Open Access Articles

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

Research paper

Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates

Kelly L. Kerr[1], Frederick C. Meinzer[2,4], Katherine A. McCulloh[3], David R. Woodruff[2] and Danielle E. Marias[1]

[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA; [2]USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331, USA; [3]Department of Botany, University of Wisconsin-Madison, Madison, WI 53706, USA; [4]Corresponding author (rick.meinzer@oregonstate.edu; fmeinzer@fs.fed.us)

First-year tree seedlings represent a particularly vulnerable life stage and successful seedling establishment is crucial for forest regeneration. We investigated the extent to which *Pinus ponderosa* P. & C. Lawson populations from different climate zones exhibit differential expression of functional traits that may facilitate their establishment. Seeds from two populations from sites with contrasting precipitation and temperature regimes east ($PIPO_{dry}$) and west ($PIPO_{mesic}$) of the Oregon Cascade mountains were sown in a common garden experiment and grown under two water availability treatments (control and drought). Aboveground biomass accumulation, vegetative phenology, xylem anatomy, plant hydraulic architecture, foliar stable carbon isotope ratios ($\delta^{13}C$), gas exchange and leaf water relations characteristics were measured. No treatment or population-related differences in leaf water potential were detected. At the end of the first growing season, aboveground biomass was 74 and 44% greater in $PIPO_{mesic}$ in the control and drought treatments, respectively. By early October, 73% of $PIPO_{dry}$ seedlings had formed dormant buds compared with only 15% of $PIPO_{mesic}$ seedlings. Stem theoretical specific conductivity, calculated from tracheid dimensions and packing density, declined from June through September and was nearly twice as high in $PIPO_{mesic}$ during most of the growing season, consistent with measured values of specific conductivity. Intrinsic water-use efficiency based on $\delta^{13}C$ values was higher in $PIPO_{dry}$ seedlings for both treatments across all sampling dates. There was a negative relationship between values of $\delta^{13}C$ 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 $\delta^{13}C$ values and photosynthetic CO_2-response curves were 6 and 28% lower, respectively, in $PIPO_{dry}$ seedlings. Leaf water potential at the turgor loss point was 0.33 MPa more negative in $PIPO_{dry}$, independent of treatment. Overall, $PIPO_{dry}$ seedlings exhibited more conservative behavior, suggesting reduced growth is traded off for increased resistance to drought and extreme temperatures.

Keywords: carbon isotope discrimination, gas exchange, genetic variation, hydraulic architecture, osmotic potential, phenology.

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 (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 can also influence the distributions of tree species (Johnson et al. 2011). Understanding the physiological mechanisms and functional traits that drive successful seedling establishment may improve our ability to predict future species distributions, especially as climate change is 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 (Germino and Smith 1999, Lewis et al. 2001, Greenwood 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 (e.g., Cui and Smith 1991, Padilla and Pugnaire 2007, Padilla et al. 2007). 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 drought, frost heave, herbivory, pathogens and trampling by animals (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, 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 influences seedling establishment in a wide variety of tree species. Temperature also influences water availability and seedling establishment because atmospheric evaporative demand, or vapor pressure deficit (VPD), increases exponentially with temperature. 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, both seed germination and establishment in 15 subarctic species were negatively affected even with an increase in soil water availability (Shevtsova et al. 2009). Similarly, exposure of first-year *Pinus flexilis* James seedlings to warmer temperatures and dry soil was negatively correlated with survival, but it was 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. Both water availability and drought sensitivity were found to directly influence distribution patterns of seedlings of woody species in central Panama (Engelbrecht et al. 2007), with evidence that variation in water availability influenced seedling population dynamics of whole plant communities and that dry

season mortality determined species' habitat associations (Comita and Engelbrecht 2009). 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 functional 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 to stem xylem area (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 genetically determined, yet the degree of phenotypic plasticity for many of these traits remains unclear. Differential 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). Variation in WUE among 14 populations of 1-year-old *Larix occidentalis* Nutt. seedlings across the species' distribution range was found to have a genetic (population) component related to the altitude of the seed sources (Zhang and Marshall 1994). In contrast, some functional traits of provenances of *Eucalyptus tricarpa* (L.A.S.Johnson) L.A.S.Johnson & K.D.Hill trees grown in their native environments and grown in two common gardens established near each end of a climate gradient exhibited complex combinations of phenotypic plasticity and genetic divergence among provenances (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 and Jain 2005). In Oregon, *P. ponderosa* 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 has 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 *P. ponderosa* 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 *P. ponderosa* seedlings and found no differences between climate groups with nearly all the genetic variation seen being partitioned within populations, leading them to conclude that their results supported little evidence of ecotypic differentiation among the populations studied. Likewise, Zhang and Cregg (2005) reported that although expression of traits varied with environmental conditions in 10 populations of 26-year-old *P. ponderosa* trees from provenance trials, there were only significant population differences in growth and morphological traits, not in physiological traits, leading them to conclude that a lack of physiological variance could be due to plastic phenotypic convergence of traits when populations are grown in a similar environment. Knowing the inherent plasticity of functional traits will vastly improve our understanding of *P. ponderosa* seedling establishment, and how this species may 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 a mesic maritime climate and a substantially drier semi-continental climate. 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: (i) drought resistance traits would be more strongly expressed in seedlings under low water availability, and (ii) that there would be population-specific differences in water-transport and photosynthetic physiology such that the drier climate population (PIPO$_{dry}$) would show greater hydraulic and stomatal constraints on leaf gas exchange.

