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WHAT CAUSES LOWER TREELINE?
CHANGES IN HYDRAULIC PARAMETERS OF DOUGLAS-FIR (*PSEUDOTSUGA*
MENZIESII) ALONG AN ELEVATION GRADIENT

by
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Committee Approval

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CHAPTER 1. GENERAL INTRODUCTION

Conifer forest ecosystems in the Western United States are an important resource. They provide many ecosystem services and are an important carbon sink for atmospheric carbon dioxide. In fact, forest ecosystems sequester approximately 70% of carbon in the Western United States (Zhu & Reed 2012). There is concern for how forest distributions will change in future climate scenarios, wherein the length of the summer dry season is predicted to increase due to earlier snowpack melt (Barnett *et al.* 2005; Klos *et al.* 2014). Trees, as sessile organisms, are particularly susceptible to rapid climate change because their migration to areas with more suitable climates can only occur through seed dispersal and establishment of the next generation of individuals (Shafer *et al.* 2001), which may or may not keep up with the pace of changing climate. Tree die-off has been observed in the Western U.S. as an apparent response to changes in climate (e.g. Allen & Breshears 1998; Bigler *et al.* 2007; Breshears *et al.* 2009; van Mantgem *et al.* 2009; Allen *et al.* 2010b) however, the first step to understanding how forest distributions will change in future climate scenarios is to understand the underlying physiological mechanisms that define how trees adapt to changes in climate.

Vegetation communities in the Intermountain West are strongly driven by elevation-based climates (Kusbach *et al.* 2014). Forest distributions are bounded by an upper (alpine) treeline and a lower treeline. The causes of alpine treeline have been heavily researched (e.g. Cairns & Malanson 1998; Körner 1998; Körner & Paulsen 2004; Brodersen *et al.* 2006a; Beckage *et al.* 2008; Dawes *et al.* 2011; Kitzberger *et al.* 2014; Lenz *et al.* 2014) and temperature seems to be the main environmental factor that limits

tree growth at alpine treelines (Richardson and Friedland, 2009). Lower treeline is thought to be associated with moisture limitations. These moisture limitations can be episodic or chronic in nature. For example, Allen and Breshears (1998) measured a mortality event at the lower treeline of a ponderosa pine forest in northern New Mexico that coincided with a 1950's drought. Radial growth and seedling recruitment in *Pinus* and *Juniper* species at lower treeline has shown to vary depending on moisture availability, where increased radial growth and episodes of seedling establishment were associated with high moisture availability (Earl & Bash 1996; League & Veblen 2006; Weber *et al.* 2007). In contrast, Ettinger *et al.* (2011) found that lower treeline conifers in the Pacific Northwest were less sensitive to climate in lower treelines vs. upper treelines based on annual growth. These few studies support the hypothesis that lower treeline is related to water availability, but changes in growth alone is not a thorough enough explanation for what is causing lower treeline.

My research aims to directly test if and how water limitations play a physiological role in determining lower treeline. To do this, I compared the water relations of Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) along an elevation gradient, with lower treeline being the lowest elevation. Because I believe that lower treeline is caused by water limitations, I utilized plant hydraulics methods to assess how hydraulic transport capacity, vulnerability to drought induced hydraulic dysfunction, and various leaf-branch morphological measurements in leaves (shoots of current year growth) and branches of Douglas-fir make adjustments with elevation.

Plant Hydraulics is the study of how plants regulate the transport of metastable water under tension and can tell us how plants respond to drought (Sperry & Love 2015).

The cohesion-tension theory states that water ascends through plants by a pulling force that is generated through transpiration at the leaves. The hydrostatic pressure through the adhesion of water to conduit walls and cohesion of water molecules to each other maintains a continuous water column under tension (Dixon 1914). However, water that is transported through xylem vessels is susceptible to cavitation—the formation of embolisms—because the tension of xylem water falls well below its vapor pressure. Plants employ a variety of strategies to either reduce or tolerate cavitation. Hydraulic vulnerability to cavitation, hereafter simply referred to as hydraulic vulnerability, is the measure of how susceptible a plant is to reductions water transport (hydraulic dysfunction) due to increased tension in the xylem.

The hydraulic conductivity of leaf and branch tissues are in part a function of tissue water potential, where conductivity decreases as tension increases (Tyree 1997). Determining vulnerability to drought-induced hydraulic dysfunction involves measuring plant tissue conductance at known intervals of water potential, as embolism are introduced either through bench-drying or the use of a pressure sleeve or centrifuge (Sperry & Saliendra 1994). The response of hydraulic conductance to decreasing water potentials is usually either sigmoidal or exponential (Cochard *et al.* 2013). Different patterns of reduced hydraulic transport capacity, such as a rapid loss over a narrow water potential range versus a gradual decline in conductance, and important ecological parameters, such as the water potential corresponding to 50% loss in conductance, can be derived from vulnerability curves and be used to make inter- and intraspecific comparisons (Pammenter & Willigen 1998; Domec & Gartner 2001). For this study we chose to measure hydraulic parameters in both leaves and branches because conifers

exhibit “hydraulic vulnerability segmentation”, where the more distal portions of trees (leaves) are more vulnerable to hydraulic dysfunction than more proximal portions (branches) (Johnson *et al.* 2016).

Conifer wood xylem is homogenous and consists only of very small similarly sized water conducting tracheid cells compared to angiosperms that possess conducting vessel elements which can vary in length and diameter and have various non-conducting tissue for water and solute storage. Thus, conifers typically exhibit a clear trade-off whereby they are less vulnerable to cavitation, but are less efficient at conducting water than angiosperms. Hydraulic vulnerability also differs among conifer taxa and these difference have been shown to play a role in how conifers are geographically distributed (e.g. Cochard 1992, Brodribb and Hill 1999, Maherali *et al.* 2004, Willson and Jackson 2006, Delzon *et al.* 2010, Barnard *et al.* 2011, Anderegg and Hillerislambers 2016). Characterizing water movement through conifer needles is complicated because it involves the movement of water outside of xylem pathways which may be more dependent on leaf mesophyll anatomy (Sack & Holbrook 2006). Thus, the response of leaf hydraulic vulnerability to drought is less clear with leaves showing both increased (Blackman *et al.* 2011, 2014) and decreased (Bucci *et al.* 2012) vulnerability with increased mean annual precipitation.

The goal of this research is to quantify intraspecific variation in various leaf and branch hydraulic parameters along an elevation gradient to see how tree water relations are related to forest elevational zones, especially lower treeline. This is an important first step to understanding how conifer forest distributions in the Intermountain West may change given future climate scenarios.

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**CHAPTER 2. WHAT CAUSES LOWER TREELINE?: CHANGES IN
HYDRAULIC PARAMETERS OF DOUGLAS-FIR ALONG AN ELEVATION
GRADIENT**

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Abstract

Lower treeline in the Intermountain West, U.S. is defined as the lowest elevation at which conifer woodlands grow (~1500-1800m) as the forest transitions to sagebrush and grassland steppe communities. Lower treeline is generally assumed to be caused by water limitations to growth and whole plant water relations, yet few studies directly show this. To describe and quantify how lower treeline might be associated with water limitations we measured changes in hydraulic transport capacity, drought-induced hydraulic vulnerability, hydraulic safety margins, and various morphological measurements in leaves and branches of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) along an elevation gradient (1600-2100m) in southeastern Idaho that included lower treeline. We hypothesized that, with decreasing elevation, leaves and branches would exhibit reduced hydraulic transport capacity, less hydraulic vulnerability, and wider hydraulic safety margins as conditions became drier. We also predicted that leaf-branch morphology would reflect increased drought tolerance. We found that, with decreasing elevation, branches became less vulnerable to hydraulic dysfunction and decreased in maximum hydraulic conductivity. Leaves showed no trends in hydraulic vulnerability, but increased in hydraulic conductance with decreasing elevation. Our results indicate that leaves and branches of Douglas-fir employ different hydraulic strategies as environmental conditions become more arid with decreasing elevation approaching lower treeline. In support of the hydraulic vulnerability segmentation hypothesis, leaves were consistently more vulnerable to hydraulic dysfunction than branches. One possible model to explain lower treeline conifer hydraulics is by trees that have hydraulically efficient leaves and branches that have relatively limited water transport capabilities, but are less

vulnerable to hydraulic dysfunction. In this case, lower treeline appears to be caused by trees' hydraulic vulnerability and safety trade-off limitations, especially in the branches.

Introduction

Montane conifer forests in the Intermountain West, USA are bounded by upper and lower treelines. The causes of upper (alpine) treeline have been heavily researched (e.g. Cairns & Malanson 1998; Körner 1998; Körner & Paulsen 2004; Brodersen *et al.* 2006a; Beckage *et al.* 2008; Dawes *et al.* 2011; Kitzberger *et al.* 2014; Lenz *et al.* 2014) and thermal constraints to photosynthetic carbon gain (i.e. Smith *et al.* 2009) and/or processing (i.e. Li *et al.* 2002) seem to be the main causal factors of alpine treeline in the Intermountain West. Lower treelines in the Intermountain West are generally assumed to be associated with water limitations to whole plant water relations and tree productivity. For example, lower treeline trees have shown to have reduced radial growth relative to individuals at higher elevations (League & Veblen 2006; Weber *et al.* 2007; Anderegg & Hillerislambers 2016). These few studies lend indirect evidence to the concept that tree growth beyond lower treeline elevation is constrained by water limitations, but an ecophysiological explanation for what is causing lower treeline is lacking in the scientific literature.

