# Investigating physiological trade-offs in North Cascades plant species

Kimberly Ertel

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Committee:

Janneke Hille Ris Lambers

Elizabeth Van Volkenburgh

Brian Harvey

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University of Washington

## Abstract

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Kimberly Ertel

Chairs of the Supervisory Committee:

Dr. Janneke Hille Ris Lambers

Department of Biology

Dr. Elizabeth Van Volkenburgh

Department of Biology

School of Environmental and Forest Sciences (Adjunct)

Determining where species are distributed and what constrains those distributions are fundamental questions in ecology, and increasingly relevant to understanding ecological responses to climate change. Despite decades of study, however, we still lack a general understanding of the relative importance of climatic and non-climatic factors in driving species distributions. Functional traits may provide a solution, offering a way to generalize the constraints on species distributions and their responses to climate change. For example, ecologists often assume that a plant species' ability to tolerate harsh conditions, like frost, comes at the cost of competing for resources, potentially explaining where species can live across climatic gradients. To explore this topic, I conducted a greenhouse experiment using 25 plant species local to the Cascades of Washington State to test for a trade-off between two functional traits, frost tolerance and competitive ability. I also tested whether species differences in frost tolerance and relative growth rate translated to their current distributions, hypothesizing that high elevation species would be frost-tolerant but slow-growing, while low-elevation species would be sensitive to frost but fast-growing. While I found the hypothesized trade-off between frost tolerance and competitive ability across our focal species, I did not find that these traits varied by species distributions (high elevation vs. low elevation) as I had expected. Alternatively, differences in life form and family of origin explained differences among species in these two traits, suggesting that life history, long-term evolutionary processes, or both may play an unappreciated role in driving differences in these traits. In total, my results suggest that although these functional traits are related and may help explain how focal plant species respond to the direct and indirect consequences of climate change, the hypothesized stress tolerance and competitive ability trade-off may not provide us the ability to generalize those responses. Future studies could explore different metrics of cold stress tolerance and competitive ability, the physiological basis of resource allocation, consider whether trait variation is driven by relatedness, and validate these results in field studies.

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# Introduction

## Background

Montane plant species in the Pacific Northwest may face significant challenges as climate change becomes more severe in this region. Climate projections suggest that the Pacific Northwest will have warmer winters and warmer, drier summers[1]. As temperatures increase plant species will likely move upslope as climatic constraints on growth decrease. However, vertical space at high elevations is finite, meaning that the highest elevation species may run out of space[2–4]. At the same time, lower elevation species may compete with and displace these higher elevation species at their lower range limits, as continued warming allows them to colonize new habitat. The mechanisms that dictate how plant species differ in what constrains their performance (e.g. climate vs. competition) may therefore drive how they respond to ongoing warming.

How individual species shift their distributions in response to changing climate has proven difficult to predict[5,6]. Despite this difficulty, functional plant traits may help us understand what constrains current species distributions and may be of particular interest in such predictions[7]. For example, plant species ranges are known to be constrained by both abiotic and biotic factors with the lower boundaries of species ranges in montane systems limited by biotic factors such as competition, disease, or predation while upper range limits are defined by climate, geography, and other abiotic factors[3–5]. This suggests functional traits related to species' tolerances of cold temperatures (abiotic) and competition (biotic) may help us understand both what drives species' current distributions as well as how these distributions might be affected by climate change[7,8]. For example, high-elevation subalpine species may be adapted to the extreme environments they live in by having a high freezing tolerance, the ability of a plant to withstand subzero temperatures[9,10]. By contrast, low elevation plant species, which occur where vegetation is more abundant, may instead have a greater competitive ability, i.e. a greater capacity to acquire limited resources. Collectively, frost tolerance and competitive ability are traits that I expect will differentially benefit species depending on where they occur geographically and in climate space.

Moreover, traits like frost tolerance and competitive ability may be related if plant species face a trade-off between their physiological tolerance for cold stress and their ability to compete. Specifically, plants may face constraints in how they allocate their resources to functional traits, like cold stress tolerance and competitive ability, that allow them to survive and thrive in different locations. Loehle (1998) proposed that this trade-off occurs because adaptations to stress (like freezing tolerance) interfere with growth[11]. Similarly, Savage and Cavender-Bares (2013) hypothesized that the cost of adapting to frost reduces a species' growth capacity, resulting in a trade-off between freezing tolerance and growth rate[12]. Regardless of the mechanism, a trade-off between frost tolerance and competitive ability could help explain montane species current distributions as well as how they may respond to warming[7,8,10–13]. For example, anticipated warming may reduce snowpack or time of snow cover, especially at high elevations, allowing more competitive low-elevation species to invade high-elevation habitats that were previously too climatically harsh for them and displace less competitive but more frost tolerant high-elevation species[1,14–16]. However, these changes in climate may also increase frost incidences, as evidenced in recent years, leaving many species susceptible to levels of damage not yet experienced[10,14–16]. Regardless, a better understanding of whether a physiological trade-off occurs between frost tolerance and competitive ability among co-occurring plant species could improve assessments of the biological impacts of climate change[8,15].

## Objectives

In this study, I explored two functional traits that may play important roles in driving species' current distributions along an elevational gradient and their responses to future climate change: freezing tolerance and competitive ability. My goal was to assess whether a trade-off of resource allocation between stress tolerance and competitive ability exists for montane plant species in the Cascade mountain range of Washington state. To do so, I addressed three interrelated questions: 1) Is there a trade-off between competitive ability and frost tolerance among focal species? 2) Do focal species differ in frost tolerance according to their current distributions, and if not, do other characteristics (lifeform or relatedness) explain species differences? 3) Do species differ in competitive ability according to their current distributions, and if not, do other characteristics (lifeform or relatedness) explain species differences?

In order to address these questions, I measured frost tolerance and competitive ability of 25 plant species native to the North Cascades region of Washington[17–19] (Table 1). I hypothesized that species with high competitive ability as seedlings would have low capacity to withstand frost events and that species that are more frost tolerant would be slow growing, i.e. that a trade-off would exist between cold stress tolerance and competitive ability (Figure 1). I also predicted that high-elevation species would have greater frost tolerance than low-elevation species. Finally, I hypothesized that species abundant at lower elevations would have faster seedling growth rates (i.e. better competitive ability) than species at higher elevations. Other studies have focused on a trade-off between stress tolerance and competitive ability along a latitudinal gradient where freezing tolerance was measured[11–13], as well as a moisture gradient[20,21], where drought tolerance was measured. However, little is known about whether a trade-off of this type exists along an elevational gradient, which is what this study addresses[15].

## Methods

### Species Information

Species for this experiment were chosen based on several factors including relevance to plant communities of the Cascades, availability of seeds, life form type, and co-occurring species from the same genus or family. A total of 25 species were chosen for this experiment from 11 different plant families representative of the region[17–19] (Table 1). I incorporated multiple life forms including herbs, shrubs and trees (coniferous and deciduous) as well as two graminoids. I grouped the graminoids (*Carex* spp.) in with the herbs for the life form analyses. I chose to use 18 species as congeneric pairs (for a total of 9 pairs) from the same genus or family as a subset for this experiment in order to control for other factors that might influence species' traits and distributions such as phylogeny and evolutionary history. One species in each pair represents a high elevation species and the other occurring at low elevation[14,22] (Table 1). Species in the Asteraceae and Saxifragaceae families were paired at the family level as genus pairs were not available. Some of the pairs I studied also had contrasting habitat moisture differences (i.e. west and east sides of the Cascades), particularly where the pairs had less of an elevational contrast, but I did not control for moisture preference in my experiment. I also chose to experiment on seedlings as they are more sensitive to extreme climate variables than adult plants[10,20].

### Experimental Design

Seeds were either ordered from seed companies within the state to ensure locally adapted seed or collected in the North Cascades during the summer of 2017. I stratified differing amounts of seed per species, based on seed size and a conservative estimate of germination rates, to result in sufficient seedling sample size. Following stratification, I sowed the seeds in the greenhouse at the University of Washington Center for Urban Horticulture. Greenhouse temperature averaged

72° F +/- 4° F, with supplemented overhead lighting using 400-watt high-pressure sodium lamps from 7:00 am to 5:00 pm daily. Relative humidity ranged from 25%-45% during the first two months of growth (mid-March to mid-May 2018), and then increased to a range of 45%-65% for the remainder of the project (mid-May to mid-July 2018). While these conditions are optimal for germination and growth for many of these plants, they are very different for species that germinate in cool wet conditions, such as winter-germinating species in lowland Puget Sound, or subalpine species that germinate before all snow cover has receded[17–19]. I used Sunshine 4 potting mix and sowed herbs and shrubs in half flats and trees in 10-inch Deepots. Plants were watered with distilled water every other day or as needed in order to retain moisture for germination. I switched to fertigation for all watering using Dynagrow fertilizer with a dilution of 1:150 once all species had more than two sets of true leaves. In all, I grew 20 individual plants per species, with the exception of one species, for which there were only 15 individuals.