Materials and methods

Study design

Ponderosa pine seeds were obtained from the Oregon Department of Forestry. The first population came from an area close to Spray, OR (44.8331°N, 119.7944°W) ~325 km from the coast, hereafter referred to as PIPO$_{dry}$. The mean annual precipitation (MAP) of Spray is 337 mm, and the 1937–2013 mean minimum and maximum temperatures for winter (December–February) are −2.7 and 8.4 °C, and those for summer (June–August) are 10.7 and 32.4 °C with annual extremes of −33 and 47 °C (WRCC, Western Regional Climate Center, 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$_{mesic}$. For this population, the climate was estimated to be similar to that of Corvallis, OR located ~120 km south of the Columbia River (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 and 8.6 °C, those for summer are 10.2 and 25.8 °C and annual extremes are −22 and 43 °C (WRCC).

This study was conducted in four unsheltered raised soil beds (18.3-m long × 1.5-m wide, ~0.75-m deep) 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 (10 km) are summarized in Table S1 available as Supplementary Data at *Tree Physiology* Online. Many places west of the Cascade Mountains in the Pacific Northwest, including Corvallis, typically experience a 'Mediterranean'-type climate with generally warm and dry summers and mild and wet winters.

Prior to planting, the raised beds were sterilized with a soil fumigant (Basamid Granular CertisUSA, Columbia, MD, USA), 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 26 March 2013. Each bed was divided into thirds and each of these bed-thirds was further divided into six sections. The two populations of ponderosa pine were randomly assigned to sections within bed-thirds for planting. Seeds were planted in six rows across the width of the section at 18 seeds per row giving a total of 106 seeds per bed section 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 to prevent seeds from becoming dislodged during irrigation, being eaten or blowing away. 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 18 July 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 3 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 3-day's total evaporation. Control beds were watered twice a week and received 50% of the previous 3-day's total evaporation. Gypsum block soil matric potential sensors (223 l, Campbell Scientific, Logan, UT, USA) were installed in the beds and values were recorded every 30 min with a data logger (CR10X, Campbell Scientific). Two soil sensors were installed one-third of the length of the bed from each bed end. At each of these locations, 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 2 August 2013 watering in these beds was reduced to 5% of the past 3-day's total evaporation once a week. Other environmental data collected at the field site between July and November 2013 included daily maximum and minimum temperatures, daily maximum VPD and precipitation (Figure 1).

Growth and phenology

Seedlings were harvested to monitor shoot biomass accumulation throughout the growing season. At each sampling period, 4–10 seedlings from each population and treatment combination were harvested. Individual shoots were sealed into plastic bags and immediately placed into a cooler for transport to the nearby laboratory. In the laboratory, shoots were rinsed in water to remove excess soil, placed into a coin envelope and kept in a 60 °C oven until dry. Only shoot biomass accumulation was
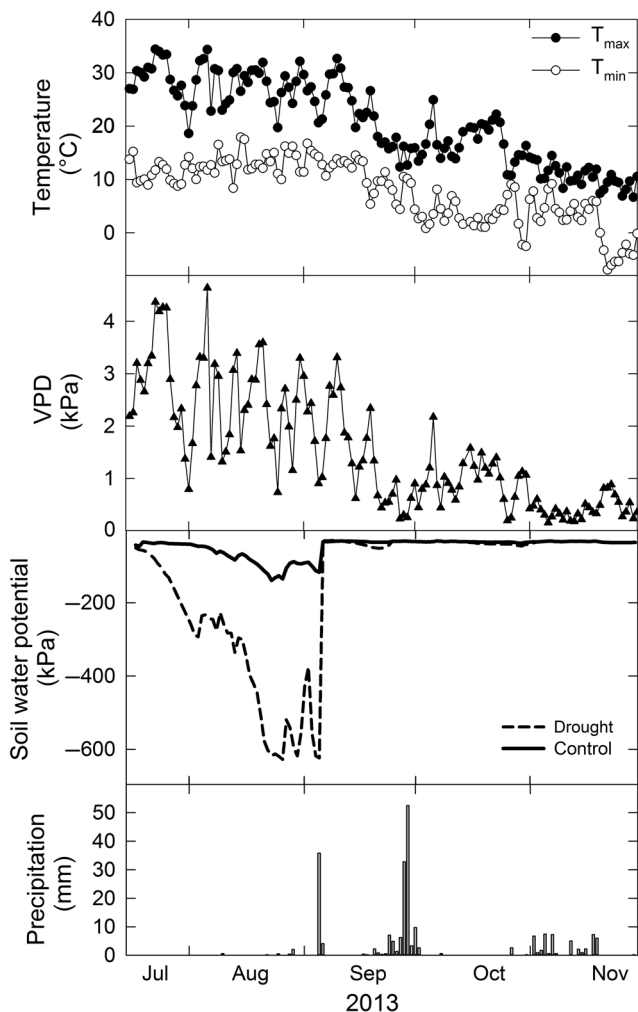

Figure 1. Maximum (T_{max}) and minimum (T_{min}) temperature, VPD, soil water potential and precipitation during the summer of 2013. Soil water potential was measured continuously at 10 cm. Large rain events in late summer effectively eliminated the drought treatment.

measured because complete intact root systems were virtually impossible to collect.

Starting in September 2013 and continuing until the end of November 2013, seedlings were visually assessed weekly for the presence of apical dormant buds and percentage (%) of seedlings with dormant buds was recorded. 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. Seedlings were sealed in plastic bags with a moist paper towel and kept in a 4 °C refrigerator until sample processing (within 2 days).

For each stem, 8–10 thin cross-sections were prepared by hand and stained with toluidine blue (T-blue), which stains cell walls, before being mounted in glycerin onto microscope slides. Images of the cross-sections were taken using a built-in 3.0 MP USB camera on a 40X-2000X digital binocular compound microscope (OMAX, Bucheon-si, Korea) using a 40× objective. Within a cross-section, a single file of tracheids, starting from its origin at the pith, was imaged. Three complete files of tracheids 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.

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 ($k_{S\text{-}theo}$; mmol m^{-1} s^{-1} MPa^{-1}) for each stem sample

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

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 molar density and the dynamic viscosity of water at 20 °C.

