

Frost Sensitivity of Subalpine Plants in the Colorado Rocky Mountains: The Effects of Seasonality, Water Content, and Phylogeny

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Abstract

Our study examined the frost sensitivities of subalpine flowering plants near the Rocky Mountain Biological Laboratory, in Gothic, CO. Most studies on the impacts of climate change have focused on the effects of temperature warming and how it affects species. Our study examined the frost sensitivities of 22 subalpine flowering species in the Colorado Rocky Mountains. To our knowledge, previous studies have only examined frost sensitivity in regards to non-flowering plants in an ecological context. Our three hypotheses were (1) seasonal – species that flower early are more resistant to frost, (2) water content – species with low water content and species with high water content are more susceptible to frost damage, and (3) phylogenetic – species that are more closely related will have similar tolerances to frost. To test the seasonal and phylogenetic hypotheses we collected plant tissue (flowers, leaves, and buds), wrapped them in aluminum foil, and placed them in a temperature chamber which simulated a nighttime frost event using liquid CO₂ as the cooling agent. In order to test for water content we collected plant tissue in the morning prior to 0800 hours, weighed samples initially for wet weight, dried for 48 hours at 60-65°C, and weighed again to acquire dry weight. Water content was found by subtracting dry weight from wet weight. Our results show a high amount of variability in the potential mechanisms for frost resistance. At the community level, neither the seasonality nor the phylogenetic hypotheses were supported. We did find support for the water content hypothesis, revealing that species containing the least and the most amount of water were more sensitive to frost than those containing intermediate amounts of water. However, among congener pairs we saw mixed support for both our seasonality and phylogenetic hypotheses, indicating that seasonality and phylogeny can play a role among some taxa but not among others. Frost appears to be highly variable among subalpine plant species in the Colorado Rocky Mountains, which has important implications for predicting the continued effects of global climate change in this ecosystem.

Introduction

Most studies on the impacts of climate change have focused on the effects of temperature warming and how it affects various aspects of species physiologies. The effects of climate change are prominently seen through melting glaciers, habitat ranges shifting, and species extinctions (Miller-Rushing et al. 2006, IPCC 2007). In particular, one of the strongest bio-indicators of a warming climate is that the timing of organisms' life history events are becoming earlier (Sparks and Menzel 2002, Parmesan and Yohe 2003), especially in high elevation and high latitude ecosystems such as the Rocky Mountain Biological Laboratory (RMBL), where this study was conducted (IPCC 2007, Iler et al. 2013, CaraDonna et al. 2014). In addition to the effect of warming temperatures, a less explored effect of climate change is climate-induced frost damage (Inouye 2000, 2008). Phenological events (e.g. flowering and migration) are tied to the date of snowmelt, especially in high altitude and latitude ecosystems, and as temperatures continue to increase, snowmelt is predicted to occur earlier in the year (Inouye 2000, IPCC 2007, Inouye 2008, Iler et al. 2013). This means that many species that would not flower until later in the growing season are now in position to flower weeks earlier than what has been normal historically. This has led to necessary research on the changes in phenology between plants and pollinators (Iler et al. 2013, CaraDonna et al. 2014), as well as the demographic consequences of flowering earlier (Iler et al. *in preparation*). However, despite the date of snowmelt occurring earlier, the potential for late spring (May-June) frost events are increasing (Miller-Rushing and Inouye 2008, Inouye 2008, Martin 2010). Earlier snowmelt increases the chance for earlier flowering plants, especially the more sensitive plant tissue, to suffer frost damage (Miller-Rushing and Inouye 2008, Inouye 2008). Although our understanding of climate induced frost damage is growing at the RMBL, we still do not have a basic knowledge of which species are sensitive to frost, at what temperatures they become damaged, and what ecological and evolutionary factors may be related to frost damage.

As a consequence of rising temperatures due to climate change, phenological events have been shown to advance in accordance with climate change (Miller-Rushing et al. 2006, Rafferty et al. 2013, CaraDonna et al. 2014). Specifically, many plants are responding to increasing temperatures and earlier snowmelt by flowering earlier in the season (Parmesan 2006, CaraDonna et al. 2014). Miller-Rushing et al. (2006) used photographic data to show that plants are flowering a month earlier in England than they were fifty years ago, and at the RMBL, CaraDonna et al. (2014) to show that at the community level, plants are flowering about 25 days earlier than they were 40 years ago. Phenological advancements are more prominently seen at higher latitudes, as was found in a study on Broad-tailed Hummingbirds (*Selasphorus platycercus*), whose first arrival near the southern limit of their breeding ranges and initial flowering have seen little to no change, but close to the northern limit of their breeding range, flowers have been trending towards earlier flowering, while the Broad-tailed Hummingbirds have not changed their arrival date to correspond with earlier flowering, leaving a narrower window for hummingbirds to find mates and build their nests (McKinney et al. 2012). Iler et al. (2013) found that although both insect-pollinators – specifically syrphid flies – and flowering time are influenced by the date of snowmelt and increasing temperatures it appears that flowering phenology is more sensitive, advancing at a faster rate than the syrphid flies. Their results reveal that while phenological synchrony at the community level is likely to be maintained, the temporal overlap between individual taxa and syrphid flies may decrease under continued climate change. Phenological changes not only interrupt evolutionary synchrony between species, it can create new temporal overlaps, introducing new relationships among

species, which can either be beneficial (mutualisms) or antagonistic, leading to changes in demographic processes and affecting plant fitness (Walther 2003, Hegland 2009, Rafferty et al 2013).

