

AN ABSTRACT OF THE THESIS OF

David M. Barnard for the degree of Master of Science in Forest Science presented on  
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Title: Stem Sapwood Water Transport and Storage Strategies in Three Conifers from Contrasting Climates

Abstract approved:

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The state of Oregon has two distinct climate types bisected by the crest of the Cascade Mountain range. The western side of the Cascades experiences high levels of precipitation and mild temperatures due to the maritime influence of the Pacific Ocean, while the eastern side lies in the rain shadow of the Cascades and is subject to more continental temperatures and arid conditions. Several tree species have subspecies or varieties adapted to the contrasting climates, including Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*) and the shore pine and lodgepole pine subspecies of *Pinus contorta*. These species provide a unique opportunity to study adaptations of tree hydraulic architecture to contrasting climates. The current study has two major foci: the first being an investigation of the water storage and transport properties and resistance to drought-induced xylem dysfunction of ponderosa pine and Douglas-fir from east and west-side populations, the second being an investigation into the sapwood radial water transport properties in east and

west-side populations of all three species listed above coupled with an analysis of ray structure and frequency in an attempt to elucidate the radial flow pathway.

Water transport efficiency was measured as specific conductivity ( $k_s$ ,  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) in both axial and radial directions at three different sapwood depths; intrinsic water storage capacity was expressed as sapwood capacitance derived from sapwood moisture release curves and normalized on a sapwood volume basis ( $\text{kg H}_2\text{O m}^{-3} \text{MPa}^{-1}$ ). Resistance to drought-induced xylem dysfunction was determined through acoustic emissions testing and is reported as percent of cumulative acoustic emissions (%UAE) as a function of xylem water potential ( $\Psi$ ). Fluorescent microscopy was used to determine ray tracheid and ray parenchyma lumen areas and a tree ring microscope was used to determine earlywood, latewood and growth ring boundary properties from the samples used to collect radial conductivity measurements.

Axial  $k_s$  values were not significantly higher in ponderosa pine than Douglas-fir (5.46 and 5.30  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$  respectively) and axial  $k_s$  was lower from western populations of both species. Resistance to drought-induced embolism expressed as the xylem water potential causing 50% loss of  $k_s$  ( $\Psi_{50}$ ) was the same in eastern populations ( $\Psi_{50}=-2.0$  MPa for both species) but was significantly higher in western populations of ponderosa pine ( $\Psi_{50}=-3.26$ ) and Douglas-fir ( $\Psi_{50}=-2.35$  MPa). Axial  $k_s$  showed a strong positive relationship with  $\Psi_{50}$  values across species and populations ( $r^2=0.40$ ,  $p<0.0001$ ). Capacitance values did not vary significantly between species of west-side populations or east-side populations, but east-side populations were

significantly higher than west-side populations. Capacitance showed a strong positive relationship with  $\Psi_{50}$  ( $r^2=0.65$ ,  $p<0.0001$ ), suggesting that capacitance acts to buffer water column tensions thereby reducing the necessity for reliance on structural adaptations to reduce  $\Psi_{50}$ .

Radial  $k_s$  values were highest in west-side populations and generally decreased with distance inward from the cambium, except for west-side ponderosa pine and Douglas-fir in which radial  $k_s$  increased with increasing depth into the sapwood. East-side populations of all species tended to have smaller ray tracheid lumen areas but ray tracheids comprised a larger proportion of total area and were more numerous per area. Ray tracheids were smaller and less numerous in Douglas-fir than in the pines, which had similar lumen areas, although ray tracheids comprised a larger proportion of total area in shore/lodgepole pine. Ray parenchyma lumen areas tended to be larger in west-side populations but ray parenchyma frequency was lower and thus comprised a smaller proportion of total area in east and west-side Douglas-fir. Total ray density was higher in west-side varieties, and correlated with radial conductivity. The strongest correlation was between radial conductivity and number of growth ring boundaries in the sample ( $p=0.002$ ,  $r^2 =0.11$ ). This result suggests that rays may not be as important in radial conductivity as thought, since growth ring boundaries should not affect water movement through ray cells.

