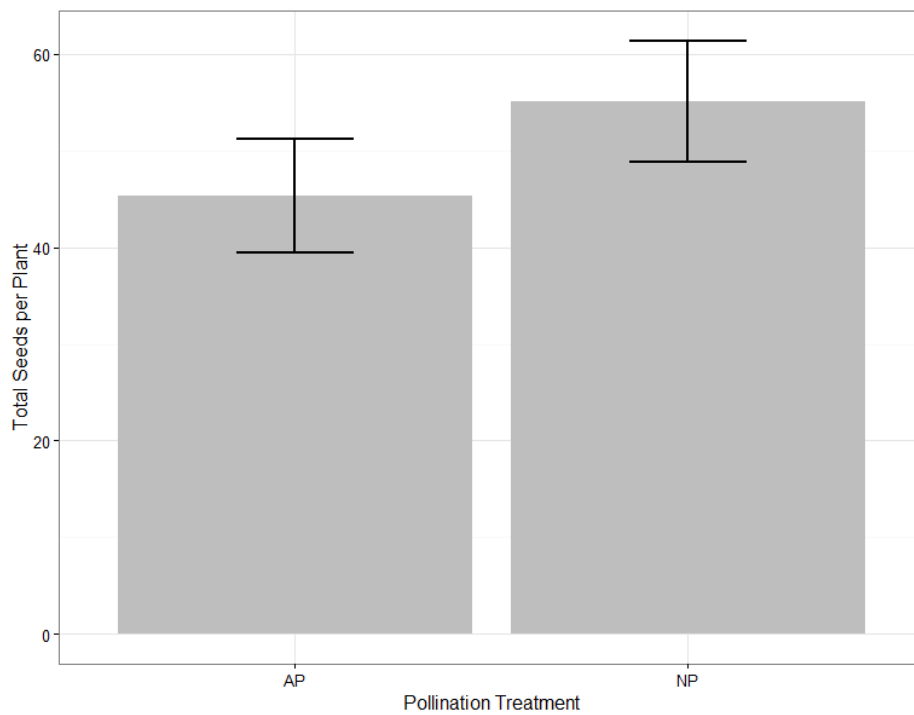


Figure 4b: Mean ($\pm$ SE) total number of *Delphinium nuttallianum* seeds per plant in natural and supplemental pollination treatments.

