

DISSERTATION

THE FUTURE OF COLORADO FORESTS: ASSESSING SEEDLING PERFORMANCE
UNDER CLIMATE CHANGE

Submitted by

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ABSTRACT

THE FUTURE OF COLORADO FORESTS: ASSESSING SEEDLING PERFORMANCE UNDER CLIMATE CHANGE

Warming temperatures as a primary manifestation of climate change will dramatically alter the forests of the Rocky Mountain region as species will either adapt in place, migrate to suitable habitats, or face extirpation. Due to high levels of seedling local adaptation and covariance between temperature and precipitation in the Rocky Mountains, predictions of future tree performance are plagued by confounding variables leading to large uncertainties about how the principal drivers of climate will shape the forests of the future. To address this concern, I established three experimental gardens along a consistent temperature gradient with similar precipitation patterns at each garden in an effort to control the water inputs to these systems and address the role of temperature on seedling performance. This allowed me draw inference about how climate change will impact one of the most vulnerable age-classes of trees under real-world conditions.

My dissertation focuses on the impacts of rising temperature on four of the dominant tree species of the Colorado Rocky Mountains, specifically focusing on how performance assessed from the physiological to the whole-plant level will be affected by investigating three main questions: (1) how are three critical anatomical, physiological, and phenological leaf traits impacted by 3°C to 6°C of warming? (2) How will warming impact whole-plant growth, prioritization of resources, and survivorship? (3) How will warming temperatures exacerbate water stress, and how plastic can the response be to that stress? In Chapter 2, I examine the

impact of warming on leaf size, the temperature of optimal photosynthesis, and the timing of leaf bud burst to assess how plastic these traits are to warming. I found divergent patterns where the deciduous angiosperm performed best at the site closest in elevation to its local seed-source and declined performance with either warming or cooling, while the conifer species were less sensitive to ambient conditions. In Chapter 3, I focus on whole-plant growth, prioritization of growth to either height or basal area, and survivorship. I show that growth was unambiguously accelerated by warming across the gardens, favoring fast-growing angiosperm over the conifers. Each species preferentially allocated resources to basal area over height, an effect that accelerated with warming, and survivorship was largely attributed to stochasticity as no clear patterns between growth rate and survivorship were determined. Finally, in Chapter 4 I explored the water-stress conditions and plastic responses across the gardens focusing on osmotic adjustments and shifts in leaf structural components to tolerate water stress, however those shifts in resources necessary to tolerate unfavorable conditions may come at the expense of efficient growth. In sum, my dissertation highlights the importance of incorporating temperature directly into forecasts of seedling performance in the future by assessing their performance at different scales. Investigating seedling tolerance of warming at a single level of inference – be it at the physiological, anatomical, or whole-plant level – can lead to incomplete interpretations and predictions. I advocate for the expanded use of experimental gardens to isolate to the best degree possible the impacts of the main drivers of climate, and test using real-world conditions the impact of warming on tree seedlings in the future.

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I would first and foremost like to thank my advisors Alan Knapp and Patrick Martin who not only helped me every step of the way on this research but provided an atmosphere that allowed me to grow into the scientist I have become. I would also like to thank Bill Bauerle and Peter Brown for their work on my committee steering this project and providing guidance throughout. Over the years I have had the joy of working with many people who have come through the Knapp and Martin labs who have all impacted my research and given me countless ideas and avenues to explore. I want to particularly mention Robert Griffin-Nolan, Ingrid Slette, Alison Post, Melissa Johnston, Elsie Denton, Joshua O'Malley, Kevin Wilcox, Dave Hoover, and Maddie Shields for their help over the years and their friendship. I would like to thank Charlie Canham for his efforts to expand my statistical approaches which has opened new ways of thinking about problems and Troy Ocheltree for encouraging me to keep exploring the ecophysiology of trees. I am extremely grateful for the many people who have worked for the Colorado Forest Restoration Institute since 2014 who have aided me from the very beginning of establishing this project throughout many years of sampling including Marin Chambers and Kevin Barrett. Boyd Lebeda, Joshua Stolz, Kathryn Seville, and Pat Rastall were vital in getting this project up and running and for allowing the project to continue in the future. My parents Jane and Charles, and friends both here in Fort Collins and further afield are owed a great deal of thanks for their encouragement and for being there to listen about many problems and complaints throughout the years. I want to especially thank my wife Courtney Larson for always being there for me from the beginning of this project to the end from helping in the field, to statistical advice, and countless discussions of the best way to interpret my data and tell a convincing story. I could

not have done this without you. It is customary to write a dissertation using words such as I, mine, and my, however it is apparent that many people are owed a great deal of thanks suggesting that this dissertation should instead have used words such as we, and our. I have had tremendous support and mentorship throughout my time at CSU and I am extremely grateful for all of it.

DEDICATION

*In memory of Phil Wolff,
without whom I'm not sure the Adirondacks would have been quite so impactful*

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CHAPTER 1: INTRODUCTION

Ecological systems are the result of the complex interactions of biotic communities and the abiotic environment in which they reside. The scale at which we understand and observe our surroundings infer a sense of permanence to the systems around us, yet in reality they are far from stable. The notion that forests are forests, and thus will remain forests in the future is pervasive, yet the abiotic drivers of these systems are rapidly changing and the forests of today will no doubt experience substantial changes in the near future. One potential driver of these changes is global climate change, due largely to anthropogenic emissions of greenhouse gases, with the most certain manifestation being an increase in air temperature. Efforts are underway to limit the amount of warming, yet we have substantially altered the planet ushering in a new epoch in the Anthropocene (IPCC 2013), which will have substantial impacts on the existing ecosystems of the planet and potentially result in the generation of novel ecosystems.

Colorado's forests have a long history of community change and shifts in species demographics (Jiménez-Moreno and Anderson 2012) as well as alterations in range distributions (Fall 1997; Jackson *et al.* 2005) through temperature and precipitation fluctuations throughout geological history. As the climate shifts, so do forest communities due to alterations of disturbance regimes, local extirpations, physical barriers to migration, and the capacity of individual species to migrate at their own rates (Davis and Shaw 2001; Chen *et al.* 2011). Yet shifts in climate are occurring at a rate expected to outpace the migration potential of trees as adult and juvenile thermal tolerance zones are diverging rapidly in some regions (Zhu *et al.* 2012; Bell *et al.* 2013; Dobrowski *et al.* 2015) and may lead to substantial disruptions of communities. This is particularly true in mountainous regions where migration can be limited by

a decline in suitable habitat with increasing elevation, and competition from more heat tolerant species (Bell et al 2014).

The two principal components of climatic regimes, precipitation and temperature are both shifting as a result of climate change. Global temperatures are expected to rise dramatically in the next century (IPCC 2013), with estimates in Colorado reaching 3°C of warming by the end of the 21st Century (Gross *et al.* 2016). The central Rocky Mountains in general is expected to experience an increase in the number of extreme warm days and a decline in cold extremes (Pederson 2010). Significant warming has occurred along the Front Range of Colorado within the past 50 years both within the mountains and the abutting plains (McGuire *et al.* 2012) which has shifted montane and subalpine forest communities and abundances (Bretfield *et al.* 2016; Renwick *et al.* 2016). This has altered disturbance regimes in the region (Westerling *et al.* 2006), and exacerbated the effects of pests and disease, particularly mountain pine beetle (*Dendroctonus ponderosae*) which impacts species composition (Collins *et al.* 2011), and the economic productivity of forests in Colorado.

Predicting how shifts in these drivers will impact future plant function is complicated by the fact that precipitation and temperature typically negatively covary in mountainous regions (ex. as temperature decreases with elevation, precipitation often increases) (Fig 1.1). This complicates understanding of how shifts in either of these variables impact plant demography, species composition, and individual tree performance. Longitudinal or elevational analyses along temperature gradients typically have confounding interactions between temperature and precipitation as well as other shifting abiotic conditions such as herbivory patterns, disturbance regimes, and underlying edaphic properties obscuring prediction and forecasts of future behavior.

Martin *et al.* (*in review*) investigated diameter growth rates of seedlings in similar light environments throughout their range across the Rocky Mountains within the United States. Martin *et al.* surprisingly found growth rates of many dominant western forest tree species to be insensitive to mean annual temperature (MAT) gradients. Considering the clear delineations in species distributions across elevation bands between the montane and subalpine zones throughout the Rocky Mountains, this was quite an intriguing result. These findings presented a potential paradox, where warming experiments typically result in increased growth in plants (Way and Oren 2010), this study suggested that following seedling establishment, temperature had no impact on radial growth at this geographic scale. Two broad conclusions from this research motivated this dissertation: (1) while seedlings grow at consistent rates across diverse habitats, the underlying temperature and precipitation gradients are shifting, potentially masking the impact of temperature on growth, and (2) local adaptation is playing a considerable role in seedling performance post-establishment.

The high degree of local adaptation characteristic of tree species has complicated prediction of future performance particularly in the Rocky Mountains. Efforts to link the current distribution and the abiotic environment occupied by those species have provided forecasts of future distribution and performance that are dependent on extrapolating future climate and estimating migration potentials, and thus are limited by the simplifications of leaving out biotic interactions (Pearson and Dawson 2003) and in many cases the role of local adaptation. Recently, bioclimatic models have begun to focus on individual populations (see Gray and Hamann 2013) in an effort to account for local adaptation but they still rely on similar techniques to estimate future tree demography. In contrast, my dissertation simulates the effect of warming using *in-situ* conditions to monitor plant performance from the leaf to the entire plant using experimental

gardens arrayed across a temperature gradient. Typically, common gardens transplant individuals from diverse provinces and compare how they perform in a single environment. Instead, my gardens contain seedlings collected from a single stand per species and then out-planted to three experimental gardens each separated by approximately 3°C MAT along an elevation/temperature gradient with similar precipitation levels. By placing each garden at locations with similar levels of precipitation, the impact of temperature on seedling performance can be better assessed.

We located each garden in areas exposed to full sun on flat east-facing slopes and transplanted homogenized soil from the intermediate-temperature site to the coldest and warmest sites to minimize edaphic differences. Comparisons between a garden and the next warmer provide a snapshot of how future warming will impact individuals both from a whole-plant perspective focusing on growth and survivorship, as well as from a ecophysiological traits-based perspective. Many ecophysiological traits (ie. plant traits such as leaf thickness that are impacted by the surrounding environment and provide some degree of information about how an organism interacts with its environment) typically display some degree of plasticity along natural temperature gradients (Royer *et al.* 2009) and with experimental warming (Gunderson *et al.* 2010; McCulloh *et al.* 2016). Additionally, inter and intra-specific variability can be comparable within the same system (Messier *et al.* 2010), necessitating clear controls on variability and isolating the causal factors. I hypothesized that warming from a native seed-source elevation to a warmer garden should induce a shift in traits that will shed light on individual tree performance in the future (Fig. 1.2). While much focus is typically expended on forest stands and broader-scale populations for assessing the status of a species, warming temperatures will impact populations through effects on individuals, therefore understanding and testing in real-world conditions the implications of rising temperatures on the physiology and growth of the dominant

tree species provides a unique method of forecasting how seedlings will fare in Colorado by the end of the 21st century.

Beginning in 2014 I planted four species, one deciduous angiosperm: quaking aspen (*Populus tremuloides*), and three gymnosperms: ponderosa pine (*Pinus ponderosa* subsp. *scopulorum*), lodgepole pine (*Pinus contorta* var. *latifolia*), and subalpine fir (*Abies lasiocarpa*). Aspen, lodgepole pine, and ponderosa pines are montane species in Colorado that typically occupy a region between 1650-2750m, while subalpine fir is typically found at higher elevations in the central Rocky Mountains (~2750m – 3350m). Seeds were collected from single stands, germinated, and grown for two years by the Colorado State Forest Service Nursery in Fort Collins, CO before field planting. The montane species: quaking aspen, lodgepole pine, and ponderosa pine were collected in Roosevelt National Forest at 2,600m, 2,450m, and 2,250m respectively, while subalpine fir was collected at approximately 2750m in Arapaho National Forest. These four species comprise approximately 54% of the forested acres in Colorado, and the vast majority of the biomass in the mountainous regions of the state (Colorado State Forest Service 2018).

My dissertation uses experimental gardens to determine key parameters that will accurately predict seedling performance in a warmer future. In the next three chapters, I first examine how key anatomical, physiological, and phenological leaf traits are impacted by 3°C to 6°C of warming. I then explicitly explored whole-tree growth and survivorship, examining how warming differentially impacts each species and how individuals in each garden prioritized growth to either height or basal area. Lastly, I investigated how rising temperatures will impact water stress responses of one dominant and wide-spread tree species that is currently under threat of considerable range retraction in the future. I conclude with a synthesis of these results and

some suggestions for the kinds of studies needed to further our capabilities for forecasting Colorado's future forests.

1.1 CHAPTER OVERVIEWS

In Chapter 2, I investigate how warming may impact the temperature of photosynthetic optima, leaf size, and the timing of spring bud break at my experimental gardens. I hypothesized that one of the key traits that will be impacted by warming is photosynthetic rate as previous work has investigated shifts in photosynthetic output at varying temperatures and found evidence for plasticity of temperature optima with warming (Gunderson *et al.* 2003; Smith and Dukes 2013). Using a portable photosynthetic measurement system, I altered leaf temperature in 5°C increments from 15-30°C and monitored shifts in photosynthetic rate. Specifically, I tested two hypotheses: (1) that warmer adapted (lower elevation) montane species would have a warmer temperature where photosynthesis was optimal, while colder-adapted species would have a colder optimal temperature, and (2) that each species would display some degree of plasticity to shift in optimal temperature based on its planting environment. Additionally, broad scale analyses across taxa found striking trends in how anatomical structures and physiological attributes shift based on warming temperatures (Way and Oren 2010; Reich *et al.* 2014). I tested the plasticity of leaf size across the gardens and hypothesized that the gymnosperms would experience larger shifts in leaf size with changes in temperature than the deciduous species. Lastly, I explored phenotypic plasticity in the gardens as a third measure of performance. The trend of increasingly earlier springs is widespread (Roberts *et al.* 2015), and while the overall direction of warming temperatures increasing growing season length was to be expected, I sought to test the relative effect of warming induced increases in growing season among the species. I hypothesized that the deciduous angiosperm, quaking aspen, would undergo the largest

shift in growing season length with warming as angiosperms typically profit from early season accumulation of photosynthate (Cannell 1989). Recent warming has led predominantly to earlier springs rather than later onset of senescence (Lee *et al.* 2003), therefore I expected that earlier springs would be most beneficial to the species that photosynthesizes the most during that time period.

In Chapter 3, I investigate the role of warming temperatures on whole-plant growth, survivorship, and resource allocation. Since planting the gardens in June 2014, I have been taking monthly measurements of height and diameter at 5 and 10 cm above the soil on every living individual as well as recording the timing of mortality. I summarize 4 seasons of growth data focusing on basal area rather than height or volume as basal area tends to increase continuously while height can be temporarily reduced via stem die-back or stochastic ice and wind damage in the winter. I tested two hypotheses, (1) that each species would experience the highest growth rates at the garden closest in elevation to its local seed source. This would suggest a high degree of local adaptation to native conditions with declines in growth accompanying either warming or cooling (only applicable to the montane species), and (2) increases in MAT would correspond with increased allocation to basal area over height to increase xylem production and water transport. I suspected that the ratio of height to basal area would be plastic among the gardens and would favorably shift towards basal area over height where water availability was lower due to warm temperatures exacerbating atmospheric evaporative water demand. I also investigated the relationship between seedling survivorship and three different metrics of growth to assess what roles slower growth and stochasticity (randomness) had on seedling survival under different temperatures.

In Chapter 4, I focus solely the effects of water stress on aspen performance at the experimental gardens, specifically how midday leaf water potential and osmotic potential shift seasonally and under varying amounts of available soil moisture. I sought to quantify the impacts of warmer temperatures on water stress under comparable levels of precipitation to investigate how future warming will exacerbate water stress even under comparable ambient precipitation to current levels. I explore how leaf structural components shift seasonally, and how high temperatures and low available soil moisture impact growth at the warmest site, while cold temperatures and preparing for freezing-events impact growth at the coldest site. I specifically tested four hypotheses (1) seedlings growing at the warmest and coldest sites will experience the largest osmotic adjustment in response to low soil moisture at the warmest site and cold temperatures at the coldest site. (2) As individuals at these sites will undergo the largest amount of osmotic adjustment, I expect they will also demonstrate the greatest resistance to freezing. Additionally, I hypothesize (3) that allocation of biomass to leaf structural components will be positively correlated with freezing tolerance, and that (4) seedlings growing at the intermediate-temperature site will have the greatest growth efficiency as resources will be available for growth rather than being used in osmotic adjustments.

1 FIGURES

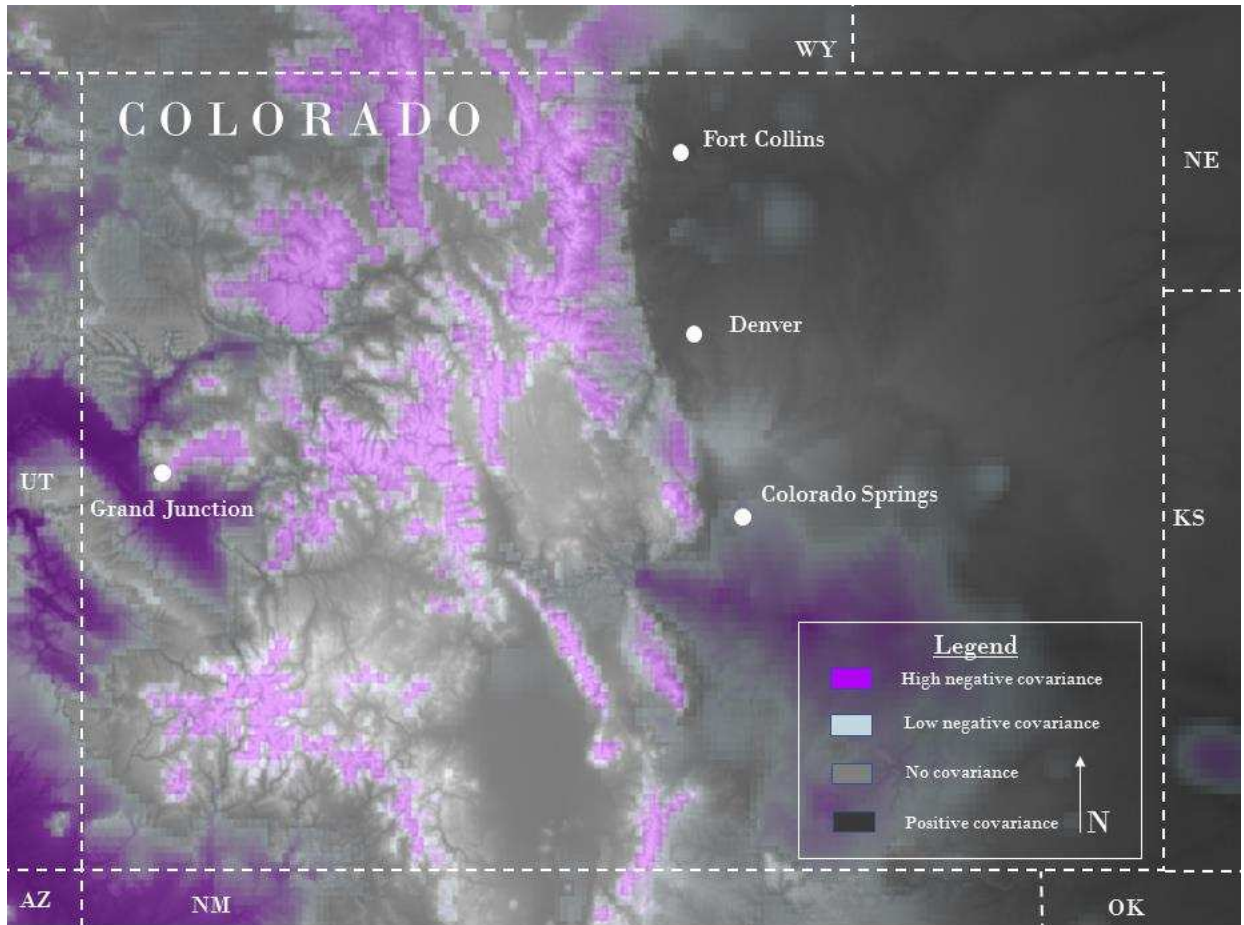


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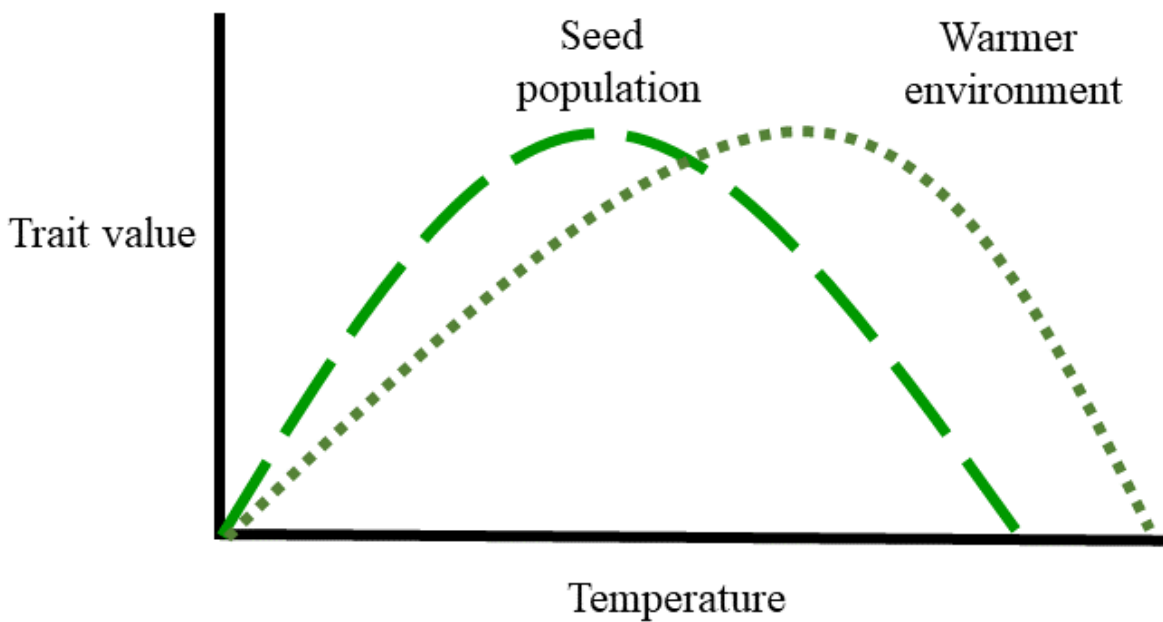


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CHAPTER 2: DOMINANT TREE SPECIES OF THE COLORADO ROCKIES HAVE DIVERGENT PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES TO WARMING¹

2.1 INTRODUCTION

There is growing evidence that many forests worldwide are undergoing rapid and substantial change as temperatures increase and drought frequency and duration intensify, leading to disruptions such as warming-induced mass mortality events (Allen *et al.* 2010; Peng *et al.* 2011; McDowell *et al.* 2016) and incipient shifts in tree species' distributions (Woodall *et al.* 2009; Zhu *et al.* 2012; Buma and Barrett 2015; Miller *et al.* 2017). Yet, our mechanistic understanding of how global change drivers are impacting forests is lacking, as empirically based experimental studies of climate impacts in forests are uncommon and have lagged behind other biomes (e.g. grasslands, Knapp *et al.* 2002; alpine ecosystems, Walker *et al.* 2006). A host of models have been developed to address the impacts of climate change on forests, mainly correlative “climate envelope” models (*e.g.* Box *et al.* 1999; Iverson *et al.* 2004) and ecophysiological process models (*e.g.* VEMAP 1995; Bugmann 1996), and both predict large and rapid shifts in tree species ranges. Yet, there is little consensus on how systems such as forests will respond given the assumptions in these models (Pearson and Dawson 2003; Guisan and Thuiller 2005; Canham and Murphy 2016), especially that species distributions remain in equilibrium with climate.

