

Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers

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Abstract

Frost is an important episodic event that damages plant tissues through the formation of ice crystals at or below freezing temperatures. In montane regions, where climate change is expected to cause earlier snow melt but may not change the last frost-free day of the year, plants that bud earlier might be directly impacted by frost through damage to flower buds and reproductive structures. However, the indirect effects of frost mediated through changes in plant–pollinator interactions have rarely been explored. We examined the direct and pollinator-mediated indirect effects of frost on three wildflower species in southwestern Colorado, USA, *Delphinium barbeyi* (Ranunculaceae), *Erigeron speciosus* (Asteraceae), and *Polemonium foliosissimum* (Polemoniaceae), by simulating moderate (−1 to −5°C) frost events in early spring in plants in situ. Subsequently, we measured plant growth, and upon flowering measured flower morphology and phenology. Throughout the flowering season, we monitored pollinator visitation and collected seeds to measure plant reproduction. We found that frost had species-specific direct and indirect effects. Frost had direct effects on two of the three species. Frost significantly reduced flower size, total flowers produced, and seed production of *Erigeron*. Furthermore, frost reduced aboveground plant survival and seed production for *Polemonium*. However, we found no direct effects of frost on *Delphinium*. When we considered the indirect impacts of frost mediated through changes in pollinator visitation, one species, *Erigeron*, incurred indirect, negative effects of frost on plant reproduction through changes in floral traits and pollinator visitation, along with direct effects. Overall, we found that flowering plants exhibited species-specific direct and pollinator-mediated indirect responses to frost, thus suggesting that frost may play an important role in affecting plant communities under climate change.

KEYWORDS

climate change, frost, phenology, plant reproduction, plant–pollinator interactions, pollination services

1 | INTRODUCTION

The abiotic environment plays a critical role in shaping plant survival, growth, and reproduction (Chapin, Bloom, Field, & Waring, 1987; Iler et al., 2013a). Plant survival and performance rely heavily on

temperature, precipitation, and photoperiod (Harte & Shaw, 1995); thus, any change in the abiotic environment can have serious effects not only on plant reproduction but also on plant community composition. One abiotic event that can have particularly detrimental effects on plants is episodic spring frosts. Frost, which we define as

the effects of subfreezing temperatures on living organisms (Inouye, 2000), occurs on clear, dry nights when heat is radiated into the atmosphere faster than it can be replaced from nearby warming sources (Leuning & Cremer, 1988; Sakai & Larcher, 1987), or when a cold air mass below freezing moves into an area. The formation of ice crystals due to frost can damage plant tissue, and in some cases, damage can be severe, resulting in the loss of leaves, flower buds, and ovaries, and even plant death (Ågren, 1988; Sutinen et al., 2001).

Frost has become a paradox in the context of climate change. Theoretically, increasing spring temperatures should lead to a decrease in plant damage from frost events, yet, this may not be the case in some montane regions (Bannister et al., 2005; Inouye, 2008). Many plant species in montane regions use spring snow melt as their primary phenological cue, emerging soon after the ground is bare. Recent changes in climatic conditions are causing thinner snowpack and earlier melting (Inouye, 2000; Wipf, 2010), resulting in earlier spring flowering phenology and the missed benefits of snow insulation during freezing temperatures (Baptist, Flahaut, Streb, & Choler, 2010; Ladinig, Hacker, Neuner, & Wagner, 2013). For example, in the Rocky Mountains of Colorado, USA, the date of first flowering for 30 plant species has advanced by approx. 3.3 days per decade since the 1970s (CaraDonna, Iler, & Inouye, 2014), but the probability that a spring frost will occur on a particular calendar date may not be changing as rapidly (Inouye, 2000). As a result, plants may be increasingly exposed to episodic frost by growing and flowering at times when nighttime temperatures still drop below freezing (Hanninen, 1991; Inouye, 2000; Baptist et al., 2010; Augspurger, 2013; but see Iler, Høye, Inouye, & Schmidt, 2013b; Walsh et al., 2014). For example, the timing of budbreak in conifers in Northern Ontario has advanced due to increasing spring temperatures; yet, the frequency of nighttime temperatures below freezing has remained stable over the last 90 years (Man, Kayahara, Dang, & Rice, 2009), thus resulting in an increase in needle damage and bud injury due to frost.

Early spring frost may have both direct and indirect effects on plant species, with direct effects studied most commonly (Hufkens, Friedl, Keenan, & Sonnentag, 2012; Lindow, 1983; Miller-Rushing, Høye, Inouye, & Post, 2010; Taschler & Neuner, 2004), especially in agricultural systems (Gu et al., 2008; Zheng, Chapman, Christopher, Frederiks, & Chenu, 2015). Frost can directly damage leaves, flower buds, and ovaries, thus reducing plant growth and reproduction (Burke, Quamme, & Weiser, 1976; Pearce, 2001). For example, in *Helianthella quinquenervis* (Asteraceae), bud mortality due to spring frost ranged from 65%–100% over a 7-year period (Inouye, 2008). Furthermore, frost can indirectly affect plant growth and reproduction via changes in the frequency and magnitude of plant–animal interactions. Previous studies have examined how frost affects animal abundance (Boggs & Inouye, 2012; Ehrlich, Breedlove, Brussard, & Sharp, 1972; Nixon & McClain, 1969; Thomas, Singer, & Boughton, 1996), but few have studied how changes in plant traits due to frost influence plant attractiveness to animals that use the plants for food resources. For example, frost events have the potential to modify plant–pollinator interactions if frost results in flowers