Flats in the greenhouse were rotated weekly in order to ensure uniform germination. Once I had enough specimens with two sets of true leaves or more from initial sowing, I transplanted 20 individuals to 4-inch rose pots and randomized specimens by life form (i.e. herbs, shrubs, and trees) using large flats as blocks containing 18 rose pots for herbs and shrubs, and Deepots racks holding 50 tree cells. Trees did not need to be transplanted, but I did not randomize them until all species were at the appropriate size in order for consistency with the desired experimental design. Block locations were also randomized on the growing bench and rotated once per week. In order to keep the plants from becoming root bound which can affect growth rates, shrubs and herbs were transplanted a second time to 1-gallon pots when they outgrew the rose pots. All but two herbs were transplanted to this size, as well as transplanted within the same week. Because of space restrictions, I could not keep these larger containers in randomized blocks.

## Data Collection

### *Relative Growth Rate*

I measured relative growth rate as a proxy for competitive ability in this experiment. Acquiring adequate resources from the surrounding environment involves competition between plants, and relative growth rate in high resource conditions in a greenhouse is thought to reflect a plant species' relative competitive ability[12,13]. To quantify relative growth rate, I utilized 5 individuals per species in a destructive harvest at approximately 4 weeks following germination (or when individuals had at least two sets of true leaves, as close to this time period as possible) in order to measure dry biomass. Plants were divided into roots, shoots and leaves, and roots were cleaned in order to prepare for drying. All tissue samples were placed in paper envelopes and set to dry in a drying oven at 50° C for three days. Dried samples were then weighed in grams using a lab scale. I harvested 5 additional specimens in order to gather the same biomass measurements after 5 more weeks of growth. Following data collection for relative growth rate components, I calculated total dry biomass, root to shoot ratio, and relative growth rate for each individual as well as the mean per species. I calculated relative growth rate using the difference of biomass over time on a log scale[23,24], as follows:

$$RGR = (\log B_2 - \log B_1) / (t_2 - t_1) ,$$

where B represents total dry biomass and t represents the corresponding time point. Other studies have used height relative growth rates as a proxy for competitive ability, which may reflect competition for light[11,12]. However, many of these studies focused on trees, and because my study included understory species of short stature, I chose to use whole plant biomass as a more comparable measure of growth across life forms.

I additionally measured the height, leaf count, and stem count at the time of initial harvest for each species once they had more than two sets of true leaves (about 3-6 weeks after germination depending on the species) for additional measures of size. Height was measured in the greenhouse using a centimeter ruler from root crown to the top of the stem. For plants with basal leaves and no true stem at this phase, height was measured to the top of the tallest leaf (relaxed). I obtained the same size measurements at the time of final harvest for the other set of 5 specimens.

***Frost Tolerance***

I used a freezing assay to calculate an index of stress or damage for the remaining 10 individuals of all focal species[9,12,23,25]. When leaf tissue starts to freeze, ice crystals form intercellularly. Frost can break cells, releasing cellular fluid and contents, which also leads to dehydration. Several mechanisms exist for protection against ice crystals, such as increased membrane stabilization or increased accumulation of solutes and proteins in intercellular spaces[15,26–28]. I measured conductivity of leaf samples in distilled water, since damaged cells release electrolytes, allowing the measurement of maximum ion conductivity from the leachate[25,29]. Specifically, I measured ion conductivity of leaf tissues subjected to freezing and leaf tissues held at a constant temperature of 5° C (for the control group). Then, I measured ion conductivity again after autoclaving samples to measure total cell damage. These values were used to calculate a comparable index of frost injury[29]:

$$I = 100 * ((F_{ratio}-C_{ratio})/(1-C_{ratio})) ,$$

where ($F_{ratio}$) represents the ratio of ion conductivity of frost damaged and autoclaved samples, and ($C_{ratio}$) represents the ratio of ion conductivity of the control group relative to autoclaved samples. Note that I calculated average index of frost injury per species as denoted above and then converted it to frost tolerance by subtracting I (frost injury) from 100.

Frost tolerance experiments were conducted after relative growth rate measurements were completed, using mature leaves from the middle of the stem (3-4 nodes down from the top of the stem, or as close to this as possible). Leaf samples for freezing experiments were taken from ten plants per species. I collected two leaf samples from one leaf for each plant: one for the freezing treatment and one for the control group. Samples were taken using a cork borer with 18 mm diameter in the center or bottom half of the leaf (closer to the petiole) in order to avoid veins and edge effects. For conifers, I used 20 1-inch needle samples per plant from the middle of the stem. Tissue samples were then placed in 50 mL VWR centrifuge tubes with 1 mL of deionized water. Control group samples were held in a cold room at 5° C for 10 hours. Freezing group samples were placed in a growth chamber at 20° C and slowly acclimated to -8° C over 7 hours at a rate of 4° C/hour. Tissues were then held at -8° C for 3 hours. After the freezing phase, I added 20 mL deionized water to each tube and placed all samples from both groups in a cold room at 5° C for 20 hours in order to collect leachate. At this point I removed all samples from the cold room, agitated tubes for 1 minute in the lab, and measured ion conductivity (in micro Siemens, µS) of each solution using a Spectrum ExStik II electrolyte conductivity meter by pouring the leachate into a 50 mL glass cylinder. Care was taken in order to ensure that no leachate was lost in this process. In order to calculate the index of damage, I poured the solutions back into each tube, respectively, autoclaved all samples at 100° C for 15 minutes, and then placed all tubes back in the cold room at 5° C for another 20 hours. The electrolyte conductivity measurements were then repeated for each group for total damage[25,29].

## Statistical Methods

I used a Pearson's correlation to explore how relative growth rates and frost tolerance were related, i.e. to test whether there was a trade-off between stress tolerance and competitive ability. Specifically, I calculated the average relative growth rate and frost tolerance for each species and then ran a Pearson's correlation of per-species frost tolerance against relative growth rate across all species to test for linearity. A negative and significant correlation would support my hypothesis. I also compared the Pearson's correlation coefficient to Spearman's rho in order to assess monotonicity.

To explore how frost tolerance varies among species and whether it varies predictably by the elevational distribution of species, I used mixed effects models and model selection. Specifically, I fit generalized linear mixed effects models with frost tolerance as the response variable, family, life form, or elevation as explanatory variables, and species as a random effect. I created seven models for this analysis using single or multiple variables (Table 2). I was unable to fit more complex models with additional interactions due to a small sample size and collinearity between the life form and family explanatory variables (7 out of the 8 trees I used in the experiment all come from the same family lineage). For each model, I ran a one-way ANOVA test to see if explanatory variables could explain the variation in frost tolerance. I additionally computed Tukey's post-hoc tests to explore which groups in explanatory variables differed. Because I could not compare significance and effect sizes of the three explanatory variables from a common model, I also used model selection with AIC values to determine which of the seven models best explained differences of frost tolerance.

To control for phylogenetic history, I tested whether the elevational distribution of congeneric pairs (low vs. high) could explain differences in frost tolerance between paired species. Specifically, I applied a paired t-test to species-specific frost tolerance values of the 9 species pairs, asking whether species within a genus (or family, in the case of Asteraceae and Saxifragaceae) differed depending on whether they were from high or low elevation. Phylogenetic relationships can have significant effects on functional traits, as some plant traits can be preserved over time[22,30].

I similarly explored how relative growth rates varied among species by using mixed effects models and model selection to see if each seedling's relative growth rate varied among species as well as elevational distribution of species. I created the same seven generalized linear mixed effects models that I used for evaluating frost tolerance, with relative growth rate as the response variable and species as a random effect (Table 3). For each of these models, I ran one-way ANOVA tests to see if explanatory variables could explain the variation in relative growth rate. I additionally computed Tukey's post-hoc tests to explore which groups in explanatory variables differed. Unable to compare significance and effect sizes of the three explanatory variables from a common model, I used model selection with AIC values in order to find the best model for relative growth rate. Finally, I applied a paired t-test to species-specific relative growth rate values of the 9 congeneric pairs, inquiring whether species within a genus differed depending their classification of high or low elevation.

