VARIATION IN MORPHOLOGICAL, PHENOLOGICAL, AND PHYSIOLOGICAL TRAITS AMONG SOUTHWESTERN PONDEROSA PINE PROVENANCES: INSIGHTS FROM FIELD AND GREENHOUSE COMMON GARDENS

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ABSTRACT

VARIATION IN MORPHOLOGICAL, PHENOLOGICAL, AND PHYSIOLOGICAL TRAITS AMONG SOUTHWESTERN PONDEROSA PINE PROVENANCES: INSIGHTS FROM FIELD AND GREENHOUSE COMMON GARDENS

AALAP HIMANSHUBHAI DIXIT

Ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests of the southwestern U.S. are threatened by climate change. Drought-related tree mortality is already a matter of concern in southwestern ponderosa pine forests and this mortality is expected to intensify over the next century as atmospheric temperature and drought severity increase. In addition, droughts and wildfires have caused a reduction in seedling regeneration and establishment. These recent losses of southwestern ponderosa pine forests may be compensated through artificial regeneration by planting drought tolerant seed sources under increasingly arid conditions. We used field and greenhouse common gardens to investigate provenance variations in survival, growth, budburst phenology, and drought-adapted morphological and physiological traits in ponderosa pines seedlings. Twenty-one provenances from a range of elevations across Arizona and New Mexico were planted in three field common gardens across an elevation gradient: low elevation site in pinyon juniper woodland (1930 m), mid elevation site in ponderosa pine forest (2200 m), and high elevation site in aspen and mixed conifer forest (2780 m). Ten out of the 21 provenances from different elevations were planted in a greenhouse common garden at the Northern Arizona University Greenhouse Facility in Flagstaff, Arizona. We evaluated the overall hypothesis that seedlings traits would vary with provenance environmental characteristics and that low elevation, warmer provenances would have traits more conducive to dry and warm conditions than high elevation, cooler provenances. Results from field common gardens suggest that seedling survival and performance depends on planting location and that low elevation provenances should be considered for planting at trailing-edge and core sites. The importance of biotic agents of seedling mortality increased with elevation of the planting site suggesting that high mortality from biotic agents should be anticipated for plantings at high elevations during assisted migration. Results suggest adaptation of low elevation provenances to warm spring temperatures due to earlier budburst of provenances from low elevations under field and greenhouse conditions. Results from the mid-elevation site suggested a trade-off between growth and water use efficiency under unusually dry conditions. Results from greenhouse common garden suggest: adaptation of low elevation provenances to aridity (as indicated by a lower specific leaf area); faster growth of provenances from wet locations; and greater allocation to roots in western provenances. Such information about environmental and geographical patterns of provenance variation may be useful for developing specific seed transfer guidelines and effective assisted migration strategies to maintain ponderosa pine in a changing climate.

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Dedicated

To

My wife: Kelsey Louise Dixit

And my entire family

Preface

This dissertation consists of a general introduction (chapter 1), four data-based chapters (chapters 2-5), and conclusions and management implications (chapter 6). The data chapters are written in format intended for publication in peer-reviewed journals which might have led to some redundancy between chapters. In all data chapters, the use of pronoun “we” instead of “I” denotes multiple authors for each publication. The first data chapter, titled “Variation in Seedling Budburst Phenology and Structural Traits Among Southwestern Ponderosa Pine Provenances”, is formatted for the *Canadian Journal of Forest Research*. The second data chapter, “Provenance geographical and climatic characteristics influence budburst phenology of southwestern ponderosa pine seedlings”, is formatted for the journal *Forests*. The third data chapter, “Variation in survival, growth, and carbon isotope discrimination among southwestern ponderosa pine provenances in common gardens across an elevational gradient”, is formatted for *Forest Ecology and Management*. The fourth data chapter, “Relationship Between Growth Rate and Water Use Efficiency of Ponderosa Pine Seedlings Under Dry Field Conditions”, is formatted for *Tree Physiology*.

Chapter I: Introduction

The persistence of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests in the southwest United States (US) is threatened due to a changing climate. In the southwest US, these forecasted climatic changes are likely to include increases in air temperature, severe droughts, and large wildfires (Liu et al. 2010; Williams et al. 2012; Garfin et al. 2013). Over the past few decades and in its current state, ponderosa pine forests have experienced high levels of mortality due to a combination of abiotic and biotic stressors such as droughts, wildfires, and bark beetle attacks (Williams et al. 2010; Hicke et al. 2016). These disturbances coupled with a warming climate have negatively impacted natural regeneration and establishment of ponderosa pine in the southwest (Puhlick et al. 2012; Davis et al. 2019; Rodman et al. 2020).

Climate change may cause maladaptation of tree populations from local environmental conditions to which they were historically adapted (Kremer et al. 2012). These populations will need to adapt or migrate to these changes to avoid extirpation (Aitken et al. 2008; Williams et al. 2010). Due to the climate change induced maladaptation, southwestern ponderosa pine provenances from lower elevation and warmer conditions experienced higher mortality than provenances from higher elevations and cooler conditions (McDowell et al. 2009; Negron et al. 2009), a pattern shown by many tree species around the world (Anderegg et al. 2019). However, these low-elevation, warmer and drier provenances may be useful for planting at higher elevations or more northern latitudes due to recent evolution of these provenances under warmer and drier conditions and the climate at higher elevations or northern latitudes may be more suitable for these provenances as climate warming continues (Rehfeldt et al. 2014; Kolb et al. 2019; Gomez-Pineda et al. 2020).

The genetic basis of population variation in drought tolerance for conifers is still unclear (Moran et al. 2017). This is particularly true for southwestern ponderosa pine where the taxonomic classification is currently being reconsidered (Willyard et al. 2017). In ponderosa pine, previous common garden studies did not include trailing-edge provenances (Rehfeldt 1993), were limited in geographical scope in sampling of provenances (Kolb et al. 2016) or were carried out on trees planted under cooler and wetter conditions in the past (DeWald and Mahalovich 2008). Based on climate envelope models of southwestern ponderosa pine, Rehfeldt et al. (2014) recommended using seed sources of provenances 220-400 m lower in elevation than the location of the planting site. Such movement may have a positive impact as lower elevation provenances may be more drought tolerant and help in mitigating negative impacts of climate warming or have a negative impact due to early budburst causing spring frost damage as suggested by Grady et al. (2015).

In this dissertation, we used field and greenhouse common gardens to investigate provenance variations in survival, growth, budburst phenology, and drought-adapted morphological and physiological traits in ponderosa pines seedlings. We planted seedlings from 21 southwestern ponderosa pine provenances from a range of elevations across Arizona and New Mexico in three field common gardens. The field sites were planted across an elevation gradient at a low elevation site in pinyon juniper woodland (1930 m), a mid-elevation site in ponderosa pine forest (2200 m), and a high elevation site in aspen and mixed conifer forest (2780 m) in July 2018. We planted 10 out of the 21 provenances from different elevations in a greenhouse common garden at the Northern Arizona University Greenhouse Facility in Flagstaff, Arizona in February 2018. We evaluated the overall hypothesis that seedlings traits would vary with provenance environmental characteristics and that low elevation, warmer provenances would have traits more conducive to dry and warm conditions than high elevation, cooler provenances.

In chapter II, we conducted a greenhouse common garden study to investigate provenance variation in spring budburst phenology, growth, and structural traits of ponderosa pine seedlings from 10 provenances of different elevations from Arizona and New Mexico. Seedlings were grown under resource rich conditions at the Northern Arizona University greenhouse facility. We measured the date at which new needles emerged (budburst date) at the start of the second growing season, and seedling growth, biomass, and specific leaf area at the end of the second growing season to determine if traits varied with provenance elevation and if provenances from lower elevations had traits more conducive to drought and heat adaptation than higher elevation provenances.

In chapter III, we examined provenance variation in budburst phenology of ponderosa pine seedlings using a field common garden study. We used seedlings from 21 provenances of different elevations from Arizona and New Mexico that were planted in a ponderosa pine dominated field site in northern Arizona in July 2018. Field budburst was monitored weekly on all seedlings in spring of 2019 to determine if budburst varied among provenances and if low elevation provenances broke bud sooner due to adaptation to warm spring temperatures. We compared field budburst with budburst timing of the same provenances measured under greenhouse conditions in chapter II to determine if differences in budburst timing among provenances are consistent for seedlings grown under greenhouse and field environments.

In chapter IV, we investigated provenance variation in survival, growth, carbon isotope discrimination, and leaf nitrogen concentration of ponderosa pine seedlings from 21 provenances using common gardens across an elevational gradient to evaluate adaptation to environmental stress. We planted the seedlings in three common gardens at a high elevation site in aspen and mixed conifer forest, a mid-elevation site in ponderosa pine forest, and a low elevation site in pinyon juniper woodland. We measured seedling survival, growth, mortality agents, carbon isotope discrimination, and leaf nitrogen over the first two years after planting in 2018 to determine the impacts of planting site, provenance, and the interaction between planting site and provenance on the measured traits and to determine the relationship between provenance environmental characteristics and field performance.

In chapter V, we measured seedling growth, leaf level gas exchange, carbon isotope discrimination, leaf nitrogen concentration and tip moth damage on ponderosa pine seedlings from 21 provenances planted at a ponderosa pine dominated field site in northern Arizona. Seedling growth was measured for three years (2018-2020), leaf level gas exchange was measured in June 2020, and carbon isotope discrimination and leaf nitrogen concentration were measured on needles developed in the year 2020, to determine if low-elevation, drier, and warmer provenances had traits more conducive to drought tolerance than high-elevation, wetter, and cooler provenances.

References

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1, 95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Anderegg, W.R.L., Anderegg, L.D.L., Kerr, K.L., Trugman, A.T., 2019. Widespread drought-induced tree mortality at dry range edges indicates that climate stress exceeds species' compensating mechanisms. Global Change Biology 25, 3793–3802. https://doi.org/10.1111/gcb.14771

Davis, K.T., Dobrowski, S.Z., Higuera, P.E., Holden, Z.A., Veblen, T.T., Rother, M.T., Parks, S.A., Sala, A., Maneta, M.P., 2019. Wildfires and climate change push low-elevation forests across a critical climate threshold for tree regeneration. Proceedings of the National Academy of Sciences 116, 6193–6198. https://doi.org/10.1073/pnas.1815107116

DeWald, L.E., Mahalovich, M.F., 2008. Historical and contemporary lessons from ponderosa pine genetic studies at the Fort Valley Experimental Forest, Arizona. In: Olberding, Susan D., and Moore, Margaret M., tech. coords. Fort Valley Experimental Forest-A Century of Research 1908-2008. Conference Proceedings; August 7-9, 2008; Flagstaff, AZ. Proc. RMRS-P-55. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 150-155 055.

Garfin, G., Jardine, A., Feldman, D.L., 2013. Overview, in: Garfin, G., Jardine, A., Merideth, R., Black, M., LeRoy, S. (Eds.), Assessment of Climate Change in the Southwest United States: A Report Prepared for the National Climate Assessment, NCA Regional Input Reports. Island Press/Center for Resource Economics, Washington, DC, pp. 21–36. https://doi.org/10.5822/978-1-61091-484-0_2

Gómez-Pineda, E., Sáenz-Romero, C., Ortega-Rodríguez, J.M., Blanco-García, A., Madrigal-Sánchez, X., Lindig-Cisneros, R., Lopez-Toledo, L., Pedraza-Santos, M.E., Rehfeldt, G.E., 2020. Suitable climatic habitat changes for Mexican conifers along altitudinal gradients under climatic change scenarios. Ecological Applications 30, e02041. https://doi.org/10.1002/eap.2041

Grady, K.C., Kolb, T.E., Ikeda, D.H., Whitham, T.G., 2015. A bridge too far: Cold and pathogen constraints to assisted migration of riparian forests. Restoration Ecology 23, 811–820. https://doi.org/10.1111/rec.12245

Hicke, J.A., Meddens, A.J.H., Kolden, C.A., 2016. Recent tree mortality in the western United States from bark beetles and forest fires. Forest Science 62, 141–153. https://doi.org/10.5849/forsci.15-086

Kolb, T.E., Dixit, A.H., Burney, O., 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern United States. Tree Planters' Note 62, 104–112.

Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A., 2016. Local-scale drought adaptation of ponderosa pine seedlings at habitat ecotones. Forest Science 62, 641–651. https://doi.org/10.5849/forsci.16-049

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S., Schueler, S., 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters 15, 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

Liu, Y., Stanturf, J., Goodrick, S., 2010. Trends in global wildfire potential in a changing climate. Forest Ecology and Management, Adaptation of Forests and Forest Management to Changing Climate 259, 685–697. https://doi.org/10.1016/j.foreco.2009.09.002

McDowell, N.G., Allen, C.D., Marshall, L., 2009. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. Global Change Biology 16, 399–415. https://doi.org/10.1111/j.1365-2486.2009.01994.x

Moran, E., Lauder, J., Musser, C., Stathos, A., Shu, M., 2017. The genetics of drought tolerance in conifers. New Phytologist 216, 1034–1048. https://doi.org/10.1111/nph.14774

Negrón, J.F., McMillin, J.D., Anhold, J.A., Coulson, D., 2009. Bark beetle-caused mortality in a drought-affected ponderosa pine landscape in Arizona, USA. Forest Ecology and Management 257, 1353–1362. https://doi.org/10.1016/j.foreco.2008.12.002

Puhlick, J.J., Laughlin, D.C., Moore, M.M., 2012. Factors influencing ponderosa pine regeneration in the southwestern USA. Forest Ecology and Management 264, 10–19. https://doi.org/10.1016/j.foreco.2011.10.002

Rehfeldt, G.E., 1993. Genetic Variation in the Ponderosae of the Southwest. American Journal of Botany 80, 330–343. https://doi.org/10.2307/2445357

Rehfeldt, G.E., Jaquish, B.C., Sáenz-Romero, C., Joyce, D.G., Leites, L.P., Bradley St Clair, J., López-Upton, J., 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Reforestation. Forest Ecology and Management 324, 147–157. https://doi.org/10.1016/j.foreco.2014.02.040

Rodman, K.C., Veblen, T.T., Chapman, T.B., Rother, M.T., Wion, A.P., Redmond, M.D., 2020. Limitations to recovery following wildfire in dry forests of southern Colorado and northern New Mexico, USA. Ecological Applications 30, e02001. https://doi.org/10.1002/eap.2001

Williams, A.P., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., Cook, E.R., Gangodagamage, C., Cai, M., McDowell, N.G., 2012. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change 3, 292–297. https://doi.org/10.1038/nclimate1693

Williams, A.P., Allen, C.D., Millar, C.I., Swetnam, T.W., Michaelsen, J., Still, C.J., Leavitt, S.W., 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Sciences 107, 21289–21294. https://doi.org/10.1073/pnas.0914211107

Willyard, A., Gernandt, D.S., Potter, K., Hipkins, V., Marquardt, P., Mahalovich, M.F., Langer, S.K., Telewski, F.W., Cooper, B., Douglas, C., Finch, K., Karemera, H.H., Lefler, J., Lea, P., Wofford, A., 2017. *Pinus ponderosa*: A checkered past obscured four species. American Journal of Botany 104, 161–181. https://doi.org/10.3732/ajb.1600336

Chapter II: Variation in Seedling Budburst Phenology and Structural Traits Among Southwestern Ponderosa Pine Provenances

Abstract

We used a common garden study to investigate genetic variation in spring budburst phenology, growth, and structural traits of ponderosa pine seedlings from 10 Arizona and New Mexico provenances from different elevations. Seedling were grown with ample resources for two growing seasons in a greenhouse in northern Arizona. Budburst date was measured at the onset of the second growing season; seedling growth, biomass, biomass ratios (shoot/root ratio, root weight ratio, stem weight ratio, leaf weight ratio), and specific leaf area were measured at the end of the second season. Low-elevation provenances (< 2000 m) had earlier budburst and lower specific leaf area than middle- (2000-2500 m) and high-elevation (> 2500 m) provenances. Height, leaf length, biomass, and biomass ratios were similar for elevational groups. Total biomass was positively correlated (r=0.824) with provenance mean annual precipitation. Shoot/root ratio was positively correlated (r=0.652) with longitude. Results suggest adaptation of low-elevation provenances to warm spring temperatures (early budburst) and aridity (low specific leaf area); inherently faster growth of provenances from wet locations; and greater allocation to shoots by eastern provenances. Such information about geographic patterns of genetic variation may be useful for selecting seed sources for planting in a changing climate.

Keywords: Climate change, drought, genetic variation, phenology, *Pinus ponderosa*

Introduction

Climate warming and more frequent extreme events such as long droughts and large severe wildfires have been predicted for the future in the southwestern US (Liu et al. 2010, Williams et al. 2012). Ponderosa pine (*Pinus ponderosa* var. *scopulorum* Lawson and C. Lawson) forests of the southwestern US have experienced unusually high amounts of deforestation in the last several decades due to interactive effects of climate warming, drought, wildfires, and bark beetle attacks (Hicke et al. 2016), which are expected to intensify in the future. In the southwestern US, droughts often reduce establishment of ponderosa pine seedlings (Puhlick et al. 2012, Savage et al. 2013), especially following high severity fires (Davis et al. 2019, Rodman et al. 2019). Consequently, the current range of ponderosa pine is expected to shrink in the southwestern US (Rehfeldt et al. 2006) if the loss of mature trees is not compensated by successful regeneration (Bell et al. 2014).

Climate change is causing maladaptation of some tree populations, which are thought to be adapted to past local environmental conditions (Kremer et al. 2012). For example, recent mortality of ponderosa pine in the southwestern US is greater at the low-elevation warm edge of its range than at the higher-elevation cold edge (McDowell et al. 2009, Negron et al. 2009), a pattern shown by many tree species globally (Anderegg et al. 2019). Constraints on ponderosa pine regeneration imposed by climatic and environmental stresses are expected to increase throughout its range over the next century (Petrie et al. 2017). Efforts to promote future ponderosa pine regeneration during increasing aridity include the planting of drought-adapted seed sources, including the assisted migration of such sources (Williams and Dumrose 2013, Rehfeldt et al. 2014).

The magnitude and genetic basis of intra-specific differences in drought tolerance are poorly understood for conifers (Moran et al. 2017). This is particularly true for ponderosa pine in the southwestern US, where its taxonomic classification is currently being reconsidered (Willyard et al. 2017). Previous common garden tests of ponderosa pine in the southwestern US did not include low-elevation trailing-edge provenances (Rehfeldt 1993) or were limited in geographical scope to comparisons of seed sources from a small region (Kolb et al. 2016). Low-elevation trailing-edge provenances may be more drought-adapted and thus particularly valuable for future plantings (Alberto et al. 2013, Kolb et al. 2019). For ponderosa pine in the southwestern US, Rehfeldt et al. (2014) recommended, based on climate-envelop models of populations, the planting of seed sources from 220 to 400 m lower in elevation than the planting site to help mitigate detrimental impacts of climate warming. Such movement of low-elevation populations, however, may have negative impacts such as spring frost damage due to early budburst (Grady et al. 2015). Consequently, we need more information about genetic variation in drought adaptive and phenological traits for southwestern ponderosa pine in order to inform efforts to mitigate future negative impacts of increasing aridity by seed source selection, and to revise seed transfer guidelines (Schubert and Pitcher 1973).

We used a greenhouse common garden study to investigate variation in seedling budburst phenology, growth, and structural traits among provenances of ponderosa pine from Arizona and New Mexico. The sampled provenances vary in elevation, temperature and precipitation, and consequently allowed us to investigate fine-grained patterns of genetic variation. We evaluated the hypothesis that seedling traits would vary with provenance elevation, and that low elevation provenances would have traits more conducive to warm temperatures and drought adaptation than provenances from high elevations. We focused on the structural traits of specific leaf area and seedling biomass ratios that assess growth allocation, such as shoot-root ratio. Low specific leaf area (SLA) and shoot-root ratio are potentially important drought adaptive traits as low SLA indicates thicker and denser leaves leading to conservation of acquired resources (Ackerly et al. 2002) and low shoot-root ratio indicates higher growth allocation to roots which might prolong survival under drought conditions (Cregg 1994).

Materials and Methods

Provenance Locations

We obtained seeds of ponderosa pine from collections at the Northern Arizona University Greenhouse Facility in Flagstaff, Arizona and the John T. Harrington Forestry Research Center in Mora, New Mexico. The collection years ranged from 1981 to 2012. The seeds were stored at -10 ^{o}C until they were sown. We used seeds from 10 provenances from a wide range in elevation (Figure 2.1). Each provenance was represented by seed from three to five mother trees. We did not consider within-provenance variation in this study because several provenances were represented by pooled seed collections without identification of mother trees.

The provenances varied in environmental characteristics including mean annual temperature (MAT) and mean annual precipitation (MAP). We obtained climatic information (30-year normal, 1981-2010) for each provenance from PRISM (Table 2.1) (www.prism.oregonstate.edu/normals/). MAT ranges among provenances between 5.7 °C at Green’s Peak and 14.3 °C at Cherry Road; MAP ranges between 364 mm at HH Ranch and 793 mm at Rim District; elevation ranges between ~1600 m at Cherry Road and ~2800 m at Mount Taylor. Provenance elevation was negatively correlated with MAT ($r=-0.979$, $p<0.0001$) and had a weak positive trend with MAP ($r=0.528$, $p=0.116$); MAT had a weak negative trend with MAP (r=-0.490, p=0.150). Longitude had a weak positive trend with elevation (r=0.491, p=0.149) and weak negative trend with MAT (-0.526, p=0.118).

Greenhouse Environment

We grew 80 seedlings from each of the 10 provenances (800 total) between February 2018 and June 2019 in the Northern Arizona University Greenhouse Facility. In February 2018, we surface-sterilized seeds using 10% bleach solution and planted two seeds per container. In cases when two seeds germinated in a container, one was clipped or transplanted to an empty container. Seedling containers were placed in 40 racks (30.5 cm x 37.6 cm each), with each rack containing 20 seedlings from the same provenance. One rack of seedlings from each provenance was placed together in a group on half of a greenhouse bench, resulting in four groups, each containing 10 racks. All four groups were located on two adjacent benches (4.5 m^2 each) in the same greenhouse. We rotated the position of racks within each group, and groups over the two benches once a month to minimize potential effects of location in the greenhouse.

The greenhouse environment, and water, fertilization and growth schedules were intended to produce fast seedling growth with little stress. For the first 11 months, we used D-40 containers (655.4 cm^3 volume, 6.3 cm diameter, 25.4 cm depth), and then transplanted seedlings to D-60 containers (983.2 cm^3 volume, 6.3 cm diameter and 35.5 cm cell depth) at the start of the second growing season in January 2019 to provide additional space for root growth. The soil medium consisted of one-part shredded peat moss to two parts vermiculite. We watered seedlings to saturation three times each week, except during the winter dormancy period (mid-November through mid-January) when the seedlings were watered once per week. Also, we fertilized seedlings twice per week with 20-20-20 NPK solution during the growing period as part of the watering. However, during the winter dormancy period, fertilization stopped. Air temperature in the greenhouse ranged from 21 °C to 26 °C, except during the winter dormancy months (mid-November through mid-January) when the greenhouse temperature ranged between 5 and 10 °C.

Budburst Phenology

We assessed budburst on all seedlings (40 racks; 800 seedlings) between January and February 2019 twice per week by visual assessment of budburst stage. We used six budburst stages following Martinez-Berdeja et al. (2019) (Supplementary Figure 2.1).

Seedling Biomass and Structural Traits

We destructively sampled seedlings from two groups (20 racks; 10 seedlings from each rack) at the end of second growing season, which occurred in early June 2019 because seedlings broke bud and started growing in early February. We selected seedlings for sampling from a rack equally over mother trees for provenances where we had mother-tree information; for the other provenances, we randomly selected seedlings for sampling from a rack. Leaves, stems, and roots were separated, washed, oven-dried at 70° C for 48 h, and then weighed using an analytical balance (Ohaus Explorer; Ohaus Corporation, Parsippany, NJ). Height, total biomass, root weight ratio (RWR=root biomass/total biomass), stem weight ratio (SWR=stem biomass/total biomass), leaf weight ratio (LWR=leaf biomass/total biomass) and shoot-root ratio (S/R=leaf + stem biomass/root biomass) were measured or calculated for each sampled seedling.

We measured needle length and specific leaf area (leaf area/leaf mass) on seedlings from two groups (20 racks; 10 seedlings from each rack) on two fascicles produced in the first flush of the second growing season. For specific leaf area, we used WinFOLIA (Regent Instruments) and a flat-bed scanner (Epson 4990) to measure projected area of each fascicle, followed by measurement of oven-dry weight.

Data Analysis

The primary objective of the analysis was to evaluate the influence of provenance elevation on budburst timing, growth, and structural traits. To assess the role of elevation, we combined provenances into three elevation groups for analysis (Table 2.1). The low elevation group consisted of four provenances with an elevation below 2000 m. The middle elevation group consisted of three provenances with an elevation between 2000 and 2500 m. The high elevation group included three provenances with an elevation above 2500 m. We used analysis of variance (ANOVA) to test for differences among elevation groups. These analyses were conducted on rack-level means to avoid pseudoreplication because individual seedlings were the observational units and racks were the experimental units. Thus, the sample size for ANOVA was 40 rack-level means for budburst, and 20 rack-level means for all other measurements. All data approximated a normal distribution. We used Tukey HSD tests to show significant differences among elevation group means. We defined budburst as the Julian date when new needles emerged from the terminal bud (stage 5; Supplementary Figure 2.1). Secondly, we explored relationships between seedling traits and environmental characteristics of provenance locations with correlation and regression analyses on provenance means ($n = 10$). JMP Pro version 14 was used for all the analyses (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989-2019).

Results

Budburst Phenology

Budburst date differed significantly among elevation groups ($p<0.05$) (Table 2.2; Figure 2.2). Mean budburst was about two days earlier for low elevation provenances (41.9 days) than for middle (43.6 days) and high elevation provenances (44.0 days). Mean budburst date of provenances was significantly ($p<0.05$) correlated with two provenance characteristics: a positive correlation with elevation ($r=0.78$) and a negative correlation with MAT ($r=-0.77$) (Table 2.3; Figure 2.3).

Seedling Biomass and Structural Traits

Of all structural traits, only SLA differed significantly ($p<0.05$) among elevation groups (Table 2.2). SLA was lower for low elevation provenances than middle- and high-elevation provenances (Figure 2.4). Correlation analysis revealed several significant relationships between mean provenance traits and environmental characteristics (Table 2.3). Total biomass of provenances was significantly correlated ($r=0.82$; $p=0.003$) with MAP (Figure 2.5). Provenance longitude was positively correlated with S/R and LWR ($r=0.652$; $p=0.041$, $r=0.677$; $p=0.031$, respectively) and negatively correlated ($r=-0.648$; $p=0.042$) with RWR (Figure 2.6). Provenance SLA showed a positive trend ($r=0.631$; $p=0.059$) with elevation (Table 2.3; Supplementary Figure 2.2).

Discussion

We investigated variation in seedling budburst timing, growth, and structural traits among elevation groups of southwestern provenances of ponderosa pine in a greenhouse common garden with ample resources. Although our study did not investigate all possible controls over seedling traits (e.g., maternal effects, epigenetic influences), the differences we observed in a common greenhouse environment suggest genetic differences among elevation groups for some seedling traits relevant to climatic adaptation. In addition, correlations between traits and environmental characteristics of provenances revealed unexpected geographic patterns of genetic variation beyond elevation.

We hypothesized that traits would differ among elevation groups because the recent evolution of low elevation provenances was under warmer and drier conditions than higher elevation provenances, and because tree populations are assumed to be locally adapted (Alberto et al. 2013). Our results partly support this hypothesis as we found significant differences among elevation groups in budburst timing and SLA, but not for biomass growth or biomass ratios. The pattern of differences among elevational groups in budburst timing and SLA is consistent with local adaptation of low elevation provenances to an earlier start to the growing season and more aridity compared with higher elevation provenances. Overall, our results are consistent with earlier reports by Rehfeldt (1993) of genetic differentiation among southwestern populations of ponderosa pine that differ in elevation.