Assessing warming impacts on the ecophysiology of tree seedlings in controlled environmental conditions is a promising area to address this gap, as ecophysiological responses

¹ Carroll, C.J.W., Knapp, A.K., and Martin, P.H. 2017. Dominant tree species of the Colorado Rockies have divergent physiological and morphological responses to warming. *Forest Ecology and Management*. 402: 234-240.

develop quickly and seedlings are small enough for manipulations, but also because the responses and resilience of forests will depend strongly on the regeneration ecology of tree species under current and future climates. Given rapid rates of environmental change and inherent limits on dispersal – most studies report mean tree seed dispersal of less than 20 m for all but small-seeded species (e.g. Ribbens *et al.* 1994; Clark *et al.* 1998; LePage *et al.* 2000; Svenning and Skov 2007; Martin and Canham 2010; Siefert *et al.* 2015) – I argue that tolerance and acclimation to changing conditions *in situ* via a combination of fixed and plastic traits will be as important as range shifts in determining how tree species are impacted by climate change. How a plant species acclimates to future conditions will depend on traits such as species-specific predispositions for maintaining minimal hydraulic safety buffers (Choat *et al.* 2012), limitations in leaf plasticity to acclimate to carbon seeding (Tjoelker *et al.* 1998), and inherent constraints on shifts in phenology (Roberts *et al.* 2015).

The effects of warming temperatures on plant carbon relations may be ameliorated foremost by the acclimation of photosynthetic rates to altered conditions (Smith and Dukes 2013). Photosynthetic acclimation can occur via shifts in the instantaneous response of net photosynthesis to temperature, as well as through changes in the shape and/or base rate of the response, often resulting in a shift in the temperature optimum (Smith and Dukes 2013). Indeed, evidence suggests that temperate deciduous tree species have an ability to rapidly acclimate photosynthesis to local conditions (Gunderson *et al.* 2000). However, more studies conducted *in situ* over longer periods are needed to assess how established seedlings respond to novel growing conditions. A key issue in studying warming effects *in situ* on tree ecophysiology is controlling environmental factors that usually co-vary with temperature (e.g. moisture). These co-varying factors can alter observed rates of photosynthesis and the acclimation response in particular.

Empirical efforts to study the direct effects of climate on tree species *in situ* have helped address some of these concerns (e.g. Buechling *et al.* 2017; Canham and Thomas 2010), but such gradient approaches on extant trees lack controls and compare across individual trees over the species' range. Given the prevalence of adaptation to local climate conditions (e.g. Leimu and Fischer 2008; Hereford 2009), treating a species as ecologically equal across its range is likely to bias predictions of growth under future climates (O'Neill *et al.* 2008; Angert *et al.* 2011). Thus, while it is clear that forests are in flux, uncertainty remains with respect to how these dynamics will unfold, as replicated controlled experimental studies of the effects of climate on tree performance have lagged behind the models (Petrie *et al.* 2016).

In this study, I established a series of experimental gardens along an elevation gradient to investigate the effects of temperature on tree seedling performance while controlling differences in precipitation, light, soil fertility, soil moisture, topography, exposure, and local adaptation. This approach enables the examination of how markedly different temperature regimes alter the physiological, phenological, and morphological performance of tree seedlings of three Rocky Mountain dominant species – two conifers and a deciduous angiosperm. I focused on the response of three key processes expected to determine the main response and acclimation of tree seedlings to warmer temperatures: photosynthesis, the phenology of spring bud break (Saxe *et al.* 2001), and leaf morphology (Mahan 1997). These three traits exhibit comparatively strong plasticity to changes in the environment (Jurik *et al.* 1988; Körner 2003; Roberts *et al.* 2015), and identifying trade-offs in these traits is important for predicting whole-plant performance. Warmer temperatures have been shown to result in increased chlorophyll content and thus photosynthetic output (Ormrod *et al.* 1999). Alternatively, warming can restrict photosynthesis via stomatal closure due to water stress, yet this relationship is highly species-specific (Saxe *et*

al. 2001). Overall, acclimation of photosynthetic optima has been shown to be plastic and can shift dramatically depending on conditions and species (Battaglia *et al.* 1996). I hypothesized that the ecophysiology of tree species adapted to warm temperatures would be less impacted by higher temperatures than species adapted to cooler environments, but that each species will have some capacity to shift its optimal temperature of photosynthesis based on local conditions. I predicted increases in growing days with higher temperatures will result in the greatest shifts in phenology for the deciduous species, as light capture is maximized by early leaf production rather than retention in the fall (Cannell *et al.* 1989). Lastly, global analyses have suggested that gymnosperms increase foliar mass to total mass ratios with warming temperatures at a higher rate than angiosperms (Reich *et al.* 2014) and that leaf size increases with warming temperatures for both groups (Way and Oren 2010). Thus, I predicted the conifers would increase leaf size the most with increasing temperature.

2.2 METHODS

Common Gardens - In the summer of 2014, I established three experimental seedling ‘gardens’ along a 1,200 m elevation gradient along the Front Range of Colorado. The 3 sites range from the lower prairie–treeline ecotone at 1560 m to high elevation forests at 2750 m near the upper alpine–treeline, and encompass 3 major forest types in the region. There is a $\sim 3^{\circ}\text{C}$ difference between each site for both mean annual temperature (MAT) and growing season temperature (GST, Table 2.1). At each site, I selected a flat area with full-sun exposure, removed preexisting vegetation and excavated the top 30 cm of soil from two 18.5 m² plots. To homogenize soils in each garden, 30 cm of topsoil was collected from the intermediate-temperature site and transported to both the warmest and coldest sites to fill in the excavated plots and tilled using a

front-tine tiller. The soil in the intermediate-temperature site was excavated in the same manner, returned to the plots, and tilled. Large rocks were removed but no other treatments were performed on the transplanted soil.

I studied 3 tree species characteristic of the montane forest zone (1650-2750 m) – lodgepole pine (*Pinus contorta* var. *latifolia*), ponderosa pine (*Pinus ponderosa*), and quaking aspen (*Populus tremuloides*). Seeds from these tree species were gathered from a single stand for each species in National Forests by the Colorado State Forest Service Nursery (Fort Collins, CO) and germinated in the spring of 2013. Seeds of lodgepole pine and aspen were gathered at 2450 m and 2600 m, respectively, and ponderosa pine seed was collected at 2250 m. By selecting populations from a single source at mid-elevation, I controlled for the effects of local adaptation allowing my study to mimic how climate change actually operates – with an altered climate for individual trees – rather than studying climate’s effect across individuals along environmental gradients as is done in observational studies where local adaptation to climate might confound the results.

The planting pattern at each garden was identical. One year-old seedlings within a species-specific height range were randomly selected from the nursery stock. Each 18.5 m² plot was planted in a 9 x 9 grid with ~23 cm separating each seedling to reduce effects of initial root competition and shading amongst seedlings. The shortest species at time of planting were placed in the southern-most row of each plot followed in order of increasing height to minimize shading. Each plot repeats this pattern twice and staggers one of those sets to allow a total of 81 seedlings per plot (162 seedlings per site). Seedlings were planted in the last week of June 2014, mulched 5cm deep with untreated chipped wood, and watered to field capacity. Seedlings were watered bi-weekly for the rest of the first growing season to isolate temperature effects and to

reduce transplant shock, and plots were weeded bi-annually. The same watering regimen was repeated in 2015, and then it was reduced to approximately once per month in the summer of 2016. Mulch was reapplied once in 2015 after snowmelt to reduce weeds. Beginning in June 2015, data-logging meteorological stations were established at each site to measure air temperature, rainfall, and soil temperature and moisture. The stations had 4 soil volumetric water content and temperature probes per site placed 5cm deep in the soil (Decagon 5TM Soil Moisture and Temperature Sensor), as well as one tipping bucket and air temperature probe per site (Onset HOBO RG3-M).

Photosynthetic Performance - Photosynthetic responses to temperature was assessed from July to early September 2016. From sunrise to midday, I manipulated temperature and assessed photosynthetic rate using a LI-COR 6400 Portable Photosynthesis System on a minimum of 10 individuals per species per site. Under saturating light conditions ($1000 \mu\text{mol m}^{-2} \text{sec}^{-1}$, LI-COR 6400-02B LED Light Source) and a relative humidity of 40-60%, leaf temperature was increased in 5°C increments from $15\text{-}30^{\circ}\text{C}$ and photosynthesis was allowed to stabilize before repeated measurements were taken for 2 minutes and then averaged. The most recent fully expanded leaves were used – 2016 leaves from aspen and a cluster (typically 6) of 2015 needles from the conifers. Leaves were marked between sampling temperatures to ensure the same sets of leaves were used in each estimate of photosynthesis (Ps) and removed from the plant at the end of analysis. The aspen leaves filled the leaf chamber while the projected leaf area of the excised conifer needles was measured using ImageJ software to determine leaf area–corrected Ps estimates. A site-level mean was calculated for each species from every measurement of a species taken at a site, providing a typical photosynthetic response across reasonable daytime temperatures. Additionally, differences in photosynthetic output at each temperature were

evaluated within species across the sites. As well as absolute photosynthetic responses, I report a percent of maximum observed photosynthesis where all values are scaled to the highest value for that species for that site (i.e., if the maximum average value for lodgepole at the warmest site was $10 \mu\text{mol m}^{-2} \text{sec}^{-1}$ at a leaf temperature of 20°C and $4 \mu\text{mol m}^{-2} \text{sec}^{-1}$ at all other leaf temperatures, I would report 100% at 20°C and 40% at all other temperatures).

Spring Bud Break - Beginning in early April 2016, seedlings were assessed at least twice a week for signs of bud break. I recorded the first date where new leaf tissue was observed from an opening terminal or axillary bud to assess initiation of annual growth (Shepherd *et al.* 1983 – development stage 4), and then estimated growing season days for each species at each site. The start of the growing season was calculated from the date when 50% of the seedlings at a site had broken dormancy while the end of the growing season was arbitrarily set as September 30th. I focused on spring bud break as it displays a dynamic response to climate and is a readily observable phenological response across both deciduous and evergreen species. In contrast, end of season leaf senescence is strongly correlated with photoperiod rather than climate (Lee *et al.* 2003), though the mechanisms driving dormancy remain unresolved (Richardson *et al.* 2013).

Leaf Size - Leaf size was estimated via measuring length and width of 10 randomly selected mid-canopy leaves in full sunlight per seedling using microcalipers. The most recent cohort of fully expanded leaves was used – 2016 for aspen, 2015 for conifers. Leaf size per seedling was determined by multiplying length by width for each of 10 leaves/needles then averaged. I did not measure specific leaf area as only non-destructive sampling was appropriate given the sensitivity of seedlings to any defoliation.

Statistical Analyses - Absolute photosynthetic rates and leaf size were both analyzed using one-way ANOVAs using site as the grouping variable. To test for influence of Julian date or air

temperature on estimates of photosynthetic rates, I used a multiple linear regression using site, species, air temperature, and Julian date as predictors. These analyses were performed using R software (R Core Team, 2016), the package ‘dplyr’ (Wickham *et al.* 2017). Differences between sites within species were further investigated using Tukey’s HSD comparisons of means with significance values set at $p < 0.05$.

2.3 RESULTS

Photosynthetic performance across sites - Comparing across sites, I observed that average Ps rates differed for aspen and the conifers. The coniferous species showed low variation in mean Ps across the range of elevations (Fig. 2.1) (lodgepole: $F = 0.733$, p -value < 0.482 ; ponderosa: $F = 0.26$, p -value < 0.772), though lodgepole pine displayed a higher Ps rate than ponderosa pine in each garden. In contrast, Ps in aspen displayed a clear maximum ($15.7 \mu\text{mol m}^{-2} \text{sec}^{-1}$) at the intermediate-temperature site– which is closest to its source location ($F = 61.35$, $p < 0.001$). Non-manipulated ambient factors (soil moisture and time of day) were not significant predictors of photosynthetic rate, while air temperature outside the chamber and calendar date were significant ($p < 0.05$) but not predictive ($R^2 = 0.02$ and 0.009 respectively).

Photosynthetic performance within sites - Within-site Ps of aspen displayed marked sensitivity to temperature variation only at the coldest site, with the Ps rates being significantly higher at 15 and 20°C than 25 or 30 °C ($p < 0.001$) (Figure 2). Similarly, lodgepole pine only displayed within-site sensitivity to temperature variation at the coldest site, with a significant decrease in Ps rate at 30 °C ($p < 0.01$). At the intermediate and warmest sites, none of the species showed significant responses to variation in temperatures. However, there was a non-significant trend for aspen of declining Ps with increasing temperatures at the warmest site. Ponderosa pine did not exhibit significant photosynthetic sensitivity to temperature at any of the sites, but showed a

trend of declining Ps with increasing temperatures at the coldest site and a decline in Ps at 30°C at the warmest site.

Spring Bud Break - The number of growing days increased with mean growing season temperature for all species (Fig. 2.3). The estimated growing season length was identical for all species at the coldest site. The conifers displayed a strong response in growing season length to the 3°C of warming between the coldest and intermediate sites (40.5 days increase on average), yet experienced a smaller increase with an additional 3°C moving between the intermediate and warmest sites (6 additional days for both species). Aspen's phenology increased by 22 days between the coldest and intermediate sites, and 14 days between the intermediate and warmest sites, yet its growing period was shorter than both conifers at the intermediate and warmest sites. These results indicate divergent responses to the effects of warming temperatures on spring bud break per species.

Leaf Size - Needle size varied non-significantly for the coniferous species across the sites, except at the warmest site where ponderosa pine needles were significantly larger (Fig. 2.4) (lodgepole: $F = 2.084, p < 0.135$; ponderosa: $F = 11.81, p < 0.001$). In contrast, aspen leaf size was significantly different at each site ($F = 25.74, p < 0.001$), with the largest size at the intermediate-temperature site and the smallest size at the warm site indicating a clear temperature sensitivity in foliar morphology.

2.4 DISCUSSION

Facing a rapidly changing environment and shifts in climatic niches in the western US (Lenoir *et al.* 2009; Bell *et al.* 2013; Redmond *et al.* 2015), tree species must migrate with climate, tolerate new conditions, and/or acclimate to novel environments to survive (Aitken *et al.*

2008). Increasingly, large-scale models predicting species distributions are incorporating within-species variability due to trait plasticity and local adaptation (Buechling *et al.* 2017, Valladares *et al.* 2014), and finding that disentangling those two drivers of trait fluidity are both critical for accurate predictions and require controlled replicated *in situ* experiments (Moran *et al.* 2016). By minimizing differences in local adaptation in my experimental gardens I provide evidence for trait plasticity and the underlying temperature sensitivities of some of the key drivers of seedling growth. I observed three key findings that suggest, as expected, species will not respond uniformly to climate change (Hamann and Wang 2006). First, aspen displayed a strong degree of photosynthetic plasticity among sites and in leaf size, with both traits achieving their highest values at the intermediate elevation site closest to their seed source location. In contrast, there were no strong Ps patterns for lodgepole pine or ponderosa pine across the sites, though ponderosa pine did increase leaf size at the warmest site. Second, I observed a nearly uniform within-site Ps temperature response for all species between 15 and 30°C suggesting that fluctuations corresponding to within-day variation in leaf temperature will have minimal effects on plant performance; rather, my data suggest that it is long-term site level temperatures that will impact seedling photosynthetic rates and presumably whole plant performance. Last, the two conifer species demonstrated greater phenological plasticity to temperature than aspen. A warming of 3°C from the coldest to the intermediate-temperature site increased the growing season for the conifers by nearly three weeks more than for aspen, while the next 3°C of warming resulted in limited increases for all species.

I originally hypothesized that I would see clear temperature optima that were plastic and responsive to shifts in the growing environment. I found no evidence to suggest this was the case for the species investigated here as I was unable to detect site-specific differences in

instantaneous Ps over a range of 15-30°C (Fig. 2.2). Interestingly I did observe differences between sites for aspen but not for lodgepole pine or ponderosa pine (Fig. 2.1), suggesting plasticity is impacting the photosynthetic process at the site level but not to instantaneous or short-term changes in leaf temperature. Turnbull *et al.* (2002) found a similar pattern in that warming during the day had minimal effects on photosynthetic or respiration rates in *Populus deltoides*, yet warming during both day and night resulted in plastic responses to temperature due primarily to upregulation of the photosynthetic pathway (VC_{max} and J_{max}). Additionally, the weak site-level acclimation of the Ps response I observed in aspen was also found in previous studies of temperature sensitivity and acclimation of Ps in the genus. These studies reported minimal evidence of photosynthetic acclimation – particularly to cooler temperatures – in *P. deltoides* in growth chambers (Ow *et al.* 2008), or in *P. tremuloides* in common gardens (Dillaway and Kruger 2010). For acclimation in conifers, Teskey and Will (1999) reported that Ps shifted directionally with temperature in loblolly pine (*Pinus taeda*) grown in growth chambers, with peak Ps occurring at the temperature at which they were established, indicating strong acclimation potential. Likewise, mature Norway spruce (*Picea abies*) exposed to warmer temperatures in whole tree chambers at the Flakaliden research site in Sweden experienced relatively flat temperature photosynthesis response curves (Hall *et al.* 2013).

As expected, growing days increased with increasingly warmer temperatures. My results support my hypothesis, although there were some important differences in the effects on warming between the species. A warming of 3°C from the coldest to the intermediate-temperature site started the conifers' growing season nearly three weeks earlier than for aspen – perhaps because an evergreen life form allows for a more rapid end to dormancy as conditions change, at least compared to a deciduous species – while the 3°C of additional warming resulted

in limited increases for all species. This trend suggests marginal increases in temperatures would benefit the montane conifers, rejecting my hypothesis that aspen would benefit the most from increasing temperatures. These findings are similar to the 3°C warming experiments at Flakaliden which found a two to three week increase in the growing season of Norway spruce due to earlier spring activity (Slaney *et al.* 2007), and this longer growing season was considered the main reason annual leaf carbon uptake increased 44% in the warmed conifers (Hall *et al.* 2013). This increased carbon uptake was significant over the growing season despite changes in other costs at higher temperatures including increased respiratory demand and non-photochemical quenching. Bud break also has been shown to be strongly regulated by temperature, rather than genotypic differences, in lodgepole pine (Liepe *et al.* 2016) and *Populus tremula* (Luquez *et al.* 2008), suggesting that the differential responses of these species are a question of plasticity rather than local adaptation.

While leaf size generally increased with warming as I hypothesized, the response was species-specific with the highest level of leaf plasticity in aspen. Aspen leaf width and length have been shown to be a highly heritable trait between populations (Kagana *et al.* 2008). As my design controlled for local adaptation by using seedlings from a single stand, my results suggest that not only is this trait heritable, but it is highly plastic within a population as well. This is in contrast to another study in Colorado, however, where aspen displayed minimal plasticity across an elevation/aridity gradient while ponderosa pine decreased in leaf size with decreasing elevation (Anderegg and HilleRisLambers 2016). These contradictory results may arise from different study designs, where co-variation in temperature and aridity along gradients in Anderegg and HilleRisLambers (2016) may exert a counteracting influence on leaf traits. That said, Olszyk *et al.* (2005) found no changes in leaf area or leaf perimeter in Douglas fir

(*Pseudotsuga menziesii*) when they isolated the impact of temperature on leaf size in outdoor growth chambers for 4 years. These results mirror my findings for lodgepole pine, reinforcing the conclusion that species responses to environmental change likely will be species-specific and idiosyncratic even in controlled studies.