During the growing period, I was only able to successfully grow 15 seedlings of *Abies grandis*. I chose to forego the initial harvest and instead used 5 seedlings for final biomass harvests and 10 seedlings for the freezing assay. In order to calculate relative growth rates for this species, I used biomass measurements for all 15 harvested *Abies grandis* and *Abies* *lasiocarpa* seedlings (5 and 10 seedlings, respectively) and measurements of seedling height and needle number to create an allometric model allowing me to predict initial *Abies grandis* biomass values for 5 individuals. The allometric model predicted biomass measurements for *Abies* seedlings well (adjusted $R^2 = 0.8012$ between predicted versus observed values, $p = 2.447e-05$), so I feel confident that this approach did not qualitatively affect my results. For all of my analyses, I used R Studio version 1.1.463 including the following packages: lattice, nlme, mvtnorm, multcomp, olsrr, and scales.

## Results

Frost tolerance and relative growth rate were negatively correlated across the 25 focal species (Pearson's r = -0.4293 and p = 0.03223; Figures 2a, 2b). Spearman's rho was similar to the Pearson's coefficient for this relationship (ρ = -0.4815 and p = 0.0158). Visual inspection of the correlation indicated that herb species were clustered together, as all had relatively low frost tolerance and moderate to high relative growth rates (Figure 2c). Shrub species were slightly slower growing, but also clustered. All 7 conifer trees were clustered together having high frost tolerance and low relative growth rates. High and low elevation species were scattered in the correlation with no apparent trend (Figure 2d). When highlighted by family, nearly all paired species were found close to each other (i.e. had similar correlations of the two measured traits; Figure 2e).

Individual mixed effects models for frost tolerance suggested that species, family, and life form explanatory variables better explained frost tolerance differences among individual plants than did elevation (Table 2; Figures 3, 4, 5). Post-hoc analysis indicated that the tree life form had significantly higher frost tolerance than did shrubs (p = 0.0015) and herbs ($p < 0.001$), but shrubs and herbs did not differ significantly from each other (p = 0.9875; Table 4; Figure 4). However, AIC values indicated that the mixed effects model with family and elevation as explanatory variables best predicted frost tolerance (Table 2). Although this model had the lowest AIC score, the second best model included family only. I also found that the mixed effects model with family only was better at explaining frost tolerance than a null model with only species random effects (AIC score: 2073 vs. 2177, respectively). Because family and life form are highly correlated (i.e. 7 out of the 8 tree species are from the same family lineage), I could not fit a mixed effects model with family and life form together as the explanatory variables.

For relative growth rate, I found that individual mixed effects models suggest that species, family, and life form explanatory variables better explain relative growth rate differences among individual plants than does elevation (Table 3; Figures 6, 7, 8). Post-hoc analysis indicated that that tree life form had significantly slower relative growth rates than did herbs (p = 0.0156), but that trees did not differ significantly from shrubs (p = 0.3506). There was no significant difference between herbs and shrubs (p = 0.4484; Table 5). AIC values indicated that the mixed effects model with only species as a random effect best predicted relative growth rate for the focal species (Table 3). Specifically, the mixed effects model with only species random effects was better at predicting relative growth rate than the model with plant family as an explanatory variable (AIC score: -714.7 vs. -674.9, respectively).

A paired t-test indicated that frost tolerance did not differ between high and low elevation species pairs (p = 0.2496; Figure 9), and the direction of the difference, while not significant, was in the opposite direction from what I had predicted. A second paired t-test also demonstrated that relative growth rate did not differ between high and low elevation paired species (p = 0.2033; Figure 10).

## Discussion

I originally hypothesized a trade-off between frost tolerance and relative growth rate (Figure 1) and I did find a significant negative correlation (Figure 2), suggesting a trade-off between the two assessed traits across the 25 species in this study. This is consistent with past studies of the same trade-off[11–13]. This finding suggests that resource allocation may constrain the ability of focal species to simultaneously grow fast and tolerate frosts[11,13]. However, despite finding the hypothesized trade-off between frost tolerance and growth rates, I found no evidence that these traits were associated with the elevational distribution of species as predicted (Figures 2d, 5, 8); neither frost tolerance nor relative growth rates differed significantly between low and high elevation species. Instead, my analysis suggested that these traits differed consistently amongst life forms or families. Below, I discuss these results in more detail.

### Why do frost tolerance and relative growth rate not vary with elevation as predicted?

I did not find that frost tolerance and relative growth rate varied by species according to their elevational distributions. Instead, I found differences in frost tolerance related to life form or family (Figures 3, 4, 5). Similarly, I found differences in relative growth rate between species that could be explained by life form or family, but not elevation (Figures 6, 7, 8). These findings suggest that elevational distributions (at least within genera or family) of focal species are not necessarily predicted by these two traits. Thus, frost tolerance and relative growth rate do not necessarily describe where species thrive along an elevational gradient, even when controlling for phylogeny and evolutionary history (Figures 9, 10). My results also imply that these traits are more heavily influenced by relatedness than location[14,22,30].

I was surprised to find that species at very different elevations did not differ in frost tolerance nor relative growth rates; in fact, the effect of elevation (although small) was in the opposite direction than I predicted for frost tolerance, i.e. lower elevation species actually have a greater frost tolerance (Table 2; Figure 9). This finding could imply that frosts are a more important environmental variable at lower elevations, which might occur due to the high amounts of snow that accumulate in the mountains of the Pacific Northwest. Snow is an excellent thermal buffer and can remain well into the summer at high elevations[15,16]. Therefore, low elevation species in the Pacific Northwest may have evolved a better ability to tolerate spring frost events than species living at high elevations, as they may actually experience more frosts due to the different lengths of growing seasons and climatic conditions. It is also worth noting that other studies involving growth differences of high and low elevation species have found significant differences between the two[31] (or differences between latitudinal warmer and colder climates[11,13]). Thus, these results are not necessarily consistent with other studies, obscured by unmeasured explanatory variables.

Although functional traits did not differ by elevational distributions as expected, I did find that related species often displayed similar values of both traits (Figures 3, 6, 9, 10). In fact, model selection suggested that frost tolerance was best explained by family and relative growth rate was best explained by species. This suggests that characteristics shared by closely related species but not measured in this experiment, such as genetics, morphological traits or germination, may play an important role in driving species differences, at least in frost tolerance[14,22,30]. Several families that exhibited significant differences in frost tolerance from other families included Asteraceae, Berberidaceae, Ericaceae and Pinaceae. For relative growth rate, Asteraceae, Betulaceae, Cyperaceae and Pinaceae were significantly different from other families.

Frost tolerance was quite variable among species, with means varying between 0-60% tolerance (Figure 3), suggesting that user error or methodological issues did not obscure hypothesized patterns in the data. One-way ANOVAs imply that family and life form accounted for a great deal of this variability (i.e. frost tolerance was also variable among families and life forms), which is consistent with phylogenetic and morphological differences among species. Frost tolerance also varied greatly within some species, such as with *Berberis aquifolium* and *Anemone occidentalis*. These species may be more sensitive in how they respond to stress, or small sample size may not have provided a large enough picture of how these species behave under freezing stress. Intraspecific variation of functional traits may have directly affected our results, and more constraints in experimental design or larger sample sizes could remedy this[32].

Although I found wide variation in frost tolerance that correlated with family membership as well as with life form type (Tables 2, 4; Figures 3, 4), I could not determine which of these two variables played a larger role due to collinearity in explanatory variables. Specifically, 7 out of the 8 tree species included in this experiment are coniferous trees from the same family, Pinaceae[17,18] (*Alnus rubra* is the only tree not in this family). Thus, while it is important to speculate on what factors caused families or life forms to be so similar in their frost tolerance (e.g. needle morphology; see below), additional experiments and tests would be needed to unequivocally test which of these factors more heavily influenced frost responses. More generally, it would be beneficial to examine a wider range of tree life forms at the family level when conducting similar studies in the future.

## Physiological mechanisms underlying observed patterns

Frost tolerance and relative growth rates may differ between trees and the other two life forms due to morphological and anatomical leaf differences[7,15,21,28,32] (Figure 4). Specifically, conifers may have greater leaf-level frost tolerance due to differences in cambium anatomy and seed size as well as needle size and shape[28]. However, because conifer seedlings grow significantly slower than herbs and shrubs of the same age and therefore may take longer to establish, they may also be vulnerable to frosts for a longer period of time. Similarly, anatomical similarities in woody stem tissue may explain why trees and shrubs did not differ from each other in their relative growth rates, while trees and herbs did. Exact anatomical and morphological traits not studied here that affect expression of both frost tolerance and relative growth rate should be explored in future research in order to understand how they may affect the proposed trade-off[28].