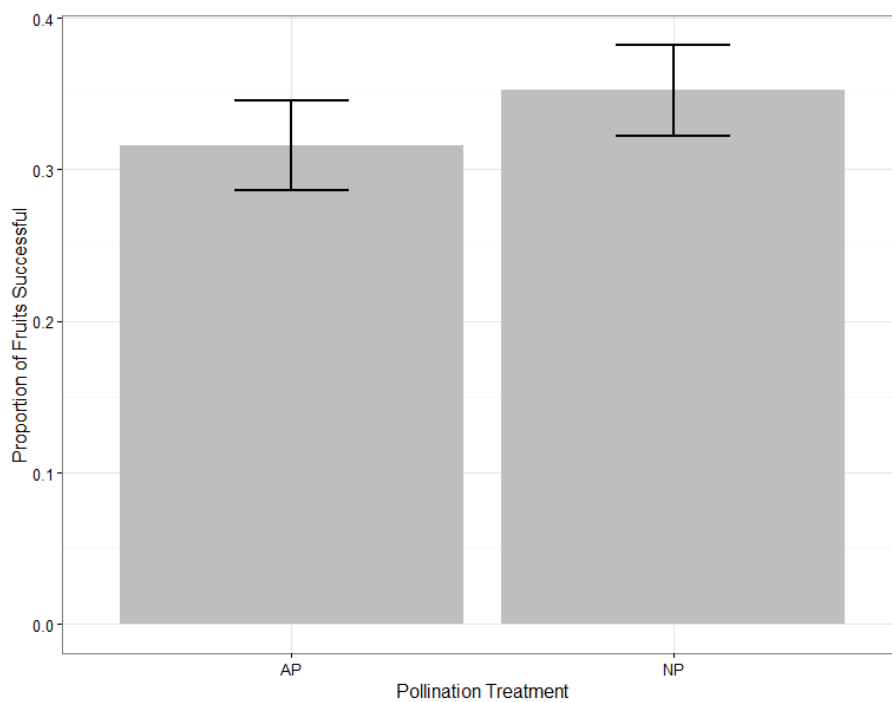


Figure 4c: Mean ($\pm$ SE) proportion of *Delphinium nuttallianum* fruits successful per plant in natural and supplemental pollination treatments.

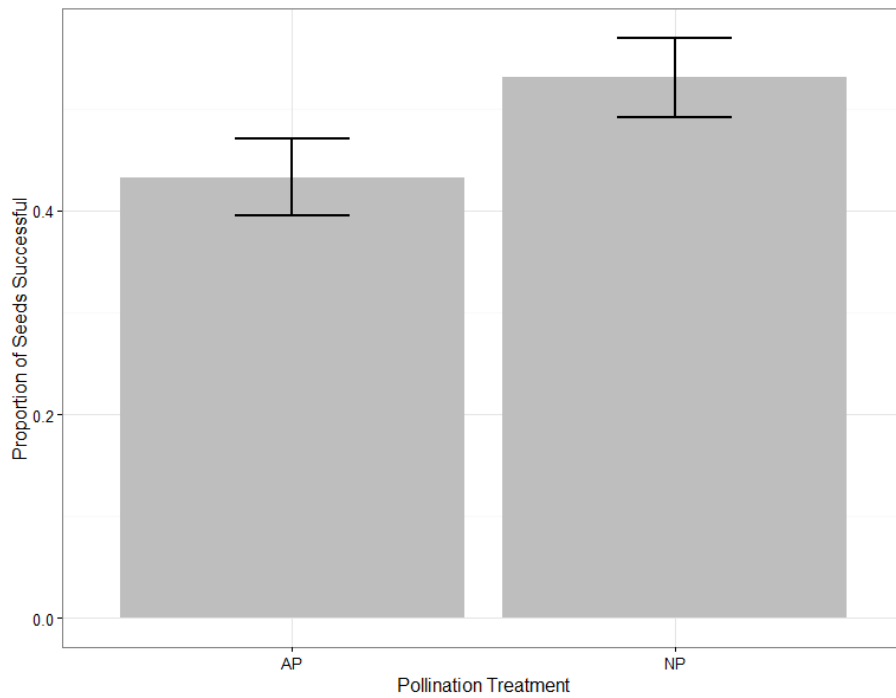


Figure 4d: Mean ($\pm$ SE) proportion of *Delphinium nuttallianum* seeds successful per plant in natural and supplemental pollination treatments.