Transpiration in leaves creates tension that pulls a continuous water column from the roots, through the plant, to the atmosphere (Cohesion-Tension Theory; Dixon 1914). When evaporative demand creates negative pressure (tension) in the xylem water that falls below its vapor pressure, cavitation can occur. Embolisms formed by cavitation limit water transport and may reduce growth and survival (Sperry & Tyree 1990). As moisture becomes limiting to plant growth, plants use a variety of structural and physiological

mechanisms to maintain tissue hydration and water transport. This includes reduced tracheid size (e.g. Brodribb & Hill 1999; Hacke & Sperry 2001; McCulloh *et al.* 2014), increased leaf mass per area (Mitchell *et al.* 2008), and increased stomatal regulation (e.g. Tardieu and Simonneau 1998, Kavanagh *et al.* 1999, Mitchell *et al.* 2008, Meinzer *et al.* 2009) to effectively transport water while minimizing cavitation. With severe cavitation, however, hydraulic transport is sufficiently compromised, and mortality can occur (Allen *et al.* 2010b; Anderegg *et al.* 2014; McDowell *et al.* 2015).

Previous studies have shown that various metrics of branch vulnerability (susceptibility to reduced water transport with increased tension) and associated morphological traits in conifers change along elevation and climate gradients, with branches from dry sites exhibiting less vulnerability to drought (more negative P50 values- the water potential associated with 50% loss in hydraulic vulnerability (Pammenter & Willigen 1998)) (e.g. Maherali & DeLucia 2000b; Sparks & Black 2000; Maherali *et al.* 2002; Martinez-Vilalta *et al.* 2009; Anderegg & Hillerislambers 2016). For example, Sparks and Black (2000) found that two lower treeline conifer species (*Larix occidentalis* and *Pinus contorta*) in northern Idaho were less sensitive to tension induced xylem cavitation than two upper treeline species (*Larix lyallii* and *Pinus albicaulis*) in northern Montana. Leaf-level vulnerability to drought has also been shown to decrease with aridity, with woody plant species exhibiting more negative leaf P50 values at sites that receive less precipitation (Blackman *et al.* 2011, 2014). Thus, leaf- and branch-level vulnerability to drought-induced hydraulic dysfunction represent a direct metric of drought tolerance of various conifer tree species (e.g. Brodribb and Holbrook, 2006; McDowell *et al.*, 2008; Meinzer *et al.*, 2009; Blackman *et al.*, 2014; Martínez-

Vilalta et al., 2014; Anderegg and Hillerislambers, 2016), and may correspond well with species' ranges. At the dry edges of species' ranges, such as lower treeline, one might expect leaves and branches to be less vulnerable (more tolerant) to drought-induced hydraulic dysfunction. However, there are limited case studies that measure intraspecific vulnerability to drought in conifers along elevation gradients, inclusive of lower treeline (Anderegg, 2016).

Here, we tested the hypothesis that lower treelines of conifer forests in the Intermountain West are associated with limitations in plant water transport by measuring intraspecific variations in various hydraulic parameters in leaves and branches Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*)—a common lower treeline species in the Intermountain West— along an elevation gradient in southeastern Idaho. Specifically, we measured: (1) leaf and branch vulnerability to drought-induced hydraulic dysfunction ($P50_{\text{leaf}}$, $P50_{\text{branch}}$, and the air entry point (P_e)), (2) leaf cell water relations (the water potential at turgor loss point (Ψ_{TLP})), (3) various leaf and branch hydraulic safety margins ($\Psi_{\text{min}} - P50_{\text{branch}}$, $P_e - P50_{\text{branch}}$, $\Psi_{\text{min}} - P50_{\text{leaf}}$, and $\Psi_{\text{min}} - \Psi_{\text{TLP}}$), (4) maximum hydraulic branch conductivity and leaf conductance (K_{branch} and k_{leaf}), and (5) morphological traits associated with drought tolerance/avoidance (leaf mass per area (LMA) and leaf area to sapwood area ($A_l:A_s$)) at 5 sites along an elevation gradient in southeastern Idaho. Since the environment becomes more arid with decreasing elevation, we predicted that with decreasing elevation approaching lower treeline: (1) leaves and branches would be less vulnerable to drought-induced hydraulic dysfunction ($P50_{\text{branch}}$ and $P50_{\text{leaf}}$ would decrease), (2) leaf cell water relations would reflect greater drought tolerance (Ψ_{TLP} would decrease), (3) hydraulic safety margins would become wider, (4)

maximum hydraulic transport capacity would be reduced (K_{branch} and k_{leaf} would decrease), and (5) LMA would increase and $A_l:A_s$ would decrease. Additionally, we predicted leaves would be more vulnerable to hydraulic dysfunction at all elevations, in support of the hydraulic vulnerability segmentation hypothesis (Tyree & Ewers 1991; Johnson *et al.* 2016).

Materials and Methods

Plant Material Collection and Study Design

Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) leaf and branch samples were collected from stands on northwest facing slopes on Scout Mountain (42°43'00'' - 42°40'50'' N and 112°42'56'' - 112°21'31'' W, Bannock Mountain Range, Caribou-Targhee NF) in southeast Idaho. Five collection sites were established along an elevation gradient (1696m, 1807m, 1895m, 2015m, and 2109m). For simplicity, the sites will hereafter be referred to as 1700m, 1800m, 1900m, 2000m, and 2100m throughout the paper. Within sites, collection elevation did not differ more than 5 m in elevation. Total annual precipitation, mean annual temperature, and mean annual maximum vapor pressure deficit at each site were estimated from modeled data from 30-year climate normals (PRISM Climate Group) (Table 1).

At each site, leaves about 5 cm in length and branches 7-10 mm in diameter were collected on the south side of trees at approximately 3.5 meters above the ground from trees whose trunk diameter was 45-55 cm in diameter at 1.5 meters from the ground. For the purpose of this study, a “leaf” is considered the branch tip of current year’s growth. Unique sample collections for each site and measurement type were carried out from

mid-July to early-November 2016. All plant materials collected in the field were double-bagged with a wet paper towel to minimize water loss, placed in a cooler, and transported back to the lab for analysis immediately after being collected. Leaf samples were collected predawn and were not rehydrated prior to measurement in order to prevent artifacts in measures associated with rehydration (Trifilò *et al.* 2014).

Leaf Cell Water Relations: Pressure-Volume Curves

Pressure-volume (P-V) curves were plotted for 5-7 leaf samples from each site based on methods described by Turner (1981) and Brodribb and Holbrook (2003). In the laboratory, P-V curves were derived by periodically measuring leaf mass using a balance and water potential with a Scholander pressure chamber (PMS, Albany, OR, USA) as leaves bench-dried. Dry mass was determined following the completion of each curve by drying leaves in an oven at 65°C for over 60 hours. The parameters of interest included the water potential at turgor loss point (Ψ_{TLP}), osmotic water potential at full turgor (Ψ_{osm}), relative water content at full turgor (RWC_{TLP}), bulk modulus of elasticity (ϵ), and absolute capacitance.

Leaf-level Hydraulic Conductance and Vulnerability Curves

At each site, about 30 leaves from 10-12 trees were collected for leaf hydraulic conductance (k_{leaf}) and leaf hydraulic vulnerability curves. Following methods used in Blackman *et al.* (2011) leaf vulnerability curves were constructed by measuring changes in k_{leaf} in leaves as they dried on a bench top. k_{leaf} was determined using the kinetics of leaf water potential (Ψ_{leaf}) relaxation upon leaf rehydration, as described by Brodribb and Holbrook, (2003). Three three-pronged shoots (i.e. branch tips with three stems of the current year's growth) were cut predawn while leaf water potential (Ψ_{leaf}) was high (least

negative for that day) from 10-15 individuals of Douglas-fir per site. In the laboratory, the shoots were bench dried for various time increments, ranging from 0 to 10 hours, to a target range of leaf water potentials. After bench-drying, leaves were bagged for at least 2 hours to prevent water loss and ensure water potential equilibrium throughout the entire three-pronged shoot. This resulted in a set of shoots covering a range of water potentials of approximately -0.4 to -3.5 MPa. Initial leaf water potential (Ψ_{leaf}^0) was measured on one of the “prongs” of the shoot using a Scholander pressure chamber (PMS, Albany, OR, USA). Another “prong” of the leaf was cut under deionized water (filtered to 0.2 μm), allowed to rehydrate for 60 s, and double-bagged for 2 m to allow water potentials to equilibrate throughout before measuring final leaf water potential (Ψ_{leaf}^f) with the pressure chamber. k_{leaf} ($\text{mmol m}^{-2} \text{MPa}^{-1} \text{s}^{-1}$) was calculated using the ratio of the Ψ_{leaf}^0 , Ψ_{leaf}^f , and the absolute capacitance of the leaf (C_{leaf})

$$k_{\text{leaf}} = \frac{C_{\text{leaf}} \ln\left(\frac{\Psi_{\text{leaf}}^0}{\Psi_{\text{leaf}}^f}\right)}{t},$$

where Ψ_{leaf}^0 = initial leaf water potential (MPa); Ψ_{leaf}^f = final leaf water potential (MPa); t = duration of rehydration (s); and C_{leaf} = average absolute leaf capacitance ($\text{mmol m}^{-2} \text{MPa}^{-1}$) determined from P-V curves.

Based on methods used in Blackman et al. (2011), leaf vulnerability curves were created by fitting a three-parameter sigmoidal regression function to the k_{leaf} versus Ψ_{leaf}^0 data from each site (equation coefficients in Supplemental Information Table 1). $P50_{\text{leaf}}$ is defined as the Ψ_{leaf}^0 at which k_{leaf} had declined by 50% from maximum values and was determined visually using Sigma Plot (version 12.5, Systat Software, Inc., San Jose California USA). Leaf hydraulic safety margins for each site were calculated as Ψ_{min} -

$P50_{\text{leaf}}$, where Ψ_{min} the average mid-day water potential of leaves at each site ($n=7$) in late August and early September. $\Psi_{\text{min}} - \Psi_{\text{TLP}}$ was also used as a type of leaf hydraulic safety margin. Maximum leaf conductance ($k_{\text{leaf-max}}$) was defined for each site independently as the mean of the 5 maximum k_{leaf} values that were measured before or shortly after the bench-drying process began.

