

## **Impacts of earlier snowmelt on fruiting phenology and seed success of Rocky Mountain wildflowers**

### **Abstract**

Phenology, or the timing of biological events, has been found to shift in response to changing climates and key environmental cues such as spring temperatures and snowmelt date. These phenological shifts have been observed to be particularly extreme in mid-to-high latitude alpine environments, like Rocky Mountain ecosystems, where a variety of spring wildflowers have already been observed to be changing their flowering phenology. While flowering has been extensively studied, fruiting phenology remains largely unexplored, despite this stage being arguably more telling of reproductive output as the culmination of successful flowering and pollination events. Understanding how mean fruiting time and duration are impacted by shifting environmental cues will be critical in understanding the effects of future climate change on wildflower reproduction and population. To more thoroughly understand exactly how these potential shifts in fruiting phenology may be impacting wildflower reproductive output and future populations, seed success - assessed through seed mass, total fruit count, and viable seed counts - will be examined. The results of this study found there to be an advance in mean fruiting time for three of the five study species in response to the early snowmelt treatment, but this shift in fruiting mean did not translate to an observed change in fruiting duration with the exception of one species. Seed characteristics and success were found to be trait-responsive to the early snowmelt treatment. For example, *Delphinium nuttallianum*, was found to have a lower seed mass for individuals in the early snowmelt treatment plots, but total fruit count or viable seed counts were unaffected. These trait-specific responses highlight the importance of more holistic assessments of seed quality and success as using only one determinant could tell a different story than including multiple.

### **Introduction**

Phenology is the timing of cyclical, recurring biological events as they relate to environmental factors. For example, the date of first shoot emergence, peak flowering or fruit maturation in plants is known to be affected by precipitation and temperature (Forrest et al. 2010, CaraDonna et al. 2014, Keller et al. 2020). Being so intimately tied to environmental conditions, phenological shifts have become increasingly documented and investigated as anthropogenic activity has rapidly changed both local and global climates (Menzel et al. 2006, Forrest et al. 2010, CaraDonna et al. 2014). These phenological shifts are particularly important to consider at the population level because timing is critical for successful reproduction and survival. Individual flowering time, for example, plays a vital role within plant populations because it determines the number of potential mates available to reproduce with. Thus, changes in the timing of such reproductive events can have resounding impacts on plant population makeup and structure (Inouye et al. 2003).

In particular, phenological shifts due to climate change have been found to be more significant and have greater impacts in ecosystems that possess distinct seasonality, such as high- to mid-latitude montane environments (Kudo et al. 2020). For instance, a variety of studies have already investigated alpine plant communities' sensitivity to climate change and illustrated shifts in flowering phenology in response to changing environmental conditions (Inouye et al. 2003, Molau et al. 2005, Iler et al. 2013). Many other higher elevation species have also been recorded advancing their life events, such as first flowering time,

in accordance to increasing temperatures and earlier snowmelt cues, with there being some variability among species and taxa to the degree in which they are doing so (Inouye et al. 2003, Iler et al. 2013).

While there is a plethora of literature investigating how changing climates, particularly earlier snowmelt, are impacting flowering phenology, the potential effects on fruiting phenology remain largely unexplored. As the name would suggest, fruiting phenology is simply the specific timing of fruit production of plants. Although flowering is an important aspect of wildflower reproduction and changes in its timing can have resounding impacts on species' population structure and status, fruit production is arguably more telling of actual reproductive output as the culmination of successful flowering and pollination events. Although sequential in nature, it remains unclear whether fruiting phenology is also shifting as a direct consequence of environmental change and/or changes in flowering time and duration (Post et al. 2008, Sander et al. 2021). Investigating how shifting key phenological cues, like advanced snowmelt and increased spring temperatures, are impacting fruiting time and duration will be critical in understanding how these plant communities will be impacted under future climate change.

Furthermore, many studies looking at phenological shifts use only seed sets as an indicator of reproductive success. However, only considering this one metric fails to provide much detail into the quality of seeds produced; for instance, a decreased number of seeds could coincide with positive traits such as higher viability, germination, and/or recruitment due to energy allocation trade-offs (Goszka and Snell 2020). Thus, seed count as the sole determinant can lead to inaccurate conclusions of total fitness and reproductive output (Wen and Simons 2020). Including a more comprehensive set of seed traits, such as mass, total fruits produced, and viability, would not only aid in illustrating a more complete picture of reproductive success for this study, but would also help fill the knowledge gap surrounding regenerative seed traits (Jiménez-Alfaro et al. 2016). Mass can be used as a measure of potential viability as seeds with a more developed, and thus more viable, embryo are heavier than seeds with undeveloped or no embryo. This assumption is consistent with a variety of studies that have shown correlations between seed size and mass, and germability (Goszka and Snell 2020). Ultimately, establishing an understanding of how shifts in environmental cues impact fruiting time and, in turn, seed production and seed quality is essential for expanding our knowledge on how climate change is affecting the entirety of wildflower reproduction.

To investigate potential shifts in subalpine fruiting phenology and what those changes mean for successful seed production, I will be studying five spring-flowering wildflower species at the Rocky Mountain Biological Laboratory in Gothic, CO. These species include; *Claytonia lanceolata*, *Mertensia fusiformis*, *Androsace septentrionalis*, *Delphinium nuttallianum*, and *Geum triflorum*. I will compare an early snowmelt treatment and a control to answer the two questions of a) *how does fruiting phenology of the five species differ between the treatments* and b) *when fruiting phenology does differ, does this coincide with a difference in reproductive output?*

## **Methodology**

### Study Area:

To investigate my two research questions, I will collect fruiting phenology data and seeds from the five study species (*Claytonia lanceolata*, *Mertensia fusiformis*, *Androsace septentrionalis*, *Delphinium nuttallianum*, and *Geum triflorum*) in plots established at the Rocky Mountain Biological Laboratory in Gothic, Colorado (38°57'26.9"N, 106°59'10.7"W, 2,900 a.s.l). This region of Colorado is characterized as

subalpine, with a short and varying growing season that is initiated by spring snowmelt. The beginning of the growing season - early summer - tends to be dry while the mid- to later portion generally has summer monsoons.

### Study Species:

The study species *Claytonia lanceolata*, *Mertensia fusiformis*, *Androsace septentrionalis*, *Delphinium nuttallianum*, and *Geum triflorum* are all spring-flowering forbs. This characterization means they are particularly influenced by snowmelt as it works as a phenological cue for emergence. Another driving factor that led to the selection of these specific species is their abundance within the pre-established plots.

### Experimental Design:

The study area possesses eight total blocks in which the study species are established and data is collected from. The entirety of the research meadow is separated into an 'upper' and 'lower' meadow in which the upper meadow consists of blocks 4,5,6 while the lower meadow possess blocks 1,2,7,3,8 (Fig. 1). Each block consists of two adjacent 5x5 m plots that were paired based on similar plant community compositions. For example, Block 3 consists of two separate plots, a treatment and a control, that are similar in vegetation makeup.

Additionally, each 5x5m plot is broken in three subplots for ease of monitoring - labelled 1,2,3 for control and 4,5,6 for treatment for IDing purposes. Within each block, each plot was randomly assigned to be the early snowmelt treatment or control. The treatment plots are distinguishable from the control plots due to the 3m rebar stakes covered by PVC poles located at each corner of the treatment plots. These poles are used to suspend a shade cloth over the treatment plots when the snowpack is ~1m in depth, which generally occurs in April or early May. The shade clothes melt out the snow in the treatment plots roughly 5-7 days sooner than the control plots, and are removed once all the snow in the treatment plots has been melted.