Shifts in phenology can directly affect species by exposing them to unfavorable abiotic conditions which they may not be adapted to, such as early season frost events and late season drought (Inouye 2008, Forrest and Miller-Rushing 2011, Rafferty et al 2013). In high altitude environments such as the RMBL, freezing can occur during most parts of the year (b. barr, *unpublished data*). As snowmelt occurs earlier in the year, so too does the growing season, increasing flowering plants' exposure to frost events (Inouye 2008, CaraDonna et al 2014). Frost is defined as the effects of temperatures below freezing (ca. $< 0^{\circ}\text{C}$) have on both the biotic and abiotic components of an ecosystem (Inouye 2000). Such exposure increases the risk for plants to suffer frost damage during early development, thereby inhibiting seasonal growth or reproduction (Inouye 2000, Inouye 2008, Wheeler et al 2013). A previous study examined freezing resistance in alpine dwarf shrub species found that frost resistance varied across species, but did not vary along the elevation or snowmelt gradient (Wheeler et al 2013).

Among the physiological effects of frost on plants is the formation of ice crystals in the plant tissue, within or between the cells, typically resulting in the death of some critical parts of the plants, such as the flower buds, ovaries, and leaves (Inouye 2000). Specifically, at the cellular level, the cells lose water to ice via osmosis, acquiring greater solute concentrations, and become surrounded by an inflexible solid with the ability to cause mechanical damage and obstruct gas exchange (Warren 1998). However, the effects of frost range beyond destroying plant tissue. Frost can trigger ice nucleation bacteria in plant tissue to expedite frost damage by catalyzing ice crystal formation, resulting in the loss of food sources for animals, including the possible loss of floral resources for many important pollinator species, and even increase competition between species (Inouye 2000). The effects of frost on abiotic factors include congeliturbation (the impacts of frost on soil), in which frost disrupts the stability of the matrix in which plants are growing, destroying seedlings and creating stretches of bare soil called "frost deserts" (Inouye 2000). Even if the air temperature is above freezing, under the right conditions plants can suffer frost damage through radiative cooling (losing heat to the open night sky) (Inouye 2000). Leaf structure can play a role in a plant's ability to resist radiative frost damage (Jordan and Smith 1995, Inouye 2000). Jordan and Smith (1995) noticed that plants that grow in exposed habitats tend to have different leaf structure and physiology versus those that grow in sheltered habitats, and that plants with larger leaves and greater exposure to the sky increases the susceptibility of the leaves to radiation frost, indicating that smaller leaf sizes may be adaptive for limiting temperature extremes.

Frosts have prompted noteworthy adaptations in species that are affected by them. Some species migrate away from the location during the main seasons frost occurs, while others have employed phenological, physiological, or morphological traits to deal with frost or avoid frost damage (Inouye 2000). Specifically for flowering plants, some species avoid frost damage by delaying their time of flowering, which, while beneficial for missing the frequent early season frosts, may put them in a position to encounter late season frosts or droughts (Inouye 2000). A previous study found that plants growing in the Alps have adapted to low temperatures and constant snow cover by undergoing a physiological change in which the plants go through a hardening process to become highly resistant to freezing damage (Martin et al 2010). The effects of frost events are different because they can occur when the plants are physiologically active, or dehardened, which is when they are at their most susceptible to frost damage (Martin et al 2010).

Under elevated CO₂ conditions, frost sensitivity has been shown to increase in plants without shifting their phenology, increasing the amount of tissue damage caused by frost events (Martin et al 2010). As long as late spring frost events remain constant, or even increase in frequency, earlier plant development due to earlier snowmelt will increase the probability that sensitive plant tissue will get damaged (Inouye 2008, Martin et al 2010).

Knowing that climate change is pushing the date of snowmelt earlier in the year, and causing plants to flower earlier, we sought to learn which plants are susceptible to frost damage, at what temperatures they become damaged, and the potential factors that allow some plants to be resistant while others get damaged. We tested three hypotheses in this study: (1) Earlier flowering plant species will display greater resistance to frost than the species that flower later (seasonality hypothesis). (2) Plant tissue water content will dictate how much the species is resistant to frost (water content hypothesis). (3) Closely related taxa will exhibit similar frost resistance and sensitivities, compared to more distantly related taxa (phylogeny)

Methods

Study Site

Plant tissue samples were collected near RMBL in Gothic, CO, USA, (38° 57.5'N, 106° 59.3'W, 2900 m.a.s.l) (Fig. 5). The terrain near Gothic is comprised of mesic and wet meadows, dry rocky meadows, aspen forests, and mixed conifer forests. The growing season at this site is short, lasting typically 3–5 months, depending on the timing of snowmelt (CaraDonna et al. 2014). Snowmelt has ranged from 22 April to 19 June, with a mean date of 20 May. Data on the timing of snowmelt, the first date of bare ground, were acquired from b. barr's snowpack dataset collected in Gothic since 1975. Flowering phenology data for all plant species in our frost study comes from David Inouye's ongoing long-term phenology study (Inouye 2008, Aldridge et al. 2011, and Iler et al. 2013).