Branch-level Hydraulic Conductivity and Vulnerability Curves

Five to eight 7-10 mm diameter branches were collected for branch hydraulic conductivity measurements (K_{branch}) and branch hydraulic vulnerability curves from each site. In the laboratory, branches were cut to a 10-16 cm length segment, and trimmed of all leaves and lateral branches. Cyanoacrylate glue was applied to the sides of the branch where lateral branches had been trimmed to prevent water loss during measurements. The ends of the branches were re-cut with a fresh razor blade under deionized water (filtered to $0.2 \mu\text{m}$ and acidified to $\text{pH}=2$) and submerged in the same filtered and acidified water in a container where a partial vacuum was applied overnight to refill all embolized tracheids. Before measurements began, the cut ends of the branch segments were inspected while still under partial vacuum to ensure no bubbles were still being pulled from the xylem.

After the segments were flushed of embolisms, branch hydraulic conductance measurements using the air injection method (Cochard *et al.* 1992; Sperry & Saliendra 1994) were used to construct vulnerability curves. We repeatedly alternated between measuring branch hydraulic conductance and introducing emboli to the branches at applied pressures from 1-7 MPa at 1 MPa increments using a pressure sleeve. Hydraulic conductance (k_{branch}) ($\text{cm}^3 \text{s}^{-1} \text{MPa}^{-1}$) was measured using a Sperry apparatus by timing

intervals for water to reach successive gradations on a pipette attached to tubing to the distal end of the branch segment, and dividing this volume flow rate by the hydrostatic pressure gradient along the stem created by a hydrostatic pressure head (approximately 55 cm high) connected to the proximal end of the branch with tubing. Sap-wood specific hydraulic conductivity (K_{branch}) ($\text{g m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was calculated by multiplying conductance by the length of the stem segment (m) and then dividing by branch sapwood area (m^2).

Branch vulnerability curves were created by graphing the percentage loss in conductivity (PLC) against applied pressure. PLC was calculated as

$$\text{PLC} = 100 \times \left(1 - \left(\frac{K_{\text{branch}}}{K_{\text{branch-max}}} \right) \right) ,$$

where $K_{\text{branch-max}}$ is the conductivity measured after the branch had been flushed of all embolisms.

Data for each elevation were fit with a three-parameter sigmoidal regression function of the form of

$$y = \frac{a}{\left(1 + e^{\frac{1}{b}(x-P50)} \right)} ,$$

where a is the y-intercept, b is an indicator of the slope, and $P50$ is the water potential at which 50% loss in conductivity occurred ($P50_{\text{branch}}$) (Pammenter & Willigen 1998; Domec & Gartner 2001) (equation coefficients in Supplemental Information Table 2). Parameters derived from these curves include $P50_{\text{branch}}$, the air entry point (P_e , threshold water potential where conductivity decreases rapidly), and two types of safety margins

(P_e - $P50_{\text{branch}}$, an estimate of the steepness of the vulnerability curve between P_e and $P50_{\text{branch}}$ and a type of safety margin (Domec & Gartner 2001; Meinzer *et al.* 2009), and Ψ_{min} - $P50_{\text{branch}}$)

Leaf and Branch Morphology

To measure leaf mass per area (LMA), needles were arranged on a bench and photographed with a scale bar, and analyzed for total leaf area using ImageJ software (Schneider *et al.* 2012). Then needles were dried at 65°C for at least 60 hours. To measure the ratio of leaf area to sapwood area ($A_l:A_s$), branch diameter was measured with calipers twice and averaged. All needles distal to the location of the branch diameter measurement were removed, subsampled for leaf area and dry mass. Total leaf area was calculated by dividing the subsampled LMA by the total dry mass of all needles distal to the sapwood area measurement.

Statistics

Least squares regression analysis was used to fit curves to data. For leaf and branch vulnerability curves, 3-parameter sigmoidal equations were used. To correlate the hydraulic parameters derived from leaf and branch vulnerability curves to elevation, linear regression was used for all parameters except $A_l:A_s$, $P50_{\text{branch}}$, and $\Psi_{\text{min}} - P50_{\text{branch}}$, which were fit using a quadratic function. SigmaPlot (Version 12.5, Systat Software, San Jose, CA) was used for all statistical analysis.

Results

Leaf Cell Water Relations: Pressure-Volume Curves

There was no variation in parameters estimated from pressure-volume curves, except for Ψ_{TLP} (Table 2). Ψ_{TLP} decreased significantly with decreasing elevation (Figure 1) and was between 0.23-0.74 MPa less than Ψ_{min} at all sites.

Leaf-level hydraulic parameters

Maximum leaf hydraulic conductance ($k_{\text{leaf-max}}$) increased significantly with decreasing elevation and ranged from 0.27 – 15.06 mmol m⁻² MPa⁻¹ (Figure 2). On average, $k_{\text{leaf-max}}$ at the 1700 m site at lower treeline was about 9 times greater than the high elevation 2100 m site (Table 3). The ratio of maximum leaf conductance to maximum branch conductivity ($k_{\text{leaf-max}} : K_{\text{branch-max}}$) increased significantly with decreasing elevation (Figure 2). Leaf hydraulic vulnerability did not significantly correlate with elevation (Figure 3); $P50_{\text{leaf}}$ (estimated from leaf vulnerability curves shown in Figure 4) ranged from -1.09 MPa to -2.0 MPa and did not significantly vary with elevation (Tables 2 and 3). $\Psi_{\text{min}} - P50_{\text{leaf}}$ was not significantly correlated with elevation (Figure 5), however, $\Psi_{\text{min}} - \Psi_{\text{TLP}}$, (another type of safety margin) showed a marginally significant increase ($R^2=0.68$, $p=0.09$) with decreasing elevation (Figure 5).

Branch-level hydraulic parameters

Exhibiting an opposite trend compared to leaves, maximum branch hydraulic conductivity ($K_{\text{branch-max}}$) decreased significantly with decreasing elevation (Figure 2), ranging from 256 to 567 g m⁻¹ MPa⁻¹ s⁻¹ (Table 3). The $K_{\text{branch-max}}$ at the lower treeline 1700 m site was 26% and 32% less than the two highest elevation sites (2100 m and 2000 m, respectively). $P50_{\text{branch}}$ (estimated from branch vulnerability curves in Figure 6) generally decreased with decreasing elevation, showing a quadratic relationship (Figure 3). $P50_{\text{branch}}$ values ranged from -5.92 MPa to -4.30 MPa (Figure 3) with the lowest $P50_{\text{branch}}$ being at lower treeline. $P_e - P50_{\text{branch}}$ (estimated from Figure 6) increased with decreasing elevation (Figure 5). $\Psi_{\text{min}} - P50_{\text{branch}}$ generally increased with decreasing elevation, showing a quadratic relationship

with elevation similar to $P50_{\text{branch}}$ (Figure 5). On average, $P50_{\text{leaf}}$ was about 3 times greater than $P50_{\text{branch}}$ at all sites (-1.56 MPa versus -4.89 MPa) (Figure 3 and Table 3). Branch safety margins ($P_e - P50_{\text{branch}}$ and $\Psi_{\text{min}} - P50_{\text{branch}}$) were 2.47 – 3.17 MPa greater than leaf safety margins ($\Psi_{\text{min}} - P50_{\text{leaf}}$ and $\Psi_{\text{min}} - \Psi_{\text{TLP}}$), with $\Psi_{\text{min}} - P50_{\text{leaf}}$ consistently having a negative value (Figure 5 and Table 3).

Morphology

Leaf mass per area (LMA) increased with decreasing elevation (Figure 7). The ratio of leaf area to sapwood area ($A_l : A_s$) generally increased with decreasing elevation, showing a quadratic relationship with elevation (Figure 8).

Discussion

We found that Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) made adjustments in various hydraulic and morphological parameters at the leaf- and branch-level with decreasing elevation that are characteristic of drought tolerance. Our study site in southeastern Idaho is characterized by decreasing precipitation and increasing vapor pressure deficit with decreasing elevation (Table 1). These gradients in elevation-based climate had a clear effect on branch and leaf hydraulic transport ability and morphological traits. While leaf-level hydraulic vulnerability did not correlate significantly with elevation (Figure 3), branch hydraulic vulnerability parameters and the Ψ_{TLP} show to decrease with elevation (Figures 3 and 1). Branches at lower treeline were more resistant to hydraulic dysfunction, but exhibited a trade-off by limiting water transport efficiency (Figure 2). Unexpectedly, for leaves, we observed increased hydraulic transport (Figure 2) and no trend in hydraulic vulnerability with decreasing elevation (Figure 3).

Branch water relations

Branch-level hydraulic parameters varied with changing elevation as predicted, where clinal patterns were consistent with increasing drought tolerance. Branches appeared to be able to transport more water at high elevations per sapwood area (Figure 2). Such trends of increasing $K_{\text{branch-max}}$ with elevation have also been observed in conifers along elevation gradients (Sáenz-Romero *et al.* 2013; Anderegg & Hillerislambers 2016). In contrast, others have found increasing or no change in intraspecific branch-specific conductivity in conifers at drier locations (Maherali & DeLucia 2000b; Martinez-Vilalta *et al.* 2009; Barnard *et al.* 2011). However, branch conductivity alone may not be the best parameter for explaining drought tolerance, as it is not always associated with site water availability in conifers (e.g. Maherali *et al.* 2004). Instead, branch vulnerability has consistently shown to decrease with decreased water availability (e.g. Brodribb and Hill, 1999; Sparks and Black, 2000b; Stout and Sala, 2003; Willson and Jackson, 2006). Consistent with this, branches from lower elevation sites were generally less vulnerable to drought-induced hydraulic dysfunction as indicated by more negative $P50_{\text{branch}}$ values (Figure 3). Overall, $P50_{\text{branch}}$ values were much more negative than Ψ_{min} (Table 3). This is due to the isohydric nature of Douglas-fir, in which stomata strongly regulate xylem water potential as a response to increases in vapor pressure deficit (Meinzer 1982). We found that the decline of $P50_{\text{branch}}$ with elevation was explained by quadratic relationship (Figure 3), with the lowest $P50_{\text{branch}}$ value measured at lower treeline and the second lowest $P50_{\text{branch}}$ value measured at the highest elevation site (Table 3). Our data do not explain the quadratic relationship between $P50_{\text{branch}}$ and elevation. This observation may be explained by the occurrence of the

highest and lowest elevation sites at the ecotones of the Douglas-fir dominant forest community where the Douglas-fir tree stands become patchy as the community transitions to other dominant species. Changes in biotic factors such as plant-plant interactions (Loehle 1998; Ettinger *et al.* 2011) and abiotic factors like leaf area index (Schultz 2003) that occur at forest have been shown to influence plant water status.