**Figure 1.** Map of plot locations and treatments within the Research Meadow study site. Location of Research Meadow within Gothic, CO context (bottom left).

Within these blocks, a subset of each of the plant species was randomly selected in order to assume an even distribution per plot and block. Due to the varying compositions across blocks, not every block contains each study species (Table 1). Each selected plant was assigned a unique ID number that was written on an aluminum tag with their species code - which consists of the first two letters of the genus name and the first two letters of the species name. To aid with locating the plants in the field, the ID tag was always placed south of the plant while a flag was placed north of the plant (Table 1).

| Species                          | Blocks Monitored     | Marking method              |
|----------------------------------|----------------------|-----------------------------|
| <i>Claytonia lanceolata</i>      | 8, 4, 5, 6           | Skewer with green ribbon    |
| <i>Mertensia fusiformis</i>      | 2S, 7, 8S, 3C, 4, 5C | Triangle light green flag   |
| <i>Androsace septentrionalis</i> | 2, 7C, 8, 3, 4, 5S   | Triangle medium green flag  |
| <i>Delphinium nuttallianum</i>   | 2, 7, 8              | Rectangle dark green flag   |
| <i>Geum triflorum</i>            | 3, 4, 5              | Rectangle medium green flag |

**Table 1.** Species marker key and blocks in which to find them.

#### Phenological Data Collection:

To investigate the question, *how does fruiting phenology of the five species differ between the treatments*, mean fruiting time and fruiting duration will be considered as response variables to the early snowmelt or control treatments. Roughly 30-35 individuals per treatment per species will be monitored. This means we will be observing and collecting data from approximately 60-70 individuals for 5 species, making a total of ~325 study plants. Three days a week - Mondays, Wednesdays and Fridays - flowering and fruiting statuses (i.e. “open flowers”, “fruits”) are monitored and recorded across all individuals and species until they have completed their reproductive cycle and are no longer observed to be fruiting. The status of individual plants is recorded as separate binary variables; “0” for No and “1” for Yes for each of the previously listed stages.

#### Seed Characteristic Data Collection:

To investigate the question, *when fruiting phenology does differ, does this coincide with a difference in reproductive output*, seed set (fruits per plant), seed mass, and viable seeds per fruit will be considered as response variables to the early snowmelt or control treatments.

#### *Fruit collection*

Fruit collection varies across species. For *M. fusiformis*, *D. nuttallianum*, and *G. triflorum*, green mesh jewelry bags were put on the stalks of individuals to cover the fruits and ultimately collect the seeds after they matured and dehisced. For *C. lanceolata* and *A. septentrionalis*, the bagging method was not used. Rather, fruits are manually plucked and collected from the plants when deemed mature but before the fruit skeleton is cracked enough to let the seeds escape. This collection process varies from the other species because these plant species are too small and fragile to be bagged. Once collected, fruits and seeds are put into labeled, individual coin envelopes and brought back to the lab for counts of seed set, seed masses, and viable seeds per fruit.

#### *Seed Mass*

For seed mass measurements, a subset of 10 random, viable seeds from the total viable seed set was used to get an average seed mass using a precision balance. If 10 viable seeds were not produced, then a minimum of 6 seeds can be used to get an average.

### *Counts and Viability*

Seeds and fruits that have been collected are taken back to the lab to count and assess for viability. Seed viability is generally assessed visually; fuller, darker brown seeds are considered to be more mature and thus more likely to contain a developed embryo. Another test of viability used is the application of slight physical pressure to the seed to assess firmness. The harder the seed, the fuller it is assumed to be. Thus, a firm seed would likely be considered viable. Fruit counts, which are used to estimate total seed set and seeds per fruit, are generally done in the field while collecting the phenology data with the expectation of *M. fusiformis*. Due to their high fruit production, *M. fusiformis* stalks are instead collected at the end of their fruiting cycle and the inflorescence is assessed for counts of fruit scars, which can be used to determine how many fruits were produced by the plant.

### Analysis:

For phenology, we used R to transform our binary phenological data into the mean (day of year) and duration of fruiting (# days) for each species and treatment. The difference between each metric, mean and duration of fruiting, will be compared using linear mixed effects models or its equivalent when the date is found not to be normally distributed. The early snowmelt treatment was the categorical predictor while fruiting duration and mean will be continuous response variables. As such, the models will be:

$$\begin{aligned} \text{fruiting duration} &\sim \text{treatment} \\ \text{mean fruiting date} &\sim \text{treatment} \end{aligned}$$

For analyzing seed characteristics and viability, the difference between each trait by treatment for each species was compared using linear mixed-effects models or its equivalent when the data is found to be not normally distributed. The early snowmelt treatment was included as a categorical predictor and the seed characteristic was the continuous response variable in the analysis. As such, the models will follow:

$$\text{seed characteristic} \sim \text{treatment}$$

### TMS Dataloggers:

TMS Dataloggers were used to measure soil moisture and temperature to provide environmental context throughout the 2025 field season. These loggers were originally placed in 2022-2023, first one in each plot and then two more later. For this season, data was taken from all three TMS dataloggers in each plot (48 total TMS loggers). These loggers are lithium powered and have a lifespan of ~10 years. They have one soil moisture sensor and three temperature sensors (MAXIM/DALLAS Semiconductor DS7505U+; resolution of 0.0625 °C, accuracy of  $\pm 0.5$  °C) at -6cm, +2cm, and +15cm. It should be noted that there is no formal inclusion of this information as a predictor variable; it is solely used to provide environment context.

### **Timeline**

| Week                    | Jun. 8 - 14 | Jun. 15 - 21 | Jun. 22 - 28 | Jun. 29 - Jul. 5 | Jul. 6 - 12 | Jul. 13 - 19 | Jul. 20 - 26 | Jul. 27 - Aug. 2 | Aug. 3 - 9 | Aug. 10 - 11 |
|-------------------------|-------------|--------------|--------------|------------------|-------------|--------------|--------------|------------------|------------|--------------|
| Data Collection         |             |              |              |                  |             |              |              |                  |            |              |
| Data Analysis           |             |              |              |                  |             |              |              |                  |            |              |
| Writing                 |             |              |              |                  |             |              |              |                  |            |              |
| Final Paper/Presentaion |             |              |              |                  |             |              |              |                  |            |              |