that are less attractive to pollinators due to changes in floral morphology or reductions in floral and pollen abundance, both of which could aggravate pollen limitation of plant reproduction. Previous studies on fruit trees have found that tissue and cell damage due to frost results in smaller flowers and damage to the pistil and style (Rodrigo, 2000). Alternatively, pollinators could help plants recoup lost fitness from frost-damaged flowers if they increase visitation rates and seed set in remaining, undamaged flowers. While the direct effects of episodic frost in montane regions have been studied for several plant species (Bannister et al., 2005), the indirect effects via changes in pollination are relatively unexplored.

We studied the effects of frost events on floral morphology and flowering traits, plant–pollinator interactions, and plant reproduction for three montane plant species. We experimentally manipulated frost to isolate the direct effects of frost on plants and indirect effects mediated through changes in plant–pollinator interactions. Studying how frost directly affects pollinators (e.g., Boggs & Inouye, 2012) was beyond the scope of this study, but can be assessed in future research. We addressed the following questions: (i) How do plant growth, floral morphology, and flowering traits respond to early season frost events? (ii) To what degree do pollinators respond to plants that have been exposed to frost? and (iii) How does frost impact plant reproduction? We predicted that plants exposed to early season frost would have a lower probability of flowering and, for those that did flower, would produce fewer, smaller flowers (Gezon, Inouye, & Irwin, 2016). Assuming that frost affects floral or flowering traits, we also predicted that pollinators would avoid frost-damaged plants, resulting in decreased plant reproduction. Given the potential for climate change to increase the incidence of frost damage (Man et al., 2009), this research provides important insight into how frost impacts plant fitness in natural communities and some of the mechanisms involved.

2 | METHODS

2.1 | Study system and focal species

This study was conducted in the spring and summer of 2014 in montane meadows around the Rocky Mountain Biological Laboratory (RMBL), in Gothic, Colorado, USA (elev. 2950 m). The meadows surrounding the RMBL host a diversity of flowering plants that rely on pollinators, especially bees (Gezon et al., 2016), for successful reproduction. The climate around the RMBL exhibits decadal variation in precipitation (Ault & St. George, 2010). It is predicted that climate change in this region will disrupt this pattern, causing temperatures to increase and precipitation to decrease (McCabe, Betancourt, & Hidalgo, 2007; Stewart, Cayan, & Dettinger, 2005). We have already seen a trend toward an increase in spring temperatures by $0.34 \pm 0.21^\circ\text{C}$ per decade over the last 40 years (Iler et al., 2013b), which has led to earlier snow melt and consequently earlier plant bloom. These changes may result in an increased incidence of plant damage from episodic spring frost events, especially if the last frost-free day of the year is not changing at the same rate (Inouye, 2000).

We studied three focal plant species that flower mid-summer and are long-lived perennials: *Erigeron speciosus* (Asteraceae), *Delphinium barbeyi* (Ranunculaceae), and *Polemonium foliosissimum* (Polemoniaceae). Hereafter, we refer to each species by genus. We chose three mid-summer flowering species because they likely preform their buds (Inouye, Morales, & Dodge, 2002), and their leaves and other tissues have been shown to be more frost sensitive than their earlier season congeners (CaraDonna & Bain, 2016); thus, their floral and flowering traits may be susceptible to early season frost damage. *Erigeron* produces light pink-to-purple, hermaphroditic flowers, with 1–11 flower heads per stem (Robertson, 1999), and on average 15 stems per plant (G.L. Pardee, *per. obs.*). *Erigeron* is weakly self-compatible, and the flowers are visited by a wide range of pollinators, including bees, flies, moths, butterflies, and beetles (Kearns, 1992; Pohl, Van Wyk, & Campbell, 2011; Wilson, Wilson, Loftis, & Griswold, 2010). *Delphinium* produces dark purple, protandrous flowers on racemes that can grow up to 2 m in height (Dodson & Dunmire, 2007). Up to 20 flowering stalks can grow from a single root system (Dodson & Dunmire, 2007), each stalk producing approximately 25 flowers (Elliott & Irwin, 2009). *Delphinium* is weakly self-compatible, and the flowers are primarily pollinated by bumble bees (*Bombus* spp.; Elliott & Irwin, 2009). *Polemonium* has purple, campanulate flowers, and populations are either hermaphroditic or gynodioecious (Brody & Waser, 1995; Campbell, Forster, & Bischoff, 2014). In the population we used for our study, 96% of the plants were hermaphroditic. *Polemonium* produces an average of seven stalks per plant and 28 flowers per stalk (G.L. Pardee & R.E. Irwin, *per. obs.*). *Polemonium* is self-incompatible, and the flowers are pollinated by bees and flies (Campbell et al., 2014). *Polemonium* and *Delphinium* have been shown to be pollen limited for reproduction (see Zimmerman & Pyke, 1988 for *Polemonium*, and Elliott and Irwin, 2009 for *Delphinium*). The buds and flowers of *Erigeron* and *Delphinium* are directly sensitive to the effects of frost (Inouye, 2008), but the indirect effects of frost via changes in pollination are unknown.