Hydraulics

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; mmol m s^{-1} MPa^{-1}) as the flow

rate divided by the pressure gradient across the stem segment, and root hydraulic conductance (mmol s^{-1} 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 near the soil surface using a razor blade, sealed in a plastic bag and stored at 4 °C in a refrigerator until a basal stem segment below the lowest needles could be excised in the laboratory the following day. The segments were re-cut under water using a razor blade in order to ensure a flat, even surface for measurement and to minimize the influence of potentially embolized tracheids. Water-filled Tygon tubing was placed ~2.5 cm over the stem segment in the laboratory 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. Intact root system conductances were measured in situ immediately after removal of the shoot. 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 20–30 min 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, mmol m^{-1} s^{-1} 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; mmol m^{-1} s^{-1} MPa^{-1}) and root system leaf-specific conductance (K_{R-L}; mmol m^{-2} s^{-1} MPa^{-1}). The mean ratio of leaf area (A_L; m^2) to xylem area (A_X; cm^2) was calculated for each population-treatment combination. Root and stem hydraulic measurements were conducted on sunny mornings towards the end of the growing season well after the treatments were implemented.

Shoot water relations

The pressure–volume (P–V) technique (Tyree and Hammel 1972) was used to estimate shoot osmotic potential. Toward the end of the growing season, complete shoots (~14-cm-tall) were excised and sealed in plastic bags for transport to the laboratory where they were subjected to a 1.5–3 h hydration treatment after re-cutting their bases under water. Shoot water potential (Ψ) measured using a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA) and weight to 0.0001 g were recorded periodically while shoots were allowed to dry on the laboratory bench. Sample saturated weights necessary for determination of relative water content or relative water deficit (RWD) for P–V analyses were estimated by extrapolating linear regressions fitted to the pre-turgor loss portions of plots of sample fresh weight versus Ψ to $\Psi = 0$. 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 (e.g., Parker and Pallardy 1987, Kubiske and Abrams 1990). 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 RWD = 0 and the 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_{md}) 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 11 : 30 and 13 : 00 h and measured at the field site using a Scholander-type pressure chamber.

Carbon isotope discrimination

Needles from randomly selected seedlings were collected for stable carbon isotope analysis twice before treatments were imposed and twice afterwards. At each sampling period, 5–6 seedlings per population and treatment combination were collected. Aboveground portions of seedlings were placed into plastic bags for transport to the laboratory where they were rinsed with water to remove soil and then dried in a 60 °C oven. The dried foliage was then separated and 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 standard:

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

$\delta^{13}C$ 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 ±0.1‰ or better were determined by repeated measures of internal quality control standards and from sample replicates.

The $\delta^{13}C$ of whole leaf tissue ($\delta^{13}C_{leaf}$) incorporates the $\delta^{13}C$ of CO_2 in the atmosphere ($\delta^{13}C_{air}$), 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):

$$\delta^{13}C_{leaf} = \delta^{13}C_{air} - a - (b - a)\frac{C_i}{C_a} \qquad (3)$$

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 bisphosphate carboxylase-oxygenase (RuBisCO) during CO_2 fixation (27‰) (Farquhar et al. 1989). Values of $\delta^{13}C_{leaf}$ can be converted to discrimination ($\Delta^{13}C$) to remove the effect of variation of $\delta^{13}C_{air}$ on $\delta^{13}C_{leaf}$ values over time:

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}} \qquad (4)$$

Carbon isotope discrimination is also related to the ratio of CO_2 assimilation to stomatal conductance (A/g), a measure of intrinsic water-use efficiency (iWUE), whereby decreased $\Delta^{13}C$ reflects increased iWUE (Farquhar and Richards 1984, Zhang et al. 1993). Because all seedlings experienced the same values of $\delta^{13}C_{air}$ in this study, differences in $\delta^{13}C_{leaf}$ between populations and treatments would reflect their rankings in terms of $\Delta^{13}C$ and iWUE.

Gas exchange

Gas exchange measurements were made in July and November 2013 to construct photosynthetic CO_2-response ($A–C_i$) curves using a portable open gas exchange system (Li-6400, LiCor, Lincoln, NE, USA) with a 6 cm^2 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 at 1200 μmol m^{-2} s^{-1}. Photosynthesis was initially measured at ambient CO_2 (400 ppm), and then the concentration of CO_2 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 A and g_s were also calculated from foliar $\delta^{13}C$ values and $A - C_i$ curves (Woodruff et al. 2009). Discrimination values ($\Delta^{13}C$) were first calculated from values of foliar $\delta^{13}C$ (Eq. (4)) using $\delta^{13}C_{air} = -8.4$‰. Values of $\Delta^{13}C$ were then used to estimate values for C_i by solving Eq. (5) for C_i at $C_a = 400\ \mu$mol mol^{-1}:

$$\frac{C_i}{C_a} = \frac{\Delta^{13}C - a}{b - a} \qquad (5)$$

Values of C_i were then used to estimate integrated 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 integrated A were used to estimate values of integrated g_s with Eq. (6):

$$g_s = \frac{1.6A}{C_a - C_i} \qquad (6)$$

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, $K_{R\text{-L}}$, $A_L : A_X$ and $\delta^{13}C$) within and across *P. ponderosa* populations. 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 examining plots of the residuals.

Model selection for each analysis was conducted by minimizing Akaike's information criterion. 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 ($\Psi_{\pi100}$), the turgor loss point (Ψ_{TLP}), predawn (Ψ_{pd}) and mid-day (Ψ_{md}) 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, USA) and in R version 3.0.2 (R Core Team 2014) using mixed models ('nlme' package; Pinheiro et al. 2014). $\alpha < 0.05$ was considered statistically significant. Error bars represent one standard error (SE).