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Stem Sapwood Water Transport and Storage Strategies in Three Conifers from  
Contrasting Climates

by  
David M. Barnard

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

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David M. Barnard, Author

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## CONTRIBUTION OF AUTHORS

Dr. Meinzer and Dr. Lachenbruch were co-authors and assisted with the design and implementation of Chapter 2, 'Relationship between sapwood capacitance, vulnerability to embolism and water transport in ponderosa pine and Douglas-fir from contrasting habitats,' and Dr. McCulloh assisted with editing and logistical support in data collection. Dr. Lachenbruch and Dr. Meinzer were co-authors and assisted with the design and implementation of Chapter 3, 'Xylem ray characteristics and radial conductivity in three species of conifers with populations adapted to contrasting climates,' and Dr. McCulloh assisted with editing.

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## **Chapter 1 - Introduction**

The cohesion-tension theory (Dixon and Joly 1894) states that water moves continuously through plants following a tension gradient induced by transpirational water loss. Atmospheric conditions can vary widely over short time periods and may result in fluctuations in transpiration and therefore tension that could be propagated immediately throughout the water column. High xylem tensions can lead to the formation of embolism via air-seeding through conduit pit membranes (Tyree and Zimmerman 2003). Embolized conduits reduce the overall conductivity of the sapwood in trees and can potentially lead to the formation of a negative feedback loop (runaway embolism), which can result in catastrophic loss of water transport capacity and ultimately tree mortality (Tyree and Zimmerman 2003). Trees have developed a variety of structural and physiological adaptations for avoidance of drought-induced xylem dysfunction and ultimately tree mortality. Structural adaptations for coping with high water column tensions include decreased pit membrane pore diameter in angiosperms and decreased pit aperture and margo pore diameter in conifers (Hacke 2003, Domec *et al.* 2006, 2008). Although it may be beneficial for trees to develop a transport system that is less vulnerable to embolism, this often occurs at the expense of a tradeoff against overall transport efficiency (Sperry *et al* 2006), which has the potential to limit biomass production (Hubbard *et al* 1999, McDowell *et al* 2008). A potential alternative mechanism for the avoidance of embolism is capacitive discharge of water stored in stem sapwood, which has been shown to contribute significantly to

daily water use as well as, and maybe even more importantly, to buffering daily fluctuations in xylem tension (Waring and Running 1978 and 1979, Phillips *et al.* 2003, Čermák *et al.* 2007, Meinzer 2003, 2006 and 2008, James *et al.* 2003). Furthermore, water storage and discharge is largely dependent on sapwood anatomical properties.

The sapwood of conifers is more homogenous than that of angiosperms. Conifer wood is typically comprised of axial tracheids (that are simultaneously responsible for water transport and structural support), axial parenchyma and ray parenchyma (Panshin and de Zeeuw 1973). In certain genera of the Pinaceae and select species of the Taxodiaceae and Cupressaceae xylem rays also contain ray tracheids (Hoadley 1991), which, due to their structural similarity to axial tracheids, are assumed to aid in radial transport of water (Tyree and Zimmerman 2003).

Several conifer species are found in Oregon with subspecies or varieties adapted to the contrasting climates of the eastern and western sides of the Cascade Mountains, namely Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.) and shore pine (*Pinus contorta* Doug ex. Loud var. *contorta*) and lodgepole pine (*Pinus contorta* Doug ex. Loud var. *murrayana* (Balf.) Engelm.). These tree species have a range of sapwood thicknesses and ray properties that present a unique opportunity to investigate the different strategies used for transport and storage of water in the stem and the manner in which they access water that is available to the entire sapwood profile by radial and axial

water flow. In this study we have two goals: to determine the water transport and storage strategies utilized by east and west-side populations of ponderosa pine and Douglas-fir and to investigate differences in ray anatomy and their impacts on radial conductivity and sapwood depth of east and west-side populations of Douglas-fir, ponderosa pine and lodgepole (shore) pine.