## The Role of Resource Allocation

An assumption underlying the hypothesized the trade-off between relative growth rate and frost tolerance is that plants use the same physiological resources to protect their leaves from frost and to stimulate growth. Indeed, plants require energy and nutrients to create more tissues, and they also require energy and resources to protect tissues from freezing[11–13,28]. However, I did not isolate nutrients or mechanisms specific to these processes in my experiments and analyses. There is ample research available surrounding mechanisms of plant growth, but research regarding the mechanisms specific to freezing protection is less common[15]. Dehydration is a major part of frost damage and turgidity, which could affect growth[27]. It is also thought that membrane stabilization is partially responsible for frost tolerance, which requires lipid proteins[15,26–28]. The creation of these proteins comes from photosynthetic production, another possible link between the two studied traits. Savage and Cavender-Bares (2013) noted that cold acclimation induces physiological changes which require carbon and nutrients, such as thickening of cell walls and increased concentrations of cellular proteins, implying the possibility of a limited growth rate capacity[12]. These connections suggest that further study is needed to identify the major resources a plant requires for frost tolerance and if these resources are exchangeable with those that plants use for growth.

## The Role of Moisture

Loehle (1998) emphasized the importance of factoring in the moisture regimes species experience when considering tolerance to frost because the damage incurred in response to frost and drought is very similar[11]. In this study, I considered species with different altitudinal distributions but not their moisture preferences, and so was unable to determine how significant wet versus dry habitat preferences were. Because focal plant species are located on both the wet and rain shadow sides of the Cascade mountains, differences among species in their moisture preferences could have influenced my results. Additionally, focal species pairs sometimes differed in their moisture preferences, but often in different ways. For example, *Abies grandis* is dominant at low elevations on the dry east side of the Cascade Range, while *Abies lasiocarpa* occurs at high elevations both on the wet west and dry east side[17–19]. Other species pairs, such as *Rubus spp.*, both grow on the wet west side while both *Pinus spp.* grow on the dry east side[17–19]. Repeating this study while constraining focal species to those that either occur only on the east or only on the west side of the Cascades may help to control for habitat moisture preference, and thus explain additional variation of frost tolerance. Moreover, plasticity in resource allocation due to a broad distribution across temperature and moisture gradients could also have affected results. For example, *Pseudotsuga menziesii* grows in many habitat types and elevations in Washington and may be able to allocate resources towards either one of these traits more strongly to thrive in various places, so the results from this species may depend more on seed origin.

## Limitations and Future Research

Although unlikely, it is possible that the methods used in this study were not sufficiently accurate in measuring the two traits of interest. Because the methods I used to measure relative growth rate (i.e. biomass over two time periods) have been often used, I believe such errors were more likely to arise during freezing assays[23,24]. For example, seedlings grew faster than anticipated, which meant that some of the leaves were older than ideal and may therefore have been heat-stressed (due to summer sun and heat in the greenhouse) or developed pest infestations. Additionally, the equipment I used to measure ion conductivity had a variability of +/- 0.5% - 1.1% error for each leachate measurement, which could have introduced error in my frost index estimates, especially considering the relatively small samples sizes I used. This seemingly small amount of error in ion conductivity measurements can make a large difference when measuring very small amounts of leachate such as with the control group samples. Thus, it is possible that more standardized use of healthy leaf tissue, larger sample sizes, and the use of a more precise conductivity meter might have allowed me to better uncover a physiological trade-off[32].

My choice to analyze frost tolerance in leaf tissue including the desiccation and damage to leaf cells after freezing may also have limited my findings[10–12,16]. I made this choice because leaves are the main anatomical structured used for photosynthesis and energy production in the types of plants I studied. While leaves are essential to growth, plants might also require energy to protect other tissues from frost damage. Savage and Cavendar-Bares (2013), for example, studied leaf tissue, stem tissue, and budburst, arguing that examining a suite of traits related to frost tolerance would provide a better understanding of the trade-off between frost tolerance and relative growth rate[12]. Other studies have also examined frost damage to buds or flowering tissues, which has major repercussions related to reproduction and population sustainability[10,11,14,16]. Using freezing tolerance on multiple tissue types in one study would likely provide a fuller picture of how species vary in their ability to tolerate cold, harsh conditions.

Still, other studies have found similar trade-offs across other species and systems, suggesting there is still more to learn about this physiological trade-off. For example, Loehle (1998) explored a trade-off between relative height growth and frost tolerance in buds for North American tree species along a latitudinal gradient and found evidence for one[11]. The subtle differences between this study and mine (height vs. biomass; buds vs. leaves) could explain differences in results of our two studies. Others, including Koehler et al. (2012), also studied tree species along a latitudinal gradient, which may provide greater overall variation in the frost events to which focal species are exposed[12,13]. Thus, expanding this study to include more species occurring at different latitudes in the Cascades to explore how functional traits relate to elevational and latitudinal distributions could provide more insight. Currently, more studies have found evidence that this trade-off is related to latitudinal rather than elevational distributions, but this study is the only one that I am aware of that explores elevation.

Given the strong effect of relatedness on both frost injury and growth, repeating this experiment with a greater number of congeneric pairs that represent a greater variety of life history traits could be beneficial. For example, congeneric pairs with contrasting moisture preferences, soil types, seed size, seed dispersal mechanisms, or trait-lineage combinations could be selected[14,22]. Carefully choosing species that vary more strongly in these characteristics may also reduce variation. For example, some of the low-high elevation species pairs actually have overlapping distributions, which may explain the lack of significant differences between frost tolerance or relative growth rate between these pairs. Including a wider array of species would also eliminate the collinearity between family and life form in this study[4,14,22].

## Conclusion

In revisiting my three objectives for this experiment, the most compelling outcomes were evidence for the proposed trade-off between frost tolerance and relative growth rate and the discovery that plant family and life form have more influence on differences in each trait among species than elevation of origin. Follow-up studies on the physiological basis of this trade-off could help us understand the physiological responses of the focal species to climate change, even if the traits do not define current distributions[10,12,13,20]. A major implication from these conclusions is that relatedness of species is a major contributing factor to focal species' responses to frost as well as their growth[22]. A phylogenetic analysis would add strength to the efficacy of these conclusions and is necessary for understanding distribution-limiting functional traits[22,30]. At the same time, knowing that closely related species have similar frost tolerances and growth rates, as suggested by this experiment, could allow researchers to apply information gathered about these traits for particular species to other related species. Both of these findings may allow us to better predict if and how these species will be influenced by changing climates, information beneficial for researchers and land managers in decision making about management, preservation and restoration of the Cascade mountain ecosystems and how to best support each species.

# Appendix 1- Tables

Table 1. This table includes all 32 plant species originally included in the experiment. Species are listed in pairs by family, but most were paired at the genus level. Species denoted by * indicate a single species with no pair. Species denoted by + indicate species that did not germinate or ultimately provide a large enough sample size to use in the experiment.

| **Species** | **Family** | **Life Form** | **Elevation** |
|---|---|---|---|
| *Acer circinatum+* | Aceraceae | Tree | Low |
| *Acer glabrum+* | Aceraceae | Shrub | High |
| *Erigeron peregrinus* | Asteraceae | Herb | High |
| *Eriophyllum lanatum* | Asteraceae | Herb | Low |
| *Berberis aquifolium* | Berberidaceae | Shrub | High |
| *Berberis nervosa+* | Berberidaceae | Shrub | Low |
| *Alnus rubra* | Betulaceae | Tree | Low |
| *Alnus viridis* | Betulaceae | Shrub | High |
| *Sambucus cerulea* | Adoxaceae | Shrub | High |
| *Sambucus racemosa* | Adoxaceae | Shrub | Low |
| *Carex spectabilis* | Cyperaceae | Graminoid | High |
| *Carex stipata* | Cyperaceae | Graminoid | Low |
| *Vaccinium deliciosum* | Ericaceae | Shrub | High |
| *Vaccinium parvifolium+* | Ericaceae | Shrub | Low |
| *Maianthemum dilatatum+* | Liliaceae | Herb | Low |
| *Maianthemum racemosum+* | Liliaceae | Herb | High |
| *Abies grandis* | Pinaceae | Tree | Low |
| *Abies lasiocarpa* | Pinaceae | Tree | High |
| *Picea engelmannii* | Pinaceae | Tree | High |
| *Picea sitchensis* | Pinaceae | Tree | Low |
| *Pinus contortoa* | Pinaceae | Tree | High |
| *Pinus ponderosa* | Pinaceae | Tree | Low |
| *Rubus parviflorus* | Rosaceae | Shrub | High |
| *Rubus ursinus* | Rosaceae | Shrub | Low |
| *Tellima grandiflora* | Saxifragaceae | Herb | High |
| *Tolmiea menziesii* | Saxifragaceae | Herb | Low |
| *Anaphalis margaritacea** | Asteraceae | Herb | Low |
| *Arnica latifolia** | Asteraceae | Herb | High |
| *Lupinus latifolius** | Fabaceae | Herb | Low |
| *Xerophyllum tenax*+* | Liliaceae | Herb | High |

| | | | |
|---|---|---|---|
| *Pseudotsuga menziesii** | Pinaceae | Tree | Low |
| *Anemone occidentalis** | Ranunculaceae | Herb | High |