Taken together, my results suggest warming in the next century will benefit ponderosa pine and lodgepole pine based on the temperature sensitivities and plasticity of the traits investigated herein. Minimal reductions in photosynthetic rates with warming coupled with a higher degree of phenological plasticity suggest that these species will fare better under a warmer climate than aspen. Recent trends in aspen distribution and stand dominance in Colorado support a hypothesis of aspen decline, as numerous studies have found substantial declines in aspen abundance across the state (Worrall *et al.* 2010; Worrall *et al.* 2013; Coop *et al.* 2014; Bretfield *et al.* 2016), with the decline most directly linked to increased aridity, droughts in particular.

Broader extrapolation of these results must be done with some caution, as the long-term implications of this study are unclear. For example, phenological plasticity does not necessarily equate to increases in tolerance or range shifts in all cases (Duputié *et al.* 2015), nor do mean temperatures explain all phenological shifts as other factors like photoperiod (Marchin *et al.* 2015) or chilling temperatures (Roberts *et al.* 2015) can contribute substantially. More fundamentally, this study isolates temperature, but other variables (e.g. moisture) will interact with warming temperatures to determine the net impact of environmental change on these species, and the inability of the gymnosperms to shift photosynthetic rates and needle size could be detrimental under some combinations of environmental change. In this context, plasticity in aspen leaf sizes could be interpreted as a drought avoidance mechanism, where smaller leaves improve water transport efficiencies in drier conditions. Finally, natural disturbances – such as

fire and insect outbreaks, which may be increasing in conjunction with climate change – have been shown to increase aspen abundance in Colorado (Buma and Wessman 2012; Kulakowski *et al.* 2013), while increases in fire intensity (Harris and Taylor 2015) are reducing regeneration, particularly at lower elevations, of ponderosa pine (Chambers *et al.* 2016), Douglas fir and lodgepole pine (Rother and Veblen 2016).

Conclusions and Future Considerations - My results suggest that of these species, aspen is likely to be the most sensitive to future climate change, as its highest Ps occurred unambiguously in the location and climate of its current seed source and declined markedly at higher temperatures. Despite a longer growing season at higher temperatures, I predict that the strong declines in leaf size and photosynthetic output for aspen at higher temperatures will lower or even restrict growth in this species. In contrast, I predict that conifer performance will benefit from earlier phenology in the warmest site (Figure 3), as these conifer species appear to be insensitive in leaf size and photosynthetic output across the range of temperatures. This suggests the possibility of divergent trajectories for these species under warming temperatures, favoring the coniferous species while limiting aspen with considerable implications on forest composition and species distributions in Colorado.

Overall, my common garden approach has allowed me to explore the temperature sensitivities of three of the dominant tree species in Colorado in a controlled yet real-world abiotic environment. I observed two contrasting response patterns which mirror the known life-history characteristics of these species. Aspen displayed strong temperature sensitivities in Ps and in leaf size to warming while the coniferous species benefited substantially via increased growing season length. Accurate predictions of forest distributions and composition will require

inputs of whole-plant growth as well as physiological and anatomical characteristics of the primary drivers of seedling performance.

2 TABLES

Table 2.1. Climate and soil data for the experimental sites. Annual and growing season values for the year 2016 were calculated directly from a weather station and soil probes at each site. Growing season values were calculated May-September. Long term means (calculated for the period 1986-2011) were from 800-m resolution PRISM climate data. Soil data are only available for the growing season.

| Site | High Elevation | Intermediate Elevation | Low Elevation |
|--|-------------------|---------------------------|------------------|
| Elevation (m) | 2750 | 2470 | 1567 |
| Long term mean annual air temperature (°C) | 3.7 | 6.6 | 9.6 |
| Long term mean annual precipitation (mm) | 447 | 567 | 409 |
| Mean annual air temperature for 2016 (°C) | 4.3 | 8.0 | 9.5 |
| Mean annual precipitation for 2016 (mm) | 484.8 | 452.6 | 426.8 |
| Growing season – mean daily air temperature (°C) | 10.1 | 13.3 | 16.7 |
| Growing season – precipitation (mm) | 183 | 219 | 171 |
| Growing season – mean soil moisture (%) | 16 | 14 | 13 |
| Growing season – mean soil temperature (°C) | 13.8 | 16.5 | 24.5 |

2 FIGURES

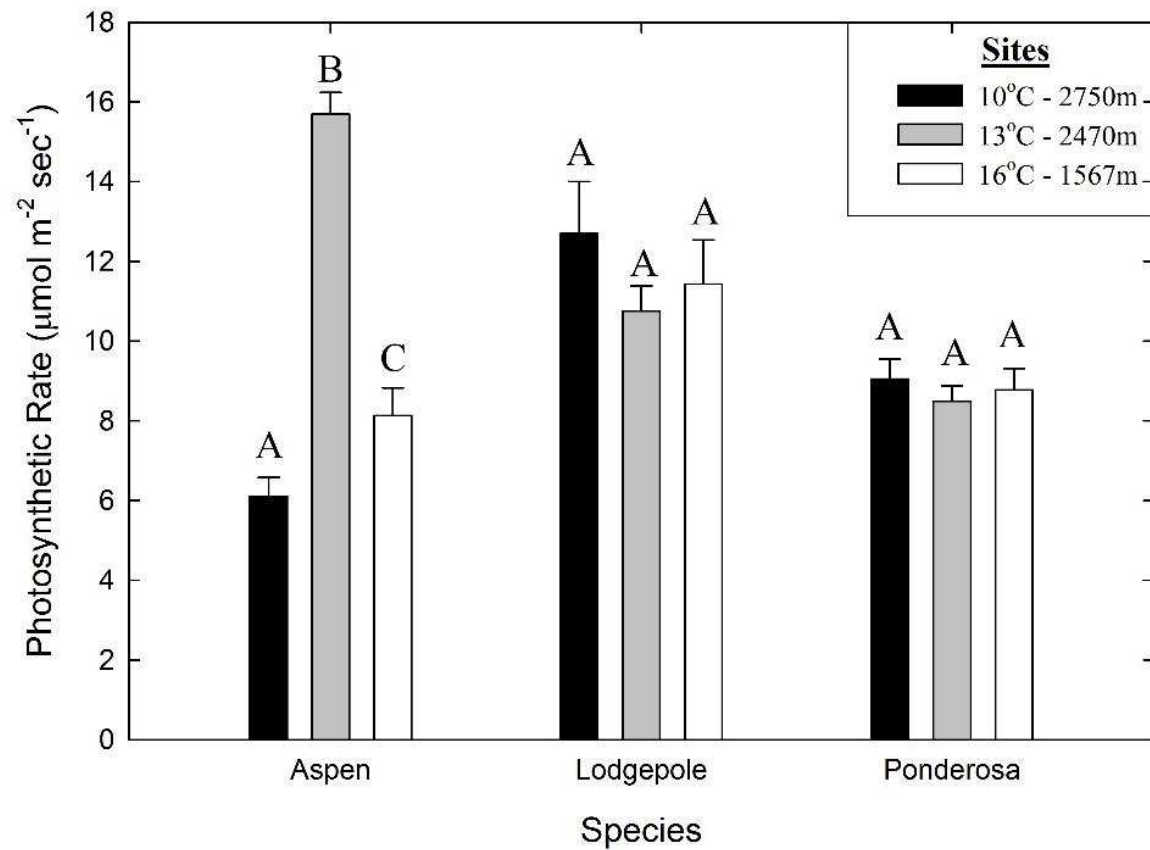


Figure 2.1. Mean photosynthetic rates for seedlings of three tree species growing experimentally at three montane sites spanning a range of 6°C. Photosynthetic rates were measured over a fixed range in experimental temperatures (15-30 °C) in each site. Site temperatures are the mean growing season temperature in 2016. Letters denote significant differences within a species based upon Tukey's HSD and bars indicate 1 standard error.

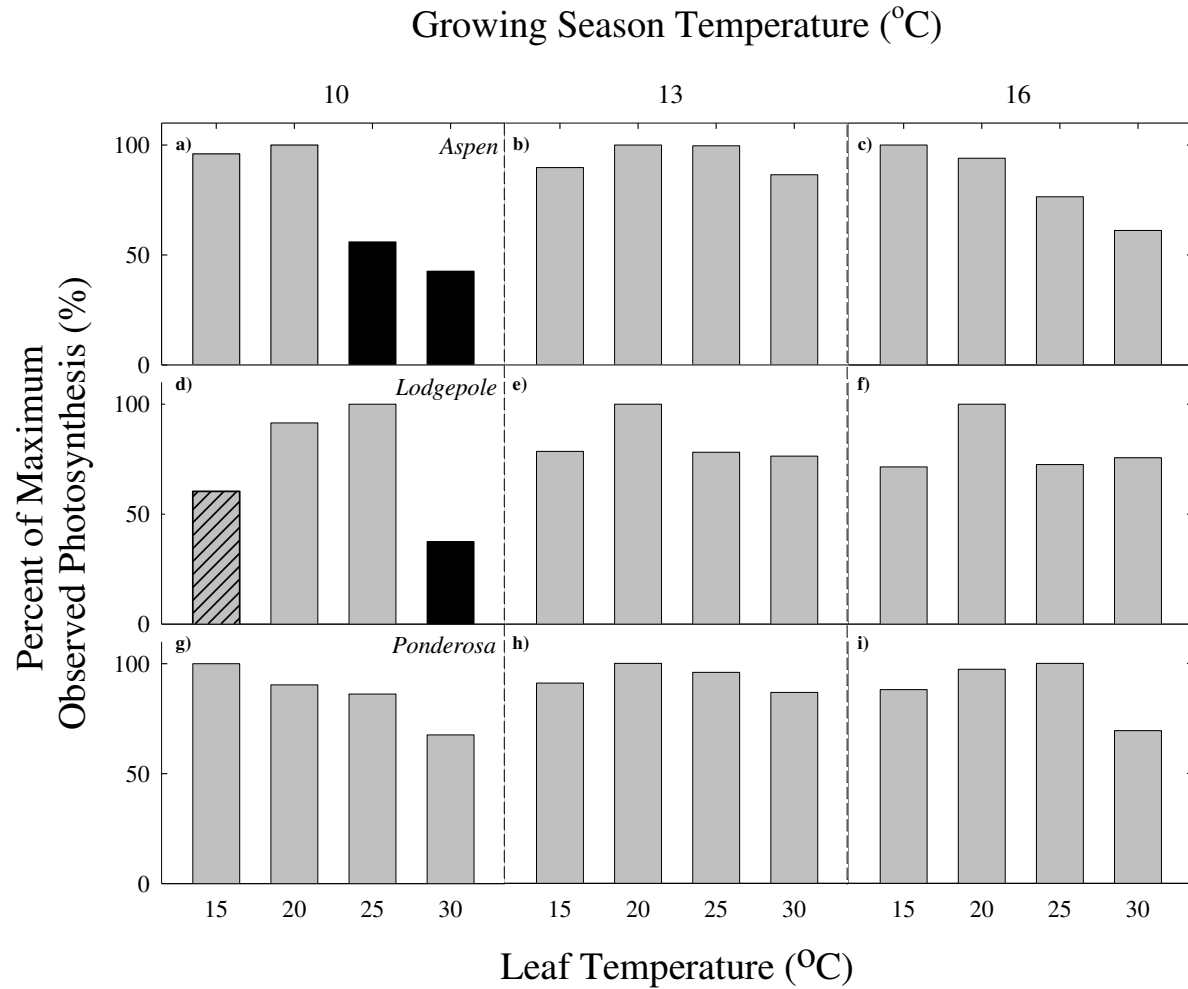


Figure 2.2. Mean percentage of maximum photosynthesis measured over a fixed range in experimental temperatures (15-30°C) at three sites. Different shading of bars denotes statistically significant differences (panels a and d) while cross-hatching (panel d) denotes an intermediate response. Mean absolute photosynthetic responses were calculated per temperature per species per site, and then scaled to be a percentage of the largest average per species per temperature per site combination. Columns denote site moving from coldest to warmest (left to right). Rows indicate species alphabetically.

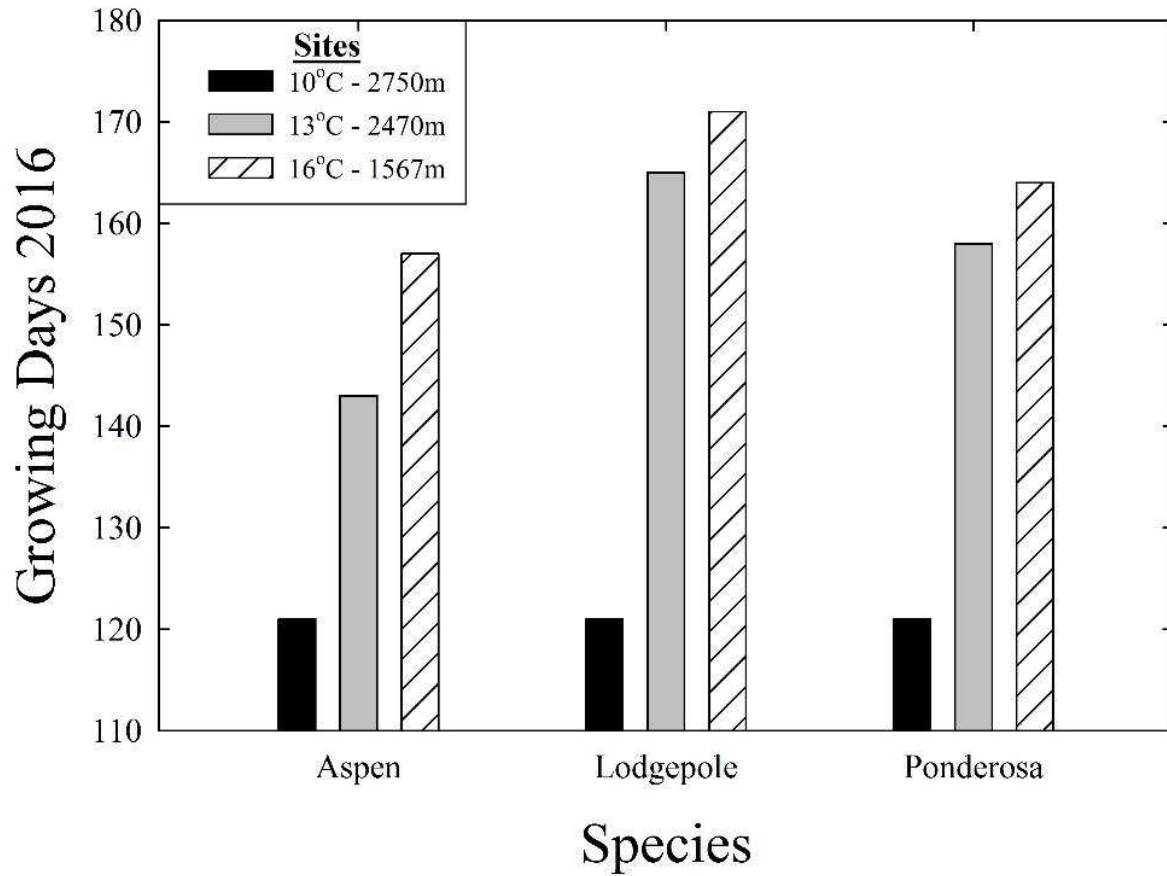


Figure 2.3. Growing days by species by site in 2016. Growing days were calculated as the number of days between the date of bud burst (when at least 50% of the individuals of a species at a garden had bud burst) and the last day of September. The estimate of growing days at the warmest site for lodgepole pine may be an under-estimate as nearly all lodgepole had already initiated bud burst on the first day of sampling.

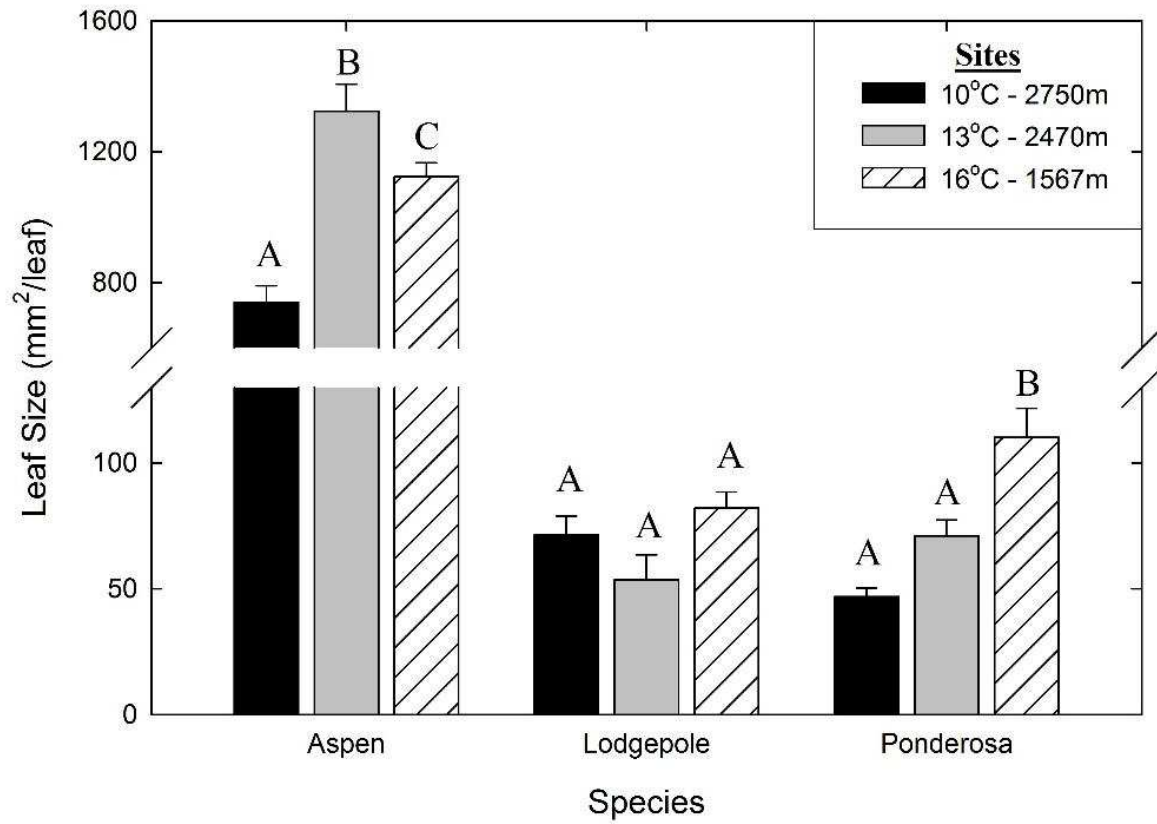


Figure 2.4. Mean leaf sizes of fully expanded leaf tissue. Letters denote significant differences within a species based upon Tukey's HSD and bars denote 1 standard error.

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CHAPTER 3: TREE GROWTH RESPONDS STRONGLY TO WARMING TEMPERATURES THROUGH TIME DESPITE STATIC SPATIAL PATTERNS

3.1 INTRODUCTION

Warming temperatures as a manifestation of climate change are expected to dramatically impact forests, yet we lack vital information on how temperature will drive and interact with future tree growth and demography. Despite the importance of accurately predicting the effects of warming on ecological processes, temperature effects on tree growth has received little study in realistic conditions independent of covarying and confounding environment conditions. Moreover, a contradiction has developed recently concerning how higher temperatures effect tree growth patterns, with different approaches providing opposing results. Likewise, we have limited knowledge of how acclimation to shifts in temperature may alter the thermal optima of species (Ghannoum and Way 2011), particularly when acclimation to warmer temperatures has led to both positive and negative effects on photosynthesis and overall plant performance in different situations and taxa (Way and Yamori 2014; Reich *et al.* 2015; de Castro *et al.* 2017). This suggests that we lack sufficient predictive power concerning tree species capacity to respond and adapt to a warming environment as even the direction of the response is context dependent.

Determining how temperature interacts with the underlying mechanisms of growth is critical for scaling up from individuals (Way 2013), and for informing future management and bioclimatic niche modeling as climate change continues to impact forests. Observational space-for-time approaches have become increasingly popular for predicting future species performance under climate change. Recently, such studies have found that dominant, widespread tree species have largely constant growth rates over a wide range in ambient temperatures when other

variables are held constant (Canham and Thomas 2010; Buechling *et al.* 2017; Martin *et al. in review*; Coomes *et al.* 2014; Canham and Murphy 2016). (Fig. 3.1a). In contrast however, controlled warming experiments typically show a strong effect of warming temperatures on tree growth rates (Melillo *et al.* 2011; Way *et al.* 2013), and the distribution of trees along elevation gradients is highly responsive to temperature (Fig. 3.1b). Why do we see strong effects of warming in field and laboratory experiments and not in natural observational settings across large gradients?

I expect that an important cause of this contradiction is that analyses along climate gradients are typically so broad – needed to capture a wide range in temperature – that the effects of local adaptation and non-climatic conditions like soils influence the results. Tree species with large ranges typically show strong evidence of local adaptation to different climate conditions in various portions of their range (Rehfeldt *et al.* 2001; Saxe 2001; Way and Oren 2008), muddying the predictions of tree demography under novel future climate and highlighting the necessity of determining the role of temperature in multi-year, controlled conditions to isolate its role independent of the extraneous factors which confound latitudinal studies. Conversely, warming experiments often lack spatial and temporal replication and are rarely conducted under *in situ* conditions.