Our finding of earlier budburst of low elevation provenances compared with higher elevation provenances of ponderosa pine in a common garden experiment differs from a recent report of no significant difference among California ponderosa pine provenances from different elevations in field common gardens (Martinez-Berdeja et al. 2019). We found a difference in mean budburst of two days between low elevation and high elevation provenances under greenhouse conditions. In order to understand if this difference is ecologically significant, additional studies are required to assess whether this difference in the greenhouse is muted, maintained, or amplified under field conditions. While early budburst is a likely adaptation to warm spring temperatures at low elevations, it could have positive or negative impacts when provenances from low elevation and high MAT are moved to higher elevations during assisted migration. The impact could be positive if early budburst of low elevation provenances allows them to take advantage of warming spring temperatures when planted at a higher elevation.

Alternatively, early budburst of low elevation provenances may predispose them to spring frost damage when moved to high elevation sites (Grady et al. 2015).

The low SLA of low-elevation provenances suggests greater drought tolerance compared with high-elevation provenances. Wright et al (2004) considered leaf dry mass/area (LMA; inverse of SLA) to be a key trait in the leaf economics spectrum, with species inhabiting dry environments having lower SLA. In evergreen species, low SLA and associated traits such as thicker leaf blade and denser tissue are associated with longer leaf survival under arid conditions (Wright et al. 2004). Our finding of differences in SLA among ponderosa pine provenances differs from previous reports of little variation in SLA in common-garden investigations of provenances of the Rocky Mountain variety of ponderosa pine (var. *scopulorum*) from a wide area from New Mexico to South Dakota (Cregg 1993, Cregg 1994). Additional research is needed to assess the importance of genetic variation in SLA to drought tolerance for southwestern provenances of ponderosa pine.

We also found an unexpected longitudinal pattern of variation where provenances from Arizona had lower S/R, lower LWR, and higher RWR as compared to provenances from New Mexico. This longitudinal gradient may be due to genetic variation among provenances, adaptation to differences in the seasonality of precipitation between Arizona and New Mexico, or may have been driven by other factors correlated with longitude. This pattern suggests a difference in seedling architecture between the provenances we included from Arizona and those from New Mexico that may be relevant to drought tolerance. While low S/R might be assumed to be best for surviving drought, Cregg (1994) reported a non-linear relationship between S/R and survival of ponderosa pine provenances, with the longest survival at intermediate values of S/R.

Our finding of a positive association between provenance MAP and seedling biomass has implications for selection of seed sources for future plantings with increasing aridity. Slow-growing provenances of ponderosa pine may be inherently more drought tolerant. Kerr et al. (2015), for example, reported a trade-off between growth rate and drought resistance in a study of several populations of ponderosa pine in Oregon. We found that provenances from areas with higher precipitation had greater seedling biomass growth than provenances from low precipitation areas when all seedlings were grown at near maximum growth rates in the greenhouse, a pattern consistent with an earlier study of ponderosa pine provenances grown in outdoor nurseries (Rehfeldt 1993). Assuming a trade-off between growth rate and drought resistance, our results suggest that maximum growth rate in resource-rich environments might be used to indirectly select seed sources for drought tolerance. This idea, however, needs to be rigorously tested with additional investigations of physiological and survival responses of ponderosa pine provenances to drought.

In summary, our results provide evidence of genetic differences between low and high elevation provenances of southwestern ponderosa pine consistent with local climatic adaptation. Additionally, we found evidences of longitudinal and growth patterns as populations from western (lower S/R) and drier parts (lower total biomass) showed traits conductive to drought tolerance as compared to populations from eastern and moister parts of the southwest region. Our finding that populations from low elevation, warm sites have low SLA, an indirect trait of drought tolerance, supports earlier recommendations that low elevation provenances of ponderosa pine should be planted at higher elevations in reforestation projects to mitigate negative impacts of future increasing aridity (Rehfeldt et al. 2014b). We caution, however, that this recommendation should be evaluated further with studies under natural field conditions to assess the extent to which early budburst of low elevation populations predisposes them to spring frost damage. Additional studies also should focus on provenance performance in field plantings over gradients of elevation and water availability, as well as on genetic controls over drought-tolerance mechanisms.

References

Ackerly, D., Knight, C., Weiss, S., Barton, K., Starmer, K. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia 130: 449–457. https://doi.org/10.1007/s004420100805

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O. 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology 19: 1645–1661. https://doi.org/10.1111/gcb.12181

Anderegg, W.R.L., Anderegg, L.D.L., Kerr, K.L., Trugman, A.T. 2019. Widespread drought-induced tree mortality at dry range edges indicates that climate stress exceeds species' compensating mechanisms. Global Change Biology 25: 3793–3802. https://doi.org/10.1111/gcb.14771

Bell, D.M., Bradford, J.B., Lauenroth, W.K. 2014. Early indicators of change: divergent climate envelopes between tree life stages imply range shifts in the western United States. Global Ecology and Biogeography 23: 168–180. https://doi.org/10.1111/geb.12109

Cregg, B.M. 1993. Seed-source variation in water relations, gas exchange, and needle morphology of mature ponderosa pine trees. Canadian Journal of Forest Research 23: 749–755. https://doi.org/10.1139/x93-098

Cregg, B.M. 1994. Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. Tree Physiology 14: 883–898. https://doi.org/10.1093/treephys/14.7-8-9.883

Davis, K.T., Dobrowski, S.Z., Higuera, P.E., Holden, Z.A., Veblen, T.T., Rother, M.T., Parks, S.A., Sala, A., Maneta, M.P. 2019. Wildfires and climate change push low-elevation forests across a critical climate threshold for tree regeneration. Proceedings of the National Academy of Sciences U.S.A. 116: 6193–6198. https://doi.org/10.1073/pnas.1815107116

Grady, K.C., Kolb, T.E., Ikeda, D.H. and Whitham, T.G. 2015. A bridge too far: cold and pathogen constraints to assisted migration of riparian forests. Restoration Ecology 23: 811-820. doi:10.1111/rec.12245

Hicke, J.A., Meddens, A.J., & Kolden, C.A. 2016. Recent tree mortality in the western United States from bark beetles and forest fires. Forest Science 62(2): 141-153. https://doi.org/10.5849/forsci.15-086

Kerr, K.L., Meinzer, F.C., McCulloh, K.A., Woodruff, D.R., and Marias, D.E. 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. Tree physiology 35(5): 535-548. https://doi.org/10.1093/treephys/tpv034

Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A. 2016. Local-scale drought adaptation of ponderosa pine seedlings at habitat ecotones. Forest Science 62(6): 641-651. https://doi.org/10.5849/forsci.16-049

Kolb, T.E., Dixit, A.H., Burney O. 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern U.S. Tree Planters' Notes 62: 104-112.

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S., Schueler, S. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters 15: 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

Liu, Y., Stanturf, J., Goodrick, S. 2010. Trends in global wildfire potential in a changing climate. Forest Ecology and Management 259: 685-697. https://doi.org/10.1016/j.foreco.2009.09.002

Martinez-Berdeja, A., Hamilton, J., Bontemps, A., Schmitt, J., Wright, J. 2019. Evidence for population differentiation among Jeffrey and Ponderosa pines in survival, growth and phenology. Forest Ecology and Management. 434: 40-48. 10.1016/j.foreco.2018.12.009

McDowell, N.G., Allen, C.D., Marshall, L. 2009. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. Global Change Biology 16: 399-415. https://doi.org/10.1111/j.1365-2486.2009.01994.x

Moran, E., Lauder, J., Musser, C., Stathos, A., Shu, M. 2017. The genetics of drought tolerance in conifers. New Phytologist 216: 1034–1048. https://doi.org/10.1111/nph.14774

Negron, J.F., Mcmillin, J.D., Anhold, J.A., Coulson, D. 2009. Bark beetle-caused mortality in a drought-affected ponderosa pine landscape in Arizona, USA. Forest Ecology and Management 257: 1353-1362. http://dx.doi.org/10.1016/j.foreco.2008.12.002

Petrie, M.D., Bradford, J.B., Hubbard, R.M., Lauenroth, W.K., Andrews, C.M. and Schlaepfer, D.R. 2017. Climate change may restrict dryland forest regeneration in the 21st century. Ecology 98: 1548-1559. doi:10.1002/ecy.1791

Puhlick, J.J., Laughlin, D.C., Moore, M.M. 2012. Factors influencing ponderosa pine regeneration in the southwestern USA. Forest Ecology and Management 264: 10-19. https://doi.org/10.1016/j.foreco.2011.10.002

Rehfeldt, G.E. 1993. Genetic variation in the ponderosae of the southwest. American Journal of Botany 80: 330-343. doi:10.1002/j.1537-2197.1993.tb13807.x

Rehfeldt, G.E., Crookston, N.L., Warwell, M.V., Evans, J.S. 2006. Empirical analyses of plant-climate relationships for the western United States. International Journal of Plant Science 167(6): 1123-1150. https://doi.org/10.1086/507711

Rehfeldt, G.E, Jaquish, B.C., Saenz-Romero, C., Joyce, D.G., Leites, L.P., St. Clair, J.B., Lopez-Upton, J. 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: reforestation. Forest Ecology and Management 324: 147-157. http://dx.doi.org/10.1016/j.foreco.2014.02.040

Rodman, K.C., Veblen, T.T., Chapman, T.B., Rother, M.T., Wion, A.P., Redmond, M.D. 2019. Limitations to recovery following wildfire in dry forests of southern Colorado and northern New Mexico, USA. Ecological Applications. https://doi.org/10.1002/eap.2001

Savage, M., Mast, J.N., Feddema, J.J. 2013. Double whammy: high-severity fire and drought in ponderosa pine forests of the Southwest. Canadian Journal of Forest Research 43: 570-583. https://doi.org/10.1139/cjfr-2012-0404

Schubert, G.A., Pitcher, J.A. 1973. A provisional tree seed-zone and cone-crop rating system for Arizona and New Mexico. USDA Forest Service Research Paper RM-105.

Williams, A.P., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., … Mcdowell, N.G. 2012. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change *3*(3): 292–297. doi:10.1038/nclimate1693

Williams, M.I., and Dumroese, R.K. 2013. Preparing for climate change: forestry and assisted migration. Journal of Forestry 111: 287-297. https://doi.org/10.5849/jof.13-016

Willyard, A., Gernandt, D.S., Potter, K., Hipkins, V., Marquardt, P., Mahalovich, M.F., Langer, S.K., Telewski, F.W., Cooper, B., Douglas, C., Finch, K., Karemera, H.H., Lefler, J., Lea, P. and Wofford, A. 2017. *Pinus ponderosa*: A checkered past obscured four species. American Journal of Botany 104: 161-181. doi:10.3732/ajb.1600336

Wright, I., Reich, P., Westoby, M., Ackerly, D., Baruch, Z., Bongers, F., Cavender-Bares, J., Cornelissen, J., Diemer, M., Flexas, J., Garnier, E., Groom, P., Gulias, J., Hikosaka, K., Lamont, B., Lee, T., Lee, W., Lusk, C., Villar, R. 2004. The world-wide leaf economics spectrum. Nature 428: 821–827. https://doi.org/10.1038/nature02403

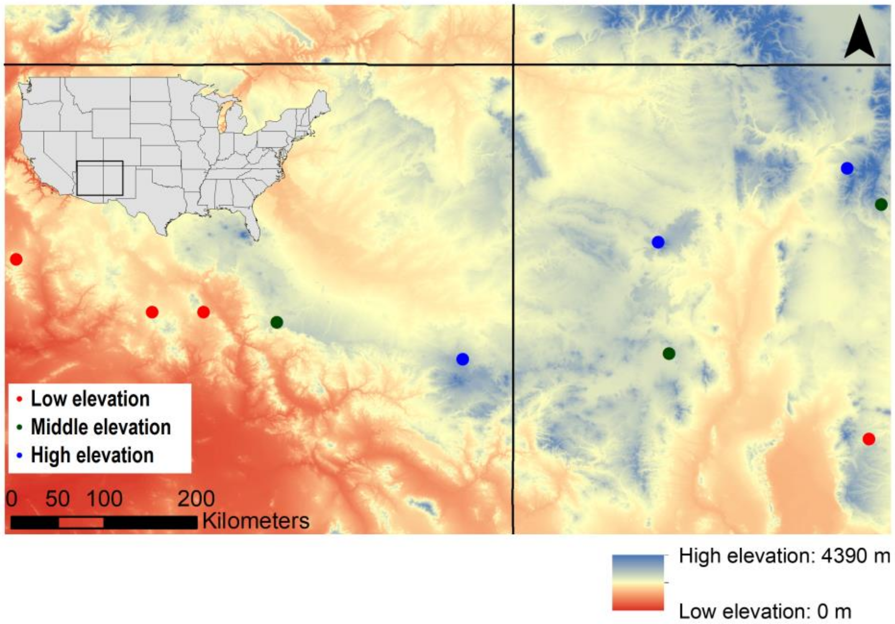


Figure 2.1: Provenance locations in Arizona and New Mexico shown on the elevational gradient of the landscape. Elevation group is shown by different colors.

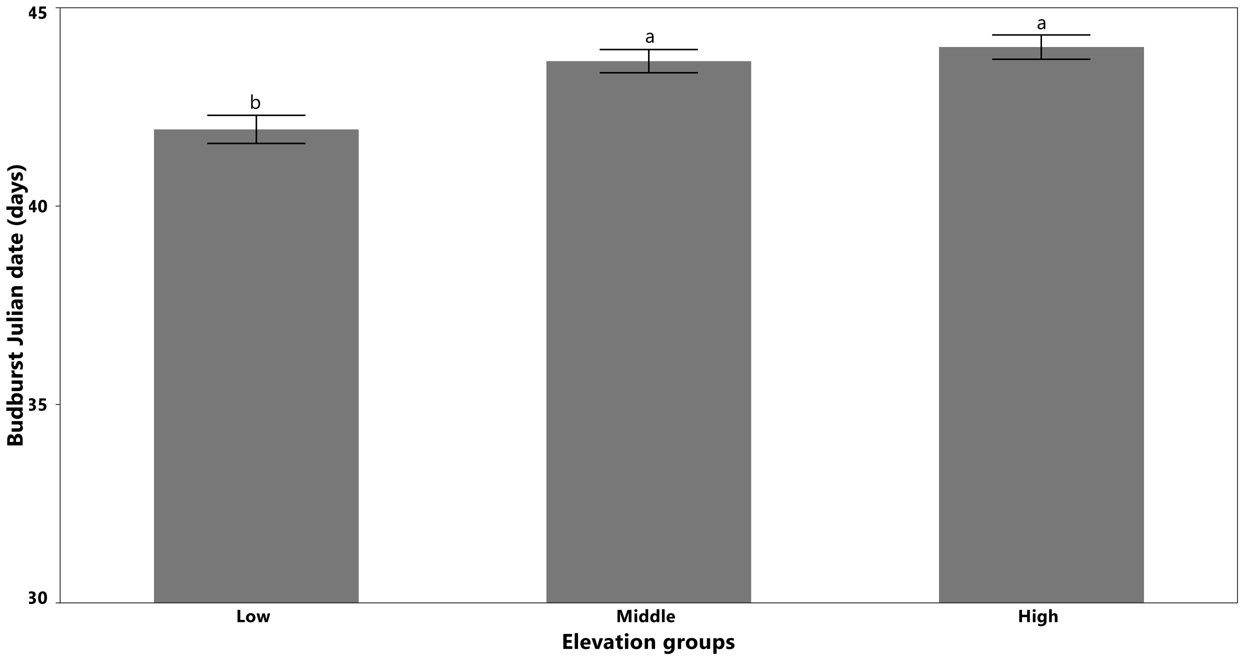


Figure 2.2: Mean budburst date and standard error for low, middle, and high elevation provenance groups. Means with different letters differ significantly (Tukey's HSD test; p<0.05).

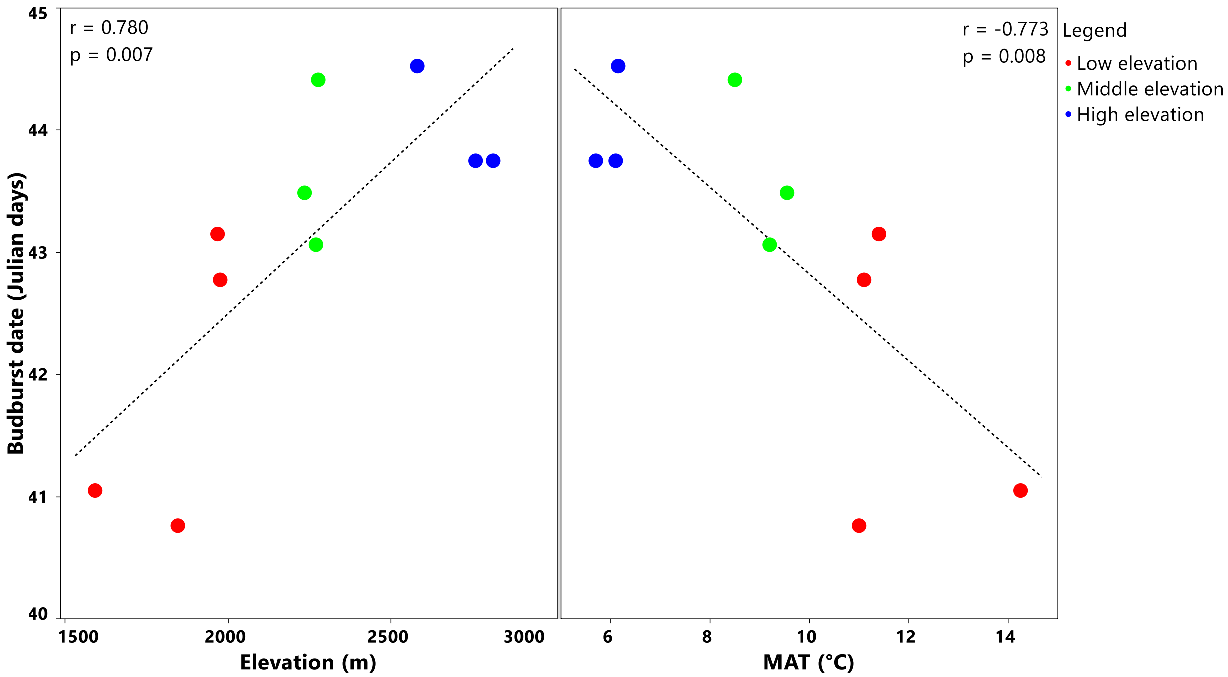


Figure 2.3: Relationships between provenance mean budburst date, elevation, and mean annual temperature (MAT). Elevation group is shown by different colors.

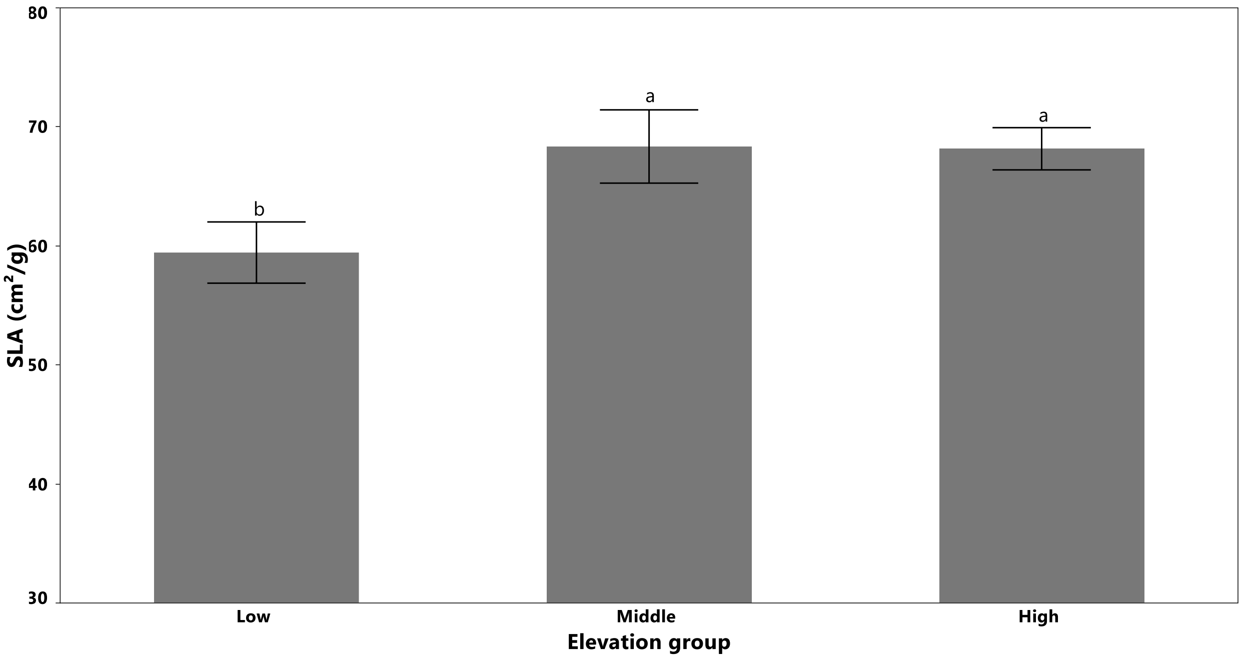


Figure 2.4: Mean specific leaf area (SLA) with standard error for low, middle, and high elevation provenance groups. Means with different letters differ significantly (Tukey's HSD tests; $p<0.05$).

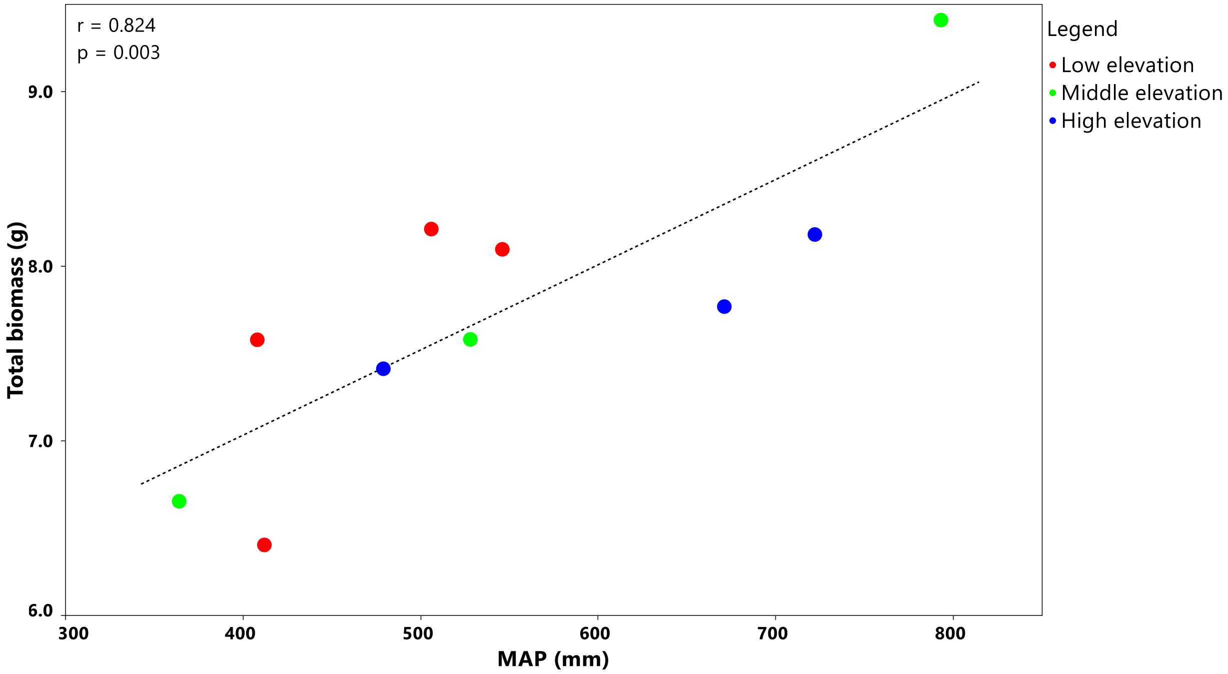


Figure 2.5: Relationship between provenance mean annual precipitation (MAP) and seedling biomass. Elevation group is shown by different colors.

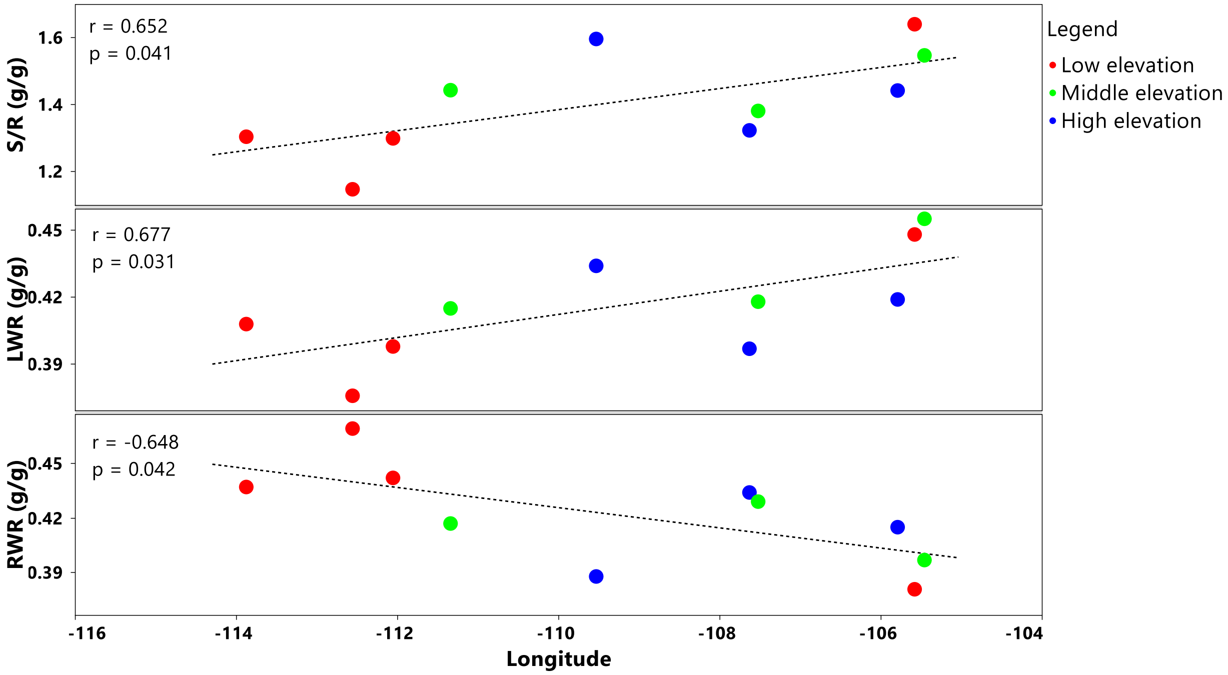
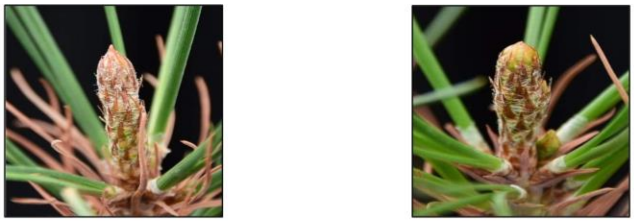


Figure 2.6: Relationships between provenance longitude and mean shoot-root ratio (S/R), leaf weight ratio (LWR), and root weight ratio (RWR). Elevation group is shown by different colors.