Study Species

We studied the frost sensitivity of a range of subalpine plants near RMBL, collected based on their individual timing of reproduction (e.g., early, mid, and late season). The early season included: *Delphinium nuttallianum*, *Mertensia fusiformis*, *Fragaria virginiana*, *Claytonia lanceolata*, *Erythronium grandiflora*, *Hydrophyllum capitatum*, *Senecio integerrimus*, and *Viola praemorsa*; the mid-season species are *Mertensia ciliata*, *Erigeron flagellaris*, *Lathyrus leucanthus*, *Potentilla gracillis*, *Cardamine cordifolia*, *Hydrophyllum fendleri*, and *Lupinus argenteus*; the latest flowering group of species collected are *Delphinium barbeyi*, *Erigeron speciosus*, *Geranium richardsonii*, *Arenaria congensta*, *Heterotheca villosa*, and *Campanula rotundifolia*.

Seasonality and Phylogeny Hypotheses

We studied frost sensitivity in 22 subalpine plant species that ranged in mean flowering time, physiology, and taxonomic membership. Overall, we conducted 3 series of frost manipulations across the season: the first frost manipulation included 8 early season species; the second frost manipulation included 7 mid-summer species; and the third frost manipulation included 7 late-summer species. 100 leaves, flower buds, and flowers were collected non-destructively from each species, and 20 of each were used in each individual frost treatment. During the collection process, plants were kept in plastic bags and stored in a cooler.

Frost Manipulation

Once tissue collection was complete, the bags were stored at 3-5°C until the frost treatment. Frost manipulations were conducted using the Sigma M26-C4 programmable temperature chamber; each species' tissue was wrapped in aluminum foil to prevent excess water loss and CO₂ poisoning during frosting. Two foil wrapped plants of each species were placed in ten plastic bags, which were then put into the temperature chamber. Three series of tests were run on species that grow throughout the majority of the summer (8 June – 15 August). The first tests were run on the species that flower early in the growing season, the second set of tests were run on those flowering in the middle of the season, while the final set of tests were run on the late flowering species, relative to the established timeframe. Each species of plant underwent five separate experimental frost treatments: 0°C, -2.5°C, -5.0°C, -7.5°C, and -10°C. In order to simulate an actual frost event, each treatment started at 5°C, and then the temperature was lowered to the target temperature over a timeframe of six hours. The target frost temperature was held for two hours, and then was brought up to 5°C in one hour. Each treatment lasted a total of nine hours, which mimics the rate and duration of nighttime frost events occurring in the field at our site. Once the each frost treatment was complete, the specimen were removed from the temperature chamber, and visually examined for frost damage. Frost damage was first scored according to three damage categories: (1) no frost damage to focal plant tissue; (2) at least some frost damage to focal plant tissue; and (3) complete frost damage to focal plant tissue. Specimen processing occurred within 24 hours of each frost treatment.

Water Content Hypothesis

In order to test the water content hypothesis, twenty samples of each species (flowers and leaves) were collected in the mornings prior to 0800 hours, which is when plants contain the greatest water content. Plant specimen were placed into a plastic bag with a wet paper towel, and then placed inside a cooler to retard further water loss. Once we returned to the Gothic Research Center, samples were stored at 3-5°C until measurements were to be made. Water content for the samples was determined by subtracting dry weight from wet weight. Wet weight was found by drying the specimen and then weighing it on a scale. Weighed samples were placed in coin envelopes and then placed in a drying oven for 48 hours at 60 - 65°C. Samples were then removed and weighed again, constituting dry weight. Initial processing of samples occurred within six hours of collection.

Analyses

In this experiment, target frost temperature was the independent variable, and it was manipulated using the temperature chamber. Frost damage to plant tissue was the dependent variable, and it was scored accordingly: no frost damage (0%), some frost damage (100% < damage < 0%), and complete frost damage (100%). These values were then transformed into a binary scale, where frost damage = 1 and no frost damage = 0. We then analyzed the relationship between target frost temperature using logistic regression models, which were implemented in R using the glm function (R core team). For some species, standard logistic models could not be fit due to perfect separation of data; in these cases we used a Bayesian logistic regression model (this issue was limited to 3 out of 22 species). We tested for phylogenetic signal by using Blomberg's K statistic (Blomberg et al. 2003) and a previously published molecular phylogeny for which our taxa are included (Davies et al. 2013, Fig. 3a). Water content was tested for using a linear regression model (R core team, Fig.2a) and a polynomial regression model (R core team,

Fig. 2b). Although flowers, flower buds, and leaves were collected, we have only analyzed results for flowers at this time. Buds and leaves will be analyzed at a later time.

Results

Seasonality Hypothesis

Frost tolerance varied across the different flowering times (Table 1). Our logistic regression models were highly significant, indicating that frost sensitivity varies significantly with temperature. The temperature at which 50% of the individuals for a given species in the treatment were damaged by frost (FD 50%) ranged from -2.5°C (*Claytonia lanceolata*) to -10.41°C (*Erigeron flagellaris*) (Table 1). At the community level, there was no significant pattern to indicate that seasonality (e.g., when in the season a plant flowered) played a role in plant frost sensitivity (seasonality linear regression: slope = -0.002 ; $R^2 = 0.0004$; $P = 0.928$, $n=22$).

Water Content Hypothesis

Water content percent in species was variable, ranging from 0.6634 (*Arenaria congesta*) to 0.8822 (*Claytonia lanceolata*) with a mean of 0.8083 (Table 2). Using a linear regression model did not detect any significant correlation between water content in plants and frost sensitivity (Fig. 2a; water content linear regression: slope = 3.51, $R^2 = 0.013$, $P = 0.608$, $n = 22$). However, using a polynomial regression revealed a significant relationship between plant water content and frost sensitivity (Fig. 2b; polynomial regression: $R^2 = 0.394$, $P = 0.009$, $n = 22$).