In the first part of this study we investigated how Douglas-fir and ponderosa pine populations from sites on the eastern and western sides of the Cascade crest store and transport water in their sapwood. The sapwood of ponderosa pine is three to four times thicker than that of Douglas-fir. According to Darcy's Law, materials with similar conductivity may transport equal amounts of water through a greater cross sectional area under less tension. This has been proposed as a possible reason why ponderosa pine is able to extend into drier regions than Douglas-fir even though it produces wood that is more vulnerable to embolism (Stout and Sala, 2003). A positive relationship has been identified between water storage capacity and vulnerability to embolism (Pratt et al 2007, Meinzer 2008a) implying that sapwood water storage may effectively buffer tensions in the water column by daily release of water into the transpiration stream thereby reducing the need for wood that is anatomically less vulnerable to embolism and more costly to produce (Scholz *et al.* 2007, Sperry *et al.* 2008, Meinzer 2003, 2006, 2008a). A positive relationship has also been shown between increasing axial specific conductivity ( $k_{s\text{-}ax}$ ) and greater vulnerability to embolism in conifers (Piñol and Sala 1999, Stout and Sala 2003).

In the second part of this study we investigated differences in stem sapwood characteristics and how they have been shown to vary within and among species (Mencuccini and Grace 1996). The manner in which trees utilize the entire sapwood profile for sap flow differs among species as well (Phillips *et al.* 1996, Gartner and Meinzer 2005). Tree species such as lodgepole pine and ponderosa pine conduct the bulk of their sap flow about one-third of the distance inward from the cambium (Mark and Crews 1974, Meinzer unpublished data) whereas Douglas-fir conducts the majority of sap flow immediately inward from the cambium and declines farther inward (Domec *et al.* 2006a. In addition to different sap flow strategies, these three species also have varying sapwood depths and ray anatomy (Hoadley 1993, Panshin and de Zeeuw 1973). Maton and Gartner (2005) showed that leaf trace connections in these three species were supplied with water from the newest one to three years of xylem, indicating that water must radially traverse a substantial portion of sapwood from the trunk to the branches and into the leaves. Also, a steady supply of water to the vascular cambium is required to maintain radial growth as well as water and nutrients to ray and axial parenchyma deeper in the sapwood. In order for this to occur there must be a suitable pathway that allows for radial transport of water with minimal resistance. This pathway would be particularly important in lodgepole and ponderosa pine considering that the bulk of sap flow can occur in xylem that is upwards of 100 years old in old-growth trees. Due to similarity with their axial counterparts it has been assumed that ray tracheids function to facilitate radial water movement (Tyree and Zimmerman 2003). Ray parenchyma has also been proposed as

a potential pathway for radial water movement. However, Kitin *et al.* (2009) proposed that radial water movement may also occur through inter-cellular spaces in the rays as well as through bordered pits on the tangential walls of axial tracheids at the growth ring boundaries. Little work has been done to develop a relationship between radial conductivity and specific anatomical characteristics, yet the results would apply to studies in physiology and wood technology studies alike.

**Relationship between sapwood capacitance, vulnerability to  
embolism and water transport in ponderosa pine and Douglas-fir  
from contrasting habitats**

David M. Barnard, Frederick C. Meinzer, Barbara Lachenbruch, Katherine A. McCulloh

## Chapter 2 - Relationship between sapwood capacitance, vulnerability to embolism and water transport in ponderosa pine and Douglas-fir from contrasting habitats