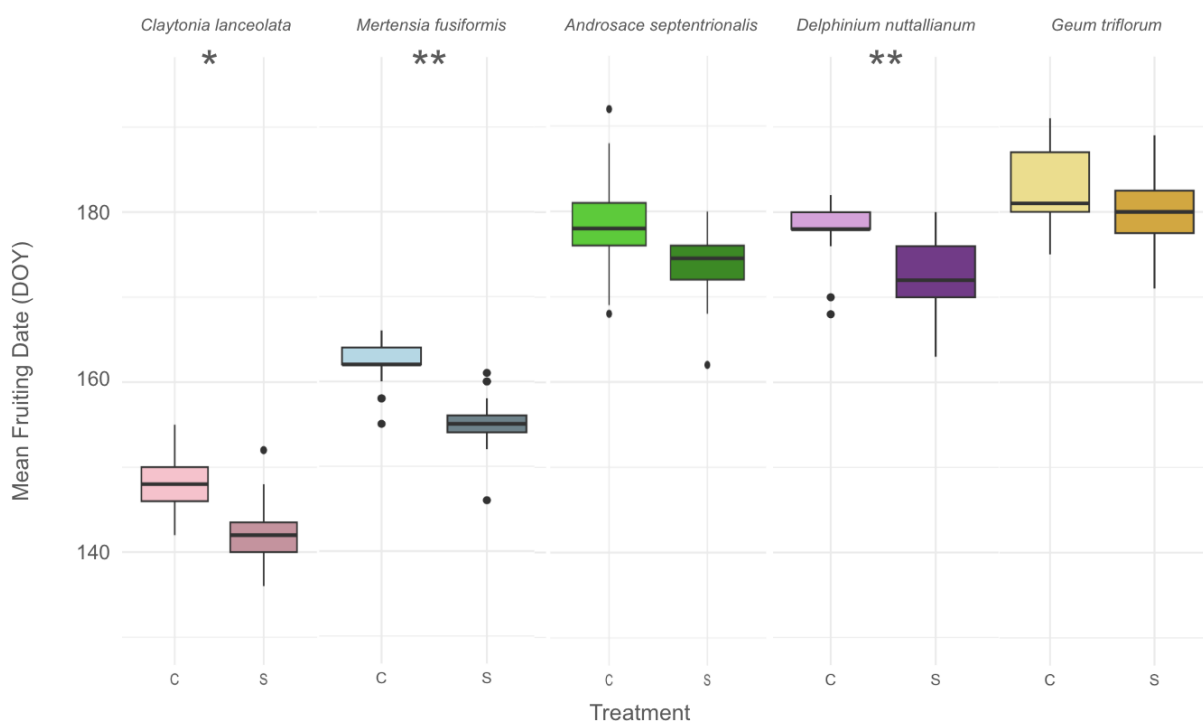
**Table 2.** General timeline of major components of the project for the 2025 summer field season.

## Results

Below, mean values of responses between treatments will be reported where [S] denotes the response under the early snowmelt treatment and [C] denotes the response under the control treatment.

### Mean Fruiting Time

The early snowmelt treatment was found to have a significant effect on the mean fruiting time of *M. fusiformis* ( $p = 0.019$ ; Table 3) and *D. nuttallianum* ( $p = 0.045$ ; Table 3). The *M. fusiformis* in the early snowmelt plots fruited an average of 8 days before those in the control plots (June 4th [S] vs. June 12th [C]; Table 3) while the *D. nuttallianum* in the snowmelt plots fruited an average of 6 days before those in the control plots (June 21st [S] vs. June 27th [C]; Table 3). Additionally, the treatment was found to have a marginally significant ( $p < 0.10$ ) effect on fruiting time of *C. lanceolata* ( $p = 0.080$ ; Table 3), where individuals in the early snowmelt plots fruited an average of 6 days before those in the control plots (May 22nd [S] vs. May 28th [C]; Table 3). Both *G. triflorum* ( $p = 0.62$ , June 29th [S] vs. July 1st [C]; Table 3) and *A. septentrionalis* ( $p = 0.16$ , June 23rd [S] vs. June 27th [C]; Table 3) mean fruiting time was not found to be affected by the treatment

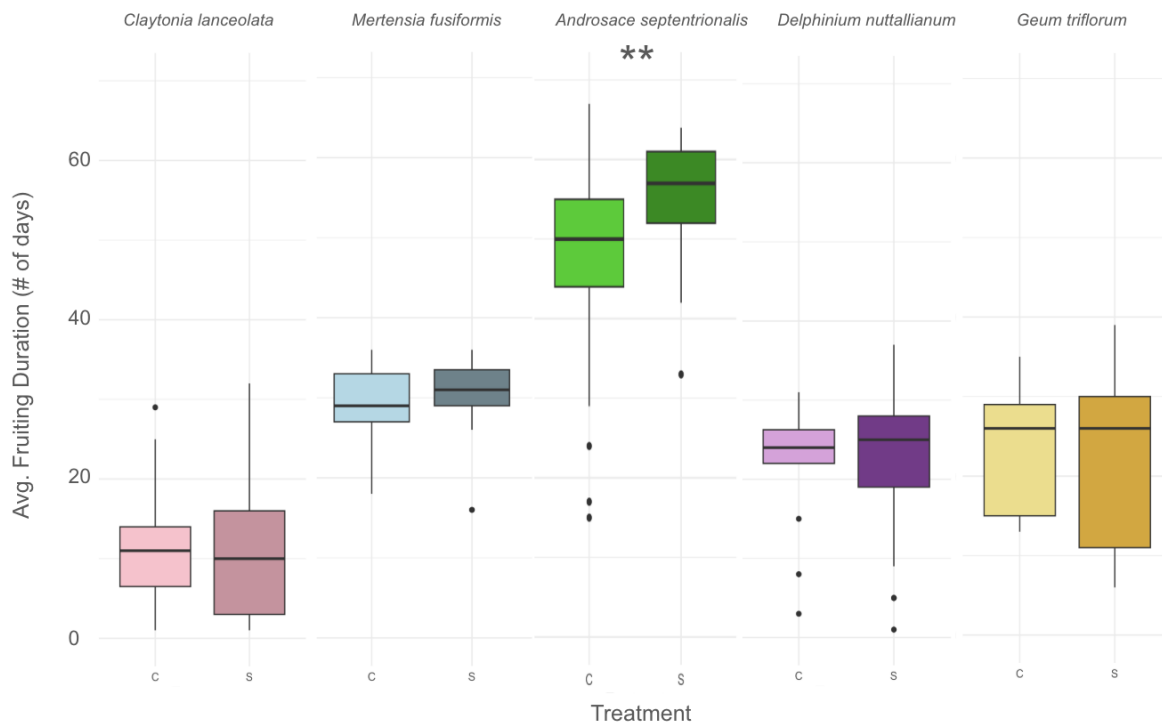


**Figure 2.** Mean fruiting date (Julian date, DOY) of all study species (left to right: *Claytonia lanceolata* (pink), *Mertensia fusiformis* (blue), *Androsace septentrionalis* (green), *Delphinium nuttallianum* (purple), *Geum triflorum* (yellow)). The control individuals are denoted by the 'C' on the x-axis and lighter shade boxplots while the early snowmelt individuals are denoted by the 'S' and darker color. The \* symbol denotes statistical significance where one (\*) is marginally significant and two (\*\*) is significant.