2.2 | Experimental design

We selected 60 plants of each species within a meadow (*Erigeron* 38°58'00.0"N, −106°59'23.0"W; *Polemonium* 38°51'25.1"N, −107°03'19.4"W; *Delphinium* 38°57'56.6"N, −106°59'36.5"W) and randomly assigned them to a frost treatment or no-frost treatment ($n = 30$ plants per treatment per species). To simulate frost events, we created frost chambers using foam coolers (43.1 × 36.5 × 30.4 cm) that were separated into two sections (upper and lower) by steel mesh. We created below-freezing temperatures by placing 100 g of dry ice and an ice:salt mixture (400 g: 40 g ratio) in plastic bags in the upper chamber to achieve temperatures between −1° and −5°C, typical episodic freezing temperatures postsnow melt in the spring at the RMBL (D.W. Inouye, unpublished data). Each of the frost chambers contained a temperature logger that was attached to the side near the top of the plant (iButtonLink LLC, Whitewater, WI, USA), that recorded the temperature every 3 min throughout the duration of the frost event. In addition, we used a nearby weather

station that recorded temperature every 5 min to determine the ambient temperature throughout the duration of the frost simulation. We used ANOVAS to compare the cooler and ambient air temperature conditions among the three plant species. All analyses (here and below) were performed in R (version 3.2.4).

For plants in the frost treatment, we placed the frost chambers over the entire plant (all stalks) as soon as buds were visible in mid/late June between 05:30 and 09:30 hours, as this is the time of day when frost events are most likely to occur (Bissonette, 1978). All plants from all three species were <30 cm in height at the time of the frost manipulation, and so the coolers were tall enough to fit over the plants. We performed the frost event one time for each plant to simulate a single, episodic frost event. Each cooler contained one plant, and we used 15 coolers over 2–3 days to frost each individual plant of each species; all three species were frosted over a 10-day span. Plants in the no-frost treatment received a chamber without coolant. Because no natural frost events occurred during the course of the experiment, we did not need to protect the frost or no-frost plants from natural frost. We recorded aboveground inflorescence survival within each treatment, and then used chi-square tests to assess whether frost affected survival. We note that it is unlikely that our treatments affected root survival, and they did not result in soil and root disturbance via frost heaving; instead, our treatments only likely affected aboveground plant parts, and thus our measurements only included aboveground buds and stalks that may have died back following treatment application. Separate analyses (here and below) were performed for each plant species.

2.2.1 | Plant and floral measurements

To determine whether frost affected plant growth, we measured the height of the tallest flowering stalk. The floral traits we measured were specific to each plant species based on their floral morphologies (Fig. S1; Galen, 1989; Ishii & Harder, 2006). Measurements were made using digital calipers to the nearest 0.01 mm on three flowers per plant and then averaged per plant. For *Erigeron*, we measured petal length, disk width, and head width. For *Delphinium*, we measured spur length, width of the flower opening, petal width, and petal length. For *Polemonium*, we measured corolla length, corolla diameter (distance from tip to tip of opposite corolla lobes), petal length, and petal width. We also counted total flowers produced per plant and the number of flowers in bloom every 2–3 days throughout the bloom period to assess whether frost affected flower production and phenology. To test whether frost treatment affected floral and flowering traits for each species, we first used a MANOVA, followed by ANOVAS for each response variable if the MANOVA was significant (Scheiner, 1993).

2.2.2 | Pollinator visitation

Upon flowering, we monitored pollinator visitation to test for indirect effects of frost on plant–pollinator interactions. To observe pollinators, we conducted observations for 1 hour each in the morning

and afternoon every 2–3 days throughout the blooming period. We only recorded visits by insects that contacted the sexual organs of flowers. For each observation period, we haphazardly walked among the focal plants, stopping when we saw a pollinator visiting a flower. Using a voice recorder, we noted the identification of each pollinator on the wing to the lowest taxonomic level possible, duration of each floral visit, and the number of flowers visited on each plant. We followed the visitor until it left the area. After each observation period, we netted for pollinators for 10 min to collect voucher specimens.

Across all floral visitors per plant species, we first ran a generalized linear mixed model using a log-link function and Poisson distribution in the R package LME4 (Bates, Maechler, Bolker, & Walker, 2015), comparing the total number of flowers visited per plant with frost treatment (frost vs. no-frost) and date of observation as fixed factors, and plant as a random effect. For this analysis, we included plants that were not visited, but were flowering on the day of the pollinator observations. Next, we ran a generalized linear mixed model using a gamma distribution, comparing the effect of frost on the average time spent per flower with frost treatment and date of observation as fixed factors, and plant as a random effect. For this analysis, we excluded all the plants that were not visited, regardless of whether they were flowering that day. We assessed the fit of the model by calculating the dispersion ratio. We then used least squares ratio tests of nested models to measure significance. Finally, we conducted the same analyses but separated the visitors into three different pollinator guilds: flies and Lepidoptera, bumble bees, and other bees to test for differences in visitation patterns among pollinator groups. Our “other bee” group contained bees from the following genera: *Halictus*, *Osmia*, *Megachile*, *Panurginus*, *Pseudopanurgus*, *Andrena*, and *Hylaeus*. Because we found significant effects of the frost treatment on pollinator visitation to *Erigeron* (but not to *Delphinium* or *Polemonium*, see Results), for *Erigeron* we conducted an additional analysis including floral and flowering traits in the model to assess the degree to which differences in pollinator visitation were associated with frost-driven changes in floral traits.