In this study, I directly investigated tree growth and survivorship under a markedly warmer climate by comparing seedling performance across experimental gardens. I have minimized many of the extraneous factors that limit the interpretation of seedling performance under warmer temperatures: each garden is separated by 3°C mean annual temperature (MAT) – a reasonable estimate of warming in Colorado by 2100 (Gross *et al.* 2016) – has comparable precipitation levels, and consists of seedlings from a single population growing on identical soils

(Carroll *et al.* 2017; Fig. 3.1c,d). Comparison of one site and the next warmer site provides a 4-year snapshot of expected seedling performance by 2100. I elected to investigate tree seedlings that have overlapping ranges, are highly habitat-dependent, and are rapidly undergoing adult and seedling niche divergence, i.e. where the current climatic conditions are suitable for adult tree persistence but are becoming inhospitable for seedling germination and establishment (Bell 2013).

3.2 METHODS

Study Site - Three experimental gardens were established in 2014 along a 1,200 m elevation gradient in the Front Range of Colorado. The three locations are each separated by approximately 3°C MAT and have similar precipitation patterns (Carroll *et al.* 2017). At each site, I selected a flat area with full-sun exposure and removed preexisting vegetation. To provide an identical soil in each garden, I removed the top 30 cm of soil from two 18.5 m² plots in each site, collected soil from the intermediate-temperature site, transported it to the warmest and coldest sites, filled the excavated plots, and tilled the transported soil. Soil in two 18.5 m² plots at the intermediate-temperature site was excavated in the same manner, returned to the plots, and tilled. We added a 2m deer fence around each garden in 2015. We planted one-year old seedlings of quaking aspen (*Populus tremuloides*), lodgepole pine (*Pinus contorta* var. *latifolia*), ponderosa pine (*Pinus ponderosa* subsp. *scopulorum*) and subalpine fir (*Abies lasiocarpa*) germinated by the Colorado Forest Service Nursery in Fort Collins Colorado from seeds collected from a single stand from National Forests nearby in Colorado to minimize differences in local adaptation. Monthly assessments of height (root collar to tip of the terminal bud) as well as diameter at 5cm and 10cm above the ground were collected at planting and end of the first growing season (2014). Starting in 2015 and continuing through 2017, height and diameter were

recorded from April through September at each garden at approximately monthly intervals. Stems were marked with a dot of paint to ensure that diameter measurements were conducted at the same point on repeated visits.

Data Analysis - While, herbivory was minimal throughout the project, there was some mortality and damage done during the experiment. As herbivory would impact estimates of growth I excluded any data collected on individuals following herbivory. Additionally, any tree that died subsequent to herbivory was removed for calculations of survivorship rates between gardens. The percentages of surviving trees are therefore based solely on trees that were either alive at the end of 2017 or died from causes independent of herbivory. Basal area was estimated from the measurements of diameter at 5cm and thus care should be taken when comparing these results to other studies. I estimated relative growth rate based on basal area and the number of months between sampling efforts (Equation 3.1). Relative growth rate of height was calculated in the same manner substituting height for basal area in Equation 3.1. Differences amongst the experimental gardens were compared using Tukey HSD pairwise differences ($\alpha=0.05$).

Equation 3.1.
$$RGR = \frac{\ln(BA_{t+1}) - \ln(BA_{t_0})}{t_{+1} - t_0}$$

Linear mixed effects models were used to test for differences in percent change in basal area using site and time as fixed effects and individual as a random effect to account for repeated measures of individuals over time. Each species was analyzed separately. In addition, height to basal area ratios were analyzed using linear mixed effect models with the same design as above for data gathered in the growing season of 2016 and 2017. Height to basal area trends were assumed to have stabilized during the first real growing season (2015), with subsequent measurements providing estimates based on environmental cues rather than transplant shock.

Survivorship was investigated for each species at locations where mortality was greater than 0 but less than 100% to determine effects of ambient growing temperature and three variables that characterize healthy trees: relative growth rate, percent change in basal area, and height to basal area ratio. Traits collected from September 2015 (the first full year after planting) were used to predict survivorship at the end of 2017 (the last month of sampling to date). A simulated annealing function was used to estimate global optimization of parameters fitting logistic regression curves in a likelihood framework. Candidate models were compared using AICc (a sample size correction for Akaike's Information Criteria) and assessed for model fit using McFadden's Pseudo R^2 . Additionally, projecting growth and survivorship in these gardens forward using an Integral Projection Model (IPMs) was attempted (see Rees *et al.* 2014) relying on post-establishment data (2015-2017). IPMs are designed to independently assess two kernels of information concerning a population: growth and fecundity both of which are impacted by survivorship. As the experimental gardens are comprised of seedlings, fecundity data was unavailable resulting in modeling the growth kernel without estimates of future cohorts. All statistics were calculated using R (3.3.3), as well as the 'likelihood' (Murphy 2015), 'lme4' (Bates *et al.* 2015), 'lsmeans' (Lenth 2016), and 'dplyr' (Wickham *et al.* 2017) packages.

3.3 RESULTS

Based on ~19,000 measurements combined across 486 individuals, I observed a strong and consistent increase in growth rate with increasing MAT in the three montane species – aspen, lodgepole pine, and ponderosa pine – even at a mean temperature higher than any found in their current distribution, signifying that future warming alone will not restrict growth in these species (Fig. 3.2). Interspecific differences in growth rates at a given temperature were significant ($\chi^2 = 28.232$, $p < 3.247 \times 10^{-6}$), but small compared to between-site temperature effects

on growth ($\chi^2 = 203.283$, $p\text{-value} < 2.2\text{e-}16$). The lone subalpine species, subalpine fir, grew at a similar rate in the gardens between its local native elevation and the next warmer garden but did not experience any sustained increases in growth at the warmest site. For each species, competing linear mixed effects models were compared testing for differences by site (Table A1-1). For aspen, lodgepole, and ponderosa pines ΔAIC of the model without site were significantly poorer ($\Delta\text{AIC} > 60$) in each case, therefore we used outputs from the best model for Tukey pairwise comparisons of differences among sites. Aspen and lodgepole pine were significantly larger at the warmest site, while the other two sites did not differ (Table A1-2). Ponderosa pine was significantly different at each site, increasing in basal area with increasing MAT. Subalpine fir was not significantly different at any site, nor were there differences between models.

In conjunction with steady increases in growth of montane species with increasing temperature, I also observed a steady decline in the height to basal area ratio, a metric of growth prioritization. For all species, models including site were preferentially selected by AICc (Table A1-3) and all site by species combinations were significantly different from one another based on Tukey pairwise comparisons ($\alpha=0.05$) with the exceptions of aspen between the coldest and intermediate-temperature site and subalpine fir between the intermediate and warmest site (Table A1-4). Allocation patterns favoring basal area over height increased with higher MAT for all four species (Fig. 3.3). The shifts in allocation are predominantly due to relatively larger increases in diameter growth at higher temperatures rather than decreases in height growth (Fig. 3.4). For both height and basal area, individuals at the warmest garden were always growing at a significantly faster rate than at any other garden.

Survivorship differed markedly across the gardens and species (Table 3.1). Lodgepole was the sole species where it was possible to assess survivorship across all three gardens.

Ponderosa pine and subalpine fir only experienced mortality rates between 0 and 100% after 2015 at the intermediate-temperature site and thus were assessed there exclusively, while aspen survivorship was investigated at the coldest and intermediate-temperature sites. Subalpine fir experienced complete mortality at the warmest site ($+6^{\circ}\text{C}$) by year three (2016) and 47.5% survivorship at the intermediate site, indicating that while mild warming ($+3^{\circ}\text{C}$ MAT) may not prove detrimental to this species, significant future warming ($+6^{\circ}\text{C}$) may prove fatal at least for seedlings. For the sites where an analysis was possible, I surprisingly found no evidence that relative growth rate post-establishment was predictive of survivorship after four years for any species (Table A1-5). Additionally, none of the variables investigated were predictive of ponderosa pine mortality. Allocation of resources (height to basal area) in 2015 led to a detectable signal in seedling mortality at the end of 2017 for aspen, lodgepole, and subalpine fir in each case leading to decreased survivorship with prioritization in height over basal area; however the predictive power of these models were weak in two cases (McFadden Pseudo R^2 : lodgepole 0.14 and subalpine fir 0.18) and only somewhat predictive in aspen (McFadden Pseudo $R^2 = 0.33$). Lastly, percent change in basal area was weakly predictive of survivorship in lodgepole and subalpine fir (McFadden Pseudo $R^2 = 0.09$ and 0.13) with increased growth yielding a higher survivorship. Additionally, for aspen and lodgepole pine models fitting separate slopes per site were selected based on AICc indicating that temperature was interacting with the predictor variables. Overall, I concluded that these growth and allocation traits were not effective at predicting survivorship, suggesting that with the exception of subalpine fir, seedling survivorship was driven primarily by stochastic or unknown factors rather than growth and temperature during this four-year period.

Predictions of population growth across the different gardens were attempted for each species, however a lack of correlation between growth rates and seedling size proved to be insufficient for useful extrapolation. Additionally, due to the relative inability to accurately predict survivorship for each species at each garden, IPMs proved to be ineffective. Mean growth rate (increase in basal area – cm²) per year were determined from 2015-2017 (Table 3.2), however more complex model predictions were not feasible at this time due to a non-existent relationship between past size and future growth. If growth patterns stabilize in the future this will be re-investigated.

3.4 DISCUSSION

Warming unambiguously increased growth across the three montane species (aspen, lodgepole pine, and ponderosa pine) from their local seed-source elevation. Moderate warming did not negatively impact subalpine fir growth (warming from the coldest to the intermediate-temperature site), however severe warming of 6°C led to 100% mortality of the species at the warmest garden. This finding supports previous work on this species which found a strong sensitivity to warming, perhaps endangering its persistence in the southern and central Rocky Mountain regions (Kulakowski *et al.* 2012; Hansen and Philips 2015). While overall shifts in growth rates differed for the montane and subalpine species, they shared an increased allocation of resources to basal area over height as they aged as well as increased basal area prioritization with increasing MAT. Higher growth rates in basal area are potentially associated with increased xylem production and water transport capabilities. Warming increases evaporative demand and plant water-stress even at consistent levels of soil moisture, and thus my results suggest that current warming projections will be compensated for with increased basal area in seedlings without deleterious effects on overall growth in these montane species assuming comparable

precipitation levels to current conditions. This allocation prioritization is supported by similar work on Douglas Fir (*Pseudotsuga menziesii*) seedlings, a common co-occurring species in this region, in growth chambers (Olszyk *et al.* 1998), as well as a potted aspen study that saw a faster increase in diameter than height under simulated warming (Way *et al.* 2013).

Two widely investigated traits that are associated with growth are photosynthetic rate and spring phenology – specifically bud-break as it largely controls shifts in growing season length. They are relatively straight forward to measure, can serve in many contexts as accurate measures of seedling performance and the effect of the ambient environment on growing conditions, and thus have been widely used to estimate future growth. A previous assessment of photosynthetic and phenological responses to warming in these gardens found a peak in average photosynthesis for aspen at the intermediate-temperature site with declines at the warm and cold extremes (Carroll *et al.* 2017). Lodgepole pine and ponderosa pine seedlings in contrast, displayed flat photosynthetic responses among the gardens, indicating erratic effects of ambient temperature on photosynthesis for these montane species. However, my current results do not show this same pattern (peaking for aspen seedlings at the intermediate-temperature site and flat across all site for the pines) in basal area production (Fig. 3.2). This discrepancy suggests that differences in average photosynthetic rate alone do not predict whole plant, aboveground seedling growth. This is surprising due to the intuitive relationship between photosynthesis and growth and the previously reported abilities of some temperate and boreal trees to acclimate photosynthetically to novel conditions (Gunderson *et al.* 2010; Sendall *et al.* 2015). Nonetheless, it appears that forecasting efforts should focus instead on other factors such as site-specific ambient growing temperatures to predict seedling performance.

Growth for the montane species correlates well with increasing growing season length – a common and easily calculated input for ‘Forest Gap’ models (Bugmann 2001). There were substantial differences in growth season length among the gardens (Carroll *et al.* 2017) and while I found a strong correlation between ambient site temperature and basal area, the previously reported differences in growing season length and increases in basal area in ponderosa pine and aspen are not adequately explained if growing degree days are the primary driver. I observed a stronger response to warming in ponderosa pine than aspen (approximately 3 additional weeks of growth for 3°C of warming), yet comparisons of percent change in basal across the three gardens revealed nearly identical slopes (Fig. 3.2), suggesting that differences in phenological response are not solely driving growth. These two underlying components of growth – growing season length and photosynthetic rates – alone do not accurately predict the linear growth responses found in this analysis, suggesting that temperature must be explicitly accounted for in future growth models as using these traits has been shown to be ineffective at predicting temperature responses in isolation.

Growth and its allocation however, are not the sole variables that regulate forest demography. Survivorship of forest seedlings under higher temperatures also has important implications for future forest dynamics. Survivorship is an important control that has often been attributed to two pathways: stress and stochasticity. Stress driven mortality estimates are an important sub-model in vegetation models (Pacala *et al.* 1996; Bugmann 2001) and thus we expected to see evidence of increased mortality in slow-growing individuals in my gardens. However, survivorship was insensitive to relative growth rate for any of the four species. Height to basal area and percent change in basal area from the year following establishment until the most recent sampling period proved to be largely ineffective at predicting mortality with the

exception of aspen (Table A1-5). In this case survivorship in the fastest growing species was inversely related to height allocation suggesting that prioritization to basal area is advantageous as was observed by the increased allocation to basal area over height for each species over time. Inconsistent growth rates prevented meaningful forecasting of population dynamics at this time.

Through the use of experimental gardens, I demonstrated that warming temperatures unequivocally increased seedling growth for montane tree species, with the subalpine species tolerating mild warming. Recent work has suggested that respiration may prove to be a more suitable trait to determine the interaction between warming and growth (Anderson-Teixeira *et al.* 2011; Reich *et al.* 2016) and my work supports investigating other processes as my previously reported photosynthetic rates and growth rates did not match. In conjunction with this explicit increase in growth with warming in the montane species I observed a clear allocation preference to basal area at the expense of vertical growth, a process that prioritizes xylem and water transport capabilities over light capture. Combined with largely insensitive relationships between growth and survivorship, I speculate that the future forests of Colorado will be driven by punctuated periods of establishment. My findings suggest that warming does not negatively impact growth for the montane species and that allocating resources to basal area appears to be a successful strategy with warming. The lack of predictive power of growth on survivorship suggests that seedlings will be generally able to tolerate a warmer future as survivorship appeared to be largely stochastic. Indeed, this suggests that germination could be the main limitation of future cohort establishment and that periods of favorable conditions (e.g. soil moisture) will largely control this relationship. This has already been observed in subalpine species in Colorado (Andrus *et al.* 2018) and my findings suggest that this may be the case for montane species as well.

Future projections of forest composition and range shifts must utilize thermally explicit datasets to estimate accurate temperature responses in growth as I have demonstrated a high degree of plasticity to ambient temperature within individuals sourced from a single population. In summary, I observed a strong forcing of warming temperatures on montane tree performance without an accompanying increase in mortality suggesting moderate warming will benefit montane species while subalpine species will be under extreme risk of extirpation. Only through the increased use of experimental gardens will we be able to provide the data necessary for broad-scale predictions of forest dynamics while minimizing the effects of confounding factors that plague other efforts.

3 TABLES

Table 3.1. Cumulative survivorship (%) at the end of each growing season from 2014-2017 for quaking aspen, lodgepole pine, ponderosa pine, and subalpine fir across the three experimental gardens.

| <i>Warmest Site</i> | | <i>End of Season Survivorship</i> | | | |
|----------------------|-------------|-----------------------------------|-------------|-------------|--|
| <i>Species</i> | <i>2014</i> | <i>2015</i> | <i>2016</i> | <i>2017</i> | |
| <i>Aspen</i> | 97.6 | 83.3 | 83.3 | 83.3 | |
| <i>Lodgepole</i> | 97.6 | 90.5 | 88.1 | 71.4 | |
| <i>Ponderosa</i> | 97.2 | 94.4 | 94.4 | 94.4 | |
| <i>Subalpine fir</i> | 94.7 | 68.4 | 31.6 | 0 | |

| <i>Intermediate Site</i> | | <i>End of Season Survivorship</i> | | | |
|--------------------------|-------------|-----------------------------------|-------------|-------------|--|
| <i>Species</i> | <i>2014</i> | <i>2015</i> | <i>2016</i> | <i>2017</i> | |
| <i>Aspen</i> | 100 | 97.3 | 91.9 | 89.2 | |
| <i>Lodgepole</i> | 97.5 | 47.5 | 30 | 30 | |
| <i>Ponderosa</i> | 100 | 96.3 | 85.2 | 85.2 | |
| <i>Subalpine fir</i> | 100 | 61.9 | 52.4 | 47.6 | |

| <i>Coldest Site</i> | | <i>End of Season Survivorship</i> | | | |
|----------------------|-------------|-----------------------------------|-------------|-------------|--|
| <i>Species</i> | <i>2014</i> | <i>2015</i> | <i>2016</i> | <i>2017</i> | |
| <i>Aspen</i> | 89.5 | 86.8 | 81.6 | 78.9 | |
| <i>Lodgepole</i> | 95.2 | 52.4 | 50 | 47.6 | |
| <i>Ponderosa</i> | 100 | 91.4 | 91.4 | 91.4 | |
| <i>Subalpine fir</i> | 100 | 85.7 | 85.7 | 85.7 | |

Table 3.2. Mean and standard error of growth rate of basal area (cm²) per year from 2015-2017 for quaking aspen, lodgepole pine, ponderosa pine, and subalpine fir. Rates are provided at each experimental garden along with mean growing season temperature.

| <i>Aspen</i> | <i>Mean</i> | <i>Se</i> |
|---------------------------|-------------|-----------|
| Warmest Site -16 °C | 19.54 | 1.27 |
| Intermediate Site – 13 °C | 9.81 | 1.81 |
| Coldest Site – 10 °C | 5.6 | 1.38 |

| <i>Lodgepole pine</i> | | |
|---------------------------|------|------|
| Warmest Site -16 °C | 12.8 | 0.59 |
| Intermediate Site – 13 °C | 2.4 | 2.60 |
| Coldest Site – 10 °C | 4.49 | 0.92 |

| <i>Ponderosa pine</i> | | |
|---------------------------|------|------|
| Warmest Site -16 °C | 8.96 | 0.36 |
| Intermediate Site – 13 °C | 5.10 | 0.30 |
| Coldest Site – 10 °C | 3.34 | 0.41 |

| <i>Subalpine fir</i> | | |
|---------------------------|------|------|
| Warmest Site -16 °C | - | - |
| Intermediate Site – 13 °C | 2.95 | 0.44 |
| Coldest Site – 10 °C | 3.90 | 0.36 |

3 FIGURES

Is growth sensitive to temperature?

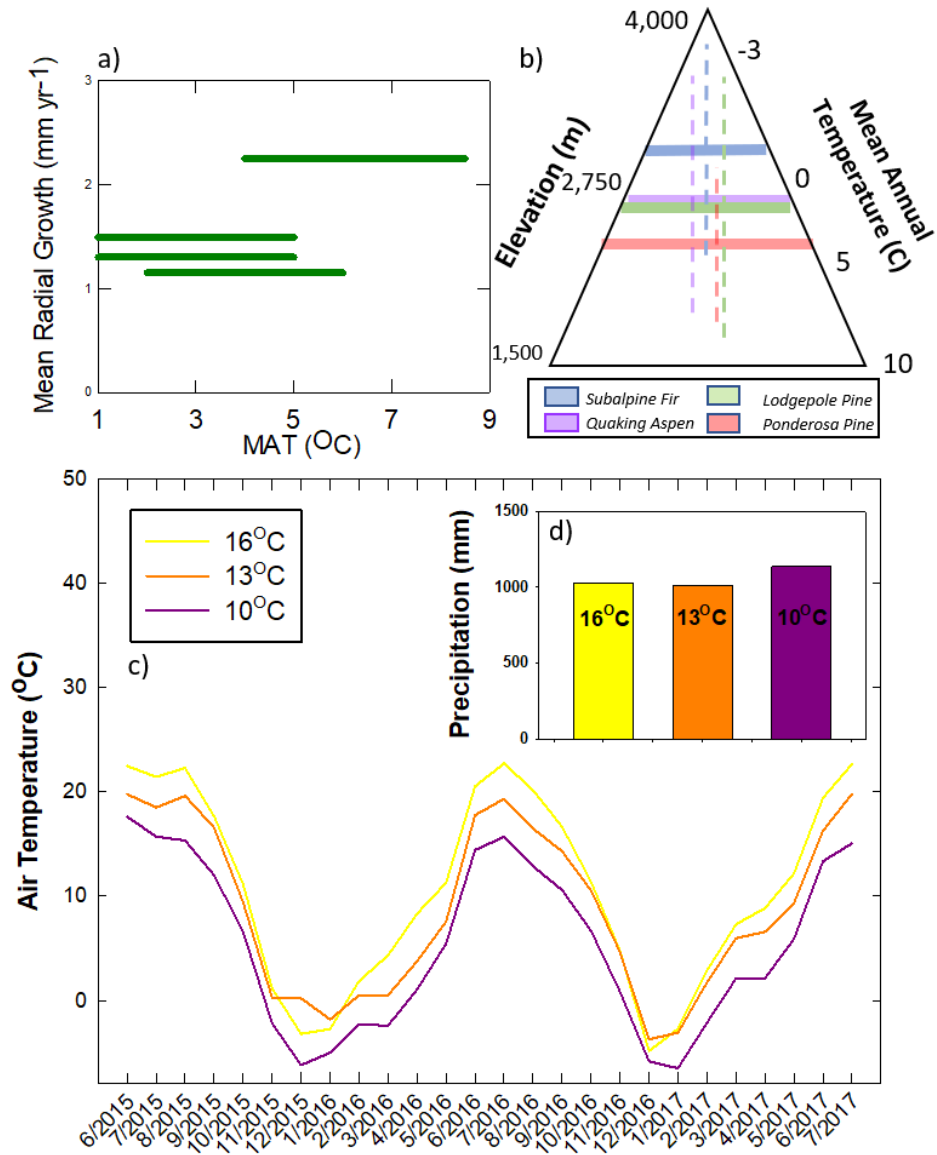


Figure 3.1. a) Recreation of Martin *et al. in review* demonstrating flat growth to temperature relationships with the species in question in this study b) Approximation of the temperature and elevation ranges of species used in this analysis c) Mean daily air temperature (°C) of the three experimental gardens d) Total cumulative precipitation mid 2015 – September 2017.