### Fruiting Duration

While there were observed differences in mean fruiting time across treatments, this did not translate into an impact on fruiting duration for four of the five study species; *C. lanceolata* ( $p = 0.64$ , 10.5 days [S] vs. 11.5 days [C]; Table 3), *M. fusiformis* ( $p = 0.40$ , 30.8 days [S] vs. 29.6 days [C]; Table 3), *D. nuttallianum* ( $p = 0.46$ , 22.2 days [S] vs. 23.7 days [C]; Table 3), and *G. triflorum* ( $p = 0.62$ , 21.7 days [S] vs. 23.4

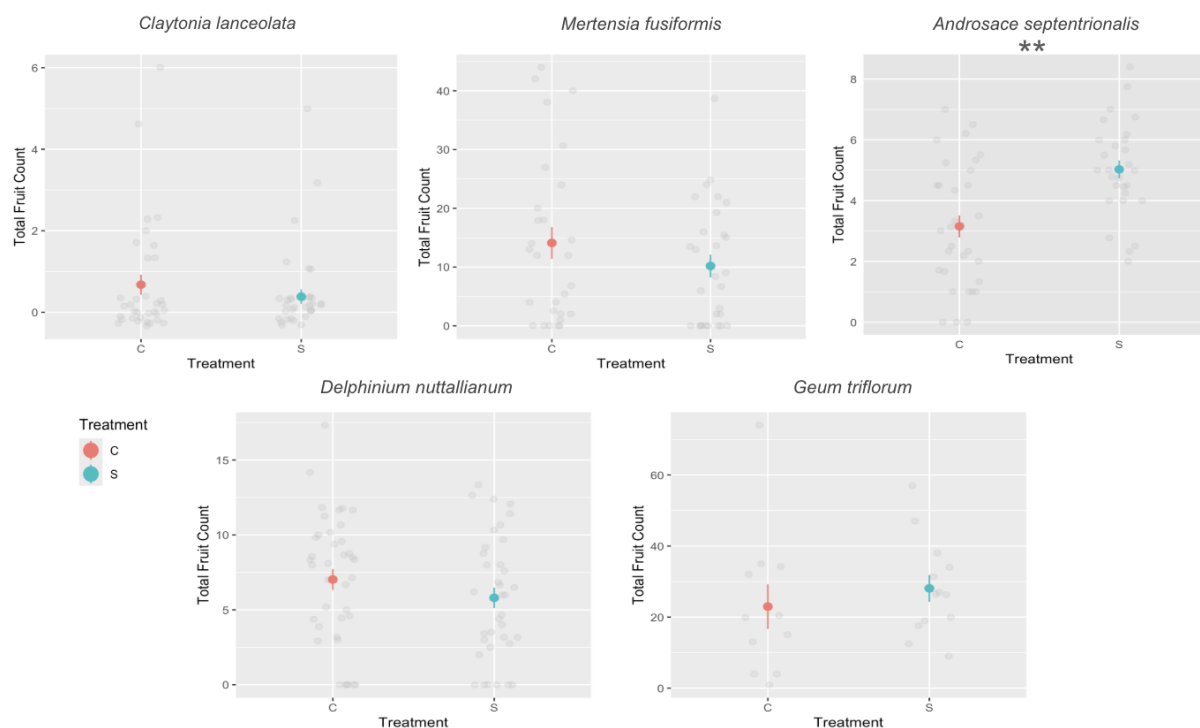
days [C]; Table 3). However, fruiting duration was found to be affected by treatment for *A. septentrionalis* fruiting duration ( $p = 0.0082$ ; 54.8 days [S] vs. 46.6 days [C]; Table 3).



**Figure 3.** Fruiting duration (Julian date, DOY) of all study species (left to right: *Claytonia lanceolata* (pink), *Mertensia fusiformis* (blue), *Androsace septentrionalis* (green), *Delphinium nuttallianum* (purple), *Geum triflorum* (yellow)). The control individuals are denoted by the 'C' on the x-axis and lighter shade boxplots while the early snowmelt individuals are denoted by the 'S' and darker color. The \* symbol denotes statistical significance where one (\*) is marginally significant and two (\*\*) is fully significant.

#### Total Fruit Count

Treatment was only found to have a significant impact on total fruits produced for *Androsace septentrionalis* ( $p = 0.0002$ ; Table 3), where early snowmelt individuals produced a mean of roughly 2 seeds more than their control counterparts (5.03 fruits [S] vs. 3.15 fruits [C]; Table 3). There was no significant effect of the treatment on total fruits produced for the remainder of the study species; *C. lanceolata* ( $p = 0.37$ , 0.38 fruits [S] vs. 0.68 fruits [C]; Table 3), *M. fusiformis* ( $p = 0.37$ , 10.21 fruits [S] vs. 14.11 [C]; Table 3), *D. nuttallianum* ( $p = 0.21$ , 5.80 fruits [S] vs. 7.03 [C]; Table 3), and *G. triflorum* ( $p = 0.48$ , 28.09 fruits [S] vs. 22.96 fruits [C]).



**Figure 4.** Total fruit count for all species (Top row, left to right; *C. lanceolata*, *M. fusiformis*, *A. septentrionalis*. Bottom row, left to right; *D. nuttallianum*, *G. triflorum*). The control individuals are denoted by the 'C' and the red color on the x-axis while the early snowmelt individuals are marked by the 'S' and blue color. The \* symbol denotes statistical significance where one (\*) is marginally significant and two (\*\*) is fully significant.

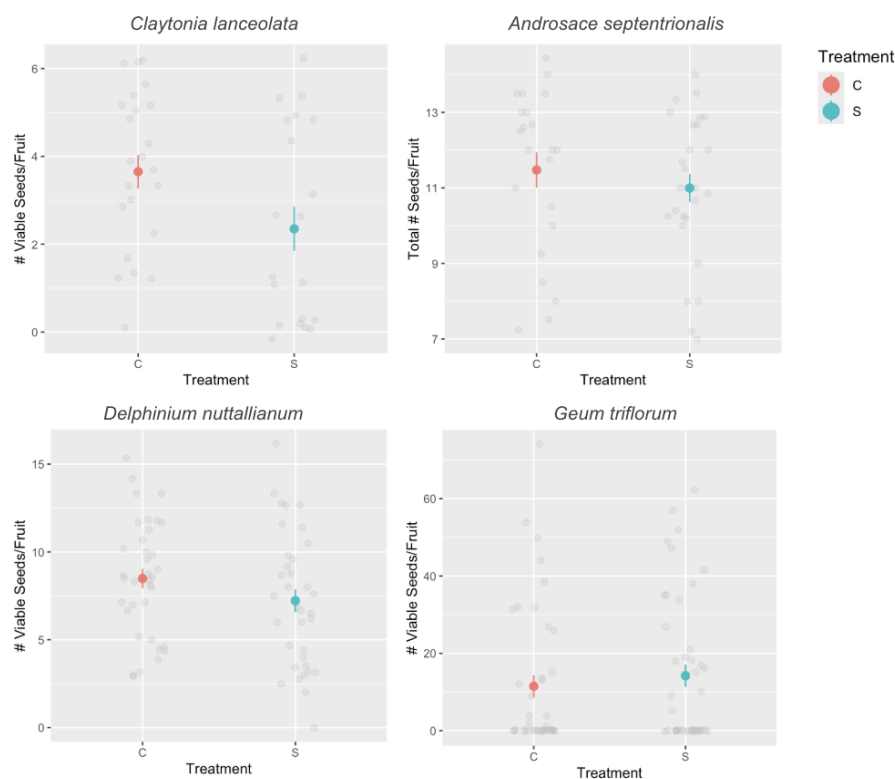
### Seed Production per Fruit

The treatment was not found to have a significant effect on viable seeds per fruit produced for *C. lanceolata* ( $p = 0.33$ , 2.35 viable seeds/fruit [S] vs. 3.65 viable seeds per fruit [C]; Table 3), *D. nuttallianum* ( $p = 0.14$ , 7.23 viable seeds per fruit [S] vs. 8.48 viable seeds per fruit [C]; Table 3), and *G. triflorum* ( $p = 0.70$ , 14.2 viable seeds per fruit [S] vs. 11.5 viable seeds per fruit [C]; Table 3). This was further compounded by the analysis also illustrating no significant effect of the treatment on nonviable seeds per fruit for the same species; *C. lanceolata* ( $p = 0.25$ , 2.90 nonviable seeds per fruit [S] vs. 1.44 nonviable seeds per fruit [C]; Table 3), *D. nuttallianum* ( $p = 0.27$ , 2.71 nonviable seeds per fruit [S] vs. 4.19 nonviable seeds per fruit [C]; Table 3), and *G. triflorum* ( $p = 0.72$ , 27.2 nonviable seeds per fruit [S] vs. 29.7 nonviable seeds per fruit [S]; Table 3).