2.2.3 | Plant reproduction

Once the flowers senesced, we measured female plant reproduction by collecting fruits and counting the seeds produced per fruit up to three stalks per plant. We recorded the number of aborted fruits (fruits producing no seeds) and counted the seeds produced in all seed-bearing fruits. Furthermore, for any fruits receiving predispersal seed predation, we recorded the number of fruits partially or fully destroyed and also the number of insects found within the fruits (when they occurred). We estimated female reproduction using the following response variables: total seed set per stalk (number of mature seeds), proportion fruit set (number of seed-bearing fruit/total flowers), and mean seeds per seed-bearing fruit.

We examined the effects of frost treatment on total seed set and proportion fruit set for all of the plants that flowered with plant as a random effect by conducting generalized linear mixed models with a zero-inflated negative binomial distribution in the R package

GLMMADMB (Fournier, Skaug, & Ancheta, 2012; Skaug, Fournier, Nielsen, Magnusson, & Bolker, 2013). This analysis allowed us to assess in general how frost affected plant reproduction. Furthermore, because we found a significant effect of frost on pollinator visitation rate and seed set for *Erigeron* (but not *Polemonium* or *Delphinium*, see Results), we ran an additional analysis on each metric of plant reproduction with frost treatment, the covariates pollinator visitation and time spent per flower, and their interaction using linear models.

3 | RESULTS

3.1 | Frost chamber and ambient air temperature conditions

The average lowest temperature the frost chambers reached was $-3.98 \pm 2.45^{\circ}\text{C}$ (mean \pm SD) for *Erigeron*, $-2.71 \pm 1.24^{\circ}\text{C}$ for *Polemonium*, and $-3.14 \pm 1.95^{\circ}\text{C}$ for *Delphinium*, showing that for all three plant species, temperatures dipped below freezing. Examination of the temperature profiles in the frost chambers document a gradual decline in temperature over approx. 50 min until the chambers were below freezing, and a holding of that below-freezing temperature for at least 60 min., followed by a gradual increase in temperature (Figure 1). During the nights of the frost simulations, the average lowest ambient air temperature was $2.75 \pm 0.74^{\circ}\text{C}$ (mean \pm SD) for *Erigeron*, $2.14 \pm 1.38^{\circ}\text{C}$ for *Polemonium*, and $2.13 \pm 1.45^{\circ}\text{C}$ for *Delphinium* (Figure 1). There were no significant differences in the temperature conditions among the three species within the frost chambers or in the ambient air temperatures ($F < 2.6$, $p > .08$ for both the frost chamber and ambient air temperatures).

3.2 | Aboveground inflorescence survival and growth

We found no effect of the frost treatment on aboveground inflorescence survival for *Erigeron* ($\chi^2 = 2.41$, $df = 1$, $p = .12$) or *Delphinium*

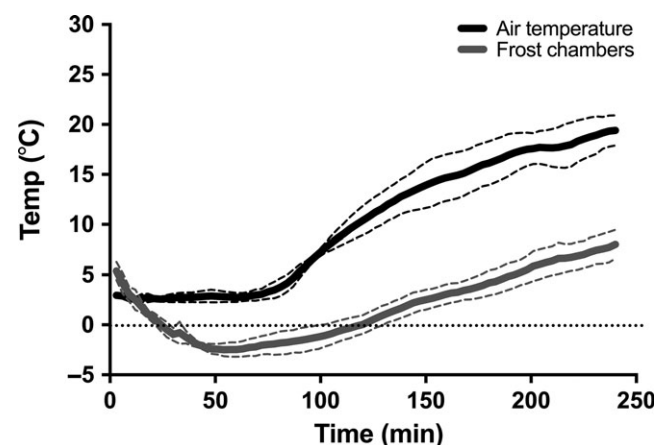


FIGURE 1 Temperature profiles for ambient air temperature (black lines) and frost chambers (gray lines) throughout the duration of the frost simulation for all three species combined. The dotted lines represent the standard deviation

($\chi^2 = 0.07$, $df = 1$, $p = .79$). For both species, survival was 80%–100% across both treatments. However, for *Polemonium*, frost reduced aboveground inflorescence survival by 12% ($\chi^2 = 6.249$, $df = 1$, $p = .01$).

When we examined plant and floral morphology, we found significant differences between the frost and no-frost treatments, but only for *Erigeron* (MANOVA: $F_{4,46} = 6.0$, Wilks' $\lambda = 0.65$, $p < .01$). We found that frost reduced *Erigeron* plant height by 29% ($F_{1,49} = 29.26$, $p < .001$), the size of the flower head by 7.8% ($F_{1,49} = 4.91$, $p = .03$), and the total number of flowers produced per plant by 40% ($F_{1,49} = 6.20$, $p = .01$). In contrast, the frost treatment had no effect on plant height, flower morphology, or flower production for *Delphinium* or *Polemonium* (MANOVA's: *Delphinium*: $F_{5,25} = 1.92$, Wilks' $\lambda = 0.72$, $p = .12$; *Polemonium*: $F_{4,31} = 0.74$, Wilks' $\lambda = 0.08$, $p = .56$). Finally, we did not find a difference in flowering phenology, measured as the first flowering date and flowering duration, between the frost and no-frost treatments for any of the species ($F < 0.98$, $p > .33$ in all cases).

