

AN ABSTRACT OF THE THESIS OF

Kelly. L. Kerr for the degree of Master of Science in Forest Ecosystems and Society presented on December 8, 2014.

Title: The Expression of Functional Traits During Seedling Establishment in Two Populations of *Pinus ponderosa* from Contrasting Climates.

Abstract approved:

Frederick C. Meinzer

Katherine A. McCulloh

Vegetation patterns and species distributions are strongly linked to soil moisture regimes, and populations within a species from contrasting climatic regimes could exhibit differences in the degree to which certain functional traits related to establishment are expressed. Tree seedling survival is crucial for forest regeneration, and thus may be a more important factor in determining species distributions than performance of adult trees. This study investigated the extent to which species populations from different climate zones exhibit differential expression of functional traits that facilitate their establishment. Seeds from two populations of ponderosa pine (*Pinus ponderosa*) from sites with contrasting climate regimes east (PIPO_{EAST}) and west (PIPO_{WEST}) of the Oregon Cascade Mountains were sown in raised soil beds in a common garden and grown under two water availability treatments (control and drought). Aboveground biomass accumulation, bud phenology, xylem anatomy, hydraulic architecture, foliar stable carbon isotope ratios ($\delta^{13}\text{C}$), gas exchange and water relations characteristics were measured in both populations and treatments. At the end of the first growing season,

PIPO_{WEST} seedlings had grown more than PIPO_{EAST} seedlings in both the control ($p < 0.001$) and drought ($p = 0.042$) treatments, while PIPO_{EAST} seedlings formed dormant buds and ceased growing much earlier in the fall than PIPO_{WEST} seedlings, suggesting that phenological differences between the two populations could partly explain differences in their performance. Vapor pressure deficit (VPD) and low water availability likely resulted in reduced seedling water potential, turgor and rates of expansion of developing tracheids in both populations, resulting in smaller tracheid diameters at maturity, which potentially affected maximum hydraulic conductivity. Xylem-area specific hydraulic conductivity was higher in PIPO_{WEST} seedlings compared to PIPO_{EAST} seedlings in both the control ($p = 0.028$) and drought ($p = 0.031$) treatments. Intrinsic WUE based on $\delta^{13}\text{C}$ values was higher in PIPO_{EAST} seedlings for both control and drought treatments ($p < 0.001$ to $p = 0.027$) across all sampling dates. There was a negative relationship ($r^2 = 0.95$) between values of $\delta^{13}\text{C}$ and leaf-specific hydraulic conductivity across populations and treatments, consistent with greater stomatal constraints on gas exchange with declining seedling hydraulic capacity. Integrated growing season assimilation and stomatal conductance estimated from foliar $\delta^{13}\text{C}$ values and photosynthetic CO_2 response curves were 7% and 38% greater in PIPO_{WEST} seedlings. There were no significant differences found in predawn or midday water potentials between the two populations. PIPO_{EAST} seedlings had significantly more negative osmotic potential at full turgor and water potential at the turgor loss point compared to PIPO_{WEST} seedlings, suggesting that osmotic adjustment occurs in this population. Overall, PIPO_{EAST} seedlings exhibited more conservative behavior, suggesting that a reduction in growth is traded off for an increased resistance to drought. Results from this

investigation will advance our understanding of the mechanisms involved in seedling establishment.

©Copyright by Kelly L. Kerr
December 8, 2014
All Rights Reserved

The Expression of Functional Traits During Seedling Establishment in Two Populations
of *Pinus ponderosa* from Contrasting Climates

by
Kelly L. Kerr

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 8, 2014
Commencement June 2015

Master of Science thesis of Kelly L. Kerr presented on December 8, 2014.

APPROVED:

Co-Major Professor, representing Forest Ecosystems and Society

Co-Major Professor, representing Forest Ecosystems and Society

Head of the Department of Forest Ecosystems and Society

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Kelly L. Kerr, Author

ACKNOWLEDGEMENTS

I would first like to express my sincere appreciation to my co-major advisors, Rick Meinzer and Kate McCulloh, for their unyielding guidance during my Master's program. Your collected support and enthusiasm for this project was extremely motivating and aided greatly in my research efforts. To Dave Woodruff, thank you for your assistance in my research project, for your willingness to serve as a committee member, and for imparting invaluable plant physiology knowledge. To Charlotte Wickham, thank you for your willingness to serve as my minor advisor, as well as for the extensive guidance on my statistical analysis and writing. To Chris Still, thank you for your willingness to serve as a committee member and for your enthusiastic support of the project. I would like to thank Chris Poklemba for his help with field set-up, and Jennifer McKay for her help with the isotope samples. I would also like to extend a big thanks to Dan Ott, Joshua Petitmermet, Carla Aponte Lopez, Jessica Kessinger, Alicia Magedman, John Crandall, Kristen Falk and Danielle Marias for their extensive contributions to lab and field work. To my family, thank you all so much for the unconditional love and support throughout the years and for always being there for me. And finally, to my friends and colleagues in the College of Forestry and greater Corvallis area, I must extend a very special thank you for your encouragement, advice, and companionship as well as for the countless adventures we've shared.

CONTRIBUTION OF AUTHORS

Chapter 2: Frederick C. Meinzer, Katherine A. McCulloh, and David R. Woodruff provided guidance in data collection, analysis, writing, and editing. Danielle E. Marias provided assistance in field and lab work.

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction.....	2
Overview of Seedling Physiology and Stress Responses	5
Predicting Seedling Stress Tolerance and Species Distributions	9
Project Overview	13
References.....	16
2. The expression of functional traits during seedling establishment in two populations of <i>Pinus ponderosa</i> from contrasting climates.....	22
Introduction	23
Methods.....	29
Study Design.....	29
Growth and Phenology	31
Anatomy	32
Hydraulics.....	33
Carbon isotope discrimination.....	35
Gas Exchange	37
Shoot Water Relations	38
Statistics.....	40
Results	42
Discussion	47
Acknowledgements	55
References	56
Figures.....	64
Tables	71
3. Conclusions.....	74
References	85

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 1. Maximum (T_{\max}) and minimum (T_{\min}) temperature, vapor pressure deficit (VPD), soil water potential and precipitation during the summer of 2013.....	64
Figure 2. Mean shoot biomass (A) and mean percentage of plants with dormant buds (B) for seedlings from two populations of <i>Pinus ponderosa</i> sampled in 2013 and early 2014. Seedlings measured prior to implementation of the drought treatment are denoted by the gray-filled symbols	65
Figure 3. Mean tracheid lumen diameters (A) and mean $k_{S\text{-theo}}$ (B) of seedlings from two populations of <i>Pinus ponderosa</i> sampled in 2013	66
Figure 4. Mean xylem area-specific hydraulic conductivity (k_S ; A), mean leaf-specific conductivity (k_L ; B), mean leaf-specific root conductance (K_{R-L} ; C) and mean ratio of leaf area (A_L) to xylem area (A_X) (D) from two populations of <i>Pinus ponderosa</i> sampled in 2013.....	67
Figure 5. $\delta^{13}\text{C}$ values for two populations of <i>Pinus ponderosa</i> seedlings sampled in 2013. For seedlings sampled after the drought treatment was implemented (Sept, Nov) no statistical differences were found between treatments within either population	68
Figure 6. $\delta^{13}\text{C}$ values (pooled from July and September data points) plotted against leaf-specific conductivity (k_L) for two populations of <i>Pinus ponderosa</i> seedlings sampled in 2013. $r^2 = 0.95$	69
Figure 7. Photosynthetic CO_2 response curves ($A-C_i$) representing pooled data for two populations of <i>Pinus ponderosa</i> seedlings sampled in July and November 2013.....	70

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 1. Climate data (precipitation, maximum and minimum temperatures) for Corvallis taken by the Hyslop Field Lab Weather Station, presented as monthly totals (precipitation) and monthly means (temperature) during selected months of 2013	71
Table 2. Mean (\pm SE) xylem area (mm^2) and hydraulic diameter, d_H (μm) for two populations of <i>Pinus ponderosa</i> seedlings sampled in 2013	72
Table 3. Mean (\pm SE) predawn and midday water potential (Ψ_{pd} , Ψ_{md}), osmotic potential at full turgor ($\Psi_{\pi 100}$) and water potential at the turgor loss point (Ψ_{TLP}) for two populations of <i>Pinus ponderosa</i> seedlings sampled in 2013	73

The expression of functional traits during seedling establishment in two populations of
Pinus ponderosa from contrasting climates

1. INTRODUCTION

On a global scale, climate change is estimated to decrease low elevation snowpack, cause earlier snowmelt and increase the duration and severity of droughts (Chmura et al. 2011). Forest growth and productivity are expected to decline (Allen et al. 2010) leading to changes in the structure and composition of forest ecosystems at the landscape scale (Chmura et al. 2011). Distributions of plant species are partially determined by functional traits (Creese et al. 2011) and have been correlated with climatic factors, such as precipitation regimes (Stephenson 1990, Engelbrecht et al. 2007, Poorter and Markesteijn 2008, McLean et al. 2014). However, functional traits can vary within species and many plant species maintain wide geographic ranges across varying climate regimes. Thus, populations within a species from contrasting climate regimes could exhibit different functional traits that facilitate their establishment in a particular region (Giuliani et al. 2013).

The establishment of seedlings is crucial to the survival of tree species and for future forest and stand regeneration (Johnson and Smith 2005). Distributions of tree species change when seedlings either establish beyond current population boundaries or fail to establish within current population boundaries (Johnson et al. 2011). Understanding the mechanisms and traits that drive seedling establishment may improve our ability to predict future species distributions, especially as climate change-driven shifts in precipitation regimes are predicted to alter the dynamics, composition and diversity of many plant ecosystems (Delucia et al. 2000, Comita and Engelbrecht 2009, Shevtsova et al. 2009). However, this requires a thorough knowledge of seedling

physiology and there have been relatively few studies regarding the ecophysiology of tree seedlings during their first year of growth.

Recent studies that have documented large-scale forest die-offs have attributed these mortality events to water limitation, or more commonly to global change-type droughts (McDowell et al. 2008, Anderegg et al. 2012). Drought-induced shifts in plant distributions have been reported for many landscapes (Allen and Breshears 1998, Kelly and Goulden 2008). However, future climate change projections predict increases in temperature for many ecosystems, in addition to increased severity and duration of drought events (Stocker et al. 2013). Temperature is expected to have an impact on water availability because atmospheric evaporative demand, or vapor pressure deficit (VPD), increases exponentially with temperature (Williams et al. 2013). Increased VPD generally leads to an increase in plant transpiration, thus creating a more rapid depletion of water supply that intensifies the effects of drought. Under a 3°C increase in surface temperature, Shevtsova et al. (2009) found that both seed germination and seedling establishment in fifteen species of subarctic plant species were negatively affected even with an additional increase in water availability. Results from this study also showed that increased temperature affected specific periods during the establishment phase due to species-specific time of germination during the growing season. Thus, both temperature and water availability may be important factors that influence successful seedling establishment under future climates.

Overview of Seedling Physiology and Stress Responses

Seedlings represent a particularly vulnerable life stage of a tree and generally exhibit high rates of mortality due to a range of both abiotic and biotic factors, such as herbivory, trampling by animals, frost heave, pathogens and drought (Grossnickle 2012). Upon germination, seedlings are dependent on seed reserves until the leaf photosynthetic machinery is developed enough to become self-sufficient and begin exporting fixed carbon (Kitajima 1992). In many ecosystems, seedling survival and establishment are major bottlenecks in the recruitment of species (Shevtsova et al. 2009) and seedling abundance alone may not guarantee the regeneration of a species (Cui and Smith 1991). Climate change influences on temperature and water supply, and thus soil moisture, have also been shown to impact seed germination (Walck et al. 2011), further emphasizing the importance of survival during these early life-stages for the continued existence of a species.

Water limitation is an important driver of vegetation mortality (McDowell et al. 2008), and as such, water availability has a significant effect on photosynthetic carbon gain (Johnson et al. 2004) and is a primary factor governing seedling survival (Cui and Smith 1991, Kennedy and Sousa 2006, Engelbrecht et al. 2007). Water, taken up by the root system, travels along a gradient in negative pressure via a continuous water column to the leaves through the xylem. Seedlings have very shallow root systems, as they are still developing, that cannot access deep-water reserves underground. Drake et al. (2011) showed that, compared to adult trees, *Eucalyptus gomphocephala* seedlings primarily utilized surface water and water from recent rainfall events. Once taken up by the root

system, the efficiency of water movement through the seedling can be measured as hydraulic conductance, which is the ratio of water flow rate to the driving force for water movement (Tyree and Zimmermann 2002). During photosynthetic gas exchange large quantities of water evaporate through the stomata concurrent with the uptake of carbon dioxide (CO₂). Thus, readily available water is important for sustaining photosynthesis, but many ecosystems experience seasonal or consistent drought, leading to water scarcities.

There are many anatomical and physiological traits that seedlings might exhibit to sustain carbon dioxide uptake as water availability becomes increasingly scarce. The hydraulic architecture of a seedling, which is determined to a large extent by the structure and properties of the xylem tissue, can impact many physiological parameters that affect a seedling's vulnerability or tolerance to water stress (Tyree and Ewers 1991, Christensen-Dalsgaard and Ennos 2012). In conifers, the individual water-conducting conduits that make up the xylem tissue are called tracheids that are connected by bordered pits with a torus-margo pit membrane (Boyd and Foster 1974, Hacke et al. 2004). The hydraulic conductance of conifer xylem is affected by the structure and arrangement of tracheids. Xylem containing longer tracheids with larger diameters and a greater frequency of inter-tracheid pits will have lower resistance to water flow (Tyree and Zimmermann 2002, Pittermann et al. 2006). However, under periods of water stress, air bubbles may enter the xylem tissue through pit membranes due to increased tension in the water column (Tyree and Ewers 1991). These air bubbles can expand until an entire tracheid is filled with water vapor or air, which results in the loss of a functioning

conduit, and are known as embolisms (Tyree and Ewers 1991). Loss of hydraulic conductance associated with massive xylem embolism can result in plant mortality (Kursar et al. 2009). Resistance to embolism formation is an important parameter for determining species' drought tolerance (Tyree and Ewers 1991, Maherali et al. 2004). Air-water menisci become trapped through capillary action that aspirates the torus-margo pit membrane and the torus seals the pit aperture to prevent movement of embolisms to neighboring tracheids (Hacke et al. 2004). The structure of bordered pits strongly influences the movement of water in tracheids, whereby differences in bordered pit morphology determines the water tension at which the tracheids become vulnerable to embolism (Hacke et al. 2004, Domec et al. 2006). Thus, the structure and function of xylem can influence a plant's vulnerability to embolism and its response to desiccation. Hacke et al. (2001) examined the role of wood density with respect to resistance of the water transport system to implosion by negative pressure. There was a strong positive relationship between conduit wall reinforcement and embolism resistance; however, increased resistance can also result in decreased hydraulic conductivity leading to a safety-efficiency tradeoff. Brodribb and Cochard (2009) investigated conifers with different resistances of stem xylem to water-stress-induced embolisms to see if xylem function plays a role in response to desiccation. They found that hydraulic function did play a large role in recovery from and survival of water stress in the conifers tested and that hydraulic limitations governed gas exchange recovery from drought.

By regulating the opening and closing of the stomata, plants can control the amount of water loss at the cost of limiting carbon dioxide uptake. Seedling growth rate

is tightly linked to stomatal regulation of water loss (Creese et al. 2011), yet seedlings may not have well-developed stomatal regulation as compared to their adult counterparts. In C_3 plants, relative stomatal limitation of photosynthetic gas exchange is reflected in the stable carbon isotope composition of their tissues. There are two stable isotopes of carbon, ^{12}C and ^{13}C , and atmospheric CO_2 is made up of approximately 1% $^{13}\text{CO}_2$ (Farquhar et al. 1989). During photosynthetic CO_2 fixation, discrimination against $^{13}\text{CO}_2$ occurs as a result of its slower diffusion into the leaf and preferential fixation of $^{12}\text{CO}_2$ by ribulose biphosphate carboxylase oxygenase (RuBisCO). When stomata partially close to reduce water loss, the intercellular CO_2 concentration decreases and plants begin to discriminate less against $^{13}\text{CO}_2$ as its abundance relative to $^{12}\text{CO}_2$ in the intercellular spaces increases. The stable carbon isotope ratio ($\delta^{13}\text{C}$) of plant tissues is determined by the isotopic composition of the ambient CO_2 as well as the diffusional and biochemical fractionation processes described above (Cernusak et al. 2013). Carbon isotope discrimination (Δ) factors out the influence of variation in the $\delta^{13}\text{C}$ of ambient CO_2 and thus reflects only biological fractionation processes (Cernusak et al. 2013). Carbon isotope ratios and discrimination values can be used to understand how a plant's physiology, as well as its environment, influence gas exchange over a period of time (Farquhar and Richards 1984, Farquhar et al. 1989, Cernusak et al. 2013). Carbon isotope discrimination is directly related to the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) and to the ratio of CO_2 assimilation to stomatal conductance (A/g) a measure of intrinsic water-use efficiency (iWUE) whereby decreased Δ reflects increased iWUE (Farquhar and Richards 1984, Zhang et al. 1993, Pastorino et al. 2012). Zhang and

Marshall (1994) compared the effect of drought on Δ and iWUE among 14 populations of *Tsuga heterophylla* seedlings and discovered that Δ decreased (iWUE increased) across all populations, with population differences in Δ associated with altitude of the seed source. Therefore, iWUE, Δ and $\delta^{13}\text{C}$ can all be used to measure and compare the physiological performance among different species and between species populations from different environments.

Predicting Seedling Stress Tolerance and Species Distributions

Morphological attributes have been shown to play a key role in defining the hydraulic architecture of a plant (Grossnickle 2012) and hydraulic architecture has been shown to differ among species (Pineda-Garcia et al. 2011) and populations (Barnard et al. 2011). Therefore, differences in morphological or physiological traits at the seedling life stage may explain why certain species survive drought, while others do not. Yet these traits (such as hydraulic architecture, root to shoot ratio, vulnerability to embolism, and gas exchange regulation) may be somewhat ephemeral as some have been shown to exist due to induction by the environment (e.g. water stress), not by genetic inheritance (Kavanagh et al. 1999, Grossnickle 2012). Vitasse et al. (2013) showed that environmental conditions had a greater influence on phenological traits, such as leaf unfolding and budset timing, than genetic effects when seedlings from seven deciduous tree species from different elevations in the Alps were grown in a common garden experiment. In addition, Zhang and Cregg (2005) compared growth, morphology, and physiological traits among 10 populations of *Pinus ponderosa* grown in three range-wide provenance trial sites along an environmental gradient, and reported that among sites

there were significant differences in morphological and physiological traits due to the varied environments at each site. However, within sites the authors did not see any significant population differences in physiological traits, and they attributed this lack of population variance in physiology to phenotypic plasticity. In contrast, Barnard et al. (2011) investigated hydraulic traits in *Pseudotsuga menziesii* and *Pinus ponderosa* individuals including sapwood thickness, axial and radial conductivity, resistance to embolism, and sapwood water storage capacity. These authors reported differences in expression of these traits due to environment: vulnerability of sapwood to embolism, capacitance and axial hydraulic conductivity were highest for populations growing in the most arid sites, suggesting the phenotypic plasticity of these traits may play an important role in avoiding high levels of xylem tension under dynamic conditions of high water loss from transpiration. Differences in xylem traits and hydraulic structure expressed among species at the seedling stage may be apparent in adult trees (Creese et al. 2011, Vitasse et al. 2013), which could help explain what traits a seedling might employ for its successful establishment and growth beyond germination.