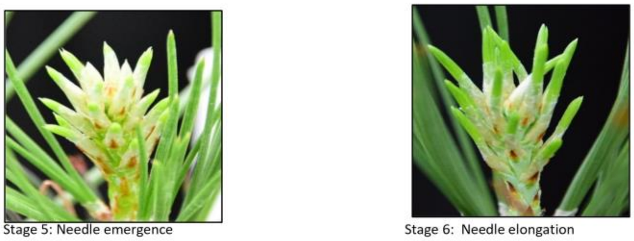


Stage 1: Tight bud

Stage 2: Early bud swelling

Stage 3: Advanced bud swelling

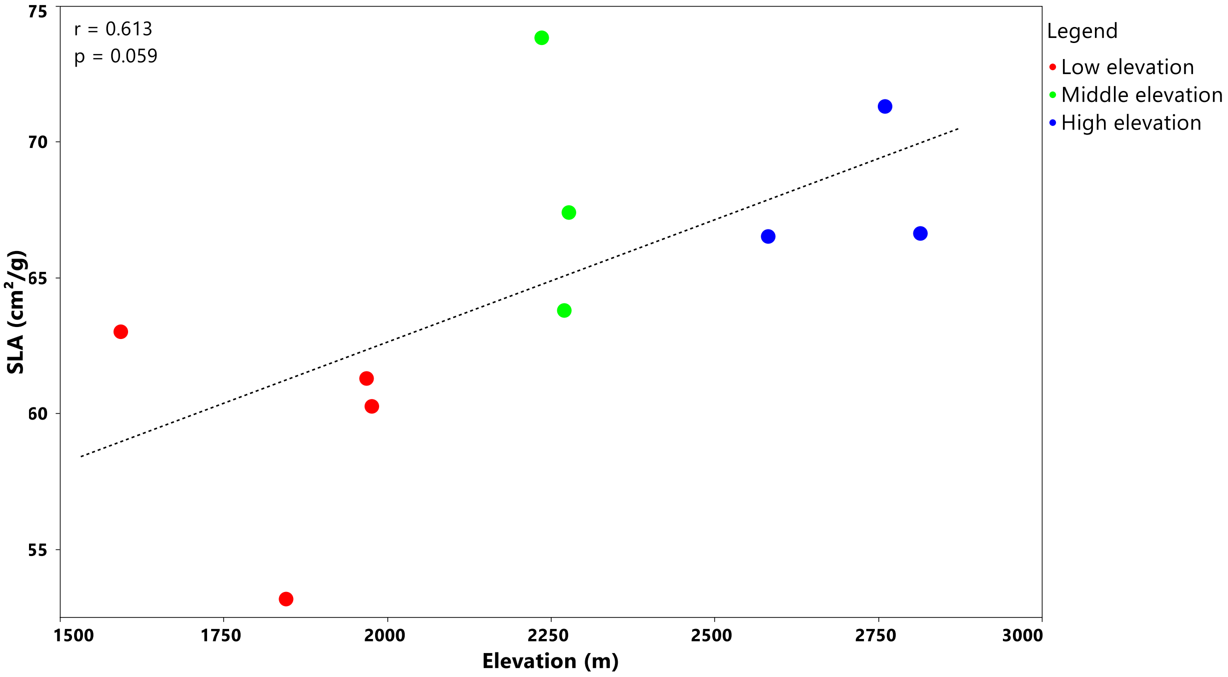
Stage 4: Early shoot and needle elongation



Stage 5: Needle emergence

Stage 6: Needle elongation

Supplementary Figure 2.1: Budburst stages of *Pinus ponderosa* seedlings.



Supplementary Figure 2.2: Relationship between provenance mean specific leaf area (SLA) and elevation. Elevation group is shown by different colors.

Table 2.1: Provenance name, code, state, elevation group, latitude, longitude, elevation, mean annual temperature (MAT) and mean annual precipitation (MAP) ordered by increasing elevation.

Provenance (code)	**State**	**Elevation group**	**Latitude**	**Longitude**	**Elevation (m)**	**MAT (ºC)**	**MAP (mm)**
Cherry Rd (CR)	AZ	Low	34.586	-112.057	1592	14.2	408
Prescott-Iron Springs Rd (PIS)	AZ	Low	34.585	-112.559	1846	11.0	546
Hualapai Mtns (HM)	AZ	Low	35.101	-113.878	1968	11.4	412
Ruidoso Service Office (RSO)	NM	Low	33.350	-105.583	1976	11.1	506
Rim District (RD)	AZ	Medium	34.487	-111.343	2235	9.6	793
HH Ranch (HHR)	NM	Medium	34.183	-107.525	2270	9.2	364
Mineral Hill (MH)	NM	Medium	35.633	-105.461	2277	8.5	528
Borrego Mesa (BM)	NM	High	35.990	-105.794	2581	6.2	479
Green’s Peak (GP)	AZ	High	34.126	-109.535	2760	5.7	671
Mount Taylor (TAY)	NM	High	35.266	-107.633	2814	6.1	722

Table 2.2: Seedling traits for low, middle, and high elevation provenance groups.

Elevation group	**Total biomass (g)**	**Height (cm)**	**S/R (g/g)**	**LWR (g/g)**	**SWR (g/g)**	**RWR (g/g)**	**SLA (cm^2/g)**	**Needle length (cm)**	**Budburst date (Julian days)**
Low	7.5 a (0.33)	18.1 a (0.63)	1.3 a (0.084)	0.40 a (0.017)	0.15 a (0.008)	0.43 a (0.015)	59.4 b (2.57)	16.2 a (0.35)	41.9 b (0.35)
Middle	7.8 a (0.75)	17.1 a (0.79)	1.4 a (0.098)	0.43 a (0.026)	0.15 a (0.010)	0.41 a (0.016)	68.3 a (3.07)	15.6 a (0.42)	43.6 a (0.29)
High	7.8 a (0.24)	16.6 a (0.40)	1.4 a (0.085)	0.41 a (0.022)	0.16 a (0.011)	0.41 a (0.014)	68.1 a (1.77)	15.8 a (0.55)	44.0 a (0.30)

Data are means (1 SE). Means in the same column followed by same letter do not differ significantly ($p \leq 0.05$; Tukey's HSD tests when elevation group main effect $p \leq 0.05$). S/R, shoot/root ratio; LWR, leaf weight ratio; SWR, stem weight ratio; RWR, root weight ratio; SLA, specific leaf area.

Table 2.3: Correlation coefficients (p values) between provenance environmental characteristics and seedling phenological and structural traits (n=10); Bold values indicate p<0.05.

Trait	**Elevation**	**MAT**	**MAP**	**Latitude**	**Longitude**
Total biomass (g)	0.102 (0.778)	-0.093 (0.796)	**0.824** **(0.003)**	-0.222 (0.536)	0.033 (0.927)
Height (cm)	-0.548 (0.100)	0.497 (0.143)	0.210 (0.559)	-0.253 (0.479)	-0.340 (0.335)
S/R (g/g)	0.374 (0.286)	-0.367 (0.296)	0.189 (0.600)	-0.270 (0.450)	**0.652** **(0.041)**
LWR (g/g)	0.256 (0.474)	-0.269 (0.452)	-0.009 (0.978)	-0.127 (0.725)	**0.677** **(0.031)**
SWR (g/g)	0.486 (0.154)	-0.430 (0.214)	0.575 (0.081)	-0.361 (0.305)	0.158 (0.661)
RWR (g/g)	-0.398 (0.253)	0.388 (0.267)	-0.198 (0.582)	0.239 (0.505)	**-0.648** **(0.042)**
SLA (cm^2/g)	0.613 (0.059)	-0.538 (0.108)	0.567 (0.086)	0.162 (0.654)	0.236 (0.510)
Needle length (cm)	-0.148 (0.682)	0.129 (0.722)	-0.076 (0.834)	-0.067 (0.853)	0.144 (0.690)
Budburst date (Julian days)	**0.780** **(0.007)**	**-0.773** **(0.008)**	0.285 (0.423)	0.443 (0.198)	0.608 (0.061)

S/R, shoot/root ratio; LWR, leaf weight ratio; SWR, stem weight ratio; RWR, root weight ratio; SLA, specific leaf area.

Chapter III: Provenance Geographical and Climatic Characteristics Influence Budburst Phenology of Southwestern Ponderosa Pine Seedlings

Abstract

Ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests of the southwestern US are threatened by climate change and deforestation. Information about geographic patterns of provenance variation in budburst phenology is needed to make decisions about selecting seed sources for future planting. In this study, provenance variation in budburst phenology of ponderosa pine seedlings was examined using common garden studies. Seedlings from 21 provenances, representing an elevational gradient in Arizona and New Mexico, were planted in July 2018 at a ponderosa pine-dominated field site in northern Arizona. Field budburst was monitored weekly on all seedlings in the spring of 2019. Field budburst was compared with budburst timing of the same provenances measured under greenhouse conditions. The hypotheses for this study were that 1) budburst varies among provenances, with earlier budburst in low elevation provenances and 2) differences in budburst timing among provenances are consistent for seedlings grown in greenhouse and field environments. Field results show that provenances vary in budburst date and that low and middle elevation provenances break bud sooner than high elevation provenances. Field budburst date had a moderate positive correlation with provenance mean annual precipitation ($r = 0.522$) and a moderate negative trend with latitude ($r = -0.413$). Budburst date of provenances in the greenhouse had a moderate positive trend with budburst date in the field ($r = 0.554$), suggesting application of greenhouse results to field plantings. Such information about provenance variation and environmental and geographic trends in budburst timing will be useful for developing species-specific seed transfer guidelines and effective assisted migration strategies in a changing climate.

Keywords: budburst; phenology; provenance variation; common garden; elevation; latitude; precipitation; *Pinus ponderosa*

Introduction

Tree species have considerable genetic variation among provenances and are generally adapted to local climate [1,2]. However, locally adapted provenances are likely to become locally maladapted [3,4] due to the inability to adapt or acclimate to rapid climatic change and associated disturbances. Forests of the southwest US are already experiencing increases in warming, drought and tree-killing disturbances [5]. An increase in temperature of over 1 °C occurred in the southwest US between 2001-2010 and mean annual temperature is expected to increase between 3-5 °C by the end of this century [6].

Climate warming has already caused an earlier onset of spring in western North America forests [7,8] because tree budburst timing responds to many factors including temperature [9]. Earlier budburst due to warmer spring temperature can have a positive impact on tree performance if early budburst promotes growth by lengthening the growing season, or a negative impact if early budburst results in spring frost damage that kills stems, buds, and leaves [9,10]. Spring frost damage has been predicted to increase in frequency with future climate warming due to early budburst [11]. Interactions among multiple factors such as chilling requirements, temperature, photoperiod, and plant genotype determine budburst timing [12]. Budburst can vary among tree populations due to local adaptation to thermal environment [13]. In *Pinus*, genetic differentiation and clines in growth and phenology have been previously reported [14-16], including among populations from different elevations of ponderosa pine (*Pinus ponderosa* Doug. Ex. Laws) [17,18].

Ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests in the southwest US are threatened by large-scale mortality due to drought, bark beetle attacks and wildfires [19], with higher mortality at the low-elevation warm edge of the range

[20]. Regeneration and establishment of ponderosa pine seedlings following drought and wildfires has been sparse [21,22]. In order to promote ponderosa pine regeneration in a warming climate, planting low-elevation trailing-edge provenances has been recommended when natural regeneration fails [18, 23], as low-elevation provenances have been shown to have traits of drought adaptation [24,25]. However, movement of populations from low elevations to higher elevations could result in earlier budburst and a risk of spring frost damage due [26]. Therefore, more information is needed about provenance variation in phenological processes in southwestern ponderosa pine under field conditions to make informed decisions about selecting seed sources for out planting in a changing climate.

Results of studies performed in controlled greenhouse environments may or may not be scalable to field conditions [27]. Direct comparisons of results from greenhouse and field-based studies are required to determine the predictability of field performance from greenhouse studies. A study of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) found a similar pattern of variation in budburst timing among provenances in greenhouse and field environments [28]. This suggests that greenhouse results for budburst timing of provenances are relevant to field performance, but studies are needed for other species such as ponderosa pine.

A field common garden study was used to investigate variation in budburst phenology among 21 southwestern ponderosa pine provenances obtained from different elevations across Arizona and New Mexico. The hypotheses for this study were that 1) budburst varies among provenances, with earlier budburst in low elevation provenances and 2) differences in budburst timing among provenances are consistent for seedlings grown in greenhouse and field environments. The objective was to determine if the pattern of earlier budburst by low elevation provenances found in an earlier greenhouse study was maintained, muted, or amplified under field conditions.

Materials and Methods

Provenance information

Ponderosa pine seeds from 21 provenances were used in this study representing a wide elevational range from Arizona and New Mexico (Figure 3.1). Seeds were obtained for 19 out of the 21 provenances from collections at the Northern Arizona University (NAU) Greenhouse Facility located in Flagstaff, Arizona and New Mexico State University's John T. Harrington Forestry Research Center located in Mora, New Mexico. Seeds were collected for the remaining two provenances in the year 2017. Each provenance consisted of seeds from 3-6 mother trees or a pooled collection without mother tree level information. The selected provenances varied in elevation, mean annual temperature (MAT) and mean annual precipitation (MAP) (Table 3.1). Climatic information for each provenance was obtained from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 19 Sep 2020). Provenance origins ranged in MAT from 5.7 °C to 14.2 °C; in MAP from 364 mm to 767 mm; and in elevation from ~1600 m to ~2800 m. Provenance elevation had a moderate positive correlation with MAP ($r = 0.500$, $p = 0.020$) and a strong negative correlation with MAT ($r = -0.902$, $p < 0.0001$); MAT had a moderate negative correlation with MAP ($r = -0.434$, $p = 0.048$). Latitude had a moderate negative trend with MAT ($r = -0.406$, $p = 0.067$) and longitude had a moderate positive trend with elevation ($r = 0.370$, $p = 0.098$) (Table 3.2).

Field planting

Seedlings used for field planting were grown at the John T. Harrington Forestry Research Center with New Mexico State University in Mora, NM. Seedlings began as seeds that were sown in March 2018 into 164 mL containers and placed in racks with 98 container capacities (Ray Leach Cone-tainers-SC10 Super, RL98 Tray, Stuewe & Sons, Inc., Tangent, OR, USA). Media was a 2:1:1 mixture of sphagnum peat, perlite, and vermiculite (v:v:v). Seedling emergence rates were uniform across sources with the majority of germination occurred within 14 days after sowing. For the initial three weeks, seeds and germinates were misted 5 times daily, followed by overhead irrigation after total soil moisture dropped to approximately 75% of container capacity based on gravimetric weights. Supplemental lighting (metal halide lamps; range of 75-125 1.55 µmmol m^{-2} s^{-1}) was provided when necessary to maintain an 18-hour photoperiod. Daytime and nighttime temperatures were maintained at 21-26 °C and 18-22 °C, respectively. A starter fertilizer (Peters Professional 25-30 ppm 10-30-20 N-P-K) was applied once per week after planting for a total of 5 weeks, followed by a grower fertilizer (Peters Professional 75-150 ppm 21-5-20 N-P-K) applied once per week, and finally a finisher fertilizer (Plant Marvel Nutriculture 4-25-35 N-P-K) that was applied once a week.

In July 2018, the greenhouse grown seedlings were planted in a field common garden study at the Arboretum Forest site of the Southwest Experimental Garden Array (SEGA; https://sega.nau.edu; Figure 3.1) located 10 kms from Flagstaff, Arizona (latitude 35.16, longitude -111.73, and elevation 2200 m). The mean annual temperature at the study site is 7.6 °C and mean annual precipitation is 555 mm (1981-2010). During the year of assessment (2019) the mean annual temperature was 6.9 °C and annual precipitation was 636 mm. Seedlings were planted in a randomized complete block design at a spacing of 1.2 m between seedlings. Forty-eight seedlings from 21 provenances each were planted across 4 blocks (1008 total seedlings). Each provenance was planted as a 12-tree linear plot, randomly positioned within each block.

Budburst phenology

Budburst timing was visually assessed on all live seedlings (658 total; 350 omitted from analysis due to rabbit herbivory) in each block of the field planting once a week between May and June 2019. The protocol used to define budburst was same as used in an earlier greenhouse study, where budburst was defined as the Julian date when new needles emerged from the terminal bud [25]. Results from this earlier study [25] were used to compare provenance differences in budburst between our field planting and the greenhouse. In the earlier greenhouse study, seedlings were grown from seed at the Northern Arizona University Greenhouse Facility starting February 2018. Seedlings were watered thrice a week, fertilized twice a week, exposed to temperature between 21 to 26 °C, except during the winter dormancy period (mid-November through mid-January), when the seedlings were watered once a week, not fertilized and exposed to temperature between 5 and 10 °C. Ten of the 21 provenances used for the field study were included in the greenhouse study (Table 3.1). For these ten provenances in the greenhouse, budburst was visually accessed on 80 seedlings per provenance (800 total), twice a week, between January and February 2019.

Data analysis

Analysis of variance (ANOVA) was used to test for differences in budburst date among the 21 provenances in the field study. We used mixed-effects model with provenance as fixed effect and block as a random effect. In order to examine the influence of provenance elevation on budburst phenology, provenances were assigned to elevational groups, which were included in the ANOVA. Elevational groups were defined following [25]: low elevation < 2000 m; middle elevation = 2000 to 2500 m; high elevation > 2500 m. The analysis was conducted on block-level means because individual seedlings were regarded as observational units and row plots of provenances within blocks were regarded as experimental units. Tukey's Honest Significant Difference (HSD) test was used to detect significant differences between means (α = 0.05) among provenances and elevation groups. The relationship between field-measured and greenhouse-measured budburst date was investigated on a subset of field provenances (10 total; Table 3.1) that was also used in an earlier greenhouse common garden study. The relationships between field and greenhouse budburst dates (n = 10) and between field budburst date and environmental characteristics of provenance locations (n = 21) were evaluated on provenance means with correlation and regression analyses. Strength of relationship was interpreted as weak when the absolute value of the correlation coefficient (r) was between 0 and 0.3; moderate between 0.3 and 0.7; and strong between 0.7 and 1.0 [29]**.** JMP Pro version 14 (SAS Institute Inc., Cary, N.C., USA) was used to perform all analyses.

Results

Budburst date in the field planting differed significantly among provenances (p = 0.0009; Table 3.3), ranging from 161.4 Julian days for SKT to 169.4 Julian days for MAG. Budburst date in the field planting also differed significantly among elevation groups (p = 0.0026; Table 3.4). Low and middle elevation provenances had similar budburst dates of about 164 Julian days. However, high elevation provenances broke bud about 3 days later (mean budburst = ~167 Julian days) than low and middle elevation provenances.

Correlation analysis revealed some interesting relationships between mean provenance budburst date in the field planting and environmental and geographical characteristics (Table 3.2). Specifically, budburst date had a moderately positive correlation with elevation (r = 0.528, p = 0.013) and MAP (r = 0.522, p = 0.015), and a moderately negative trend with latitude (r = -0.413, p = 0.062) (Figure 3.2).

A moderate positive trend (r = 0.554, p = 0.096; Figure 3.3) was found between budburst date measured in the field planting and measured in greenhouse for the same 10 provenances (Table 3.1). Budburst occurred much earlier in the greenhouse (Julian days 41 to 45), which was warmed up in mid-January to promote early growth, compared with the field where budburst occurred in late June (Julian days 163 to 169). Also, for the same 10 provenances, the budburst range of about 4 days in the greenhouse was less than the range of about 6 days in the field.

Discussion

This study investigated variation in budburst phenology among 21 provenances of southwestern ponderosa pine using observations of a field common garden study in the first spring after planting, and of a greenhouse study of 10 of the same provenances. The goal of this study was to test the hypotheses that 1) timing of budburst would vary among provenances in a field planting, and low elevation provenances would break bud sooner because budburst timing is often genetically controlled and associated with spring frost hardiness [30,31]; and 2) provenance variation in budburst timing measured under field conditions would be related to budburst timing of the same provenances measured under greenhouse conditions. In addition, correlations between field budburst date and provenance environment and geographic characteristics were examined. While the results strongly suggest genetic differences in budburst phenology among southwestern provenances of ponderosa pine, other explanations such as epigenetic and maternal influences, were not investigated.

The first hypothesis was supported by significant differences in budburst date among provenances and elevation groups, along with a moderate positive correlation between budburst date and provenance elevation. The maximum difference in field budburst date among provenances was 8 days. To understand the ecological significance of this difference, additional field common garden studies at elevations higher than the current study are needed to see if this difference is amplified or maintained. Amplification of this difference could predispose seedlings to spring frost damage. On the contrary, if this difference is not amplified, it suggests an opportunity for management in the form of flexibility for seed transfer guidelines pertaining to drought and/or heat resistance. Also, the current study used observations on seedlings in only the first spring after planting in a year when spring frost damage did not occur. More complete understanding of the ecological importance of this amount of variation in budburst will require observations over more years [32], especially years with pronounced late-spring frosts.

The pattern of low elevation provenances breaking bud earlier than high elevation provenances is likely a result of local adaptation to warm spring temperatures at lower elevations and has been shown to be a highly species-specific response [32]. Similar elevational influence on budburst has been reported in an earlier study showing provenance variation and a negative correlation between elevation and growth potential and duration of shoot elongation in ponderosa pines from Colorado [13]. In addition, the finding in the field study of earlier budburst of low-elevation provenances is consistent with an earlier greenhouse study of some of the same provenances [25]. However, results in this study are different from a recent field common garden study in California showing no significant difference in budburst phenology among four provenances of ponderosa pine from different elevations [14], suggesting that findings from one region of ponderosa pine are not always applicable to other regions. Elevation of provenances in that study ranged from ~145 m to ~1920 m as compared to ~1600 m to 2800 m in this study.

In addition to an elevational trend, a moderate positive correlation between provenance MAP and budburst timing was found in the current study, showing that provenances from drier areas broke bud sooner than provenances from wetter areas. This result is similar to a study of

Douglas-fir that reported earlier budburst of provenances from areas with low summer rainfall [33]. However, the result is different from a study involving 35 provenances of cork oak (*Quercus suber* L.) where no correlation was found between budburst and provenance precipitation and elevation [34]. Such information may have implications for assisted migration of seed sources from drier areas to high elevation colder sites that are expected to become drier in the future with climate warming.

An interesting latitudinal trend of earlier budburst by northern provenances was also found in the current study. The results of this latitudinal pattern are different from a study involving beech (*Fagus sylvatica* L.) provenances in northern Poland showing later flushing by northern provenances [35]. The latitudinal pattern could be the result of earlier fulfillment of the chilling hour requirement by northern provenances, as days to budburst have been shown to decrease as chilling hour accumulation increases in ponderosa pine [36].

The lack of significant correlation between budburst date and MAT ($p = 0.116$) was surprising considering the strong negative correlation between elevation and MAT ($p < .0.0001$) and a moderate positive correlation between budburst date and elevation ($p = 0.013$). This result may be due to the lack of direct temperature and precipitation data measured on-site for the provenances. The provenance environmental data were obtained using PRISM from an interpolation equation that predicts temperature and precipitation largely from elevation in a particular region [37]. This approach likely does not capture all ecologically relevant microsite climatic variation.

A moderate positive trend was documented between budburst timing in field and greenhouse environments for the same ten provenances. This relationship is consistent with our second hypothesis and may have application for investigating provenance variation in phenology in greenhouse experiments. However, the correlation for the relationship had a p value of 0.096 and a sample size of only 10 provenances, and therefore must be interpreted with caution. To our knowledge, this is the first report for ponderosa pine provenances of a direct comparison of budburst phenology between field and greenhouse studies. Similar results were reported in a study of Douglas-fir provenances, which suggests that greenhouse studies have potential for investigating provenance differences in budburst and predicting patterns in field plantings [24].

Conclusions

In conclusion, budburst timing varies among provenances of southwestern ponderosa pine. These variations are mainly related to provenance elevation, precipitation, and latitude; low elevation, drier, and high-latitude provenances break bud sooner than higher elevation, wetter and lower latitude provenances. Also, geographic patterns in budburst timing from greenhouse experiments may be applicable to field plantings. More information about budburst timing and risk of spring frost damage is needed for developing species-specific seed transfer guidelines and effective assisted migration strategies in a changing climate.

References

Savolainen, O., Pyhäjärvi, T., Knürr, T., 2007. Gene flow and local adaptation in trees. Annual Review of Ecology Evolution and Systematics. 38, 595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Hereford, J., 2009. A quantitative survey of local adaptation and fitness trade-offs. The American Naturalist. https://doi.org/10.1086/597611

O’Neill, G.A., Hamann, A., Wang, T., 2008. Accounting for population variation improves estimates of the impact of climate change on species’ growth and distribution. Journal of Applied Ecology 45, 1040–1049. https://doi.org/10.1111/j.1365-2664.2008.01472.x

Grady, K.C., Ferrier, S.M., Kolb, T.E., Hart, S.C., Allan, G.J., Whitham, T.G., 2011. Genetic variation in productivity of foundation riparian species at the edge of their distribution: implications for restoration and assisted migration in a warming climate. Global Change Biology 17, 3724–3735. https://doi.org/10.1111/j.1365-2486.2011.02524.x

Garfin, G., Jardine, A., Merideth, R., Black, M., LeRoy, S. (Eds.), 2013. Assessment of climate change in the southwest United States: A report prepared for the national climate assessment. Island Press/Center for Resource Economics, Washington, DC. https://doi.org/10.5822/978-1-61091-484-0

Melillo, J.M., Richmond, T. (T. C.), Yohe, G.W., 2014. Climate change impacts in the United States: The third national climate assessment. U.S. Global Change Research Program. https://doi.org/10.7930/J0Z31WJ2

Schwartz, M.D., Ahas, R., Aasa, A., 2006. Onset of spring starting earlier across the Northern Hemisphere. Global Change Biology 12, 343–351. https://doi.org/10.1111/j.1365-2486.2005.01097.x

Ault, T.R., Macalady, A.K., Pederson, G.T., Betancourt, J.L., Schwartz, M.D., 2011. Northern hemisphere modes of variability and the timing of spring in western north America. Journal of Climate 24, 4003–4014. https://doi.org/10.1175/2011JCLI4069.1

Caffarra, A., Donnelly, A., 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. International Journal of Biometeorology 55, 711–721. https://doi.org/10.1007/s00484-010-0386-1

Hufkens, K., Friedl, M.A., Keenan, T.F., Sonnentag, O., Bailey, A., O’Keefe, J., Richardson, A.D., 2012. Ecological impacts of a widespread frost event following early spring leaf-out. Global Change Biology 18, 2365–2377. https://doi.org/10.1111/j.1365-2486.2012.02712.x

Richardson, A.D., Hufkens, K., Milliman, T., Aubrecht, D.M., Furze, M.E., Seyednasrollah, B., Krassovski, M.B., Latimer, J.M., Nettles, W.R., Heiderman, R.R., Warren, J.M., Hanson, P.J., 2018. Ecosystem warming extends vegetation activity but heightens vulnerability to cold temperatures. Nature 560, 368–371. https://doi.org/10.1038/s41586-018-0399-1

Howe, G.T., Aitken, S.N., Neale, D.B., Jermstad, K.D., Wheeler, N.C., Chen, T.H., 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany 81, 1247–1266. https://doi.org/10.1139/b03-141

Rehfeldt, G.E., 1990. Genetic differentiation among populations of *Pinus ponderosa* from the Upper Colorado River Basin. Botanical Gazette 151, 125–137. https://doi.org/10.1086/337812

Martínez-Berdeja, A., Hamilton, J.A., Bontemps, A., Schmitt, J., Wright, J.W., 2019. Evidence for population differentiation among Jeffrey and Ponderosa pines in survival, growth and phenology. Forest Ecology and Management 434, 40–48. https://doi.org/10.1016/j.foreco.2018.12.009

Wang, T., A. Hamann, A. Yanchuk, G. A. O’neill, and S. N. Aitken., 2006. Use of response functions in selecting lodgepole pine populations for future climates. Global Change Biology 12, no. 12, 2404–16. https://doi.org/10.1111/j.1365-2486.2006.01271.x

Chmura, D.J., Rożkowski, R., Chałupka, W., 2012. Growth and phenology variation in progeny of Scots pine seed orchards and commercial seed stands. European Journal of Forest Research 131, 1229–1243. https://doi.org/10.1007/s10342-012-0594-9

Rehfeldt, G.E., 1993. Genetic variation in the Ponderosae of the Southwest. American Journal of Botany 80, 330-343. https://doi.org/10.2307/2445357

Rehfeldt, G.E., Jaquish, B.C., Sáenz-Romero, C., Joyce, D.G., Leites, L.P., Bradley St Clair, J., López-Upton, J., 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Reforestation. Forest Ecology and Management 324, 147–157. https://doi.org/10.1016/j.foreco.2014.02.040

Hicke, J.A., Meddens, A.J.H., Kolden, C.A., 2016. Recent tree mortality in the western United States from bark beetles and forest fires. Forest Science 62, 141–153. https://doi.org/10.5849/forsci.15-086

McDowell, N.G., Allen, C.D., Marshall, L., 2010. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. Global Change Biology 16, 399–415. https://doi.org/10.1111/j.1365-2486.2009.01994.x

Davis, K.T., Dobrowski, S.Z., Higuera, P.E., Holden, Z.A., Veblen, T.T., Rother, M.T., Parks, S.A., Sala, A., Maneta, M.P., 2019. Wildfires and climate change push low-elevation forests across a critical climate threshold for tree regeneration. Proceedings of the National Academy of Sciences of the United States of America 116, 6193–6198. https://doi.org/10.1073/pnas.1815107116

Rodman, K.C., T.T. Veblen, M.A. Battaglia, M.E. Chambers, P.J. Fornwalt, Z.A. Holden, T.E. Kolb, J.R. Ouzts, M.T. Rother. 2020. A changing climate is snuffing out post-fire forest recovery in montane forests. Global Ecology and Biogeography: DOI:10.1111/geb.13174

Kolb, T., Dixit, A., Burney, O., 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern United States. Tree Planters' Notes 62: 104-112.

Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A., 2016. Local-scale drought adaptation of ponderosa pine seedlings at habitat ecotones. Forest Science 62, 641–651. https://doi.org/10.5849/forsci.16-049

Dixit, A., Kolb, T., 2020. Variation in seedling budburst phenology and structural traits among southwestern ponderosa pine provenances. Canadian Journal of Forest Research 1–8. https://doi.org/10.1139/cjfr-2019-0333

Grady, K.C., Kolb, T.E., Ikeda, D.H., Whitham, T.G., 2015. A bridge too far: cold and pathogen constraints to assisted migration of riparian forests. Restoration Ecology 23, 811–820. https://doi.org/10.1111/rec.12245

Bacilio, M., Moreno, M., Lopez-Aguilar, D.R., Bashan, Y., 2017. Scaling from the growth chamber to the greenhouse to the field: Demonstration of diminishing effects of mitigation of salinity in peppers inoculated with plant growth-promoting bacterium and humic acids. Applied Soil Ecology 119, 327–338. https://doi.org/10.1016/j.apsoil.2017.07.002

Malmqvist, C., Wallin, E., Lindström, A., Säll, H., 2017. Differences in bud burst timing and bud freezing tolerance among interior and coastal seed sources of Douglas fir. Trees 31, 1987–1998. https://doi.org/10.1007/s00468-017-1603-x

Ratner, B. 2009. The correlation coefficient: Its values range between +1/−1, or do they? Journal of Targeting, Measurement and Analysis for Marketing 17, 2, 139–42. https://doi.org/10.1057/jt.2009.5

Ducousso, A., Guyon, J., Krémer, A., 1996. Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt) Liebl). Annals of Forest Science 53, 775–782. https://doi.org/10.1051/forest:19960253

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Vitasse, Y., Delzon, S., Bresson, C.C., Michalet, R., Kremer, A., 2009. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Canadian Journal of Forest Research 39, 1259–1269. https://doi.org/10.1139/X09-054

White, T.L., Ching, K.K., Walters, J., 1979. Effects of provenance, years, and planting location on bud burst of Douglas-fir. Forest Science 25, 161–167. https://doi.org/10.1093/forestscience/25.1.161

Sampaio, T., Branco, M., Guichoux, E., Petit, R.J., Pereira, J.S., Varela, M.C., Almeida, M.H., 2016. Does the geography of cork oak origin influence budburst and leaf pest damage? Forest Ecology and Management 373, 33–43. https://doi.org/10.1016/j.foreco.2016.04.019

Chmura DJ, Rozkowski R. 2002. Variability of beech provenances in spring and autumn phenology. Silvae Genetica 51, 2–3: 123-127.

Wenny, D.L., Swanson, D.J., Dumroese, R.K., 2002. The chilling optimum of Idaho and Arizona ponderosa pine buds. Western Journal of Applied Forestry 17, 117–121. https://doi.org/10.1093/wjaf/17.3.117

Di Luzio, M., Johnson, G.L., Daly, C., Eischeid, J.K., Arnold, J.G., 2008. Constructing retrospective gridded daily precipitation and temperature datasets for the conterminous United States. Journal of Applied Meteorology and Climatology 47, 475–497. https://doi.org/10.1175/2007JAMC1356.1

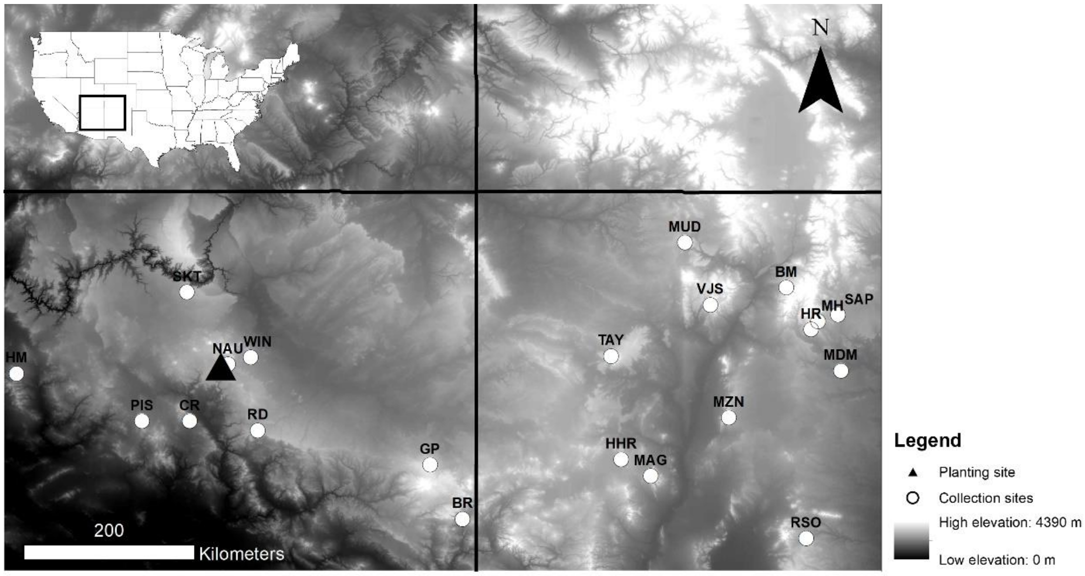


Figure 3.1: Location map of 21 provenance collection sites (white circles) of *Pinus ponderosa* and the location of the field common garden (black triangle).

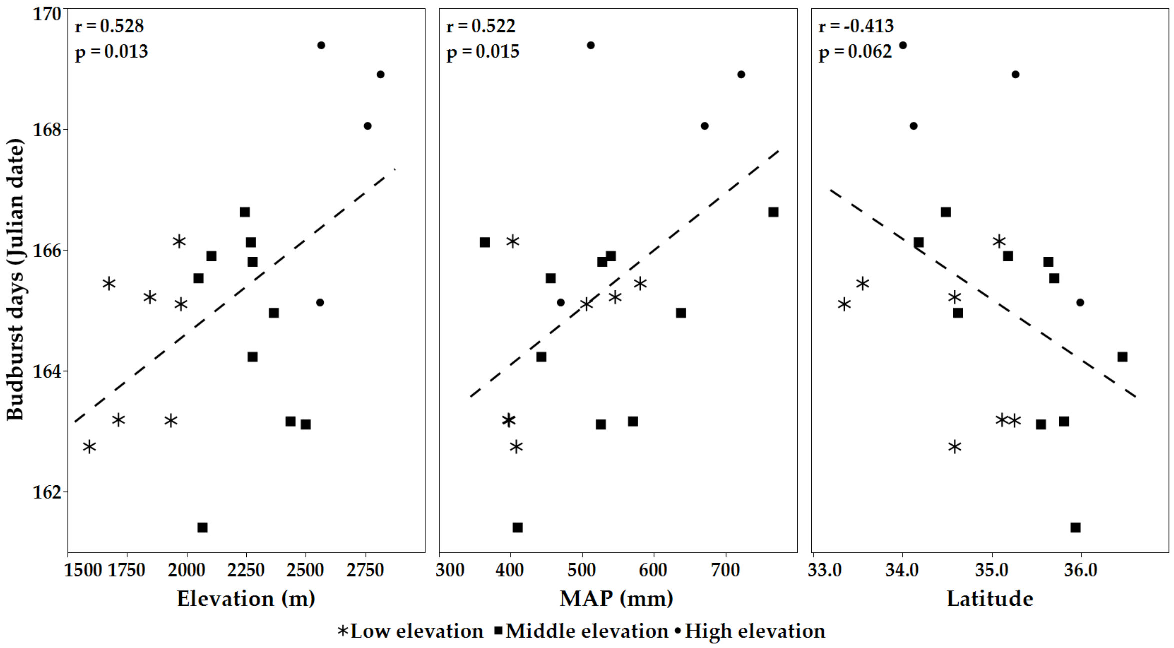


Figure 3.2: Relationships between provenance mean budburst date and elevation, mean annual precipitation (MAP) and latitude (n = 21). Elevation group is shown by different symbols.

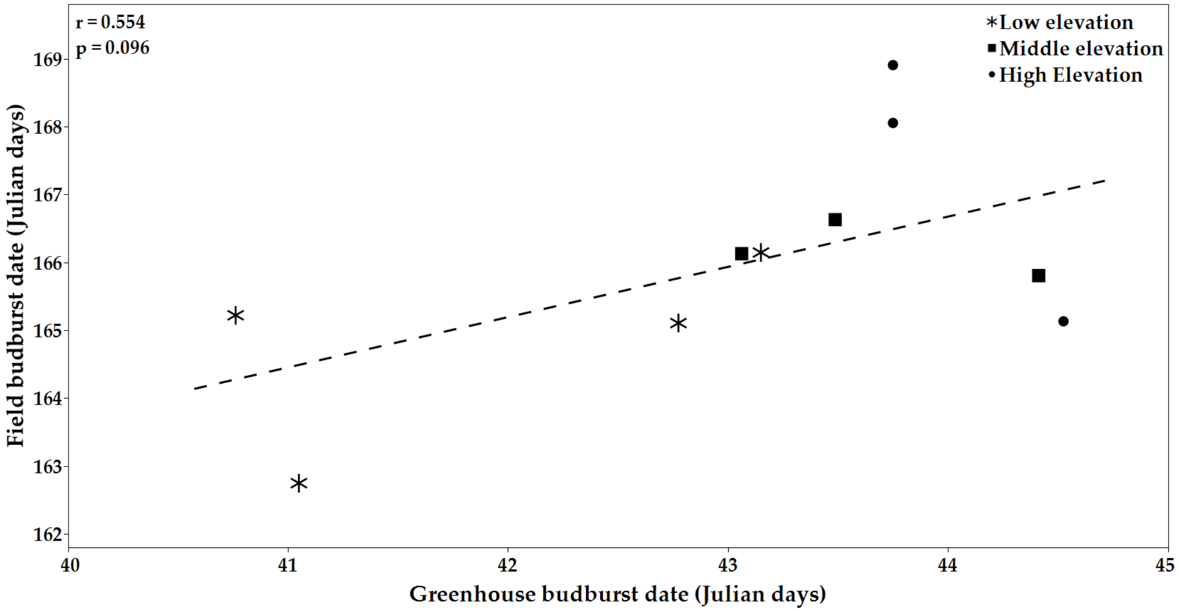


Figure 3.3: Relationships between mean budburst date measured under greenhouse and field conditions for the same provenances (n = 10).

Table 3.1: Provenance name, code, latitude, longitude, elevation, mean annual temperature (MAT), and mean annual precipitation (MAP) ordered by increasing elevation. Climate data (30-year normal, 1981-2010) are from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 19 Sep 2020). Values are means over mother trees at each provenance.

Provenance (code)	**Latitude/Longitude**	**Elevation (m)**	**MAT (ºC)**	**MAP (mm)**
Cherry Road (CR)[1]	34.586/-112.057	1592	14.2	408
Blue River (BR)	33.555/-109.193	1674	11.8	581
Mesa Del Medio (MDM)	35.116/-105.217	1714	12.3	397
Prescott – iron Springs Road (PIS)[1]	34.585/-112.559	1846	11	546
Townsend Winona (WIN)	35.254/-111.415	1934	10.2	398
Hualapai Mountains (HM)[1]	35.084/-113.875	1969	11.5	403
Ruidoso Service Office (RSO)[1]	33.350/-105.583	1976	11.1	506
Sapello Rt. 3 (SAP)	35.700/-105.250	2050	9.8	456
South Kaibab Tusayan Dist. (SKT)	35.939/-112.084	2067	8.9	410
Northern Arizona University (NAU)	35.182/-111.655	2104	8.4	540
Rim District (RD)[1]	34.487/-111.343	2244	9.4	767
HH Ranch (HHR)[1]	34.183/-107.525	2270	9.2	364
Mineral Hill (MH)[1]	35.633/-105.461	2277	8.5	528
Mud Springs (MUD)	36.463/-106.859	2277	6.9	443
Manzano Mountains (MZN)	34.623/-106.400	2366	8.6	638
Vallecitos-Jemez Springs (VJS)	35.809/-106.589	2436	6.8	571
Hartman Ridge (HR)	35.550/-105.533	2500	8.8	526
Borrego Mesa (BM)[1]	35.990/-105.794	2560	6.3	470
Magdalena Mountains (MAG)	34.006/-107.215	2565	8.9	512
Green's Peak (GP)[1]	34.126/-109.535	2760	5.7	671
Mount Taylor (TAY)[1]	35.266/-107.633	2814	6.1	722

[1] Provenances used in earlier greenhouse study [25].

Table 3.2: Correlation coefficients between provenance environmental characteristics and budburst date (n = 21). Values in parentheses are p values. Boldface type indicates significance ($p < 0.05$).

	Budburst date	Elevation	MAT	MAP	Latitude
Budburst date					
Elevation	**0.528 (0.013)**				
MAT	-0.353 (0.116)	**-0.902 (<.0001)**			
MAP	**0.522 (0.015)**	**0.500 (0.020)**	**-0.434 (0.048)**		
Latitude	-0.413 (0.062)	0.203 (0.375)	-0.406 (0.067)	-0.243 (0.287)	
Longitude	0.058 (0.802)	0.370 (0.098)	-0.324 (0.151)	0.029 (0.898)	0.139 (0.546)

Table 3.3: Mean budburst for each provenance, with standard errors in parentheses (Ordered by increasing elevation). Means followed by the same letter do not differ significantly ($p \leq 0.05$; Tukey's honestly significant difference (HSD) tests; provenance main effect p=0.0009).

Provenance	**Number of seedlings**	**Budburst date (Julian days)**
CR	24	162.7 ab (1.05)
BR	29	165.4 ab (1.05)
MDM	31	163.2 ab (1.37)
PIS	36	165.2 ab (0.84)
WIN	33	163.2 ab (1.60)
HM	41	166.1 ab (0.74)
RSO	28	165.1 ab (0.69)
SAP	30	165.5 ab (0.84)
SKT	39	161.4 b (1.34)
NAU	30	165.9 ab (0.39)
RD	27	166.6 a (0.84)
HHR	39	166.1 ab (1.30)
MH	36	165.8 ab (0.83)
MUD	30	164.2 ab (1.20)
MZN	26	165.0 ab (0.49)
VJS	36	163.2 ab (0.52)
HR	26	163.1 ab (1.47)
BM	30	165.1 ab (0.50)
MAG	18	169.4 a (2.13)
GP	37	168.1 a (1.06)
TAY	32	168.9 a (0.93)

Table 3.4: Mean budburst for each elevation group, with standard errors in parentheses. Means followed by the same letter do not differ significantly ($p \leq 0.05$; Tukey's honestly significant difference (HSD) tests; provenance main effect $p = 0.0026$).

Elevation group	**Budburst date (Julian days)**
Low elevation	164.6 b (0.39)
Middle elevation	164.6 b (0.22)
High elevation	167.7 a (0.52)

Chapter IV: Variation in Survival, Growth, and Carbon Isotope Discrimination Among Southwestern Ponderosa Pine Provenances in Common Gardens Across an Elevational Gradient

Abstract

We investigated survival, growth, and carbon isotope discrimination of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) seedlings from different provenances using common gardens across an elevational gradient. Twenty-one provenances from a range of elevations across Arizona and New Mexico were planted in three common gardens: high elevation site in aspen and mixed conifer forest, mid elevation site in ponderosa pine forest, and low elevation site in pinyon juniper woodland. We assessed seedling survival, growth, mortality agents, carbon isotope discrimination ($\Delta^{13}C$), and leaf nitrogen (%N) over the first two years after planting in 2018. Survival was highest at the mid elevation site (54%), low at the high elevation site (1.5%), and lowest at the low elevation site (0%) where almost all seedlings died in the first two months after planting. At the low elevation site, provenances from low elevation survived longer than provenances from mid and high elevations, but this pattern was not seen at the other sites. Mortality agents changed from abiotic to biotic factors with increase in elevation across sites. Seedlings had significantly higher growth rates, $\Delta^{13}C$, and lower %N at the mid elevation site compared with the high elevation site. Provenances differed significantly in height, diameter, and $\Delta^{13}C$, but not in height growth rate and %N. The provenance x site interaction was not significant for any trait at the mid and high elevation sites. Results show that seedling survival and performance depends on planting location and provenance; low elevation provenances should be considered for planting under increasingly arid conditions; impacts of biotic agents should be considered while planning a large-scale reforestation and assisted migration of ponderosa pine. Overall, such information about site and provenance variations in survival and performance of seedlings planted under different site conditions is needed to develop strategies and seed transfer guidelines to maintain ponderosa pine during changing climate.

Keywords: Common gardens; provenance variation; elevation gradient; carbon isotope discrimination; survival; growth; ponderosa pine; climate change

Introduction

Climate warming and associated high-severity fires and extended droughts have caused large scale tree mortality in the southwestern United States (Williams et al. 2012; Hicke et al. 2016). In southwestern ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests, severe wildfires and droughts have also reduced seedling establishment from natural regeneration (Savage et al. 2013; Rodman et al. 2020). A continuation of high tree mortality and meager regeneration threatens to reduce the geographical distribution of southwestern ponderosa pine forests (Rehfeldt et al. 2006). Range contraction of southwestern ponderosa pine forests after severe drought has been observed in both past and recent studies (Allen and Breshears 1998; Minott and Kolb 2020). Climatic constraints on natural regeneration of ponderosa pine (e.g. drought and high temperature) are expected to increase in the future (Petrie et al. 2017).

In the absence of sufficient natural regeneration, it is important to explore the use of artificial regeneration (i.e., tree planting or direct seedling) as a possible tool to compensate for recent losses of ponderosa pine forests (Kolb et al. 2019). Tree populations that are adapted to past local climatic conditions may become maladapted due to rapidly changing climate (Aitken et al., 2008; Kremer et al., 2012). Planting seedlings from provenances that are pre-adapted to future arid conditions might mitigate negative impacts of a warming climate (Williams and Dumroese 2013; Rehfeldt et al. 2014). In addition to abiotic stress, impacts of biotic agents should be considered while developing assisted migration strategies (Bucharova 2017). In ponderosa pine, damage to seedlings due to mammals such as gophers and rabbits have been previously reported (Pearson 1950; Schubert et al. 1970; Schubert 1974). This damage by small mammals may be particularly severe in planted areas fenced for protection against large herbivores, inadvertently excluding access by predators (Shepperd and Mata 2005).

Previous attempts to evaluate ponderosa pine seed sources for future harsh conditions in the southwestern U.S. did not specifically include low elevation provenances (Rehfeldt 1993) which may be arid adapted (Alberto et al. 2013), did not cover a wide environmental and geographic range (Kolb et al. 2016), or only studied seedlings growing under resource rich greenhouse conditions (Dixit and Kolb 2020). More studies testing provenance performance in field conditions over environmental gradients are needed in order to determine impacts of abiotic and biotic factors on planted seedlings and to establish seed transfer guidelines based on a changing climate.

Survival, growth, carbon isotope discrimination, and nitrogen concentration were the primary response variables assessed in this study. In ponderosa pine, a trade-off between growth rate and drought tolerance has been previously reported (Kerr et al. 2015). In addition, carbon isotope discrimination ($\Delta^{13}C$) and water use efficiency are closely related in ponderosa pine (Cregg et al. 2000; Olivas-Garcia et al., 2000). Carbon isotope discrimination is determined by the ratio of net photosynthesis to stomatal conductance integrated over time and is related to leaf internal CO_2 concentration (Farquhar et al. 1982), a potentially important mechanism of drought tolerance. Leaf nitrogen concentration is positively related to photosynthetic capacity as it is essential for proteins of the photosynthetic apparatus (Wright et al. 2004).

In this study, a total of 21 provenances from Arizona and New Mexico were evaluated for their response to environmental stressors (i.e., high temperatures and water stress) at three planting sites that varied in elevation, temperature, and precipitation to address the following questions (1) How does planting site, provenance, and their interaction influence survival, growth, and carbon isotope discrimination of ponderosa pine seedlings? (2) How does seedling performance relate to provenance environmental characteristics?

Methods

Common garden sites

The study was conducted in three common gardens across an elevation gradient. The low elevation site was planted on 21st July 2018 in an open area within a pinyon-juniper woodland in northern Arizona at an elevation of 1930 m. The soil at the site is a clay loam derived from basalt parent material. The year of planting (2018) was 1.4 °C warmer and received only 33% of average annual precipitation compared to 30-year normals (1981-2010; https://prism.oregonstate.edu). The middle elevation site was planted on 20th July 2018 in an opening in a ponderosa pine forest in northern Arizona at an elevation of 2200 m. The soil type at this site is clay loam derived from basalt parent material. At this site, the planting year was 0.2 °C warmer and received only 66% of average annual precipitation compared to 30-year normals. Conditions at this site were particularly dry in the year 2020 with only 33% of average annual precipitation. The high elevation site was planted on 24th July 2018 in a grassy meadow within an aspen and mixed conifer forest in southern Utah at an elevation of 2780 m. The soil type at this site is a silty clay loam derived from volcanic parent material. The planting year was 1.5 °C warmer and received about 85% of average annual precipitation compared to 30-year normals. Conditions at this site were particularly dry in the year 2020 with about 40% of 30-year average annual precipitation. Overall, the study period (2018-2020) was drier compared to the long-term average at all three sites (Supplementary figures 4.1 and 4.2). During this period, temperatures were warmer at all sites except for the year 2019 when the mean annual temperatures were 0.8 °C and 0.6 °C lower than long term average at the mid and high elevation sites, respectively (Supplementary figure 4.1). Each site was equipped with a weather station and a datalogger (Campbell Scientific, Logan, Utah, USA) to measure air temperature and precipitation. The planted areas at all sites were protected against ungulate herbivory by fences that prevented access by large (e.g., cattle, deer, elk) but not small animals.

Experimental design and plant material

At each of the three sites, 1008 seedlings were planted in a randomized complete block design. We planted a 1.2 m buffer around the periphery with a row of ponderosa pine seedlings to reduce potential edge effects. Existing vegetation within the fenced exclosures at the mid and high elevation sites was reduced using herbicide (Ranger Pro, Bayer Crop Science, Chesterfield, MO, USA) prior to planting. Herbicide was not needed at the low elevation site due to sparse grass cover. At each site, seedlings were planted in 4 blocks, each consisting of one 12-tree linear plot for each provenance. The arrangement of provenance plots was randomized for each block. Spacing among seedlings was 1.2 m. Each of the 21 provenances was represented by 48 seedlings from between 3 and 6 mother trees or a pooled collection of seeds in cases where mother tree level information was not available (5 provenances). To produce the seedlings, seeds were sown in March 2018 into 164 mL containers (Ray Leach Cone-tainers SC10 Super, Stuewe & Sons, Inc., Tangent, OR, USA) at the John T. Harrington Forestry Research Center with New Mexico State University located in Mora, NM. Seedlings were grown in the greenhouse nursery under standard operational protocols for approximately 4 months until planting in July 2018. Detailed greenhouse growing conditions, and seed and provenance information are described in Dixit et al (2020). The provenances are located over a gradient of elevation, temperature and precipitation in Arizona and New Mexico (Table 1). Provenances ranged from ~1600 m to ~2800 m in elevation, 364 mm to 767 mm in mean annual precipitation, and 5.7 °C to 14.2 °C in mean annual temperature. Provenance elevation had a strong negative correlation with mean annual temperature (MAT) ($r = -0.902$, $p < 0.0001$) and a moderate positive correlation with mean annual precipitation (MAP) ($r = 0.500$, $p = 0.020$); provenance MAT and MAP had a moderate negative correlation ($r = -0.434$, $p = 0.048$). Latitude and MAT had a moderate negative trend ($r = -0.406$, $p = 0.067$) and longitude and elevation had a moderate positive trend ($r = 0.370$, $p = 0.098$).

Survival and growth measurements

At the high and mid elevation sites, survival was assessed once every fall and summer each year between 2018 and 2020. Height and diameter at the soil surface (ground line diameter) were measured every fall (October or November) between 2018 and 2020. At the low elevation site, survival was assessed once per week between August and September 2018. We did not measure seedling growth at the low elevation site because almost all seedlings died within the first two months after planting. Seedlings were classified as dead when they were 100% brown or were removed from planting locations by herbivores. We also observed potential causes of mortality and signs of mortality agents such as scats, gopher holes, etc.

Carbon isotopic discrimination

In November 2019 at the end of the second full growing season after planting, we collected current year true needles from between 7 and 13 seedlings from each provenance at each of the mid and high elevation sites. At each site, seedlings were selected for sampling from each provenance-block combination (row plot) using stratified random sampling. We used mother trees from each row plot as strata to ensure balanced sampling over all surviving mother trees. Provenance rows in some of the blocks had no survival and thus, samples were not taken. For 5 provenances without mother tree information, we randomly sampled 3 seedlings from each row plot per block. The needles were oven-dried (Sheldon Manufacturing, INC, Cronelius, OR) at 65 °C for 72 hours and ground to homogenous powder using a ball mill grinder (Mixer Mill MM200, Retsch, Haan, Germany). The samples were analyzed for carbon stable isotopic composition and nitrogen concentration at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University, Flagstaff, AZ using a DELTA V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) which is configured through a Finnigan ConFlo III for automated continuous-flow analysis of δ^{13}C and %N using a Carlo-Erba NC2100 elemental analyzer for combustion and separation of carbon and nitrogen. Carbon isotope discrimination (Δ^{13}C) was calculated using the following equation (Farquhar et al. 1989) where δ^{13}C is the isotopic ratio of ^{13}C to ^{12}C in needle tissues, and -0.008 is the approximate δ^{13}C of atmospheric CO_2, compared with the Pee Dee Belemnite standard.

$$\Delta^{13}\mathrm{C} = \frac{-0.008 + [(\delta^{13}\mathrm{C})/(-1000)]}{1 - [(\delta^{13}\mathrm{C})/(-1000)]} \times 1000$$

Data analysis

We used a mixed-effects model with provenance, site, and the interaction between provenance and site as fixed effects and blocks nested within sites as a random effect. We conducted the analyses on block-level provenance means and regarded the row plot of seedlings from same provenance within a block as the experimental unit. We used post-hoc Tukey's Honestly Significant Difference (HSD) comparison of means to evaluate significant site and provenance differences ($\alpha = 0.05$). Provenance effect on growth was not tested for the year 2020 due to very low survival at the high elevation site at the end of the year. We used relative height growth to assess growth since planting estimated as: (Fall height – Height after planting)/Height after planting. We used survival analysis to assess the impact of planting site on seedling survival between July 2018 and October 2020. In order to examine the influence of provenance elevation on survival at each of the three sites, provenances were assigned to elevation groups, which were included in ANOVA as fixed effects. Elevational groups were defined as follows: low elevation < 2000 m; middle elevation = 2000 to 2500 m; high elevation > 2500 m (Dixit and Kolb 2020; Dixit et al. 2020). All data approximated a normal distribution. The relationships between provenance traits and provenance environmental characteristics were evaluated on provenance means with correlation and regression analyses. We interpreted the strength of relationship based on the value of the correlation coefficient (r) as: weak between 0 and 0.3, moderate between 0.3 and 0.7, and strong between 0.7 and 1.0 (Ratner 2009). JMP Pro version 14 (SAS Institute Inc., Cary, NC, USA) was used to perform all analyses.