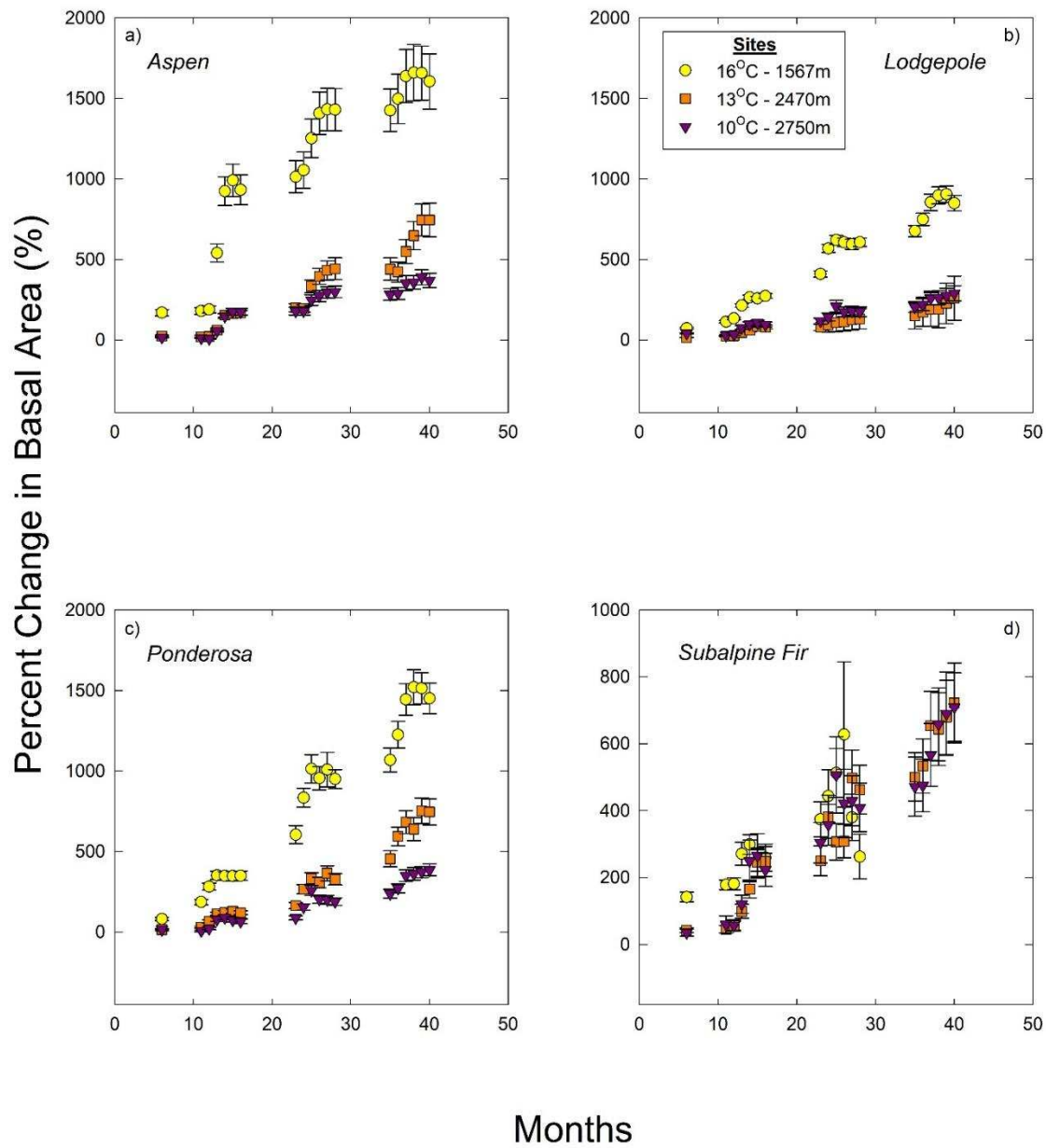


Figure 3.2. Percent change in basal area (%) for a) quaking aspen, b) lodgepole pine, c) ponderosa pine, and d) subalpine fir across the three experimental gardens. Mean values for percent change in basal area from initial planting are reported with bars corresponding to 1 standard error.

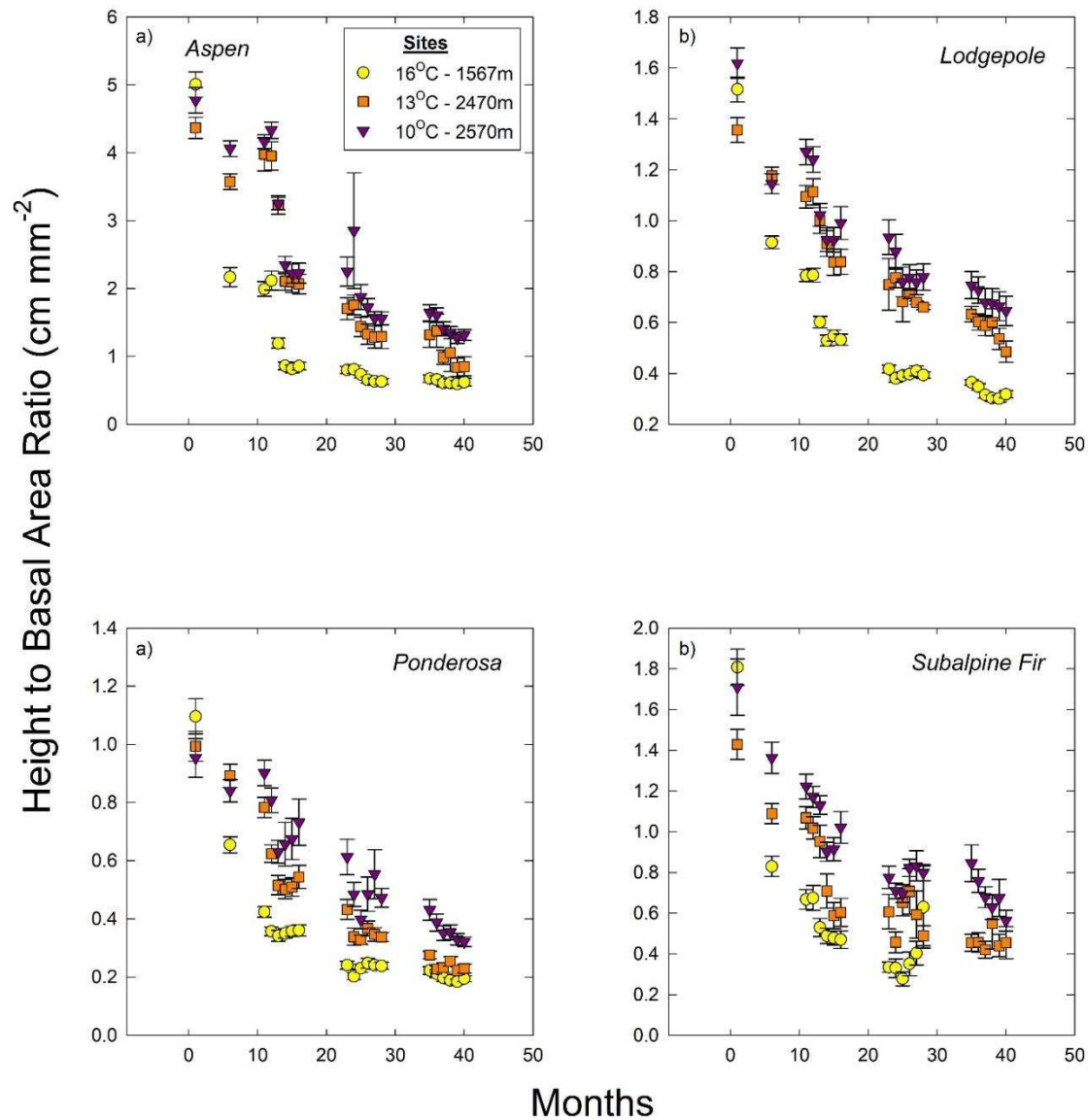


Figure 3.3. Height to basal area ratio (cm mm⁻²) for a) quaking aspen, b) lodgepole pine, c) ponderosa pine, and d) subalpine fir across the three experimental gardens. Mean values for height to basal area ratio from 2014 to 2017 are reported with bars corresponding to 1 standard error.

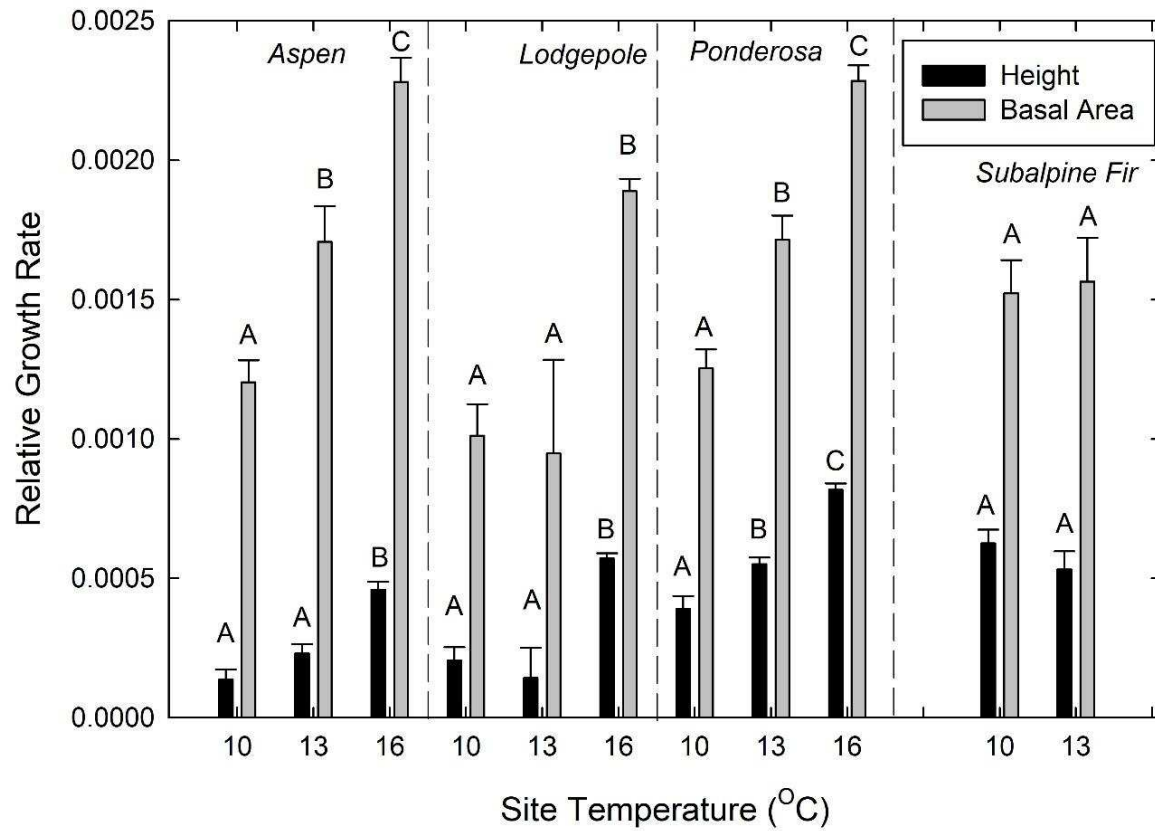


Figure 3.4. Relative growth rate of height and basal area for quaking aspen, lodgepole pine, ponderosa pine, and subalpine fir. Each garden is listed by its growing season temperature from the coldest site to the warmest site. Subalpine fir does not have an estimate at the warmest site (16°C) due to mortality of all individuals at that site. Letters denote significant differences within a species based on Tukey's HSD and bars denote 1 standard error.

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CHAPTER 4: RESPONSES OF LEAF TRAITS AND OSMOTIC REGULATION TO CLIMATE CHANGE MAY REDUCE GROWTH IN ASPEN

4.1. INTRODUCTION

The forests of the Rocky Mountain region are changing dramatically as a combination of forces restructure plant communities. In particular, temperatures are rising rapidly in the montane forests of Colorado where air temperatures increased $\sim 0.4^{\circ}\text{C}$ per decade over the second half of the 20th century (McGuire *et al.* 2012). These forests are expected to experience an additional 3°C of warming by the end of the 21st century (Gross *et al.* 2016). Rising temperatures effect trees directly (e.g. causing higher respiration rates), and indirectly by altering their water balance. The water-status of a plant is strongly affected by higher temperatures as the evaporative demand of hotter air increases exponentially with linear increases in temperature, causing drying and intensifying water stress. While warming temperatures may improve plant performance in some instances, hotter conditions combined with decreased precipitation or drier conditions typically reduce tree growth (Wu *et al.* 2010). This is particularly true of systems in the western U.S. where water budgets are already limited due to high evaporative demand from warm temperatures and low precipitation during the growing season. Thus, even if precipitation patterns remain unchanged, plants in the future will experienced increased water stress due to warming temperatures alone.

Quaking aspen (*Populus tremuloides*) is a dominant component of Rocky Mountain forests, but recently has experienced substantial population declines across parts of its range due to a suite of factors referred to as Sudden Aspen Decline (SAD), including severe drought, warming temperatures, and pests and pathogens (Worrall 2010; Michaelian *et al.* 2011). The combined effect of warming temperatures and drought have recently contributed to dramatic aspen declines in montane (Breshears *et al.* 2005; Dudley *et al.* 2015) and subalpine forests (Bretfeld *et al.* 2016) throughout the region. Deciduous angiosperms in mountainous regions often face a particular challenge: avoiding damage from hydric stress due to low soil

water availability during the summer growing season while photosynthesizing enough for growth and survivorship before the onset of frost and leaf senescence in the autumn. As soil moisture declines, plants respond in one of two general ways. Plants can maintain a constant leaf water potential (Ψ_{leaf}) to maintain the hydration of cells in the leaf but at the cost of reduced carbon uptake and reduced growth.

Alternatively, plants can maintain positive growth, but Ψ_{leaf} values will decline and endanger the tissues of dehydration. One way to avoid dehydration in the leaf is to increase the solute concentration of cells (osmotic adjustment), thus allowing cells to maintain positive turgor as Ψ_{leaf} declines. Seasonal osmotic adjustment (becoming increasingly negative in the fall/winter) has been correlated with increases in frost tolerance (Pramsohler and Neurner 2013), can delay frost damage as addition of solutes can reduce the freezing temperature of symplastic water (see Wisniewski *et al.* 2003), and extenuating the fall growing season (Webster and Ebdon 2005). The decreasing soil moisture availability that is expected in the future may limit aspen growth unless it is able to acclimate and adapt to these new conditions. Quantifying the range of osmotic adjustment that aspen can attain in response to variability in soil moisture and air temperature will be required to predict how this species will respond to future environmental conditions.

The interaction between warming and shifts in available moisture will be critical for future plant performance across the region. Recent efforts to include plant hydraulic traits in landscape level projections of tree performance highlight the value of this approach in improving estimates of plant mortality (Anderegg *et al.* 2015), particularly in aspen, which has been shown to primarily rely on shallow soil moisture (<10 cm, Anderegg *et al.* 2013). As most trees – and angiosperms in particular – operate with limited hydraulic safety margins (Choat *et al.* 2012), investigating these traits at xeric range-limits have proven to be highly predictive of drought induced mortality (Tai *et al.* 2017), yet there continues to be a lack of information directly relating hydraulic stress and plant performance at the whole plant level (Zwieniecki and Secchi 2015). To address these issues, I established three experimental gardens at sites with similar

levels of precipitation which span a range of 6°C mean annual temperature (MAT). By comparing seedlings across these gardens, I investigated the role of warming temperatures on *in situ* tree seedling responses to water stress. I hypothesized that (1) plants growing at the coldest and warmest sites will experience the largest amount of osmotic adjustment in response to cold temperatures (at the coldest site) or reduced soil moisture (at the warmest site). As osmotic adjustment can enhance both drought and freezing tolerance, I expect (2) these two sites to exhibit the greatest resistance to damage in response to freezing. I also hypothesize that (3) adjustments in the allocation of biomass to leaf structural components could also provide freezing tolerance regardless of osmotic adjustment. Finally, osmotic adjustment requires investment of resources by the plant and may alter the growth efficiencies of plants, and so I hypothesize that (4) plants growing at the intermediate-temperature site will have the greatest growth efficiency due to a reduction in resources invested in osmotic adjustments.

4.2 METHODS

Field Site - From June through September 2017, measurements were conducted on 5-year-old aspen seedlings (diameter at 5cm above the ground ranged from: 6.2-30.3 mm) planted in three experimental gardens located along a 1,200 m elevation gradient in the Front Range of Colorado. These gardens were established in 2014 and are identically orientated on flat east-facing slopes. Each garden is separated by approximately 3°C MAT but have similar precipitation patterns at each site (~475 mm, see Carroll *et al.* 2017 for full site description). Seedlings were grown in a greenhouse from seed collected in a single stand in the Roosevelt National Forest. When 1 year old, these seedlings were planted in homogenous soil excavated and transplanted from the intermediate-temperature site. Environmental conditions have been continually monitored since

2015 including air temperature and precipitation (Onset HOBO RG3-M), and soil temperature and moisture (Decagon 5TM Soil Moisture and Temperature Sensor) (Fig. 4.1; Table 4.1).

Seasonal Water Availability: Midday Water Potential and Osmotic Potential - Leaves were excised monthly from south-facing fully-exposed mid-canopy stems on sunny days. Sampling began at 11h and continued no later than 13h. One leaf from 7-14 individuals was randomly selected and immediately placed in a Scholander-style pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA) to measure midday water potential (Ψ_{mid}). Leaves were then suspended in water and transported back to the lab and allowed to rehydrate for ~24 hr before being frozen and stored in a freezer for later analysis of osmotic potential (Ψ_{osm}). Frozen leaves were used to estimate Ψ_{osm} by cutting 6mm leaf discs from leaf lamina tissue, being sure to avoid the mid-vein. Leaf disc initial weight was measured and then leaves were placed in an osmometer (model 5520, Vapro Co. Vapor Pressure Chamber, Wescor, Inc. Logan, UT, USA). The Ψ_{osm} of the leaf disc was repeatedly measured, without opening the chamber, until two readings differed by < 0.01 MPa. Ψ_{mid} values were regressed with volumetric water content (VWC) values collected during sampling and compared among sites. It was determined that the gardens had a common relationship and thus were combined to generate an overall relationship. Daily values of midday soil moisture at each garden were then compiled and used to predict Ψ_{mid} throughout the growing season at each site.

Leaf Dry Matter Content & Electrolyte Leakage - After Ψ_{osm} was determined, leaf discs were dried at 55°C until reaching a constant weight and then reweighed. Leaf Dry Matter Content (LDMC) – a common measurement of leaf water content and solute quantity – was then calculated by dividing the dry weight (g) by the initial weight (g). Additionally, I calculated Leaf

Sugar Matter Content (LSMC) to determine if changes in leaf weight were accounted for by accumulation of sugars or increased structural components. I first determined leaf water mass content (LWMC) by subtracting LDMC from 1. I then multiplied LWMC by the Ψ_{osm} and the molecular weight of sucrose to estimate the leaf sugar matter content (LSMC). A study of *Populus* clones (*P. trichocarpa* x *detooides*) found 70% of osmotic adjustment was accounted for by sucrose (Gebre *et al.* 1998). Although the assumption that all osmolytes have the molecular weight of sucrose is unrealistic, I assumed that sucrose approximates the molecular mass of upregulated compounds used as osmolytes if they comprise the majority of those compounds. Shifts in LSMC and LDMC were compared over time with deviations from a 1:1 line indicative of prioritization of either sugar production or structural components.

During the final sampling period in early September pairs of leaves were randomly sampled from five individuals at each site, sealed in separate plastic bags containing a damp paper towel and returned to the lab in a cooler. One leaf of each pair was refrigerated overnight while the other leaf was placed in the freezer (-10°C) to simulate a nightly freezing event. Leaves were frozen for approximately 18 hours followed by two hours in the refrigerator to reach similar temperatures as the samples stored in the refrigerator. Both sets of leaves were then allowed to warm to room temperature for approximately two hours. After warming, leaves were individually submerged in ~40ml of deionized water and conductance was determined (μS , Accumet Model 50 pH/conductivity meter, Fisher Scientific, Pittsburgh, PA. USA.). After the initial conductance was measured, samples were remeasured after: 15, 30, 45, 60, 180, and 360 minutes to detect maximum electrolyte leakage. Samples were then autoclaved (Steris Steam Sterilizer Model 20VS) at 121°C for 20 minutes to induce complete cell lysis and final

conductance was determined (Sánchez Viveros *et al.* 2010). Finally, electrolyte leakage was calculated for each time period as:

$$\text{Eq. 1 - Electrolyte Leakage (EL)} = \left(\frac{(S_t - S_0)}{S_F} \right) * 100$$

Where electrolyte leakage equals the change from initial conductance (S_0) to conductance at a time point (S_t), scaled by the final conductance (S_F) and multiplied by 100.