Table 2. This table details all of the generalized linear mixed effects models used to evaluate which variables best explained frost tolerance results including their AIC values. The model using elevation and family as fixed effects and species as a random effect had the lowest AIC value. However, since we found elevation to be so insignificant on its own, the model using only family should also be considered.

| **Model** | **F-value** | **p-value** | **AIC** |
|---|---|---|---|
| No effects | n/a | n/a | 2303 |
| Species random effect | 273 | <.0001 | 2177 |
| Family fixed effect | 29.5 | <.0001 | 2073 |
| Life Form fixed effect | 9.19 | 0.0013 | 2155 |
| Elevation fixed effect | 0.00188 | 0.9658 | 2173 |
| Elevation and<br>Life Form fixed effects | 0.0033<br>9.3402 | 0.9548<br>0.0013 | 2151 |
| Elevation and<br>Family fixed effects | 0.029<br>29.766 | 0.8672<br><.0001 | 2070 |

Table 3. This table details all of the generalized linear mixed effects models used to evaluate which of variables best explained relative growth rate results including their AIC values. The model using only species as a random effect had the lowest AIC value.

| **Model** | **F-value** | **p-value** | **AIC** |
|---|---|---|---|
| No effects | n/a | n/a | -533.4 |
| Species random effect | 195 | <.0001 | -714.7 |
| Family fixed effect | 16.3 | <.0001 | -674.9 |
| Life Form fixed effect | 3.82 | 0.0378 | -703.4 |
| Elevation fixed effect | 0.415 | 0.5257 | -705.9 |
| Elevation and<br>Life Form fixed effects | 0.527<br>4.12 | 0.4757<br>0.0310 | -695.1 |
| Elevation and<br>Family fixed effects | 3.31<br>17.4 | 0.0921<br><.0001 | -665.8 |

Table 4. This table includes the results of the Tukey's post-hoc test for frost tolerance according to life form. Trees had significantly higher frost tolerance (i.e. lower injury) compared to both shrubs and herbs. There was no significant difference between shrubs and herbs.

| Comparison | Estimate | Std. Error | z-value | p-value |
|---|---|---|---|---|
| Shrubs vs. Herbs | -1.207 | 7.982 | -0.151 | 0.9875 |
| Trees vs. Herbs | -30.19 | 7.669 | -3.937 | <0.001 |
| Trees vs. Shrubs | -28.98 | 8.373 | -3.461 | 0.0015 |

Table 5. This table includes the results of the Tukey's post-hoc tests for relative growth rate according to life form. Trees had significantly slower relative growth rates compared to herbs but not shrubs. There was also no significant difference between shrubs and herbs.

| Comparison | Estimate | Std. Error | z-value | p-value |
|---|---|---|---|---|
| Shrubs vs. Herbs | -0.01423 | 0.01179 | -1.207 | 0.4484 |
| Trees vs. Herbs | -0.03132 | 0.01134 | -2.763 | 0.0156 |
| Trees vs. Shrubs | -0.01709 | 80.01238 | -1.381 | 0.3506 |

## Appendix 2- Figures

Figure 1. This graph represents the hypothesized trade-off between two functional traits, freezing tolerance and competitive ability, among 25 species of plants studied from the North Cascades, Washington.

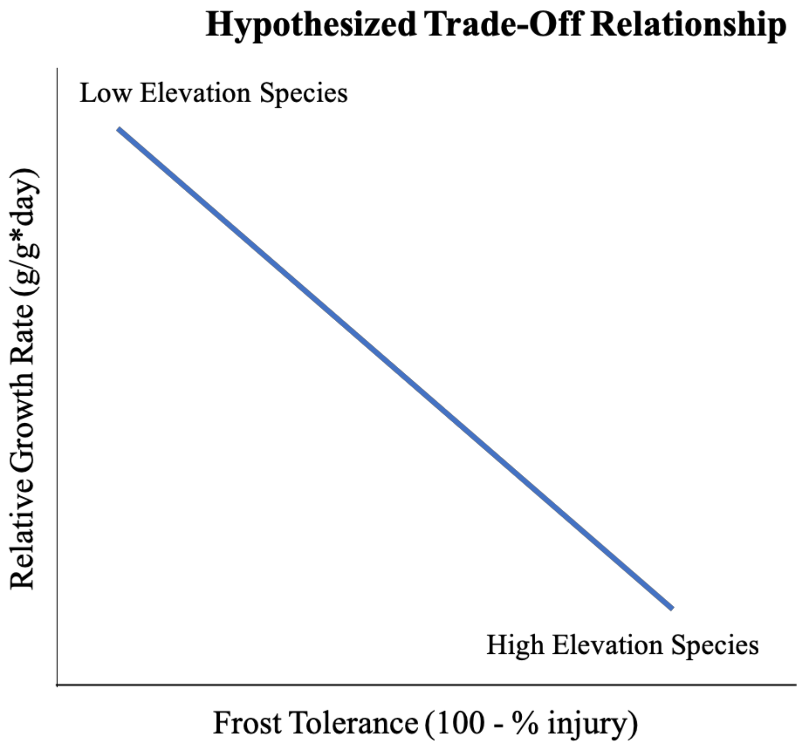
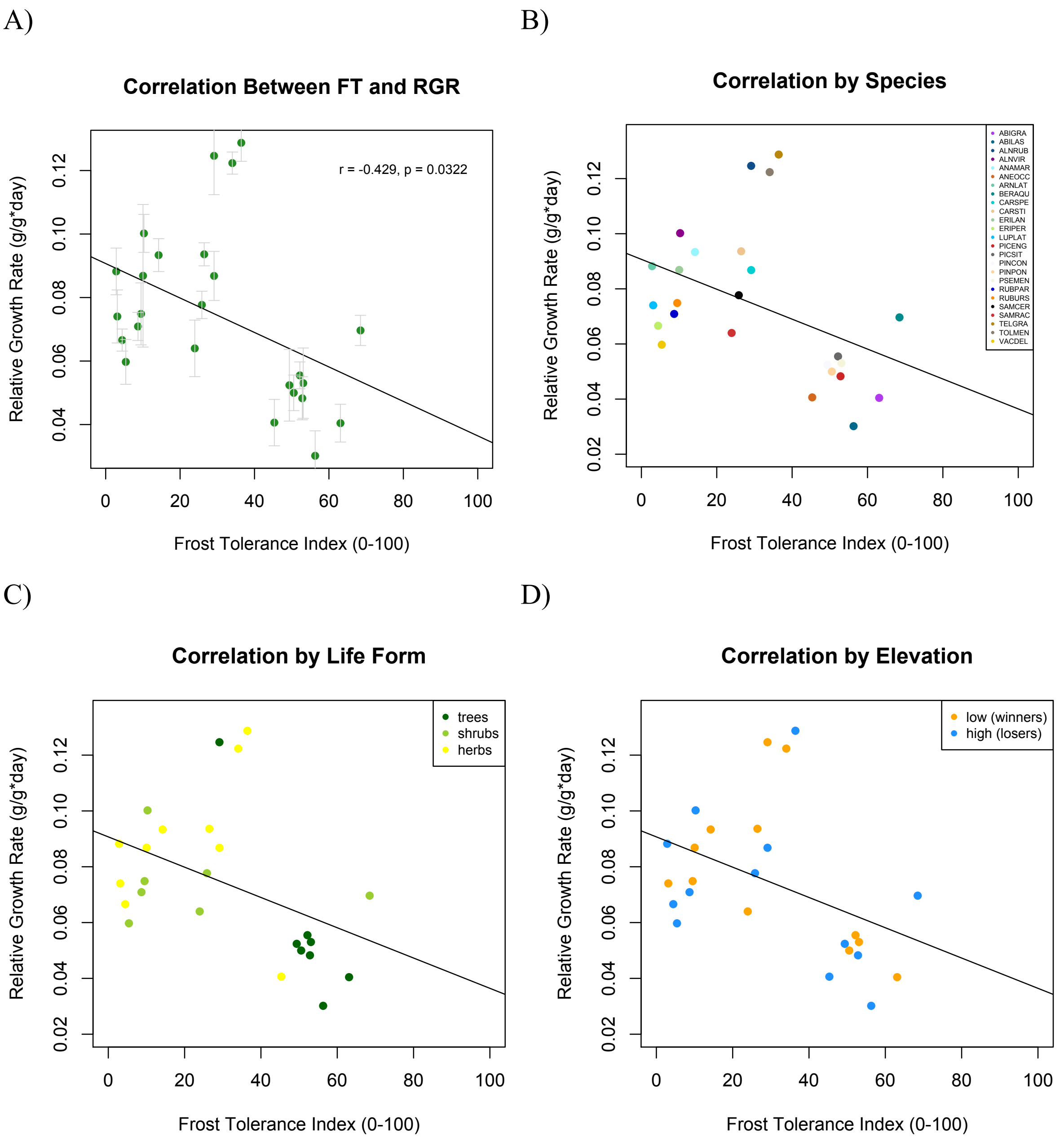