Results

Survival analyses

Seedling survival differed significantly ($p < 0.0001$) among the three common garden sites (Figure 4.1). By Fall 2020, survival at the mid elevation site was much higher than the other two sites; 54% seedlings survived at mid elevation site compared to 1.5% and 0% at the high and low elevation sites, respectively. At the low elevation site, seedlings from low elevation provenances survived longer than seedlings from mid and high elevation provenances ($p = 0.0015$; Figure 4.2). At this site, most seedlings (80%) died in the first month after planting and survival was 0% at the end of fall 2018. At the mid and high elevation sites, survival at the end of 2020 did not differ significantly among elevation groups ($p = 0.3169$ and 0.1081, respectively). Two major mortality events occurred at the mid elevation site; summer 2019 (30% mortality) and fall 2020 (10% mortality). At the high elevation site, survival decreased continuously between 2018 and 2020 with the largest decrease (40%) in summer 2020.

Mortality agents changed from abiotic to biotic factors with an increase in elevation over the site elevational gradient. At the low elevation site, 99.7% of seedling mortality was attributed to desiccation (visually observed dry seedlings with intact roots). The only exception was herbivory-induced mortality of three seedlings that were planted close to ant colonies. At the mid elevation site, important mortality agents were rabbit herbivory (~30% seedlings; obvious scat and feeding patterns) and desiccation (~10% seedlings). Rabbit herbivory occurred over an 8-week period in the first spring after planting (2019). At the high elevation site, most seedling mortality (90%) was attributed to pocket gopher (*Thomomys bottae*) herbivory as evidenced by gopher holes, complete absence of seedlings, and seedlings with missing roots, with a smaller amount (10%) attributed to desiccation.

Growth

Provenances differed significantly in height immediately after planting and in fall 2019 after the first full growing season. Across the mid and high elevation sites, height at planting ranged among provenances from 90.6 mm for SAP to 135.6 mm for BR. Provenances did not vary significantly in relative height growth between planting and fall 2019 (Tables 4.2 and 4.3). However, relative height growth was affected by planting site for years 2019 and 2020 and was significantly higher at the mid elevation site than the high elevation site for both years (Figure 4.3). Provenance effect on growth was not tested for the year 2020 because only 1.5% of seedlings survived at the high elevation site during that year. Overall, seedlings grew more at the mid elevation site compared with the high elevation site (Figure 4.3). Site differences were consistent over provenances as indicated by the non-significant provenance x site interaction (Table 4.3).

Correlation analysis revealed several moderate significant correlations ($r = 0.3 - 0.7$; $p < 0.05$) between site-specific mean provenance growth traits and provenance environmental characteristics (Table 4.4). At the mid and high elevation sites, height after planting was negatively correlated with provenance longitude ($r = -0.60$ and -0.54, respectively) and elevation ($r = -0.55$ and -0.45, respectively); height in fall 2019 was negatively correlated with provenance longitude ($r = -0.52$ and -0.44, respectively). At the mid elevation site, height after planting and provenance MAT were positively correlated ($r = 0.46$); relative height growth had a positive correlation with provenance elevation ($r = 0.56$) and provenance MAP ($r = 0.54$), and a negative correlation with MAT ($r = -0.51$). At the high elevation site, GLD was correlated negatively with provenance latitude ($r = -0.53$) and positively with provenance MAP ($r = 0.60$). At both sites, we also found a few interesting moderate trends ($r = 0.3 - 0.7$; $p = 0.05 – 0.1$) between site-specific provenance growth traits and environmental characteristics. At the mid elevation site, fall 2019 height had a negative trend with provenance elevation ($r = -0.42$) and a positive trend with MAT ($r = 0.39$). Also, a positive trend between GLD and provenance MAP ($r = 0.43$) occurred at the same site. At the high elevation site, provenance latitude had a negative trend with height after planting ($r = -0.41$), longitude had a negative trend with GLD ($r = -0.40$), and MAT had a positive trend with height after planting ($r = 0.37$).

Carbon isotope discrimination

Provenances ($p = 0.006$; Figure 4.4) and sites ($p = 0.0001$; Figure 4.5) varied significantly in $\Delta^{13}C$, whereas the provenance x site interaction was not significant ($p = 0.239$) (Table 4.3). $\Delta^{13}C$ was about 1‰ higher in seedlings growing at the mid elevation site compared with the high elevation site. $\Delta^{13}C$ ranged among provenances from 18.4 ‰ for SKT to 20.2 ‰ for PIS (Figure 4.4). Correlations between mean $\Delta^{13}C$ and provenance environmental characteristics were weak and non-significant at the mid elevation site. However, at the high elevation site, provenance latitude had a moderate negative trend with $\Delta^{13}C$ (r = -0.42; Table 4.4).

Leaf nitrogen

Leaf %N differed between planting sites (p = 0.0004) but not among provenances (p = 0.333) (Table 4.3; Figure 4.4). The interaction between provenance and site was not significant (p = 0.177). %N was about 0.3% higher in seedlings growing at the high elevation site than at the mid elevation site. At the mid elevation site, %N and provenance longitude were negatively correlated (r = -0.48). At the high elevation site, %N had a negative trend with provenance latitude (r = -0.37) and longitude (r = -0.40), and a positive trend with provenance MAP (r = 0.42; Table 4.4).

Discussion

Our results show that seedling survival was highest at the mid elevation site in the core of the species current range and decreased substantially at sites outside the current range with no survival at the low elevation site and almost none at the high elevation site (1.5%). Our finding of differences in survival among sites is similar to a recent report of significant site effects for survival of Jeffery (*Pinus jeffreyi* Grev. & Balf.) and ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) seedlings planted at three different elevations in California (Martínez-Berdeja et al. 2019); however, the pattern of survival percentage at different elevations was not consistent. The ~2 ^{o}C warmer MAT of the low elevation site than the mid elevation site is an example of the future warmer climate in the current range of ponderosa pine in the southwestern U.S. by the end of this century (Garfin et al. 2013). Our result of 0% survival at this low elevation site implies future challenges in successful planting of ponderosa pine within the core of its current geographical range due to high temperature and water stress. In the future, reforestation via planting might need to be timed with particularly wet years, if possible.

Longer survival of provenances from low elevations (<2000 m) than mid (2000-2500 m) and high elevation (>2500 m) provenances at the hot, low elevation site (Figure 4.2) suggests better adaptation of low elevation provenances to warmer and drier conditions. This result is consistent with previous studies that reported better performance of pine seedlings from provenances from arid locations at warm field sites (Taibi et al. 2014) and under experimental drought in the greenhouse (Kolb et al. 2016). While we found a difference in mean survival of about 3 days between low elevation provenances and mid and high elevation provenances under extreme field conditions, it is important to note that no provenances had successful establishment at the low elevation site. Provenances from low elevation and drier locations have been shown to have traits related to drought adaptation in ponderosa pine such as lower specific leaf area and higher growth allocation to roots (Kolb et al. 2016; Dixit and Kolb 2020) and have been recommended for future planting to mitigate impacts of climate warming (Williams and Dumroese 2013; Rehfeldt et al. 2014). However, mechanisms of drought adaptation in such provenances are not fully understood and should be investigated in future studies.

At the mid and high elevation sites, the most important source of seedling mortality was herbivory followed by desiccation. Herbivory and drought have been recognized as major challenges in outplanting success of nursery grown seedlings for reforestation (Burney and Jacobs 2013; Fargione et al. 2021). At the mid elevation site ~30% of seedlings died due to rabbit herbivory. At the high elevation site, gophers were the most important mortality agent, accounting for ~90% seedling mortality. Our finding of seedling mortality due to rabbits and gophers is consistent with ponderosa pine mortality agents previously reported by Schubert

(1974). However, the use of fences at our sites to prevent ungulate herbivory may have indirectly caused an increase in herbivory by small mammals due to restricted access of fenced area by predators (Shepperd and Mata 2005). As of fall 2020, about 10% seedlings died due to desiccation at the mid and high elevation sites each. This could be attributed to the dry conditions in summer 2020 at both sites (supplementary figures 4.1 and 4.2). Overall, our results of high seedling mortality due to herbivory suggests severe biotic constraints to planting success at higher elevations. The use of protective measures such as tree shelters or tubes may alleviate much of the herbivory damage while simultaneously improving plant water relations (Oliet et al. 2018).

Growth of provenances after planting was primarily determined by early provenance differences in height growth in the greenhouse, not relative growth rate after planting. Provenance differences in relative growth rate in the field might emerge in wetter years, which will require further investigation. Relative height growth differed significantly across sites with the seedlings at the mid elevation site having a higher growth rate than the seedlings at high elevation site (Figure 4.3). Overall, we found that seedling growth was constrained by the high elevation environment, perhaps due to a shorter frost-free season and cooler temperature. At both sites, low-elevation western provenances were taller at planting suggesting faster growth of these provenances under resource rich greenhouse conditions. This result is similar to an earlier study of some of the same provenances conducted in a different greenhouse (Dixit and Kolb 2020). At the mid elevation site, provenances from high elevation, cooler, and wetter areas had a higher relative height growth rate than low elevation, warmer, and drier provenances. This result shows that high elevation provenances can take advantage of warmer conditions, which in our study were created by planting these high elevation provenances at the mid elevation common garden site.

Results for leaf Δ^{13}C and leaf %N indicate an increase in water use efficiency and photosynthetic capacity with increasing elevation of the planting site (Figure 4.5). Hultine and Marshall (2000) suggested that a decrease in Δ^{13}C with increasing elevation in conifer species may result from a decrease in stomatal conductance with elevation due to lower temperature. Therefore, a greater photosynthetic capacity as suggested by higher leaf %N and a lower stomatal conductance might explain the lower Δ^{13}C and higher water use efficiency at the high elevation site as compared to the mid elevation site in our study. Our results of a decrease in Δ^{13}C with elevation of planting site are similar to an earlier study involving ten populations of ponderosa pine growing at three sites in Nebraska (Zhang and Cregg, 2005). We also found a significant provenance effect on Δ^{13}C but not on %N suggesting a lack of genetic variation in photosynthetic capacity. The provenance effect on Δ^{13}C was mainly due to provenance SKT which had the lowest Δ^{13}C among all provenances across both sites and needs to be further investigated as it might be useful for future reforestation due to its higher water use efficiency. Provenances from more southern latitudes had higher Δ^{13}C than northern provenances at the high elevation site. This latitudinal pattern is similar to a greenhouse study involving 21 provenances of *Populus balsamifera* L. (Soolanayakanahally et al., 2009) but differs from a common garden study of *Pinus strobiformis* seedlings (Goodrich et al. 2016). At the mid elevation site, western provenances had high %N suggesting higher photosynthetic capacity of these provenances as compared to the eastern ones. These environmental patterns could be a result of genetic variation among provenances or due to correlations with other environmental factors such as temperature and precipitation.

In summary, our results provide evidence of site and provenance effects on survival and performance of planted southwestern ponderosa pine seedlings growing at different elevations. Our findings of longer survival of low elevation provenances under dry, hot field conditions support previous recommendations for planting ponderosa pine provenances from lower elevations in the same geographic region in reforestation projects to promote seedling establishment in a warming climate (Rehfeldt et al. 2014). In addition to taking site environment and provenance origin into consideration, our study highlights the importance of accounting for mortality from biotic agents in reforestation projects especially at high elevation meadow sites. Additional studies should focus on influence of biotic agents on different provenances over a longer period of time and the interactions between abiotic and biotic stresses on seedling survival and performance (Sáenz-Romero et al. 2021). Overall, our findings enhance understanding of constraints on the success of planted seedlings and thus inform strategies to maintain ponderosa pine during changing climate by active reforestation.

References

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1, 95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Allen, C.D., Breshears, D.D., 1998. Drought-induced shift of a forest–woodland ecotone: Rapid landscape response to climate variation. Proceedings of the National Academy of Sciences 95, 14839–14842.

Bucharova, A., 2017. Assisted migration within species range ignores biotic interactions and lacks evidence. Restoration Ecology 25, 14–18. https://doi.org/10.1111/rec.12457

Burney, O.T., Jacobs, D.F., 2013. Ungulate herbivory of boreal and temperate forest regeneration in relation to seedling mineral nutrition and secondary metabolites. New Forests 44 (5):753-768. https://doi.org/10.1007/s11056-013-9381-9

Cregg, B.M., Olivas-García, J.M., Hennessey, T.C., 2000. Provenance variation in carbon isotope discrimination of mature ponderosa pine trees at two locations in the Great Plains. Canadian Journal of Forest Research. https://doi.org/10.1139/x99-226

Dixit, A., Kolb, T., 2020. Variation in seedling budburst phenology and structural traits among southwestern ponderosa pine provenances. Canadian Journal of Forest Research. https://doi.org/10.1139/cjfr-2019-0333

Dixit, A., Kolb, T., Burney, O., 2020. Provenance Geographical and Climatic Characteristics Influence Budburst Phenology of Southwestern Ponderosa Pine Seedlings. Forests 11, 1067. https://doi.org/10.3390/f11101067

Fargione, J., Haase, D.L., Burney, O.T., Kildisheva, O.A., Edge, G., Cook-Patton, S.C., Chapman, T., Rempel, A., Hurteau, M.D., Davis, K.T., Dobrowski, S., Enebak, S., De La Torre, R., Bhuta, A.A.R., Cubbage, F., Kittler, B., Zhang, D., Guldin, R.W., 2021. Challenges to the Reforestation Pipeline in the United States. Frontiers in Forests and Global Change 4. https://doi.org/10.3389/ffgc.2021.629198

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Functional Plant Biology. 9, 121–137. https://doi.org/10.1071/pp9820121

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon Isotope Discrimination and Photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443

Garfin, A.; Jardine, R.; Merideth, M. Black; LeRoy, S. 2013. Assessment of climate change in the southwest United States: a report prepared for the National Climate Assessment. Washington, DC: Island Press. 531 p.

Goodrich, B.A., Waring, K.M., Kolb, T.E., 2016. Genetic variation in *Pinus strobiformis* growth and drought tolerance from southwestern US populations. Tree Physiology 36, 1219–1235. https://doi.org/10.1093/treephys/tpw052

Hicke, J.A., Meddens, A.J.H., Kolden, C.A., 2016. Recent Tree Mortality in the Western United States from Bark Beetles and Forest Fires. Forest Science 62, 141–153. https://doi.org/10.5849/forsci.15-086

Hultine, K.R., Marshall, J.D., 2000. Altitude trends in conifer leaf morphology and stable carbon isotope composition. Oecologia 123, 32–40. https://doi.org/10.1007/s004420050986

Kerr, K.L., Meinzer, F.C., McCulloh, K.A., Woodruff, D.R., Marias, D.E., 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. Tree Physiology 35, 535–548. https://doi.org/10.1093/treephys/tpv034

Kolb, T.E., Dixit, A.H., Burney, O., 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern United States. Tree Planters' Note 62, 104–112.

Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A., 2016. Local-Scale Drought Adaptation of Ponderosa Pine Seedlings at Habitat Ecotones. Forest Science 62, 641–651. https://doi.org/10.5849/forsci.16-049

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S., Schueler, S., 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters 15, 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

Martínez-Berdeja, A., Hamilton, J.A., Bontemps, A., Schmitt, J., Wright, J.W., 2019. Evidence for population differentiation among Jeffrey and Ponderosa pines in survival, growth and phenology. Forest Ecology and Management 434, 40–48. https://doi.org/10.1016/j.foreco.2018.12.009

Minott, J.A., Kolb, T.E., 2020. Regeneration patterns reveal contraction of ponderosa forests and little upward migration of pinyon-juniper woodlands. Forest Ecology and Management 458, 117640. https://doi.org/10.1016/j.foreco.2019.117640

Oliet, J.A., Blasco, R., Valenzuela, P. et al. Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments? New Forests 50, 267–282 (2019). https://doi.org/10.1007/s11056-018-9659-z

Olivas-García, J.M., Cregg, B.M., Hennessey, T.C., 2000. Genotypic variation in carbon isotope discrimination and gas exchange of ponderosa pine seedlings under two levels of water stress. Canadian Journal of Forest Research 30, 10. https://doi.org/10.1139/x00-080

Pearson. G.A. 1950. Management of ponderosa pine in the Southwest. U.S. Department of Agriculture Monograph 6, 218 p.

Petrie, M.D., Bradford, J.B., Hubbard, R.M., Lauenroth, W.K., Andrews, C.M., Schlaepfer, D.R., 2017. Climate change may restrict dryland forest regeneration in the 21st century. Ecology 98, 1548–1559. https://doi.org/10.1002/ecy.1791

Ratner, B., 2009. The correlation coefficient: Its values range between +1/−1, or do they? J Target Meas Anal Mark 17, 139–142. https://doi.org/10.1057/jt.2009.5

Rehfeldt, G.E., 1993. Genetic Variation in the Ponderosae of the Southwest. American Journal of Botany 80, 330–343. https://doi.org/10.2307/2445357

Rehfeldt, G.E., Crookston, N.L., Warwell, M.V., Evans, J.S., 2006. Empirical Analyses of Plant-Climate Relationships for the Western United States. International Journal of Plant Sciences 167, 1123–1150. https://doi.org/10.1086/50771

Rehfeldt, G.E., Jaquish, B.C., Sáenz-Romero, C., Joyce, D.G., Leites, L.P., Bradley St Clair, J., López-Upton, J., 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Reforestation. Forest Ecology and Management 324, 147–157. https://doi.org/10.1016/j.foreco.2014.02.040

Rodman, K.C., Veblen, T.T., Chapman, T.B., Rother, M.T., Wion, A.P., Redmond, M.D., 2020. Limitations to recovery following wildfire in dry forests of southern Colorado and northern New Mexico, USA. Ecological Applications 30, e02001. https://doi.org/10.1002/eap.2001

Sáenz-Romero, C., O'Neill, G., Aitken, S.N., Lindig-Cisneros, R., 2021. Assisted Migration Field Tests in Canada and Mexico: Lessons, Limitations, and Challenges. Forests 12, 9. https://doi.org/10.3390/f12010009

Savage, M., Nystrom, M., J, F., 2013. Double whammy: high-severity fire and drought in ponderosa pine forests of the Southwest. Canadian Journal of Forest Research. https://doi.org/10.1139/cjfr-2012-0404

Schubert, G.H., 1974. Silviculture of southwestern ponderosa pine: The status of our knowledge. Res. Pap. RM-123. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 71 p. 123.

Schubert, Gilbert H., L.J. Heidmann, and M.M. Larson. 1970. Artificial reforestation practices for the Southwest. U.S. Department of Agriculture, Handbook 370, 25 p.

Shepperd WD, Mata SA (2005) Planting aspen to rehabilitate riparian areas: a pilot study. United States Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Soolanayakanahally, R.Y., Guy, R.D., Silim, S.N., Drewes, E.C., Schroeder, W.R., 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment 32, 1821–1832. https://doi.org/10.1111/j.1365-3040.2009.02042.x

Taïbi, K., del Campo, A.D., Mulet, J.M., Flors, J., Aguado, A., 2014. Testing Aleppo pine seed sources response to climate change by using trial sites reflecting future conditions. New Forests 45, 603–624. https://doi.org/10.1007/s11056-014-9423-y

Williams, A.P., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., Cook, E.R., Gangodagamage, C., Cai, M., McDowell, N.G., 2012. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change 3, 292–297. https://doi.org/10.1038/nclimate1693

Williams, M.I., Dumroese, R.K., 2013. Preparing for Climate Change: Forestry and Assisted Migration. Journal of Forestry 111, 287–297. https://doi.org/10.5849/jof.13-016

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827. https://doi.org/10.1038/nature02403

Zhang, J., Cregg, B.M., 2005. Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. Forest Ecology and Management 219, 1–12. https://doi.org/10.1016/j.foreco.2005.08.038

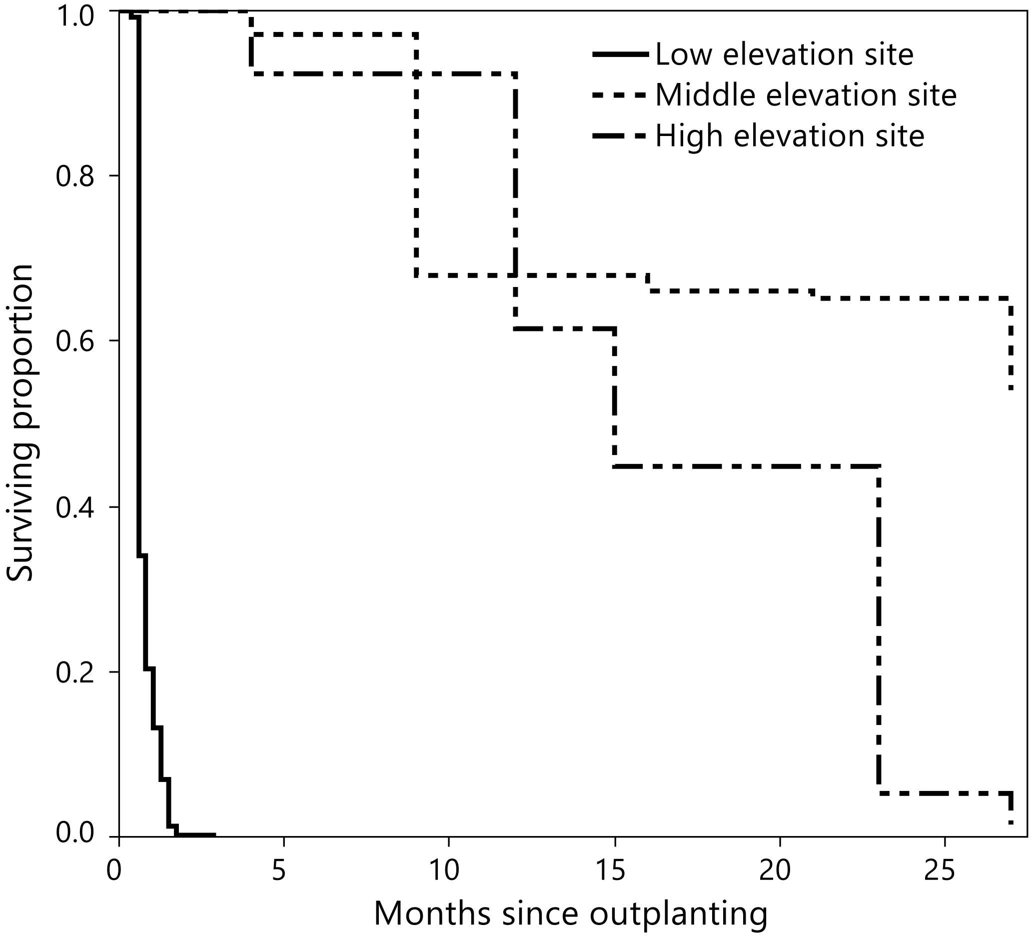


Figure 4.1: Surviving proportion of ponderosa pine seedlings at the three common garden sites over 27 months (July 2018 to October 2020).

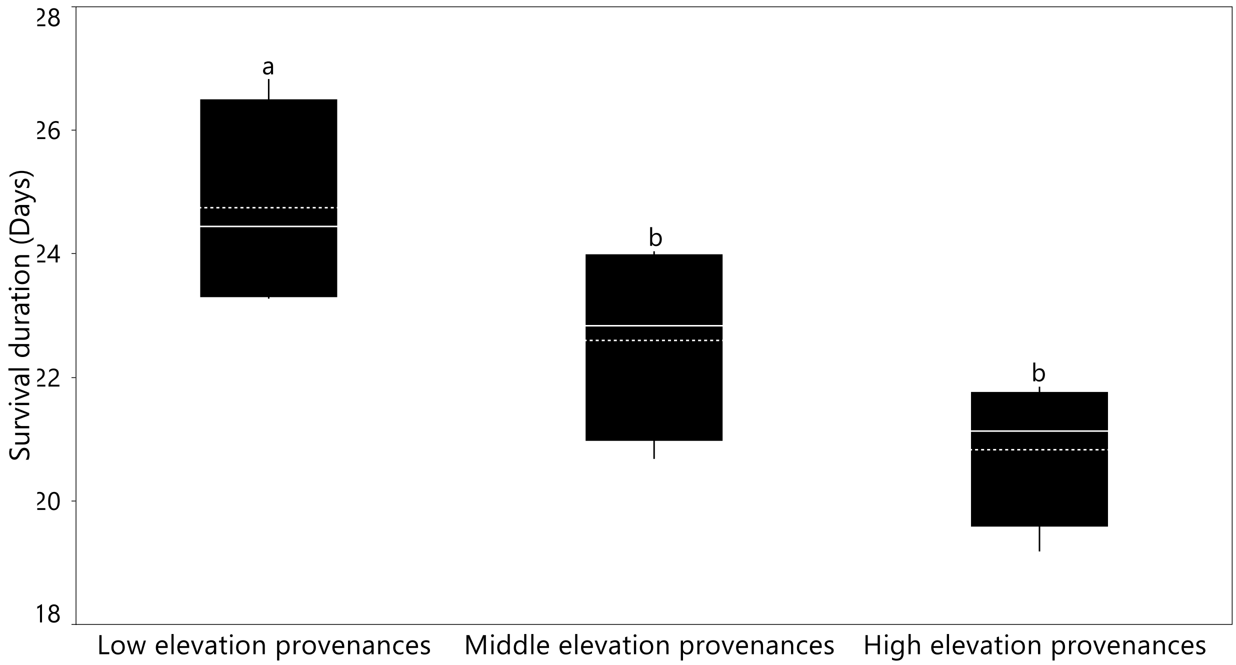


Figure 4.2: Surviving days since outplanting by provenance elevation groups at the low elevation site. Elevation groups: Low (<2000 m), Mid (2000 – 2500 m), and High (>2500 m). Dashed lines are mean and solid lines are the median. Means followed by the same letter do not differ significantly (Tukey's HSD tests; α = 0.05).