Growth Implications- To quantify the efficiency of growth at each experimental garden, the relative change in basal area was determined from the beginning of the growing season (April) to the end of the season (September). Additionally, initiation of bud-burst was tracked in the experimental gardens in spring 2017 to estimate growing season length (see Carroll *et al.* 2017 for methods). Percent change in basal area was then divided by the number of growing days at each site. Basal area was calculated using estimates of diameter at 5cm above the root collar using microcalipers. Stems were marked with paint at 5cm to ensure accurate measurements each month.

Statistical Analyses- Linear mixed-effects models comparing electrolyte leakage over time were compared fitting time as a fixed effect and individual as a random effect, with the full model including site as a fixed effect. Pairwise comparisons were calculated when appropriate using Tukey's HSD ($\alpha=0.05$). All other relationships were determined using simulated annealing global parameter estimation for curve fitting using a likelihood framework. AICc model comparison was used to determine best-fit models in all cases. All statistics were calculated using R (3.3.3) and the 'likelihood' (Murphy 2015), 'dplyr' (Wickham *et al.* 2017), 'lme4' (Bates *et al.* 2015), and 'lsmeans' (Lenth 2016) packages.

4.3 RESULTS

Seasonal Water Availability: Midday Water Potential and Osmotic Potential - Soil VWC varied considerably among the three gardens early in the experiment (June and July) declining with increasing Growing Season Temperature (GST) (Fig. 4.1). In August, a series of substantial rain events increased VWC to comparable levels amongst the gardens followed by the sites drying again in September with faster drying at the warmer sites. I sampled Ψ_{mid} across the entire range of soil moisture experienced at each site throughout the study period and found a strong relationship between Ψ_{mid} and VWC (Fig. 4.2 a and b). By quantifying the relationship between Ψ_{mid} and VWC ($R^2 = 0.83$; Table A2-1) I could estimate the number of days seedlings in each garden experienced Ψ_{mid} in different ranges of water stress (Fig. 4.2d). I estimated that individuals at the coldest site never experienced any days where Ψ_{mid} was more negative than -1.5 MPa, whereas the warmest site experienced the majority of days during the growing season at those ranges. Individuals at the intermediate-temperature site experienced the most even distribution of Ψ_{mid} with lowest number of days in between -1 and -1.5 MPa. These differences in Ψ_{mid} occurred despite similar precipitation through September 2017 at all three sites (Fig. 4.2c) suggesting that the effect of warmer temperatures on evaporative demand was an important driver on plant water relations.

I found a strong relationship ($R^2 = 0.72$; Table A2-2) between osmotic Ψ_{osm} and Ψ_{mid} with Ψ_{osm} becoming more negative with declining water potentials (Fig. 4.3). I provided both a linear and sigmoidal curve ($\Delta\text{AIC} = 6.1$), as the linear model was selected by AICc comparison, however based on *a priori* knowledge I suggest that the sigmoidal curve is appropriate but is not favored due to limited sample size. I also observed a strong seasonal effect on Ψ_{osm} with values

becoming more negative throughout the growing season (Fig. 4.4a), although differences in absolute osmotic adjustment were minimal among the three gardens. Individuals at the coldest, intermediate-temperature, and warmest sites reduced Ψ_{osm} by 1.95, 1.88, and 2.1 MPa respectively.

The intermediate and warmest sites experienced increasingly more negative Ψ_{osm} between June and July and then maintained comparable values throughout the remainder of the experiment. In contrast, individuals at the coldest site experienced more negative Ψ_{osm} each month, with osmotic adjustment peaking in August. Ψ_{osm} and VWC were also highly correlated as expected (Fig. 4.4b). Overall, there was strong evidence of Ψ_{osm} becoming more negative with declining soil moisture ($R^2 = 0.69$, Table A2-2). These trends suggest that shifts in Ψ_{osm} undergo both a strong seasonal effect (Fig. 4.4a), and a tightly coupled influence of water availability (Fig. 4.4b). Correlations between soil and air minimum temperatures were tested but determined to be not significant in any cases (Fig. A2-1).

Frost tolerance - To investigate the relationship between osmotic regulation and frost tolerance I tested the stability of cell membranes to freezing and the resultant loss of electrolytes from within the symplast. AICc model comparison of linear mixed-effects models comparing the effect of site found a substantial improvement over a model without site ($\Delta\text{AIC} = 55$). Additionally, plants from all sites differed in the amount of electrolyte leakage in response to a simulated freezing event (Tukey HSD for all pairwise comparisons <0.001 ; Fig. A2-2), and as expected the coldest site experienced the least damage due to freezing (the least electrolyte leakage). Surprisingly, the intermediate-temperature site experienced the most damage via freezing over the 6 hours with the warmest site experiencing moderate leakage relative to the other sites. As freezing tolerance has been shown to be a product in part of internal leaf water

content and exposure to non-freezing temperatures (Juntilla *et al.* 2002), I compared electrolyte leakage with the change in LDMC over the season and found a decline in electrolyte leakage with increases in LDMC (Fig. 4.5a). Additionally, daily minimum temperatures throughout the study period were found to reflect the same trend as electrolyte leakage (Fig. A2-3). Individuals at the intermediate-temperature site had the smallest increase in LDMC over the season coupled with nights on average 1.5°C warmer than the warmest site. These factors resulted in the plants from this site being the most susceptible to freezing-induced membrane damage and thus the greatest electrolyte leakage among the three gardens.

Growth Efficiency - Lastly, I predicted that a tradeoff may exist between how efficiently a tree grows and how resources are allocated to leaf structural tissues, osmotic regulation, or frost hardiness. I examined the efficiency of growth at the different sites by calculating the percent change in basal area per growing day in 2017 (Fig. 4.5b). The intermediate-temperature site had the highest growth rate based on Tukey HSD pairwise comparisons indicating the most efficient growth of well-established seedlings ($F = 13.47$, $p\text{-value} < 0.001$), followed by the coldest site, and finally the warmest site. Thus, the intermediate-temperature site experienced the greatest leakage, the smallest shift in seasonal LDMC (Fig. 4.5a), and the highest growth efficiency, while the other two sites experienced larger shifts in LDMC, less electrolyte leakage, and declines in growth efficiency compared to the intermediate-temperature site.

Leaf Dry Matter Content - I expected the observed decline in Ψ_{osm} through the season would impact both water uptake and freezing tolerance which results from increased solute concentration in cells and/or differences in cell membrane characteristics. To distinguish the two, I separated cellular adjustments into its' constituent parts: sugars and structural components (i.e. water transport tissues). I expected that LDMC and LSMC should predominantly vary together

seasonally (as LDMC and non-structural carbohydrates have been shown to covary in certain deciduous angiosperms, Palacio *et al.* 2008), but that the growing environment would dictate which would be prioritized over the other. As samples were collected after leaf maturation, increases in LDMC without comparable increases in LSMC would indicate structural augmentation within the leaf rather than simply the accumulation of additional sugars and other osmolytes within the existing leaf tissues. I observed a clear increase throughout the growing season in LDMC at all gardens with early season (June) values being the lowest and significantly increasing later in the growing season based on Tukey HSD pairwise comparisons (Fig. 4.6a). While LSMC trended positively throughout the growing season like LDMC in all the gardens, I did not observe any significant temporal pairwise differences.

To further investigate the relationship between LDMC and LSMC, I compared the rate of increase in LDMC and LSMC to determine if preferential allocation to one variable over the other varied seasonally. I observed a large increase in LDMC at the warmest site; between June and July LDMC shifted substantially faster than LSMC (above the 1:1 line Fig. 4.6b). This suggests that additional leaf structural components were developed as the increases in LDMC cannot be explained from increases in solute concentration. Not only was this the largest increase in LDMC relative to LSMC, but it was also the largest observed LDMC value observed throughout the experiment. Estimates of error are not possible around this estimate.

4.4. DISCUSSION

When faced with a decrease in available soil moisture, my results show that aspen appears to allow leaf water potential to decline with soil moisture, yet my results suggest that they are able to maintain turgor by adjusting osmotically as indicated by the relationship between soil volumetric water content and osmotic potential (Fig. 4.4b). However, in a manner that

appears unrelated to water availability, the coldest site displayed a late seasonal decrease in Ψ_{osm} that I hypothesize is in response to minimal nightly temperatures. In contrast, the warmest and intermediate-temperature sites decreased Ψ_{osm} earlier in the year (July) than the coldest site suggesting that water limitation largely drives this relationship.

I hypothesized that osmotic regulation would be the most plastic at extremes of the gradient in response to high levels of heat induced water stress at the warmest site and cold limitation at the coldest site. I observed evidence that the timing of osmotic adjustment was variable, but the amount of overall seasonal osmotic adjustment was similar ~ 2 MPa among individuals at each garden (Fig. 4.4a). This was surprising based on the differences in estimated water stress levels among individuals at each site. I estimated that individuals at the coldest garden experienced zero days of Ψ_{mid} more negative than -1.5 MPa (Fig. 4.2b). Below this water potential, vital functions such as stomatal conductance, leaf hydraulic conductance, and photosynthetic rate begin to be impacted (Boyer 1970; Bartlett 2016) and can eventually lead to mortality via carbon starvation or desiccation (Parker and Patton 1975, McDowell *et al.* 2008). The warmest site, which has an annual temperature 3°C higher than the area where the seeds were collected, increased the number of estimated days spent at greater than -1.5 MPa by 33% indicating greater water stress despite similar levels of precipitation during the experiment.

As osmotic adjustment may improve not only drought but frost tolerance as well, I expected that the warmest and coldest sites would exhibit the highest degree of frost tolerance when subjected to a discreet freezing event. My findings support my hypothesis that these two sites would have higher freezing tolerance than the local seed-source site (Fig. 4.5), yet it appears that osmotic adjustment is not the mechanism explaining this relationship as each site experienced similar levels of osmotic adjustment seasonally. Furthermore, the coldest site

exhibited the least negative Ψ_{osm} in September (when the electrolyte leakage test occurred), followed by the intermediate-temperature site. This further highlights the lack of predictive power of Ψ_{osm} for predicting leaf hardiness. I did however observe that damage to freezing was inversely proportional to the amount of seasonal increase in LDMC (Fig. 4.5). By increasing investment in leaf structural tissues throughout the growing season, seedlings at the coldest site increased leaf hardiness and experienced the least amount of freezing damage. Individuals at the intermediate-temperature site, which is closest to the seed-source elevation acted similarly to those at the warmest site in terms of Ψ_{osm} throughout the season (Fig. 4.4a) yet underwent the lowest seasonal percent change in LDMC (Fig. 4.5) had the highest amount of electrolyte leakage suggesting a lack of investment in membrane durability. I hypothesize that the plants were mitigating the impact of low water availability by increasing internal water transport capabilities as increased production in LDMC.

Two main factors have previously been isolated as major contributing factors for aspen cold tolerance. Welling *et al.* (2002) using knock-out *Populus tremula* x *tremuloides* hybrids determined that both day length and lower temperatures led to cold acclimation. My results agree with these findings as the site with the lowest average and minimum temperatures during the study – the coldest site – (Table 4.1, Fig. A2-3), underwent the least amount of cell damage to a freezing event (Fig. 4.5). My findings also agree with previous work again on *Populus tremula* x *tremuloides* hybrids in which electrolyte leakage was lower in cold-adapted individuals along with a production of biochemical compounds associated with frost tolerance (Renault *et al.* 2005). The coldest site underwent the largest overall increase in LDMC throughout the experiment (June-September; Fig. 4.6), never experienced Ψ_{mid} or VWC conditions as low as the other sites but increased Ψ_{osm} throughout the season indicating that other factors such as

potentially increasing leaf hardness were impacting seasonal Ψ_{osm} increases. Across this temperature gradient, my results suggest that electrolyte leakage was not associated with osmotic adjustments but were rather indicative of the amount of dry matter allocated the leaf production as well as other abiotic factors. I hypothesized that these investments either in osmotic adjustment or leaf structural components would come at a tradeoff with growth.

I observed the highest growth efficiency (growth per day of the growing season) of any of the gardens at the intermediate-temperature garden (Fig. 4.5) suggesting that aspen grows most efficiently in the local conditions it originated from and that it allocates resources away from growth at the other sites. Allocation of resources to increasing LDMC for improved frost tolerance at the cold-limited higher elevation site and increasing water transport at the water and temperature limited warmest site correspond to declines in whole-plant growth efficiency. When both water and temperature combine to limit performance of aspen I observed an alternative growth strategy develop wherein growth efficiency was reduced to the lowest of the three gardens and I observed the largest absolute LDMC value suggesting that individuals are responding to environmental conditions by altering carbon allocation to offset abiotic stressors (Fig. 4.5).

Comparisons of LDMC and LSMC suggested minimal differences among the gardens to the timing of accumulation and production of these components of leaf weight. Of note however, I observed a significantly higher production of structural components at the warmest site in July relative to changes in sugar content when plant water stress was high due to low water availability (lowest observed value across the study) and high temperatures exacerbating evaporative demand (Fig. 4.6b). Similar work assessing drought tolerance at the dry range of aspen observed an increased allocation of carbon to structural components but not a shift in

water use efficiency or other behaviors shifts (Anderegg and HilleRisLambers 2016). They also found that such allocation patterns lead to a decline in basal area growth suggesting that offsetting drought with structural carbon is possible but may come at a cost to whole-tree performance.

Preliminary analyses of growth prioritization in the gardens suggests that higher temperatures result in trees prioritizing radial and stem growth over height growth (Chapter 3; unpublished data). Reduced growth efficiencies, increased resources in leaf structural components (LDMC), and prioritization of stem area to height all suggest that aspen are responding to the combined effect of water stress via increased evaporative demand at the warmest site and may considerably restrict performance of this species with future warming. Recent work by Rodgers *et al.* (2018) in an old field system in the northeast US, found an interaction between warming and precipitation availability on mortality in seedlings of (*Populus grandidentata*) where any warming x precipitation level resulted in increased mortality above ambient conditions. Even with a considerable increase in precipitation (+50%), survival decreased by roughly half with 4°C of warming. While my temperature increase is not as extreme, I have not increased precipitation and thus I expect similar declines in aspen performance where they are increasingly becoming limited by warming temperatures under similar precipitation levels. This is reflected in my growth efficiency results (Fig. 4.5b) and is similar to other studies relating drought to reduced historical aspen growth (Chen *et al.* 2017), forecasting *Populus* performance declines with future warming (Rehfeldt *et al.* 2009), and considerable shifts in optimal range migrations in the Rocky Mountains (Gray and Hamann 2013).

Predictions of future shifts in precipitation and soil moisture in the western U.S. remain uncertain based in part on the challenges in modeling cloud microphysics and precipitation levels (Kang *et al.* 2015), however, uncertainty around warming trends in the region is low (Gross *et al.* 2016). My experimental approach allowed me to mimic an expected 3°C of warming by the end of the century. However, warming temperatures resulted in decreased available soil moisture and increased days at higher water-stress levels despite similar precipitation levels highlighting the importance of incorporating temperature directly into estimates of plant water budgets in the future to generate accurate predictions of plant performance. While it is well established that rising temperature exponentially increases plant water stress due to rising evaporative demand, I have been able to document in an experimental framework the importance of 3°C of warming on plant function in Colorado. I tracked seasonal changes in Ψ_{osm} and found tight relationships with water availability with other factors impacting seasonal adjustment at the cold-limited site. I saw some evidence that shifts in temperature away from the local seed source resulted in reductions in growth efficiency to either increase water transport or improve frost tolerance depending on the environmental constraints. My results suggest that future aspen performance will be reduced in these regions impacting future aspen distribution and abundance throughout the western U.S.

4 TABLES

Table 4.1. Climate and soil data for the experimental sites. Long-term means were derived from 800-m resolution PRISM climate data (PRISM Climate Group, 2004) based on 1986-2011. Experimental data were calculated based on a time period from 6/1/17 through 9/31/17.

| Site | Coldest | Intermediate | Warmest |
|---|---------|--------------|---------|
| Elevation (m) | 2750 | 2470 | 1567 |
| Long term mean annual air temperature (°C) | 3.7 | 6.6 | 9.6 |
| Long term mean annual precipitation (mm) | 447 | 567 | 409 |
| Experimental* mean daily air temperature (°C) | 12.5 | 16.3 | 19.2 |
| Experimental* min daily air temperature (°C) | 2.9 | 10.9 | 9.4 |
| Experimental* precipitation (mm) | 170.6 | 127.4 | 137.2 |
| Experimental* mean soil moisture (%) | 16.3 | 12.3 | 10.8 |
| Experimental* mean soil temperature (°C) | 13.9 | 18.1 | 22.5 |

4 FIGURES

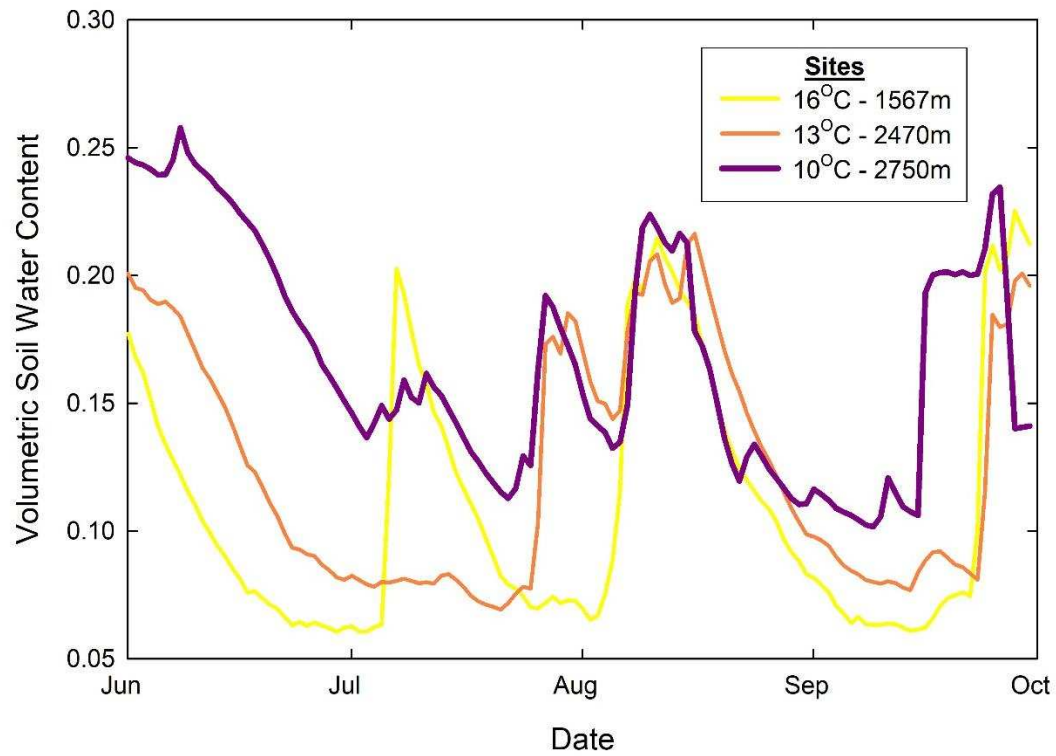


Figure 4.1. Volumetric Water Content (VWC) of the soil (5 cm) through the duration of the experiment June-September 2017. Colors correspond to the mean growing season temperature of each site.

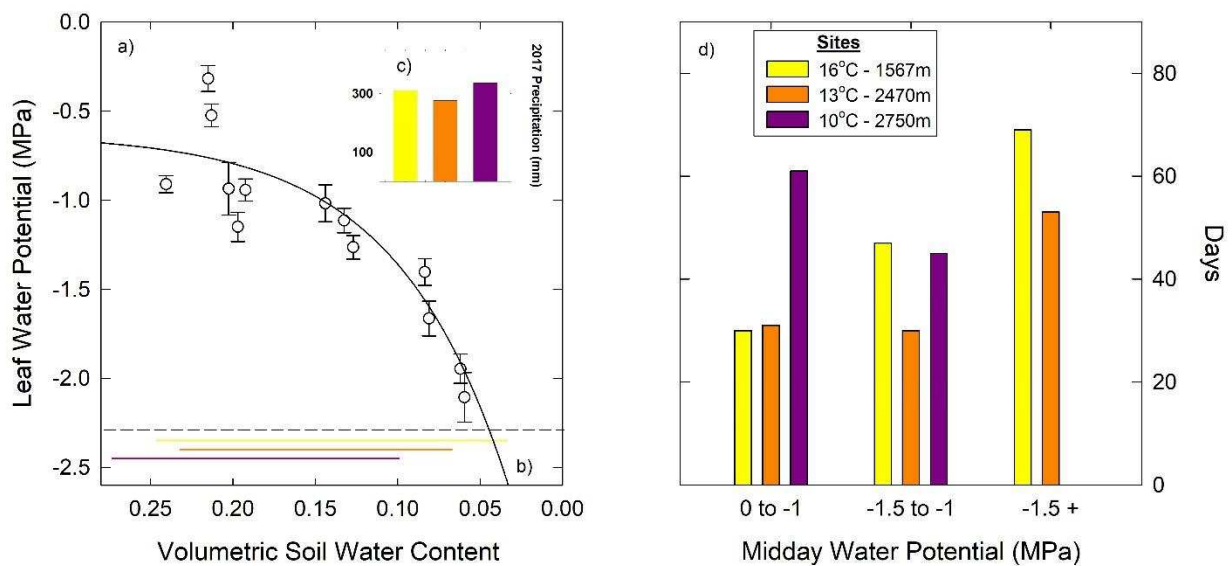


Figure 4.2. a) Midday leaf water potentials (Ψ_{mid}) vs soil volumetric water content (VWC) Bars are 1 standard error. b) Total observed range of VWC during the study period, with the colors corresponding to the mean growing season temperature of that site. c) Total precipitation (mm) in each garden during the experiment (June-September). d) Estimated number of days at each site where the Ψ_{mid} fell within one of the ranges of MPa.