Branch hydraulic safety margins generally increased with decreasing elevation, where $\Psi_{\min} - P50_{\text{branch}}$ showed a quadratic relationship to elevation (driven by clinal changes in $P50_{\text{branch}}$, which were also quadratic) and $P_e - P50_{\text{branch}}$ increased linearly with decreasing elevation (Figure 5). Since there were no significant differences in midday water potential between elevation (Table 3), $\Psi_{\min} - P50_{\text{branch}}$ shows a similar trend as $P50_{\text{branch}}$, generally increasing with decreasing elevation (Figure 5). The increase in $P_e - P50_{\text{branch}}$ with decreasing elevation indicates that branches at low elevations are able to maintain water transport after the air entry point (P_e) over a greater range of decreasing water potentials. Meinzer *et al.* (2009) show that hydraulic safety margins increase with decreasing species-specific values for $\Psi_{\min}$, however we did not observe changes in $\Psi_{\min}$ with decreasing elevation. In this case, we observed a safety efficiency trade off in Douglas-fir branches with increasing aridity. A recent review by Gleason *et al.* (2016) has shown that the efficiency-safety tradeoff generally lacks supporting evidence among most woody species, however they, along with Piñol and Sala (2000), show that Douglas-fir is one of the few gymnosperm species that does exhibit the efficiency-safety trade off in branches. The species-specific nature of branch hydraulic trade-offs may somewhat limit the application of our findings to broad geographic scales, but is important to understand for Douglas-fir as a common lower treeline species.

Cell and leaf water relations

For tissue-water relations, the water potential at turgor loss point (Ψ_{TLP}) was the only parameter derived from the P-V curves that was significantly correlated with elevation (Table 2), and it declined (became more negative) with decreasing elevation (Figure 1). This indicates that individuals at low elevations exhibit leaf cell water relations characteristic of plants that are more adapted to arid conditions, i.e. decreasing Ψ_{TLP} with decreasing elevation. Briefly, the Ψ_{TLP} is the water tension in the leaf tissue corresponding to the point at which leaf-tissue cells begin to lose water—and therefore turgor—as extra-cellular water depletes as leaves dry. Interestingly, Ψ_{TLP} and Ψ_{min} values were close, with the Ψ_{TLP} being 0.23 to 0.74 MPa more negative than Ψ_{min} . Ψ_{TLP} has shown to correspond to mean minimum xylem tensions of ponderosa pine (Maherali & DeLucia 2000a), to correlate with stomatal closure (Brodribb & Holbrook 2003), and be correlated to the leaf water potential where k_{leaf} falls to zero (Brodribb & Holbrook 2006). Thus, the dynamics of maintaining leaf cell turgor and associated water potentials may be an important governor of lower treeline.

Our prediction that leaf hydraulic vulnerability would decrease with decreasing elevation was based off of previous studies that show an increase in $P50_{\text{leaf}}$ with increasing precipitation in Australian and Peruvian woody plant species (Blackman *et al.* 2011, 2014). However, we did not see any relationship between $P50_{\text{leaf}}$ and elevation (Figure 3) or leaf safety margin (Table 5). It may be that $P50_{\text{leaf}}$ is trivial functional trait for Douglas-fir. Additionally, our study site was characterized not only by a precipitation gradient, but also by temperature and VPD gradients, which might be more important abiotic factors that drive leaf hydraulic functioning (Meinzer 1982). Bouche *et al.* (2016)

recently found that assessing leaf hydraulic vulnerability using the rehydration kinetics method may only be valid at the whole-needle level and that decline of hydraulic conductance in *Pinus pinaster* was not due to embolism in the xylem but instead to hydraulic dysfunction of extra-xylary needle tissue. Thus, ground tissue water dynamics derived from pressure-volume analysis (i.e. Ψ_{TLP}) may be better for assessing fine scale adjustments in leaf hydraulic vulnerability and water transport capacity. Still, even if $P50_{leaf}$ is overestimated, comparisons using the same method among sites is still a useful tool for assessing leaf hydraulic vulnerability.

Opposite from what was hypothesized, we observed an increase in maximum leaf conductance $k_{leaf-max}$ with decreasing elevation (Figure 2) and an overall increase in $A_l:A_s$ (Figure 8). Though we cannot fully explain this we believe it may be related to the need for transpirational cooling at warmer low elevation sites. We did not directly measure leaf stomatal conductance, but it has been shown to be positively correlated with leaf conductance in temperate conifers (Brodribb *et al.* 2012). Thus, one explanation for greater $k_{leaf-max}$ with decreasing elevation is that trees at low elevations must make hydraulic adjustments to increase water transport to the leaves due to a longer growing season and increased solar radiation and temperature at lower treeline. Similarly, Mitchell *et al.* 2008, found plants at drier sites in southwestern Australia showed smaller reductions in stomatal conductance and Ψ_{TLP} , and had species with greater LMA. We suspect that the timing of bud burst and shoot elongation coincided with favorable spring growing conditions for our sample collection year (2016) (i.e. high soil moisture availability, mild air temperatures and vapor pressure deficit) and may explain the increase in $A_l:A_s$ at lower treeline. We observed an increase in LMA with decreasing

elevation, which is common in more xeric environments (i.e. Mitchell *et al.* 2008; Scoffoni *et al.* 2011). Moreover, leaf size and venation density have been shown to be coordinated with leaf conductance (Scoffoni *et al.* 2011), but the relationships between entire leaf conductance, mesophyll conductance, stomatal conductance, and leaf morphological traits are difficult to study and methodologies and ideas are still being developed (Flexas *et al.* 2013). Nevertheless, our findings from this study indicate that Douglas-fir are making adjustments in their hydraulic transport capacity and morphology to adjust to increased aridity at lower treeline.

Comparing Leaves to Branches

As hypothesized, branches of Douglas-fir at all elevations had a much more negative $P_{50_{\text{branch}}}$ than $P_{50_{\text{leaf}}}$ (Figure 3), indicating that branches are much less vulnerable to drought than leaves. Similar results were observed by Mcculloh *et al.* (2014) in coastal Douglas-fir trees in Washington and by Johnson *et al.* (2016) in *Pinus pinea* and *Juniperous ashei*. Additionally, Douglas-fir consistently had $P_{50_{\text{leaf}}}$ values greater than Ψ_{min} while $P_{50_{\text{branches}}}$ were consistently less than Ψ_{min} (Table 3). Thus, leaves regularly lost >50% of hydraulic conductivity while branches never lost more than 15% of maximum hydraulic conductivity. Distal leaves most likely act as circuit breakers to protect proximal branch xylem networks in support of the hydraulic vulnerability segmentation hypothesis (Tyree & Ewers 1991; Johnson *et al.* 2016), highlighting the importance of leaves as a hydraulic bottleneck (Sack & Holbrook 2006).

Our results indicate that leaves and branches employ very different hydraulic strategies as environmental conditions become more xeric approaching lower treeline. One possible model to explain lower treeline hydraulics in Douglas-fir is trees that have

hydraulically efficient leaves and branches that have relatively limited water transport capabilities, but are less vulnerable to hydraulic dysfunction. In this case, leaves and branches are on different ends of a safety-efficiency trade-off spectrum, where the leaf's role as the main driver of water transport is emphasized. Similar results of coordinated leaf and branch hydraulic functioning were observed in *Nothofagus* species from various climates in South America, where there was a positive relationship across species between percent loss in conductivity in the stems and percent loss in conductance in the leaves; and greater leaf conductance and wider safety margins were correlated with greater branch wood density (Bucci *et al.* 2012).

What causes lower treeline?

We found clinal changes in various leaf and branch hydraulic parameters with decreasing elevation, and our data set supports the common postulation that water relations limit tree growth at lower treeline. Approaching lower treeline from above elevations, hydraulic safety margins (e.g., $P_e - P_{50_{\text{branch}}}$) increased, vulnerability to drought (e.g., $P_{50_{\text{branch}}}$) decreased, branch water transport capacity ($K_{\text{branch-max}}$) decreased, and Ψ_{TLP} in leaves decreased and was always greater than Ψ_{min} . Hydraulic safety margins increase, and hydraulic transport efficiency decreases, with greater drought exposure/decreased water availability. Additionally, the $P_{50_{\text{branch}}}$ values we report (-5.9 MPa) for the lower treeline site are the lowest of any values P_{50} reported in the literature for Douglas-fir (-4.35 to -5.75 MPa) (Maherali *et al.* 2004). Finally, Bond & Kavanagh (1999) discuss the existence of a threshold leaf water potential for Douglas-fir based on literature values of -2.1 to -2.4 MPa and that tight stomatal regulation associated with this

range of threshold water potentials is important for hydraulic functioning. In agreement, we observed minimum midday water potentials in Douglas-fir *in situ* ranging from -1.96 to -2.21 MPa. For this study, minimum midday water potential was always 0.23 to 0.74 MPa greater than the water potential at turgor loss point. Therefore, it may be that Douglas-fir's inability to maintain leaf cell turgor at elevations below lower treeline is a cause. Collectively, our data suggest that Douglas-fir-dominated lower treelines may be caused by hydraulic safety-efficiency trade-off limitations in the branches. However, other considerations related to the ecology, population biology, and ecophysiology of lower treelines are needed. This includes more research on the limitations of seedling establishment and photosynthetic carbon gain and transport, and biotic interactions at the lower treeline ecotone.