3.3 | Pollinator visitation

The frost treatment significantly affected pollinator visitation but again, only to *Erigeron* and not to *Delphinium* or *Polemonium*. For *Erigeron*, plants in the frost treatment received 48% fewer visits from pollinators ($\chi^2 = 4.20$, $df = 1$, $p = .04$; Figure 2a) but pollinators spent 33% more time per flower compared to plants in the no-frost treatment ($\chi^2 = 5.91$, $df = 1$, $p = .02$; Figure 2b). Across both treatments, 37% of the floral visits to *Erigeron* were by flies from the Syrphidae and Muscidae families and Lepidoptera, 36% were by bumble bees, and 23% were by other bees. When we conducted analyses by pollinator group, we found that plants in the frost treatment received 66% fewer visits by flies and Lepidoptera ($\chi^2 = 7.17$, $df = 1$, $p = .007$) but not by bumble bees ($\chi^2 = 0.04$, $df = 1$, $p = .83$) or other bees ($\chi^2 = 0.68$, $df = 1$, $p = .41$). Furthermore, we found that flies and Lepidoptera spent, on average, 65% more time on flowers from plants in the frost compared to no-frost treatment (Flies and Lepidoptera: $\chi^2 = 4.95$, $df = 1$, $p = .03$), but bumble and other bees did not vary in the time they spent per flower ($p > .37$ in both cases). When we included floral traits in the statistical models for

Erigeron, we found that frost treatment ($\chi^2 = 4.15$, $df = 1$, $p = .04$) and floral traits were important for affecting pollinator visitation rates. Plants with smaller floral display ($\chi^2 = 379.13$, $df = 1$, $p < .01$; Figure 3a) and smaller flower heads ($\chi^2 = 108.76$, $df = 1$, $p < .01$; Figure 3b) attracted fewer visits from pollinators, with no interaction between frost treatment and either covariate ($p > .06$ in both cases). However, we did not find any association between floral traits and the amount of time visitors spent on each flower ($p > .30$ for each trait measured).

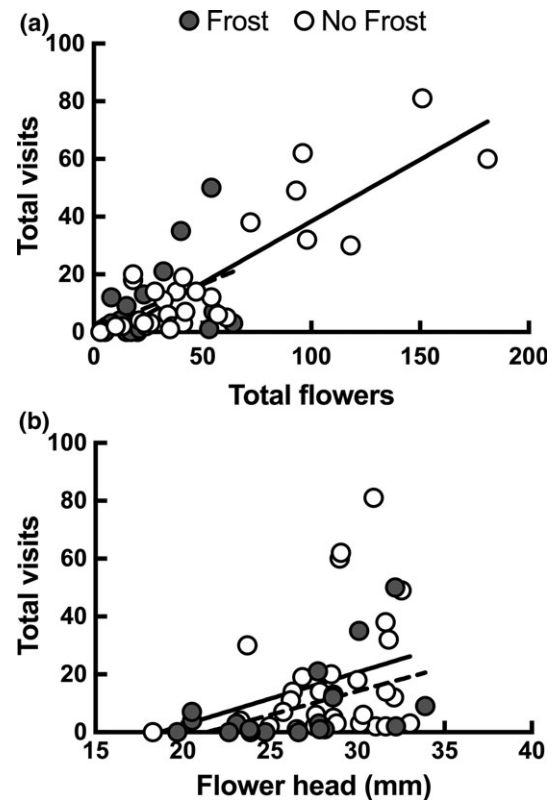


FIGURE 3 For *Erigeron speciosus*, there was a significant positive relationship ($p < .01$ in all cases) between total pollinator visits and (a) floral display and (b) flower head size, with no interaction between frost treatment and either covariate. The gray circles and dashed lines represent the frost treatment and the open circles and solid lines represent the no-frost treatment

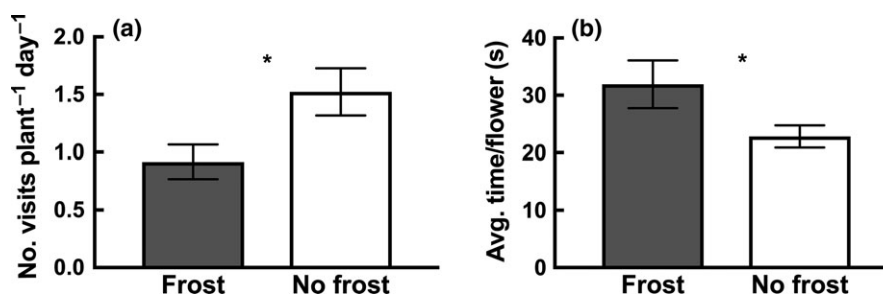


FIGURE 2 Pollinator visitation to *Erigeron speciosus* measured as (a) mean number of pollinator visits per plant per day and (b) mean time spent per flower (in seconds) in the frost (gray) and no-frost (white) treatments. Pollinators visited significantly fewer plants per day in the frost treatment but spent more time on average on flowers in the frost treatment. Bars represent means \pm SE. The asterisks denote statistical significance at $p < .05$

For *Delphinium* and *Polemonium*, we found no differences in any measures of pollinator visitation or by pollinator group composition between the frost and no-frost treatments ($p > .35$ in all cases).