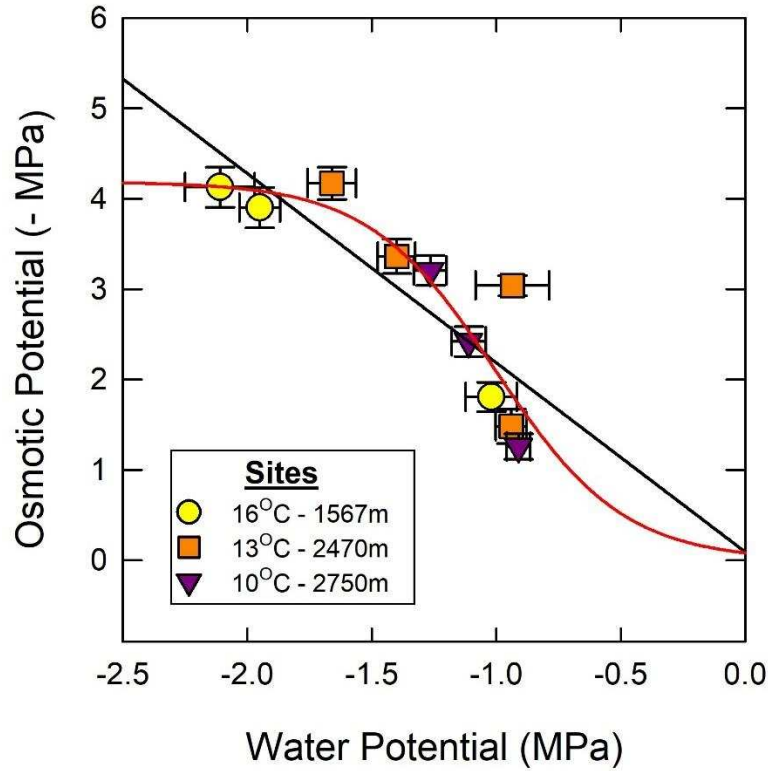


Figure 4.3. Mean osmotic potential (Ψ_{osm}) and the corresponding midday water potential (Ψ_{mid}) sampled over the study period. Bars denote 1 standard error. Linear ($\Delta\text{AICc} = 0$) and sigmoidal curves (red; $\Delta\text{AICc} = 6.25$) are provided.

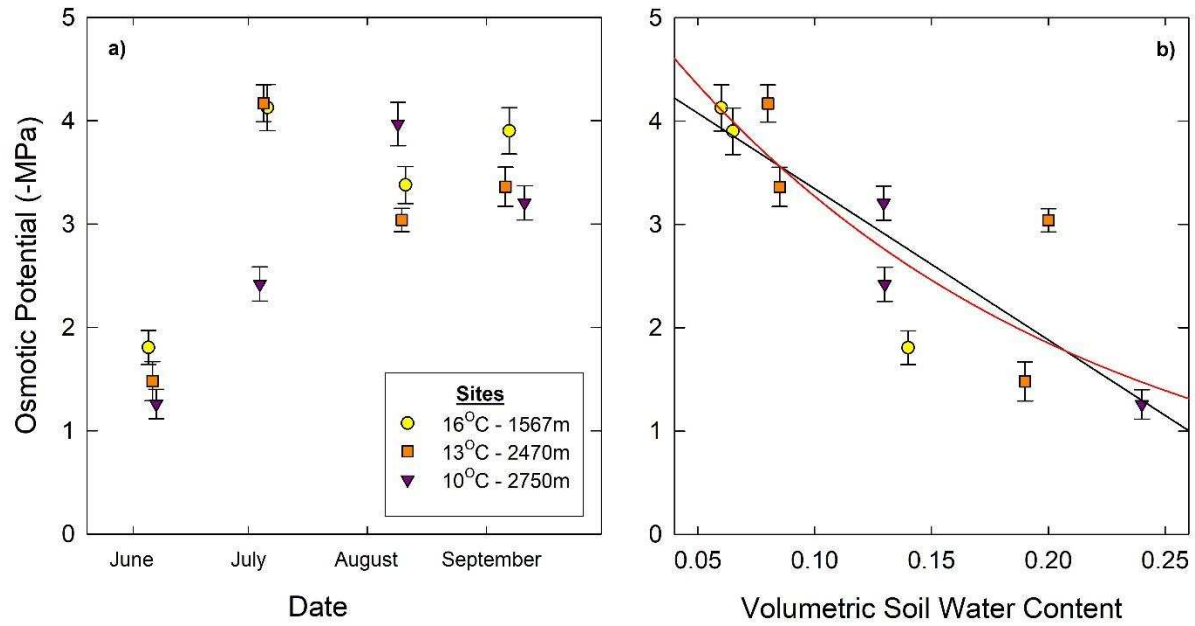


Figure 4.4. a) Osmotic potential (Ψ_{osm}) at each site by sample month. b) Ψ_{osm} and soil volumetric water content (VWC). Bars indicate 1 standard error. Linear ($\Delta\text{AICc} = 0$) and exponential curves (red; $\Delta\text{AICc} = 1.58$) are provided.

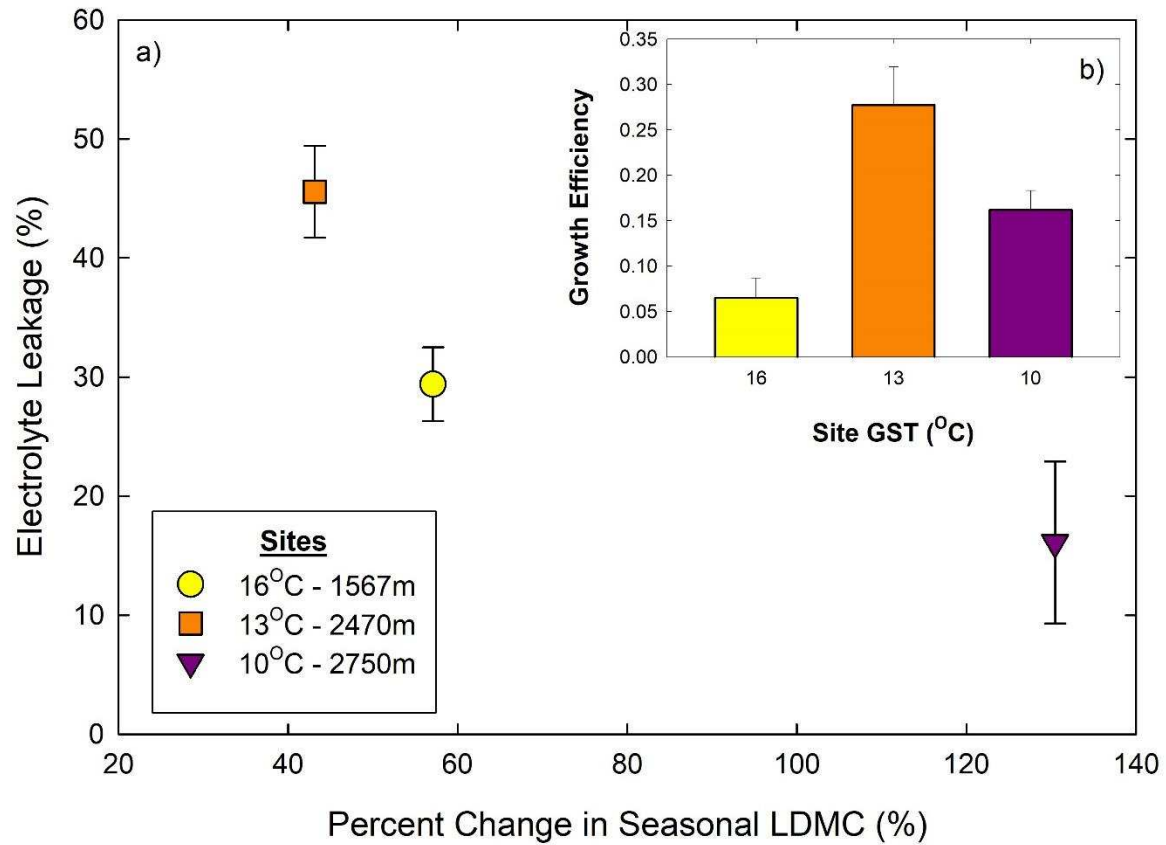


Figure 4.5. a) Percent electrolyte leakage from frozen leaves after a period of 6 hours vs the percent change in LDMC throughout the entire sample period. Bars denote 1 standard error. b) Growth efficiency (percent change in basal area per possibly growing day) for each site. Bars denote 1 standard error.

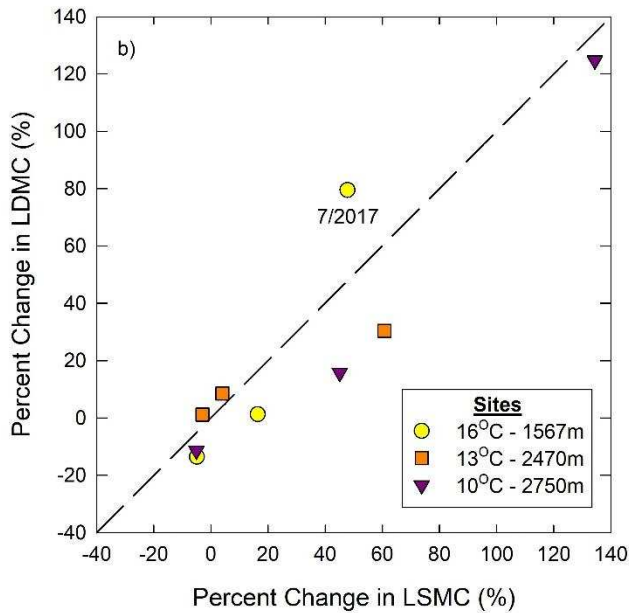
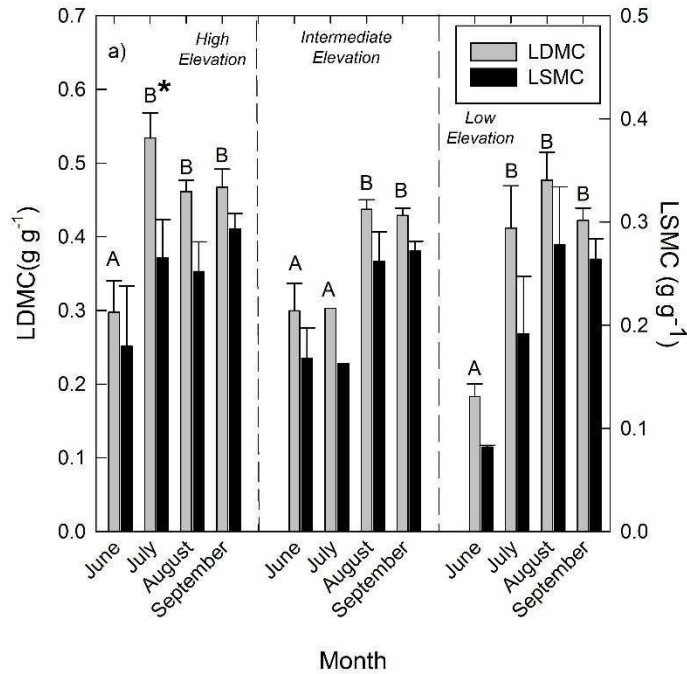


Figure 4.6. a) LDMC (Leaf Dry Matter Content) and LSMC (Leaf Sugar Matter Content) values throughout the season. Differences based on Tukey HSD pairwise comparisons are denoted by different letters. Stars denote when either LDMC or LSMC increased at a faster rate than the other trait b) Percent changes in LDMC and LSMC between consecutive months in 2017. Shapes denote each site, where circles are the warmest site (1567m, the squares are the intermediate-temperature site (2470m), and the triangles are the coldest site (2750). b) Growth efficiencies of each garden for the growing season of 2017 (April-September) - percent change in

basal area per growing day. Bars denote 1 standard error and letters indicate significant differences based on Tukey HSD pairwise comparisons.

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CHAPTER 5 CONCLUSIONS

Isolating and investigating the effects of key drivers of climate on tree seedling performance is vital for providing accurate estimates of how forests will fare in the future. Without mechanistic examinations of how temperature and precipitation impact forest distribution and community assemblages, researchers will be forced to rely upon bioclimatic relationships to forecast future changes. This is potentially problematic as bioclimatic envelope models often lack key inputs such as biotic interactions (Pearson and Dawson 2003), until recently have avoided differences among populations (Gray and Hamann 2013), and assume some level of niche stability for organisms within a community. This is problematic because shifting temperature and precipitation levels will most likely result in novel forest communities (Hobbs *et al.* 2006) as species migrate independently at rates based on physiological and demographic limitations (Davis and Shaw 2001), further confounding the use of bioclimatic forecasts. In this dissertation I provided an alternative strategy for forecasting species performance. The use of experimental gardens provides insight into the effects of temperature on some of the vital components of tree seedling performance by subjecting seedlings to realistic representations of future climate while controlling many extraneous and confounding factors and largely isolating the impact of temperature.

By assessing performance in experimental gardens and utilizing *in situ* growing conditions, I was able to address three main questions: (1) How do warming temperatures impact the photosynthetic optima, phenological timing of spring bud burst, and leaf size of tree seedlings of three dominant montane species in Colorado? (2) How do warming temperatures effect the overall growth and survivorship of montane and subalpine tree species in Colorado,

and does warming impact the prioritization of growth to height or basal area within those species? (3) How does warming interact with available soil moisture to impact water stress within the wide-spread quaking aspen (*Populus tremuloides*), and to what degree does the plasticity of hydraulic traits influence aspen's ability to tolerate suboptimal conditions?

Throughout the rest of this chapter I address each of these questions relying upon the results presented in Chapters 2 through 4. I highlight the main conclusions of this dissertation and discuss their implications for informing future research and management efforts as well as argue for the proliferation of additional experiments using this same framework to provide vital information on tree seedling performance in the future.

Physiological, phenological, and anatomical sensitivity to temperature – My first question was centered on comparing the sensitivities of three dominant montane species to warming temperatures across my experimental gardens. Photosynthetic rate and growing season length are typically associated with seedling health and thus served as barometers for the broader viability of these species under warming conditions. The first intriguing finding was that the temperature optima of photosynthesis did not shift when I manipulated leaf temperature in 5 °C increments from 15-30 °C. While within species temperature optima were not determined, I did observe differences in photosynthetic output between gardens for quaking aspen. Across individuals, the average site-level photosynthetic rate peaked at the intermediate-temperature site – which is closest to the local seed source location of this species – and declined with either warming or cooling suggesting a large degree of local adaptation and optimization of individuals to their local planting site. In contrast, the two conifer species I examined, lodgepole pine and ponderosa pine experienced no differences in average photosynthetic output across the gardens indicating less of a pre-adaptation to one growing environment.

I also examined the impact of warming on growing season length. Not surprisingly, warming extended the growing season for all species; however, the aspen growing season length increased consistently with each increase in 3 °C, while seedlings of the two conifer species increased their growing seasons substantially with the first 3 °C of warming and less with the next 3 °C. They also displayed an overall larger total increase with either 3 or 6 °C of warming than quaking aspen. These findings are supported by long-term warming experiments at the Flakaliden Experimental Site in Sweden that found comparable increases in growing season length with similar levels of warming (Hall *et al.* 2013) suggesting that these findings are not only applicable to the Rocky Mountains. Lastly, I investigated the effects of warming of leaf size and found that the trend in leaf size plasticity mirrored that of photosynthesis with aspen seedlings peaking at the local seed-source elevation and declining with either warming or cooling while the conifers largely remained constant. In conjunction, these findings speak to the relative stability of the coniferous species with the ability to maintain comparable photosynthesis rates and leaf size across all experimental gardens, while aspen was highly sensitive to shifts in temperature and clearly optimized for its native seed-source location. The relative stability of the conifers in addition to their large increase in growing season suggest 3 °C of warming will favor these species at the expense of aspen and could suggest a shift in species composition throughout the mixed forests of Colorado.

Whole-plant growth and carbon allocation – In light of the findings from Chapter 2, I investigated the response of whole-plant growth to warming in Chapter 3. Across my three experimental gardens I found that the three montane species responded similarly; warming increased growth. In contrast, subalpine fir seedlings experienced similar growth rates between the coldest site and the intermediate-temperature site suggesting 3 °C of warming will not

dramatically reduce growth rates; however, 6 °C of warming led to the complete extirpation of subalpine fir from the warmest garden. Quaking aspen experienced the fastest growth rates across montane species at the warmest site followed by ponderosa pine and then lodgepole pine. This result was surprising as the results from Chapter 2 strongly indicated aspen would experience peak performance at the intermediate-temperature site and decline with either warming or cooling. Quaking aspen is an early successional specialist that colonizes disturbed areas and quickly grows to occupy the canopy but has a short life-expectancy. I speculated that despite the accelerated growth rates with warming, quaking aspen will not be able to sustain the growth rate, and despite a lack of evidence for increased mortality, I expect decreased survivorship in the future for aspen.

A second main conclusion from Chapter 3 was that all species preferentially prioritized resources to basal area over height and that this trend accelerated over time and with increasing mean annual temperature (MAT). This is partially a scaling effect of seedling growth patterns where increases in height must be supported by increases in basal area or the seedling will become unstable and weak, however the correlation with increasing resources to basal area with warming MAT suggested that hydraulic function was prioritized particularly at the warmest site. Higher MAT increases evaporative demand by increased vapor pressure deficit leading to more rapid declines in available soil water, exacerbating water stress. I speculated that increased basal area with warming MAT was a direct response to this decline in available soil moisture, particularly at the warmest site. These findings suggest that future studies of performance should account for both ecophysiological traits as well as whole-plant growth as they together provide a more complete picture of seedling health than one alone.

Water stress responses of quaking aspen – The finding that seedlings at the warmer sites preferentially prioritized basal area production over height in Chapter 3 led to me investigate how warming temperatures impact water stress in quaking aspen seedlings in the experimental gardens in Chapter 4. By first determining the relationship between volumetric soil water content and midday water potential (Ψ_{mid}) throughout the growing season I was able to estimate the number of days each site experienced various levels of water stress. The warmest site experienced the most days with Ψ_{mid} more negative than -1.5 MPa suggesting the highest level of water stress (decline in stomatal conductance and tissue conductance – Bartlett *et al.* 2016) despite comparable levels of precipitation. The increased water stress was due to non-linear increases in evaporative demand with linearly rising temperatures. One method to control hydraulic function is for plants to adjust the amount of osmolytes within their cells. I found both a seasonal trend explaining the accumulation of osmolytes as well as an impact of available soil moisture acting in conjunction to effect leaf osmotic potential (Ψ_{osm}). I also observed an increase in leaf structural tissues that were correlated with higher frost tolerance at both the warmest and coldest sites. Increases in leaf tissues at the warmest site were indicative of high water stress where temperature and soil moisture colimit growth, while increased leaf tissues at the coldest site appear to be predominantly a function of prolonged growing season. While these adaptations are beneficial for tolerating the constraints of the local environment, it may come at the cost of efficient growth. I observed that growth efficiency peaked at the intermediate-temperature site and declined at the coldest and warmest sites. I speculated that this decline in growth efficiency is related to the prioritization of limited resources to improving leaf tolerance of either warm and dry or cold conditions and may explain why the photosynthetic and leaf size data similarly peaked at the intermediate-temperature site and declined at the other gardens. This apparent

tradeoff has been observed previously in this species where dry conditions led to increases in structural tissues but not with shifts in water use efficiency and declines in growth (Anderegg and HilleRisLambers 2016) under dry soil conditions. These findings suggest that while aspen is growing the fastest at the warmest site, it is growing less efficiently than would otherwise be expected due to increased resource allocation away from either height or basal area and into leaf structural components to counteract high water stress.

Experimental manipulations of forests designed to produce meaningful estimates of how individual seedlings are impacted by climate are rare (Canham and Thomas 2010) but increasing particularly within the last decade. Without these efforts our understanding of climate impacts on forests rely upon “conjecture, observation, and intuition” (Rehfeldt *et al.* 2008). More information is clearly needed including seedling competition and germination success rates under warming (Kroiss and HilleRisLambers 2015), nursery-plant dynamics, the impacts of canopy gaps on growth, and differential migration rates across a species range (Renwick and Rocca 2015). Experimental gardens provide a unique method for determining realistic outcomes while largely controlling input variables and specifically targeting the effects of specific climate drivers. Common gardens successfully implemented can address questions that simply cannot be tackled in other ways without sacrificing either expense or the tractability of an accurate representation of *in situ* conditions. As climate change continues to impact forest health, deriving mechanistic understandings of seedling performance will become even more vital for determining accurate predictions of forest viability in the future. In particular, understanding the interaction between rising temperatures and precipitation will expand upon the recent improvements in understanding seedling mortality and growth (Anderegg *et al.* 2015) especially as additional traits are identified that link shifts in climate to changes in seedling performance.