Phylogenetic Hypothesis

Using our 22 species community, we did not detect any phylogenetic patterns in frost tolerance; in other words, more closely related taxa are not more likely to exhibit similar frost tolerance, compared to a random pattern of evolution (Blomberg's $K = 0.56$, $P = 0.537$).

Analyses of frost sensitivity of congener pairs

For *Delphinium* and *Erigeron* taxa pairs, the earlier flowering species were significantly more resistant to frost when compared to the later flowering species (glm model with different intercepts for each species pair; *Delphinium* pair, $P < 0.001$, *Erigeron* pair, $P < 0.001$ intercept; Fig. 4a, b). On the other hand, frost sensitivity for *Mertensia* and *Hydrophyllum* taxa pairs was similar for early and late flowering species (glm model with different intercepts for each species pair: *Mertensia* pair, $P = 0.219$; *Hydrophyllum* pair, $P = 0.551$; Fig. 4c,d).

Discussion

We experimentally investigated the frost sensitivity of 22 subalpine flowering plant species from the Colorado Rocky Mountains. Species used in the analyses differed in phenology, water content, and phylogeny. We tested three hypotheses: (1) seasonality – early season species would be more adapted to frost and thus have higher resistances, (2) plant tissue water content – species that contain low water content and high water content would be more susceptible to frost, and (3) phylogeny – closely related taxa would have similar resistances to frost. Overall, when examining all 22 species, we found no support for our seasonality and phylogenetic hypotheses (Figs. 1 and 3). In other words, when during the season a species flowers is not predictive of its frost sensitivity; similarly more closely related taxa do not tend to

resemble each other in frost sensitivities. However, we did find support for the water content hypothesis: species with the lowest *and* highest floral tissue water content were most sensitive to frost (Fig. 2b). These results suggest that frost sensitivity and tolerance are quite variable among our 22 species, but that water content does help to explain on average approximately 40% of this variation (Fig. 2b).

Our water content results make sense in light of the physiological mechanisms underlying frost sensitivity in plants. Plants with low water content are likely easier to freeze because their cellular water content is lost to extracellular ice via osmosis at a faster rate than plants with high water content, leaving them in a state of desiccation (Warren 1998). This means that plants with low water content would likely dehydrate faster than those with high water content. Plants containing large amounts of water would also lose water to extracellular ice by osmosis, but larger amount of ice would afflict greater mechanical damage to the cell (Warren 1998).

We also examined the seasonality and phylogenetic hypotheses more closely using four sets of congener pairs (Fig. 3). Contrary to our findings with all 22 species, we found mixed, but clear support for seasonality and phylogeny at this more refined scale. First, for both the *Delphinium* and *Erigeron* taxa pairs, the early-flowering species was significantly more resistant to frost than the later-flowering species. On the other hand, for the *Mertensia* and *Hydrophyllum* taxa pairs there was no difference in frost sensitivity among early and late flowering species. Our results confirm the findings of Inouye (2000), which reported that *Delphinium nuttallianum* and *Erigeron flagellaris* (early season species) were among the least sensitive species to frost (Table 1, Fig. 4a, b). These results suggest, that while seasonality and phylogeny may not be ubiquitous predictors of frost sensitivity, they may be present among particular clades of taxa.

Despite early flowering species tending to be the most sensitive to climate warming, indicating their phenologies are thermal sensitive (Hegland et al 2009), we found that frost sensitivities of floral tissue were highly variable across the entire growing season (Table 1, Fig.1), meaning the timing of reproduction for a plant may not be a definite predictor of its sensitivity to frost. These results show that no single hypothesis explains all of the variation we've seen in frost resistance. It appears there is likely a combination of factors explaining frost resistances among the subalpine plant communities within the Colorado Rocky Mountains. As temperatures continue to increase, and plants continue to flower sooner due to earlier snowmelt, not only is there predicted to be an increase in frost damage to plant tissue, but the geographical distribution of some species will be influenced by late spring frost events (Walther 2003, Inouye 2000). Since frost events do not generate the same responses as daily exposure during winter does (Inouye 2000), it seems plausible that the highly frost resistant species will be competitively dominant in ecosystems that receive a higher frequency of frost events, while those species more sensitive to frost will tend toward lower elevation and latitude areas where the local climate is less volatile.

Plant frost tolerance is expected to decrease under future CO₂ conditions (Obrist et al 2001). This could have significant consequences for plant communities: established mutualisms between plants and pollinators will likely be affected (Hegland 2009, Iler et al. 2013, CaraDonna et al 2014), the distribution and abundance of some species will be affected (Walther 2003), as well as an increased risk of losing species with inherently weak frost tolerances (Obrist et al 2001). From the early flowering species we looked at in our study, we would expect *Claytonia lanceolata*, *Hydrophyllum capitatum*, and *Erythronium grandiflorum* to be significantly impacted by late spring frost events while other species such as *Delphinium nuttallianum* and *Erigeron flagellaris* to suffer little impacts (Table 1).