Acknowledgements

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Figures for Chapter 2

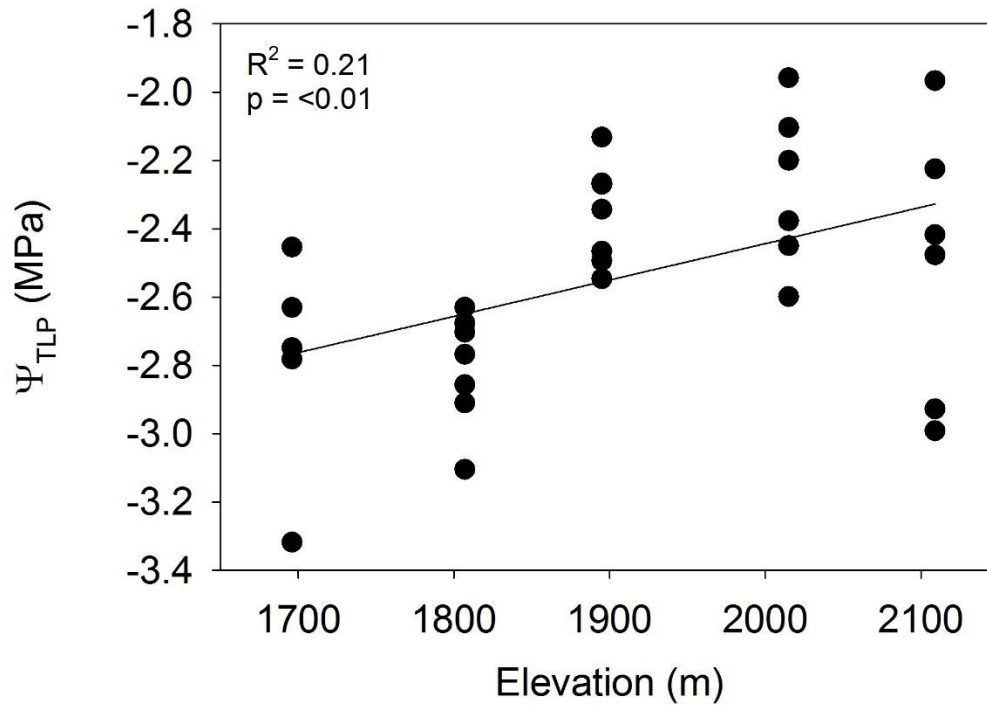


Figure 1. The osmotic potential at the turgor loss point (Ψ_{TLP}) of Douglas-fir leaves (n=5-7) measured at 5 sites along an elevation gradient in southeastern Idaho.

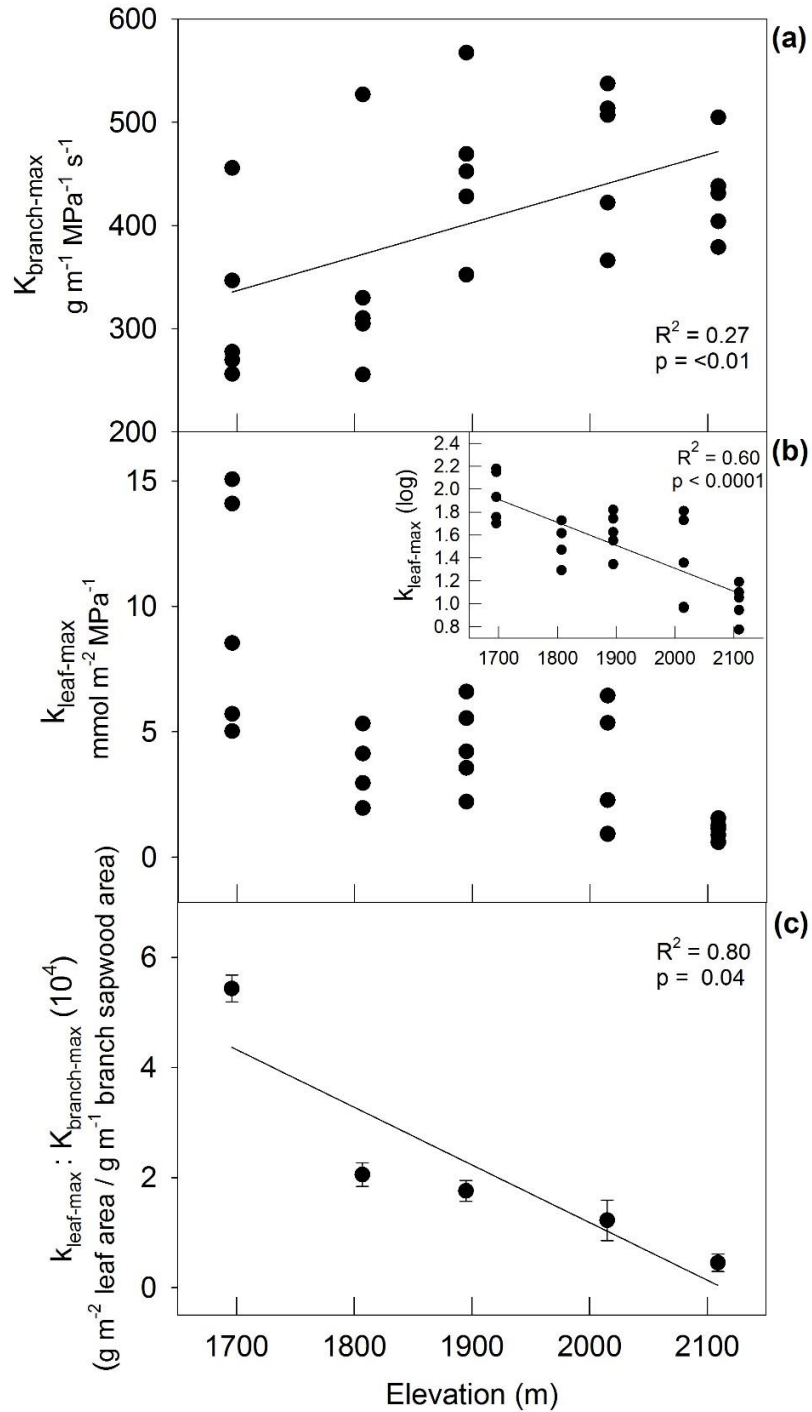


Figure 2. Maximum hydraulic (a) branch conductivity ($K_{\text{branch-max}}$), (b) leaf conductance ($k_{\text{leaf-max}}$), and (c) the ratio of maximum leaf conductance to maximum branch conductivity ($k_{\text{leaf-max}} : K_{\text{branch-max}}$) of Douglas-fir ($n=5$) measured at 5 sites along an elevation gradient in southeastern Idaho. To obtain homogeneity of variance, $k_{\text{leaf-max}}$ data were log transformed prior to analysis.

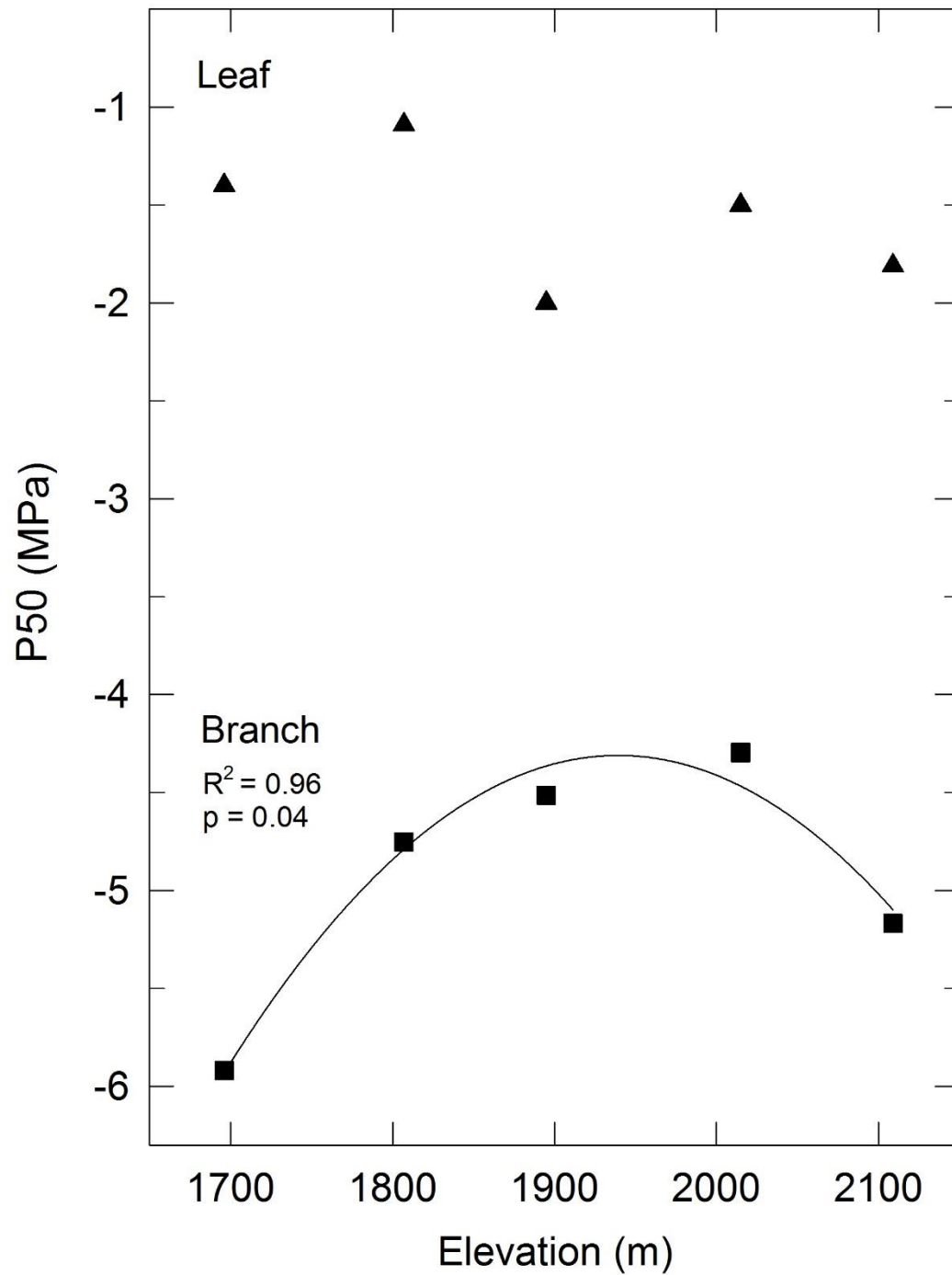


Figure 3. The water potential at 50% loss of leaf hydraulic conductance ($P50_{leaf}$) and at 50% loss of hydraulic conductivity in branches ($P50_{branch}$) of Douglas-fir leaves measured at 5 sites along an elevation gradient in southeastern Idaho. $P50$ values for each elevation are derived from the leaf and branch vulnerability curves (Figures 4 and 6). $P50_{branch}$ data were fit with a quadratic function.