The intensity of plant responses to water stress seems to be species-specific and is dependent upon hydraulic strategy (Ayup et al. 2012). There is current discussion on hydraulic strategies, but one aspect of these strategies relates to a continuum of stomatal regulation of xylem tension, with the extremes being labeled as isohydric and anisohydric behavior (Tardieu and Simonneau 1998, West et al. 2012). Extreme isohydric species close their stomata under water stress to maintain constant midday water potential, while anisohydric species allow midday water potential to decline as the soil dries or VPD

increases (Tardieu 1993, Tardieu and Simonneau 1998, McDowell et al. 2008). Hubbard et al. (2001) showed that as leaf specific hydraulic conductance (K_L) declined, *Pinus ponderosa* seedlings maintained leaf water potential (Ψ_L) around -1.45 MPa, which suggested a response of stomatal conductance to K_L to maintain Ψ_L . These authors posited that *P. ponderosa* seedlings exhibited isohydric behavior and that the reduction in stomatal conductance in response to K_L maintains Ψ_L at its original level. Although isohydric and anisohydric species often co-occur on the landscape level, anisohydric species tend to occupy more drought-prone areas and have xylem that is more resistant to embolism (McDowell et al. 2008). Understanding the variation in hydraulic strategies can help identify the mechanisms that cause mortality, and help predict which species may be vulnerable to widespread mortality events under a changing climate. McDowell et al. (2008) discuss the carbon starvation-hydraulic failure paradigm as it relates to mechanisms of mortality in plants subjected to drought events. The carbon starvation hypothesis predicts that stomatal closure to prevent desiccation will result in diminished uptake of photosynthetic carbon and, because of the continued metabolic demand for photosynthates, the plant will starve to death. The hydraulic failure hypothesis predicts that xylem conduits will embolize in response to reduced soil water and high evaporative demands, which will result in reduced water flow and desiccation of plant tissues. While evidence exists for both hypotheses as drivers of vegetation mortality for a wide range of species (McDowell and Sevanto 2010, McDowell et al. 2011, Anderegg et al. 2012, Poyatos et al. 2013), the hydraulic strategies employed by plants may ultimately determine how a plant will die. For example, isohydric plants are expected to succumb to

prolonged drought as a result of carbon starvation due to the stomatal restriction of photosynthesis. In addition to hydraulic strategies, hydraulic safety margins, the difference between the minimum xylem pressure normally experienced by a plant organ such as a stem or root and a given point on its hydraulic vulnerability curve (Meinzer and McCulloh 2013), vary among species and may be related to species-specific differences in the ability to potentially survive drought. Conifer stems have been shown to often have larger safety margins than angiosperm stems (Meinzer et al. 2009, Johnson et al. 2012), but conifers and angiosperms co-exist in many ecosystems. Resistance to xylem embolism is determined by many morphological and physiological traits and there is a wide range of resistances that plants exhibit (Maherali et al. 2004). Expression of these traits at the seedling life stage may reflect the ability of a species to withstand periods of water stress.

Global and regional patterns of species distributions and vegetation patterns have been linked to rainfall regimes (Engelbrecht et al. 2007, Poorter and Markesteijn 2008, Comita and Engelbrecht 2009). Although species with different resistances to xylem embolism co-occur (Maherali et al. 2004), vulnerability to embolism may also limit plant distributions because it plays a role in determining the drought tolerance of species across a range of habitats (Pockman and Sperry 2000). However, water stress can induce tree species to develop drought resistance (Grossnickle 2012) so the capacity for hydraulic acclimation to water limitation may partly determine species distributions (Creese et al. 2011). Kavanagh et al. (1999) showed that vulnerability to embolism in Douglas-fir sapling roots and shoots varied among populations and was related to the climate of

origin of each seed source, yet their results suggest that saplings were less vulnerable to embolism than mature trees. Although there may be some plasticity in traits that confer drought resistance, the degree to which traits manifested in the first year of seedling growth influence species distributions remains unclear.

Project Overview

The ability of a species to maintain its geographic distributional range may depend on many factors, including its responses to future climate change. Shifts in vegetation distributions due to climate change have been reported for many ecosystems (Allen and Breshears 1998, Kelly and Goulden 2008), but the ability of a species to migrate to new locations will depend on the survival and successful establishment of seedlings (Johnson et al. 2011). Climate change, including both increases in drought and temperature, have been predicted to impact seedling germination and establishment (Shevtsova et al. 2009, Walck et al. 2011, del Cacho et al. 2013), thus understanding the influence of climate change on seedling establishment is crucial to understanding how a species range might shift in the future. Currently, there are gaps in our knowledge as to what extent certain physiological and biological plant traits are influenced by the environment (phenotypic plasticity). Recently, Richardson et al. (2014) used vegetative responses from a common-garden experiment to assess adaptive genetic variation in blackbrush (*Coleogyne ramosissima*) and concluded that genetic variation was associated with population-specific climate variables. Richter et al. (2012) studied phenotypic plasticity and genotypic variation in *P. sylvestris* provenances to future climate conditions in a common-garden experiment and reported that certain provenances were more plastic

than others in response to decreased precipitation, but there were marginal effects of maternal lineage on biomass. Common garden experiments offer scientists the ability to understand the influence of certain environmental parameters on seedling responses while subjecting seedlings to identical environmental factors. Further, common garden designs allow scientists to compare and evaluate multiple populations, or provenances, of the same species under similar environmental conditions and thus, are critically important to furthering our understanding of the range of plasticity of certain responses within a species (Zhang et al. 1993, Maherali et al. 2002, Richter et al. 2012, Giuliani et al. 2013, Vitasse et al. 2013, McLean et al. 2014, Richardson et al. 2014).

Recently, the field of assisted migration, or assisted colonization, has gained much attention from researchers, policy makers and the public alike. Assisted migration pertains to the notion that under a changing climate species may be required to migrate to new habitats, and if certain species are unable to keep pace with a rapidly changing climate human assistance in the migration process might be necessary in order to ensure the survival of those species (McLachlan et al. 2007, Aitken et al. 2008, Stone 2010). Currently, there is a strong need for a scientifically based policy on assisted migration due to the controversial nature of the subject and its potential use as a management option for conservation purposes (McLachlan et al. 2007). Those in opposition of assisted migration claim the process could lead to unforeseen consequences including an introduced species becoming invasive in its new environment, an introduced species bringing along a new pest or disease to the new habitat, or the failure of an introduced species to establish and survive in the new environment (Marris 2008). Another related

strategy is facilitated migration, which involves human involvement in manipulating the environment so that a species, through natural spread and establishment, could potentially inhabit and survive therein (McLachlan et al. 2007). Regardless of the strategy, most authors agree that more research is needed before an effective assisted migration policy can be implemented. To date, researchers use species distribution models (SDMs) to predict changes in species distribution ranges, yet these SDMs do not account for population differences within a species distribution (Aitken et al. 2008). Assisted migration research can be further improved by including short-term seedling common garden experiments that focus on determining the physiological basis of adaptation and population tolerances to extreme climatic events (Aitken et al. 2008).

This research project will investigate physiological traits expressed during seedling establishment in two ponderosa pine populations from contrasting environments. Results from this project will: 1) improve our knowledge of the physiological mechanisms involved in seedling establishment, 2) advance our understanding of species-specific responses to limiting environmental factors, 3) provide data that can be used to model predictions of vegetation responses to future climate change, and 4) advance techniques used for measuring physiological parameters in seedlings.

References

- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl* 1:95–111.
- Allen CD, Breshears DD (1998) Drought-induced shift of a forest–woodland ecotone: rapid landscape response to climate variation. *Proc Natl Acad Sci* 95:14839–14842.
- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684.
- Anderegg WR, Berry JA, Smith DD, Sperry JS, Anderegg LD, Field CB (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proc Natl Acad Sci* 109:233–237.
- Ayup M, Hao X, Chen Y, Li W, Su R (2012) Changes of xylem hydraulic efficiency and native embolism of *Tamarix ramosissima* Ledeb. seedlings under different drought stress conditions and after rewatering. *South Afr J Bot* 78:75–82.
- Barnard DM, Meinzer FC, Lachenbruch B, McCulloh KA, Johnson DM, Woodruff DR (2011) Climate-related trends in sapwood biophysical properties in two conifers: avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. *Plant Cell Environ* 34:643–654.
- Boyd JD, Foster RC (1974) Tracheid anatomy changes as responses to changing structural requirements of the tree. *Wood Sci Technol* 8:91–105.
- Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiol* 149:575–584.
- Del Cacho M, Estiarte M, Penuelas J, Lloret F (2013) Inter-annual variability of seed rain and seedling establishment of two woody Mediterranean species under field-induced drought and warming. *Popul Ecol* 55:277–289.
- Cernusak LA, Ubierna N, Winter K, Holtum JA, Marshall JD, Farquhar GD (2013) Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytol* 200:950–965.
- Chmura DJ, Anderson PD, Howe GT, Harrington CA, Halofsky JE, Peterson DL, Shaw DC, Brad St Clair J (2011) Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *For Ecol Manag* 261:1121–1142.

- Christensen-Dalsgaard KK, Ennos AR (2012) Effects of drought acclimation on the mechanical properties of *Ochroma pyramidale*, *Betula pendula* and *Acacia karroo* tree seedling stems. *Forestry* 85:215–223.
- Comita LS, Engelbrecht BM (2009) Seasonal and spatial variation in water availability drive habitat associations in a tropical forest. *Ecology* 90:2755–2765.
- Creese C, Benschoter AM, Maherali H (2011) Xylem function and climate adaptation in *Pinus*. *Am J Bot* 98:1437–1445.
- Cui M, Smith WK (1991) Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol* 8:37–46.
- Delucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. *Glob Change Biol* 6:587–593.
- Domec JC, Lachenbruch B, Meinzer FC (2006) Bordered pit structure and function determine spatial patterns of air-seeding thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinaceae) trees. *Am J Bot* 93(11):1588-1600.
- Drake PL, Froend RH, Franks PJ (2011) Linking hydraulic conductivity and photosynthesis to water-source partitioning in trees versus seedlings. *Tree Physiol* 31:763–773.
- Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447:80–82.
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Biol* 40:503–537.
- Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct Plant Biol* 11:539–552.
- Giuliani AL, Kelly EF, Knapp AK (2013) Geographic variation in growth and phenology of two dominant Central US grasses: consequences for climate change. *J Plant Ecol*. DOI: 10.1093/jpe/rtt036.
- Grossnickle SC (2012) Why seedlings survive: influence of plant attributes. *New For* 43:711–738.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457–461.

- Hacke UG, Sperry JS, Pittermann J (2004) Analysis of circular bordered pit function II. Gymnosperm tracheids with torus-margo pit membranes. *Am J Bot* 91(3):386-400.
- Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113–121.
- Johnson DM, Germino MJ, Smith WK (2004) Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings below and above the alpine timberline. *Tree Physiol* 24:377–386.
- Johnson DM, McCulloh KA, Reinhardt K (2011) The Earliest Stages of Tree Growth: Development, Physiology and Impacts of Microclimate. In: Meinzer FCC, Lachenbruch B, Dawson TEE, Meinzer FC, Niinemets Ü (eds) *Size- and Age-Related Changes in Tree Structure and Function*. Springer Netherlands, pp 65–87.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci* 195:48–53.
- Johnson DM, Smith WK (2005) Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. *Tree Physiol* 25:1379–1387.
- Kavanagh KL, Bond BJ, Aitken SN, Gartner BL, Knowe S (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiol* 19:31–37.
- Kelly AE, Goulden ML (2008) Rapid shifts in plant distribution with recent climate change. *Proc Natl Acad Sci* 105:11823–11826.
- Kennedy PG, Sousa WP (2006) Forest encroachment into a Californian grassland: examining the simultaneous effects of facilitation and competition on tree seedling recruitment. *Oecologia* 148:464–474.
- Kitajima K (1992) Relationship between photosynthesis and thickness of cotyledons for tropical tree species. *Funct Ecol*:582–589.
- Kursar TA, Engelbrecht BM, Burke A, Tyree MT, El Omari B, Giraldo JP (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Funct Ecol* 23:93–102.
- Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85:2184–2199.

- Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence. *Funct Ecol* 16:510–521.
- Marris E (2008) Moving on assisted migration. *Nat Rep Clim Change*:112–113.
- McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends Ecol Evol* 26:523–532.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739.
- McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: how, when, or does it even occur at all? *New Phytol* 186:264–266.
- McLachlan JS, Hellmann JJ, Schwartz MW (2007) A framework for debate of assisted migration in an era of climate change. *Conserv Biol* 21:297–302.
- McLean EH, Prober SM, Stock WD, Steane DA, Potts BM, Vaillancourt RE, Byrne M (2014) Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant Cell Environ* 37:1440–1451.
- Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Funct Ecol* 23:922–930.
- Meinzer FC, McCulloh KA (2013) Xylem recovery from drought-induced embolism: where is the hydraulic point of no return? *Tree Physiol* 33:331–334.
- Pastorino MJ, Aparicio AG, Marchelli P, Gallo LA (2012) Genetic variation in seedling water-use efficiency of Patagonian Cypress populations from contrasting precipitation regimes assessed through carbon isotope discrimination. *For Syst* 21:189–198.
- Pineda-García F, Paz H, Tinoco-Ojanguren C (2011) Morphological and physiological differentiation of seedlings between dry and wet habitats in a tropical dry forest. *Plant Cell Environ* 34:1536–1547.
- Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH (2006) Inter-tracheid pitting and the hydraulic efficiency of conifer wood: the role of tracheid allometry and cavitation protection. *Am J Bot* 93:1265–1273.
- Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Am J Bot* 87:1287–1299.

- Poorter L, Markesteijn L (2008) Seedling traits determine drought tolerance of tropical tree species. *Biotropica* 40:321–331.
- Poyatos R, Aguadé D, Galiano L, Mencuccini M, Martínez-Vilalta J (2013) Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. *New Phytol* 200:388–401.
- Richardson BA, Kitchen SG, Pendleton RL, Pendleton BK, Germino MJ, Rehfeldt GE, Meyer SE (2014) Adaptive responses reveal contemporary and future ecotypes in a desert shrub. *Ecol Appl* 24:413–427.
- Richter S, Kipfer T, Wohlgemuth T, Guerrero CC, Ghazoul J, Moser B (2012) Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* 169:269–279.
- Shevtsova A, Graae BJ, Jochum T, Milbau A, Kockelbergh F, Beyens L, Nijs I (2009) Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Glob Change Biol* 15:2662–2680.
- Stephenson NL (1990) Climatic control of vegetation distribution: the role of the water balance. *Am Nat*:649–670.
- Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex B, Midgley BM (2013) IPCC, 2013: climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- Stone R (2010) Home, home outside the range. *Science* 329:1592–1594.
- Tardieu F (1993) Will increases in our understanding of soil-root relations and root signalling substantially alter water flux models? *Philos Trans R Soc Lond B Biol Sci* 341:57–66.
- Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J Exp Bot* 49:419–432.
- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360.
- Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer, Berlin.

- Vitasse Y, Hoch G, Randin CF, Lenz A, Kollas C, Scheepens JF, Körner C (2013) Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. *Oecologia* 171:663–678.
- Walck JL, Hidayati SN, Dixon KW, Thompson KEN, Poschlod P (2011) Climate change and plant regeneration from seed. *Glob Change Biol* 17:2145–2161.
- West AG, Dawson TE, February EC, Midgley GF, Bond WJ, Aston TL (2012) Diverse functional responses to drought in a Mediterranean-type shrubland in South Africa. *New Phytol* 195:396–407.
- Williams AP, Allen CD, Macalady AK, Griffin D, Woodhouse CA, Meko DM, Swetnam TW, Rauscher SA, Seager R, Grissino-Mayer HD (2013) Temperature as a potent driver of regional forest drought stress and tree mortality. *Nat Clim Change* 3:292–297.
- Zhang J, Cregg BM (2005) Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. *For Ecol Manag* 219:1–12.
- Zhang J, Marshall JD (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Can J For Res* 24:92–99.
- Zhang J, Marshall JD, Jaquish BC (1993) Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. *Oecologia* 93:80–87.

2. THE EXPRESSION OF FUNCTIONAL TRAITS DURING SEEDLING
ESTABLISHMENT IN TWO POPULATIONS OF *PINUS PONDEROSA* FROM
CONTRASTING CLIMATES

Kelly L. Kerr, Frederick C. Meinzer, Katherine A. McCulloh, David R. Woodruff,

Danielle E. Marias

Introduction

Recently, documented tree mortality around the world has been linked to climate-induced water stress and warmer temperatures (Allen et al. 2010) and has led to changes in the structure and composition of forest ecosystems at the landscape scale (Chmura et al. 2011). Distribution patterns of plant species have been correlated with climatic factors, such as precipitation regimes (Stephenson 1990, Engelbrecht et al. 2007, Poorter and Markesteijn 2008, McLean et al. 2014), but climate change is predicted to alter many of these factors including an increase in the duration and severity of droughts (Chmura et al. 2011, Stocker et al. 2013). The successful establishment of tree seedlings is crucial for future forest and stand regeneration, and distributions of tree species can be altered when seedlings either establish beyond current population boundaries or fail to establish within current population boundaries (Johnson et al. 2011). Understanding the physiological mechanisms and traits that drive successful seedling establishment may improve our ability to predict future species distributions, especially as climate change-driven shifts in rainfall regimes are predicted to alter the dynamics, composition and diversity of many plant ecosystems (Delucia et al. 2000, Comita and Engelbrecht 2009, Shevtsova et al. 2009).