### Abstract

In Oregon, the Cascade Mountain range blocks much of the precipitation and maritime influence of the Pacific Ocean dividing the state into two unique temperature and precipitation regimes. The aim of the current study was to investigate the relationships between sapwood thickness and the water storage and transport (axial and radial) properties as well as vulnerability to embolism of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) and their populations adapted to both climates. Sapwood capacitance ( $\text{kg m}^{-3} \text{ MPa}^{-1}$ ), percent cumulative acoustic emissions (%UAE), axial conductivity ( $k_{s-\text{ax}}$ ,  $\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ ) and radial conductivity ( $k_{s-\text{rad}}$ ,  $\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ ) measurements were made on wood samples collected with a 12-mm-increment-borer. The  $k_{s-\text{ax}}$  measurements were made with a new method using a high pressure flow meter (HPFM).  $k_{s-\text{rad}}$  did not vary significantly between species or populations, but  $k_{s-\text{ax}}$  was significantly higher in east-side populations and varied among species. Sapwood capacitance varied little between species at either site but was significantly higher ( $P<0.0001$ ) in east-side trees and correlated strongly with mean embolism pressure ( $\Psi_{50}$ ,  $p<0.0001$ ,  $r^2=0.65$ ) and  $k_{s-\text{ax}}$  ( $p<0.0001$ ,  $r^2=0.40$ ). These data suggest that trees maintain a tradeoff between mean embolism pressure and sapwood capacitance as a strategy for avoiding embolism.

## Introduction

Trunk sapwood plays a vital role in water transport, but also as a water storage buffer between the leaves and root system which may simultaneously experience different magnitudes of flux. Ideally, the sapwood would function as an efficient transport pathway and offer little resistance to water flow between roots and leaves. However, seasonal and diurnal fluctuations in soil moisture and evaporative demand can cause very high tensions to develop in the sapwood. Under these circumstances, it is adaptive to have xylem structural features that withstand high tensions while avoiding excessive development and propagation of embolism. Thus, the tracheids of conifer sapwood often exhibit structural features that appear to result in a trade-off between efficiency of water transport (high specific conductivity) and safety (high resistance to embolism at tensions that are likely to occur *in vivo*). The safety versus efficiency tradeoff appears to be a result of the geometry and characteristics of the bordered pit connecting two tracheids. Larger pit apertures and pore size in the margo will result in higher pit conductance but simultaneously increase the risk of air-seeding from margo rupture or stretching beyond the pit aperture (Hacke *et al.* 2003, Domec *et al.* 2006b). Coniferous plants appear to have a number of ‘design options’ beyond the physiological regulation of water uptake by the roots and water loss from the leaves for decreasing the magnitude of likely tensions. In situations of similar leaf area and evaporative demand, thicker sapwood and/or higher axial specific conductivity could result in a reduced tension gradient in the sapwood, so could

increased radial conductivity by facilitating access to the inner sapwood more readily for water transport and release of stored water, thus allowing a temporal lag between peak transpiration and peak water absorption at the roots. This study compares patterns of sapwood width, axial and radial conductivity, resistance to embolism, and sapwood water storage at opposite ends of a climatic moisture gradient for two coniferous species differing in maximum sapwood depth.

Water flows through the xylem in a metastable state following a hydrostatic pressure gradient. During periods of high transpirational demand and/or low soil moisture, the tension in the water column may reach a point at which a gas bubble can be pulled into a xylem conduit and expand to form an embolism that blocks water transport (Hacke *et al.* 2004, Domec *et al* 2006 ). The increased tension caused by the partial loss of conductivity can generate a negative feed-back loop resulting in runaway embolism (Tyree and Sperry 1988) and potentially plant death (Brodribb and Cochard 2008, McDowell *et al.* 2008). Darcy's Law describes laminar flow of a viscous fluid through a porous medium such as the xylem:

$$Q = -\frac{kA}{L} \Delta \Psi$$

where the volumetric flow rate ( $Q$ ) depends on the conductivity ( $k$ ) of the material, the pressure differential across the sample ( $\Delta \Psi$ ), the dynamic viscosity of the fluid ( $\eta$ ) and the area ( $A$ ) and length ( $L$ ) of the sample. Darcy's law also shows that,