Conclusion

Our study sought to elucidate which subalpine flowering plant species near the RMBL were sensitive to late spring frost events, at what temperatures they become damaged by frost, and the potential mechanisms underlying which plants are sensitive and which plants are not. Our first two goals were accomplished, however, further research is necessary to shed light on the specific mechanisms underlying frost sensitivity and resistance. We found that when in a season a plant reproduces does not dictate its sensitivity to frost, broadly speaking, nor does the phylogenetic relationships among species. However, at a finer scale, there appears to be mixed support for both hypotheses among congener pairs, meaning that while seasonality and phylogeny are not ubiquitous predictors of frost sensitivity in subalpine flowering species, it can be a factor among certain taxa groups. Water content was found to play a role in frost sensitivity, explaining approximately 40% of the variation in frost sensitivity we observed. This research can be expanded by addressing whether frost sensitivity is the same across a species range, or across plant communities. Further studies on the specific mechanisms that underlie frost sensitivities are needed. Future research can also examine the impacts of frost on floral abundance and diversity. Overall, this study opens the door to research that will become increasingly necessary as temperatures continue to rise.

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Table 1. Summary of floral frost sensitivity logistic regression models for 22 species from a subalpine plant community in the Colorado Rocky Mountains. *Notes:* ¹Indicates cases where a Bayesian GLM was used due to perfect separation of data. ²FD = Frost Damage Temperature; ³statistically significant slopes or intercepts at $P < 0.05$ are indicated in bold.

Species	FD 5%	FD 10%	FD 50%	FD 90%	FD 95%	Slope	P^3	Intercept	P^3
<i>Amerosedum lanceolatum</i> ¹	-3.6	-3.64	-3.75	-3.87	-3.91	-3.1865	> 0.0001	-12.42	0.0022
<i>Arenaria congesta</i>	0.02	-0.86	-3.45	-6.04	-6.92	-0.85	> 0.0001	-2.93	> 0.0001
<i>Campanula rotundifolia</i>	-1.6	-2.65	-5.76	-8.87	-9.92	-0.71	> 0.0001	-4.07	> 0.0001
<i>Cardamine cordifolia</i> ¹	-7.09	-7.18	-7.44	-7.7	-7.79	-1.61	> 0.0001	-11.59	> 0.0001
<i>Claytonia lanceolata</i>	1.25	0.19	-2.95	-6.09	-7.16	-0.7	> 0.0001	-2.07	> 0.0001
<i>Delphinium barbeyi</i>	-2.63	-3.42	-5.75	-8.08	-8.87	-0.94	> 0.0001	-5.42	> 0.0001
<i>Delphinium nuttallianum</i>	-7.65	-8.28	-10.16	-12.03	-12.67	-1.17	0.004	-11.9	0.002
<i>Erigeron flagellaris</i> ¹	-10	-10.11	-10.41	-10.72	-10.83	-0.207	0.335	-5.59	0.00146
<i>Erigeron speciosus</i>	-2.71	-3.32	-5.12	-6.92	-7.53	-1.22	> 0.0001	-6.25	> 0.0001
<i>Erythronium grandiflorum</i>	-0.54	-1.58	-4.64	-7.69	-8.73	-0.72	> 0.0001	-3.33	> 0.0001
<i>Fragaria virginiana</i>	-4.68	-5.29	-7.12	-8.94	-9.56	-1.21	> 0.0001	-8.59	> 0.0001
<i>Geranium richardsonii</i>	-5.06	-5.5	-6.78	-8.04	-8.47	-1.73	> 0.0001	-11.69	0.0002
<i>Heterotheca villosa</i>	-4.24	-4.75	-6.25	-7.75	-8.26	-1.47	> 0.0001	-9.18	> 0.0001
<i>Hydrophyllum capitatum</i>	-1.84	-2.32	-3.7	-5.11	-5.59	-1.57	> 0.0006	-5.84	0.005
<i>Hydrophyllum fendleri</i>	-1.38	-2.02	-3.88	-5.74	-6.37	-1.18	> 0.0001	-4.58	> 0.0001
<i>Lathyrus leucanthus</i>	-5.23	-5.72	-7.16	-8.6	-9.09	-1.53	> 0.0002	-10.95	> 0.0003
<i>Lupinus sericeus</i>	-5.13	-5.6	-6.97	-8.34	-8.8	-1.6	> 0.0001	-11.18	> 0.0002
<i>Mertensia ciliata</i>	-5.52	-6.02	-7.5	-8.98	-9.49	-1.49	> 0.0002	-11.15	> 0.0002
<i>Mertensia fusiformis</i>	-3.32	-4.25	-6.99	-9.74	-10.67	-0.8	> 0.0001	-10.93	> 0.0001
<i>Potentilla gracilis</i>	-5.54	-5.96	-7.45	-8.93	-9.44	-1.48	> 0.0002	-11.01	> 0.0002
<i>Senecio integerrimus</i>	-4.6	-5.33	-7.49	-9.65	-10.39	-1.02	> 0.0001	-7.62	> 0.0001
<i>Viola praemorsa</i>	-5.65	-6.5	-9.01	-11.52	-12.37	-0.88	> 0.0001	-7.89	> 0.0001

Table 2. Summary of mean water content for 22 species from a subalpine plant community in the Colorado Rocky Mountains.