Most studies involving tree seedlings typically classify this stage as encompassing the first 5 years of development (Heiner et al. 1972, Germino and Smith 1999, Lewis et al. 2001, Greenwood, O'Brien, et al. 2008, Schall et al. 2012, Moyes et al. 2013), yet few studies have investigated the ecophysiology of germinant seedlings within the first year of growth (see Cui and Smith 1991, Padilla and Pugnaire 2007, Padilla et al. 2007 for

examples). Seedlings represent a particularly vulnerable life stage of a tree and generally exhibit high rates of mortality due to both abiotic and biotic factors, such as herbivory, trampling by animals, frost heave, pathogens and drought (Grossnickle 2012). In many ecosystems, seedling survival and establishment are major bottlenecks in species recruitment and seedling abundance alone may not guarantee the regeneration of a species (Cui and Smith 1991). Climate change influences on soil temperature and moisture levels have been shown to impact seed germination (Walck et al. 2011), and compromised seed germination may result in more vulnerable seedlings. Understanding the dynamics of germinant seedlings can improve our predictions of future species distribution patterns (Aitken et al. 2008), and the ability of a species to migrate to new locations will depend on the survival and successful establishment of seedlings (Johnson et al. 2011).

Water availability has been shown to influence seedling establishment in a wide variety of tree species (Gordon et al. 1991, Cui and Smith 1991, Johnson and Smith 2005, Padilla and Pugnaire 2007, Matías et al. 2012, del Cacho et al. 2013) and seedlings have been shown to use different soil water sources compared to their adult counterparts (Drake et al. 2011). Temperature also influences water availability and seedling establishment because atmospheric evaporative demand, or vapor pressure deficit (VPD), increases exponentially with temperature (Williams et al. 2013). Increased temperature and VPD generally result in more rapid depletion of soil water, which intensifies the effects of drought. For instance, under a 3°C increase in surface temperature, Shevtsova et al. (2009) found that both seed germination and establishment in fifteen subarctic

species were negatively affected even with an increase in soil water availability. Moyes et al. (2013) evaluated the effects of warming on first-year limber pine (*P. flexilis*) seedling physiology and survival, and discovered that seedling exposure to warmer temperatures and dry soil was negatively correlated with survival. However, these authors concluded that soil moisture limitations ultimately determined seedling survival with temperature exacerbating any effects of a drought (Moyes et al. 2013).

While water availability is a crucial factor for seedling survival and establishment, it also influences species distribution patterns. Engelbrecht et al. (2007) tested if variation in drought sensitivity explained distribution patterns of tropical woody plant seedlings in central Panama and found that both water availability and drought sensitivity directly influenced species' distribution patterns of the tropical forests examined. In addition, Comita and Engelbrecht (2009) found that there was strong evidence that variation in water availability influenced seedling population dynamics of whole plant communities and that dry season mortality determined species' habitat associations. Both spatial and temporal variation in water availability have been shown to influence seedling recruitment and species' establishment (Comita and Engelbrecht 2009, Baltzer and Davies 2012, Matías et al. 2012), thus understanding the traits contributing to drought resistance of seedlings during their first few years of growth is important for predicting how future fluctuations in water availability will influence species' distribution patterns.

Plants can exhibit a range of responses to low water availability that increase their resistance and tolerance to drought events, including decreasing the ratio of leaf area (A_L)

to stem xylem area (A_X) (Mencuccini and Grace 1995, Delucia et al. 2000, Maherali et al. 2004), increasing the ratio of root tissue to shoot tissue (Greenwood et al. 2008, Schall et al. 2012), increasing the resistance of the xylem to embolism (Kavanagh et al. 1999, Christensen-Dalsgaard and Ennos 2012), and increasing water-use-efficiency (WUE) (Smit and Van Den Driessche 1992, Zhang et al. 1993). These responses may be plastic or fixed in nature, yet the degree of plasticity for many of these traits remains unclear. Differentiation in the expression of physiological traits has been shown to guide habitat partitioning among tree species (Pineda-García et al. 2011) and thus may influence population-specific establishment within a species. Habitat heterogeneity across a species' range can lead to variability in certain plant functional traits so that within a single species, genetically distinct populations of individuals represent different ecotypes (Turesson 1922, Linhart and Grant 1996, Hufford and Mazer 2003). Zhang and Marshall (1994) investigated variation in WUE between fourteen populations of 1-year-old western larch (*Larix occidentalis*) seedlings across its distribution range and found evidence of genetic (population) differences in WUE that were related to the altitude of the seed sources. Recently, McLean et al. (2014) assessed the extent of phenotypic plasticity in response to climate by comparing provenances of *Eucalyptus tricarpa* trees in their native environments to those grown in two common gardens established near each end of a climate gradient. These authors discovered that overall tree performance did not decline due to differences in climate and that functional traits varied among the populations examined, showing significant associations with mean annual precipitation

(MAP), which suggests that variation among provenances relates to functional trait plasticity (McLean et al. 2014).

Ponderosa pine (*Pinus ponderosa* P. & C. Lawson, PIPO) is a conifer species that maintains an expansive distribution in North America, ranging from southern Canada down into northern Mexico and throughout the western United States (Little 1971, Graham et al. 2005). In Oregon, ponderosa pine exists predominately in the northeastern and southwestern parts of the state, as well as along the central Cascade Mountain range (Little 1971). The physiology of both seedling and adult life stages of this species have been studied (Rehfeldt 1990, Cregg 1994, Hubbard et al. 1999, Zhang and Cregg 2005), including germinant-year seedlings (Zhang et al. 1997, Maherali et al. 2002, Creese et al. 2011). Studies that investigated whether or not the plasticity of certain functional traits varies due to within-species population differences have reached opposing conclusions. Rehfeldt (1990) assessed genetic variation among populations of 2-4 year-old ponderosa pine seedlings and reported differences in 19 out of 20 traits analyzed, which were related to geographical location and elevation of the seed source. In contrast, Maherali et al. (2002) studied climate-driven shifts in biomass allocation and hydraulics between desert and montane populations of germinant-year ponderosa pine seedlings and concluded that their results supported little evidence of ecotypic differentiation among the populations studied. In this study the authors saw no differences between climate groups and nearly all the genetic variation seen was partitioned within populations (Maherali et al. 2002). Cregg (1994) examined physiological and morphological traits of 1-year-old ponderosa pine seedlings and found that, in general, drought resistance traits did not correlate well

with the climate of origin of the seed source. However, this author reported that growth biomass and morphology did correlate with temperature of the seed source (Cregg 1994). Zhang and Cregg (2005) investigated physiological traits in ten populations of 26-year-old ponderosa pine trees from provenance trials and reported that while the traits varied due to the environmental conditions, there were only significant population differences in growth and morphological traits, not in physiological traits. These authors further concluded that a lack of physiological variance could be due to plastic phenotypic convergence of traits when populations are grown in a similar environment (Zhang and Cregg 2005). Performing common garden studies, where all populations experience the same environment, on geographically distant populations within a species allows examination of which plant functional traits are plastic to environmental changes and which populations represent genetically distinct ecotypes (Aitken et al. 2008). Knowing the inherent plasticity of functional traits will vastly improve our understanding of ponderosa pine seedling establishment, and how this species will respond to future climate changes.

In this study, we examined functional traits in two populations of *P. ponderosa* seedlings grown in a common garden under a drought and control treatment. The populations were selected from within the natural distribution of *P. ponderosa* to represent contrasting temperature regimes and amounts of precipitation received at their sites of origin. We compared functional traits of the two populations, including shoot biomass accumulation, vegetative phenology, tracheid anatomy, hydraulic architecture, carbon isotope discrimination, photosynthetic behavior and shoot water relations

components. We hypothesized that: 1) drought resistance traits would be more strongly expressed in seedlings under low water availability, and 2) that there would be population-specific differences in water-transport and photosynthetic physiology.

Methods

Study Design

Ponderosa pine (*Pinus ponderosa* P. & C. Lawson, PIPO) seeds from two populations were obtained from the Oregon Department of Forestry (ODF). The first population came from an area close to Spray, in Umatilla County, OR (44.8331° N, 119.7944° W), hereafter referred to as PIPO_{EAST}. The mean annual precipitation (MAP) of Spray is 337 mm, and the 1937-2013 mean minimum and maximum temperatures for winter (Dec-Feb) are -2.7°C and 8.4°C, and those for summer (June-Aug) are 10.7°C and 32.4°C (WRCC, Western Regional Climate Center, URL <http://www.wrcc.dri.edu/>). The second population came from a mix of seed sources from the Willamette Valley, spanning from the junction of the Columbia and Willamette Rivers to south of Eugene, OR, hereafter referred to as PIPO_{WEST}. For this population, the climate was estimated to be similar to that of Corvallis, OR (44.5667° N, 123.2833° W), where MAP is 1,043 mm, and the 1889-2013 mean minimum and maximum temperatures for winter are 1.2°C and 8.6°C, and those for summer are 10.2°C and 25.8°C (WRCC).

This study was conducted in four raised soil beds (18.3 m x 1.5 m) on the Oregon State University campus located in Corvallis, OR from March to November of 2013. Weather data during the study recorded at a nearby site are summarized in Table 1

(Station Data, Corvallis Farm Unit, Oregon State University, URL <http://agsci.oregonstate.edu/farmunit/weather>). Many places west of the Cascade Mountains in the Pacific Northwest, including Corvallis, typically experience a “Mediterranean”-type climate where summers are generally hot and dry while winters are mild and wet.

Prior to planting, all of the raised beds were treated with a soil fumigant (Basamid Granular CertisUSA, Columbia, MD), and roto-tilled to ensure even coverage. Following fumigation, the beds were leveled to create an even surface for planting and seeds were planted on March 26, 2013. Each bed was divided into three (bed-thirds) and each of these bed-thirds was further divided into sections (box). The two populations of ponderosa pine were randomly assigned to a box for planting. Seeds were planted in six rows across the width of the box at 18 seeds per row giving a total of 106 seeds per box with 10 cm spacing between all seedlings. The soil was depressed 13 mm and once a seed was placed in the depression, it was loosely covered by soil and sprinkled with turkey grit. Turkey grit was applied to prevent seeds from becoming dislodged during irrigation, being eaten by birds or blowing away during strong wind events. Each seed was lightly watered in and then a mycorrhizal inoculum (Great White Premium Mycorrhizae, Plant Success, <http://www.plant-success.com>) was applied to the beds according to the label.

The seedlings were watered daily until July 18, 2013 when the treatments were imposed. Then, two beds were randomly selected to receive the drought treatment while the remaining two beds received the water treatment (control). The amount of water

applied was calculated as a percentage of the potential evaporation over the three days prior to watering. Evaporation data were collected at the Hyslop Field Lab Weather Station located in Corvallis, OR. Drought beds were watered once a week and received 10% of the previous three-day's total evaporation. Control beds were watered twice a week and received 50% of the previous three-day's total evaporation. Gypsum block soil matric potential sensors (223L, Campbell Scientific, Logan, UT) were installed in the beds and values were recorded every 30 min with a data logger (CR10X, Campbell Scientific). Four soil sensors were installed in each bed, with two installed between the first and second bed-third and two installed between the second and third bed-third. At each location, one sensor was installed at a depth of 10 cm and the other at 20 cm.

Initial data from the soil sensors revealed no strong drought occurring in the drought beds (Figure 1), so starting on August, 2013 watering was reduced to 5% of the past three-day's total evaporation once a week. Other environmental data collected at the raised bed site between July and November 2013 included daily maximum and minimum temperatures, daily maximum vapor pressure deficit (VPD) and precipitation (Figure 1).

Growth and Phenology

Seedlings were sampled to measure shoot biomass throughout the growing season. At each sampling period, 4-10 seedlings from each population and treatment combination were sampled. Individual seedling shoots were collected, placed into plastic bags and immediately placed into a cooler for transport to the nearby laboratory. In the lab, each shoot sample was rinsed in water to remove excess soil, placed into a coin

envelope and kept in a 60°C oven until dry. Dry weights (g) were then recorded to the nearest 0.0001 g. Due to the planting density and size of the seedlings, complete intact root systems were nearly impossible to collect, thus root system measurements were not taken for biomass processing.

Starting in September 2013, seedlings were visually assessed weekly for the presence of dormant buds until the end of November 2013. The number of dormant buds present per bed-third was recorded and reported as a percentage (%) of the total number of seedlings present in the bed-third. Percentage totals from each bed-third were summed up for each population and treatment combination. Starting in February 2014, the seedlings were again visually assessed weekly for the presence of dormant buds until the end of April 2014.

Anatomy

Seedlings were sampled to measure the diameter and lumen cross-sectional area of individual tracheids in the stem xylem. Stems were collected monthly starting in June until September 2013. In June and July, three seedlings per population per bed were collected. In August and September, three seedlings per population per treatment were collected. Each seedling was placed into a plastic bag with a moist paper towel and then placed into a cooler for transport to the laboratory. Seedlings were kept in a 4°C refrigerator until sample processing (within 2 days).

For each seedling stem, 8-10 thin cross-sections were prepared by hand and stained with toluidine blue (T-blue) before being mounted in glycerin onto glass

microscope slides. T-blue darkens the cell walls making the inner lumen diameter of the tracheids easier to measure. Images of the cross-sections were taken using a built-in 3.0 MP USB camera on a compound microscope (Digital Binocular Compound Microscope, OMAX 40X-2000X, Korea) using a 40x objective. Within a cross-section a single file of tracheids, starting from its origin at the pith, was imaged. Three whole rows of cell files were randomly selected from three separate cross sections per shoot sample. Images were analyzed using the freeware software ImageJ (NIH, USA, <http://rsb.info.nih.gov/ij/>) to measure the cross-sectional area of each tracheid lumen along the file. Cross-sectional areas were converted to diameters (d ; μm) assuming the tracheids were circular, using $d = [2(\text{Area}/\pi)^{1/2}]$, where *Area* is the lumen area (μm^2).

The tracheid lumen mean hydraulic diameter (d_H) for each seedling stem sample was calculated as $d_H = (\Sigma d^4/n)^{1/4}$, where n is the total number of tracheid lumens measured in the sample. The Hagen-Poiseuille equation (Tyree and Ewers 1991) was used to calculate the theoretical xylem area-specific hydraulic conductivity ($k_{S\text{-theo}}$) for each stem sample

$$k_{S\text{-theo}} = \left(\frac{\pi\rho}{128\mu A_w} \right) \Sigma_{i=1}^n (d_i^4) \quad \text{Eqn 1}$$

where d is the lumen diameter of each individual tracheid (i), A_w is the area of the tracheids measured, and ρ and μ are the density and the dynamic viscosity of water at 20°C.

Hydraulics

Root and stem hydraulic measurements were conducted on sunny mornings towards the end of the growing season well after the treatments were implemented. A locally constructed high-pressure flow meter (HPFM), similar to that described by Tyree et al. (1993), was used to measure stem hydraulic conductivity (k_H ; $\text{mmol m s}^{-1} \text{MPa}^{-1}$) as the flow rate divided by the pressure gradient across the stem segment, and root hydraulic conductance (K_R ; $\text{mmol s}^{-1} \text{MPa}^{-1}$) as the flow rate divided by the pressure difference across the root system. For each randomly selected seedling, the aboveground portion was removed using a razor blade, sealed in a plastic bag and stored at 4°C in a refrigerator until a basal stem segment could be excised in the laboratory the following day. The shoots were re-cut under water using a razor blade in order to ensure a precise, flat even surface for measurement. Tygon tubing was placed about 2.5 cm over the segment (stem in lab or protruding root in field) and the end of the tubing was tightened with a nylon zip-tie to prevent leakage. Water flowed from the HPFM, through the tubing, into the stem segment or root system and the rate of flow was recorded every minute until the readings stabilized. This procedure was repeated on 5-6 seedlings per population per treatment. The temperature of the water was recorded for each time interval and hydraulic conductance and conductivity calculations were corrected to 20 °C. Flow rates were determined volumetrically 20-30 minutes after connection when they became steady. Stem segment length, xylem diameter and pith diameter were measured for the calculation of xylem area-specific conductivity (k_S , $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), which is k_H divided by the xylem cross-sectional area. Leaf areas distal to the stem segments and root systems were determined by scanning needles then using the total pixel count in a

calibration regression developed using ImageJ freeware software, and used to calculate stem leaf-specific conductivity (k_L ; $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and root system leaf-specific conductance (K_{R-L} ; $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). The mean ratio of leaf area (A_L) to xylem area (A_X) was calculated for each seedling by dividing A_L (m^2) by A_X (cm^2).

Carbon isotope discrimination

Throughout the growing season, needles from randomly sampled seedlings were collected for stable carbon isotope analysis. Samples were collected once before treatments were imposed and were collected once a month afterwards. At each sampling period, 5-6 seedlings per population and treatment combination were collected.

Aboveground portions of seedlings were placed into plastic bags and into a cooler for transport to the laboratory. In the laboratory, shoot samples were rinsed with water to remove excess soil and placed into coin envelopes for drying in a 60°C oven. Once the dry weight stabilized, the foliage was separated for stable carbon isotope analysis. The dried needle samples were ground to a fine powder with a tissue homogenizer.

Approximately 1 mg of powder was weighed and packed into tin capsules for carbon combustion and analysis.

Carbon isotopic results are represented by delta (δ) notation and are reported as per mil (‰, parts per thousand) expressed in relation to the Vienna Peedee Belemnite (VPDB) standard:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad \text{Eqn 2}$$

$\delta^{13}\text{C}$ analyses were conducted at Oregon State University's College of Earth, Oceanic, Atmospheric Sciences stable isotope laboratory (Corvallis, OR). Samples were combusted to CO_2 in a Carlo Erba NA1500 elemental analyzer then introduced into a DeltaPlusXL isotope ratio mass spectrometer. IAEA-600 caffeine was used as a check standard and runs were calibrated using the international standards USGS40 glutamic acid and ANU sucrose. Typical errors of $\pm 0.1\text{‰}$ or better were determined by repeated measures of internal quality control standards and from sample replicates.

The $\delta^{13}\text{C}$ of whole leaf tissue ($\delta^{13}\text{C}_{\text{leaf}}$) incorporates the $\delta^{13}\text{C}$ of CO_2 in the atmosphere ($\delta^{13}\text{C}_{\text{air}}$), and fractionation against the heavier isotope (^{13}C) is influenced by the ratio of the concentration of CO_2 inside the leaf (C_i) to that in the ambient air (C_a):

$$\delta^{13}\text{C}_{\text{leaf}} = \delta^{13}\text{C}_{\text{air}} - a - (b - a) \frac{C_i}{C_a} \quad \text{Eqn 3}$$

where a is the fractionation effect of diffusion of CO_2 through stomata (4.4‰) and b is the fractionation effect of discrimination against ^{13}C by the enzyme ribulose biphosphate carboxylase-oxygenase (RuBisCO) during CO_2 fixation (27‰) (Farquhar et al. 1989).