when all else is equal, a given amount of water will require greater pressures (or tensions) to flow through a smaller cross sectional area than a larger one. In response to these physical constraints on water flow, trees have evolved a suite of physiological, allometric, and anatomical strategies to minimize potential xylem dysfunction induced by high tensions in the water column. These include maintenance of small leaf area relative to sapwood area (Mencuccini and Grace 1995) and the ability of stomata to regulate transpiration to reduce the tension gradient (Farquhar 1982, Saliendra *et al.* 1995, Mencuccini and Grace 1994, Whitehead 1998, McDowell *et al.* 2008), diurnal and seasonal storage and capacitive discharge of water in trunk wood and other tissues (Waring and Running 1978, Waring *et al.* 1979, Holbrook 1995, Phillips *et al.* 2003, Čermák *et al.* 2007, Scholz *et al.* 2007, Meinzer *et al.* 2003, 2006 and 2008) and decreased pit membrane pore diameter and changes in pit geometry (Hacke *et al.* 2004, Domec *et al.* 2006). On the other hand, in situations in which water is not limiting, the same adaptations to protect against xylem dysfunction may be deleterious to transport efficiency and may limit tree growth (Hubbard *et al.* 1999 and 2001, Santiago *et al.* 2004).

A strong positive correlation has been shown between mean air seeding point ( $\Psi_{50}$ , MPa) and stem capacitance normalized on a sapwood volume basis ( $\text{kg m}^{-3} \text{ MPa}^{-1}$ ) in tropical angiosperms (Meinzer *et al.* 2008) and with capacitance defined as a change in relative water content (RWC) per change in pressure (MPa) in chaparral shrub species (Pratt *et al.* 2007) and conifers (Domec and Gartner

2002; Domec *et al.* 2005). Positive relationships have also been shown between maximum sapwood specific conductivity and  $\Psi_{50}$  in Norway spruce (Rosner *et al.* 2008), three pine species (Piñol and Sala 2000) and Douglas-fir (Domec *et al.* 2006a). Isotope tracer residence time has been shown to increase, while tracer velocity decreased, with increasing sapwood capacitance in both tropical angiosperms and temperate conifers (James *et al.* 2003, Meinzer *et al.* 2006) indicating that stem water storage enhances apparent efficiency, which in conjunction with wider sapwood and increased stomatal control could act to reduce the occurrence of excessive xylem tensions.

The Cascade Mountain range, traversing the Pacific Northwest states of Washington and Oregon along a north-south axis, is a major contributor to the climatic diversity of the region. The resulting mild and moist climate west of the Cascade crest and the semi-arid continental climate east in the rain shadow of the crest present an opportunity to study the strategies exhibited by species with populations adapted to both climates. Ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.) is the most widely distributed pine in North America (Burns and Honkala 1990) with a range extending from the mid-western portion of the United States west to the Willamette Valley in Western Oregon and from British Columbia down into northern Mexico. Ponderosa pine has very wide sapwood and is more tolerant of dry conditions (McMinn 1952, Burns and Honkala 1990) yet can also tolerate saturated soil conditions (Emmington *et al.* 2005). The Willamette Valley ponderosa pine is found in the Willamette Valley of western Oregon and is popular