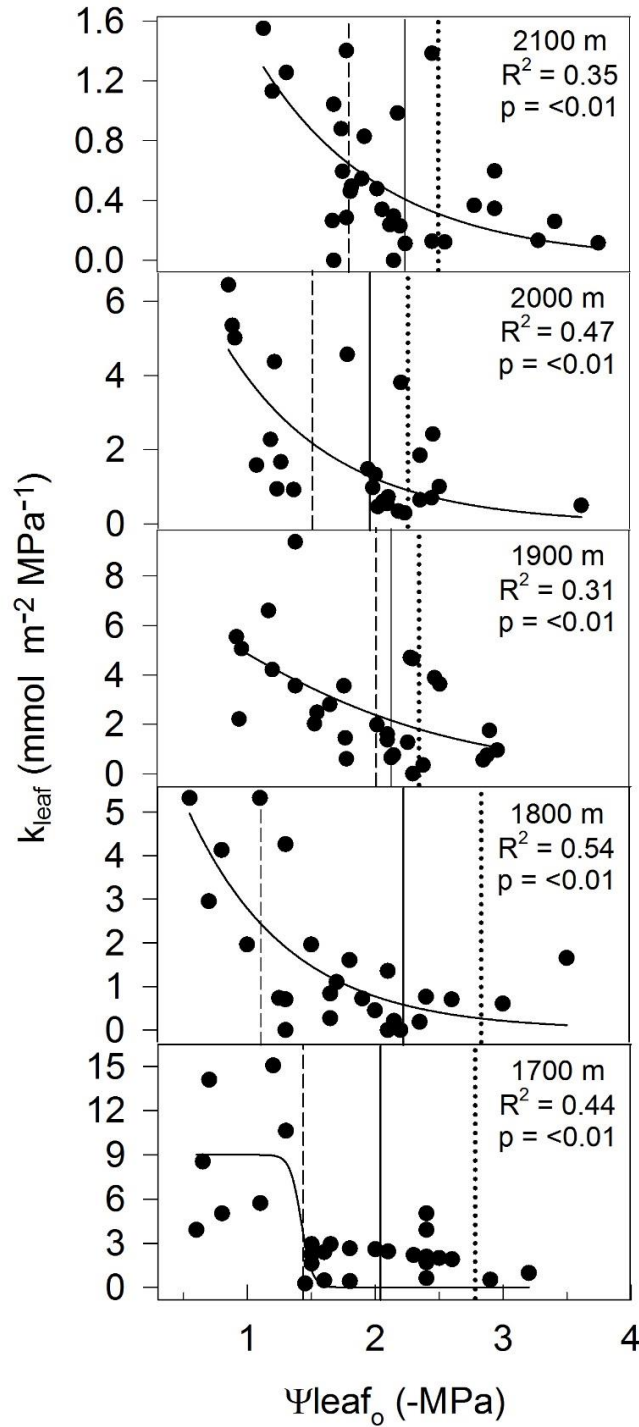


Figure 4. Leaf vulnerability curves of Douglas-fir leaves derived from about 30 leaves from 10-12 separate individuals at 5 sites along an elevation gradient (1700 m -2100 m) in southeastern Idaho. Lines were fit using a three-parameter sigmoidal function. Dashed vertical lines represent $P50_{\text{leaf}}$, solid vertical lines represent Ψ_{min} , and dotted vertical lines represent Ψ_{TLP} .

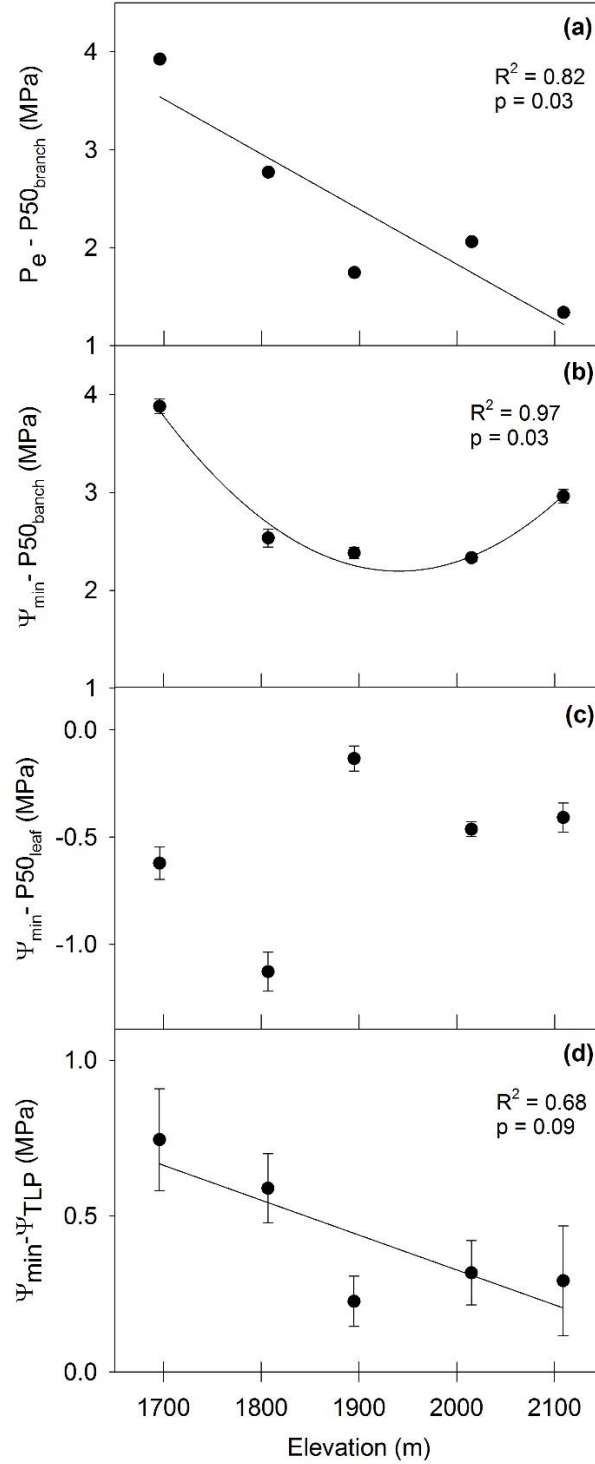


Figure 5. Hydraulic safety margins for branches: (a) $P_e - P50_{branch}$ and (b) $\Psi_{min} - P50_{branch}$, and leaves: (c) $\Psi_{min} - P50_{leaf}$ and (d) $\Psi_{min} - \Psi_{TLP}$ in Douglas-fir measured at 5 sites along an elevation gradient in southeastern Idaho. Error bars represent standard error ($n=5$). There is no standard error for $P_e - P50_{branch}$ because parameters are based on bulk data. For $\Psi_{min} - P50_{branch}$, the line is a quadratic fit to the data.

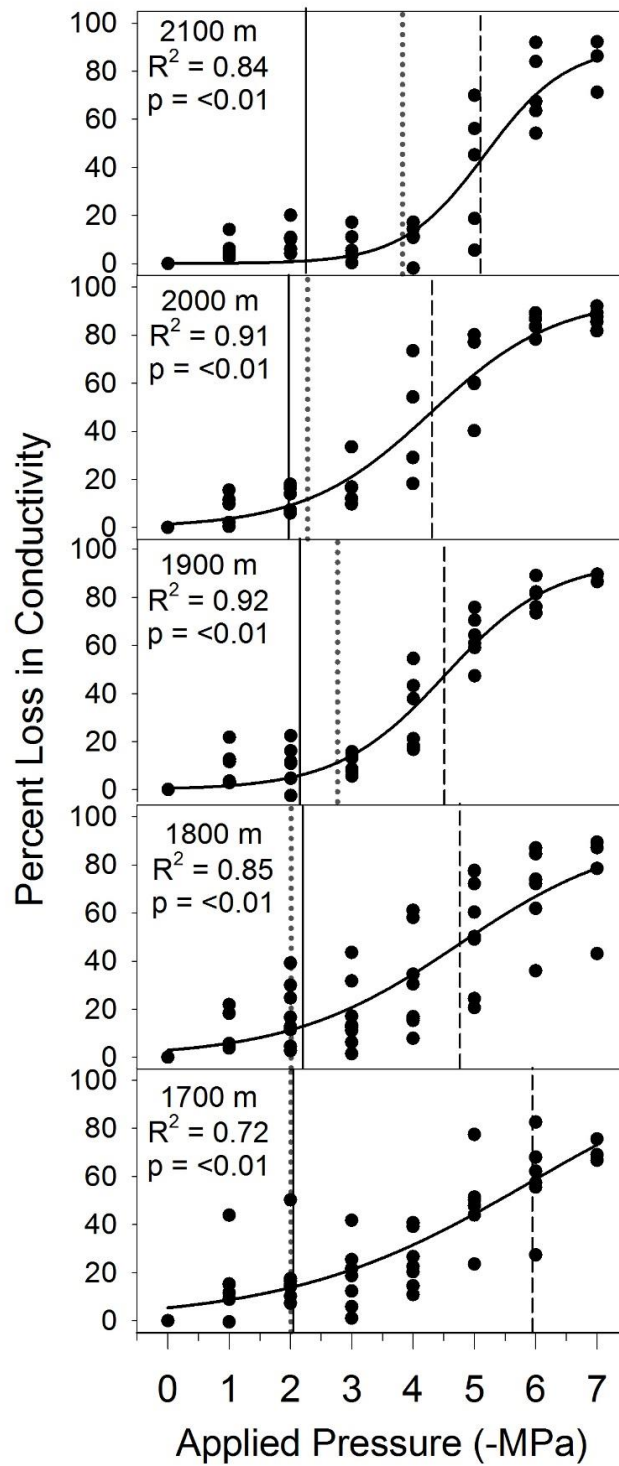


Figure 6. Branch vulnerability curves of Douglas-fir derived from 6-8 branches from separate individuals at 5 sites along an elevation gradient (1700 m -2100 m) in southeastern Idaho. Lines were fit using a three-parameter sigmoidal function. Dashed vertical lines represent $P_{50_{branch}}$, solid vertical lines represent Ψ_{min} , and dotted vertical lines represent P_e .