Results

During the growing season, there were no significant treatment or population-related differences in either predawn (Ψ_{pd}) or mid-day (Ψ_{md}) water potentials (Table 1). The lack of a significant drought effect on Ψ was due to unseasonably early rain events during the latter portion of the growing season, which reversed the decline in soil water potential in the drought treatment (Figure 1). Consistent with the water potential results, there

Table 1. Mean (±SE) predawn and mid-day water potential (Ψ_{pd}, Ψ_{md}), osmotic potential at full turgor ($\Psi_{\pi100}$) and water potential at the turgor loss point (Ψ_{TLP}) for two populations of *P. ponderosa* seedlings sampled in 2013. Both $\Psi_{\pi100}$ and Ψ_{TLP} differed significantly between populations at $P \leq 0.01$ (denoted by bold).

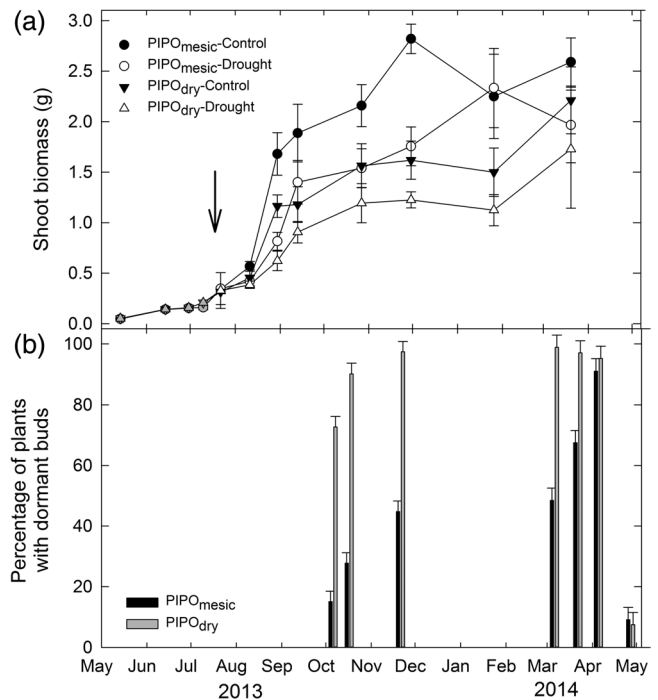| Population	Ψ_{pd} (MPa)	Ψ_{md} (MPa)	$\Psi_{\pi100}$ (MPa)	Ψ_{TLP} (MPa)
PIPO$_{mesic}$	−0.13 ± 0.04	−0.95 ± 0.03	**−1.33 ± 0.06**	**−1.59 ± 0.06**
PIPO$_{dry}$	−0.19 ± 0.07	−0.95 ± 0.07	**−1.55 ± 0.03**	**−1.92 ± 0.07**

Figure 2. Mean shoot biomass (a) and mean percentage of plants with dormant buds (b) for seedlings from two populations of *P. ponderosa* sampled in 2013 and early 2014. Measurements 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 SE.

were no significant treatment-related differences in shoot osmotic potential at full turgor ($\Psi_{\pi100}$) and water potential at the turgor loss point (Ψ_{TLP}) (data not shown). Nevertheless, at the end of the growing season, $\Psi_{\pi100}$ and Ψ_{TLP} were ~0.2 and 0.3 MPa more negative ($P \leq 0.01$), respectively, in PIPO$_{dry}$ compared with PIPO$_{mesic}$ seedlings (Table 1). A subsequent set of P–V measurements conducted when the seedlings were 1-year-old in May 2014 showed both $\Psi_{\pi100}$ and Ψ_{TLP} remaining significantly different between populations ($P \leq 0.01$), but no seasonal or age-related trends (data not shown).

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$_{mesic}$, shoot biomass was significantly greater in control than in droughted seedlings for the August through November sampling dates ($P < 0.0001$, $P = 0.042$). Within PIPO$_{dry}$, there were no statistically significant differences in shoot biomass found between the treatments. In the control treatment, shoot biomass of PIPO$_{mesic}$ seedlings was significantly greater than in PIPO$_{dry}$ seedlings on the September through November sampling dates ($P < 0.0001$, $P = 0.034$). In the drought treatment, shoot biomass of PIPO$_{mesic}$ seedlings was significantly greater than in PIPO$_{dry}$ seedlings on the September, November and January sampling dates ($P = 0.023$, $P = 0.042$). For the three sampling dates in October and November of 2013, the mean percentage of seedlings with dormant buds was ~58% greater ($P < 0.0001$) in PIPO$_{dry}$ (Figure 2b). For the March 2014 sampling dates, the mean percentage of seedlings with dormant buds was still ~40% greater in PIPO$_{dry}$ ($P < 0.0001$), but by early April nearly 100% of seedlings in both populations had dormant buds. Bud break occurred by the end of April with <10% of seedlings in both populations still having dormant buds.

During June, tracheid mean hydraulic diameter (d_H) did not differ significantly between populations, but in July, August and September d_H was substantially greater ($P < 0.001$, $P = 0.027$) in both control and droughted PIPO$_{mesic}$ seedlings (Figure 3a). Within PIPO$_{mesic}$, d_H was significantly greater in control seedlings in August and September ($P < 0.001$, $P = 0.049$). Within PIPO$_{dry}$, there were no statistically significant treatment-related differences in d_H. There were no statistically significant differences in k_{S-theo} between the two populations during June and July

(Figure 3c), but in August and September k_{S-theo} was significantly higher ($P < 0.001$, $P = 0.03$) in both control and droughted PIPO$_{mesic}$ seedlings. Within PIPO$_{mesic}$, k_{S-theo} was significantly higher in control seedlings in August and September ($P = 0.011$, $P = 0.008$). There were no statistically significant treatment-related differences found in k_{S-theo} in PIPO$_{dry}$ seedlings. The areas of the images used for estimates of d_H increased from June through September as new tracheids were produced radially (Figure 3b). Areas of radial files of tracheids did not differ significantly between populations in June, July and August, nor were there any treatment-related differences within PIPO$_{mesic}$ in August or within PIPO$_{dry}$ in August and September. Within PIPO$_{mesic}$, however, image areas for control seedlings were significantly greater than those of droughted seedlings in September ($P < 0.001$), contributing to differences in k_{S-theo} for that sampling date. Among control seedlings, image areas were significantly larger in PIPO$_{mesic}$ in September ($P < 0.001$), again contributing to differences in k_{S-theo}.