Values of $\delta^{13}\text{C}_{\text{leaf}}$ can be converted to discrimination ($\Delta^{13}\text{C}$) to remove the effect of variation of $\delta^{13}\text{C}_{\text{air}}$ on $\delta^{13}\text{C}_{\text{leaf}}$ values over time. However, in this study, both populations of seedlings were planted at the same time and the same location. Therefore, both populations experienced the same $\delta^{13}\text{C}_{\text{air}}$, so differences in $\delta^{13}\text{C}_{\text{leaf}}$ between populations would reflect their rankings in terms of $\Delta^{13}\text{C}$.

Carbon isotope discrimination is directly related to the ratio of intercellular to ambient CO₂ concentration (C_i/C_a) and to the ratio of CO₂ assimilation to stomatal conductance (A/g_s), a measure of intrinsic water-use efficiency (iWUE) whereby decreased $\Delta^{13}\text{C}$ reflects increased iWUE (Farquhar and Richards 1984, Zhang et al. 1993, Pastorino et al. 2012). Therefore, iWUE, $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}$ can all be used to measure and compare physiological performance among different species and between populations within a species from different environments.

Gas Exchange

Gas exchange measurements were made in July and November 2013 to construct photosynthetic CO₂ response ($A-C_i$) curves using a portable open gas exchange system (Li-6400, LiCor, Lincoln, Nebraska, USA) with a 6 cm² chamber aperture and a red-blue light source. Conditions in the chamber were set at: leaf temperature = 22°C, relative humidity (RH) = 70% and photosynthetic photon flux density (PPFD) at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Photosynthesis was initially measured at ambient CO₂ (400 ppm), and then the concentration of CO₂ was gradually decreased stepwise to 50 ppm, brought back to ambient and then was gradually increased stepwise until the curve reached a point where further increases in A appeared to be negligible. At each step, A , stomatal conductance to water vapor (g_s) and C_i were recorded once the readings stabilized. Needles in the gas exchange chamber were collected and their area was computed using the freeware software ImageJ. Measured projected leaf area was used to normalize A .

Estimates of integrated, assimilation-weighted A and g_s were also calculated from foliar $\delta^{13}\text{C}$ values and $A-C_i$ curves. While estimates of the conductance to CO_2 diffusion through the boundary layer and stomata have been confirmed to be valid (Sharkey et al. 1982, Mott and O'Leary 1984), there is evidence that the concentration of CO_2 at the sites of carboxylation in chloroplasts (C_c) is less than C_i and that the internal conductance to CO_2 can pose a limitation to photosynthesis independent of stomatal conductance (Warren 2006). However, Cernusak et al. (2013) reported a strong, species-independent linear relationship between leaf $\delta^{13}\text{C}$ and C_i/C_a over a broad range of C_i/C_a .

Discrimination values ($\Delta^{13}\text{C}$) were first calculated from values of foliar $\delta^{13}\text{C}$ as:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{air} - \delta^{13}\text{C}_{plant}}{1 + \delta^{13}\text{C}_{plant}} \quad \text{Eqn 4}$$

where $\delta^{13}\text{C}_{plant}$ is the $\delta^{13}\text{C}$ of the needle tissue sample. Values of $\Delta^{13}\text{C}$ were used to obtain values for C_i by solving Eqn 5 for C_i at $C_a = 400 \mu\text{mol mol}^{-1}$:

$$C_i/C_a = (\Delta^{13}\text{C} - a)/(b - a) \quad \text{Eqn 5}$$

Values of C_i were then used to estimate A using an asymptotic regression equation fitted to the pooled $A-C_i$ curve data for both populations after it was determined that there were no significant population- or sampling date-related differences in $A-C_i$ curves. Finally, values of A were used to estimate values of g_s with Eqn 6:

$$g_s = 1.6A/(C_a - C_i) \quad \text{Eqn 6}$$

Shoot Water Relations

Toward the end of the growing season, complete shoots, which were ~14 cm tall at this stage, were excised and sealed in plastic bags for transport to the laboratory. In the laboratory, shoots were subjected to a 1.5 to 3h hydration treatment after re-cutting their bases under water. After a rapid determination of initial shoot water potential (Ψ) with a Scholander-type pressure chamber (PMS Instruments, Albany, Oregon, USA) and weight to 0.0001 g, Ψ and weight (g) were recorded periodically while shoots were allowed to dry on the laboratory bench. Sample saturated weights are necessary for determination of relative water content (RWC) or relative water deficit (RWD) for P-V analyses, so saturated weights of rehydrated samples were estimated by extrapolating linear regressions fitted to the pre-turgor loss portions of plots of sample fresh weight versus Ψ to $\Psi =$ zero. The fresh weight corresponding to the initial balance pressure was not included in the regression analysis to eliminate the potential influence of the “plateau effect” on estimates of saturated weights (Dichio et al., 2003; Kubiske and Abrams, 1990; Parker and Pallardy, 1987). Regressions were fitted to the linear portions of plots of $1/\Psi$ versus RWD beginning with the three lowest values of $1/\Psi$ and adding points until the coefficient of determination reached a maximum. The P-V curves and the regressions were then used to estimate the osmotic potential at full turgor ($\Psi\pi_{100}$) from the extrapolation of the linear regression to the $1/\Psi$ axis at $\text{RWD} = 0$ and the water (and osmotic) potential at the turgor loss point (Ψ_{TLP}) from the intersection of the linear portion of the curve with a negative exponential function fitted to the non-linear portion.

Predawn (Ψ_{PD}) and mid-day (Ψ_{MID}) shoot water potentials were measured in June and August 2013. Two healthy seedlings per population per bed (June) and four healthy

seedlings per population per treatment (August) were randomly selected and collected before sunrise and between 1130 and 1300 h and measured at the field site using a Scholander-type pressure chamber.

Statistics

Generalized mixed models were used to fit the relationships between treatment (drought or control) and mean values of the response variables (shoot biomass, percent bud, tracheid diameter, $k_{S\text{-theo}}$, k_S , k_L , K_{Root} , $A_L:A_X$, and $\delta^{13}\text{C}$) within and across *P. ponderosa* populations. Eqn 7 denotes the model used:

$$Y_{ij} = \beta_0 + \beta_1 I.d_{ij} + \beta_2 I.p_{ij} + \beta_3 (I.d \times I.p)_{ij} + a_j + \varepsilon_{ij} \quad \text{Eqn 7}$$

where Y_{ij} is the mean of the response variable from the i^{th} bed and the j^{th} bed-third ($i = 1,2,3,4$; $j = 1,2,3,4,5,6,7,8,9,10,11,12$), β_0 is the mean of the response variable of the $\text{PIPO}_{\text{WEST}}$ seedlings treated with drought, β_1 is the incremental effect of the control treatment on the mean of the response variable, β_2 is the incremental effect of $\text{PIPO}_{\text{EAST}}$ on the mean of the response variable, β_3 is the further incremental effect of both the control treatment and $\text{PIPO}_{\text{EAST}}$ on the mean of the response variable, $I.d$ is 1 when the treatment is the control and 0 otherwise, $I.p$ is 1 when the population source is $\text{PIPO}_{\text{EAST}}$ and 0 otherwise, a_j is the random effect of the j^{th} bed-third on the mean of the response variable and ε_{ij} is the random error term for the i^{th} bed and the j^{th} bed-third. This model assumes an additive relationship between the mean of the response variable and treatment, as well as an additive relationship between the mean of the response variable and population. This model also assumes an interactive relationship between the mean of

the response variable with both treatment and population. Further, this model assumes that both the random effects from bed and bed-third, as well as the random errors are independent and come from a normal population. Lastly, this model assumes that random variation in a treatment group is the same. The assumptions of the model were checked by investigating plots of the residuals.

Model selection for each analysis was conducted by minimizing Akaike's information criterion (AIC). For percent dormant buds, gas exchange and water relations characteristics, there were no statistically significant differences found between treatments within populations, thus these data were pooled and a modified generalized mixed model was used to fit the relationship between populations and mean percent bud, the osmotic potential at full turgor ($\Psi\pi_{100}$), the turgor loss point (Ψ_{TLP}), predawn (Ψ_{PD}) and mid-day (Ψ_{MID}) water potentials. Simple linear regression was used to determine the coefficient of determination (r^2) between the fitted values of $\delta^{13}C$ and mean k_L .

Statistical analyses were conducted in SigmaPlot 12.3 (Systat Software, San Jose, CA) and in R version 3.0.2 (R Core Team 2014) using mixed models ("nlme" package; Pinheiro et al. 2014). We checked residuals of the models for normality and homoscedasticity, and computed false discovery rate (FDR) tests to determine which treatment combinations were significantly different from the others. FDR is less conservative than the Bonferroni-type correction procedure, and results in fewer Type II errors while the amount of Type I errors remains close to the expected values (Verhoeven

et al. 2005, Pike 2011). $\alpha < 0.05$ was considered statistically significant. Error bars represent one standard error (SE).

Results

Prior to the drought treatment, there were no significant differences in mean shoot biomass between the two populations, but after the drought treatment was implemented, there were some statistically significant differences between treatments both within and between populations (Figure 2A). Within $PIPO_{WEST}$, mean shoot biomass in control seedlings was estimated to be 112, 34, 38 and 64% greater than in drought seedlings for the August through November sampling dates ($p=0.001$ 97% CI: 0.31, 1.49 g; $p=0.057$, unadjusted. $p=0.038$ 97% CI: 0.05, 0.93 g; $p=0.042$ 97% CI: 0.10, 1.08 g; $p<0.0001$ 97% CI: 0.66, 1.52 g). Within $PIPO_{EAST}$, there were no statistically significant differences in mean shoot biomass found between the treatments. In the control treatment, mean shoot biomass of $PIPO_{WEST}$ seedlings was estimated to be 60, 38 and 7% greater than in $PIPO_{EAST}$ seedlings on the September through November sampling dates ($p=0.012$ 97% CI: 0.26, 1.15 g; $p=0.034$; 97% CI: 0.14, 1.07 g; $p<0.0001$ 97% CI: 0.80, 1.61 g). In the drought treatment, mean shoot biomass of $PIPO_{WEST}$ seedlings was estimated to be 54, 43 and 10% greater than in $PIPO_{EAST}$ seedlings on the September, November and January sampling dates ($p=0.034$ 97% CI: 0.12, 0.87 g; $p=0.042$ 97% CI: 0.09, 0.97 g; $p=0.205$, unadjusted. $p=0.023$ 97% CI: 0.39, 2.03 g).

During the fall of 2013 and the spring of 2014, statistically significant differences in mean percent of seedlings with dormant buds were found between populations (Figure

2B). For the fall 2013 dates, the mean percentage of seedlings with dormant buds was estimated to be 58, 62 and 53% greater in $PIPO_{EAST}$ than in $PIPO_{WEST}$ on the three sampling dates in October and November ($p < 0.0001$ 95% CI: 51, 63; $p < 0.0001$ 95% CI: 56, 68 %; $p < 0.0001$ 95% CI: 46, 58 %). For the spring 2014 dates the mean percentage of seedlings with dormant buds was estimated to be 50 and 29% greater in $PIPO_{EAST}$ than in $PIPO_{WEST}$ on the March sampling dates ($p < 0.0001$ 96% CI: 42, 57 %; $p < 0.0001$ 96% CI: 22, 37 %).

There were no statistically significant differences in mean tracheid lumen diameter found between populations during the month of June, but mean tracheid lumen diameter was estimated to be 20% greater in $PIPO_{WEST}$ than in $PIPO_{EAST}$ in July ($p < 0.001$ 95% CI: 1.1, 2.8 μm) (Figure 3A). Within $PIPO_{WEST}$, mean tracheid lumen diameter was estimated to be 6 and 13% greater in in control seedlings compared to droughted seedlings in August and September ($p = 0.066$, unadjusted. $p = 0.049$ 97.5% CI: 0.0, 1.6 μm ; $p = 0.027$ 96% CI: 0.3, 2.6 μm). Within $PIPO_{EAST}$, there were no statistically significant treatment-related differences in mean tracheid lumen diameter. In the control treatment, mean tracheid lumen diameter was estimated to be 22 and 30% greater in $PIPO_{WEST}$ seedlings compared to $PIPO_{EAST}$ seedlings in August and September ($p = 0.002$ 97.5% CI: 1.5, 3.1 μm ; $p = 0.003$ 96% CI: 2.4, 3.6 μm). For droughted seedlings, mean tracheid lumen diameter was estimated to be 14 and 10% greater in $PIPO_{WEST}$ seedlings compared to $PIPO_{EAST}$ seedlings in August and September ($p < 0.0001$ 97.5% CI: 1.5, 3.1 μm ; $p < 0.0001$ 96% CI: 2.4, 3.6 μm). There were no statistically significant differences in mean k_{S-theo} between the two populations during June and July (Figure 3B). Within

PIPO_{WEST}, mean $k_{S\text{-theo}}$ was estimated to be 38 and 47% higher in control seedlings than in droughted seedlings in August and September ($p=0.011$ 96.25% CI: 16,809, 79,663 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$; $p=0.008$ 96.25% CI: 20,384, 83,239 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$). There were no statistically significant treatment-related differences found in mean $k_{S\text{-theo}}$ in PIPO_{EAST} seedlings. In the control treatment, mean $k_{S\text{-theo}}$ was estimated to be 89 and 100% higher in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings in August and September ($p<0.001$ 96.25% CI: 51,066, 112,521 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$; $p<0.001$ 96.25% CI: 49,722, 111,176 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$). In the drought treatment, mean $k_{S\text{-theo}}$ was estimated to be 53 and 31% higher in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings in August and September ($p<0.001$ 96.25% CI: 24,110, 63,841 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$; $p=0.03$; 96.25% CI: 5,958, 46,663 $\text{mmol m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$).

In July, mean hydraulic diameter (d_H) was estimated to be 18 % greater in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings ($p=0.027$; 97% CI: 0.4, 3.7 μm) (Table 2). Within PIPO_{WEST}, mean d_H was estimated to be 7% greater in August ($p=0.065$, unadjusted. $p=0.049$; 97% CI: 0.0, 1.6 μm) and 14% greater in September ($p<0.001$; 97% CI: 0.9, 2.4 μm) in control seedlings compared to droughted seedlings. There were no statistically significant treatment-related differences found within PIPO_{EAST}. For droughted seedlings, mean d_H was estimated to be 13% greater in August ($p=0.002$; 97% CI: 0.6, 2.1 μm) and 10% greater in September ($p=0.013$; 97% CI: 0.3, 1.9 μm) in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings. For control seedlings, mean d_H was estimated to be 21% greater in August ($p<0.001$; 97% CI: 1.4, 3.0 μm) and 31% greater in September ($p<0.001$; 97% CI: 2.4, 4.0 μm) in PIPO_{WEST} seedlings than in PIPO_{EAST}

seedlings. There were no statistically significant differences found in mean xylem area between populations in June, July or August, nor were there any treatment-related differences within PIPO_{WEST} in August or within PIPO_{EAST} in August and September (Table 2). Within PIPO_{WEST}, control seedlings were estimated to have 79% more xylem area in September than droughted seedlings ($p < 0.001$, 98.75% CI: 0.005, 0.01 mm²). For control seedlings, mean xylem area was estimated to be twice as large in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings in September ($p < 0.001$; 98.75% CI: 0.007, 0.01 mm²).

There were no statistically significant treatment-related differences found in mean xylem area-specific hydraulic conductivity (k_S) within either population (Figure 4A). In the drought treatment, mean k_S in PIPO_{WEST} seedlings was estimated to be 72% greater than in PIPO_{EAST} seedlings ($p = 0.052$, unadjusted.p = 0.031; 99% CI: -8, 197 mmol m⁻¹ s⁻¹ MPa⁻¹). In the control treatment, mean k_S was estimated to be 76% greater in PIPO_{WEST} seedlings than in PIPO_{EAST} seedlings ($p = 0.052$, unadjusted.p = 0.028; 99% CI: -7, 221 mmol m⁻¹ s⁻¹ MPa⁻¹). There were no statistically significant treatment- or population-related differences in mean leaf-specific conductivity (k_L) (Figure 4B). For mean leaf-specific root conductance (K_{R-L}), there were no statistically significant treatment-related differences found within either population (Figure 4C). In the control treatment, mean K_{R-L} was estimated to be over two times greater in PIPO_{EAST} seedlings than in PIPO_{WEST} seedlings ($p = 0.264$, unadjusted.p = 0.027; 99% CI: 0, 15 mmol m⁻² s⁻¹ MPa⁻¹). There were no statistically significant treatment-related differences found in mean ratio of leaf area (A_L) to xylem area (A_X) within either population (Figure 4D). For control seedlings, mean

$A_L:A_X$ in $PIPO_{WEST}$ was estimated to be 33% greater than in $PIPO_{EAST}$ ($p=0.005$; 98% CI: 0.02, 0.06 m^2/cm^2).

Over the course of the growing season, $PIPO_{EAST}$ seedlings maintained less negative $\delta^{13}C$ values compared to $PIPO_{WEST}$ seedlings (Figure 5). For June and July prior to treatment implementation, mean $\delta^{13}C$ values were estimated to be =1.03 and -1.64 ‰ less negative in $PIPO_{EAST}$ seedlings than in $PIPO_{WEST}$ seedlings ($p=0.019$ 95% CI: -1.66, -0.40 ‰; $p<0.001$ 95% CI: -2.10, -1.17 ‰). For September and November, there were no statistically significant treatment-related differences in mean $\delta^{13}C$ within either population. In September, mean $\delta^{13}C$ values were estimated to be -1.31 ‰ less negative ($p=0.015$; 98% CI: -2.25, -0.37 ‰) in droughted $PIPO_{EAST}$ seedlings than in droughted $PIPO_{WEST}$ seedlings, and -0.72 ‰ less negative ($p=0.053$, unadjusted, $p=0.027$; 98% CI: -1.38, -0.06 ‰) in control $PIPO_{EAST}$ seedlings than in control $PIPO_{WEST}$ seedlings. In November, mean $\delta^{13}C$ was estimated to be -1.93 ‰ less negative ($p=0.003$; 98% CI: -2.99, -0.87 ‰) in droughted $PIPO_{EAST}$ seedlings than in droughted $PIPO_{WEST}$ seedlings. When foliar $\delta^{13}C$ values from July and September, 2013, were pooled and plotted against leaf-specific conductivity (k_L) measured in September and October, 2013, a strong linear relationship emerged across the treatments and populations (Linear regression; $p<0.001$, $r^2=0.95$) (Figure 6). The negative relationship between $\delta^{13}C$ and k_L indicated that iWUE increased with increasing treatment- and population-related hydraulic constraints on photosynthetic gas exchange. Within each population, $\delta^{13}C$ values were less negative in droughted seedlings compared to control seedlings. *P. ponderosa* seedlings that received the drought treatment exhibited greater iWUE compared to their control counterparts.