Total seeds produced per fruit was measured for *A. septentrionalis* rather than viable seeds per fruit due to plants being too small and fragile to be bagged and rapid dehiscing preventing the collection of enough fully mature fruits from the field. That being said, treatment was not found to have a significant impact on total seeds per fruit produced for *A. septentrionalis* ( $p = 0.42$ , 11.0 seeds per fruit [S] vs. 11.5 seeds per fruit [C]; Table 3).

For *M. fusiformis* each fruit produces only one seed so total fruit count is synonymous to total seed count (see *Total Fruit Count* section). In terms of viability, *M. fusiformis* is incredibly challenging to visually assess for viability as many of the “mature” looking fruits actually lack embryos (Knauf, unpublished data). Therefore, in order to get an accurate measure of seed viability, x-ray imagery would need to be

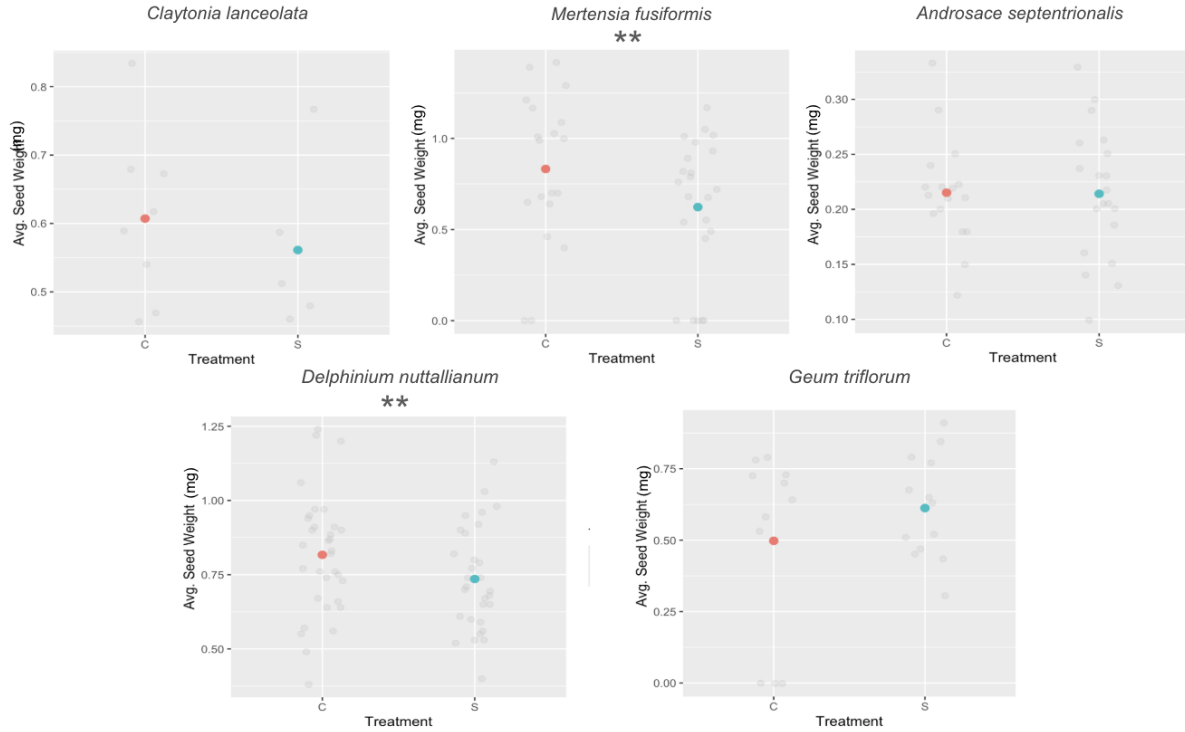
used. Unfortunately, that equipment was not available at the Rocky Mountain Biological Laboratory where this study took place.



**Figure 5.** Viable seed count per fruit for all species (Top row, left to right; *C. lanceolata*, *A. septentrionalis*. Bottom row, left to right; *D. nuttallianum*, *G. triflorum*). The control individuals are denoted by the 'C' and the red color on the x-axis while the early snowmelt individuals are marked by the 'S' and blue color. The \* symbol denotes statistical significance where one (\*) is marginally significant and two (\*\*) is fully significant.

### Seed Mass

Treatment was found to have a marginally significant effect on seed mass for *M. fusiformis* ( $p = 0.098$ ; Table 3), where the early snowmelt individuals produced seeds with a mean mass that was 0.21 mg less than the individuals in the control plots (0.62 mg [S] vs. 0.83 mg [C]; Table 3). Additionally, treatment was also found to have a marginally significant effect on seed mass of *D. nuttallianum* ( $p = 0.094$ ; Table 3), where individuals in the early snowmelt treatment produced seeds with a mean mass that was 0.08 mg less than the control individuals (0.74 mg [S] vs. 0.82 mg [C]; Table 3). The early snowmelt treatment was not found to have a significant effect on seed mass of *C. lanceolata* ( $p = 0.46$ , 0.56 mg [S] vs. 0.61 mg [C]; Table 3), *A. septentrionalis* ( $p = 0.96$ , 0.21 mg [S] vs. 0.22 mg [C]; Table 3), and *G. triflorum* ( $p = 0.29$ , 0.61 mg [S] vs. 0.50 mg [C]; Table 3).



**Figure 6.** Average seed mass for all species (Top row, left to right; *C. lanceolata*, *M. fusiformis*, *A. septentrionalis*. Bottom row, left to right; *D. nuttallianum*, *G. triflorum*). The control individuals are denoted by the 'C' and the red color on the x-axis while the early snowmelt individuals are marked by the 'S' and blue color. The \* symbol denotes statistical significance where one (\*) is marginally significant and two (\*\*) is fully significant.

#### Greenhouse *Androsace septentrionalis*

To investigate potential generational and longer-term impacts of the early snowmelt treatment on seed success, *A. septentrionalis* seeds from the previous 2024 field season were collected and grown in a greenhouse at the Chicago Botanic Garden; seeds from the 2025 field season could not be used due to time restraints. Seeds from this second generation were collected and viable seed counts and seed mass measurements were taken for analysis. The early snowmelt treatment was not found to have a significant impact on the second generation's viable seed count per fruit ( $p = 0.81$ , 13.3 viable seeds per fruit [S] vs. 13.5 viable seeds per fruit [C]; Table 3) or seed mass ( $p = 0.81$ , 0.201 mg [S] vs. 0.197 mg [C]; Table 3).

*A. septentrionalis* was the only species in which we could investigate for generational effects because the other four species included in this study are perennials whereas *A. septentrionalis* is an annual. These longer-lived species take multiple years to be mature enough to produce fruits and thus fall out of the timeline of this study.