3.4 | Plant reproduction

When we examined the effect of frost on plant reproduction, we found that the frost treatment had significant negative effects on the reproduction of *Erigeron* and *Polemonium*, but not *Delphinium*. For *Erigeron*, frost decreased the proportion of successful fruits by 8.5% ($\chi^2 = 4.28$, $df = 1$, $p = .03$; Figure 4a), the total number of seeds by 27% ($\chi^2 = 4.49$, $df = 1$, $p = .03$; Figure 4b), and the

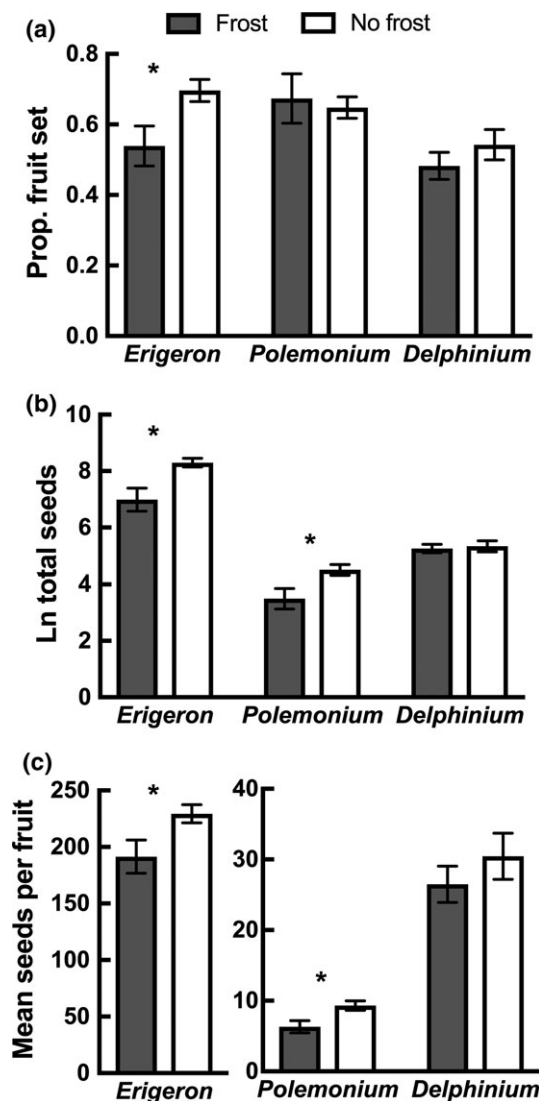


FIGURE 4 (a) Proportion fruit set, (b) total seeds produced, and (c) mean seeds per fruit in frost (gray) and no-frost (white) treatments for *Erigeron speciosus*, *Polemonium foliosissimum*, and *Delphinium barbeyi*. In (c) we report *Erigeron* separate from *Polemonium* and *Delphinium* so that differences in the y-axis scale do not affect the ability to observe patterns in mean seeds produced per fruit per species. Bars are means \pm SE. The asterisks denote statistical significance at $p < .05$

average number of seeds per fruit by 9% ($\chi^2 = 3.92$, $df = 1$, $p = .04$; Figure 4c). For *Polemonium*, frost did not have an effect on proportion fruit set ($\chi^2 = 0.63$, $df = 1$, $p = .42$; Figure 4a), but frost reduced total seeds produced by 45% ($\chi^2 = 4.39$, $df = 1$, $p = .03$; Figure 4b) and average seeds per fruit by 39% ($\chi^2 = 3.90$, $df = 1$, $p = .05$; Figure 4c). Frost had no effect on *Delphinium* reproduction ($p > .14$ for all response variables).

Because we found significant effects of frost treatment on pollinator visitation rate to *Erigeron*, we ran an additional model that included not only frost treatment but also pollinator visitation rate as a covariate to tease apart the direct effects of frost on plant reproduction vs. the effects mediated through changes in pollinator visitation. We found seed production increased with pollinator visitation ($\chi^2 = 11.04$, $df = 1$, $p = .001$; Figure 5), but the slope of this relationship was steeper for the frost compared to no-frost treatment (frost treatment \times visitation interaction: $\chi^2 = 33.95$, $df = 3$, $p < .01$). This result suggests that while frost has negative effects on plant reproduction, increasing pollinator visitation to plants in the frost treatment helps partially, but not fully, to rescue seed production. However, we did not find any effect of visitation on fruit set or average seeds per fruit ($p > .08$ in both cases).

4 | DISCUSSION

Climate change is resulting in plants being increasingly exposed to episodic spring frost events. Although previous studies have examined the direct effects of frost on tissue damage in flowers and leaves (Taschler & Neuner, 2004), the indirect effects of frost mediated through changes in pollination services have received less attention. Overall, we found species-specific effects of a single frost event on aboveground inflorescence survival, growth, pollinator visitation, and seed set. These direct and indirect effects could have long-term impacts on montane flowering species under climate change as plants may become exposed to more frost events as the snow melt date continues to advance.