Figure 2. This is a series of five graphs displaying the correlation between the two tested traits, frost tolerance and relative growth rate, for all 25 species' means. Here, r = -0.4293 and p= 0.03223, indicating a moderate negative correlation. A) Correlation graph with error bars. B) Correlation graph highlighted by species. C) Correlation graph highlighted by life form. D) Correlation graph highlighted by elevation. E) Correlation graph highlighted by family.



E)

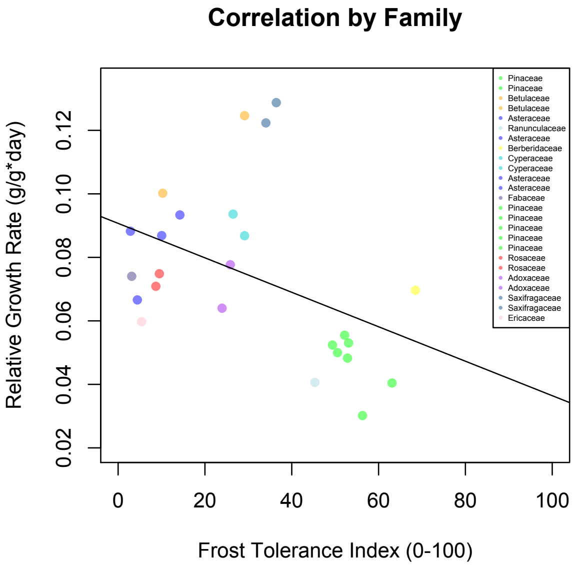

Correlation by Family
Relative Growth Rate (g/g*day)
0.02
0.04
0.06
0.08
0.10
0.12
0
20
40
60
80
100
Frost Tolerance Index (0-100)
Pinaceae
Pinaceae
Betulaceae
Betulaceae
Asteraceae
Ranunculaceae
Asteraceae
Berberidaceae
Cyperaceae
Cyperaceae
Asteraceae
Asteraceae
Fabaceae
Pinaceae
Pinaceae
Pinaceae
Pinaceae
Pinaceae
Rosaceae
Rosaceae
Adoxaceae
Adoxaceae
Saxifragaceae
Saxifragaceae
Ericaceae

Figure 3. This graph displays the distributions for frost tolerance for all 25 focal species, with the medians denoted by black dots. The families are also coded by color in order to analyze a different level of relatedness. Note that 7 out of 8 trees used in the experiment are from the same family, Pinaceae (green boxes at the top). Outliers above 100% or below 0% frost tolerance may be due to sampling error.

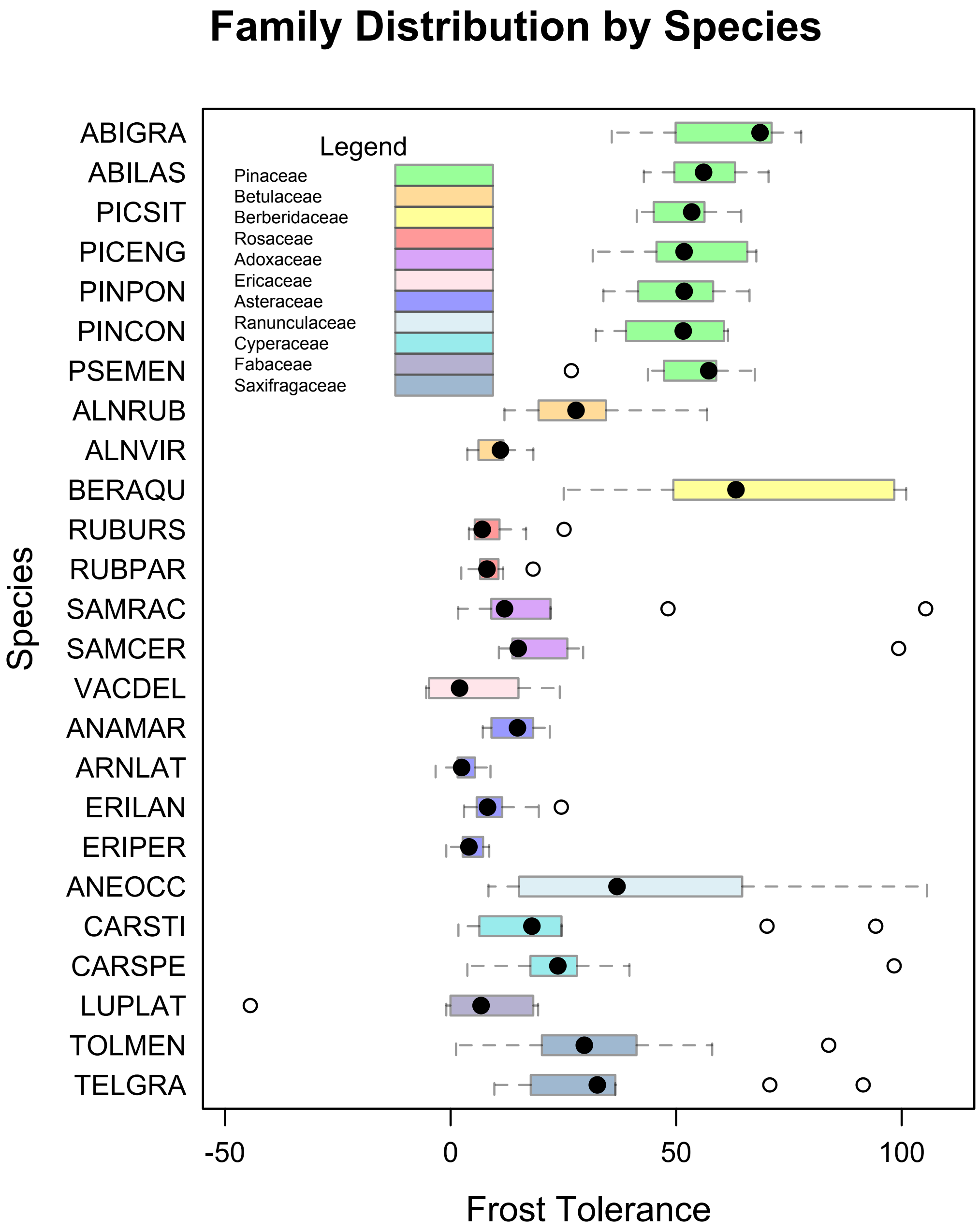


Figure 4. This graph displays the distributions for frost tolerance for life form, with thick black lines representing the median. I found life form level differences of frost tolerance, specifically between trees (b) and shrubs (a), and trees (b) and herbs (a), but no difference between herbs and shrubs. Trees exhibited a higher tolerance to frost.