Further, seedlings from PIPO_{EAST} exhibited greater iWUE compared to seedlings from PIPO_{WEST} based on the recorded $\delta^{13}\text{C}$ values from each population.

There were no statistically significant differences between photosynthetic CO_2 response ($A-C_i$) curves across sampling dates and treatments within populations. The resulting $A-C_i$ curves showed no significant difference between PIPO_{EAST} and PIPO_{WEST} (Figure 7). When mean growing season assimilation (A) and stomatal conductance (g_s) were estimated from the mean $\delta^{13}\text{C}$ values and the $A-C_i$ curve function, A was about 7% greater and g_s about 38% greater in PIPO_{WEST} (Figure 7). Statistical comparisons of estimates of A and g_s were not made owing to the approach employed for obtaining them (see methods).

During the growing season, there were no significant differences in either predawn (Ψ_{pd}) or midday (Ψ_{md}) water potentials between the two populations (Table 3). The lack of a significant drought effect was due to the unforeseen rain events during the later portion of the growing season (Figure 1). At the end of the growing season, PIPO_{EAST} seedlings had significantly more negative osmotic potential at full turgor ($\Psi_{\pi_{100}}$) and water potential at the turgor loss point (Ψ_{TLP}) values compared to PIPO_{WEST} seedlings ($p \leq 0.01$) (Table 3).

Discussion

We found large differences in the expression of key functional traits of *Pinus ponderosa* seedlings from the two populations used in this common garden study. In

agreement with our hypotheses, we observed population-specific differences in water-transport and photosynthetic physiology, and reduced water availability resulted in some population-specific differences in the expression of physiological traits. The seedling population from the site with the drier, more continental climate (PIPO_{EAST}) had higher iWUE as a result of lower g_s and greater relative stomatal limitation of A . Because the dependence of A on C_i did not differ among populations, higher iWUE in PIPO_{EAST} implied a substantial savings in transpiration at the cost of a relatively small reduction in A (Fig. 7). Although k_L did not differ significantly between populations, there was a strong negative relationship between $\delta^{13}C$ and k_L across populations indicative of a close coordination between photosynthetic gas exchange and hydraulic architecture with PIPO_{EAST} tending to be more constrained with respect to gas exchange and hydraulics (Fig. 6). More negative values of $\Psi_{\pi_{100}}$ and Ψ_{TLP} in PIPO_{EAST} imply a greater ability to maintain turgor under a drought-induced decline in plant water status. Finally, PIPO_{EAST} seedlings grew more slowly regardless of water availability, and showed earlier onset of dormant bud formation. The overall more conservative functional behavior of PIPO_{EAST} seedlings seems consistent with this population's climate of origin where plants must contend with a shorter growing season due to the earlier onset and longer duration of colder temperatures as well as about a third of the precipitation that PIPO_{WEST} seedlings typically receive. The geographical distance and isolation between these two populations imply that the transfer of genetic material between them is highly unlikely, and therefore, the findings reported here could be indicative of these populations representing distinct ecotypes within *P. ponderosa*. Common garden trials of *P. ponderosa* provenances have

been conducted since 1910 and extensive research has shown there to be five genetically distinct subspecies across its distribution range, with the North Plateau and Pacific varieties present in Oregon (Larson 1967, Wang 1977, Conkle and Critchfield 1988, Sorenson et al. 2001, Callaham 2013). The distributions of these subspecies in Oregon suggest that PIPO_{EAST} may reflect seedlings originating from the North Plateau subspecies, and PIPO_{WEST} may reflect seedlings originating from the Pacific subspecies (Conkle and Critchfield 1988, Sorenson 2001 et al., Callaham 2013), thus supporting the idea that the populations used in this study were genetically distinct.

Limiting the amount of available water reduced shoot biomass growth in all seedlings, which has been well documented in many studies (Heiner et al. 1972, Gordon et al. 1991, Padilla et al. 2007, Schall et al. 2012). There are three potential non-mutually exclusive causes for the lower shoot biomass production in PIPO_{EAST} seedlings. First, greater allocation of photosynthates to the root system would have reduced shoot biomass accumulation. Increased allocation to root biomass, or an increase in the root to shoot ratio, has been purported to be a response to drought for many plant species (Turner 2001, Greenwood et al. 2008, Schall et al. 2012). However, technical constraints in this study prevented us from quantifying root biomass production and root to shoot ratios in these seedlings. Second, greater stomatal constraints on photosynthesis would have limited the amount of photosynthates available for shoot biomass accumulation. However, greater stomatal constraints on photosynthesis in PIPO_{EAST} do not seem sufficient to account for its lower growth rate as integrated estimates of A were only 7% higher in PIPO_{WEST} (Figure 7). Third, an increased demand for energy and metabolites

for osmotic adjustment would have decreased the amount of photosynthates available for shoot biomass accumulation. Significantly more negative $\Psi_{\pi 100}$ and Ψ_{TLP} in $PIPO_{EAST}$ (Table 3) were consistent with its lower growth rate and suggest the presence of osmotic adjustment in this population. Osmotic adjustment, which consists of the accumulation of solutes in response to water stress, promotes turgor maintenance and plant growth during periods of low water availability (Hsiao et al. 1976, Morgan 1984). However, this process is dependent on the import and maintenance of solutes, including sugars, into expanding and developed leaves which is, in turn, dependent on photosynthesis (Morgan 1984). While osmotic adjustment can maintain plant function and growth during periods of water stress, the energy required in this process could result in a reduction in the amount of available resources, thus reducing biomass production (Vivin et al. 1996).

While $PIPO_{WEST}$ seedlings continued growing through October and November of 2013, $PIPO_{EAST}$ seedlings stopped growth in September (Figure 2A), as reflected in their rates of dormant bud setting (Figure 2B). The more conservative phenological behavior of $PIPO_{EAST}$ seedlings would increase their chances of surviving severe early season frosts of the climate of origin, albeit this requires a trade-off of earlier bud set against growth. The vegetative phenology of the $PIPO_{WEST}$ seedlings, as seen in this study, supports the idea that this population is better adapted to the milder thermal climate of the Willamette Valley where the risk of early autumn killing frosts is relatively low. Nevertheless, chilling temperatures during the winter appeared to induce progressive formation of dormant buds in $PIPO_{WEST}$ as spring approached (Figure 2B).

All seedlings exhibited ontogenetic changes in xylem anatomy that appeared to be associated with environmental seasonality and intrinsic cambial phenology. The initial decline in tracheid lumen diameter from June to July 2013 was likely associated with the onset of warmer temperatures and an increase in the vapor pressure deficit (VPD) (Figure 1). A VPD-driven increase in transpiration may have reduced seedling water potential and turgor and therefore the rate of expansion of developing tracheids in both populations, resulting in smaller tracheid diameters at maturity (Figure 3A). An identical mechanism was likely responsible for smaller tracheid diameters in droughted seedlings. This reduction in tracheid diameter in droughted seedlings could have influenced the hydraulic conductivity of those conduits as well as the conduits' resistance to embolism. Although resistance to embolism was not directly tested for, other studies have shown that a decline in tracheid lumen diameter and an increase in cell wall thickness in conifer xylem generally results in an increase in resistance to embolism (Hacke et al. 2001, Domec et al. 2006, Pittermann et al. 2006). These studies indicated that hydraulic safety versus efficiency trade-offs were likely involved where greater mechanical reinforcement in tracheid structure occurred at the expense of reduced hydraulic efficiency (Pittermann et al. 2006, Meinzer et al. 2010), a response which has been documented in several studies (Kavanagh et al. 1999, Santiago et al. 2004, Nardini et al. 2012). The decline in tracheid diameter observed in these seedlings in response to low water availability could have constrained hydraulic efficiency, which ultimately would have reduced gas exchange and growth. Interestingly, the leaf-specific root conductance (K_{R-L}) values were higher in control PIPO_{EAST} seedlings (Figure 4C), which could mean these seedlings

allocated more resources toward larger root systems in the first year of growth thereby improving the hydraulic conductance of the root system. However, root systems are generally intrinsically more vulnerable to drought-induced embolism than shoot systems (Kavanagh et al. 1999, Jackson et al. 2000), thus root hydraulic conductance in these *P. ponderosa* seedlings could be more sensitive to drought events. Domec et al. (2004) investigated seasonal root embolism in 15-year-old *P. ponderosa* trees and noted that as soil water became depleted, percent loss of conductivity (PLC) due to embolism in the roots increased. Further, root embolism correlated with stomatal closure, suggesting that the lower resistance of roots to embolism may be important for prevention of xylem failure and maintenance of plant water status during periods of low water availability.

Calculations of theoretical xylem-area specific conductivity ($k_{S\text{-theo}}$) showed a general decline in $k_{S\text{-theo}}$ over the growing season for both seedling populations even on dates when tracheid lumen diameters in some seedlings had widened again from their smallest values (Figure 3A, 3B). Given the fourth-power relationship between $k_{S\text{-theo}}$ and the hydraulic diameter (d_H) (Eqn 1; Tyree and Ewers 1991), wider tracheid lumen diameters should result in higher values of $k_{S\text{-theo}}$. One explanation for the trend in $k_{S\text{-theo}}$ that we observed could be the declining ratio of d_H to xylem area during the summer of 2013 (Table 2). With the exception of control PIPO_{WEST} seedlings, d_H values either decreased or remained stable while the xylem areas increased at each sampling period.

Consistently less negative foliar $\delta^{13}\text{C}$ values and therefore higher iWUE in PIPO_{EAST} resulted in a trade-off, although relatively small, of reduced A for reduced g_s ,

and transpiration, which is likely advantageous in terms of establishment and survival in its climate of origin (Chapin et al. 1987). Pronounced seasonal fluctuations in $\delta^{13}\text{C}$ in both populations (Figure 5) appeared to be related to seasonal trends in environmental variables. During the summer, longer days with higher VPD most likely lowered g_s , resulting in reduced discrimination and less negative $\delta^{13}\text{C}$ values in September. During the fall, shorter days with lower VPD most likely allowed for an increase in g_s , leading to increased discrimination and more negative $\delta^{13}\text{C}$ values in November. In order for changes in stomatal regulation of gas exchange of mature leaves to be reflected in their bulk leaf $\delta^{13}\text{C}$ values, there must have been a significant component of the leaf that turned over and was replaced frequently, such as non-structural carbohydrates (NSCs) (Farrar 1989, Chapin et al. 1990, Damesin et al. 1998). Seasonal, as well as inter-annual, trends in foliar $\delta^{13}\text{C}$ values have been documented (Damesin et al. 1998, Scartazza et al. 2013). Similar to the results reported here, Scartazza et al. (2013) analyzed seasonal and inter-annual variability in $\delta^{13}\text{C}$ in a beech forest exposed to varying summer water availability and observed that $\delta^{13}\text{C}$ in leaves increased seasonally following a decrease in soil water content. Previous studies have found that discrimination values are correlated with climate, particularly with mean annual precipitation (MAP) (Bowling et al. 2002, Diefendorf et al. 2010). Therefore, populations within a species from drier climates would be expected to exhibit lower discrimination given that the populations represent genetically distinct ecotypes.

The impacts of hydraulic constraints on photosynthetic gas exchange were clear when comparing the $\delta^{13}\text{C}$ values with the k_L values for both populations. Whereas

PIPO_{EAST} may have been more hydraulically constrained overall, the negative relationship between foliar $\delta^{13}\text{C}$ and k_L indicated that iWUE increased with decreasing k_L in both treatment and population comparisons (Figure 6). Few studies have reported on the link between plant hydraulic properties and $\delta^{13}\text{C}$ values (Panek 1996, Cernusak and Marshall 2001), but many have reported on the relationship between g_s and hydraulics in tree species (Meinzer and Grantz 1990, Sperry and Pockman 1993, Saliendra et al. 1995, Hubbard et al. 2001). In 4-5-year-old *P. ponderosa* seedlings, g_s decreased linearly with decreasing leaf-specific hydraulic conductance (Hubbard et al. 2001), while foliar $\delta^{13}\text{C}$ values became less negative with decreasing branch leaf-specific conductivity in 30-year-old Douglas-fir (*Pseudotsuga menziesii*) trees (Panek 1996). Climatic factors that reduce water movement through a plant subsequently limit carbon uptake, thereby highlighting the ecological significance of water-use efficiency. Higher iWUE in PIPO_{EAST} appears to be an important functional trait that contributes to seedling establishment in its native geographical range.

The absence of treatment- and population-related differences in predawn and midday plant Ψ prior to the heavy rain events in September (Table 3) most likely reflects the rapid growth of deep roots in *P. ponderosa*. Roots in both populations had reached a depth of up to 56 cm by the end of the growing season (personal observation). Nevertheless, both $\Psi_{\pi 100}$ and Ψ_{TLP} were inherently lower in PIPO_{EAST} seedlings (Table 3) implying a greater capacity for turgor maintenance than PIPO_{WEST} seedlings during periods of prolonged drought. Similar variation in needle osmotic properties and osmotic

adjustment related to the dryness of the climate of origin was observed among five provenances of 3-year-old maritime pine (*P. pinaster*) (Nguyen-Queyrens and Bouchet-Lannat 2003). Vance and Zaerr (1991) determined $\Psi_{\pi 100}$ for shaded and unshaded 5-month-old *P. ponderosa* seedlings and found that shaded seedlings had higher $\Psi_{\pi 100}$ and lower starch content, suggesting a reduction in the availability of metabolites required for osmotic adjustment. This finding supports our suggestion that the metabolic requirements involved in osmotic adjustment could have limited the amount of available substrates in PIPO_{EAST} seedlings that would have otherwise been used for growth.

In summary, the more conservative behavior of seedlings from the dry site population suggests growth is reduced because of the need for increased resistance to drought. Since both of these populations were grown in a common garden under similar environmental conditions, the seemingly conservative strategies seen in PIPO_{EAST} seedlings could be due to genetic variation within *Pinus ponderosa* and these geographically distant populations could represent different ecotypes. Future studies should include additional geographically distant populations of *P. ponderosa* in order to compare differences in the functional traits of seedlings across the entire range of this species and for understanding the potential adaptability of individual populations to future climate change.

Acknowledgements

Thanks to the USDA Forest Service, PNW Research Station for providing facilities. Thanks to Chris Poklemba, Jennifer McKay, Dan Ott, Joshua Petitmermet,

Carla Aponte Lopez, Jessica Kessinger, Alicia Magedman, John Crandall and Kristen Falk for their assistance with field and lab work. This project was funded by the National Science Foundation (IOS-1146751).

References

- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl* 1:95–111.
- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684.
- Baltzer JL, Davies SJ (2012) Rainfall seasonality and pest pressure as determinants of tropical tree species' distributions. *Ecol Evol* 2:2682–2694.
- Barnard DM, Meinzer FC, Lachenbruch B, McCulloh KA, Johnson DM, Woodruff DR (2011) Climate-related trends in sapwood biophysical properties in two conifers: avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. *Plant Cell Environ* 34:643–654.
- Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol Lett* 15:393–405.
- Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M (2004) Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. *Tree Physiol* 24:891–899.
- Del Cacho M, Estiarte M, Penuelas J, Lloret F (2013) Inter-annual variability of seed rain and seedling establishment of two woody Mediterranean species under field-induced drought and warming. *Popul Ecol* 55:277–289.
- Callaham RZ (2013) *Pinus ponderosa*: A Taxonomic Review With Five Subspecies in the United States. Res Pap PSW-RP-264. US Dep Agric For Serv Alba CA. 52 p.

- Cernusak LA, Marshall JD (2001) Responses of foliar $\delta^{13}\text{C}$, gas exchange and leaf morphology to reduced hydraulic conductivity in *Pinus monticola* branches. *Tree Physiol* 21:1215–1222.
- Chapin III FSC, Bloom AJ, Field CB, Waring RH (1987) Plant Responses to Multiple Environmental Factors. *BioScience* 37:49–57.
- Chapin III FSC, Schulze E-D, Mooney HA (1990) The Ecology and Economics of Storage in Plants. *Annu Rev Ecol Syst* 21:423–447.
- Chmura DJ, Anderson PD, Howe GT, Harrington CA, Halofsky JE, Peterson DL, Shaw DC, Brad St Clair J (2011) Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *For Ecol Manag* 261:1121–1142.
- Christensen-Dalsgaard KK, Ennos AR (2012) Effects of drought acclimation on the mechanical properties of *Ochroma pyramidale*, *Betula pendula* and *Acacia karroo* tree seedling stems. *Forestry* 85:215–223.
- Comita LS, Engelbrecht BM (2009) Seasonal and spatial variation in water availability drive habitat associations in a tropical forest. *Ecology* 90:2755–2765.
- Conkle MT, Critchfield WB (1988) Genetic variation and hybridization of ponderosa pine. In: Baumgartner DM, Loten JE (eds) *Proceedings, Symposium on ponderosa pine - the species and its management*. Spok WA, pp 27-43.
- Creese C, Benschoter AM, Maherali H (2011) Xylem function and climate adaptation in *Pinus*. *Am J Bot* 98:1437–1445.
- Cregg BM (1994) Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. *Tree Physiol* 14:883–898.
- Cui M, Smith WK (1991) Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol* 8:37–46.
- Damesin C, Rambal S, Joffre R (1998) Seasonal and annual changes in leaf $\delta^{13}\text{C}$ in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. *Funct Ecol* 12:778–785.
- Delucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. *Glob Change Biol* 6:587–593.
- Dichio B, Xiloyannis C, Angelopoulos K, Nuzzo V, Bufo SA, Celano G (2003) Drought-induced variations of water relations parameters in *Olea europaea*. *Plant Soil* 257:381–389.

- Domec JC, Warren JM, Meinzer FC, Brooks JR, Coulombe R (2004) Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. *Oecologia* 141:7-16.
- Domec JC, Lachenbruch B, Meinzer FC (2006) Bordered pit structure and function determine spatial patterns of air-seedling thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinacea) trees. *Am J Botany* 93(11):1588-1600.
- Drake PL, Froend RH, Franks PJ (2011) Linking hydraulic conductivity and photosynthesis to water-source partitioning in trees versus seedlings. *Tree Physiol* 31:763-773.
- Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447:80-82.
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Biol* 40:503-537.
- Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct Plant Biol* 11:539-552.
- Farrar JF (1989) The carbon balance of fast-growing and slow-growing species. *Causes Consequence Var Growth Rate Product High Plants* 1:241-245.
- Germino MJ, Smith WK (1999) Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell Environ* 22:407-415.
- Gordon DR, Rice KJ, Welker JM (1991) Soil water effects on blue oak seedling establishment. *Gen. Tech. Rep. PSW-126*. USDA For Serv. 5 p.
- Graham RT, Jain TB (2005) Ponderosa pine ecosystems. In: *Proceedings of the Symposium on Ponderosa Pine: Issues, Trends, and Management*, Gen. Tech. Rep. PSW-GTR-198. Albany CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture, Klamath Falls, OR. Citeseer, pp 1-32.
- Greenwood MS, O'brien CL, Schatz JD, Diggins CA, Day ME, Jacobson GL, White AS, Wagner RG (2008) Is early life cycle success a determinant of the abundance of red spruce and balsam fir? *Can J For Res* 38:2295-2305.
- Grossnickle SC (2012) Why seedlings survive: influence of plant attributes. *New For* 43:711-738.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457-461.