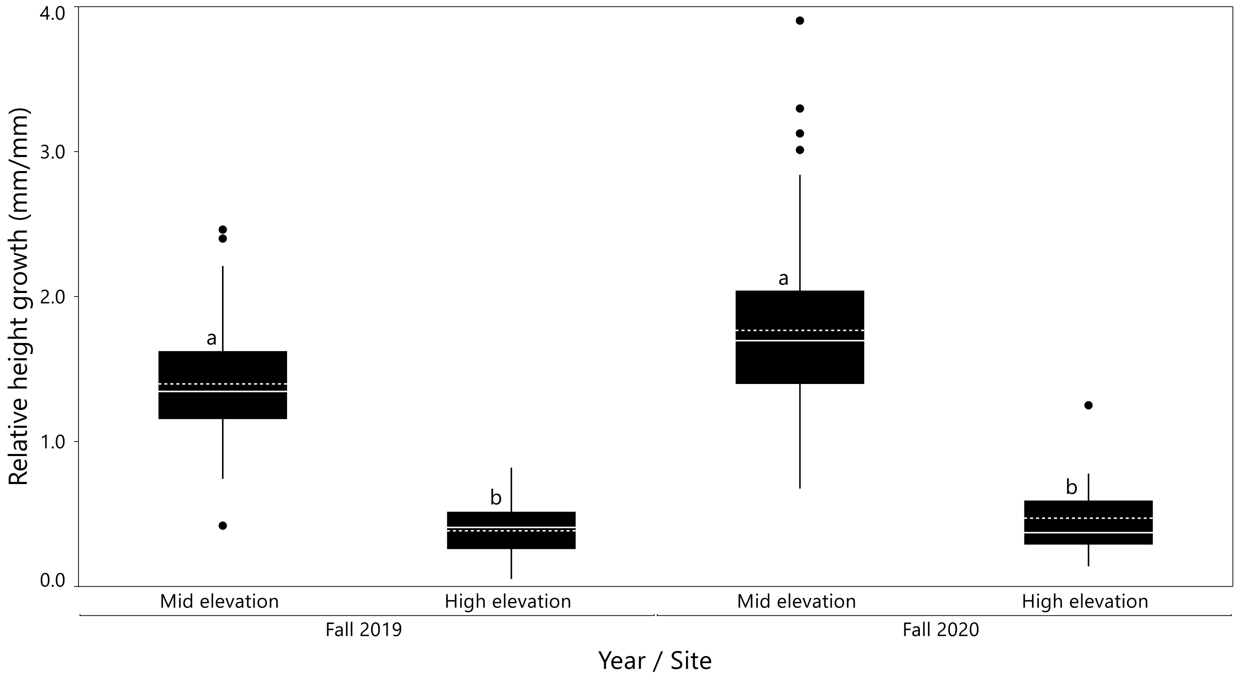


Figure 4.3: Relative height growth by planting sites for 2019 and 2020. Dashed lines are mean and solid lines are the median. Means followed by the same letter do not differ significantly (Tukey's HSD tests; α = 0.05).

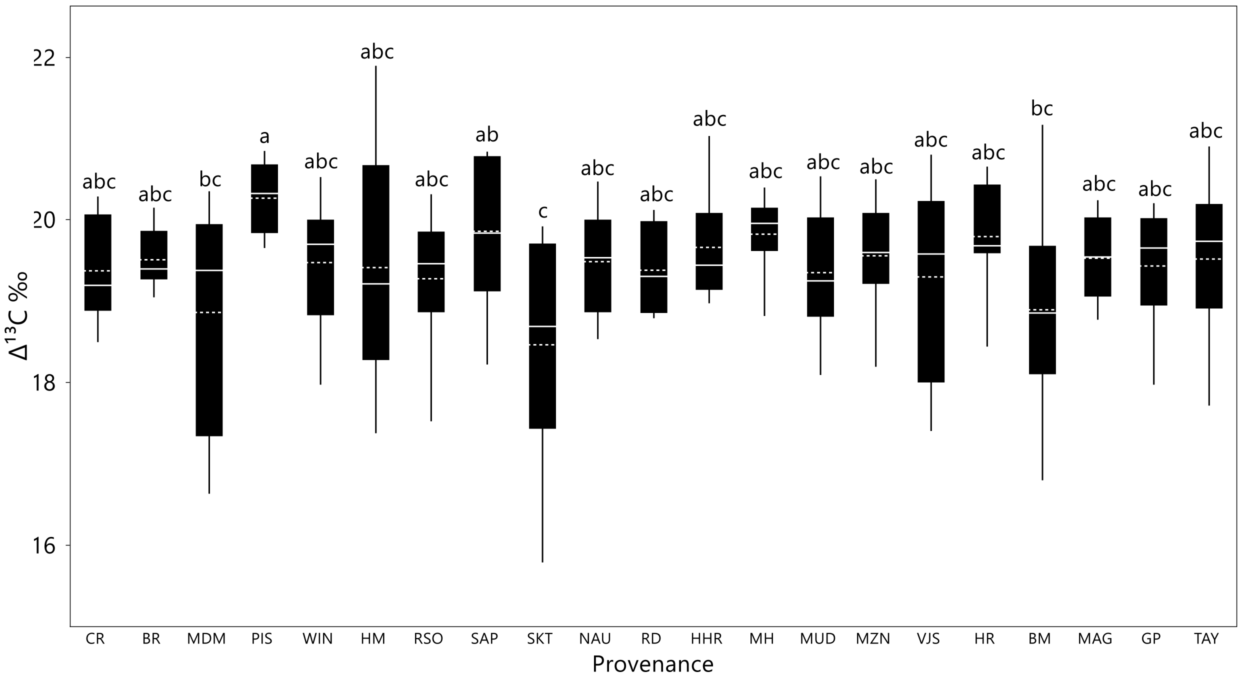


Figure 4.4: Leaf carbon isotope discrimination ($\Delta^{13}C$) by provenance averaged over the mid and high elevation sites (Ordered by elevation). Dashed lines are mean and solid lines are the median. Means followed by the same letter do not differ significantly (Tukey's HSD tests; $\alpha = 0.05$).

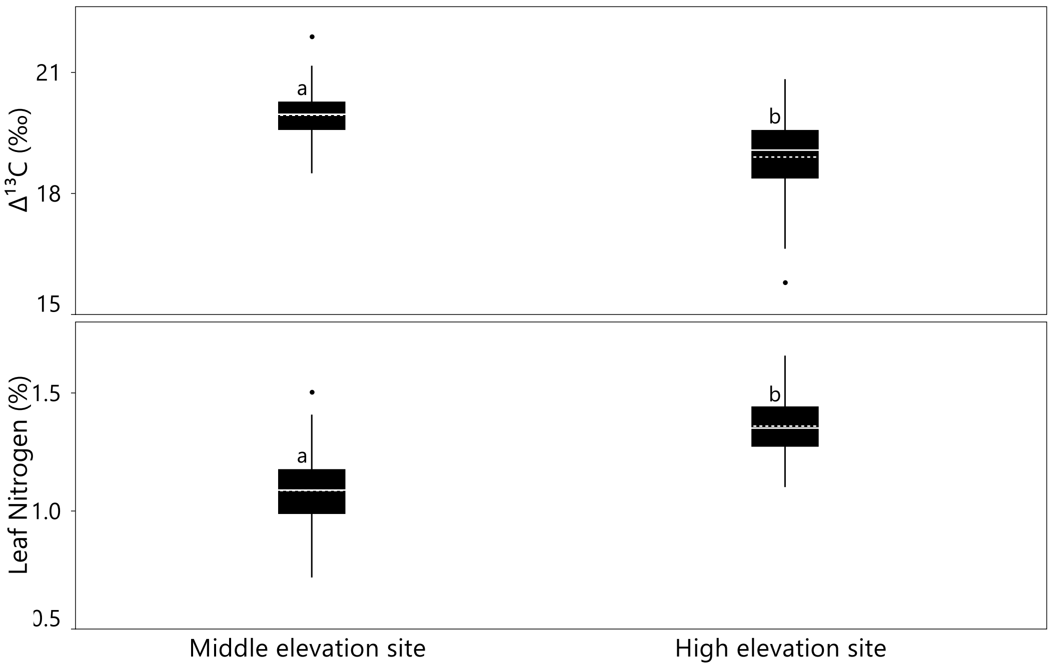
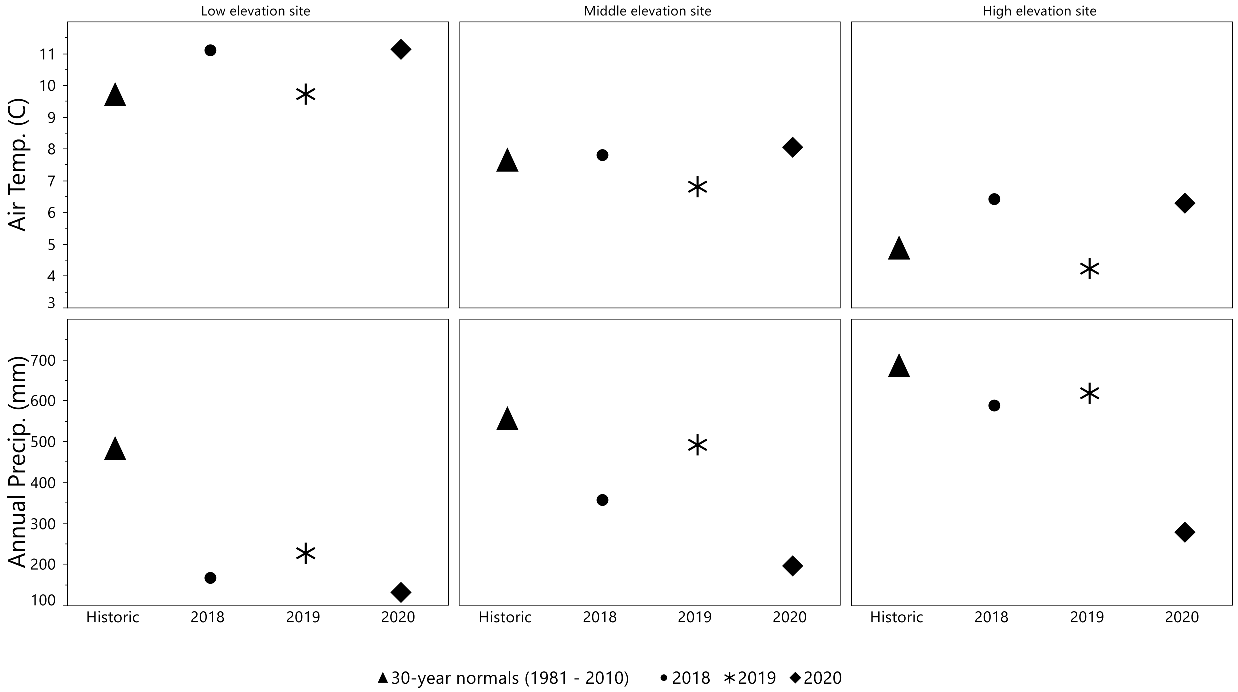
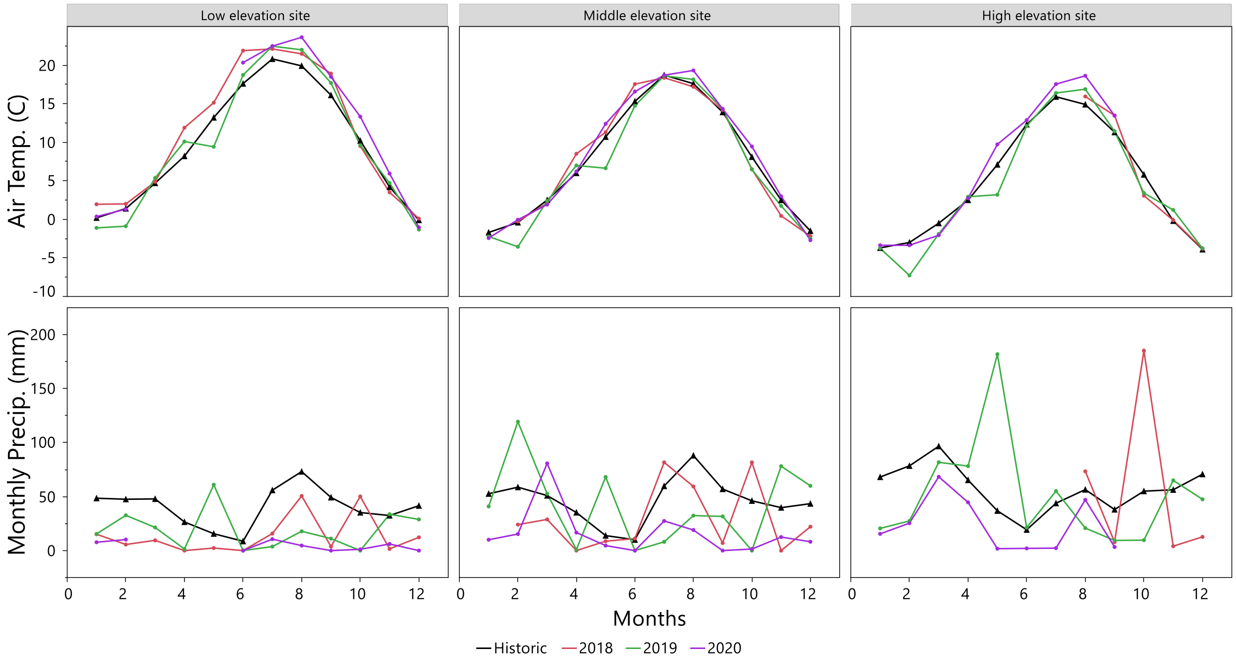


Figure 4.5: Leaf carbon isotope discrimination ($\Delta^{13}C$) and leaf nitrogen (%N) averaged over all provenances by planting site. Dashed lines are mean and solid lines are the median. Means followed by the same letter do not differ significantly (Tukey's HSD tests; $\alpha = 0.05$).



Supplementary figure 4.1: Mean air temperature (°C) and Annual precipitation for the study period (2018-2020) compared to 30-year normals (1981-2010; https://prism.oregonstate.edu/). At each site, gaps in weather station data were included via PRISM.



Supplementary figure 4.2: Monthly air temperature (°C) and precipitation for the study period (2018-2020) compared to 30-year normals (1981-2010; https://prism.oregonstate.edu/). Gaps in line represent unavailable data from weather stations.

Table 4.1: Provenance name and code (ordered by increasing elevation), planting sites, latitude, longitude, elevation, mean annual temperature (MAT), and mean annual precipitation (MAP). Climate data (30 year normal, 1981–2010) are from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 19 September 2020). Values are means over mother tree locations at each provenance.

	Latitude/Longitude	Elevation (m)	MAT (ºC)	MAP (mm)
Provenance (code)				
Cherry Road (CR)	34.586/-112.057	1592	14.2	408
Blue River (BR)	33.555/-109.193	1674	11.8	581
Mesa Del Medio (MDM)	35.116/-105.217	1714	12.3	397
Prescott – iron Springs Road (PIS)	34.585/-112.559	1846	11	546
Townsend Winona (WIN)	35.254/-111.415	1934	10.2	398
Hualapai Mountains (HM)	35.084/-113.875	1969	11.5	403
Ruidoso Service Office (RSO)	33.350/-105.583	1976	11.1	506
Sapello Rt. 3 (SAP)	35.700/-105.250	2050	9.8	456
South Kaibab Tusayan Dist. (SKT)	35.939/-112.084	2067	8.9	410
Northern Arizona University (NAU)	35.182/-111.655	2104	8.4	540
Rim District (RD)	34.487/-111.343	2244	9.4	767
HH Ranch (HHR)	34.183/-107.525	2270	9.2	364
Mineral Hill (MH)	35.633/-105.461	2277	8.5	528
Mud Springs (MUD)	36.463/-106.859	2277	6.9	443
Manzano Mountains (MZN)	34.623/-106.400	2366	8.6	638
Vallecitos-Jemez Springs (VJS)	35.809/-106.589	2436	6.8	571
Hartman Ridge (HR)	35.550/-105.533	2500	8.8	526
Borrego Mesa (BM)	35.990/-105.794	2560	6.3	470
Magdalena Mountains (MAG)	34.006/-107.215	2565	8.9	512
Green’s Peak (GP)	34.126/-109.535	2760	5.7	671
Mount Taylor (TAY)	35.266/-107.633	2814	6.1	722
Site				
Low elevation	35.586/-111.969	1930	9.7	482
Mid elevation	35.160/-111.730	2200	7.6	556
High elevation	37.620/-113.025	2780	4.9	685

Table 4.2: Provenance height, ground line diameter (GLD), and relative height growth ((Fall 2019 height – Height after planting)/Height after planting) at mid and high elevation sites (ordered by increasing elevation). Values are means (1 SE). Means in the same column followed by the same letters do not differ significantly (Tukey's HSD tests; α = 0.05).

Provenance	**Height after planting (mm)**	**Fall 2019 Height (mm)**	**Fall 2019 Relative height growth (mm/mm)**	**Fall 2019 GLD (mm)**
Mid elevation site				
CR	120.4 (1.6)abcd	244.4 (15.9)ab	1.0 (0.1)a	7.66 (0.41)a
BR	128.1 (5.1)a	283.2 (31.4)a	1.3 (0.2)a	7.96 (0.45)a
MDM	100.7 (0.5)abcde	219.2 (5.9)ab	1.2 (0.0)a	6.24 (0.06)a
PIS	122.7 (4.6)abc	287.9 (10.4)a	1.4 (0.0)a	7.27 (0.23)a
WIN	127.4 (6.3)ab	269.3 (11.9)ab	1.3 (0.0)a	7.53 (0.45)a
HM	103.5 (2.6)abcde	249.1 (1.3)ab	1.5 (0.1)a	7.00 (0.31)a
RSO	109.5 (4.3)abcde	244.5 (8.6)ab	1.4 (0.2)a	7.38 (0.33)a
SAP	92.4 (6.7)e	202.4 (8.2)b	1.4 (0.1)a	7.21 (0.21)a
SKT	123.0 (3.4)abc	252.9 (5.2)ab	1.0 (0.0)a	7.80 (0.23)a
NAU	119.2 (0.8)abcde	240.1 (10.7)ab	1.1 (0.1)a	7.75 (0.34)a
RD	120.5 (6.1)abcd	277.9 (11.0)ab	1.6 (0.1)a	8.46 (0.45)a
HHR	121.4 (2.5)abcd	263.1 (7.0)ab	1.2 (0.0)a	7.49 (0.20)a
MH	97.3 (6.6)cde	232.2 (14.8)ab	1.5 (0.1)a	7.55 (0.46)a
MUD	103.9 (8.5)abcde	217.1 (12.0)b	1.4 (0.2)a	6.50 (0.25)a
MZN	113.1 (7.0)abcde	265.4 (19.4)ab	1.2 (0.2)a	7.86 (0.36)a
VJS	100.0 (4.9)bcde	224.2 (5.3)ab	1.3 (0.1)a	7.90 (0.24)a
HR	98.1 (4.0)cde	225.8 (8.4)ab	1.5 (0.2)a	7.80 (0.22)a
BM	104.8 (8.3)abcde	225.0 (6.4)ab	1.6 (0.2)a	7.41 (0.32)a
MAG	106.2 (7.9)abcde	207.3 (2.9)b	1.1 (0.1)a	6.40 (0.52)a
GP	94.5 (3.2)de	200.3 (8.6)b	1.7 (0.3)a	7.45 (0.58)a
TAY	96.3 (2.0)cde	247.2 (10.8)ab	1.7 (0.1)a	7.19 (0.19)a
High elevation site				
CR	132.3 (5.3)abcd	149.2 (NA)ab	0.08 (NA)a	5.15 (NA)ab
BR	143.1 (6.0)a	187.6 (25.4)a	0.3 (0.0)a	5.32 (0.01)ab
MDM	104.2 (1.9)fgh	130.8 (6.3) ab	0.3 (0.1)a	4.46 (0.20)ab
PIS	137.2 (6.7)ab	183.7 (2.3) ab	0.4 (0.0)a	5.39 (0.26)ab
WIN	119.5 (6.6)abcdef	162.6 (13.4) ab	0.5 (0.0)a	5.58 (0.59)ab
HM	118.2 (4.6)bcdefg	147.8 (2.7)ab	0.3 (0.1)a	5.10 (0.09)ab
RSO	119.3 (3.7)abcdef	179.6 (21.7) ab	0.5 (0.2)a	6.01 (0.07)a
SAP	88.8 (3.7)h	130.2 (5.9) ab	0.4 (0.0)a	5.05 (0.84)ab
SKT	122.6 (3.3)abcdef	179.89 (NA) ab	0.4 (NA)a	5.32 (NA)ab
NAU	132.8 (9.7)abcd	160.6 (12.6) ab	0.3 (0.0)a	5.75 (0.22)ab
RD	135.5 (2.1)abc	173.78 (NA) ab	0.2 (NA)a	6.42 (NA)a
HHR	139.4 (3.2)ab	126.00 (NA)ab	0.3 (NA)a	3.80 (NA)ab
MH	106.6 (1.9)efgh	142.67 (NA) ab	0.3 (NA)a	4.58 (NA)ab
MUD	117.1 (2.1)bcdefg	146.71 (NA) ab	0.2 (NA)a	3.90 (NA)ab
MZN	129.9 (7.0)abcde	182.4 (3.4) ab	0.3 (0.0)a	5.70 (0.12)ab
VJS	111.5 (1.7)defgh	152.0 (NA) ab	0.6 (NA)a	4.72 (NA)ab
HR	93.1 (6.1)h	113.7 (8.3)b	0.2 (0.0)a	4.14 (0.56)b
BM	111.7 (7.1)cdefgh	145.7 (6.4)ab	0.4 (0.0)a	4.50 (0.05)ab
MAG	111.4 (6.3)defgh	100.0 (NA) ab	0.0 (NA)a	5.80 (NA)ab
GP	95.2 (5.0)gh	178.5 (NA)ab	0.6 (NA)a	6.45 (NA)a
TAY	109.6 (3.2)defgh	143.3 (18.4) ab	0.3 (0.2)a	5.60 (0.40)ab

Table 4.3: P-value of seedling heights, ground line diameter (GLD), carbon isotope discrimination ($\Delta^{13}C$), and percent nitrogen (%N) for the provenance effect, planting site effect, and provenance x site interaction. Bolded values significant at $P < 0.05$.

	Provenance	Site	Provenance x site
Height after planting	**<0.0001**	**0.024**	0.241
Height Fall 2019	**0.0002**	**<0.0001**	0.670
Relative height 2019	0.926	**<0.0001**	0.982
GLD 2019	**0.008**	**0.001**	0.143
$\Delta^{13}C$	**0.006**	**0.0001**	0.239
%N	0.333	**0.0004**	0.177

Table 4.4: Correlation coefficients between provenance environmental characteristics and trait means for mid and high elevation sites. Values in parentheses are p values. Boldface type indicates significance ($p < 0.05$). Ground line diameter (GLD); percent nitrogen (%N); carbon isotope discrimination ($\Delta^{13}C$).

	Latitude	**Longitude**	**Elevation**	**MAT**	**MAP**
Mid elevation site					
Height after planting (mm)	-0.36 (0.114)	**-0.60 (0.003)**	**-0.55 (0.009)**	**0.46 (0.036)**	-0.15 (0.518)
Height fall 2019 (mm)	-0.34 (0.123)	**-0.52 (0.014)**	-0.42 (0.053)	0.39 (0.081)	0.14 (0.534)
Rel ht. growth (mm/mm)	0.09 (0.668)	0.13 (0.561)	**0.56 (0.007)**	**-0.51 (0.015)**	**0.54 (0.010)**
GLD 2019 (mm)	-0.12 (0.586)	-0.26 (0.244)	0.001 (0.993)	-0.04 (0.843)	0.43 (0.051)
%N	0.36 (0.102)	**-0.48 (0.026)**	-0.07 (0.752)	-0.18 (0.422)	0.12 (0.593)
$\Delta^{13}C$	0.32 (0.154)	-0.05 (0.806)	-0.001 (0.996)	0.03 (0.864)	-0.09 (0.683)
High elevation site					
Height after planting (mm)	-0.41 (0.064)	**-0.54 (0.012)**	**-0.45 (0.039)**	0.37 (0.092)	0.02 (0.932)
Height fall 2019 (mm)	-0.27 (0.232)	**-0.44 (0.044)**	-0.28 (0.221)	0.08 (0.745)	0.35 (0.117)
Rel ht. growth (mm/mm)	0.09 (0.708)	0.007 (0.973)	0.10 (0.657)	-0.30 (0.175)	0.04 (0.850)
GLD 2019 (mm)	**-0.53 (0.014)**	-0.40 (0.066)	0.06 (0.789)	0.01 (0.960)	**0.60 (0.004)**
%N	-0.37 (0.091)	-0.40 (0.069)	-0.29 (0.197)	0.20 (0.371)	0.42 (0.059)
$\Delta^{13}C$	-0.42 (0.054)	0.01 (0.942)	0.02 (0.936)	0.09 (0.699)	0.31 (0.161)

Chapter V: Relationship Between Growth Rate and Water Use Efficiency of Ponderosa Pine Seedlings Under Dry Field Conditions

Abstract

We investigated growth rate, carbon isotope discrimination, leaf nitrogen concentration, leaf level gas exchange, and specific leaf area of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) seedlings from different provenances in an extremely dry year using a field common garden study. Twenty-one provenances from a range of elevations across Arizona and New Mexico were planted in year 2018 at a field site in the core of the species range in northern Arizona. We measured stem growth rate for 2019 to 2020, leaf carbon isotope discrimination ($\Delta^{13}C$), leaf nitrogen concentration (%N), and tip moth damage in fall 2020 on all 21 provenances, and leaf-level gas exchange, instantaneous water use efficiency (WUE), predawn and midday water potentials, soil-to-leaf hydraulic conductance (K_l) and specific leaf area (SLA) on nine provenances from different elevations in summer 2020. Provenances differed significantly in stem growth rate, $\Delta^{13}C$, and SLA, and several traits were correlated with provenance environmental and climatic characteristics. Warmer provenances had lower growth rate and $\Delta^{13}C$ than cooler provenances in the field during the driest year (2020) indicating a trade-off between growth rate and water use efficiency. Field growth rate in the driest year was positively associated with provenance stomatal conductance, net photosynthetic rate, and soil-to-leaf hydraulic conductance and negatively associated with WUE indicating the importance of maintaining water uptake and gas exchange to growth during drought under dry conditions. Provenances with higher %N had greater tip moth damage. Overall, our results enhance understanding of physiological mechanisms of establishment in planted ponderosa pine seedlings and provide information about provenance geographic patterns of seedling growth and drought tolerance traits that should be useful in future reforestation efforts.

Keywords: Ponderosa pine; common garden; carbon isotope discrimination; gas exchange; water use efficiency

Introduction

In the southwestern United States (U.S.), ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) forests have experienced a usually high amount of deforestation during recent climate warming due to droughts, wildfire, and bark beetle attacks (Hicke et al. 2016; Williams et al. 2010). Regeneration after deforestation is constrained by loss of seed trees, drought, high temperature, and high vapor pressure deficit (Savage et al. 2013; Davis et al. 2019; Rodman et al. 2020). Deforestation followed by poor regeneration is contracting the range of ponderosa pine forests in the southwestern U.S. (Allen and Breshears 1998; McDowell et al. 2009; Minott and Kolb 2020). Warmer and drier conditions are predicted for the southwestern U.S. (Seager et al. 2007) which is expected to further constrain natural regeneration of ponderosa pine (Petrie et al. 2017). These losses of ponderosa pine forests might be compensated to some extent by active reforestation with seed sources pre-adapted to the arid conditions of the future (Williams and Dumroese 2013; Rehfeldt et al. 2014).

Local seed sources may become maladapted due to impacts of climate change such as warming and drought (Kremer et al. 2012) and therefore may not be appropriate for planting under increasingly arid conditions. Instead, seed sources from low elevation, warmer, or drier locations may offer some degree of pre-adaptation to climate warming (Alberto et al. 2013, Kolb et al. 2019). For ponderosa pine, common-garden studies have revealed variation among provenances in growth rate, phenology, and morphology (Conkle and Critchfield 1988; Rehfeldt 1993; Zhang et al. 1996), whereas the magnitude of provenance variation in physiological traits relevant to drought tolerance is unresolved (Cregg 1994; Zhang and Marshall 1995). Recent investigations of provenances from wet and dry areas of the Pacific Northwest U.S. suggest a tradeoff between drought tolerance and physiological traits that support high growth rate such as high leaf gas exchange (Kerr et al. 2015), yet such tradeoffs have not been investigated for the extensive ponderosa pine forests of the southwestern U.S. Recent investigations of ponderosa pine provenances in the southwestern U.S. have focused on seedling growth, architecture, and phenology primarily in resource rich greenhouse conditions (Kolb et al. 2016; Dixit and Kolb 2020; Dixit et al. 2020). More studies of physiological traits of southwestern U.S. ponderosa pine provenances are needed under harsh field conditions to enhance understanding of physiological mechanisms of seedling establishment and growth, drought tolerance, and to determine geographical patterns in field survival and performance that should be useful in future restoration efforts.