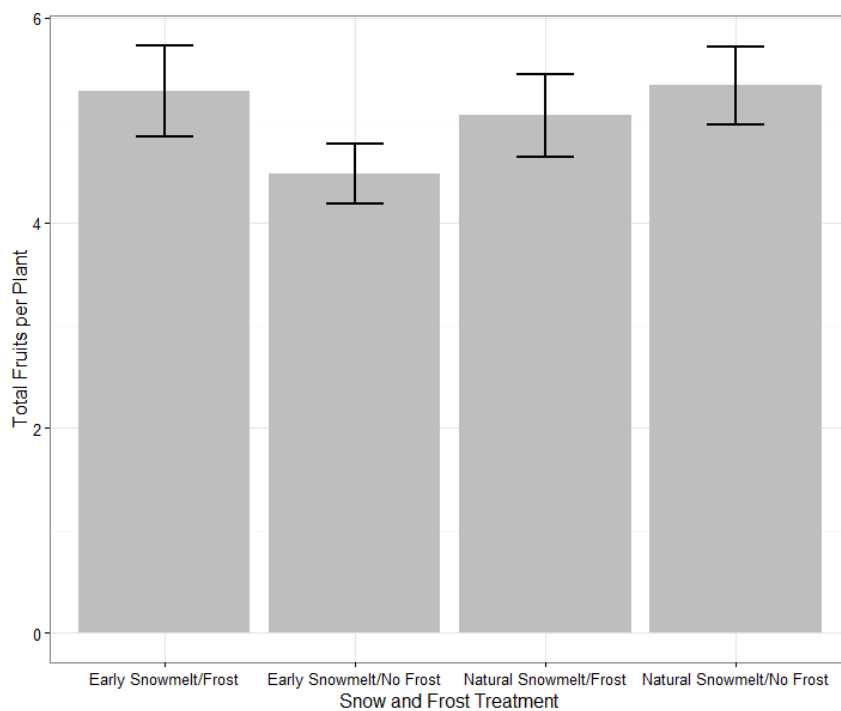


Figure 5a: Mean ($\pm$ SE) number of *Delphinium nuttallianum* fruits per plant in combined snowmelt and frost treatments.

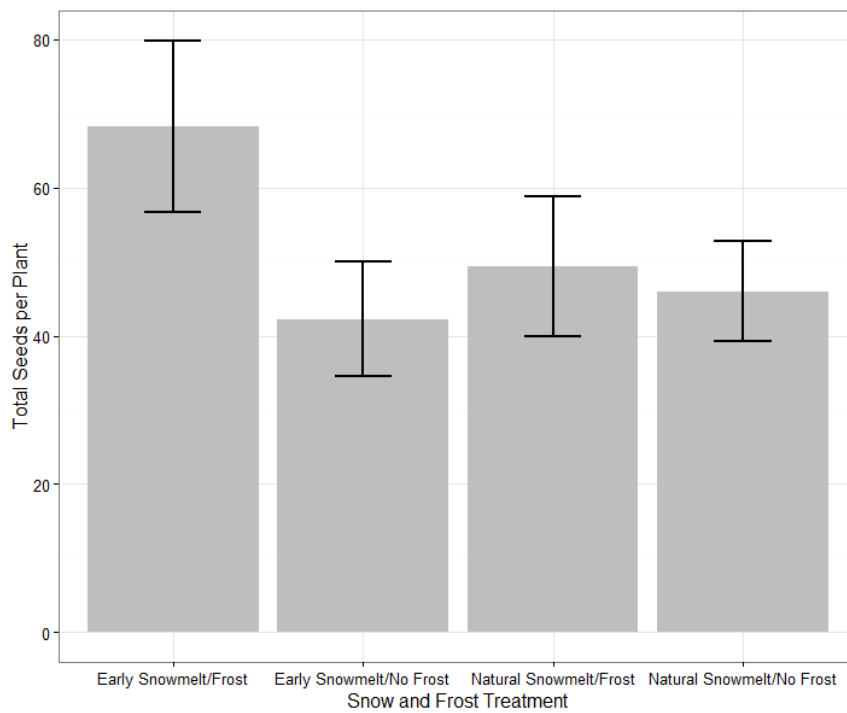


Figure 5b: Mean ($\pm$ SE) number of *Delphinium nuttallianum* seeds per plant in combined snowmelt and frost treatments.