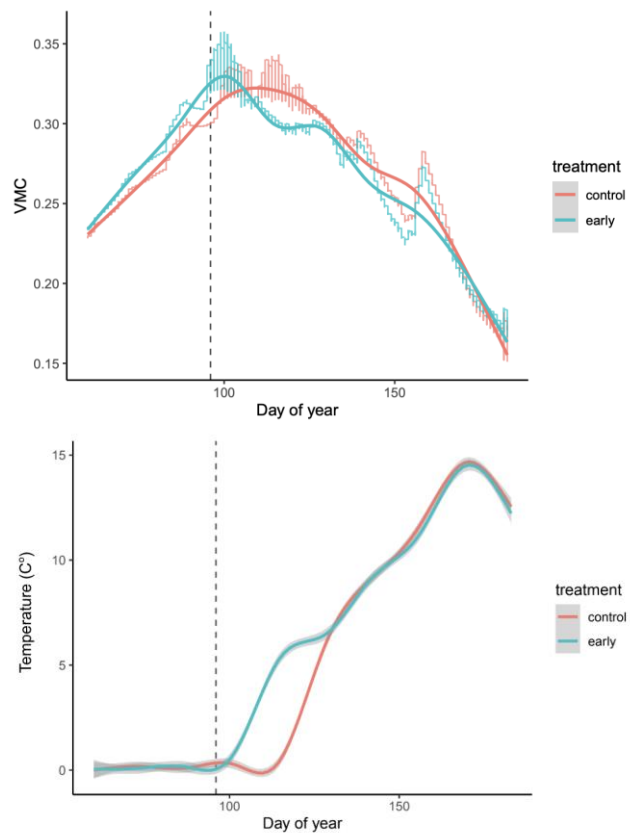
| Response                        | Predictor    | <i>Claytonia lanceolata</i>           | <i>Mertensia fusiformis</i>          | <i>Androsace septentrionalis</i>      | <i>Delphinium nuttallianum</i>        | <i>Geum triflorum</i>                 |
|---------------------------------|--------------|---------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| <b>Fruiting Duration (days)</b> | Treatment    | N (0.64)                              | N (0.40)                             | <b>Y* (0.0082)</b>                    | N (0.46)                              | N (0.62)                              |
|                                 | Values (S/C) | 10.5 (S) / 11.5 (C)                   | 30.8 (S) / 29.6 (C)                  | 54.8 (S)/ 46.6 (C)                    | 22.2 (S) / 23.7 (C)                   | 21.7 (S) / 23.4 (C)                   |
| <b>Mean Fruiting (DOY)</b>      | Treatment    | <b>Y* (0.08)</b>                      | <b>Y* (0.019)</b>                    | N (0.16)                              | <b>Y* (0.045)</b>                     | N (0.55)                              |
|                                 | Values (S/C) | 142 May 22nd (S) / 148 May 28th (C)   | 155 June 4th (S) / 163 June 12th (C) | 174 June 23rd (S) / 178 June 27th (C) | 172 June 21st (S) / 178 June 27th (C) | 180 June 29th (S) / 182 July 1st (C)  |
|                                 |              |                                       |                                      | Total Seeds/Fruit                     |                                       |                                       |
| <b>Viable Seeds/Fruit</b>       | Treatment    | N (0.33)                              | NA                                   | N (0.42)                              | N (0.14)                              | N (0.70)                              |
|                                 | Values (S/C) | 2.35 seeds/fr (S) / 3.65 seeds/fr (C) |                                      | 11.0 seeds/fr (S) / 11.5 seeds/fr (C) | 7.23 seeds/fr (S) / 8.48 seeds/fr (C) | 14.2 seeds/fr (S) / 11.5 seeds/fr (C) |
| <b>Nonviable Seeds/Fruit</b>    | Treatment    | N (0.25)                              | NA                                   | NA                                    | N (0.27)                              | N (0.72)                              |
|                                 | Values (S/C) | 2.90 seeds/fr (S) / 1.44 seeds/fr (C) |                                      |                                       | 2.71 seeds/fr (S) / 4.19 seeds/fr (C) | 27.2 seeds/fr (S) / 29.7 seeds/fr (C) |
| <b>Seed Weight (mg)</b>         | Treatment    | N (0.46)                              | <b>Y* (0.098)</b>                    | N (0.96)                              | <b>Y* (0.094)</b>                     | N (0.29)                              |
|                                 | Values (S/C) | 0.56 (S) / 0.61 (C)                   | 0.62 (S) / 0.83 (C)                  | 0.21 (S) / 0.22 (C)                   | 0.74 (S) / 0.82 (C)                   | 0.61 (S) / 0.50 (C)                   |
| <b>Total Fruit Count</b>        | Treatment    | N (0.37)                              | N (0.37)                             | <b>Y* (0.0002)</b>                    | N (0.21)                              | N (0.48)                              |
|                                 | Values (S/C) | 0.38 (S) / 0.68 (C)                   | 10.21 (S) / 14.11 (C)                | 5.03 (S) / 3.15 (C)                   | 5.80 (S) / 7.03 (C)                   | 28.09 (S) / 22.96 (C)                 |
| <b>GH Viable Seeds/Fruit</b>    | Treatment    | NA                                    | NA                                   | N (0.81)                              | NA                                    | NA                                    |
|                                 | Values (S/C) | NA                                    | NA                                   | 13.3 (S) / 13.5 (C)                   | NA                                    | NA                                    |
| <b>GH Seed Mass (mg)</b>        | Treatment    | NA                                    | NA                                   | N (0.81)                              | NA                                    | NA                                    |
|                                 | Values (S/C) | NA                                    | NA                                   | 0.201 (S) / 0.197 (C)                 | NA                                    | NA                                    |

**Table 3.** Average values by treatment and p-value results of linear-mixed effects models, or its equivalent, for each species (*C. lanceolata*, *M. fusiformis*, *A. septentrionalis*, *D. nuttallianum* and *G. triflorum*) and response variable (fruiting duration, mean fruiting, viable seeds/fruit, nonviable seeds/fruit, seed mass (mg), and total fruit count). The early snowmelt treatment is denoted by the (S) while the control treatment is denoted by the (C). The grayed out boxes and NA's indicate that no data could be collected or analyzed for those responses or species.

## Discussion

Overall, the results of this study illustrate that the most consistent response of the study species to earlier snowmelt is an advancement of mean fruiting time. Three of the five the study species, with the exception of *G. triflorum* and *A. septentrionalis*, fruited roughly a week earlier in correspondence to the snowmelt treatment (Table 3). The observed advancement of fruiting in response to the changing climate is consistent with other studies investigating potential shifts in fruiting phenology (Menzel et al. 2006, Sander et al. 2021). This shift in fruiting time suggests that this phenophase of the plant reproductive cycle is responsive to changes in environmental conditions such as warmer spring temperatures and earlier snowmelt. Such conditions were confirmed using the standard TOMST dataloggers, which measure soil moisture and temperatures at the depths of 6 cm belowground, 2 cm above ground and 15cm above ground. The TMS datalogger information illustrates an earlier peak in volumetric moisture content (VMC) and soil temperature ( $^{\circ}\text{C}$ ) in the snowmelt treatment plots compared to the control plots (Fig. 6). A plethora of other studies have found earlier flowering in response to the same environmental factors (Inouye et al. 2003, Inouye et al. 2008, Kudo 2020). Although the scope of this study solely focused on external influences on fruiting time, the results of this study conjunct with the similar advancement in flowering time found in other literature suggests biological links between flowering and fruiting for some species (Sandor et al. 2021).