as a timber species that grows well in sites that are too wet or too dry for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Fletcher 2007). The two populations of Douglas-fir adapted to the Pacific Northwest are the coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) and the Rocky Mountain, or interior, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco). Coastal Douglas-fir ranges from the western slope of the Coastal range eastward to the western slope of the Cascade Mountains where rainfall can be 3-5 times greater than in the areas to which the interior Douglas-fir is adapted (Burns and Honkala 1990). On both the western and eastern sides of the Cascade crest, Douglas-fir and ponderosa pine can be found growing together and separately, but ponderosa pine typically extends farther into drier regions (McMinn 1952, Emmingham *et al.* 2005) suggesting a more successful strategy for avoiding drought-induced xylem dysfunction. Despite the more xeric range of ponderosa pine, numerous investigations have shown it to be more vulnerable to embolism than Douglas-fir in branch segments (Piñol and Sala 2000, Stout and Sala 2003, Martinez-Vilalta *et al.* 2004), roots (Stout and Sala 2003) and in trunk sapwood (Bouffier *et al.* 2003, Domec and Gartner 2002) suggesting the existence of alternative mechanisms that prevent tensions from rising above the embolism-inducing threshold. Additionally, multiple studies have reported that individuals of a given species from sites where water is more limiting tend to produce xylem that is more resistant to embolism and have lower water transport efficiency (Kavanagh *et al* 1999, Stout and Sala 2003, Bouffier *et al.* 2002, and Ladjal *et al.*

2005). However, Maherli and DeLucia (2000) found somewhat contradictory results with ponderosa pine growing in the drier desert portions of the Sierra Nevada having slightly higher specific conductivity than their montane counterparts, but statistically indistinguishable vulnerabilities to embolism.

The aim of the current study is to investigate sapwood water transport and storage characteristics in Douglas-fir and ponderosa pine, two tree species with different sapwood thicknesses that also have populations adapted to the eastern and western sides of the Cascade Crest. Our first hypothesis was that the success of ponderosa pine in drier conditions, and thus the species' broader ecological distribution, is associated with larger cross-sectional area for water transport in the stem, and that by spreading stem water transport over a greater area, less energy is required for producing wood that is anatomically safer from drought-induced xylem dysfunction, allowing more energy to be invested in growth and reproduction. Furthermore, we believed that to use this thicker sapwood, ponderosa pine would have higher radial conductivity. Our second hypothesis was that populations east of the Cascade Crest would have thicker sapwood and greater capacitance, and thus be more vulnerable to embolism while simultaneously having higher axial and radial water transport efficiency than their west-side counterparts.

## Methods

### *Sites, Plant material and sample preparation*

This study was carried out at eight stands at five different sites in Oregon (Table 2.1). The region is characterized by dry summers both west and east of the Cascade crest with the bulk of precipitation occurring during the fall, winter and spring months, mostly as snow east of the crest. Mean annual precipitation is 283 mm across the three sites east of the Cascade crest and 1250 mm across the two sites in western Oregon. For the purpose of this study a population refers to a specific species (i.e. Douglas-fir or ponderosa pine) at a specific location (east or west). Furthermore, when referring to plant origin, “east- side” indicates east of the Cascade crest (continental and arid) and west-side indicates west of the Cascade crest (mild and moist). Within each population, two uneven-aged, natural-regenerated stands were selected. At each stand 12 trees were selected with a breast-height diameter of 20-40 cm, similar crown sizes and an overall healthy appearance (i.e. lacked visible damage or insect or disease infestation). To characterize individual tree growth (i.e. sapwood depth, age), we took two 5-mm-diameter increment cores from breast height to a depth that included the pith, for all trees. For trees without visible bending in the trunk and growing on relatively flat ground, the cores were taken at 90° angles to one another. For trees that were leaning or growing on a slope, cores were taken at 180 ° to one another and 90 ° from the axis of lean, to avoid both compression wood and opposite wood. For

determination of water transport and storage properties, we selected six of those twelve trees and took three 12-mm-diameter increment cores from breast height, at similar locations to avoid the presence of compression wood or opposite wood.

After removing the cores from the tree, we marked the heartwood/sapwood boundary (determined by the marked change in translucency when held up to light) with a pencil and then quickly sealed the cores into two nested plastic bags.

The samples were then placed in an ice-filled cooler for transport to the lab.

#### *Individual tree growth characteristics*

We used two 5 mm increment cores to estimate tree age at breast height (hereafter referred to as tree age), sapwood depth, number of rings of sapwood and average annual basal growth increment for each of the twelve trees in each stand.