- Heiner TC, Lavender DP (1972) Early growth and drought avoidance in Douglas-fir seedlings. Res. Pap. 14. Corvallis, Or.: Forest Research Laboratory, School of Forestry, Oregon State University. 9 p.
- Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. *Nature* 424:901–908.
- Hsiao TC, Acevedo E, Fereres E, Henderson DW (1976) Water Stress, Growth, and Osmotic Adjustment. *Philos Trans R Soc Lond B Biol Sci* 273:479–500.
- Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiol* 19:165–172.
- Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113–121.
- Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol Evol* 18:147–155.
- ImageJ. <http://imagej.nih.gov/ij/> (9 October 2014, date last accessed).
- Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport: using physiological processes in global predictions. *Trends Plant Sci* 5:482–488.
- Johnson DM, McCulloh KA, Reinhardt K (2011) The Earliest Stages of Tree Growth: Development, Physiology and Impacts of Microclimate. In: Meinzer FCC, Lachenbruch B, Dawson TE, (eds) *Size- and Age-Related Changes in Tree Structure and Function*. Springer Netherlands, pp 65–87.
- Johnson DM, Smith WK (2005) Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. *Tree Physiol* 25:1379–1387.
- Kavanagh KL, Bond BJ, Aitken SN, Gartner BL, Knowe S (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiol* 19:31–37.
- Kubiske ME, Abrams MD (1990) Pressure-volume relationships in non-rehydrated tissue at various water deficits. *Plant Cell Environ* 13:995–1000.
- Larson MM (1967) Effect of temperature on initial development of ponderosa pine seedlings from three sources. *Forest Science* 13(3):286-294.

Lewis JD, Lucash M, Olszyk D, Tingey DT (2001) Seasonal patterns of photosynthesis in Douglas fir seedlings during the third and fourth year of exposure to elevated CO₂ and temperature. *Plant Cell Environ* 24:539–548.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annu Rev Ecol Syst*:237–277.

Little EL (1971) Atlas of United States trees. Volume 1. Conifers and important hardwoods. Miscellaneous publication 1146. US Dep Agric For Serv Wash DC.

Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85:2184–2199.

Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence. *Funct Ecol* 16:510–521.

Matías L, Zamora R, Castro J (2012) Sporadic rainy events are more critical than increasing of drought intensity for woody species recruitment in a Mediterranean community. *Oecologia* 169:833–844.

McLean EH, Prober SM, Stock WD, Steane DA, Potts BM, Vaillancourt RE, Byrne M (2014) Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant Cell Environ* 37:1440–1451.

Meinzer FC, McCulloh KA, Lachenbruch B, Woodruff DR, Johnson DM (2010) The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency. *Oecologia* 164:287–296.

Meinzer FC, Woodruff DR, Marias DE, McCulloh KA, Sevanto S (2014) Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species. *Plant Cell Environ*. 37:2577-2586..

Mencuccini M, Grace J (1995) Climate influences the leaf area/sapwood area ratio in Scots pine. *Tree Physiol* 15:1–10.

Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annu Rev Plant Physiol* 35:299–319.

Moyes AB, Castanha C, Germino MJ, Kueppers LM (2013) Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia* 171:271–282.

Nardini A, Pedà G, Rocca NL (2012) Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytol* 196:788–798.

- Nguyen-Queyrens A, Bouchet-Lannat F (2003) Osmotic adjustment in three-year-old seedlings of five provenances of maritime pine (*Pinus pinaster*) in response to drought. *Tree Physiol* 23:397–404.
- Padilla FM, de Dios Miranda J, Pugnaire FI (2007) Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant Soil* 296:103–113.
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495.
- Panek JA (1996) Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. *Tree Physiol* 16:747–755.
- Parker, W.C., and Pallardy, S.G. (1987). The Influence of Resaturation Method and Tissue 3Quercus alba L. Seedlings. *J. Exp. Bot.* 38, 535–549.
- Pastorino MJ, Aparicio AG, Marchelli P, Gallo LA (2012) Genetic variation in seedling water-use efficiency of Patagonian Cypress populations from contrasting precipitation regimes assessed through carbon isotope discrimination. *For Syst* 21:189–198.
- Pike N (2011) Using false discovery rates for multiple comparisons in ecology and evolution. *Methods Ecol Evol* 2:278–282.
- Pineda-García F, Paz H, Tinoco-Ojanguren C (2011) Morphological and physiological differentiation of seedlings between dry and wet habitats in a tropical dry forest. *Plant Cell Environ* 34:1536–1547.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2014) *_nlme: Linear and Nonlinear Mixed Effects Models_*. <http://CRAN.R-project.org/package=nlme>
- Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH (2006) Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant Cell Environ* 29:1618–1628.
- Poorter L, Markesteijn L (2008) Seedling traits determine drought tolerance of tropical tree species. *Biotropica* 40:321–331.
- R Core Team (2014) *R: A Language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rehfeldt GE (1990) Genetic Differentiation among Populations of *Pinus ponderosa* from the Upper Colorado River Basin. *Bot Gaz* 151:125–137.

- Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff D, Jones T (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550.
- Scartazza A, Moscatello S, Matteucci G, Battistelli A, Brugnoli E (2013) Seasonal and inter-annual dynamics of growth, non-structural carbohydrates and C stable isotopes in a Mediterranean beech forest. *Tree Physiol* 33:730–742.
- Schall P, Lödige C, Beck M, Ammer C (2012) Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. *For Ecol Manag* 266:246–253.
- Shevtsova A, Graae BJ, Jochum T, Milbau A, Kockelbergh F, Beyens L, Nijs I (2009) Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Glob Change Biol* 15:2662–2680.
- Smit J, Van Den Driessche R (1992) Root growth and water use efficiency of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and lodgepole pine (*Pinus contorta* Dougl.) seedlings. *Tree Physiol* 11:401–410.
- Sorenson FC, Mandel NL, Aagaard JE (2001) Role of selection versus historical isolation in racial differentiation of ponderosa pine in southern Oregon: an investigation of alternative hypotheses. *Can J For Res* 31:1127–11239.
- Sperry JS (2000) Hydraulic constraints on plant gas exchange. *Agric For Meteorol* 104:13–23.
- Station Data | Corvallis Farm Unit | Oregon State University.
<http://agsci.oregonstate.edu/farmunit/weather> (9 October 2014, date last accessed).
- Stephenson NL (1990) Climatic control of vegetation distribution: the role of the water balance. *Am Nat*:649–670.
- Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex B, Midgley BM (2013) IPCC, 2013: climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 1535 p.
- Turesson G (1922) The species and the variety as ecological units. *Hereditas* 3:100–113.
- Turner IM (2001) The ecology of trees in the tropical rain forest. Cambridge University Press, Cambridge, United Kingdom.
- Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment: a review and evaluation. *Adapt Plants Water High Temp Stress* 1:87–103.

- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360.
- Tyree MT, Sinclair B, Lu P, Granier A (1993) Whole shoot hydraulic resistance in *Quercus* species measured with a new high-pressure flowmeter. In: *Annales des sciences forestières*. EDP Sciences, pp 417–423.
- Vance NC, Zaerr JB (1991) Influence of drought stress and low irradiance on plant water relations and structural constituents in needles of *Pinus ponderosa* seedlings. *Tree Physiol* 8:175–184.
- Verhoeven KJ, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. *Oikos* 108:643–647.
- Vivin P, Guehl JM, Clément A, Aussenac G (1996) The effects of elevated CO₂ and water stress on whole plant CO₂ exchange, carbon allocation and osmoregulation in oak seedlings. In: *Annales des sciences forestières*. EDP Sciences, pp 447–459.
- Walck JL, Hidayati SN, Dixon KW, Thompson KEN, Poschlod P (2011) Climate change and plant regeneration from seed. *Glob Change Biol* 17:2145–2161.
- Wang C (1977) Genetics of ponderosa pine. Res.Pap. WO-34. US Dep Agric For Serv Wash DC. 24 p.
- Western Regional Climate Center. <http://www.wrcc.dri.edu/> (9 October 2014, date last accessed).
- Williams AP, Allen CD, Macalady AK, Griffin D, Woodhouse CA, Meko DM, Swetnam TW, Rauscher SA, Seager R, Grissino-Mayer HD (2013) Temperature as a potent driver of regional forest drought stress and tree mortality. *Nat Clim Change* 3:292–297.
- Zhang J, Cregg BM (2005) Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. *For Ecol Manag* 219:1–12.
- Zhang JW, Feng Z, Cregg BM, Schumann CM (1997) Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. *Tree Physiol* 17:461–466.
- Zhang J, Marshall JD (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Can J For Res* 24:92–99.
- Zhang J, Marshall JD, Jaquish BC (1993) Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. *Oecologia* 93:80–87.

Figures

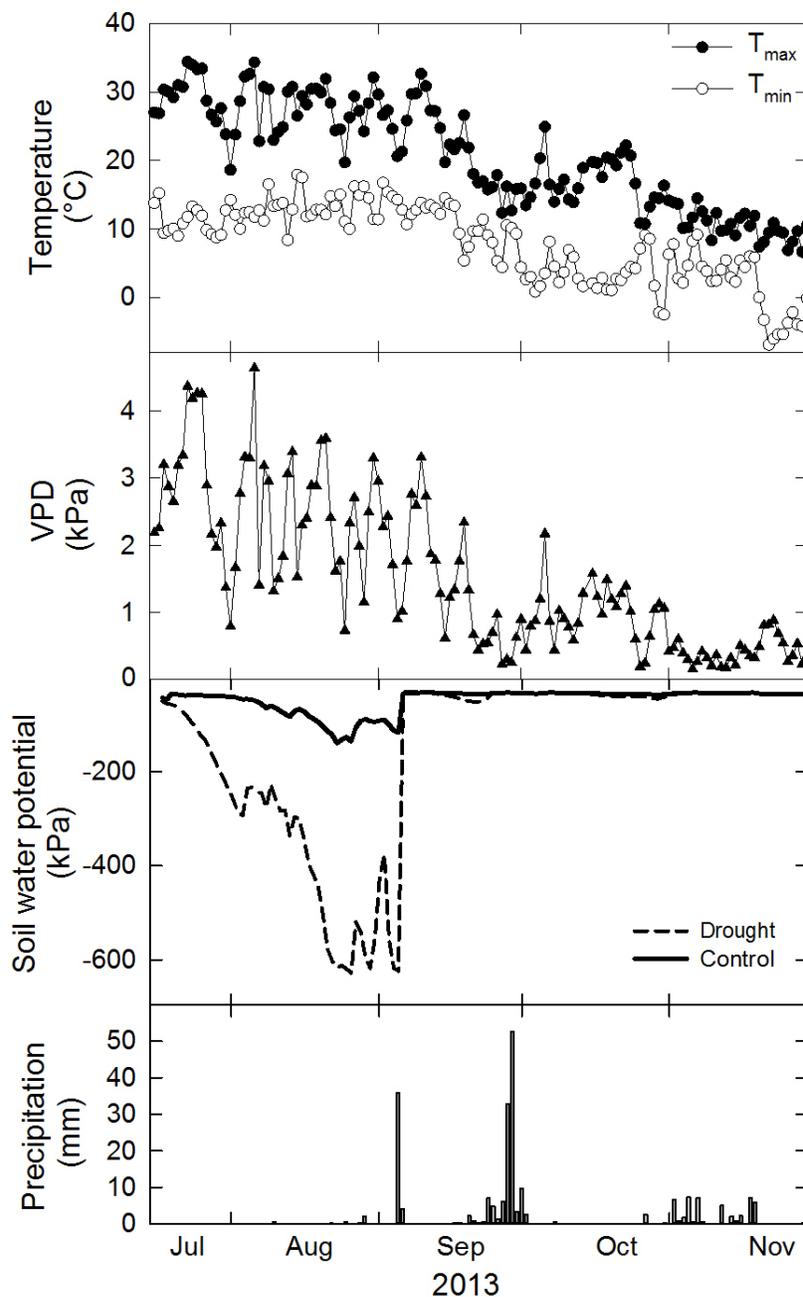


Figure 1. Maximum (T_{max}) and minimum (T_{min}) temperature, vapor pressure deficit (VPD), soil water potential and precipitation during the summer of 2013. Soil water potential was measured continuously at 10 cm. Large rain events in late summer effectively eliminated the drought treatment.

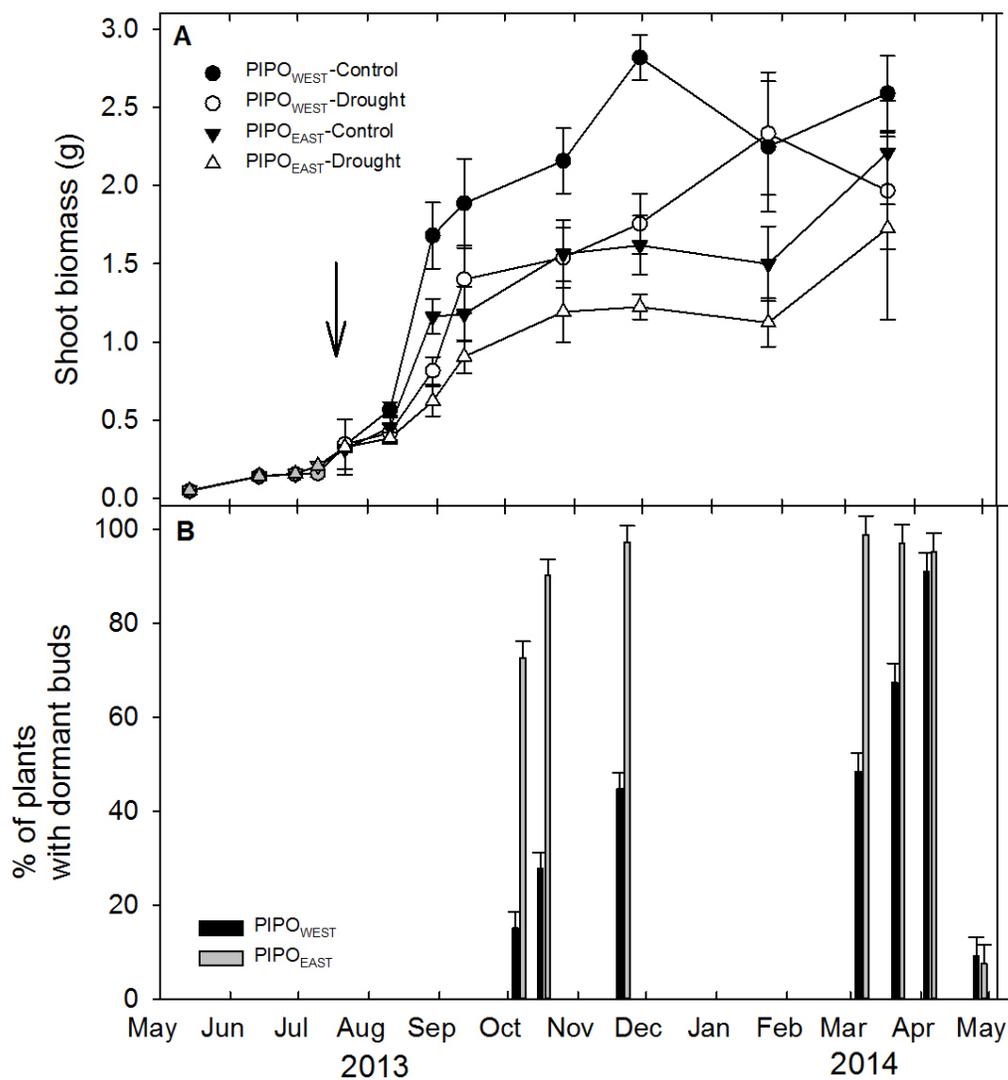


Figure 2. Mean shoot biomass (A) and mean percentage of plants with dormant buds (B) for seedlings from two populations of *Pinus ponderosa* sampled in 2013 and early 2014. Seedlings measured prior to implementation of the drought treatment are denoted by the gray-filled symbols. The arrow indicates the date the drought treatment was implemented. Statistically significant differences were found in the August, September, October, November and January sampling dates (see text for specific results). Error bars represent one standard error (SE).

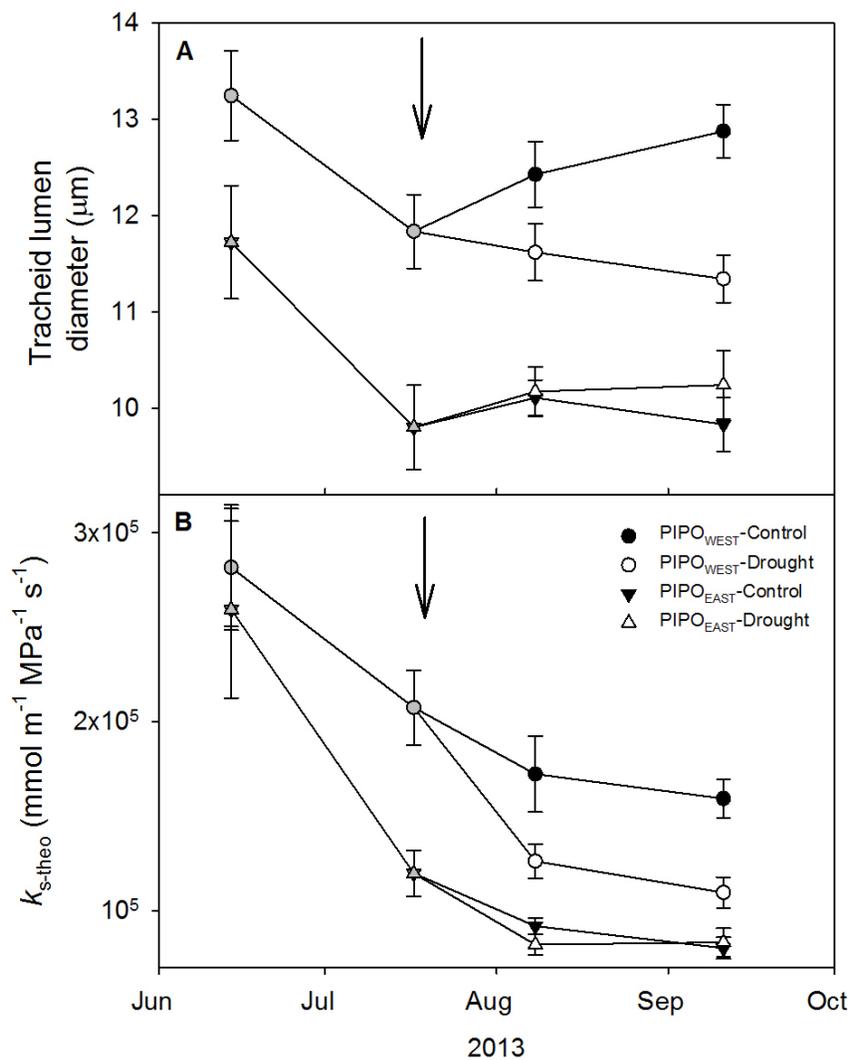


Figure 3. Mean tracheid lumen diameters (A) and mean k_{S-theo} (B) of seedlings from two populations of *Pinus ponderosa* sampled in 2013. Seedlings measured prior to implementation of the drought treatment are denoted by the gray-filled symbols. The arrows indicate the date the drought treatment was implemented. Statistically significant differences were found in the July, August and September sampling dates (see text for specific results). Error bars represent one standard error (SE).