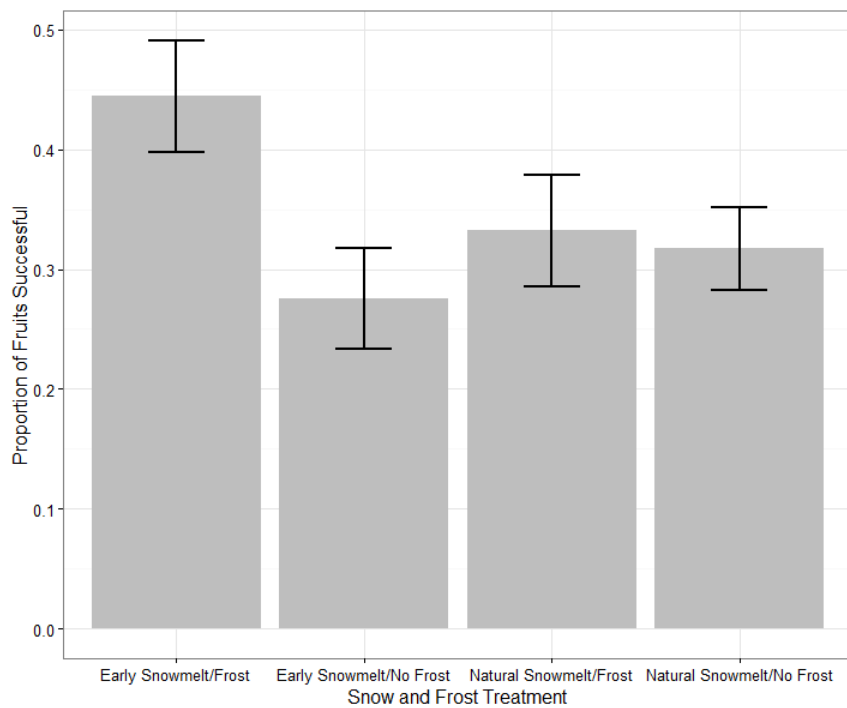


Figure 5c: Mean ($\pm$ SE) proportion of *Delphinium nuttallianum* fruits successful per plant in combined snowmelt and frost treatments.

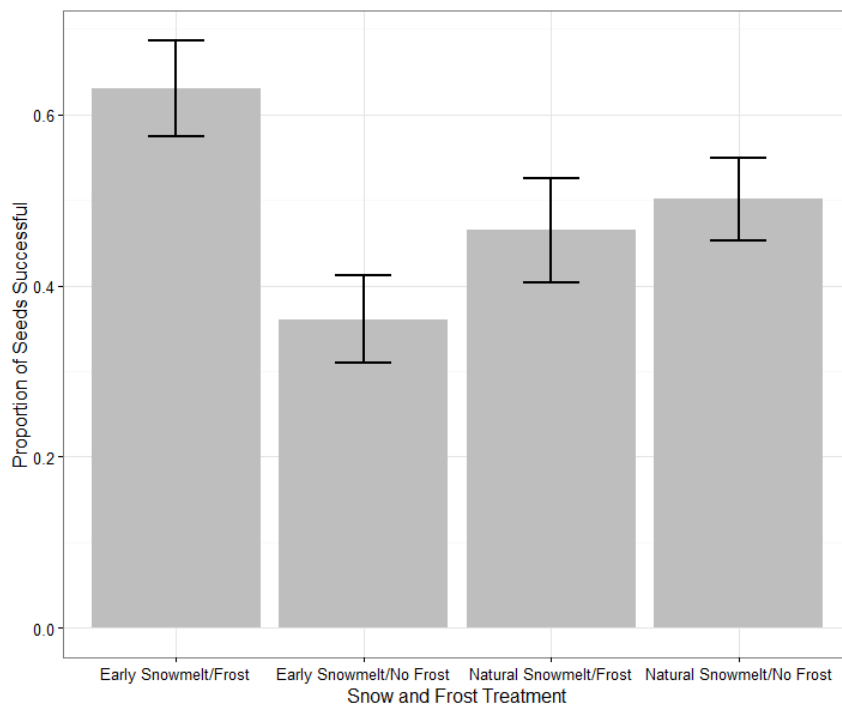


Figure 5d: Mean ($\pm$ SE) proportion of *Delphinium nuttallianum* seeds successful per plant in combined snowmelt and frost treatments.

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