There were no statistically significant treatment-related differences found in k_S, k_L, k_{R-L} or $A_L : A_X$ within either population (Figure 4). However, in both the control and drought treatments, k_S was substantially greater ($P \leq 0.031$) in PIPO$_{mesic}$ seedlings (Figure 4a). There were no statistically significant
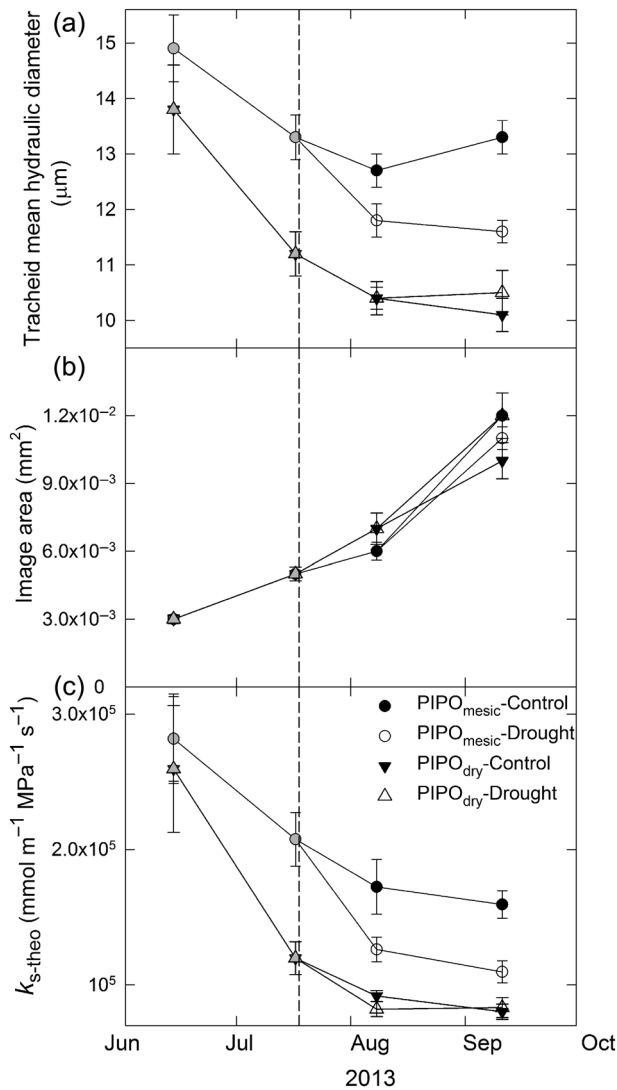
Figure 3. Mean tracheid hydraulic diameters (a), mean areas of images from which hydraulic diameters were estimated (b) and mean $k_{S\text{-theo}}$ (c) of seedlings from two populations of *P. ponderosa* sampled in 2013. Measurements prior to implementation of the drought treatment are denoted by the gray-filled symbols. The vertical dashed lines 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 SE.
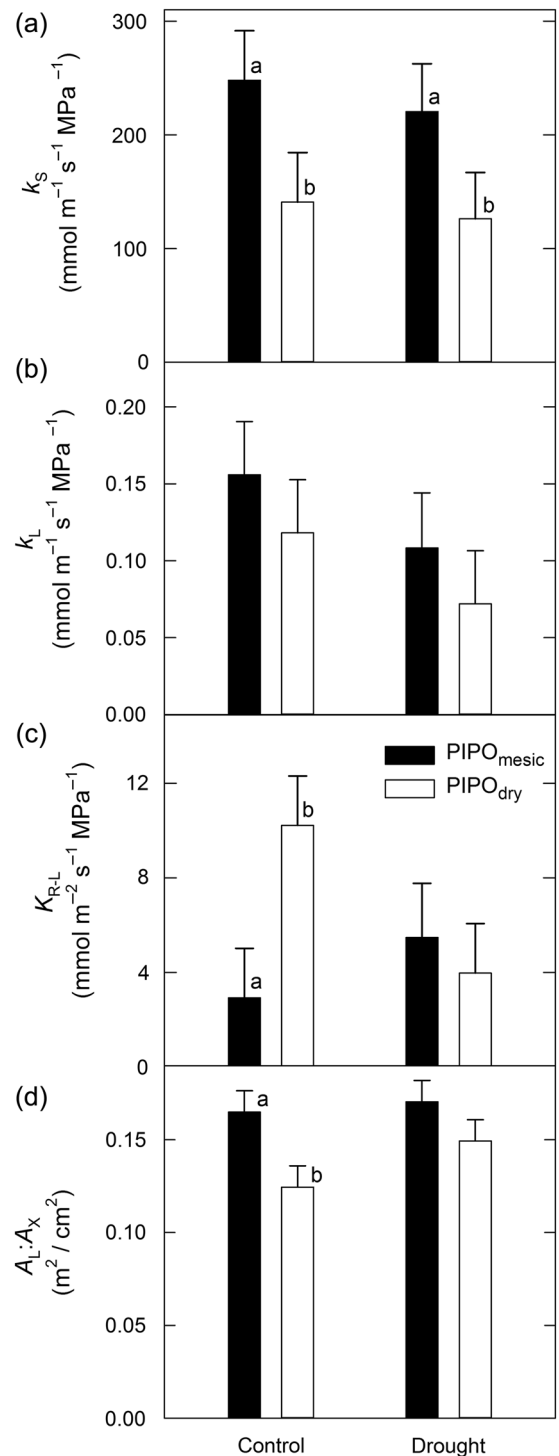