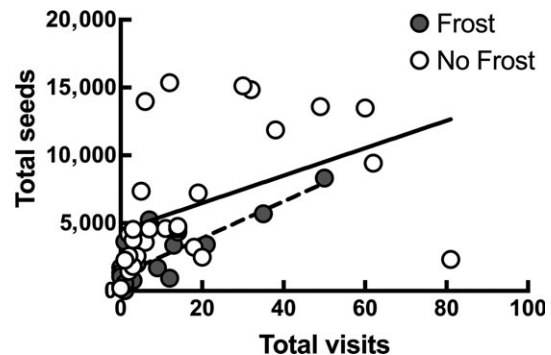


FIGURE 5 For *Erigeron speciosus*, there was a significant positive relationship ($p < .01$) between total seeds produced and pollinator visits. The gray circles and dashed lines represent the frost treatment and the open circles and solid lines represent the no-frost treatment

We found that frost had direct impacts on *Erigeron* and *Polemonium* but not on *Delphinium*. The frost treatment did not affect *Erigeron* aboveground inflorescence survival, but *Erigeron* in the frost treatment produced fewer, smaller flowers than the plants that did not undergo a frost event. For *Polemonium*, frost significantly reduced aboveground inflorescence survival by 12%. Our findings for *Erigeron* extend prior observational field studies and laboratory studies suggesting that this wildflower species is frost sensitive (Boggs & Inouye, 2012; CaraDonna & Bain, 2016; Inouye, 2008), in our case using experimental frost events in wild-growing plants. CaraDonna and Bain (2016) exposed individual leaves and flowers of *Erigeron* to different below-freezing temperatures in the laboratory. They found that 10% of the flowers showed frost damage at temperatures of -3.2°C , and 50% of flowers showed frost damage at -5°C . Between 1973 and 2005, Inouye (2008) reported that the average minimum spring temperature was -4.3°C , with some nights getting as cold as -8°C in the location where this research was conducted. Therefore, by combining these findings with our results, just one spring frost event could greatly reduce the number of *Erigeron* flowers in a summer. Although studies have suggested that *Polemonium* is frost sensitive (Brody, 1997), there are no in-depth studies to our knowledge on the effects of frost on aboveground inflorescence survival and morphology for this species. Our results suggest that *Polemonium* aboveground survival is sensitive to frost, but if plants do flower, floral traits remain unchanged.

It was surprising that we did not find any effects of frost on aboveground inflorescence survival or morphology for *Delphinium*. Previous observational studies on this species that used natural frost events as opposed to experimental frost found that frost was associated with reduced flower bud survival and damaged leaves and flowers (Inouye, 2008; Inouye et al., 2002). Moreover, in the study by CaraDonna and Bain (2016), *Delphinium* was only slightly more frost tolerant than *Erigeron*; thus, we expected to find frost-induced effects on *Delphinium* flower survival and reproduction. One hypothesis explaining the lack of effect is that there could be a period of time after the snow melts when *Delphinium* leaves shelter the small, early forming buds from radiation frost (Inouye et al., 2002). Because we subjected the plants to a single frost event soon after plant emergence, the buds could have been small enough to be protected from frost by the leaves. Or very early buds may not be as sensitive as larger, later bud stages that have been observed to have frost damage. Studies that experimentally frost plants single vs. multiple times during the spring season would help assess the degree to which *Delphinium* leaves protect the plants from frost early in the growing season.

Our study provides evidence of changes in plant–pollinator interactions due to episodic spring frost events. While other studies have alluded to changes in the frequency, behavior, and species of pollinators visiting plants following frost events (CaraDonna & Bain, 2016; Forrest, Inouye, & Thomson, 2010; Inouye, 2000), none have examined differences in pollinator visitation rates between frost-damaged and nonfrost-damaged plants. For one of the species we observed, *Erigeron speciosus*, we found that plants in the frost

treatment experienced reduced pollinator visitation. *Erigeron* was also the only species for which frost affected floral traits. Thus, one parsimonious explanation for this reduction in pollinator visitation to *Erigeron* in the frost treatment is that frost induced changes in floral traits that affected pollinator visitation. For example, we found that frost reduced the number of flowers and flower head size. In other systems, plants with fewer open flowers are often less attractive to pollinators than those with more open flowers (Makino & Sakai, 2007). In a similar vein, reductions in flower size often are associated with reduced pollinator attractiveness and pollinator visitation (Galen & Stanton, 1989). We find similar patterns with *Erigeron* as well, with plants with fewer open flowers and smaller flower heads receiving fewer pollinator visits. In our statistical model when we examined the effects of both frost treatment and floral traits on pollinator visitation, we found effects of all factors, suggesting that frost has additional effects on pollinator visitation beyond that incurred through changes in flower number and size. A more detailed documentation of frost-induced effects on plant and flower size and shape and floral rewards, and how pollinators respond to such changes, may provide additional mechanistic insight. One surprising result for *Erigeron* is that even though we found higher pollinator visitation to flowers in the no-frost treatment, the pollinators spent, on average, more time on the frost-damaged flowers. The mechanisms driving this result are unknown but could be due to frost affecting how hard it is to extract floral rewards or changes in floral rotational symmetry, factors known to affect flower handling time in other systems (Harder, 1983). Another possible explanation could be that the flowers in the frost treatment are less-visited and therefore could have more resources; thus, pollinators would spend more time at the flowers when they did visit. Finally, when we looked across different pollinator groups, we found that flies and Lepidoptera showed a preference for flowers in the no-frost treatment and spent more time on the frost-damaged flowers, but bumble and other bees did not show changes in foraging behavior. Pollinator groups often vary in how responsive they are to changes in flower morphology and floral rewards (Gomez et al., 2008; Kaczorowski, Gardener, & Holtsford, 2005). One reason that may explain these differences is that flies and Lepidoptera primarily collect nectar from flowers (Larson, Kevan, & Inouye, 2001), while bees collect nectar and pollen (Thorpe, 2000). Thus, the difference in visitation rates among the groups could be explained if frost-damaged flowers experienced a reduction in nectar but not in pollen, suggesting that flies and Lepidoptera would avoid frost-damaged flowers due to lower resource availability. For additional mechanistic insight, future studies should examine the direct effects of frost on nectar and pollen production, their extraction rates, and subsequent effects on pollinator behavior.