Species	Mean Water Content (grams)	Mean Water Content (%)
<i>Amerosedum lanceolatum</i>	0.1765	0.8716
<i>Arenaria congesta</i>	0.0639	0.6634
<i>Campanula rotundifolia</i>	0.0612	0.8023
<i>Cardamine cordifolia</i>	0.0837	0.8681
<i>Claytonia lanceolata</i>	0.074	0.8822
<i>Delphinium barbeyi</i>	0.4124	0.8113
<i>Delphinium nuttallianum</i>	0.0673	0.827
<i>Erigeron flagellaris</i>	0.0532	0.7372
<i>Erigeron speciosus</i>	0.1325	0.6948
<i>Erythronium grandiflorum</i>	0.9027	0.89
<i>Fragaria virginiana</i>	0.136	0.7752
<i>Geranium richardsonii</i>	0.1581	0.8315
<i>Heterotheca villosa</i>	0.0974	0.7038
<i>Hydrophyllum capitatum</i>	0.2641	0.8733
<i>Hydrophyllum fendleri</i>	0.3874	0.8681
<i>Lathyrus leucanthus</i>	0.0382	0.8185
<i>Lupines species</i>	0.2245	0.8538
<i>Mertensia ciliata</i>	0.0776	0.858
<i>Mertensia fusiformis</i>	0.0696	0.8664
<i>Potentilla gracilis</i>	0.123	0.7363
<i>Senecio integerrimus</i>	0.1026	0.754
<i>Viola praemorsa</i>	0.0971	0.86
Mean	0.172690476	0.808342857

Fig. 1: Results for seasonality using a linear regression model for all 22 experimental species. *Notes:* the y-axis starts at 0°C and decreases as the axis goes up. Each dot represents and individual species.

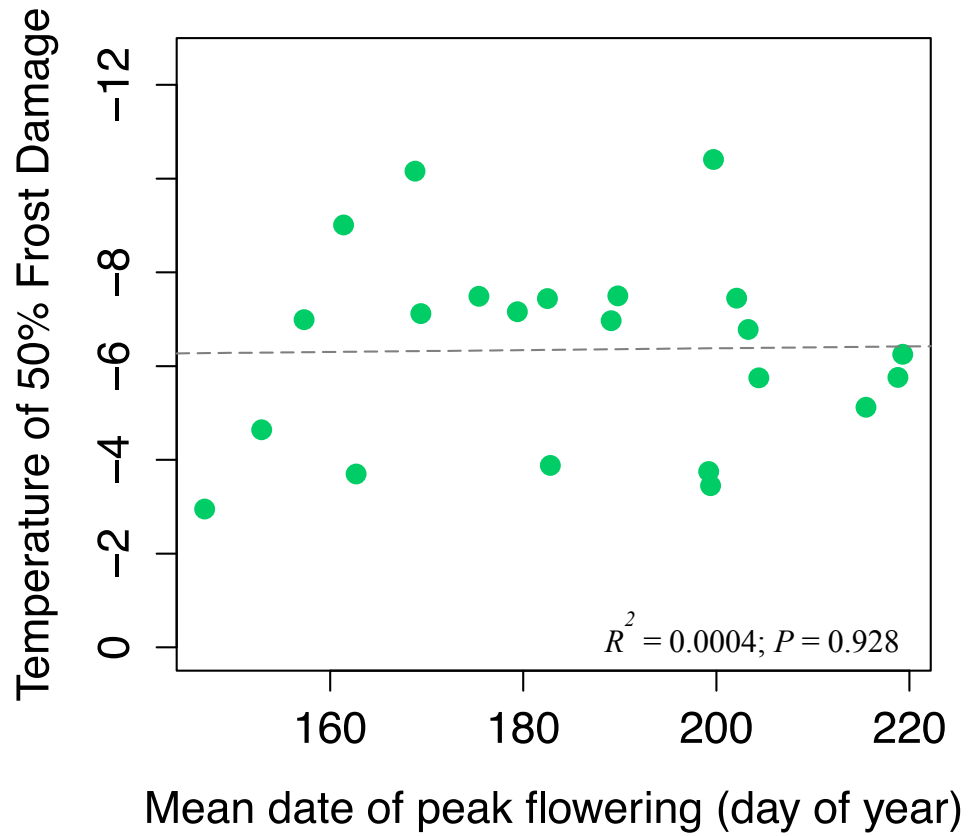


Fig. 2: a) Water content results for all 22 experimental species using a liner regression model, b) water content results for all 22 species using a polynomial regression model.
Notes: the y-axis starts at 0°C and decreases as the axis goes up. Each dot represents and individual species.