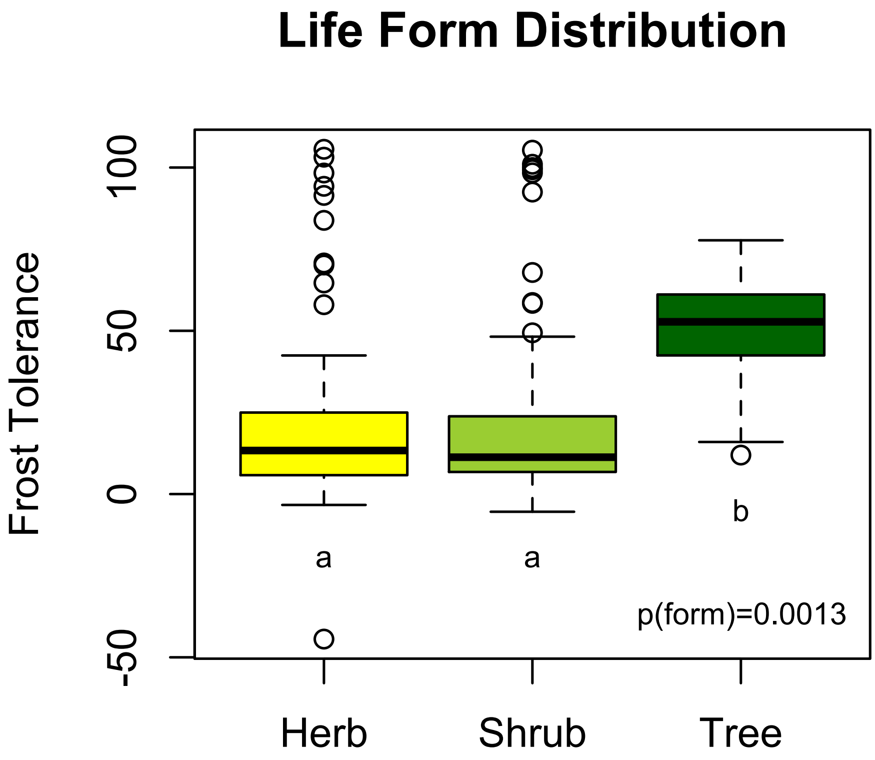


Figure 5. This graph displays the distributions for frost tolerance for high and low elevation species, with thick black lines representing the median. I did not find elevational differences in frost tolerance.

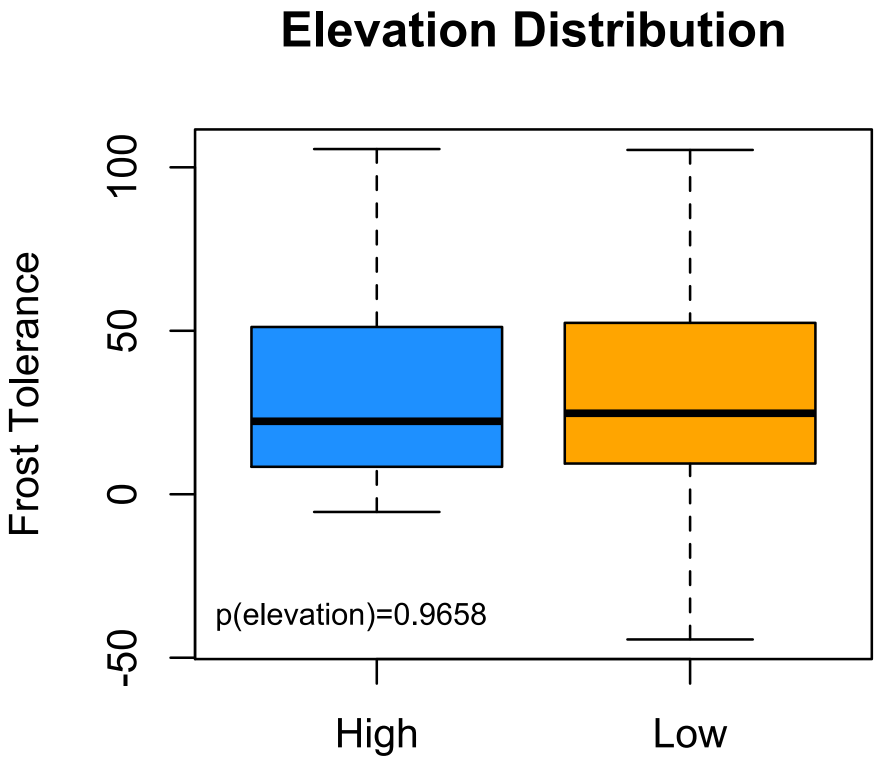


Figure 6. This graph displays the distributions for relative growth rate for all 25 focal species, with the medians denoted by black dots. The families are also coded by color in order to analyze a different level of relatedness. Note that 7 out of 8 trees used in the experiment are from the same family, Pinaceae (green boxes at the top).

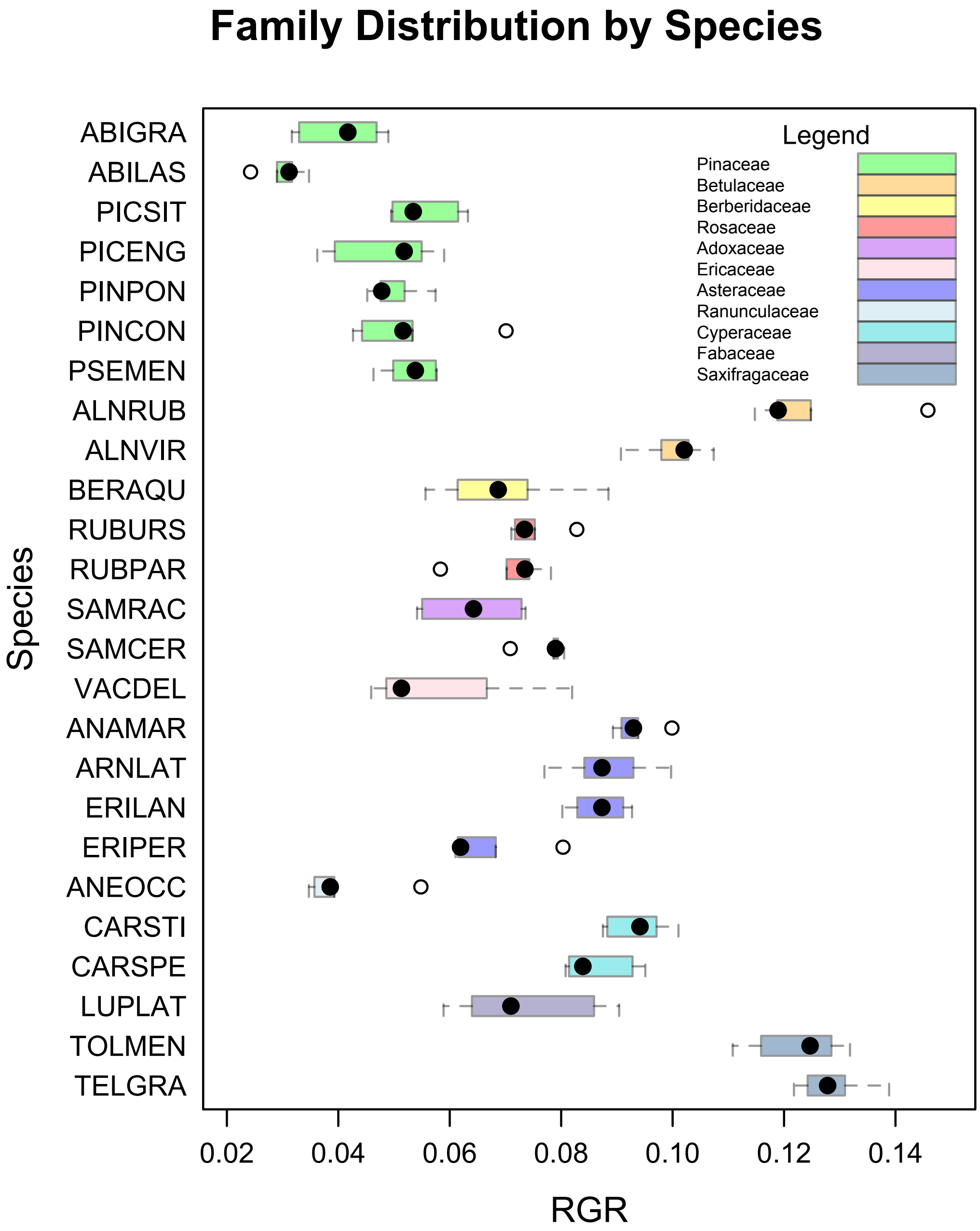


Figure 7. This graph displays the distributions for relative growth rate for life form, with thick black lines representing the median. I found life form level differences of relative growth rate between trees and herbs with trees having slower growth rates, but no difference between trees and shrubs. Similarly, there was no difference between growth rates of shrubs and herbs.