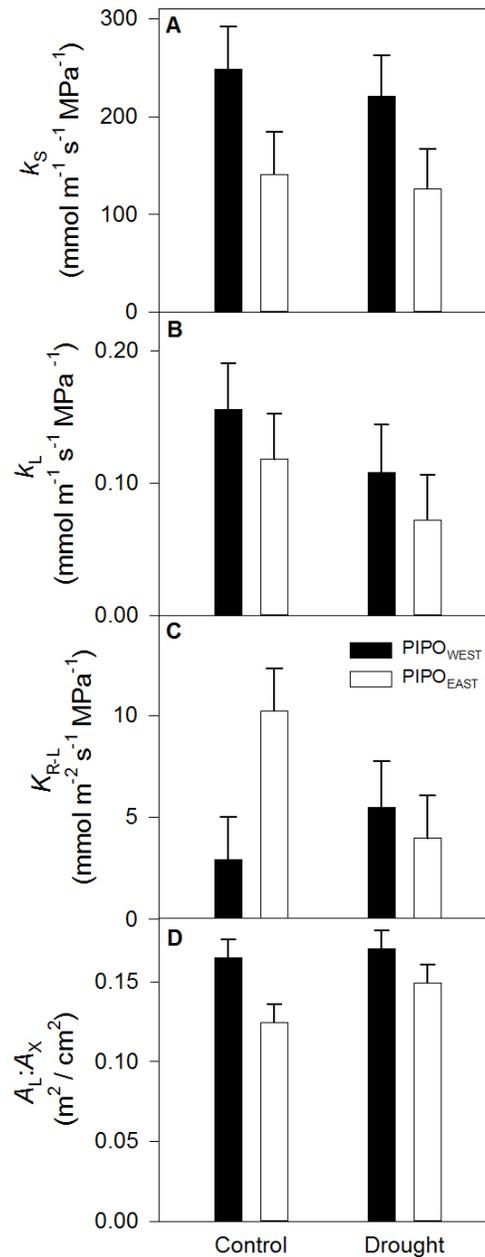


Figure 4. Mean xylem area-specific hydraulic conductivity (k_S ; A), mean leaf-specific conductivity (k_L ; B), mean leaf-specific root conductance (K_{R-L} ; C) and mean ratio of leaf area (A_L) to xylem area (A_X) (D) from two populations of *Pinus ponderosa* sampled in 2013. Statistically significant differences were found for k_S , K_{R-L} and $A_L:A_X$ measurements (see text for details). Error bars represent one standard error (SE).

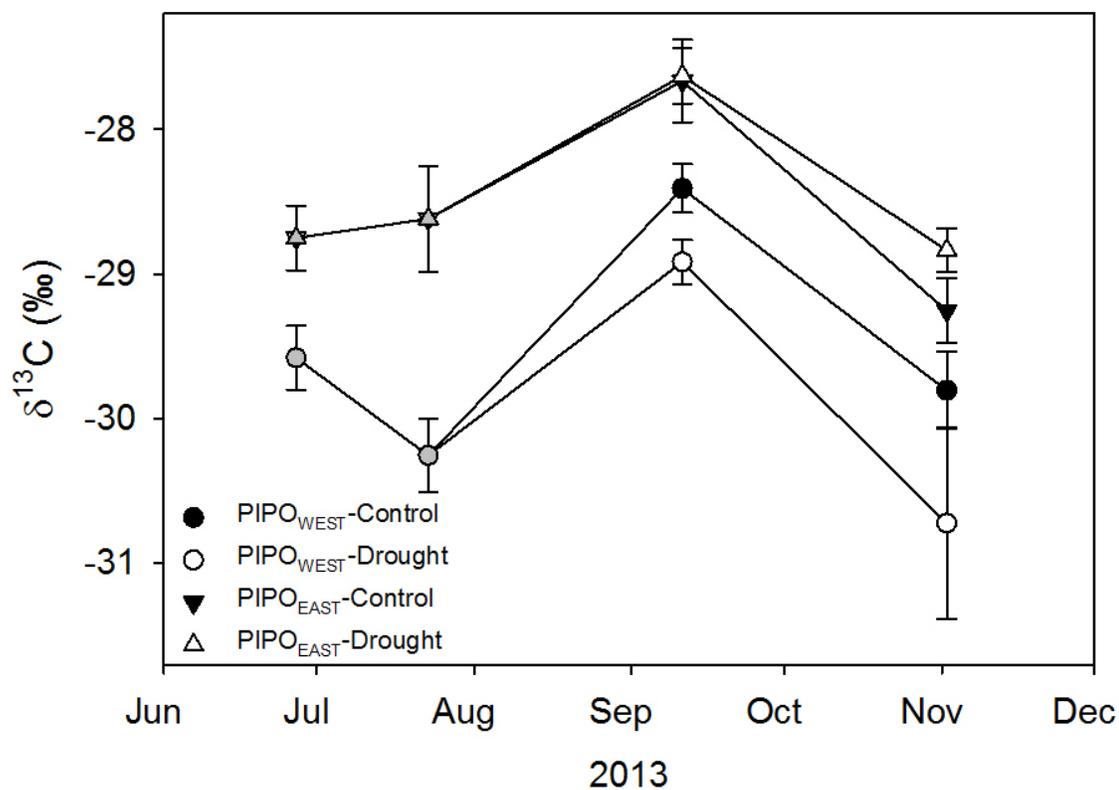


Figure 5. $\delta^{13}\text{C}$ values for two populations of *Pinus ponderosa* seedlings sampled in 2013. For seedlings sampled after the drought treatment was implemented (Sept, Nov) no statistical differences were found between treatments within either population. Statistically significant differences were found between populations of seedlings at every sampling date. Error bars represent one standard error (SE).

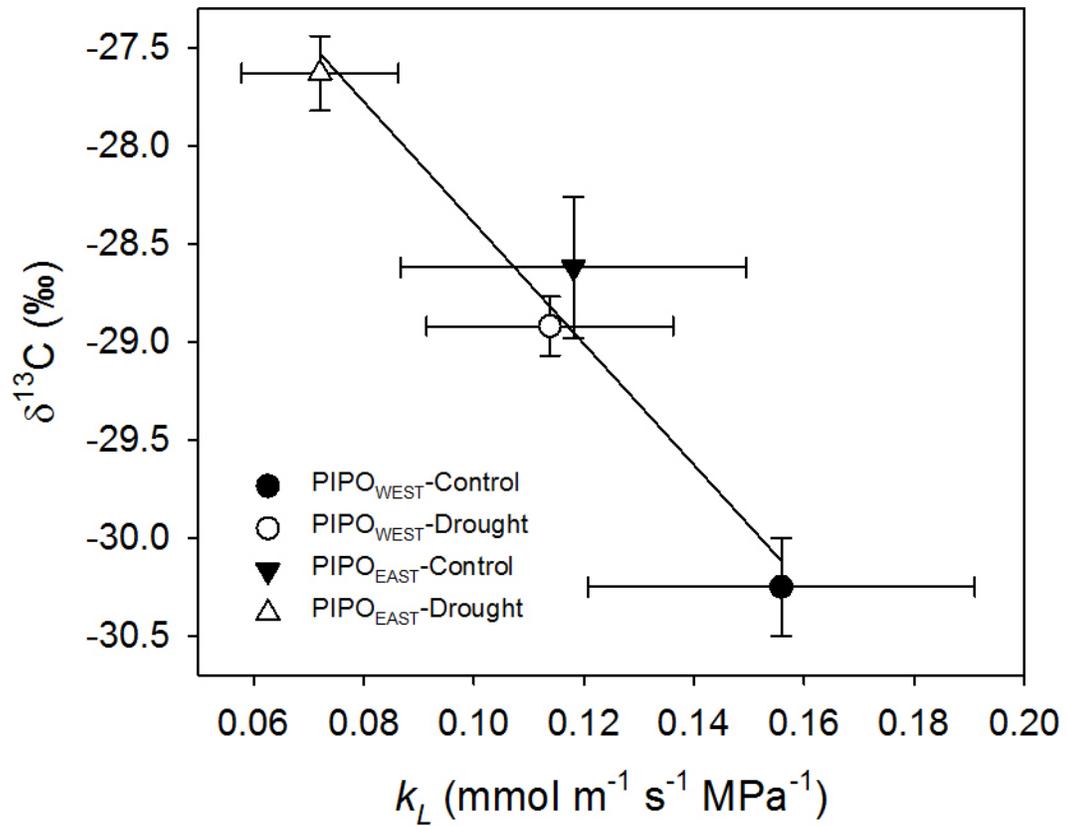


Figure 6. $\delta^{13}C$ values (pooled from July and September data points) plotted against leaf-specific conductivity (k_L) for two populations of *Pinus ponderosa* seedlings sampled in 2013. $r^2 = 0.95$. Error bars represent one standard error (SE).

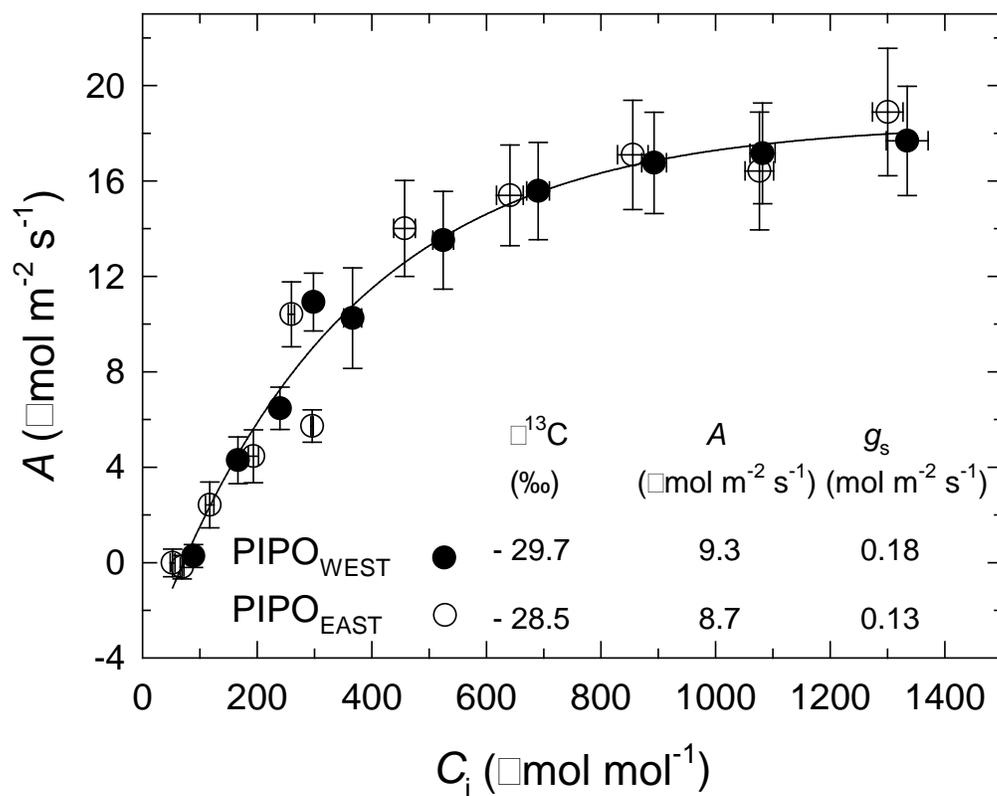


Figure 7. Photosynthetic CO_2 response curves ($A-C_i$) representing pooled data for two populations of *Pinus ponderosa* seedlings sampled in July and November 2013. $A-C_i$ curves for the two populations were indistinguishable. Error bars represent one standard error (SE).

Tables

Table 1. Climate data (precipitation, maximum and minimum temperatures) for Corvallis taken by the Hyslop Field Lab Weather Station, presented as monthly totals (precipitation) and monthly means (temperature) during selected months of 2013.

Month	Precipitation (mm)	Mean maximum temperature (°C)	Mean minimum temperature (°C)
March	65.5	13.9	2.8
April	50.5	17.2	4.8
May	57.6	20.8	8.1
June	32.5	24.2	9.8
July	0.0	30.4	11.0
August	8.4	28.5	12.0
September	179.3	23.8	11.3
October	29.7	17.3	4.2
November	69.3	11.4	3.2

Table 2. Mean (\pm SE) xylem area (mm^2) and hydraulic diameter, d_H (μm) for two populations of *Pinus ponderosa* seedlings sampled in 2013. Dashed lines (--) denote unavailable data. Significant differences were found both within and between populations at $P \leq 0.05$ (denoted by bold).

Sampling Date	PIPO _{WEST}				PIPO _{EAST}			
	Xylem area (10^3 mm^2)		d_H (μm)		Xylem area (10^3 mm^2)		d_H (μm)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
June	3 \pm 0.2	--	14.9 \pm 0.6	--	3 \pm 0.2	--	13.8 \pm 0.8	--
July	5 \pm 0.3	--	13.3 \pm 0.4	--	5 \pm 0.3	--	11.2 \pm 0.4	--
August	6 \pm 0.4	6 \pm 0.4	12.7 \pm 0.3	11.8 \pm 0.3	7 \pm 0.7	7 \pm 0.6	10.4 \pm 0.2	10.4 \pm 0.3
September	1.2 \pm 1	1.1 \pm 0.5	13.3 \pm 0.3	11.6 \pm 0.2	1.0 \pm 0.8	1.2 \pm 1	10.1 \pm 0.3	10.5 \pm 0.4

Table 3. Mean (\pm SE) predawn and midday water potential (Ψ_{pd} , Ψ_{md}), osmotic potential at full turgor ($\Psi_{\pi 100}$) and water potential at the turgor loss point (Ψ_{TLP}) for two populations of *Pinus ponderosa* seedlings sampled in 2013. Both $\Psi_{\pi 100}$ and Ψ_{TLP} differed significantly between populations at $P \leq 0.01$ (denoted by bold).

Population	Ψ_{pd} (MPa)	Ψ_{md} (MPa)	$\Psi_{\pi 100}$ (MPa)	Ψ_{TLP} (MPa)
PIPO _{WEST}	- 0.13 \pm 0.04	- 0.95 \pm 0.03	- 1.33 \pm0.06	- 1.59 \pm0.06
PIPO _{EAST}	- 0.19 \pm 0.07	- 0.95 \pm 0.07	- 1.55 \pm0.03	- 1.92 \pm0.07

3. CONCLUSIONS

Understanding the mechanisms involved in tree physiological processes is of the utmost importance for a wide variety of scientific research projects aimed at addressing the consequences of global climate change. For instance, widespread tree mortality has been linked to warming and climate change and is anticipated to impact ecosystems worldwide (Allen et al. 2010), yet the mechanisms underlying these mortality events are not well known (McDowell et al. 2008). The mortality of adult trees effectively decreases seed production and limits tree recruitment, a process that already poses a bottleneck to forest stand regeneration (Cui and Smith 1991, Walck et al. 2011). Therefore, a working knowledge of tree and seedling physiology, particularly of the mechanisms involved with mortality events, will improve predictions of how these mortality events will impact a landscape and how forests might respond to a changing climate. In addition, knowledge about seedling physiology will provide researchers an enhanced ability to understand how seedling establishment could influence future species distribution patterns. Climate change-driven shifts in rainfall regimes are predicted to alter the dynamics, composition and diversity of many plant ecosystems (Delucia et al. 2000, Comita and Engelbrecht 2009, Shevtsova et al. 2009), and for some ecosystems, will result in the increased duration and severity of droughts (Chmura et al. 2011, Stocker et al. 2013). Thus, understanding the role of seedling physiology in species establishment and distribution within ecosystems will assist in developing management and conservation plans to mitigate the impacts of future climate change.

The ability of a seedling to acclimate to drought events will depend upon the physiological responses it exhibits to periods of low water availability. In this study, I

found large differences in the expression of key functional traits between populations of *Pinus ponderosa* seedlings, as well as population-specific differences in the expression of functional traits under a low water availability treatment. While I evaluated a wide variety of physiological responses, there are still some unanswered questions regarding other functional traits and how these traits could influence the drought resistance of a seedling. Three key research questions that still need to be resolved include understanding the mechanisms involved with the continuation of root growth under drought, how and if seedlings recover from embolism and the maximum dehydration tolerance of a seedling. Seedlings tend to exhibit more plastic physiological responses than adult trees (Creese et al. 2011), thus providing this life stage with an enhanced ability to acclimate more readily to environmental changes. Expanding our knowledge about seedling physiology will therefore improve our understanding of how certain physiological mechanisms might improve their acclimation to a changing environment, particular during drought events.

Water availability has been shown to impact the seedling establishment for a wide variety of tree species (Gordon et al. 1991, Johnson and Smith 2005, Padilla and Pugnaire 2007, Matías et al. 2012, del Cacho et al. 2013). During periods of low water availability, seedlings generally experience reduced shoot biomass growth (Heiner and Lavender 1972, Gordon et al. 1991, Padilla et al. 2007, Schall et al. 2012), which was seen in the *P. ponderosa* seedlings subjected to the drought treatment in this study. Because growth occurs due to an integration of several physiological processes, growth can be seen as a physiological attribute and physiological responses to water availability will be reflected

in growth patterns. Moyes et al. (2013) evaluated how photosynthetic gas exchange in limber pine (*Pinus flexilis*) seedlings responded to soil moisture and observed seasonal declines in photosynthesis with soil drying. These authors emphasized the importance of soil moisture for seedling photosynthesis and shoot growth as they discovered that shoot growth did not fully recover after the seedlings were released from the drought period.