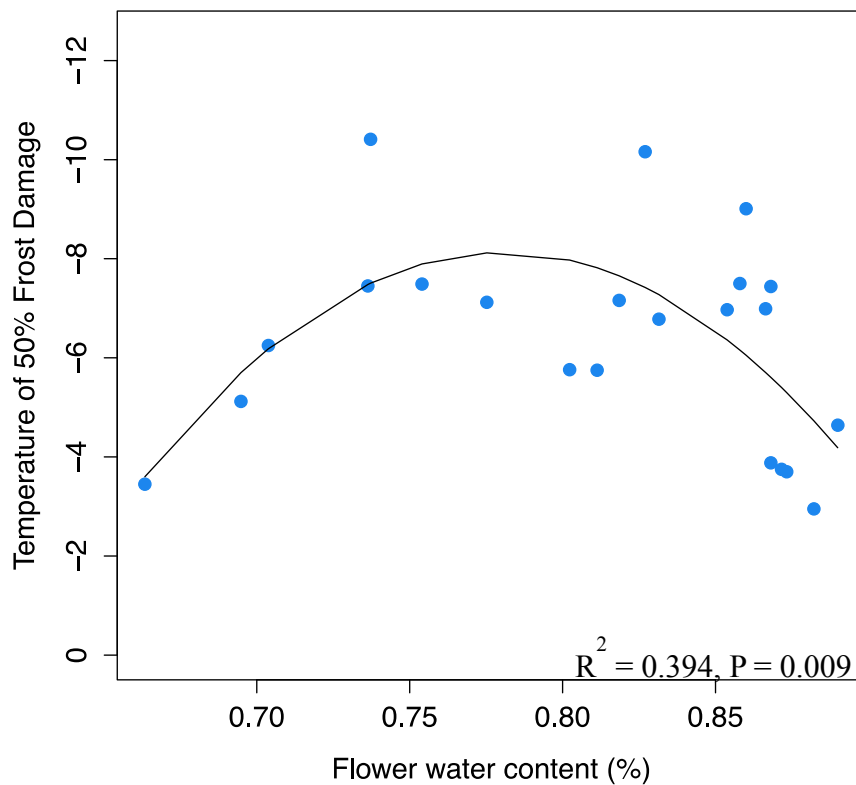
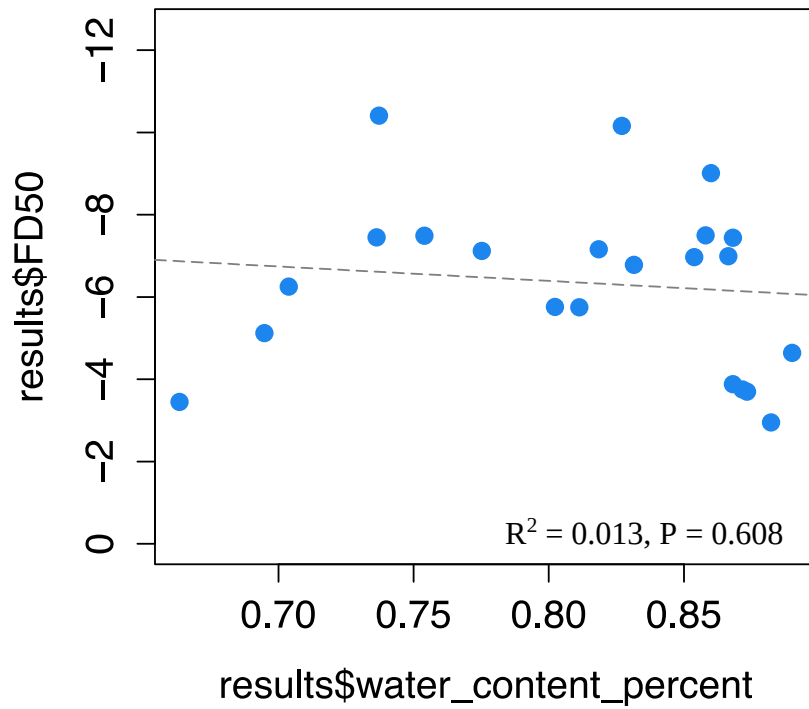


Fig.3: a) phylogenetic tree containing all 22 experimental species.
 Note: + indicates species is very sensitive to frost damage, ++ and +++ indicates species is moderately sensitive to frost damage, ++++ indicates species is not sensitive to frost damage. b) phylogenetic trait gram of all 22 experimental species.

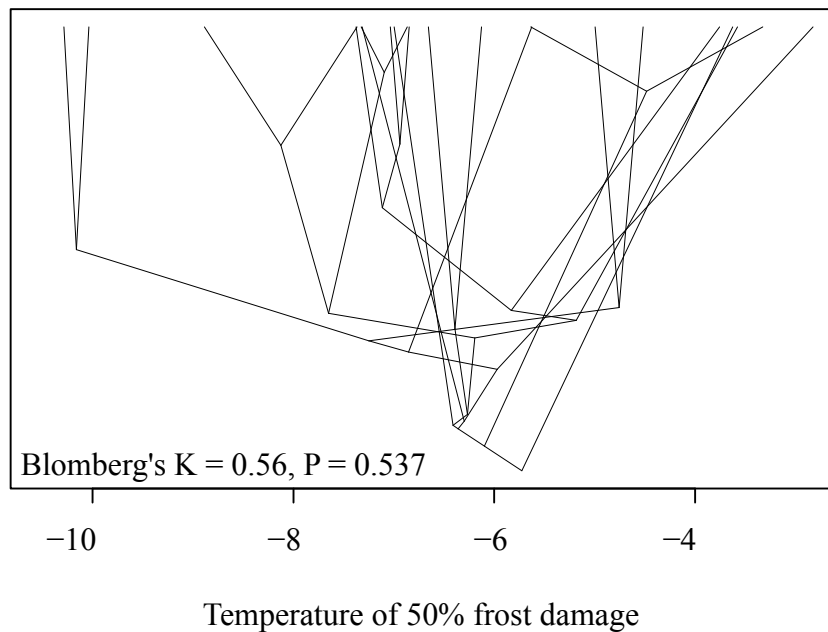
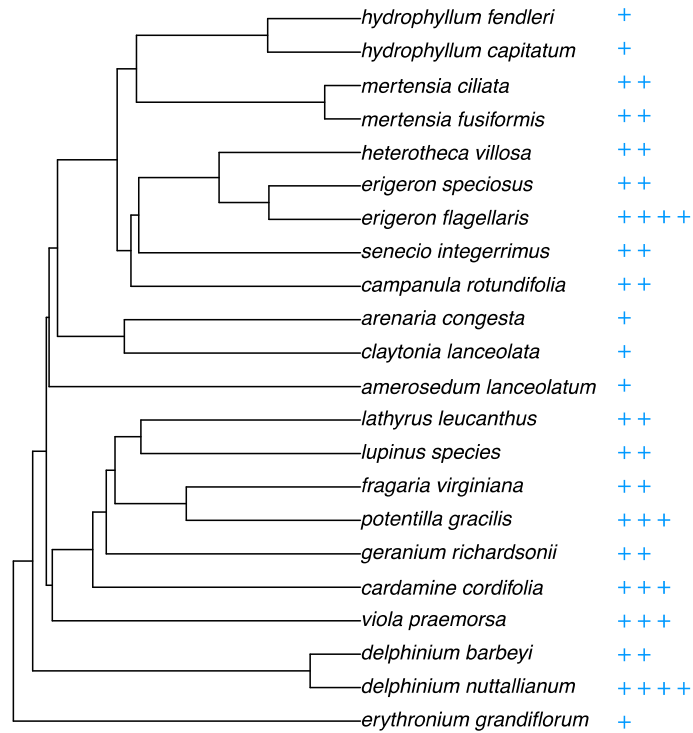