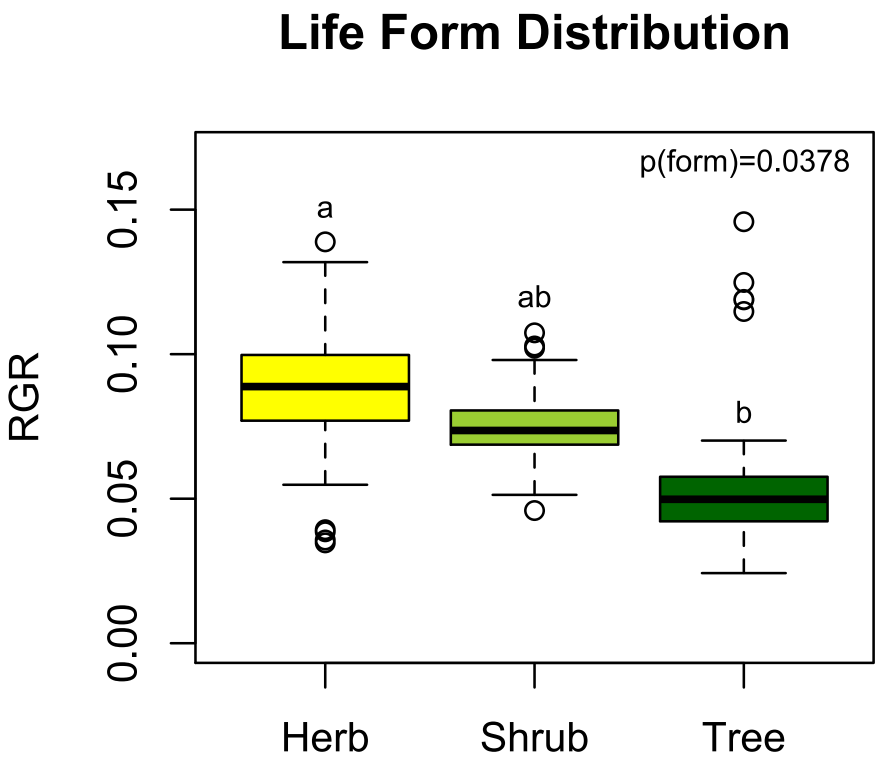


Figure 8. This graph displays the distributions for relative growth rate for high and low elevation species, with thick black lines representing the median. I did not find elevational differences in relative growth rate.

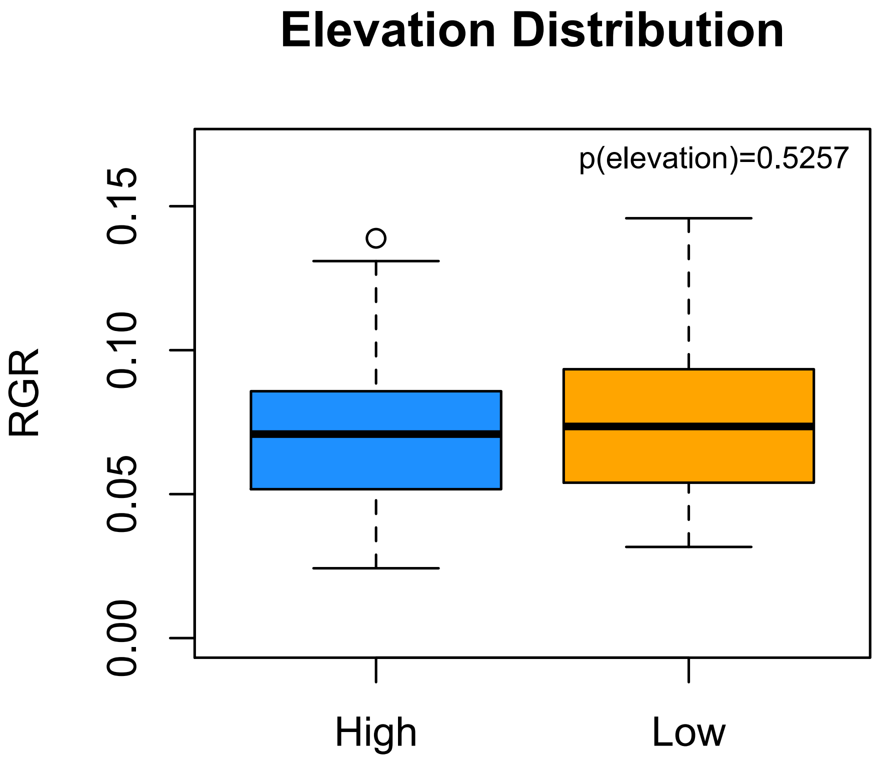


Figure 9. This graph compares the frost tolerance means for the high and low elevation congener pairs. I did not find a significant difference between the means of the pairs based on elevation of origin.

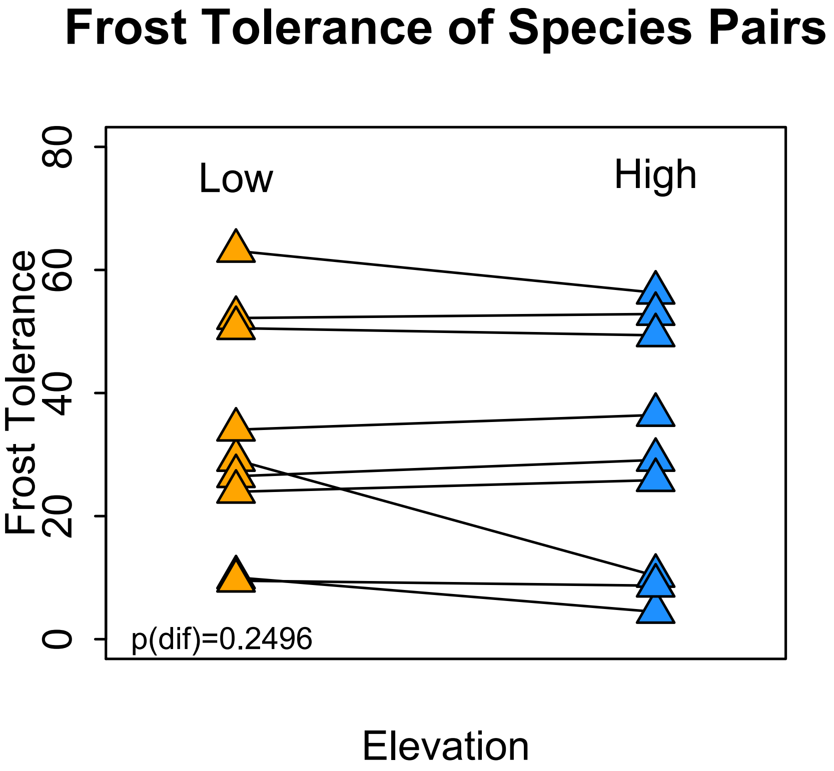
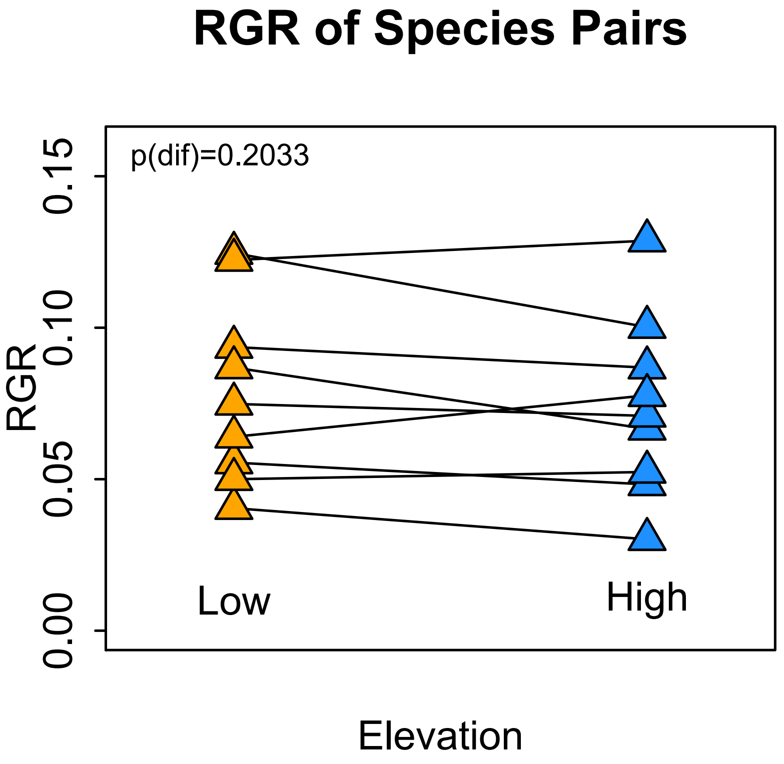


Figure 10. This graph compares the relative growth rate means for the high and low elevation congener pairs. I did not find a significant difference between the means of the pairs based on elevation of origin.



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