In contrast to reduced photosynthesis and shoot growth, an increased production of root tissue, and thereby an increased root to shoot ratio in response to low water availability, has been documented in a number of species (Stone and Jenkinson 1970, Davies and Zhang 1991, Hsaio and Xu 2000, Greenwood et al. 2008, Schall et al. 2012). However, I was unable to directly test for this response in the present study due to technical complications involved in collecting complete root systems. The extent to which a root system grows defines how much soil can be explored for water and nutrients, yet the physiological mechanisms that permit expansion in root growth during periods of low water availability are not well known. In their review, Hsiao and Xu (2000) examined and discussed the current state of the literature for potential mechanisms that allow the growth of roots during periods of water stress. These authors discovered that roots are able to rapidly adjust to water stress via osmotic adjustment and a lowering of the threshold turgor pressure required for growth, which ultimately allows root elongation to recover at reduced turgor. Further, leaf growth has been shown to be inhibited during water stress despite the continuation of photosynthesis, which results in more assimilates available for root growth and, combined with osmotic adjustment, allows for the maintenance of root growth under drought (Hsaio and Xu 2000 and

references therein). However, the majority of research papers highlighted in the Hsaio and Xu (2000) review involved non-coniferous species, such as maize, sorghum, other crop species and grasses. To the best of my knowledge, the mechanisms involved in conifer root growth responses to drought have not been studied in such detail.

Nevertheless, similar increases in root to shoot ratios in seedlings in response to drought have been documented for conifer species, including *P. ponderosa* (Stone and Jenkinson 1970, Heiner and Lavender 1972, Padilla et al. 2007, Padilla and Pugnaire 2007), suggesting that the specific mechanisms governing root growth and expansion in conifers during drought might in fact be similar to those discovered in crop species.

Adjustments in hydraulic architecture in response to increased evaporative demand, can result in an increased resistance to embolism in the xylem tissue (refs). Generally, this is achieved through a reinforcement of the tracheid cell walls in order to withstand increased tension in the soil-plant-atmosphere hydraulic continuum, and leads to a reduction in conduit diameter (refs). I did not directly test for changes in tracheid wall thickness or for changes in vulnerability to embolism within either population of *P. ponderosa*. However, the data suggested that an increase in vapor pressure deficit (VPD) likely increased transpiration and reduced water potential and turgor, which in turn resulted in reduced tracheid expansion and smaller tracheid diameters once the xylem conduits reached maturation. Studies have shown that decreased tracheid lumen diameter and thicker cell walls in conifer xylem generally resulted in increased resistance to embolism (Hacke et al. 2001, Domec et al. 2006, Pittermann et al. 2006). These studies indicated that hydraulic safety versus efficiency trade-offs were involved where greater

mechanical reinforcement in tracheid structure occurs at the expense of reduced hydraulic efficiency (Pittermann et al. 2006, Meinzer et al. 2010). Hacke et al. 2001 tested the relationships between embolism resistance, conduit wall reinforcement as measured by the ratio of wall thickness to lumen diameter, and wood density in 48 tree species and discovered a tight correlation between embolism resistance and conduit wall reinforcement, with increased wood density and resistance to embolism occurring at the expense of conduit lumen area and water conductance in conifers. Therefore, the reduction in tracheid diameter observed in the *P. ponderosa* seedlings from the present study seems likely to have influenced hydraulic conductivity and xylem resistance to emboli and may be indicative of a hydraulic safety versus efficiency trade-off (Pittermann et al. 2006). In addition, the variation in tracheid lumen diameter seen between these two populations suggests that while a hydraulic safety versus efficiency trade-off may occur for both, these populations may operate at different places along a trade-off continuum. However, additional studies that directly test for vulnerability to embolism and tracheid diameter are needed in order to know whether this type of trade-off is actually occurring within seedlings of *P. ponderosa*.

Recovery from embolism events has been documented for many plant species (Salleo et al. 1996; Salleo et al. 2009; Johnson et al. 2012) and, in particular, has been shown to be an integral process during freeze-thaw cycles and drought events in conifer species (Sobrado et al. 1992; Sperry 1993; Sperry et al. 1994; McCulloh et al. 2011), and embolism loss and recovery can even occur on a daily basis in conifers (Woodruff et al. 2007; Johnson et al. 2009; Johnson et al. 2012 and references therein). However, the

mechanisms of embolism recovery are still unclear, although there is evidence that sugars are involved in the process (Salleo et al. 1996, Salleo et al. 2009, Johnson et al. 2012). A sustained drought was not attained in the present study, so therefore, a drought-induced increase in seedling mortality was not observed. Thus, the question of why seedlings die during drought remains and whether or not runaway embolism events would lead to catastrophic xylem failure and foliage death. In addition, it is unclear whether seedlings exhibit embolism refilling and recovery from drought-induced embolism. McCulloh et al. (2011) investigated seasonal changes in native embolism and conduit refilling capabilities in the upper branches of four adult conifer species over an entire year. These authors discovered that declines in native embolism in the spring could not be explained by growth of new tracheids alone, suggesting the role of embolism refill in recovery from freeze-thaw-induced embolism. Further, embolism recovery from drought-induced embolism occurred in later summer despite continued declines in water potential in all species. Therefore, embolism refill and recovery from embolism events could be viewed as mechanisms to cope with hydraulic constraints, yet the costs of embolism refill may result in continued conservative behavior from species that exhibit a refill response (McCulloh et al. 2011). Climatic factors that reduce water movement through a plant subsequently limit carbon uptake, thereby highlighting the ecological significance of embolism events and subsequent post-drought recovery in determining the distribution pattern of a species.

The population-specific differences in water-transport and photosynthetic physiology that were observed in this study appeared to reflect each population's

adaptations to their climate of origin. The seedling population from the site with the drier, more continental climate exhibited more conservative functional behavior by having higher iWUE, more constrained gas exchange and hydraulics, a greater ability to maintain turgor under drought-induced declines in plant water status, and grew more slowly regardless of water availability. Overall, the conservative functional behavior of these seedlings seems consistent with this population's climate of origin, which is characterized by greater temperature extremes and periods of low water availability during the growing season. In agreement with this study, within-species population differences have been reported for a variety of physiological responses for a number of species (Zhang and Marshall 1994, Maherali et al. 2002, Pastorino et al. 2012). Habitat heterogeneity across a species distribution range can lead to variability in the expression of certain functional traits so that within a single species, genetically distinct populations of individuals represent different ecotypes (Turesson 1922, Linhart and Grant 1996, Hufford and Mazer 2003). While ecotypes are adapted to their local environments, the potential for migration of ecotypes to new environments, whether due to natural migration in response to climate change or to anthropogenic involvement under policies like assisted migration, requires studies involving both local and distant adaptation (Hufford and Mazer 2003). Since 1910 there has been an extensive amount of systematic research done on *Pinus ponderosa*, and many of these studies were common garden provenance trials (Larson 1967, Wang 1977, Conkle and Critchfield 1988, Sorenson et al. 2001, Callaham 2013 and references therein). Currently, there are five genetically distinct subspecies of *P. ponderosa*. (Callaham 2013). Columbia ponderosa pine (subsp.

ponderosa, formerly known as the North Plateau race) is distributed east of the Cascade Mountains and extends north to southern British Columbia, south to southern Oregon, and west to the eastern halves of Montana and Idaho (Callaham 2013). Pacific ponderosa pine (subsp. *critchfieldiana*) ranges north to northern Washington and south to southern California, with its distribution west of the Cascade Mountains in Oregon and Washington (Callaham 2013). Based on these distribution ranges, it appears that seedlings from this study could have originated from the Columbia (eastern population) and Pacific (western population) subspecies, thereby further supporting the idea that these populations are genetically distinct. Knowing the inherent plasticity of functional traits will vastly improve our understanding of the adaptability of a species to its environment, and how that species will respond to future climate changes.

Studies involving seedling physiology are important for the development of enhanced methods and techniques for physiological measurements and for improving process-based plant models. While I was able to observe many physiological aspects of germinant-year *P. ponderosa*, future studies could build upon much of the work laid out in the present study or address components of seedling establishment that I was unable to incorporate. Many of the techniques used to measure the physiology of conifer species require the presence of woody tissue, which is not yet fully developed in many germinant-year seedlings. By the end of the first growing season, *P. ponderosa* seedlings had developed woody stems, but certain measurements were not possible to make earlier in the season due to a lack of woody tissue. For example, the high-pressure flow meter (HPFM) method for measuring hydraulics was very difficult to use on seedlings without

woody stem material because the non-woody stems could not withstand the pressure required to obtain an adequate seal around the stem segment in order for the hydraulic measurement to be taken. However, it is important to make physiological measurements earlier during the ontogeny of a seedling in order to obtain a more complete picture of the mechanisms underlying establishment and survival. Thus, future studies could focus on developing new methods for measuring functional traits in this fragile life stage. In addition, future studies should attempt to include measurements of intact root systems to better understand the influence that a root system might possess in driving seedling establishment. Finally, additional studies should include populations of *P. ponderosa* across the entire range of this species in order to investigate all population-specific differences in the expression of functional traits. Knowledge about population-specific physiology would improve our understanding of how genetically distinct the subspecies of *P. ponderosa* are from one another and how these individual populations might acclimate to future climate change.

The use of models for predicting plant behavior is critical for exploring potential outcomes of climate change in an effort to better understand how ecosystems and species will react to anticipated changes in the environment (Rushton et al. 2004). However, many models currently used today lack a thorough incorporation of plant physiological parameters, which are crucial predictors of how and why a species will respond to certain climatic conditions (Prentice et al. 1992, Aitken et al. 2008). In addition, many models use generic values of parameters across multiple species that are cast into a broadly defined functional group rather than species-specific values. For example, juniper

(*Juniperus* species) and pinyon pine (*P. edulis*) have both been classified under the same functional type based on needle-leaf morphology in some models yet these species actually behave quite different physiologically (Breshears et al. 2009; Peterman et al. 2012; Meinzer et al. 2014). Also, physiological input parameters used in nearly all models are for mature plants resulting in poor modeling capabilities of the seedling cohorts within a species. A comprehensive understanding of seedling physiology will improve model predictions of future species distributions under climate change due to the influence that seedling establishment exhibits on the distribution patterns of a species (Johnson et al. 2011).

Results from the present study suggest that there were population-specific differences in physiology between the two populations of *Pinus ponderosa* under investigation. However, physiological responses in both populations of seedlings allowed us to investigate the mechanisms involved in seedling establishment and survival within the first year of growth. Since both of these populations were grown in a common garden under similar environmental conditions, the seemingly conservative strategies of seedlings from the drier site population are likely due to genetic variation within *P. ponderosa* and these geographically distant populations likely represent different ecotypes. Common garden studies are imperative for understanding the physiological mechanisms and functional traits that drive successful seedling establishment, and will improve our ability to predict future species distribution patterns (Aitken et al. 2008).

References

- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl* 1:95–111.
- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684.
- Breshears DD, Myers OB, Meyer CW, Barnes FJ, Zou CB, Allen CD, McDowell NG, Pockman WT (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Front Ecol Environ* 7(4):185–189.
- Del Cacho M, Estiarte M, Penuelas J, Lloret F (2013) Inter-annual variability of seed rain and seedling establishment of two woody Mediterranean species under field-induced drought and warming. *Popul Ecol* 55:277–289.
- Callaham RZ (2013) *Pinus ponderosa*: a taxonomic review with five subspecies in the United States. Res. Pap. PSW-RP-264. US Dept Ag For Serv, Albany CA. 52 p.
- Conkle MT, Critchfield WB (1988) Genetic variation and hybridization of ponderosa pine. In *Ponderosa Pine: The Species and Its Management*. Eds. D.M. Baumgartner and J.E. Lotan. Washington State Univ. Coop. Ext. Serv., Pullman, WA, pp 27–43.
- Chmura DJ, Anderson PD, Howe GT, Harrington CA, Halofsky JE, Peterson DL, Shaw DC, Brad St Clair J (2011) Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *For Ecol Manag* 261:1121–1142.
- Comita LS, Engelbrecht BM (2009) Seasonal and spatial variation in water availability drive habitat associations in a tropical forest. *Ecology* 90:2755–2765.
- Creese C, Benscoter AM, Maherali H (2011) Xylem function and climate adaptation in *Pinus*. *Am J Bot* 98:1437–1445.
- Cui M, Smith WK (1991) Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol* 8:37–46.
- Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol Plant Mol Biol* 42:55–76.

- Delucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. *Glob Change Biol* 6:587–593.
- Domec JC, Lachenbruch B, Meinzer FC (2006) Bordered pit structure and function determine spatial patterns of air-seeding thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinaceae) trees. *Am J Bot* 93(11):1588-1600.
- Gordon DR, Rice KJ, Welker JM (1991) Soil water effects on blue oak seedling establishment. Gen Tech Rep PSW-GTR-126. US Dept Ag For Serv, Berkeley CA. 5p.
- Greenwood MS, O'Brien CL, Schatz JD, Diggins CA, Day ME, Jacobson GL, White AS, Wagner RG (2008) Is early life cycle success a determinant of the abundance of red spruce and balsam fir? *Can J For Res* 38:2295–2305.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457-461.
- Heiner TC, Lavender DP (1972) Early growth and drought avoidance in Douglas-fir seedlings. Res Pap 14 For Res Lab, Oregon State University. 9p.
- Hsaio TC, Xu L (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J Exp Bot* 51(350):1595-1616.
- Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol Evol* 18:147–155.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: Why are conifers and angiosperms so different? *Plant Sci* 195:48-53.
- Johnson DM, McCulloh KA, Reinhardt K (2011) The Earliest Stages of Tree Growth: Development, Physiology and Impacts of Microclimate. In: Meinzer FCC, Lachenbruch B, Dawson TEE, Meinzer FC, Niinemets Ü (eds) *Size- and Age-Related Changes in Tree Structure and Function*. Springer Netherlands, pp 65–87.
- Johnson DM, Woodruff DR, McCulloh KA, Meinzer FC (2009) Leaf hydraulic conductance, measured in situ, declines and recovers daily: leaf hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. *Tree Phys* 29:879-887.
- Johnson DM, Smith WK (2005) Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. *Tree Physiol* 25:1379–1387.

- Larson MM (1967) Effect of temperature on initial development of ponderosa pine seedlings from three sources. *For Sci* 13(3,1):286-294.
- Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annu Rev Ecol Syst*:237–277.
- Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence. *Funct Ecol* 16:510–521.
- Matías L, Zamora R, Castro J (2012) Sporadic rainy events are more critical than increasing of drought intensity for woody species recruitment in a Mediterranean community. *Oecologia* 169:833–844.
- McCulloh KA, Johnson DM, Meinzer FC, Lachenbruch B (2011) An annual pattern of native embolism in upper branches of four tall conifer species. *Am J Bot* 98(6):1007-1015.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739.
- Meinzer FC, Woodruff DR, Marias DE, McCulloh KA, Sevanto S (2014) Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species. *P,C&E* 37(11):2577-2586.
- Moyes AB, Castanha C, Germino MJ, Kueppers LM (2013) Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia* 171:271–282.
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495.
- Padilla FM, Miranda JD, Pugnaire FI (2007) Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant Soil* 296:103-113.
- Pastorino MJ, Aparicio AG, Marchelli P, Gallo LA (2012) Genetic variation in seedling water-use efficiency of Patagonian Cypress populations from contrasting precipitation regimes assessed through carbon isotope discrimination. *For Syst* 21:189–198.
- Peterman W, Waring RH, Seager T, Pollock WL (2012) Soil properties affect pinyon pine - juniper response to drought. *Ecohydrology*. DOI: 10.1002/eco.1284.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH (2006) Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *PC&E* 29(8):1618-1628.

Prentice IC, Cramer W, Harrison SP, Leemans R, Monserud RA, Solomon AM (1992) Special Paper: A Global Biome Model Based on Plant Physiology and Dominance, Soil Properties and Climate. *J Biogeogr* 19:117–134.

Rushton SP, Ormerod SJ, Kerby G (2004) New paradigms for modelling species distributions? *J Appl Ecol* 41:193–200.

Salleo S, Trifilò P, Esposito S, Nardini A, LoGullo MA (2009) Starch-to-sugar conversion in wood parenchyma of field-growing *Laurus nobilis* plants: a component of the signal pathway for embolism repair? *Func Plant Biol* 36:815-825.

Salleo S, LoGullo MA, de Paoli D, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytol* 132:47-56.

Schall P, Lödige C, Beck M, Ammer C (2012) Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. *For Ecol Manag* 266:246–253.

Shevtsova A, Graae BJ, Jochum T, Milbau A, Kockelbergh F, Beyens L, Nijs I (2009) Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Glob Change Biol* 15:2662–2680.

Sobrado MA, Grace J, Jarvis PG (1992) The limits to xylem embolism recovery in *Pinus sylvestris* L. *J Exp Bot* 43(6):831-836.

Sorenson FC, Mandel NL, Aagaard JE (2001) Role of selection versus historical isolation in racial differentiation of ponderosa pine in southern Oregon: an investigation of alternative hypotheses. *Can J For Res* 31(7):1127-1139.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75(6):1736-1752.

Sperry JS (1993) Winter xylem embolism and spring recovery in *Betula cordifolia*, *Fagus grandifolia*, *Abies balsamifera*, and *Picea rubens*. In Raschi A, Borghetti M, Grace J (Eds). *Water transport in plants under climatic stress*. Cambridge University Press, Cambridge, England. pp 86-98.

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex B, Midgley BM (2013) IPCC, 2013: climate change 2013: the physical science basis.

Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. <http://41.89.141.5/dspace/handle/0/6655> (8 October 2014, date last accessed).

Stone EC, Jenkinson JL (1970) Influence of soil water on root growth capacity of ponderosa pine transplants. *For Sci* 16(2):230-239.

Turesson G (1922) The species and the variety as ecological units. *Hereditas* 3:100–113.

Walck JL, Hidayati SN, Dixon KW, Thompson KEN, Poschlod P (2011) Climate change and plant regeneration from seed. *Glob Change Biol* 17:2145–2161.

Wang CW (1977) Genetics of ponderosa pine. Res. Pap. US For Serv, Wash DC. 24 p.

Woodruff DR, McCulloh KA, Warren JM, Meinzer FC, Lachenbruch B (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *P,C&E* 30(5):559-569.

Zhang J, Marshall JD (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Can J For Res* 24:92–99.