Figure 4. Mean xylem area-specific hydraulic conductivity (k_S; a), mean leaf-specific conductivity (k_L; b), mean leaf-specific root conductance ($K_{R\text{-}L}$; c) and mean ratio of leaf area to xylem area ($A_L : A_X$) (d) from two populations of *P. ponderosa* sampled in 2013. Different letters over bars indicate statistically significant differences at $P < 0.05$ (see text for details). Error bars represent one SE.

population-related differences in k_L (Figure 4b), but in the control treatment, $K_{R\text{-}L}$ of PIPO$_{dry}$ seedlings was over twice that of PIPO$_{mesic}$ seedlings ($P = 0.027$; Figure 4c). These differences in $K_{R\text{-}L}$ were largely attributable to differences in root conductance rather than leaf area. The ratio $A_L : A_X$ was significantly greater in PIPO$_{mesic}$ than in PIPO$_{dry}$ ($P = 0.005$) in the control treatment (Figure 4d).

Over the course of the growing season, foliar $\delta^{13}C$ was consistently less negative, and therefore iWUE was higher, in PIPO$_{dry}$ seedlings (Figure 5). During June and July prior to treatment implementation, and in September, $\delta^{13}C$ values were 0.8–1.6‰ less negative in PIPO$_{dry}$ seedlings ($P < 0.001$, $P = 0.027$). For

September and November, there were no significant treatment-related differences in $\delta^{13}C$ within either population. In November, $\delta^{13}C$ of droughted seedlings was 1.9‰ less negative in PIPO$_{dry}$
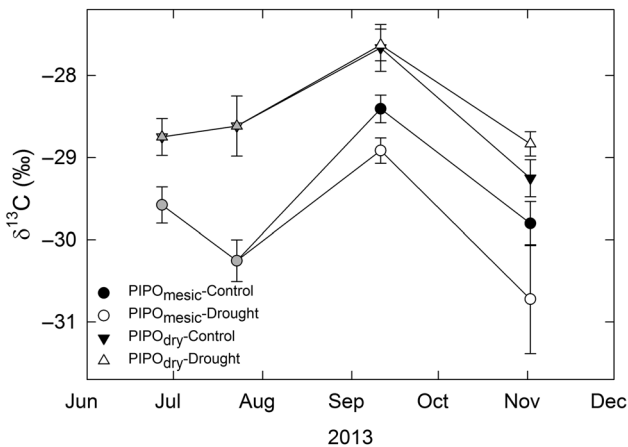
Figure 5. Foliar $\delta^{13}C$ values for two populations of *P. ponderosa* seedlings sampled in 2013. Measurements prior to implementation of the drought treatment are denoted by the gray-filled symbols. See text for details of statistical comparisons. Error bars represent one SE.

Figure 6. $\delta^{13}C$ values (pooled from July and September data points) plotted against leaf-specific conductivity (k_L) for two populations of *P. ponderosa* seedlings sampled in 2013 ($r^2 = 0.95$; $P < 0.001$). Error bars represent one SE.

$(P = 0.03)$. 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, a strong linear relationship ($P < 0.001$, $r^2 = 0.95$) emerged across the treatments and populations (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 with control seedlings. Overall, seedlings receiving the drought treatment exhibited greater iWUE compared with their control counterparts.

There were no 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 $PIPO_{dry}$ and $PIPO_{mesic}$ (Figure 7). When mean growing season assimilation (A) and stomatal conductance (g_s) were estimated from the mean $\delta^{13}C$ values and the A–C_i curve function, A was ~7% greater and g_s ~38% greater in $PIPO_{mesic}$ (Figure 7). Statistical comparisons of estimates of A and g_s were not made owing to the approach employed for obtaining them (see Materials and methods).

Figure 7. Photosynthetic CO_2 response curve (A–C_i) representing pooled data for two populations of *P. ponderosa* seedlings sampled in July and November 2013. Estimates of integrated seasonal rates of CO_2 assimilation (a) and stomatal conductance (g_s) obtained from foliar $\delta^{13}C$ values and the A–C_i curve function are shown. See text for details. Error bars represent one SE.

Discussion

We found large differences in the expression of key functional traits of *P. 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 ($PIPO_{dry}$) 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

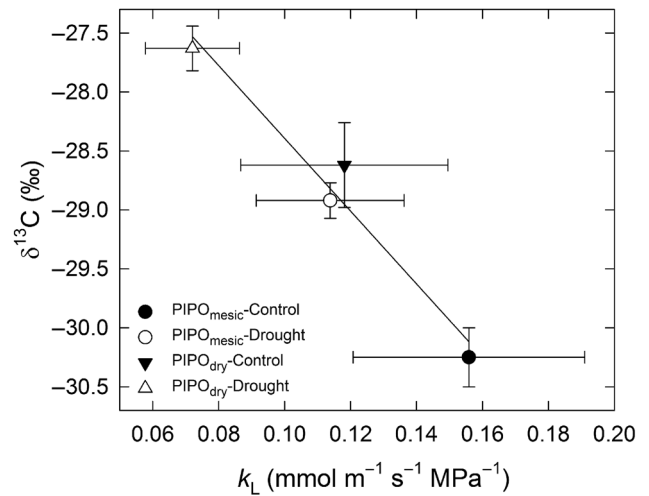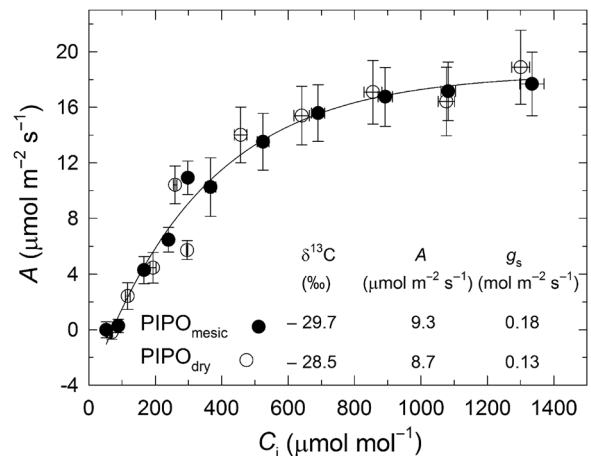among populations, higher iWUE in $PIPO_{dry}$ implied a substantial savings in transpiration at the cost of a relatively small reduction in A (Figure 7). Although k_L did not differ significantly between populations, there was a strong negative relationship between $\delta^{13}C$ and k_L across populations indicative of a close coordination between photosynthetic gas exchange and hydraulic architecture with $PIPO_{dry}$ tending to be more constrained with respect to gas exchange and hydraulics (Figure 6). More negative values of $\Psi_{\pi100}$ and Ψ_{TLP} in $PIPO_{dry}$ imply a greater ability to maintain turgor under a drought-induced decline in plant water status. Finally, $PIPO_{dry}$ seedlings grew more slowly regardless of water availability, and showed earlier onset of dormant bud formation.