We determined sapwood depth by holding fresh cores up to a light source to visually determine the heartwood-sapwood boundary. We averaged together the sapwood depths from the two cores and used a dissecting scope (Cole Parmer model 482900-00, Vernon Hills, IL, USA) to estimate tree age and total rings of sapwood based on a count of the number of growth ring boundaries. To estimate site specific basal area growth rates for each stand we calculated average annual basal growth increment as the tree diameter divided by the total number of growth rings.

### *Axial and radial specific conductivity*

We cut two of the 12 mm cores into outer, middle and inner segments.

Outer segments were taken at two growth rings inward from the cambium to ensure that only mature xylem would be tested. Middle segments were centered exactly half-way between the cambial layer and the heartwood-sapwood boundary, and the inner segments were taken at two growth rings outwards from the heartwood-sapwood to ensure that no heartwood was included in the sample. The segments from one core were used for radial conductivity measurements and were cut to a 10 mm length (radial direction). The other 12 mm cores were used to collect axial conductivity measurements and were cut to approximately 6 mm in the radial direction and ultimately 10 mm in the axial direction.

Pilot work comparing specific conductivity from sapwood segments 150-200-mm-long to that of the 10 mm segments found them to be statistically indistinguishable ( $p=0.31$ , data not shown), justifying the use of 10-mm-long segments for this study. The 6 mm (radial direction) segments, for axial conductivity, were then cut to produce a sample that was approximately 10mm x 5mm x 5mm (axial, tangential, and radial respectively) and the sharp corners were shaved to produce a cylinder. All samples were then placed into a perfusion solution consisting of 0.22 $\mu$ m filtered and degassed, distilled water, adjusted to pH of 2 with HCl (to retard bacterial and fungal growth) and placed under vacuum pressure overnight to remove any air embolism. We utilized the high pressure flow meter (HPFM) method (Tyree *et al.* 1993, Yang and Tyree 1994) to

determine axial ( $k_{s\text{-ax}}$ ) and radial ( $k_{s\text{-rad}}$ ) specific conductivities. Pilot work showed that  $k_{s\text{-rad}}$  decreased with increasing pressure up to about 0.15 MPa. However once the pressure differential stabilized, it did not decrease with time. On the other hand,  $k_{s\text{-ax}}$  increased with increasing pressure up to about 0.05 MPa, where it leveled off and decreased sharply with time. Based on this pilot work, radial samples were perfused for 10-15 minutes at a pressure of about 0.2 MPa and axial samples were perfused for 1-5 minutes at a pressure of about 0.08 MPa during which time flow rate was determined from the pressure drop across a capillary tube of known resistance between a pressurized water tank and the sample. The flow meter was previously calibrated by determining flow rates ( $\text{kg s}^{-1}$ ) through capillary tubes of various diameters over a series of known pressure differentials.

#### *Sapwood moisture release and capacitance*

For determination of sapwood moisture release and capacitance we used 10-mm-long segments (radial direction) cut from the sapwood of the third 12 mm core. Segments were cut from locations that corresponded to previously observed and published depths of peak sap flow for the two species. The peak of sap flow for ponderosa pine occurs about one third of the distance inward from the cambium to the heartwood-sapwood boundary (Meinzer, unpublished). The peak of sap flow in Douglas-fir typically occurs immediately inward from the cambial layer (Domec *et al.* 2006). Capacitance samples were then infiltrated under