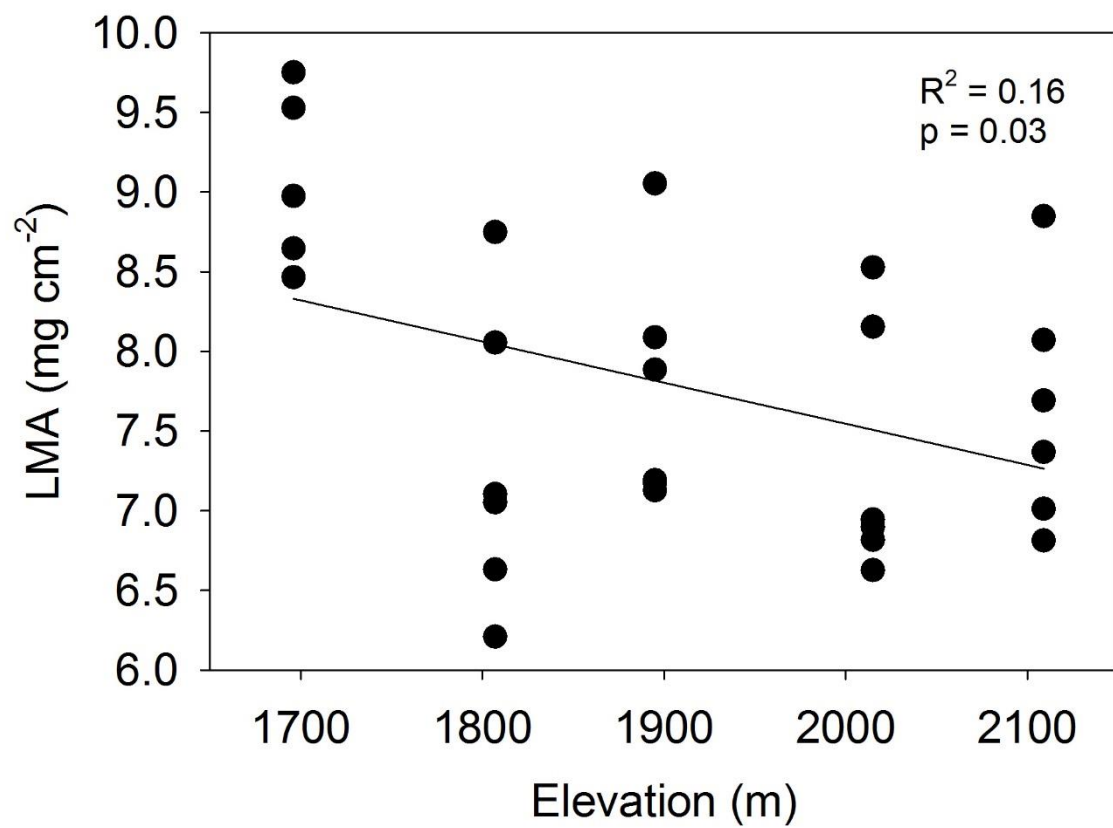


Figure 7. Leaf mass per area (LMA) measured in 5-6 Douglas-fir leaf samples at 5 sites along an elevation gradient in southeastern Idaho.

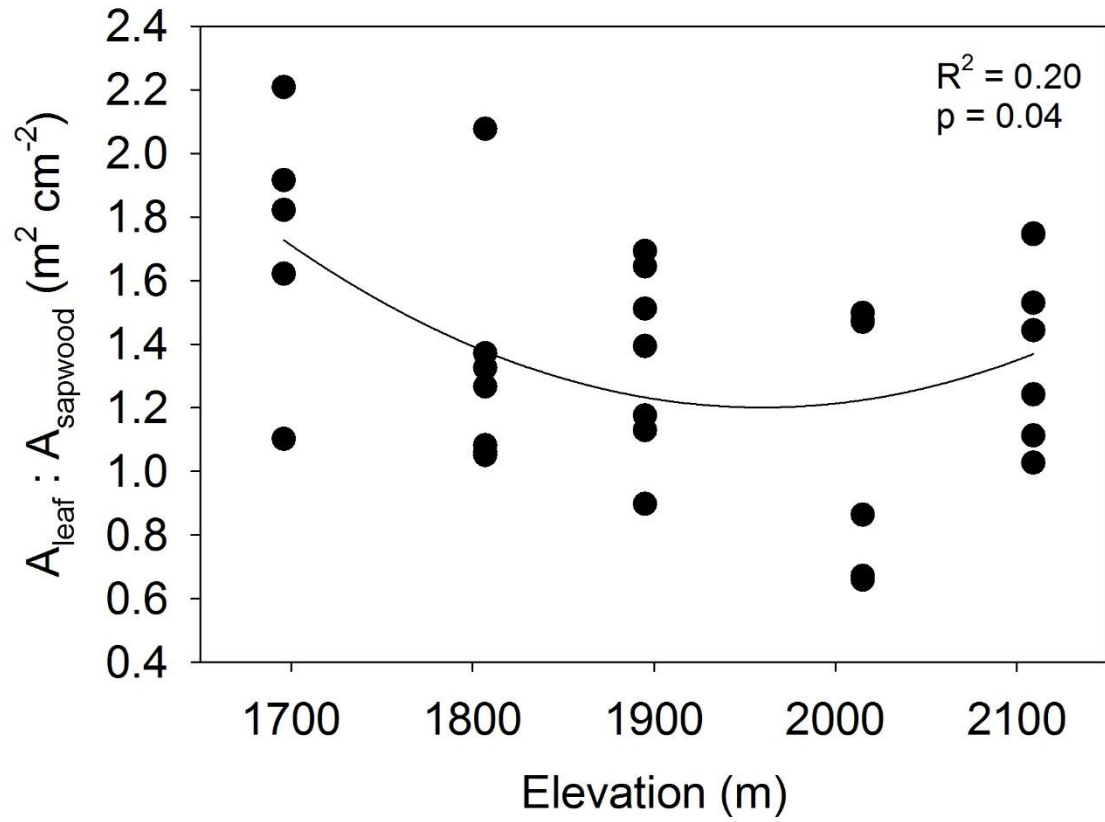


Figure 8. The ratio of leaf area to sapwood area ($A_l:A_s$) of Douglas-fir ($n=5-7$) at 5 sites along an elevation gradient in southeastern Idaho. The line is a quadratic fit to the data.

Tables for Chapter 2

Table 1. Climate (total annual precipitation, mean annual temperature, and mean monthly maximum vapor pressure deficit) at each site was estimated from modeled data from 30 year climate normals (PRISM Climate Group).

Elevation (m)	Total annual precipitation (mm)	Mean annual temperature (°C)	Mean monthly maximum VPD (kPa)
1700	580	7.6	1.41
1800	638	7.2	1.36
1900	700	6.4	1.26
2000	745	5.6	1.21
2100	798	5	1.13

Table 2. Linear and nonlinear regression equation coefficients of various cell-, leaf-, and branch-level hydraulic parameters across elevation where Y_0 is the y-intercept and a is slope. $P50_{\text{branch}}$ and $\Psi_{\text{min}} - P50_{\text{branch}}$ were fit with a nonlinear polynomic quadratic equation and therefore have a coefficient b . Abbreviations are: Π_o , osmotic potential at full turgor; Ψ_{TLP} , water potential at turgor loss; RWC_{TLP} , relative water content at turgor loss point; ϵ , bulk modulus of elasticity; C_{leaf} , absolute leaf capacitance; $k_{\text{leaf-max}}$, maximum leaf conductance; $P50_{\text{leaf}}$, water potential at 50% loss of maximum leaf conductance; $\Psi_{\text{min}} - P50_{\text{leaf}}$, minimum midday water potential minus $P50_{\text{leaf}}$; $K_{\text{branch-max}}$, maximum branch hydraulic conductivity; $P50_{\text{branch}}$, water potential at 50% loss of maximum branch conductivity; $\Psi_{\text{min}} - P50_{\text{branch}}$, minimum midday water potential minus $P50_{\text{branch}}$; $P_e -$

Coefficient	Π_o (MPa)	Ψ_{TLP} (MPa)	RWC_{TLP} (%)	ϵ (MPa)	C_{leaf} (mol m ⁻² MPa ⁻¹)	$k_{\text{leaf-max}}$ (mmol m ⁻² MPa ⁻¹)	$P50_{\text{leaf}}$ (MPa)	$\Psi_{\text{min}} - P50_{\text{leaf}}$ (MPa)
y_0	-3.57	-4.57	79.4	-49.8	0.795	5.31	0.487	2.44
a	0.000865	0.0011	0.00445	0.0381	-0.0002	-0.002	-0.0011	-0.001
R^2	0.859	0.213	0.00086	0.075	0.0106	0.60	0.248	0.193
p	0.110	0.0089	0.620	0.136	0.582	<0.0001	0.393	0.458

$P50_{\text{branch}}$, air entry point minus $P50_{\text{branch}}$; LMA, leaf mass per area; $A_l:A_s$, the ratio of leaf area to sapwood area.