The overall more conservative functional behavior of $PIPO_{dry}$ 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 $PIPO_{mesic}$ 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), thus supporting the idea that the populations used in this study were genetically distinct.

We propose four potential non-mutually exclusive causes for the lower shoot biomass production in $PIPO_{dry}$ seedlings in both the control and drought treatments. 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 shown to be a response to drought for many plant species (Greenwood et al. 2008, Schall et al. 2012). Although technical constraints prevented us from quantifying root biomass production and root-to-shoot ratios in our study, greater K_{R-L} in control $PIPO_{dry}$ seedlings was consistent with greater allocation to root biomass. Second, greater stomatal constraints on photosynthesis would have limited the amount of photosynthates available for biomass accumulation. However, greater stomatal constraints on photosynthesis in $PIPO_{dry}$ do not seem sufficient to account for its lower growth rate as integrated estimates of A were only 7% higher in $PIPO_{mesic}$ (Figure 7). Third, an increased demand for energy and metabolites for osmotic adjustment would have decreased the amount of photosynthates available for biomass accumulation. Significantly more negative $\Psi_{\pi100}$ and Ψ_{TLP} in $PIPO_{dry}$ (Table 1) 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, so 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). Finally, the earlier onset of dormancy in $PIPO_{dry}$ under temperature conditions that were still favorable for growth (Figure 2) limited its seasonal growth potential.

While $PIPO_{mesic}$ seedlings continued growing through October and November of 2013, $PIPO_{dry}$ seedlings stopped growth in September (Figure 2a), as reflected in their rates of dormant bud setting (Figure 2b). The more conservative phenological behavior of $PIPO_{dry}$ 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 $PIPO_{mesic}$ 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 $PIPO_{mesic}$ as spring approached (Figure 2b).

All seedlings exhibited ontogenetic changes in stem xylem anatomy that appeared to be associated with environmental seasonality and intrinsic cambial phenology. The initial decline in tracheid mean hydraulic diameter (d_H) from June to July 2013 was likely associated with the onset of warmer temperatures and an increase in 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. Calculations of theoretical xylem-area-specific conductivity (k_{S-theo}) showed a general decline in k_{S-theo} over the growing season for both seedling populations, consistent with generally declining values of d_H (Figure 3). Seasonal, treatment and population-related trends in k_{S-theo} were consistent with those of k_s measured directly with the HPFM (cf. Figures 3c and 4a).

The 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 assessed directly, other studies have shown that a decline in tracheid lumen diameter and an increase in cell wall thickness in conifer xylem generally result 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, 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 $PIPO_{dry}$ than in control $PIPO_{mesic}$ 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 more vulnerable to drought-induced embolism than shoot systems (Kavanagh et al. 1999, Jackson et al. 2000), thus root hydraulic conductance in *P. ponderosa* seedlings could be more sensitive to drought events than shoot conductance. 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 due to embolism in the roots increased. Further, increased root embolism correlated with stomatal closure, suggesting that it may serve as part of a signaling mechanism for maintaining shoot water status.

Consistently less negative foliar $\delta^{13}C$ values and therefore higher iWUE in PIPO$_{dry}$ 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 the climate of origin (Chapin et al. 1987). Pronounced seasonal fluctuations in $\delta^{13}C$ 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 $\delta^{13}C$ values in September. During the fall, shorter days with lower VPD most likely allowed for an increase in g_s and C_i/C_a, leading to increased discrimination and more negative $\delta^{13}C$ values in November. In order for changes in stomatal regulation of gas exchange of mature leaves to be reflected in their bulk leaf $\delta^{13}C$ values, there must have been a sizable component of the leaf carbon, such as non-structural carbohydrates, that was replaced over time (Chapin et al. 1990, Damesin et al. 1998, Woodruff and Meinzer 2011). Seasonal, as well as inter-annual, trends in foliar $\delta^{13}C$ 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 $\delta^{13}C$ in a beech forest exposed to varying summer water availability and observed that $\delta^{13}C$ in leaves increased seasonally following a decrease in soil water content. Previous studies have found that discrimination values are correlated with climate, particularly with MAP (Bowling et al. 2002, Diefendorf et al. 2010). Therefore, populations within a species from drier climates would be expected to exhibit lower discrimination provided that the populations represent genetically distinct ecotypes, which is apparently the case for the two *P. ponderosa* populations studied here.