In this dissertation, I set out to investigate the effect of temperature on tree seedling performance from complimentary perspectives to achieve a holistic view of how some of Colorado's dominant tree species will fare by the end of the 21st century. I found that the temperature of optimal photosynthesis and leaf size varied considerably in quaking aspen seedlings peaking at the intermediate-temperature site that is closest to their local seed elevation suggesting a high degree of local adaptation that resulted in declining performance with temperatures shifts, particularly warming. In contrast, lodgepole pine and ponderosa pine displayed a lack of plasticity in those traits but underwent larger increases in their growing seasons than aspen suggesting mild warming will benefit these species. I found that warming unambiguously improved growth for the montane species while leading to mortality in the subalpine species and allocation to radial growth was prioritized with increasing temperature suggesting substantial indirect effects of warming on seedling performance. Lastly, I determined that the timing of osmotic regulation is plastic within quaking aspen which may contribute to the large range of this species. The ability to both adjust osmotically and to increase leaf structural components when temperature and water availability limit growth appears to allow this species to tolerate suboptimal conditions. However, evidence suggests that this tendency to accumulate leaf structural tissues may come at the cost of reduced growth efficiency which could prove problematic for this species in the future.

In light of warming temperatures and increasingly variable precipitation regimes expected in the future, my findings indicate that the future forests of Colorado may look considerably different than today. The high degree of local adaptation and declines in growth efficiency with shifts in MAT suggest that quaking aspen may not keep pace with a shifting climate leading to decreased growth rates and potential decline in the region. Similarly, while

mild warming did not substantially impact subalpine fir growth rates, competition pressure and relative abundance of lower elevation species as well as increasingly variable precipitation regimes (Andrus *et al.* 2018) are expected to result in declines in this cold-adapted species. Surprisingly, the lack of correlation between growth and survivorship suggests that future cohort establishment may be highly episodic as climate may regulate seed germination and early establishment, but warmer temperatures alone will not restrict seedling growth post-establishment. Based upon my findings, lodgepole pine and ponderosa pine which were able to physiologically tolerate warming and capitalize on increased growing season length should profit from the warming expected by the end of the 21st century. In summary, rising temperatures will have a substantial impact on the performance of tree seedlings of the Rocky Mountain region and will likely lead to shifts in forest composition in the future.

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APPENDIX I

Table A1-1. Comparison of two linear mixed effects models testing the effect of site on percent change in basal area (% Δ BA). Site (corresponds to the experimental gardens) and Time (month since planting) are fixed effects, while individual is treated as a random effect to account for repeated sampling of individuals over time.

| Species | Model | Param# | Δ AIC | AIC Weight |
|-----------|---|----------|--------------|------------|
| Aspen | y = % Δ BA ~1 Individual + Time | 3 | 65 | <0.001 |
| | y = % Δ BA ~Site + (1 Individual) + Time | 6 | 0 | 1 |
| Lodgepole | y = % Δ BA ~1 Individual + Time | 3 | 73 | <0.001 |
| | y = % Δ BA ~Site + (1 Individual) + Time | 6 | 0 | 1 |
| Ponderosa | y = % Δ BA ~1 Individual + Time | 3 | 91 | <0.001 |
| | y = % Δ BA ~Site + (1 Individual) + Time | 6 | 0 | 1 |
| Subalpine | y = % Δ BA ~1 Individual + Time | 3 | 0 | 0.5 |
| | y = % Δ BA ~Site + (1 Individual) | | | |
| Fir | + Time | 6 | 0 | 0.5 |

Table A1-2. Tukey pairwise differences of percent change in basal area by experimental garden for each species: quaking aspen, lodgepole pine, ponderosa pine, and subalpine fir. Estimates of contrast, standard error (SE), and p-values are provided.

| | Aspen | | Lodgepole | | Ponderosa | | Subalpine Fir | |
|-------------|-----------------|---------|-----------------|---------|-----------------|---------|-----------------|--------|
| Site | Estimate (SE) | p | Estimate (SE) | p | Estimate (SE) | p | Estimate (SE) | p |
| Int. - Low | -635.56 (87.46) | <0.0001 | -223.28 (28.76) | <0.0001 | -443.56 (51.33) | <0.0001 | -104.99 (62.41) | 0.2120 |
| Int. - High | 142.24 (87.24) | 0.2315 | 56.07 (30.09) | 0.1494 | 158.48 (51.09) | 0.0055 | -9.99 (59.07) | 0.9844 |
| Low-High | 778.07 (84.33) | <0.0001 | 279.35 (27.23) | <0.0001 | 602.04 (49.45) | <0.0001 | 95.00 (61.11) | 0.2656 |

Table A1-3. Comparison of two linear mixed effects models testing the effect of site on the height to basal area ratio. Site (corresponds to the experimental gardens) and Time (month since planting) are fixed effects, while individual is treated as a random effect to account for repeated sampling of individuals over time.

| Species | Model | Param# | Δ AIC | AIC Weight |
|---------|-------|--------|--------------|------------|
|---------|-------|--------|--------------|------------|

| | | | | |
|---------------|---|---|-------|--------|
| Aspen | $y = \% \Delta BA \sim \text{Site} + (1 \text{Individual}) + \text{Time}$ | 6 | 0 | 1 |
| | $y = \% \Delta BA \sim 1 \text{Individual} + \text{Time}$ | 3 | 49.4 | <0.001 |
| Lodgepole | $y = \% \Delta BA \sim \text{Site} + (1 \text{Individual}) + \text{Time}$ | 6 | 0 | 1 |
| | $y = \% \Delta BA \sim 1 \text{Individual} + \text{Time}$ | 3 | 63.57 | <0.001 |
| Ponderosa | $y = \% \Delta BA \sim \text{Site} + (1 \text{Individual}) + \text{Time}$ | 6 | 0 | 1 |
| | $y = \% \Delta BA \sim 1 \text{Individual} + \text{Time}$ | 3 | 35.77 | <0.001 |
| Subalpine Fir | $y = \% \Delta BA \sim \text{Site} + (1 \text{Individual}) + \text{Time}$ | 6 | 0 | 0.999 |
| | $y = \% \Delta BA \sim 1 \text{Individual} + \text{Time}$ | 3 | 13.7 | 0.001 |

Table A1-4. Tukey pairwise differences of percent change in basal area by experimental garden for each species: quaking aspen, lodgepole pine, ponderosa pine, and subalpine fir. Estimates of contrast, standard error (SE), and p-values are provided.

| Site | Aspen | | Lodgepole | | Ponderosa | | Subalpine Fir | |
|-------------|-----------------|---------|-----------------|---------|-----------------|---------|-----------------|---------|
| | Estimate (SE) | p | Estimate (SE) | p | Estimate (SE) | p | Estimate (SE) | p |
| Int. - Low | 0.85 (0.14) | <0.0001 | 0.28 (0.04) | <0.0001 | 0.15 (0.04) | <0.0001 | 0.09 (0.07) | 0.3470 |
| Int. - High | -0.31 (0.14) | 0.0709 | -0.11 (0.04) | 0.0368 | -0.09 (0.03) | 0.0368 | -0.19 (0.06) | 0.0061 |
| Low-High | -1.16 (0.13) | <0.0001 | -0.39 (0.04) | <0.0001 | -0.24 (0.03) | <0.0001 | -0.28 (0.06) | <0.0001 |

Table. A1-5. Model summaries comparing survival and three separate predictor variables referred to as X_2 in the presented models while the individual gardens are X_1 . Percent change in basal area ($\% \Delta BA$), height to basal area ratio (H:BA), and relative growth rate of basal area (RGR) are compared for each species. $\Delta AICc$, and $AICc$ weights are presented. In cases where a more complex model than the null is selected (**bold**), McFadden's Pseudo R^2 is provided.

| Species | X_2 | Model | # of Params | $\Delta AICc$ | $AICc$ Weight | Pseudo R^2 |
|---------|----------------|--|-------------|---------------|---------------|--------------|
| Aspen | $\% \Delta BA$ | $y = \beta_0$ | 1 | 0 | 0.41 | |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 0.91 | 0.26 | |
| | | $logit(y) = \beta_0 X_1$ | | | | |
| | | $+ \beta_1 x_1 x_2$ | 6 | 2.27 | 0.13 | |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 2.50 | 0.12 | |
| | | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 3.12 | 0.09 | |

| | | | | | |
|---------------|-------|--|----------|----------|-------------|
| Lodgepole | H:BA | $logit(y) = \beta_0 X_1$ | | | 0.33 |
| | | $+ \beta_1 x_1 x_2$ | 6 | 0 | 0.77 |
| | | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 3.79 | 0.12 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 4.82 | 0.07 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 5.61 | 0.05 |
| | | $y = \beta_0$ | | 14.5 | |
| | RGR | | 1 | 0 | 0.00 |
| | | $y = \beta_0$ | 1 | 0 | 0.59 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.05 | 0.21 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 3.69 | 0.09 |
| | | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 4.26 | 0.07 |
| | | $logit(y) = \beta_0 X_1$ | | | |
| | | $+ \beta_1 x_1 x_2$ | 6 | 5.98 | 0.03 |
| | %Δ BA | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 0 | 0.37 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 0.84 | 0.25 |
| | | $y = \beta_0$ | 1 | 1.18 | 0.21 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.67 | 0.10 |
| | | $logit(y) = \beta_0 X_1$ | | | |
| | | $+ \beta_1 x_1 x_2$ | 6 | 3.26 | 0.07 |
| Ponderosa | H:BA | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 0 | 0.41 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 0.35 | 0.35 |
| | | $logit(y) = \beta_0 X_1$ | 6 | | |
| | | $+ \beta_1 x_1 x_2$ | | 1.51 | 0.19 |
| | | $y = \beta_0$ | 1 | 5.32 | 0.03 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 5.93 | 0.02 |
| | RGR | $y = \beta_0$ | 1 | 0 | 0.40 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 4 | 0.05 | 0.39 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.04 | 0.15 |
| | | $logit(y) = \beta_0 X_1$ | 6 | 4.66 | 0.04 |
| | | $+ \beta_1 x_1 x_2$ | | | |
| | | $logit(y) = \beta_0 + \beta_1 x_1 x_2$ | 4 | 6.29 | 0.02 |
| | %Δ BA | $y = \beta_0$ | 1 | 0 | 0.74 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.08 | 0.26 |
| | H:BA | $y = \beta_0$ | 1 | 0 | 0.74 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.34 | 0.23 |
| | RGR | $y = \beta_0$ | 1 | 0 | 0.74 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.36 | 0.23 |
| Subalpine fir | %Δ BA | $logit(y) = \beta_0 + \beta_1 x_2$ | | | 0.13 |
| | | | 2 | 0 | 0.65 |
| | | $y = \beta_0$ | 1 | 1.25 | 0.35 |
| | H:BA | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 0 | 0.79 |
| | | $y = \beta_0$ | 1 | 2.69 | 0.21 |
| | RGR | $y = \beta_0$ | 1 | 0 | 0.76 |
| | | $logit(y) = \beta_0 + \beta_1 x_2$ | 2 | 2.27 | 0.24 |

APPENDIX II

Table A2-1. AICc model comparison output of three models testing the relationship between midday leaf water potential (Ψ_{mid}) and volumetric water content.

| Model | # of Parameters | ΔAICc | AICc Weight | R^2 |
|--|--------------------|---------------------|----------------|-------------|
| $y = a + be^{(-c+x)}$ | 4 | 0 | 0.685 | 0.83 |
| $y = a + e^{(b+x)}$ | 3 | 1.55 | 0.315 | 0.74 |
| $y = \frac{a}{(1+e^{-\frac{(x-b)}{c}})}$ | 4 | 22.105 | 0 | 0.09 |

Table A2-2. AICc model comparison for osmotic potential (Ψ_{osm}) and midday leaf water potential (Ψ_{mid}), as well as Ψ_{osm} and volumetric water content (VWC).

| Ψ_{osm} and Ψ_{mid} | # of Parameters | ΔAICc | AICc Weight | R^2 |
|---|--------------------|---------------------|----------------|-------|
| $y = a + bx$ | 2 | 0 | 0.75 | 0.72 |
| $y = a + e^{(b+x)}$ | 3 | 2.50 | 0.215 | 0.64 |
| $y = \frac{a}{(1+e^{-\frac{(x-b)}{c}})}$ | 4 | 6.126 | 0.03 | 0.72 |
| Ψ_{mid} and VWC | # of Parameters | ΔAICc | AICc Weight | R^2 |
| $y = a + bx$ | 2 | 0 | 0.66 | 0.69 |
| $y = a + e^{(b+x)}$ | 3 | 1.58 | 0.30 | 0.64 |
| $y = \frac{a}{(1+e^{-\frac{(x-b)}{c}})}$ | 4 | 5.34 | 0.04 | 0.72 |

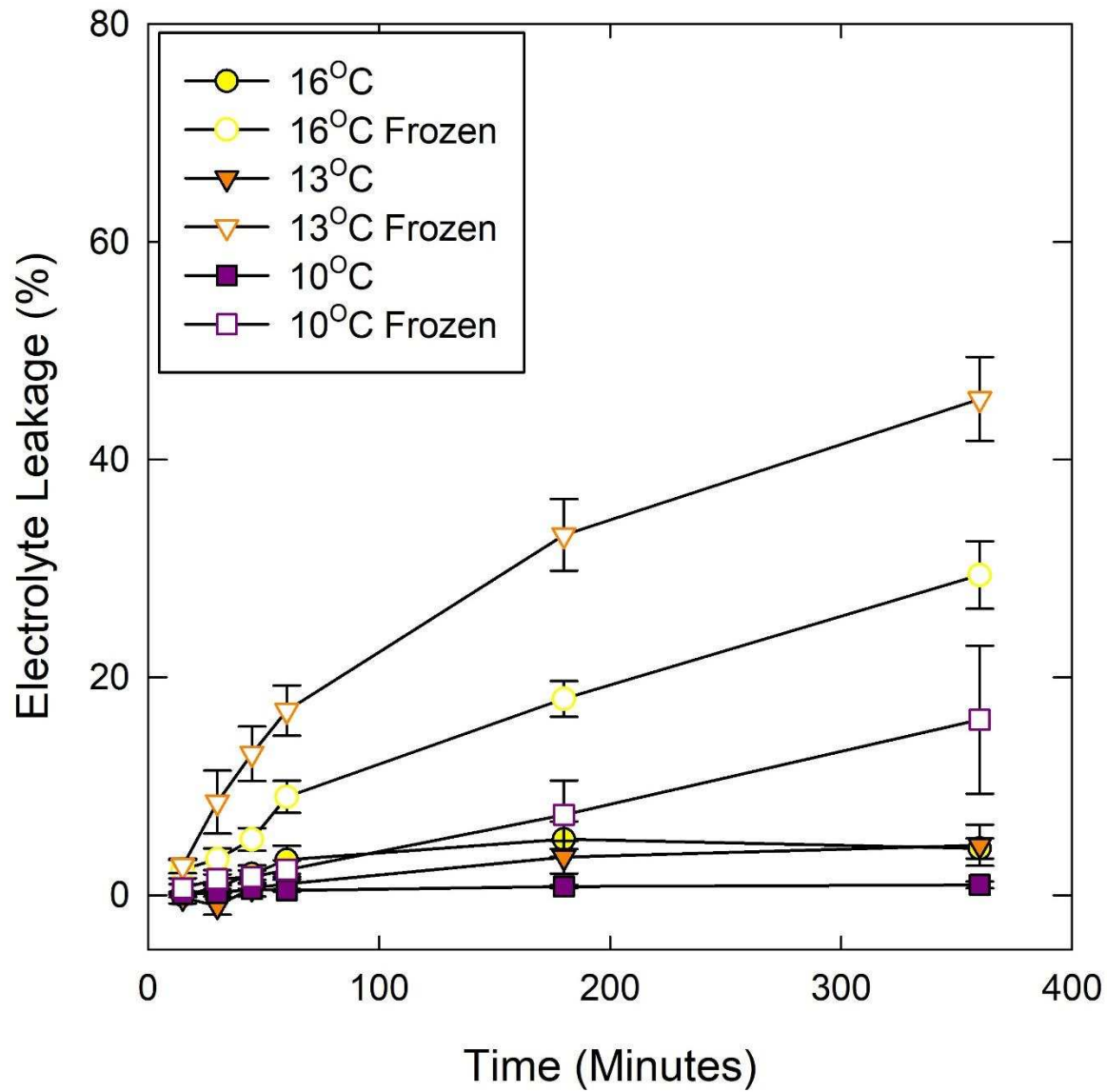


Figure A2-1. Electrolyte leakage of frozen (open symbols) and unfrozen (full symbols) leaves over time. Color and shape corresponds to the common garden location the samples were collected from. Growing Season Temperature ($^{\circ}\text{C}$) is also provided for each site.

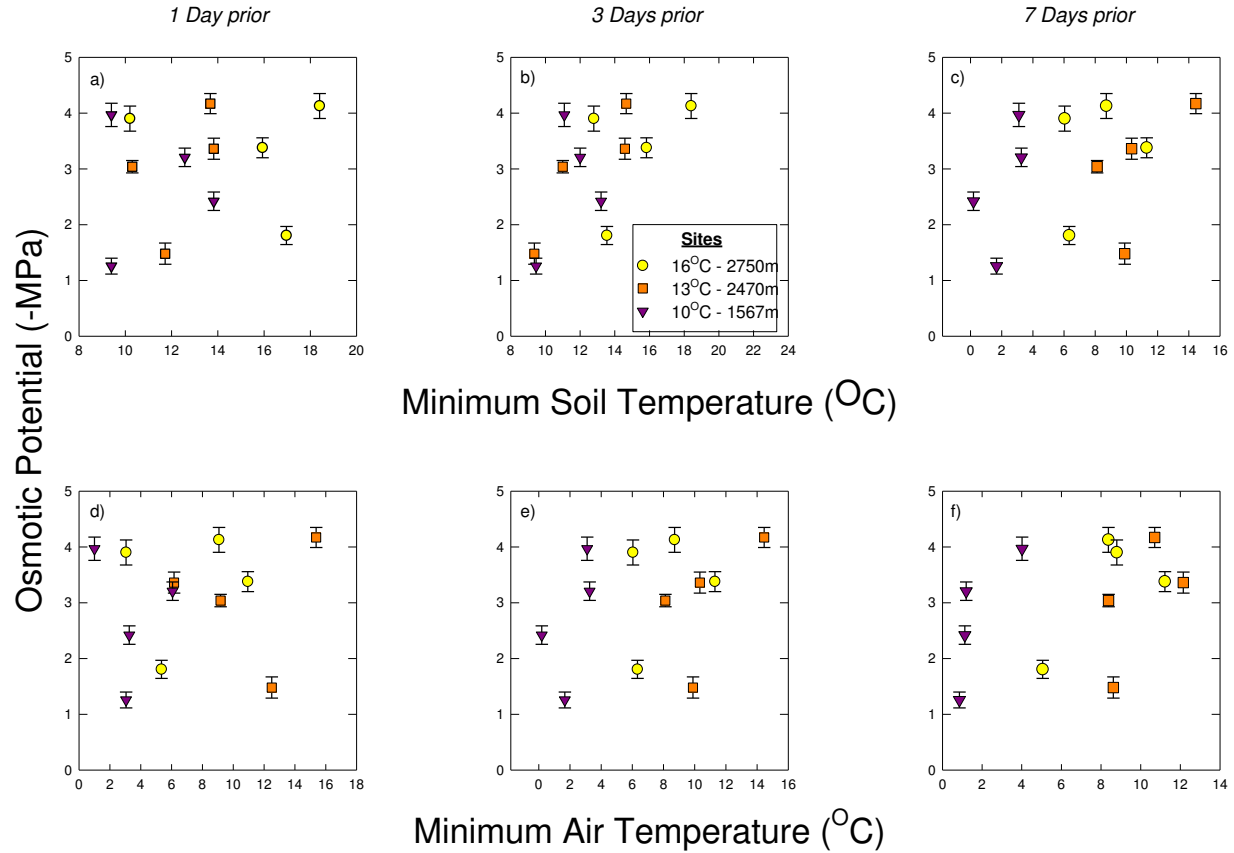


Figure A2-2. Air and soil minimum temperatures for each site 1,3, and 7 days prior to sampling versus osmotic potential (Ψ_{osm}) over the growing season. Bars denote 1 standard error.

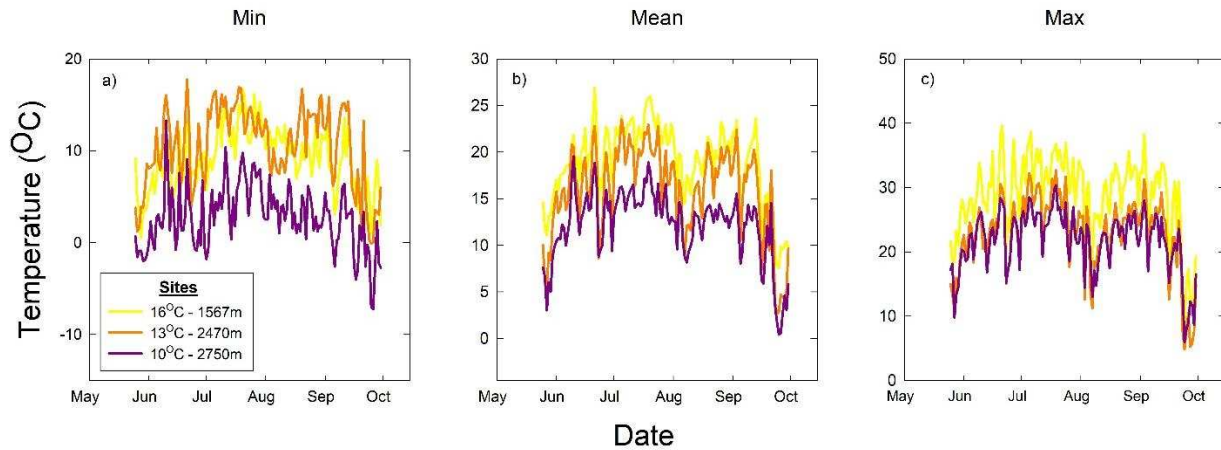


Figure A2-3. Daily minimum, mean, and maximum air temperature values at each common garden 6/1-9/30 2017. Colors correspond to each common garden.