Fig.4: Logistic regression models comparing congener pairs: a) comparison of Delphinium congener pair. b) comparison of Erigeron congener pair. c) comparison of Mertensia congener pair. d) comparison of Hydrophyllum congener pair.

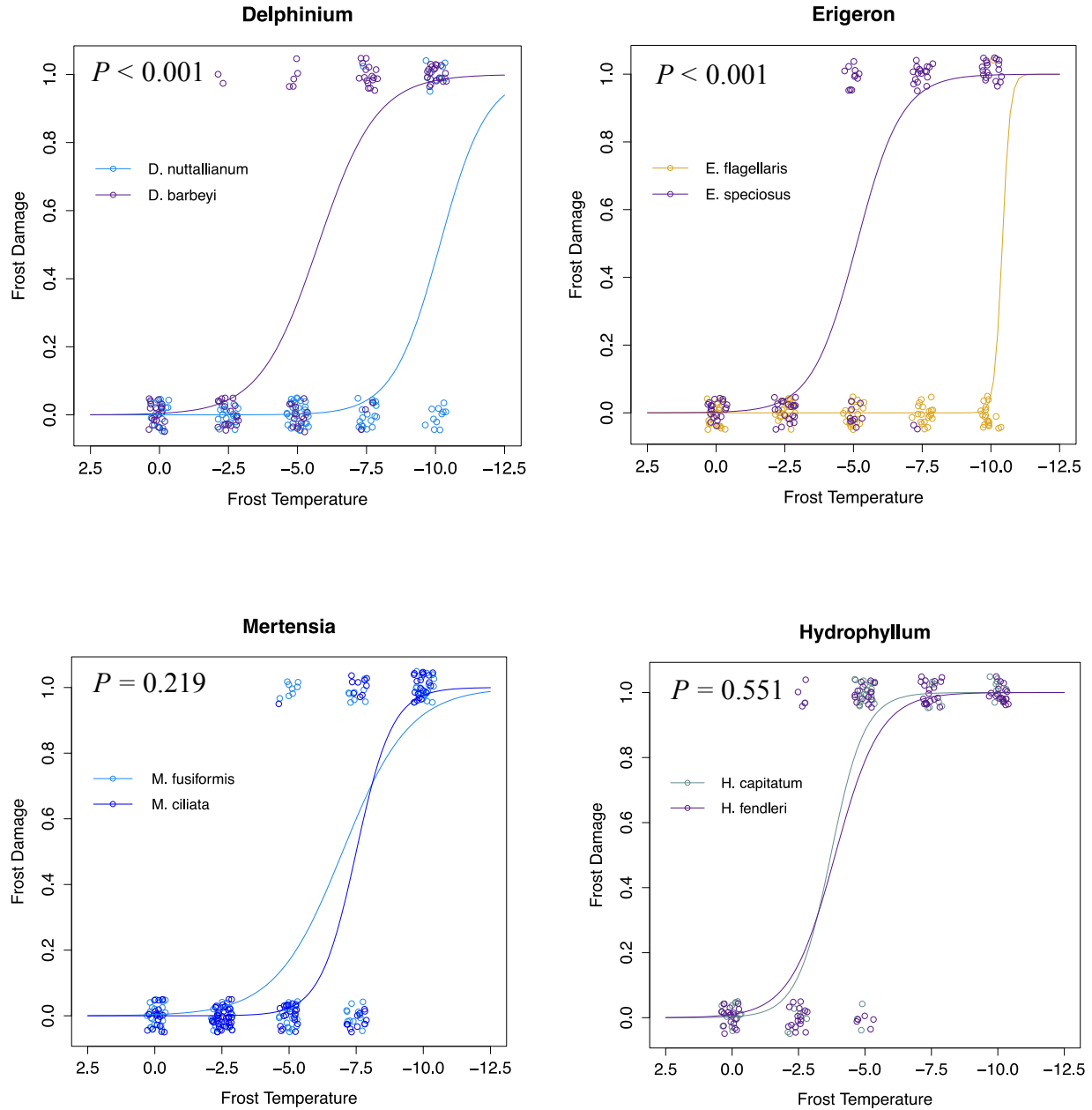


Fig.5 – Map of plant tissue collection sites. *Notes:* sites are in red. Major roads are outlined in black and rivers are outlined in blue.