The impacts of hydraulic constraints on photosynthetic gas exchange were clear when comparing the $\delta^{13}C$ values with the k_L values for both populations. Whereas PIPO$_{dry}$ may have been more hydraulically constrained overall, the negative relationship between foliar $\delta^{13}C$ and k_L indicated that iWUE increased with decreasing k_L in both treatment and population comparisons (Figure 6). Several studies have reported on the link between plant hydraulic properties and $\delta^{13}C$ values (e.g., Panek 1996, Cernusak and Marshall 2001, Martínez-Vilalta et al. 2004,

2009) and many have reported on the relationship between g_s and hydraulics in tree species (e.g., Sperry and Pockman 1993, Saliendra et al. 1995, Hubbard et al. 2001, Santiago et al. 2004). In 4–5-year-old *P. ponderosa* seedlings, g_s decreased linearly with decreasing leaf-specific hydraulic conductance (Hubbard et al. 2001), while foliar $\delta^{13}C$ values became less negative with decreasing branch leaf-specific conductivity in 30-year-old *Pseudostuga menziesii* (Mirb.) Franco 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$_{dry}$ 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 mid-day plant Ψ prior to the heavy rain events in September (Table 1) 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 (K.L. Kerr, personal observation). Nevertheless, both $\Psi_{\pi100}$ and Ψ_{TLP} were inherently lower in PIPO$_{dry}$ seedlings (Table 1) implying a greater capacity for turgor maintenance than PIPO$_{mesic}$ 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 *Pinus pinaster* Aiton (Nguyen-Queyrens and Bouchet-Lannat 2003). Vance and Zaerr (1991) determined $\Psi_{\pi100}$ for shaded and unshaded 5-month-old *P. ponderosa* seedlings and found that shaded seedlings had higher $\Psi_{\pi100}$ 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$_{dry}$ seedlings that would have otherwise been used for growth.

In summary, the more conservative behavior of *P. ponderosa* seedlings from the dry site population suggests growth is reduced because of the requirement 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$_{dry}$ seedlings are likely due to genetic variation within *P. 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.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Acknowledgments

Conflict of interest

Funding

References

Allen CD, Macalady AK, Chenchouni H et al. (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.

Bowling DR, McDowell NG, Bond BJ, Law BE, Ehleringer JR (2002) ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit. Oecologia 131:113–124.

Callaham RZ (2013) *Pinus ponderosa*: a taxonomic review with five subspecies in the United States. Research Paper. PSW-RP-264. US Department of Agriculture Forest Service, Albany, CA. 52 p.

Cernusak LA, Marshall JD (2001) Responses of foliar δ^{13}C, gas exchange and leaf morphology to reduced hydraulic conductivity in *Pinus monticola* branches. Tree Physiol 21:1215–1222.

Chapin FS III, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. BioScience 37:49–57.

Chapin FS III, 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, Lotan JE (eds) Proceedings, Symposium on ponderosa pine—the species and its management. Washington State University, Pullman, WA, pp 27–43.

Creese C, Benscoter 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 δ^{13}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.

Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH (2010) Global patterns in leaf ^{13}C discrimination and implications for studies of past and future climate. Proc Natl Acad Sci USA 107: 5738–5743.

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-seeding thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinaceae) trees. Am J Botany 93:1588–1600.

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, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Funct Plant Biol 11:539–552.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537.

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.

Graham RT, Jain TB (2005) Ponderosa pine ecosystems. In: Proceedings of the Symposium on Ponderosa Pine: Issues, Trends, and Management. General Technical Report PSW-GTR-198. Pacific Southwest Research Station, Forest Service, US Department of Agriculture, Albany, CA. 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.

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, LachenbruchDawson BTE (eds) Size- and age-related changes in tree structure and function. Springer, Dordrecht, The Netherlands, pp 65–87.

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. For Sci 13:286–294.

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 CO_2 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 27:237–277.

Little EL (1971) Atlas of United States trees. Volume 1. Conifers and important hardwoods. Miscellaneous publication 1146. US Department of Agriculture, Forest Service, Washington, DC.

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.

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

Martínez-Vilalta J, Sala A, Piñol J (2004) The hydraulic architecture of Pinaceae—a review. Plant Ecol 171:3–13.

Martínez-Vilalta J, Cochard H, Mencuccini M et al. (2009) Hydraulic adjustment of Scots pine across Europe. New Phytol 184:353–364.

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.

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, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21:489–495.

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.

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 WC, Pallardy SG (1987) The influence of resaturation method and tissue type on pressure-volume analysis of Quercus alba L. seedlings. J Exp Bot 38:535–549.

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 (21 April 2015, date last accessed).

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/ (21 April 2015, date last accessed).

Rehfeldt GE (1990) Genetic differentiation among populations of Pinus ponderosa from the Upper Colorado River Basin. Bot Gaz 151:125–137.

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in Betula occidentalis. Planta 196:357–366.

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 31:1127–1139.

Sperry JS, Pockman WT (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in Betula occidentalis. Plant Cell Environ 16:279–287.

Stephenson NL (1990) Climatic control of vegetation distribution: the role of the water balance. Am Nat 135:649–670.

Stocker TF, Qin D, Plattner GK et al. (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, UK and New York, NY, USA. 1535 p.

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

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119:345–360.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23:267–282.

Tyree MT, Sinclair B, Lu P, Granier A (1993) Whole shoot hydraulic resistance in *Quercus* species measured with a new high-pressure flowmeter. Ann Sci For 50:417–423.

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.

Vivin P, Guehl JM, Clément A, Aussenac G (1996) The effects of elevated CO_2 and water stress on whole plant CO_2 exchange, carbon allocation and osmoregulation in oak seedlings. Ann Sci For 53:447–459.

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

Wang C (1977) Genetics of ponderosa pine. Research Paper. WO-34. US Department of Agriculture, Forest Service, Washington, DC. 24 p.

Western Regional Climate Center. http://www.wrcc.dri.edu/ (9 October 2014, date last accessed).

Woodruff DR, Meinzer FC (2011) Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant Cell Environ 34:1920–1930.

Woodruff DR, Meinzer FC, Lachenbruch B, Johnson DM (2009) Coordination of leaf structure and gas exchange along a height gradient in a tall conifer. Tree Physiol 29:261–272.

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.

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.