Conversely, *G. triflorum* mean flowering time was found to be unaffected by the early snowmelt treatment. This lack of response suggests that the reproductive period of *G. triflorum* could be driven more by internal biological processes and timing rather than environmental cues; though little is currently known about the reproductive process of this species (Iler, unpublished). Additionally, *G. triflorum* is the last of the five study species to flower and fruit. Individuals that fruit later in the season are temporally the furthest away from the initial snowmelt treatment, potentially reducing the effects of the treatment and making other environmental factors such as summer precipitation more influential. This effect could explain the lack of difference between treatments as summer precipitation and other environmental events would be similar across the different treatment blocks. Although the mean fruiting time of *A. septentrionalis* was found to be more impacted by the treatment than *G. triflorum* ( $p=0.16$ ; Table 3), the difference was still not large enough to be statistically significant. That being said, it is hypothesized that



**Figure 6.** Mean volumetric moisture content (VMC; above) and temperature ( $^{\circ}\text{C}$ ; below) of blocks throughout the year (Julian days) as collected by TOMST dataloggers. Dashed line indicates the day of year in which treatment was

the low winter snowfall and summer precipitation could play a role in this finding as plants that fruit later in the season, generally the controls, could have been under more drought-stress as there was less snowmelt water available to them at this time in the season. This stress could have cut off their fruiting sooner than it would under more ideal conditions and thus pushed their mean fruit date up to be more similar to the early snowmelt individuals. In the future to confirm this, a multi-year comparison would need to be done to investigate when the *A. septentrionalis* control individuals normally fruit under non-drought conditions.

Despite mean fruiting time advancing roughly one week earlier for three of the study species (*C. lanceolata*, *M. fusiformis* and *D. nuttallianum*; Table 3), the duration of fruiting was largely unaffected by the treatment with the exception of *A. septentrionalis*. *A. septentrionalis* fruiting duration was found to extend with the treatment; as those in the early snowmelt plots were fruiting for longer than those in the control plots by ~10 days (Table 3) despite no change in mean fruiting time being observed across treatments for this species. One explanation for this could be that *A. septentrionalis* is an annual and thus displays different life strategies than the other perennial study species (Friedman and Rubin 2015). The *A. septentrionalis* could experience more pressure to successfully fruit and thus allots more energy into reproduction for longer even if conditions are not necessarily ideal. The observed consistency of duration for all the other species across treatments was unexpected as previous years of data collection (2023 and 2024 field season) found there to be an extension of fruiting for at least some of the species per year. While there is notable variability between which species were found to show a difference in duration per year, *C. lanceolata* was consistently found to show an extension of fruiting duration in the early snowmelt individuals (Knauf, unpublished). The lack of response across most species for this year could be explained by this winter season's low winter precipitation and snowpack. This last winter season (2024-2025) had not only the lowest snowpack of the last three years in which this data has been collected (2023-2025), but was the fourth lowest total winter snowfall since the start of weather data collection back in 1974 with only 573 cm of snowfall (billy barr, 2025). This lower snowpack combined with little summer precipitation throughout this flowering and fruiting season could mean a lack of prolonged soil moisture. This could potentially result in less resources and greater stress on the plants during fruit production, as well as initiation of early senescence (Miryeganeh 2021).

For the seed characteristic section of this study, *A. septentrionalis* was the only species found to have a significant difference in total fruit produced between treatments; where the individuals in the early snowmelt plot produced an average of two more fruits than those in the control plots (Table 3). This response could in-part be explained by the extension of fruiting duration observed, as the extra fruiting time under suitable conditions potentially provided the early snowmelt plants with the opportunity to amass more resources and subsequently produce more fruit (Wagner et al. 2011). The remainder of the other study species displayed no significant difference in the total fruits produced across treatments (Table 3), despite the early snowmelt individuals fruiting roughly a week earlier than the control. The observed difference in total fruits produced in *A. septentrionalis* but not the other species could be explained by the species' different life history strategies. *A. septentrionalis* was the only annual species included in the study where all four other species are long-lived perennial forbs. Annuals generally have only one reproductive event before the end of their life cycle, this means they often prioritize reproductive growth and seed production over resource partitioning, while longer lived perennials have multiple reproductive events throughout their life cycles over the course of multiple years and thus can prioritize

long-term survival (Friedman and Rubin 2015). Under environmental stress, like drought, these life history strategies could present as annuals pushing out as many fruits as possible, while perennials prioritize vegetative growth and long-term storage in hopes of being able to produce more successful fruits the next year when conditions are better. To better understand the extent of influence this season's distinctly low snowpack had on seed production, a longer-term, multi-year study should be conducted.

Seed viability counts, based on visual and physical assessment, were found to not be significantly impacted by the early snowmelt treatment for any of the study species despite the observed differences in mean fruiting. That being said, it should be noted that viability counts could not be done for *M. fusiformis* as the seeds are often visually misleading. As such, seeds would frequently lack a developed embryo despite physical size and color being similar to seeds that did possess a mature embryo (Knauf, unpublished). In the future, to get accurate counts of *M. fusiformis* seed viability, x-ray imagery would be necessary to thoroughly check the status of the seed embryo. To further support the conclusion that seed viability was unaffected by the observed shift in mean fruiting, based on our metrics, nonviable seed counts were also taken and found to have no difference across treatments (Table 3). Viable and nonviable seed counts not differing between treatments, and subsequent mean fruiting times, could also be explained by this season's conditions. Other studies have found plants to be responsive to environmental stress more in the quantity of the seeds produced rather than the viability or quality; this means less seeds may be produced, but they are still of high quality or vigor (Delouche 1980). The extreme and prolonged lack of soil moisture this season could have been more influential on seed production and overruled the shorter-term effects of the snowmelt treatment, though multi-season data would have to be collected and compared to confirm this.

Significant differences in seed mass between the early snowmelt and control treatments were found for *M. fusiformis* and *D. nuttallianum*, where the control seeds were found to be marginally heavier than the early snowmelt seeds (Table 3). Seed mass is an important characteristic to consider as the heavier the seed is, the more embryonic material available to the embryo (Goszka and Snell 2020). The more embryonic material available to the embryo increases their resources at time of germination and thus increases their chances at successful growth and establishment. The observed heavier mass of the control seeds conjunct with the found differences in mean fruiting for *M. fusiformis* and *D. nuttallianum* could indicate that later-season conditions are marginally better for seed-filling. While the lighter seeds may not be considered nonviable based on our metrics, the heavier seeds could have a competitive advantage over the lighter ones (Goszka and Snell 2020). As these responses are species-specific, the longer-term implications of this effect should it become prolonged could be a shift in the different species' ability to persist in the community and eventually result in a shift in community make-up.

To investigate any potential generational effects, *A. septentrionalis* seeds from the previous 2024 field season were collected and reared in a greenhouse. The seeds of those second-generation individuals were then collected in this year's field season and the seed traits of mass and viability were compared across first generation treatments. It should be noted that the same could not be done for the four other species included in this study as *C. lanceolata*, *M. fusiformis*, *D. nuttallianum*, and *G. triflorum* are all perennials and the multi-year scale of their reproduction fall outside the timeline of this study. The results of this study show that the treatment had no effect on either seed mass or viability counts for the second generation of *A. septentrionalis* individuals (Table 3). This illustrates that, at least on a shorter timescale,

seed traits may be more heavily influenced by environmental conditions during production and development than an internal or genetic basis; and thus may be intimately tied to changes in the climate. However, to properly disentangle the complex interactions between environmental and internal factors on the seed quality of spring-forbs, a longer-term, more comprehensive study must be done.