Coefficient	$K_{\text{branch-max}}$ (g m ⁻¹ MPa ⁻¹ s ⁻¹)	$P50_{\text{branch}}$ (MPa)	$\Psi_{\text{min}} - P50_{\text{branch}}$ (MPa)	$P_e - P50_{\text{branch}}$ (MPa)	$\Psi_{\text{min}} - \Psi_{\text{TLP}}$ (MPa)	$k_{\text{leaf-max}} : K_{\text{branch-max}}$	LMA (mg cm ⁻²)	$A_l:A_s$
y_0	-223.7	-107.2	105.1	13.1	2.56	22.12	12.7	30.28
a	0.330	0.11	-0.11	-0.0056	-0.001	-0.01	-0.003	-0.03
R^2	0.270	0.96	0.97	0.82	0.68	0.80	0.16	0.20
p	0.0076	0.036	0.025	0.034	0.086	0.041	0.034	0.038
b		-2.74 x 10 ⁻⁵	-2.73 x 10 ⁻⁵					7.57 x 10 ⁻⁶

Table 3. Mean and standard deviation of various hydraulic parameters at all sites. Parameters derived from vulnerability curves do not have a standard error associated with them because they are estimated from vulnerability curves (Figure 2 and 3) from bulk data. Abbreviations are: Π_o , osmotic potential at full turgor; Ψ_{TLP} , water potential at turgor loss; RWC_{TLP} , relative water content at turgor loss point; ϵ , bulk modulus of elasticity; C_{leaf} , absolute leaf capacitance; $k_{leaf-max}$, maximum leaf conductance; $P50_{leaf}$, water potential at 50% loss of maximum leaf conductance; $\Psi_{min} - P50_{leaf}$, minimum midday water potential minus $P50_{leaf}$; $K_{branch-max}$, maximum branch hydraulic conductivity; $P50_{branch}$, water potential at 50% loss of maximum branch conductivity; $\Psi_{min} - P50_{branch}$, minimum midday water potential minus $P50_{branch}$; $P_e - P50_{branch}$, air entry point minus $P50_{branch}$; LMA, leaf mass per area; $A_l:A_s$, the ratio of leaf area to sapwood area.

Site elevation (m)	Ψ_{min} (MPa)	$k_{leaf-max}$ ($\text{mol m}^{-2} \text{MPa}^{-1} \text{s}^{-1}$)	$K_{branch-max}$ ($\text{g m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$)	$k_{leaf-max} : K_{branch-max}$	LMA (mg cm^{-2})	$A_l : A_s$
1700	-2.04 (0.08)	9.684 (2.088)	321.17 (37.2)	5.43 (0.245)	9.07 (0.25)	101.24 (15.43)
1800	-2.22 (0.09)	3.937 (0.661)	345.49 (47.0)	2.05 (0.216)	7.30 (0.38)	127.37 (11.08)
1900	-2.13 (0.06)	4.424 (0.764)	453.87 (34.7)	1.756 (0.188)	7.75 (0.31)	114.76 (10.81)
2000	-1.96 (0.03)	3.185 (1.147)	469.22 (32.3)	1.22 (0.367)	7.33 (0.33)	79.71 (7.84)
2100	-2.21 (0.06)	1.083 (0.163)	431.41 (21.1)	0.452 (0.158)	7.63 (0.31)	91.03 (5.36)

Site elevation (m)	Π_o (MPa)	Ψ_{TLP} (MPa)	RWC_{TLP} (%)	ϵ (MPa)	C_{leaf} ($\text{mol m}^{-2} \text{MPa}^{-1}$)
1700	-2.013 (0.206)	-2.787 (0.137)	90.052 (1.022)	18.601 (4.463)	0.387 (0.05)
1800	-2.061 (0.168)	-2.807 (0.070)	81.467 (2.327)	13.299 (4.019)	0.631 (0.145)
1900	-2.072 (0.051)	-2.360 (0.063)	92.063 (1.177)	27.888 (4.515)	0.253 (0.046)
2000	-1.574 (0.171)	-2.281 (0.100)	87.991 (2.798)	19.048 (7.602)	0.480 (0.121)
2100	-1.843 (0.167)	-2.501 (0.169)	88.530 (3.736)	35.121 (15.343)	0.372 (0.112)

Site elevation (m)	$P50_{branch}$ (MPa)	$P_e - P50_{branch}$ (MPa)	$\Psi_{min} - P50_{branch}$ (MPa)	$P50_{leaf}$ (MPa)	$\Psi_{min} - P50_{leaf}$ (MPa)	$\Psi_{min} - \Psi_{TLP}$ (MPa)
1700	-5.92	3.92	3.88	-1.42	-0.62	0.745 (0.16)
1800	-4.75	2.77	2.53	-1.09	-1.13	0.589 (0.11)
1900	-4.52	1.74	2.38	-2.0	-0.13	0.226 (0.08)
2000	-4.30	2.06	2.33	-1.5	-0.46	0.318 (0.10)
2100	-5.17	1.34	2.96	-1.8	-0.41	0.292 (0.18)

Supplemental Information

Supplemental Information Table 1. Sigmoidal coefficients for all sites' leaf vulnerability curves (Figure 3), fit with a three-parameter sigmoidal equation, where the coefficient a is the y-intercept, the coefficient b is an indicator of slope, and coefficient x_0 indicates the x-value in the middle of the curve.

Elevation	a	b	x_0	R^2	p
1700	9.00	-0.043	1.418	0.438	0.0007
1800	397.48	-0.776	-2.84	0.539	<0.0001
1900	13.142	-1.033	0.451	0.308	0.0083
2000	15512	-0.852	-6.05	0.470	0.0007
2100	2455.5	-0.962	-6.13	0.346	0.0026

Supplemental Information Table 2. Sigmoidal coefficients for branch vulnerability curves (Figure 7) at all sites. Data were fit with a three-parameter sigmoidal equation, where the coefficient a is the y-intercept, the coefficient b is an indicator of slope, and coefficient x_0 indicates the x-value in the middle of the curve ($P50_{\text{branch}}$).

Elevation	a	b	x_0	R^2	p
1700	115.3	1.962	5.92	0.721	<0.0001
1800	94.1	1.385	4.753	0.728	<0.0001
1900	95.3	0.8727	4.515	0.92	<0.0001
2000	95.7	1.03	4.296	0.912	<0.0001
2100	90.64	0.669	5.169	0.847	<0.0001

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CHAPTER 3: GENERAL CONCLUSIONS

In the Intermountain West, the majority of annual precipitation falls as snow in the mountains. Climate scientists have observed warming trends that are projected to continue, causing earlier spring snow melt, reduced snowpack retention, and a rise in elevation of the rain-snow transition zone (Leung *et al.* 2004; Barnett *et al.* 2005; Klos *et al.* 2014). This will cause a longer summer dry period (Westerling *et al.* 2006), which will have great effects on montane biomes that are reliant on the retention and redistribution of snowpack throughout spring and early summer (Björk & Molau 2007). For this research, we identify the lower treeline of montane conifer forests as a potential forest boundary that is at risk in future climate scenarios.

Lower treeline is assumed to be limited by water availability. In the Intermountain West, precipitation decreases and temperature increases with decreasing elevation, which creates elevation-based distributions of plant communities (Kusbach *et al.* 2014). In order to understand how forest distributions might be affected at lower treeline with aforementioned changes in climate, I conducted a research project that sought to understand how trees make adjustments in their water relations with decreasing elevation approaching lower treeline. I measured various leaf- and branch-level hydraulic parameters in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) at five sites along an elevational cline in southeastern Idaho, with the lowest elevation site being at lower treeline. I found that branches at lower treeline were more resistant to hydraulic dysfunction, but exhibited a trade-off through a reduction in water transport efficiency. Unexpectedly, for leaves, I observed increased maximum hydraulic transport capacity and no trend in hydraulic vulnerability with decreasing elevation. We also found that the

water potential associated with loss of turgor in leaf cells decreased with decreasing elevation, indicating that lower treeline leaves can maintain turgor at greater levels of drought stress. Additionally, I found that leaf mass per area and the ratio of leaf area to sapwood area both increased with decreasing elevation, indicating that leaf growth efficiency is favored by trees at low elevations.

Overall, my research found that trees at lower treeline seem to be well adapted to more arid conditions, with branches that exhibit a safety-efficiency trade-off and leaves that exhibit no trends in hydraulic safety, but are more efficient in water transport and growth. Since lower treelines are characterized by higher temperatures and longer growing seasons, whole plant water balance may be reliant on leaves that display greater hydraulic and growth efficiency. Although I did not measure woody tissue growth, previous studies have found that conifers reduce woody growth at low elevation dry edge boundaries (League & Veblen 2006; Anderegg & Hillerislambers 2016). If this is also the case for Douglas-fir in this study, it provides another explanation that whole plant hydraulic functioning at our study area may be characterized by favored leaf vs. woody tissue growth. Although the question of “What causes lower treeline?” would benefit from more research involving more tree species and a wider array of hydraulic and growth parameters, the results from this study tell us that there is intraspecific phenotypic plasticity in tree water relations along an elevation gradient and that water limitations play a role in causing lower treeline.

We see here that different plant parts are utilizing tradeoffs in hydraulic efficiency and safety to maintain water transport while avoiding hydraulic dysfunction due to embolism. The concern with rapid climate change is that trees will not be able to adapt

quickly enough to changes in temperature and water availability, which may in turn cause range shifts due to tree mortality at lower treeline. Alternatively, we may see increased die-off at mid-elevations where trees typically receive adequate moisture and are less adapted to drought. Nevertheless, since the nature of hydraulic efficiency-safety tradeoff hypothesis is very taxa-specific (Gleason *et al.* 2016), more studies are needed to assess the water relations of different tissue types (i.e leaves, branches, and roots) of various conifer lower treelines.

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