In this study, we investigated seedling growth, leaf level gas exchange, carbon isotope discrimination, and tip moth (*Rhyacionia neomexicana*) damage on ponderosa pine seedlings from 21 provenances planted at a field site in northern Arizona. The provenances were from a wide range of elevations, temperatures, and precipitation across Arizona and New Mexico. We evaluated the hypothesis that low-elevation, drier, and warmer provenances would have traits conducive to drought tolerance compared to high-elevation, wetter, and cooler provenances. For example, we hypothesized that carbon isotope discrimination, a time-integrated measure of water use efficiency (Farquhar et al. 1982), would be lower in warm or dry provenances than in cool and wet provenances. Based on the previously reported tradeoff between drought tolerance and growth rate of ponderosa pine, we expected slower growth rates of provenances from warm and dry sites. Also, our field experiment provided the opportunity to investigate provenance variation in seedling damage from tip moths (*Rhyacionia neomexicana*) (Wagner and Chen. 2004). Our results provide insight about selecting seed sources for future planting under increasingly arid conditions.

Materials and Methods

Provenance information, field planting site, and experimental design

We used ponderosa pine seeds from 21 provenances from a wide elevational range (~1600 m to ~2800 m) from Arizona and New Mexico in this study (Table 5.1). Seeds from 19 provenances were obtained from collections at the Northern Arizona University Greenhouse Facility located in Flagstaff, Arizona and the John T. Harrington Forestry Research Center of New Mexico State University located in Mora, New Mexico. We collected seeds for two provenances in the year 2017. In March 2018, seeds from three to six mother trees per provenance or a pooled collection (mother tree level information was not available) were sown into SC-10 containers (Ray Leach Cone-tainers-SC10 Super, Stuewe & Sons, Inc., Tangent, OR, USA) at the John T. Harrington Forestry Research Center in Mora, New Mexico. Seedlings grew in the greenhouse under standard growing conditions until planting in July 2018 at a field site in northern Arizona. Detailed greenhouse growing conditions and protocol are described in Dixit et al. (2020). Provenance elevation, mean annual temperature (MAT), and mean annual precipitation (MAP) were obtained from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu) (Table 5.1). Provenance elevation had a positive correlation with MAP ($r = 0.500$, $p = 0.020$) and a negative correlation with MAT ($r = -0.902$, $p < 0.0001$); MAT had a moderate, negative correlation with MAP ($r = -0.434$, $p = 0.048$).

On 20th July 2018, forty-eight seedlings from each of the 21 provenances were planted in a clearing of approximately 0.2 ha surrounded by ponderosa pine forest near Flagstaff, Arizona . This site is part of the Southwest Experimental Garden Array (SEGA; https://sega.nau.edu; latitude 35.16, longitude −111.73; elevation 2200 m). The field site is equipped with a weather station and a datalogger (Campbell Scientific, Logan, Utah, USA) to measure air temperature and precipitation and is fenced for protection against ungulate herbivory. The 30-year normal (1981-2010) MAT and MAP at the field site are 7.6 °C and 555 mm, respectively. On-site weather station data revealed that the study period (2018-2020) was drier compared to the long-term average. The year of planting (2018) received only 66% of average annual precipitation and was 0.2 ^{o}C warmer compared to the long-term average. The year 2019 received 88% of average annual precipitation and was 0.8 ^{o}C cooler compared to the long-term average. The year of gas exchange measurements (2020) was drier compared to the long-term average receiving only 1/3rd of average annual precipitation, and had a slightly warmer spring, summer, and fall than normal (Figure 5.1).

A randomized complete block design was used for planting, where each of the four blocks contained a 12-seedling row plot from each of the 21 provenances (1008 total seedlings). The location of each provenance row plot was randomly determined for each block. Seedlings were planted at a spacing of 1.2 m, including a one-seedling buffer row of seedlings from the same greenhouse around the periphery to reduce potential edge effects. One month prior to planting, we reduced the existing herbaceous vegetation in the planted area with one application of herbicide (Ranger Pro, Bayer Crop Science, Chesterfield, MO, USA).

Trait measurements

In November 2020, three growing seasons after planting, we measured ground line diameter and stem increment on all surviving seedling (538 total seedlings) in November 2020. Stem increment was determined for the year of planting (2018) and the two subsequent years (2019, 2020), using a ruler and measured to the nearest 1 mm, based on the distance between the terminal bud scar and terminal bud for each year. We calculated relative stem increment (cm cm^{-1}) for the years 2019 and 2020 using equations 1 and 2, respectively in order to determine the relative growth rate under field conditions after taking the initial planting height into account which was influenced by resource rich greenhouse conditions and differences in planting depth. Also, in November 2020, we measured tip moth (*Rhyacionia neomexicana*) damage on each seedling. The infested seedlings had distorted and dead terminals that were dark brown in color; we scored the damage as present or absent for each seedling.

$$\text{Relative stem increment 2019 (cm cm}^{-1}\text{)} = \frac{\text{stem increment 2019} - \text{stem increment 2018}}{\text{stem increment 2018}} \tag{1}$$

$$\text{Relative stem increment 2020 (cm cm}^{-1}\text{)} = \frac{\text{stem increment 2020} - \text{(stem increment 2019)}}{\text{stem increment 2019}} \tag{2}$$

In September 2020, we collected current-year foliage from all seedlings that had produced sufficient new needles in that year from each of the 21 provenances (492 total seedlings) for measurement of carbon isotope discrimination and nitrogen concentration. We oven-dried (Sheldon Manufacturing, INC, Cronelius, OR) the needles at 65 °C for 72 hours and ground to homogenous powder using a Mixer Mill MM200 (Retsch, Haan, Germany) ball mill grinder. The samples were analyzed at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University, Flagstaff, AZ using a DELTA V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA), which is configured to a Finnigan ConFlo III, for automated continuous-flow analysis of $\delta^{13}C$ and %N using a Carlo-Erba NC2100 elemental analyzer for combustion and separation of carbon and nitrogen. Carbon isotope discrimination ($\Delta^{13}C$) was calculated using equation 3 (Farquhar et al. 1989) where $\delta^{13}C$ is the isotopic ratio of ^{13}C in needle tissues, and -0.008 is the approximate δ^{13}C of atmospheric CO_2, compared with the Pee Dee Belemnite standard.

$$\Delta^{13}\mathrm{C}\ ‰ = \frac{-0.008 + \left[\frac{\delta^{13}\mathrm{C}}{-1000}\right]}{1 - \left[\frac{\delta^{13}\mathrm{C}}{-1000}\right]} \times 1000 \tag{3}$$

We measured gas exchange and xylem water potential on four consecutive days during an abnormally dry period from 21st through 24th June (Figure 5.1) on nine of the 21 provenances from different elevations (Table 5.1). We selected three provenances each from elevations below 2000 m; between 2000 and 2500 m, and above 2500 m to assess the role of elevation on measured traits. Measurements were conducted on 13-20 seedlings per provenance (158 total seedlings). From each provenance, a maximum of 5 seedlings/block were selected for measurements using stratified random sampling with mother trees as strata. In cases where mother tree information was not available (5 provenances), we randomly selected a maximum of 5 seedlings per block. We measured gas exchange with a portable gas exchange measurement system (Li-6800, LiCor, Lincoln, NE, USA) between 10:00 and 14:00 hours on two fascicles (6 needles) developed in the year 2019. We measured one field block each day to simultaneously control for spatial and temporal variation. Within each field block, gas exchange measurements of provenances during the day were conducted evenly over hours to minimize confounding of measurement time and provenance. For example, we measured one seedling of each provenance in the first hour, then the second seedling of each provenance in the second hour, and so forth. Each time block within a day consisted of one seedling from each provenance. We measured net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and calculated instantaneous, intrinsic water-use efficiency (WUE, P_n/g_s) from the data. Needles of each seedling were spread out to avoid overlapping inside the 6 cm^2 chamber. Conditions controlled inside the chamber were: relative humidity = 50% (SE = 0.06), CO_2 = 420 ppm (SE = 0.002), temperature = 25 ^oC (SE = 0.01), and photosynthetic photon flux density = 1800 μmol m^{-2} s^{-1} (SE = 0.001), which is light saturating for individual needles of ponderosa pine (Kolb and Robberecht, 1996). The section of the needle inside the chamber was marked and removed after measurement and the projected leaf area was measured using a flatbed scanner (Epson 4990; Epson, Suwa, Nagano, Japan) and the image processing software ImageJ in order to calculate gas exchange on a leaf area basis. We measured specific leaf area (SLA; leaf area/leaf mass) on the same needles collected for gas exchange measurements. The needles were oven-dried (Sheldon Manufacturing, INC, Cronelius, OR) at 70 ^oC for 48 hours and then weighed using an analytical balance (Ohaus Explorer; Ohaus Corporation, Parsippany, N.J., USA).

We measured predawn (WP_{PD}) and midday (WP_{MD}) water potentials on two needles from each of the same seedlings used for gas exchange using a pressure chamber (PMS Instruments, Corvallis, OR, USA). WP_{MD} was measured on the same needles used for gas exchange. WP_{PD} was measured between 05:00 and 06:00 hours, whereas WP_{MD} was measured during the gas exchange measurements between 10:00 and 14:00 hours. We calculated soil-to-leaf hydraulic conductance (K_l) from gas exchange measurements and predawn and midday water potentials using equation 4 (Hubbard et al., 1999).

$$K_l\ (\mathrm{mol\ m^{-2}\ s^{-1}\ MPa^{-1}}) = \frac{E}{WP_{PD} - WP_{MD}} \qquad (4)$$

Data analysis

We used mixed-model analysis of variance to test for differences in measured traits among the 21 provenances with provenance as a fixed effect and block as a random effect. To determine the effect of year and year by provenance interaction on seedling growth, we used repeated measures analysis of variance. All the analyses were conducted on block level means of provenance row plots because row plots were the unit of randomization within each block when the experiment was planted. Relationships among provenance trait means and environmental characteristics were evaluated using correlation and regression analyses. All data approximated a normal distribution. All analyses were performed using JMP Pro version 14 (SAS Institute Inc., Cary, NC, USA).

Results

Provenance and yearly differences in seedling traits based on ANOVA

Relative stem increment, a measure of stem growth rate in each year, was much higher in 2019 (1.1 cm cm^{-1}) than 2020 (0.30 cm cm^{-1}). Relative stem increment did not vary among provenances in 2019 ($p = 0.081$); however, the effect of provenance on relative stem increment in 2020 was significant ($p < 0.001$; Table 5.2). Relative stem increment in 2020 ranged from 0.22 cm cm^{-1} for RD to 0.38 cm cm^{-1} for HR.

Specific leaf area ($p = 0.038$) and $\Delta^{13}C$ ($p = 0.018$) differed significantly among provenances, whereas leaf %N did not ($p = 0.746$). Provenances did not differ significantly ($p > 0.05$) in WP_{PD}, WP_{MD}, K_l, tip moth damage or any of the leaf level gas exchange parameters (Table 5.2). Overall, the mean WP_{PD} across all provenances was -0.8 MPa (SE = 0.2), and the mean WP_{MD} was -2.2 MPa (SE = 0.4).

Correlations of seedling traits with provenance environmental characteristics

Correlation analysis revealed relationships between seedling traits and provenance location and climate that were not always apparent from the ANOVA results. Wetter provenances had a higher relative stem increment in 2019 than drier provenances (Table 5.3), and cooler provenances had a higher relative stem increment in 2020 than warmer provenances

(Figure 5.2). Also, higher latitude provenances had higher relative stem increment in 2020 and a lower SLA (Table 5.3). Correlations between physiological traits and provenance environmental characteristics were weak and non-significant except for a significant negative correlation between %N and MAT ($r = -0.50$; $p = 0.020$; Figure 5.2); a negative trend between $\Delta^{13}C$ and MAT ($r = -0.38$; $p = 0.086$; Figure 5.2); and a positive trend between $\Delta^{13}C$ and provenance latitude ($r = 0.38$; $p = 0.087$; Table 5.3). Lastly, provenance MAP had a positive trend with tip moth damage ($r = 0.40$; $p = 0.068$; Table 5.3).

Relationships among provenance traits

Across all 21 provenances, foliar $\Delta^{13}C$ was positively correlated with relative stem increment in 2020 (Table 5.4). Foliar %N was positively correlated with ground line diameter 2020 (Table 5.4). Also, tip moth damage was positively correlated with relative stem increment 2019 and %N (Table 5.4; Figure 5.3). Across the nine provenances measured for leaf gas exchange, foliar $\Delta^{13}C$ was positively associated with P_n, g_s, E and K_l (Table 5.5).

Discussion

In order to better understand drought tolerance of ponderosa pine seed sources for planting in an increasing arid climate in the southwest US, we measured variation in growth, carbon isotope discrimination, foliar nitrogen concentration, specific leaf area, leaf level gas exchange, and tip moth damage during dry conditions after planting in a field common garden. The 21 provenances were sampled across an elevational and thermal gradient allowing investigation of fine-grained patterns of genetic variation within the southwestern U.S. The common garden was located in the core of the species range in northern Arizona and the traits were measured during an unusually dry period in 2020 allowing us to investigate differences in performance among provenances under harsh field conditions.

We found lower growth of warmer provenances compared to cooler provenances under dry field conditions in 2020 (Table 5.3). Warmer provenances also had a lower $\Delta^{13}C$ for leaves formed in 2020 compared to cooler provenances (Figure 5.2). $\Delta^{13}C$ is a time integrated measure of leaf internal CO_2 concentration (Farquhar et al. 1982), which in our study was apparently controlled primarily by g_s since photosynthetic capacity, inferred from %N, was lower in warm than cool provenances. Our results show more genetic variation in $\Delta^{13}C$ than %N (Table 5.2) suggesting that variation in water use efficiency was more strongly controlled by g_s than photosynthetic efficiency in the provenances investigated in our study.

Our finding of a positive correlation between $\Delta^{13}C$ and growth (Table 5.4) and a positive association between leaf level gas exchange and growth (Table 5.5) suggests a trade-off between water use efficiency and gas exchange and growth under dry field conditions. Our results are consistent with an earlier report of a trade-off between growth rate and drought tolerance (inferred from water potential at turgor loss point, carbon isotope discrimination, etc.) in ponderosa pine from two populations in Oregon (Kerr et al. 2015). Provenances in our study from more southern latitudes had a lower $\Delta^{13}C$ than northern provenances suggesting a higher water use efficiency of southern provenances (Table 5.3). This latitudinal pattern in $\Delta^{13}C$ is similar to a greenhouse common garden study of *Pinus strobiformis* seedlings from provenances in the southwestern U.S. (Goodrich et al. 2016). Provenances in our study with higher $\Delta^{13}C$ had a higher leaf level stomatal conductance, transpiration rate, and net photosynthetic rate which shows a coherent relationship between long term and instantaneous gas exchange measurements (Table 5.5).

Tip moth damage seemed to increase with foliar nitrogen concentration (Figure 5.3). Tip moth damage is not lethal to seedlings. Instead, it kills the terminal bud and promotes stem crooks and forks. In our study provenances with higher photosynthetic capacity (inferred from %N) grew more in 2019 and had more tip moth damage than provenances with lower photosynthetic capacity (Figure 5.3; Table 5.4). Our finding of more tip moth damage in provenances with higher nitrogen concentration is consistent with an earlier report of an increase in Nantucket pine tip moth (*Rhyacionia frustrana*) infestation in loblolly pine (*Pinus taeda* L.) plantations in Texas due to nitrogen fertilization and could be a result of nutritious plant tissue with high nitrogen content being preferred by the tip moth (Sun et al. 2001). This finding highlights the importance of considering biotic agents in provenance evaluation and assisted migration strategies (Bucharova 2017). We found that cooler and wetter provenances have higher %N suggesting higher photosynthetic capacity and higher susceptibility to tip moth damage of these provenances as compared to warmer and drier ones (Table 5.3). Therefore, a lower susceptibility to tip moth damage along with a higher water use efficiency, inferred from $\Delta^{13}C$, in warmer provenances may have implications for future performance in our field experiment as well as informing selection of seed sources for future planting.

Our findings suggest genetic variation and an influence of provenance latitude on SLA (Tables 5.2 and 5.3). SLA is a key trait in the leaf economic spectrum and a lower value is associated with longer survival under dry conditions (Wright et al. 2004). However, the difference among provenances and correlation between latitude and SLA was mainly due to provenance GP which had the highest SLA among all provenances and was from the lowest latitude among the nine provenances investigated for SLA. Overall, our finding of significant provenance variation in SLA is consistent with an earlier greenhouse study involving some of the same provenances of ponderosa pine (Dixit and Kolb 2020).

We found more evidence for provenance differences in leaf gas exchange based on $\Delta^{13}C$ than on direct but short-term measurements of leaf gas exchange during a particularly dry mid-summer period. Provenances did not vary in leaf level gas exchange parameters that were measured on nine provenances only during an extremely dry period in 2020, however, we did find significant provenance variation in $\Delta^{13}C$ which is a surrogate and time-integrated measure of gas exchange (Table 5.2). Lack of genetic variation in leaf-level gas exchange has been reported in earlier studies involving ponderosa pine (Zhang and Marshall 1995; Cregg 1994; Zhang and Cregg 2005) and could be attributed to a similar plastic response of all provenances to xeric conditions. Our findings show relationships between plant hydraulics and gas exchange parameters (Brodribb and Holbrook 2006, 2007; Bartlett et al. 2016) (Table 5.5) suggesting an influence of hydraulics in limiting net photosynthesis (Hubbard et al. 1999; Kolb and Stone 1999). Soil-to-leaf hydraulic conductance (K_l) decreased with $\Delta^{13}C$ (Figure 5.4), consistent with an earlier report of relationship between foliar carbon isotope discrimination and K_l in ponderosa pine provenances from Oregon (Kerr et al. 2015).

Our results demonstrate the importance of maintaining high soil-to-leaf hydraulic conductance for seedling growth during drought. Plants often close stomates to limit water loss water during dry periods; however, in order to maintain leaf gas exchange and growth during prolonged dry conditions, water needs to be efficiently supplied to the mesophyll (Scoffoni et al. 2018). Anderegg and HilleRisLambers (2016) reported regulation of water loss by stomatal closure in ponderosa pine suggesting a drought avoidance strategy. We found that growth under dry conditions was positively associated with provenance stomatal conductance, net photosynthetic rate, and soil-to-leaf hydraulic conductance and negatively associated with WUE, indicating the importance of maintaining water uptake and gas exchange to growth even under dry conditions (Table 5.5). However, growth under dry conditions apparently was not promoted by high water use efficiency in our study; in fact, growth was negatively related to water use efficiency. On the contrary, Garcia-Forner et al. (2016) reported longer survival of *Pinus sylvestris* L. saplings with higher photosynthetic rates during drought treatment. The mechanisms that maintain high gas exchange and hydraulic conductance of planted seedlings under dry conditions are not clear in our study, but may include osmotic adjustment, greater xylem hydraulic conductance, greater root depth, higher non-structural carbohydrate content, and lower shoot to root ratio (Garcia-Forner et al. 2016).

Conclusions

Our study of ponderosa pine provenances from the southwestern U.S. planted into a field common garden produced five key findings: First, seedlings from warmer provenances were more water-use efficient than cooler provenances. Second, growth and water use efficiency were negatively related, indicating a trade-off. Third, provenances with higher foliar nitrogen concentration had greater growth and more tip moth damage than provenances with a lower foliar nitrogen concentration. Fourth, measurements of leaf $\Delta^{13}C$ identified provenance variation in leaf gas exchange and water-use efficiency more clearly than direct short-term measurements of leaf-level gas exchange during dry conditions. Fifth, currently resolved mechanisms that maintain high soil-to-leaf hydraulic conductance and gas exchange promote growth during drought. Overall, our findings enhance understanding of mechanisms of drought adaptation and herbivory resistance in planted seedlings and inform strategies to maintain ponderosa pine during changing climate by planting more arid adapted seed sources.

References

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Allen, C.D., Breshears, D.D., 1998. Drought-induced shift of a forest–woodland ecotone: Rapid landscape response to climate variation. Proceedings of the National Academy of Sciences 95, 14839–14842

Anderegg, L.D.L., HilleRisLambers, J., 2016. Drought stress limits the geographic ranges of two tree species via different physiological mechanisms. Global Change Biology 22, 1029–1045. https://doi.org/10.1111/gcb.13148

Bartlett, M.K., Klein, T., Jansen, S., Choat, B., Sack, L., 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. Proceedings of the National Academy of Sciences 113, 13098–13103. https://doi.org/10.1073/pnas.1604088113

Brodribb, T.J., Holbrook, N.M., 2007. Forced Depression of Leaf Hydraulic Conductance in situ: Effects on the Leaf Gas Exchange of Forest Trees. Functional Ecology 21, 705–712.

Brodribb, T.J., Holbrook, N.M., 2006. Declining hydraulic efficiency as transpiring leaves desiccate: two types of response*. Plant, Cell & Environment 29, 2205–2215. https://doi.org/10.1111/j.1365-3040.2006.01594.x

Bucharova, A., 2017. Assisted migration within species range ignores biotic interactions and lacks evidence. Restoration Ecology 25, 14–18. https://doi.org/10.1111/rec.12457

Conkle, M.T., Critchfield, W.B., 1988. Genetic variation and hybridization of ponderosa pine. In: Ponderosa Pine: the species and its management, Washington State University Cooperative Extension, 1988: p. 27-43.

Cregg, B.M., 1994. Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. Tree Physiology 14, 883–898. https://doi.org/10.1093/treephys/14.7-8-9.883

Davis, K.T., Dobrowski, S.Z., Higuera, P.E., Holden, Z.A., Veblen, T.T., Rother, M.T., Parks, S.A., Sala, A., Maneta, M.P., 2019. Wildfires and climate change push low-elevation forests across a critical climate threshold for tree regeneration. Proceedings of the National Academy of Sciences 116, 6193–6198. https://doi.org/10.1073/pnas.1815107116

Dixit, A., Kolb, T., 2020. Variation in seedling budburst phenology and structural traits among southwestern ponderosa pine provenances. Canadian Journal of Forest Research. https://doi.org/10.1139/cjfr-2019-0333

Dixit, A., Kolb, T., Burney, O., 2020. Provenance Geographical and Climatic Characteristics Influence Budburst Phenology of Southwestern Ponderosa Pine Seedlings. Forests 11, 1067. https://doi.org/10.3390/f11101067

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon Isotope Discrimination and Photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Functional Plant Biol. 9, 121–137. https://doi.org/10.1071/pp9820121

Garcia-Forner, N., Sala, A., Biel, C., Savé, R., Martínez-Vilalta, J., 2016. Individual traits as determinants of time to death under extreme drought in *Pinus sylvestris* L. Tree Physiology 36, 1196–1209. https://doi.org/10.1093/treephys/tpw040

Goodrich, B.A., Waring, K.M., Kolb, T.E., 2016. Genetic variation in *Pinus strobiformis* growth and drought tolerance from southwestern US populations. Tree Physiology 36, 1219–1235. https://doi.org/10.1093/treephys/tpw052

Hicke, J.A., Meddens, A.J.H., Kolden, C.A., 2016. Recent Tree Mortality in the Western United States from Bark Beetles and Forest Fires. Forest Science 62, 141–153. https://doi.org/10.5849/forsci.15-086

Hubbard, R.M., Bond, B.J., Ryan, M.G., 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiology 19, 165–172. https://doi.org/10.1093/treephys/19.3.165

Kerr, K.L., Meinzer, F.C., McCulloh, K.A., Woodruff, D.R., Marias, D.E., 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. Tree Physiology 35, 535–548. https://doi.org/10.1093/treephys/tpv034

Kolb, P.F., Robberecht, R., 1996. *Pinus ponderosa* Seedling Establishment and the Influence of Competition with the Bunchgrass *Agropyron spicatum*. International Journal of Plant Sciences 157, 509–515

Kolb, T.E., Dixit, A.H., Burney, O., 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern United States. Tree Planters' Note 62, 104–112.

Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A., 2016. Local-Scale Drought Adaptation of Ponderosa Pine Seedlings at Habitat Ecotones. Forest Science 62, 641–651. https://doi.org/10.5849/forsci.16-049

Kolb, T.E., Stone, J.E., 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine–oak forest. Tree Physiology 20, 1–12. https://doi.org/10.1093/treephys/20.1.1

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S., Schueler, S., 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters 15, 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

McDowell, N., Allen, C.D., Marshall, L., 2009. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. Global Change Biology 16, 399–415. https://doi.org/10.1111/j.1365-2486.2009.01994.x

Minott, J.A., Kolb, T.E., 2020. Regeneration patterns reveal contraction of ponderosa forests and little upward migration of pinyon-juniper woodlands. Forest Ecology and Management 458, 117640. https://doi.org/10.1016/j.foreco.2019.117640

Petrie, M.D., Bradford, J.B., Hubbard, R.M., Lauenroth, W.K., Andrews, C.M., Schlaepfer, D.R., 2017. Climate change may restrict dryland forest regeneration in the 21st century. Ecology 98, 1548–1559. https://doi.org/10.1002/ecy.1791

Pinto, J.R., Marshall, J.D., Dumroese, R.K., Davis, A.S., Cobos, D.R., 2011. Establishment and growth of container seedlings for reforestation: A function of stocktype and edaphic conditions. Forest Ecology and Management 261, 1876–1884. https://doi.org/10.1016/j.foreco.2011.02.010

Rehfeldt, G.E., 1993. Genetic Variation in the Ponderosae of the Southwest. American Journal of Botany 80, 330–343. https://doi.org/10.2307/2445357

Rehfeldt, G.E., Jaquish, B.C., Sáenz-Romero, C., Joyce, D.G., Leites, L.P., Bradley St Clair, J., López-Upton, J., 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Reforestation. Forest Ecology and Management 324, 147–157. https://doi.org/10.1016/j.foreco.2014.02.040

Rodman, K.C., Veblen, T.T., Chapman, T.B., Rother, M.T., Wion, A.P., Redmond, M.D., 2020. Limitations to recovery following wildfire in dry forests of southern Colorado and northern New Mexico, USA. Ecological Applications 30, e02001. https://doi.org/10.1002/eap.2001

Savage, M., Nystrom, M., J, F., 2013. Double whammy: high-severity fire and drought in ponderosa pine forests of the Southwest. Canadian Journal of Forest Research. https://doi.org/10.1139/cjfr-2012-0404

Scoffoni, C., Albuquerque, C., Cochard, H., Buckley, T.N., Fletcher, L.R., Caringella, M.A., Bartlett, M., Brodersen, C.R., Jansen, S., McElrone, A.J., Sack, L., 2018. The Causes of Leaf Hydraulic Vulnerability and Its Influence on Gas Exchange in *Arabidopsis thaliana*. Plant Physiol 178, 1584–1601. https://doi.org/10.1104/pp.18.00743

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.-P., Harnik, N., Leetmaa, A., Lau, N.-C., Li, C., Velez, J., Naik, N., 2007. Model Projections of an Imminent Transition to a More Arid Climate in Southwestern North America. Science 316, 1181–1184. https://doi.org/10.1126/science.1139601

Sun, J.H., Kulhavy, D.L., Roques, A., 2000. Effects of fertilizer and herbicide application on Nantucket pine tip moth infestation (Lep., Tortricidae). Journal of Applied Entomology 124, 191–195. https://doi.org/10.1046/j.1439-0418.2000.00453.x

van den Driessche, R., 1992. Absolute and relative growth of Douglas-fir seedlings of different sizes. Tree Physiology 10, 141–152. https://doi.org/10.1093/treephys/10.2.141

Wagner, M.R., Chen, Z., 2004. Long-Term Benefits to the Growth of Ponderosa Pines from Controlling Southwestern Pine Tip Moth (Lepidoptera: Tortricidae) and Weeds. Journal of Economic Entomology 97, 6.