Ultimately, the results of this study found that mean fruiting phenology generally advances in response to earlier snowmelt for all but one of the included study species and these phenological shifts can manifest themselves in seed traits. However, these responses are species-dependent; which can have long-term consequences for the community make-up and structure as some species may be able to function and survive better with these changing conditions than others. Additionally, not only are these responses species-specific, but there was also observed variability in trait-responses. In other words, the earlier snowmelt may be impacting a seed in one trait, like mass, but not another, like total fruit count. This highlights the importance of using more holistic assessments of seed traits and supports the work of others, such as Goszka and Snell (2020) as well as Wen and Simons (2020), that argue using only one seed characteristic as the sole determinant of seed quality or reproductive output could lead to completely different conclusions than including multiple. While this study worked to bridge that gap as best it could given the equipment and time constraints, more work could be done in the future for viability counts as these metrics were the most subjective. X-ray imagery or seed germination measurements would be best suited to most accurately determine seed viability. In addition, a longer-term study investigating these responses to earlier snowmelt across years would be greatly beneficial in exploring the extent of influence the historically low snowpack and drought conditions of the 2025 field season had on these results. In addition a longer-term study would also potentially explore any generational effects on the perennial species in addition to the one annual included here.

## Citations

- barr, billy. “Gothic weather”. *Gothic Weather* (2025). <https://www.gothicwx.org/>
- CaraDonna, Paul J., Amy M. Iler, and David W. Inouye. “Shifts in Flowering Phenology Reshape a Subalpine Plant Community.” *Proceedings of the National Academy of Sciences* 111 Forrest et al. 2010, no. 13 (April 2014): 4916–21. <https://doi.org/10.1073/pnas.1323073111>.
- Delouche, James C. . “Environmental Effects on Seed Development and Seed Quality”. *Hort Science*, no. 15 (1980): 13-18.
- Forrest, Jessica, and Abraham J. Miller-Rushing. “Toward a Synthetic Understanding of the Role of Phenology in Ecology and Evolution.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, no. 1555 (October 12, 2010): 3101–12. <https://doi.org/10.1098/rstb.2010.0145>.
- Friedman, Jannice, and Matthew J. Rubin. “All in Good Time: Understanding Annual and Perennial Strategies in Plants.” *American Journal of Botany* 102, no. 4 (2015): 497–99. <https://doi.org/10.3732/ajb.1500062>.
- Goszka, Abigail R., and Rebecca S. Snell. “Seed Quality and Seed Quantity in Red Maple Depends on Weather and Individual Tree Characteristics.” *Ecology and Evolution* 10, no. 23 (December 2020): 13109–21. <https://doi.org/10.1002/ece3.6900>.
- Iler, Amy M., Toke T. Høye, David W. Inouye, and Niels M. Schmidt. “Nonlinear Flowering Responses to Climate: Are Species Approaching Their Limits of Phenological Change?” *Philosophical Transactions of the Royal Society B: Biological Sciences* 368, no. 1624 (August 19, 2013): 20120489. <https://doi.org/10.1098/rstb.2012.0489>.
- Inouye, David W., Francisca Saavedra, and Wendy Lee-Yang. “Environmental Influences on the Phenology and Abundance of Flowering by *Androsace Septentrionalis* (Primulaceae).” *American Journal of Botany* 90, no. 6 (June 2003): 905–10. <https://doi.org/10.3732/ajb.90.6.905>.
- Inouye, David W. “EFFECTS OF CLIMATE CHANGE ON PHENOLOGY, FROST DAMAGE, AND FLORAL ABUNDANCE OF MONTANE WILDFLOWERS.” *Ecology* 89, no. 2 (February 2008): 353–62. <https://doi.org/10.1890/06-2128.1>.
- Jiménez-Alfaro, Borja, Fernando A.O. Silveira, Alessandra Fidelis, Peter Poschlod, and Lucy E.

- Commander. “Seed Germination Traits Can Contribute Better to Plant Community Ecology.” *Journal of Vegetation Science* 27, no. 3 (2016): 637–45. <https://doi.org/10.1111/jvs.12375>.
- Keller, Markus. “Phenology and Growth Cycle.” In *The Science of Grapevines*, 61–103. Elsevier, 2020. <https://doi.org/10.1016/B978-0-12-816365-8.00002-6>.
- Kudo, Gaku. “Dynamics of Flowering Phenology of Alpine Plant Communities in Response to Temperature and Snowmelt Time: Analysis of a Nine-Year Phenological Record Collected by Citizen Volunteers.” *Environmental and Experimental Botany* 170 (February 2020): 103843. <https://doi.org/10.1016/j.envexpbot.2019.103843>.
- Menzel, Annette, Tim H. Sparks, Nicole Estrella, Elisabeth Koch, Anto Aasa, Rein Ahas, Kerstin Alm-Kübler, et al. “European Phenological Response to Climate Change Matches the Warming Pattern.” *Global Change Biology* 12, no. 10 (October 2006): 1969–76. <https://doi.org/10.1111/j.1365-2486.2006.01193.x>.
- Miryeganeh, Matin. “Senescence: The Compromised Time of Death That Plants May Call on Themselves.” *Genes* 12, no. 2 (2021): 143. <https://doi.org/10.3390/genes12020143>.
- Molau, Ulf, Urban Nordenhäll, and Bente Eriksen. “Onset of Flowering and Climate Variability in an Alpine Landscape: A 10-year Study from Swedish Lapland.” *American Journal of Botany* 92, no. 3 (March 2005): 422–31. <https://doi.org/10.3732/ajb.92.3.422>.
- 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. “Impact of Disturbed Desert Soils on Duration of Mountain Snow Cover.” *Geophysical Research Letters* 34, no. 12 (June 2007): 2007GL030284. <https://doi.org/10.1029/2007GL030284>.
- Post, Eric S., Christian Pedersen, Christopher C. Wilmers, and Mads C. Forchhammer. “PHENOLOGICAL SEQUENCES REVEAL AGGREGATE LIFE HISTORY RESPONSE TO CLIMATIC WARMING.” *Ecology* 89, no. 2 (February 2008): 363–70. <https://doi.org/10.1890/06-2138.1>.
- Sandor, Manette E., Clare E. Aslan, Liba Pejchar, and Judith L. Bronstein. “A Mechanistic Framework for Understanding the Effects of Climate Change on the Link Between Flowering and Fruiting Phenology.” *Frontiers in Ecology and Evolution* 9 (December 6, 2021): 752110. <https://doi.org/10.3389/fevo.2021.752110>.

Steltzer, Heidi, Chris Landry, Thomas H. Painter, Justin Anderson, and Edward Ayres.

“Biological Consequences of Earlier Snowmelt from Desert Dust Deposition in Alpine Landscapes.” *Proceedings of the National Academy of Sciences* 106, no. 28 (July 14, 2009): 11629–34. <https://doi.org/10.1073/pnas.0900758106>.

Wagner, Johanna, Ursula Ladinig, Gerlinde Steinacher, and Ilse Larl. “From the Flower Bud to the Mature

Seed: Timing and Dynamics of Flower and Seed Development in High-Mountain Plants.” In *Plants in Alpine Regions*, edited by Cornelius Lütz. Springer Vienna, 2012. [https://doi.org/10.1007/978-3-7091-0136-0\\_10](https://doi.org/10.1007/978-3-7091-0136-0_10).

Wen, Lina, and Andrew M. Simons. “Delusions of Grandeur: Seed Count Is Not a Good Fitness Proxy under Individual Variation in Phenology.” *Journal of Evolutionary Biology* 33, no. 8 (2020): 1039–49. <https://doi.org/10.1111/jeb.13633>.