We found evidence for both direct and indirect, pollinator-mediated negative effects of frost on plant reproduction, but the responses were species-specific. For *Polemonium*, we only found evidence for direct, negative effects of frost on plant reproduction, given that frost had no effect on either floral traits or pollinator visitation. For *Erigeron*, however, we found evidence for both direct and

indirect, pollinator-mediated effects of frost. In our statistical model including both frost treatment and pollinator visitation on *Erigeron* seed production, we found effects of both factors, suggesting that not only does visitation affect plant reproduction but frost also has additional direct effects on plant reproduction beyond those incurred through changes in pollination. Surprisingly, we also found a significant interaction between frost treatment and pollinator visitation with increasing pollinator visitation more strongly affecting seed set in plants in the frost compared to no-frost treatment. This result suggests that increasing pollinator visitation may help partially, but not fully, rescue the negative effects of frost on seed production. Why increasing pollinator visitation more strongly benefits seed production in the frost compared to no-frost treatment is unknown, but could be due to resource reallocation within the plant. These results lend support to the importance of the abiotic environment having not only direct effects on plant reproduction but also indirect effects via changes in pollination (Carroll, Pallardy, & Galen, 2001; Potts et al., 2003) and suggest that changes in species interactions may be critically important in assessing the mechanistic response of plants to climate change.

Five caveats are important to consider in the interpretation of our study. First, we only examined the effects of frost on three mid-blooming species, and not on any early blooming species. Plant species that bloom soon after snow melt might be at a greater risk to frost damage because their buds and flowers might undergo several frost events before the later blooming plants even begin to germinate and produce buds. Alternatively, early blooming plants might have a higher tolerance to frost as a means of withstanding harsh, early spring weather conditions (CaraDonna & Bain, 2016). Future experiments should be conducted to examine the direct and indirect effects of frost on early blooming species in the field. Second, we only exposed the plants in our experiments to one moderate frost event. Unless plants are hit with a hard frost ($<-5^{\circ}\text{C}$), they might be able to withstand one or two moderate frost events before any damage to the buds occur (Augspurger, 2009; Thomson, 2010). Studies that have examined the direct effects of frost on plant reproduction have found that frost damage to seed set varies from year-to-year depending on the frequency and severity of frost events (Ågren, 1988; Kudo & Hirao, 2006). Although we only conducted our experiment for 1 year, it gives insight as to how seed set can be affected by just one moderate frost episode. We may expect seed set to be even lower in years with more severe frost events, which could possibly lead to long-term consequences for *Erigeron* and *Polemonium* populations. Third, we did not expose pollinators to frost events; yet, frost likely directly affects pollinator survival (Boggs & Inouye, 2012), phenology, and/or behavior and could have long-term implications for feedbacks between plants and their pollinators. The direct effects of climate change, including frost, on most pollinator groups are understudied and require further observational and experimental work (Forrest, 2014). Fourth, the three plant species we studied are long-lived perennials, and for *Erigeron* and *Polemonium*, it is possible that they may compensate for frost damage in 1 year with higher reproduction in the next, assuming they are not

hit again with frost. Studies that experimentally manipulate frost over multiple seasons and that assess the degree of compensatory responses to changes in plant reproduction will provide additional ecological insights. Fifth, our experiment did not consider that frost might indirectly affect pollination and plant fitness via changes in the aboveground survival and floral display of co-flowering species, which could lead to changes in competition or facilitation for pollinators (Feldman, Morris, & Wilson, 2004; Ghazoul, 2006) if some species are more frost tolerant than others, which is the case at our study site. Understanding the community-level implications of species-specific frost tolerance has important implications for how this episodic event could affect webs of interactions between plants and pollinators.

Declining snowpack and earlier snow melt due to climate change (Mote, Hamlet, Clark, & Lettenmaier, 2005; Solomon et al., 2007; Stewart, 2009) can lead to changes in plant communities. Studies that have examined how plant communities have become reshaped under climate change have primarily focused on abiotic conditions such as snow melt timing, increasing temperatures, and loss of precipitation as indicators (Cleland, Chuine, Menzel, Mooney, & Schwartz, 2007; Hanson & Weltzin, 2000; Henry & Molau, 1997). Given our results, frost may also likely play an important role in shaping flowering communities under climate change (Inouye, 2008). We found that frost has strong species-specific effects on plant reproduction, and effects can be both direct and indirect via changes in floral traits and pollinator visitation. How pollinators will respond to such community-level changes in floral communities and food resources, and how frost will directly affect their survival and health, has important implications for the stability of pollination mutualisms and warrants further investigation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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