Williams, A.P., Allen, C.D., Millar, C.I., Swetnam, T.W., Michaelsen, J., Still, C.J., Leavitt, S.W., 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Sciences 107, 21289–21294. https://doi.org/10.1073/pnas.0914211107

Williams, M.I., Dumroese, R.K., 2013. Preparing for Climate Change: Forestry and Assisted Migration. Journal of Forestry 111, 287–297. https://doi.org/10.5849/jof.13-016

Zhang, J., Cregg, B.M., 2005. Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. Forest Ecology and Management 219, 1–12. https://doi.org/10.1016/j.foreco.2005.08.038

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827. https://doi.org/10.1038/nature02403

Zhang, J., Marshall, J.D., Fins, L., 1996. Correlated Population Differences in Dry Matter Accumulation, Allocation, and Water-Use Efficiency in Three Sympatric Conifer Species. Forest Science 42, 242–249. https://doi.org/10.1093/forestscience/42.2.242

Zhang, J.W., Feng, Z., Cregg, B.M., Schumann, C.M., 1997. Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. Tree Physiology 17, 461–466. https://doi.org/10.1093/treephys/17.7.461

Zhang, J.W., Marshall, J.D., 1995. Variation in Carbon Isotope Discrimination and Photosynthetic Gas Exchange Among Populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in Different Environments. Functional Ecology 9, 402–412. https://doi.org/10.2307/2390003

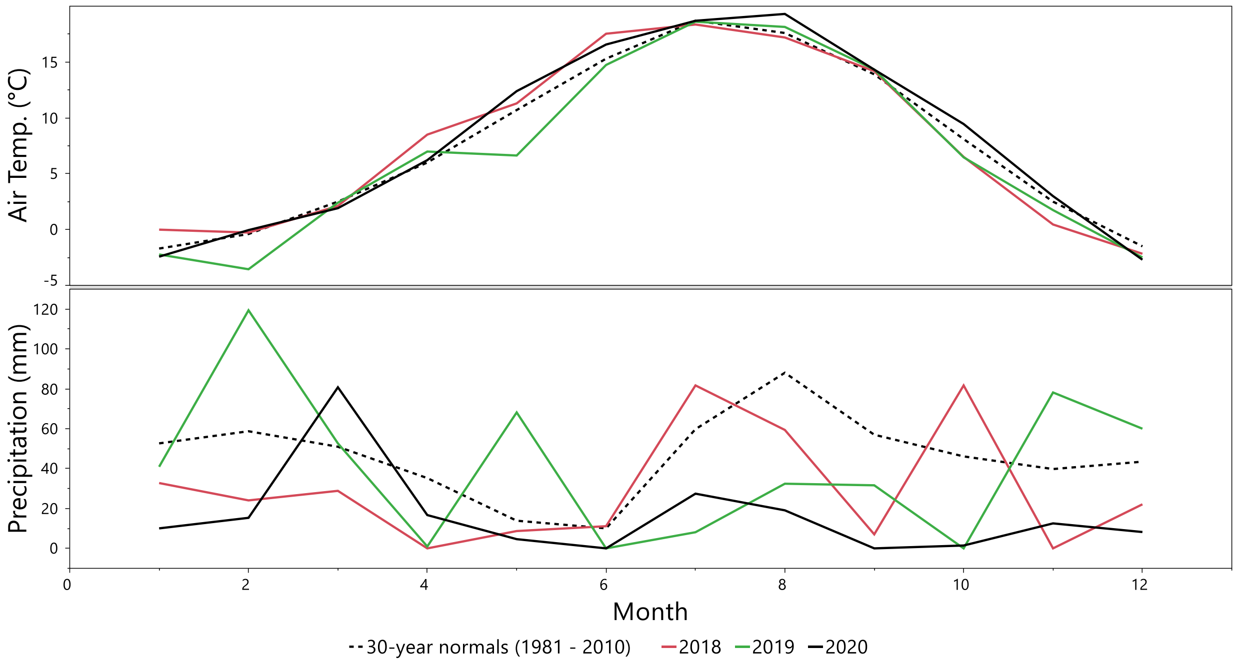


Figure 5.1: Monthly air temperature (°C) and precipitation (mm) for the years of growth assessment (2018-2020) compared to 30-year normals (1981-2010; https://prism.oregonstate.edu/).

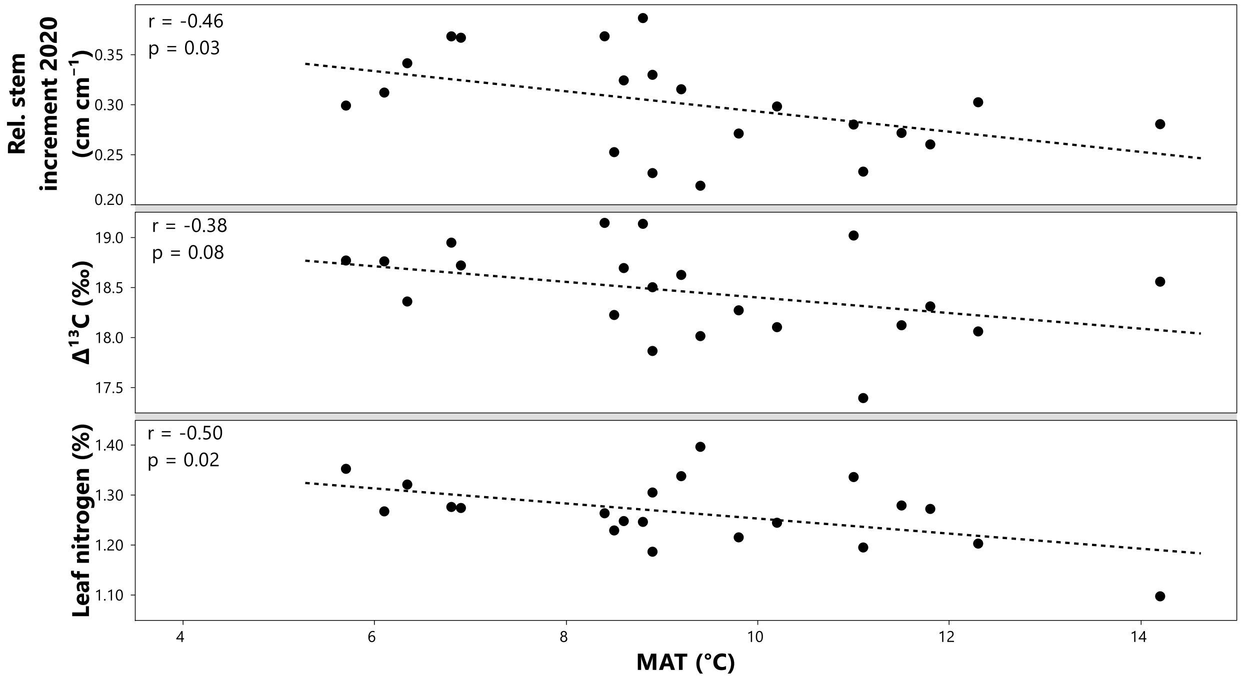


Figure 5.2: Relationships between provenance mean annual temperature (MAT) and relative stem increment 2020, carbon isotope discrimination (Δ^{13}C), and leaf nitrogen concentration (%N).

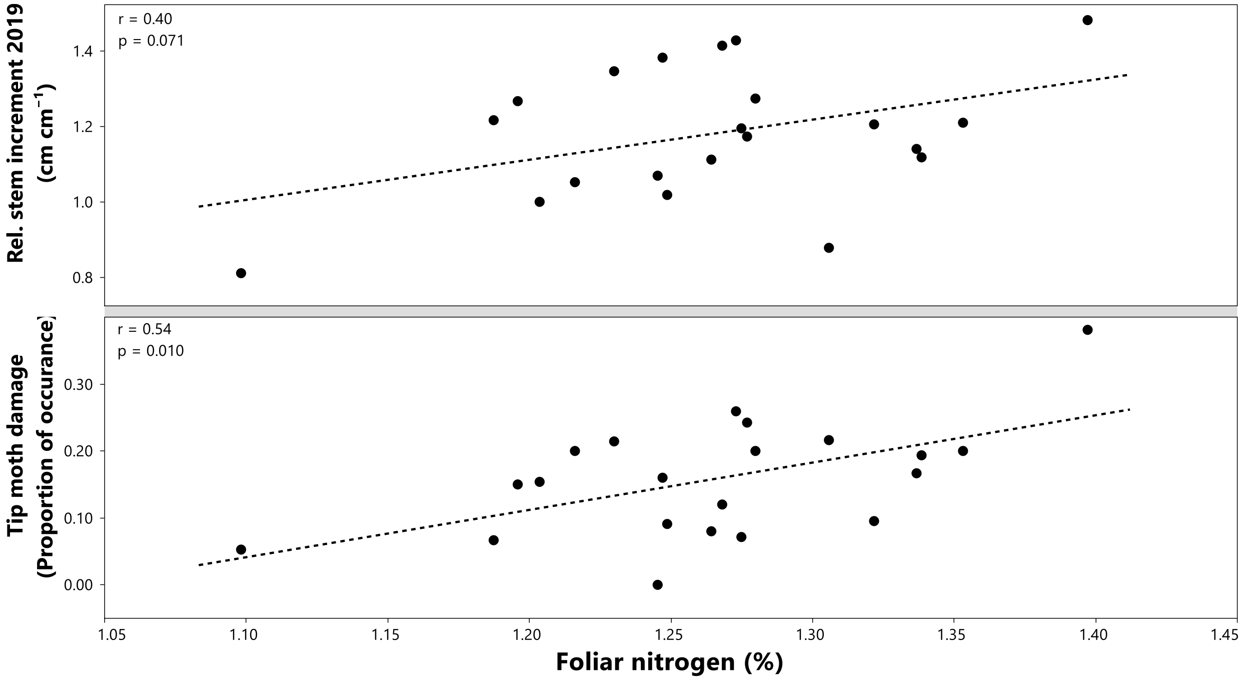


Figure 5.3: Relationships between provenance foliar nitrogen and tip moth damage and relative stem increment 2019.

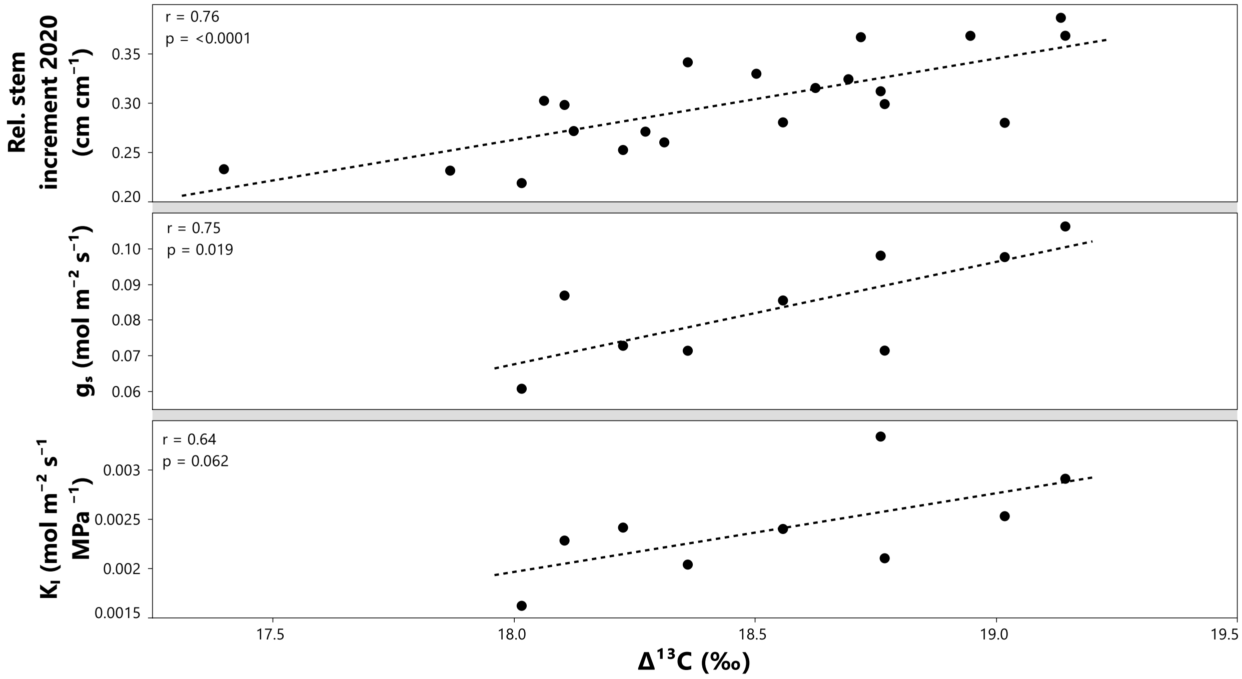


Figure 5.4: Relationships between provenance carbon isotope discrimination ($\Delta^{13}C$) and relative stem increment 2020, stomatal conductance (g_s), and soil-to-leaf hydraulic conductance (k_l).

Table 5.1: Provenance name, code, latitude, longitude, elevation, mean annual temperature (MAT), and mean annual precipitation (MAP) ordered by increasing elevation. Climate data (30-year normal, 1981-2010) are from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu). Values are means over mother tree locations at each provenance.

	Latitude/Longitude	**Elevation (m)**	**MAT (ºC)**	**MAP (mm)**
Provenance (code)				
Cherry Road (CR) [1]	34.586/-112.057	1592	14.2	408
Blue River (BR)	33.555/-109.193	1674	11.8	581
Mesa Del Medio (MDM)	35.116/-105.217	1714	12.3	397
Prescott – iron Springs Road (PIS) [1]	34.585/-112.559	1846	11.0	546
Townsend Winona (WIN) [1]	35.254/-111.415	1934	10.2	398
Hualapai Mountains (HM)	35.084/-113.875	1969	11.5	403
Ruidoso Service Office (RSO)	33.350/-105.583	1976	11.1	506
Sapello Rt. 3 (SAP)	35.700/-105.250	2050	9.8	456
South Kaibab Tusayan Dist. (SKT)	35.939/-112.084	2067	8.9	410
Northern Arizona University (NAU) [1]	35.182/-111.655	2104	8.4	540
Rim District (RD) [1]	34.487/-111.343	2244	9.4	767
HH Ranch (HHR)	34.183/-107.525	2270	9.2	364
Mineral Hill (MH) [1]	35.633/-105.461	2277	8.5	528
Mud Springs (MUD)	36.463/-106.859	2277	6.9	443
Manzano Mountains (MZN)	34.623/-106.400	2366	8.6	638
Vallecitos-Jemez Springs (VJS)	35.809/-106.589	2436	6.8	571
Hartman Ridge (HR)	35.550/-105.533	2500	8.8	526
Borrego Mesa (BM)[1]	35.990/-105.794	2560	6.3	470
Magdalena Mountains (MAG)	34.006/-107.215	2565	8.9	512
Green’s Peak (GP) [1]	34.126/-109.535	2760	5.7	671
Mount Taylor (TAY) [1]	35.266/-107.633	2814	6.1	722

[1] Provenances used for leaf level gas exchange measurements.

Table 5.2: Seedling traits, number of provenances, F ratio, and the associated p-value for the provenance effect. Bolded values are significant at $p < 0.05$. Carbon isotope discrimination ($\Delta^{13}C$); foliar nitrogen concentration (%N); specific leaf area (SLA); predawn water potential (WP_{PD}); midday water potential (WP_{MD}); net CO_2 uptake (P_n); stomatal conductance (g_s); transpiration rate (E); instantaneous water-use efficiency (WUE); soil-to-leaf hydraulic conductance (K_l).

Traits	**Units**	**Number of provenances**	**F Ratio**	**p-value**
Rel. stem increment (2019)	cm cm^{-1}	21	1.60	0.081
Rel. stem increment (2020)	cm cm^{-1}	21	2.82	**0.001**
Ground line diameter (2020)	mm	21	1.27	0.235
$\Delta^{13}C$	‰	21	2.04	**0.018**
%N	%	21	0.76	0.746
Tip moth damage	Proportion	21	0.85	0.643
SLA	cm^2 g^{-1}	9	2.51	**0.038**
WP_{PD}	MPa	9	0.92	0.513
WP_{MD}	MPa	9	0.71	0.678
E	mol m^{-2} s^{-1}	9	0.84	0.574
P_n	µmol m^{-2} s^{-1}	9	0.55	0.800
g_s	mol m^{-2} s^{-1}	9	0.81	0.599
WUE	P_n/g_s	9	1.01	0.451
K_l	mol m^{-2} s^{-1} MPa^{-1}	9	0.79	0.614

Table 5.3: Correlation coefficients (p values) between provenance environmental characteristics and traits. Carbon isotope discrimination ($\Delta^{13}C$); foliar nitrogen concentration (%N); specific leaf area (SLA); predawn water potential (WP_{PD}); midday water potential (WP_{MD}); net CO_2 uptake (P_n); stomatal conductance (g_s); transpiration rate (E); instantaneous water-use efficiency (WUE); soil-to-leaf hydraulic conductance (K_l); mean annual temperature (MAT); mean annual precipitation (MAP). Bolded values are significant at $p < 0.05$.

Traits	**Latitude**	**Longitude**	**Elevation**	**MAT**	**MAP**
Rel. stem increment (2019)	-0.18 (0.422)	0.17 (0.442)	0.40 (0.070)	-0.31 (0.162)	**0.59 (0.004)**
Rel. stem increment (2020)	**0.62 (0.002)**	0.14 (0.521)	0.28 (0.203)	**-0.46 (0.035)**	-0.15 (0.499)
Ground line diameter (2020)	0.18 (0.434)	-0.20 (0.370)	0.16 (0.484)	-0.32 (0.156)	0.30 (0.182)
$\Delta^{13}C$	0.38 (0.087)	-0.13 (0.558)	0.27 (0.235)	-0.38 (0.086)	0.17 (0.442)
%N	0.05 (0.809)	-0.24 (0.294)	0.36 (0.101)	**-0.50 (0.020)**	0.39 (0.073)
Tip moth damage	-0.15 (0.511)	-0.05 (0.818)	0.001 (0.994)	-0.01 (0.956)	0.40 (0.068)
SLA	**-0.67 (0.045)**	-0.11 (0.767)	0.08 (0.823)	0.03 (0.931)	0.12 (0.744)
WP_{PD}	-0.37 (0.323)	-0.65 (0.056)	-0.45 (0.213)	0.28 (0.456)	-0.02 (0.959)
WP_{MD}	0.04 (0.910)	0.44 (0.230)	0.15 (0.696)	-0.03 (0.924)	0.23 (0.536)
E	0.08 (0.822)	-0.35 (0.346)	-0.26 (0.488)	0.16 (0.669)	-0.22 (0.555)
P_n	0.22 (0.567)	-0.29 (0.441)	-0.36 (0.330)	0.25 (0.503)	-0.31 (0.410)
g_s	0.07 (0.844)	-0.36 (0.335)	-0.26 (0.498)	0.16 (0.671)	-0.23 (0.547)
WUE	-0.33 (0.381)	0.24 (0.530)	0.42 (0.253)	-0.31 (0.416)	0.56 (0.113)
K_l	0.22 (0.553)	0.06 (0.878)	0.11 (0.767)	-0.12 (0.751)	-0.01 (0.995)

Table 5.4: Correlation coefficients (p values) among provenance traits (n = 21). Carbon isotope discrimination ($\Delta^{13}C$); foliar nitrogen concentration (%N). Bolded values are significant at $p < 0.05$.

Traits	Rel. stem increment (2019)	Rel. stem increment (2020)	Ground line diameter (2020)	$\Delta^{13}C$	%N
Rel. stem increment (2019)					
Rel. stem increment (2020)	-0.22 (0.326)				
Ground line diameter (2020)	0.05 (0.815)	0.38 (0.089)			
$\Delta^{13}C$	-0.10 (0.637)	**0.76 (0.00005)**	**0.52 (0.013)**		
%N	0.40 (0.071)	0.11 (0.618)	**0.62 (0.002)**	0.26 (0.253)	
Tip moth damage	**0.45 (0.036)**	-0.31 (0.171)	0.28 (0.207)	-0.08 (0.707)	**0.54 (0.010)**

Table 5.5: Correlation coefficients (p values) between physiological and growth traits on provenances used for gas exchange measurements (n = 9). Carbon isotope discrimination ($\Delta^{13}C$); foliar nitrogen concentration (%N); specific leaf area (SLA); predawn water potential (WP_{PD}); midday water potential (WP_{MD}); net CO_2 uptake (P_n); stomatal conductance (g_s); transpiration rate (E); instantaneous water-use efficiency (WUE); soil-to-leaf hydraulic conductance (K_l). Bolded values are significant at $p < 0.05$.

Traits	SLA	WP_{PD}	WP_{MD}	E	P_n	g_s	WUE	K_l
Rel. stem increment (2019)	-0.16 (0.677)	-0.20 (0.605)	0.32 (0.399)	-0.36 (0.342)	-0.36 (0.341)	-0.36 (0.328)	0.51 (0.154)	-0.07 (0.851)
Rel. stem increment (2020)	-0.38 (0.303)	0.12 (0.758)	-0.44 (0.229)	0.59 (0.087)	0.54 (0.128)	0.59 (0.088)	-0.57 (0.105)	0.51 (0.153)
Ground line diameter (2020)	-0.27 (0.477)	0.66 (0.052)	-0.37 (0.324)	0.05 (0.890)	0.04 (0.916)	0.03 (0.926)	0.09 (0.809)	-0.24 (0.524)
$\Delta^{13}C$	-0.12 (0.744)	0.58 (0.098)	-0.11 (0.764)	**0.76 (0.015)**	0.66 (0.052)	**0.75 (0.019)**	-0.51 (0.158)	**0.64 (0.062)**
%N	-0.006 (0.987)	0.21 (0.586)	-0.29 (0.443)	-0.33 (0.377)	-0.43 (0.236)	-0.34 (0.358)	0.53 (0.139)	-0.38 (0.302)

Chapter VI

Conclusions and Management Implications

The results of my dissertation suggest differences among provenances from different elevations for seedlings traits that are relevant to climatic adaptation. Since our sampling of provenances represented an elevation and thermal gradient, we were able to investigate fine grained patterns of genetic variation under greenhouse and field conditions. Consistent with the concept of local adaptations of tree populations (Alberto et al. 2013), we found evidence of differences between provenances from different elevations in specific leaf area and budburst phenology in the greenhouse. An earlier budburst and lower specific leaf area could be the result of adaptation of low elevation provenances to longer growing season and aridity compared to high elevation provenances. During assisted migration, an earlier budburst by low elevation provenances could have a positive or negative impact as it would allow for a longer growing season if frost damage does not occur or it may predispose them to spring frost damage when planted at higher elevations (Grady et al. 2015) and should be tested under field conditions for longer durations. Species in dry environments have been reported to have a lower specific leaf which have been associated with conservation of acquired resources and an increase in survival under dry conditions (Wright et al. 2004); this suggests greater drought tolerance in provenances from low elevations under greenhouse conditions. We also show an association between provenance mean annual precipitation and total biomass under resource-rich greenhouse conditions. Considering a trade-off between growth rate and drought tolerance (Kerr et al. 2015), this result may be used to indirectly select for drought tolerant sources. However, further studies based on field survival and physiology are required to fully understand this mechanism of higher drought tolerance in provenances with a lower growth rate.

My work highlights the importance of comparing field and greenhouse results from the same provenances to determine if greenhouse findings are applicable to field performance. We measured spring budburst at the mid elevation site and compared that will budburst measured on 10 provenances in the greenhouse. Result suggests applicability of greenhouse results to field performance as the pattern of earlier budburst by low elevation provenances was similar. Difference between provenances in budburst date was about eight days. From the management standpoint, this could either provide flexibility to plant low elevation sources at higher elevations if this difference is maintained or cause frost damage if the difference is amplified after planting at higher elevations. However, more research is needed examining long term difference in budburst phenology among provenance as an unusually cold spring once in 15-20 years could still cause frost damage.

At this mid-elevation site, we saw higher survival across the study period (2018-2020) compared to the low and high elevation sites. Interestingly, the majority of mortality at the mid and high elevation sites was due to rabbit and gopher herbivory respectively, emphasizing the importance of considering impacts of biotic agents while planning a large-scale reforestation and assisted migration of ponderosa pine (Bucharova, 2017). We found longer survival of low elevation provenances under dry, hot conditions at the low elevation site where almost all seedlings died in the first two months after planting due to desiccation. This finding supports the recommendation of planting low elevation provenances in reforestation projects in a warming climate (Rehfeldt et al. 2014; Kolb et al. 2019). This low elevation site was located below the current range of ponderosa pine and is an example of future warmer climate in the current range in that region by the end of this century. Lack of survival at this site suggests that reforestation by planting might need to be timed with particularly wet years in future, if possible. My work also found tip moth damage on provenances with higher foliar nitrogen at the mid-elevation site further emphasizing the importance of biotic agents in seedling performance in the field. At this site, we found differences among provenances in growth under usually dry conditions in the year 2020; cooler provenances grew more under dry field conditions compared to warmer provenances. A trade-off between growth and drought tolerance have been reported (Kerr et al. 2015) suggesting that slow growing provenances of ponderosa pine may be more drought tolerant. These warmer provenances also had a lower carbon isotope discrimination (higher water use efficiency) than cooler provenances.

Overall, the field and greenhouse common garden studies produced some results that may have implications for selection of seed sources for planting under arid conditions and support the recommendation that low elevation and warmer sources should be considered for planting at higher elevation sites (Rehfeldt et al. 2014). Results also show that information about budburst timing and long-term risk of spring frost damage is needed for developing species-specific seed transfer guidelines and effective assisted migration strategies in a changing climate. Results from the field common gardens provide evidence of site and provenance effects on survival and performance of planted southwestern ponderosa pine seedlings growing at different elevations and highlights the importance of planning for biotic agents in reforestation projects and need for studies focusing on the interactions between abiotic and biotic stresses on seedling survival and performance (Sáenz-Romero et al. 2021). Results also suggest a potential trade-off between growth rate under harsh field conditions and water use efficiency. Overall, our findings enhance understanding of constraints on the success of planted seedlings and thus inform strategies to maintain southwestern ponderosa pine during changing climate by active reforestation.

References

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Bucharova, A., 2017. Assisted migration within species range ignores biotic interactions and lacks evidence. Restoration Ecology 25, 14–18. https://doi.org/10.1111/rec.12457

Grady, K.C., Kolb, T.E., Ikeda, D.H., Whitham, T.G., 2015. A bridge too far: cold and pathogen constraints to assisted migration of riparian forests. Restoration Ecology 23, 811–820. https://doi.org/10.1111/rec.12245

Kerr, K.L., Meinzer, F.C., McCulloh, K.A., Woodruff, D.R., Marias, D.E., 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. Tree Physiology 35, 535–548. https://doi.org/10.1093/treephys/tpv034

Kolb, T.E., Dixit, A.H., Burney, O., 2019. Challenges and opportunities for maintaining ponderosa pine forests in the southwestern United States. Tree Planters' Note 62, 104–112.

Rehfeldt, G.E., Jaquish, B.C., Sáenz-Romero, C., Joyce, D.G., Leites, L.P., Bradley St Clair, J., López-Upton, J., 2014. Comparative genetic responses to climate in the varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Reforestation. Forest Ecology and Management 324, 147–157. https://doi.org/10.1016/j.foreco.2014.02.040

Sáenz-Romero, C., O'Neill, G., Aitken, S.N., Lindig-Cisneros, R., 2021. Assisted Migration Field Tests in Canada and Mexico: Lessons, Limitations, and Challenges. Forests 12, 9. https://doi.org/10.3390/f12010009

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827. https://doi.org/10.1038/nature02403