vacuum overnight with the perfusion solution described above. The following morning the samples were removed from the water and segment volume, using the immersion method, was recorded. The samples were then quickly blotted with a paper towel to remove excess water, weighed and placed into the cap of screen cage thermocouple psychrometer chambers (83 series; JRD Merrill Specialty Equipment, Logan , UT, USA). The cap was then joined with the rest of the chamber and sealed tightly. All psychrometer chambers were then placed into nested polyethylene plastic bags and into an insulated water bath for 2 h to allow equilibration. Following the equilibration, a 12-channel digital psychrometer meter (85 series; JRD Merrill Specialty Equipment, Logan, UT, USA) was used for determination of water release isotherms. Measurements were recorded every 20 min until water potential values stabilized. The samples were then removed from the chambers, weighed and allowed to partially air dry. Air drying intervals were determined by the rate at which water evaporated from the samples, based on measurements of mass. As the samples approached the presumed inflection point of the moisture release curves, the drying periods we used became much shorter (i.e. 30-45 minutes) in an effort to clearly define the shape of the curve. Following the drying periods, we resealed the samples in their chambers and the whole procedure was repeated until water potential readings reached approximately - 4MPa. The samples were then removed and placed into a drying oven overnight at 70°C, for determination of oven dry mass and sample density. Recorded psychrometer values were transformed to water potential values based on

calibration curves of solutions of known water potential. Relative water content (RWC) was then determined for each sample at each recorded mass by the following equation

$$RWC = \frac{W_f - W_d}{W_s - W_d} \quad (1)$$

where  $W_f$  is the fresh mass of the sample,  $W_d$  is the oven dry mass and  $W_s$  is the fully saturated mass of the sample. The values of relative water deficit ( $RWD = 1 - RWC$ ) were then plotted against measured values of water potential and fit with a modified hyperbola using the following equation

$$\Psi_{\text{mean}} = \frac{a * RWD}{1 + b * RWD} \quad (2)$$

to determine moisture release parameters (Table 2.2) and used to determine water potential of samples subjected to acoustic emissions analysis of xylem vulnerability curves (see below).

Capacitance ( $C$ ) values were determined from sapwood moisture release curves for individual trees (Figure 2.3) following the method described in Meinzer *et al.* (2003) where  $C$  is expressed on a sapwood volume basis ( $\text{kg m}^{-3} \text{ MPa}^{-1}$ ).

$$C = \frac{dW}{d\Psi_{\text{sw}}} \quad (3)$$

where  $dW$  is the cumulative weight of water released and  $d\Psi_{\text{sw}}$  is the change in sapwood water potential. Capacitance values were calculated by fitting a linear

regression line to the initial nearly linear portion of the cumulative weight of water lost versus tissue water potential curve (Figure 2.4). Cumulative weight of water released initially increases rapidly over a range of sapwood water potentials corresponding to those normally experienced *in vivo* then slows down and levels off at water potentials that typically correspond to the onset and rapid accumulation of embolism (Meinzer *et al.* 2003, 2008; Scholz *et al.* 2007).

#### *Acoustic emissions and vulnerability to embolism parameters*

For determination of sapwood vulnerability to embolism, we cut 2-mm-thick discs from a section of the 12 mm core that corresponded to the depth of peak sap flow (see description above). The disc was then examined to determine the axial plane and a smaller sliver, approximately 6 mm in length (axial) was cut from the edge of the disc resulting in a piece of sapwood rounded on one side with a flat area of the radial plane exposed. These samples were vacuum infiltrated overnight in the perfusion solution. Prior to detection of acoustic emissions, the radial plane was placed flush against an acoustic sensor (R15α, Physical Acoustics Corporation, Princeton Junction NJ, USA) which was connected to an ultrasonic acoustic emission (UAE) specific data logger (either Pocket AE or USB AE node; Physical Acoustics Corporation, USA). The acoustic sensor and sample were then held together with a clamp and an initial mass measurement was taken. The sample, sensor and clamp were then connected to the data logger and acoustically

isolated from ambient noise with insulated foam packing material. The acoustic sensitivity threshold was set to a value of 45 dB. We allowed the samples to air-dry for a period ranging from 3 to 8 h while acoustic events were continuously recorded. At intervals ranging from 15 to 45 min (intervals between measurements were shorter around presumed inflection points of what would ultimately be a sigmoidal curve) the clamp, sensor and sample assembly were disconnected from the data logger and mass and time from beginning of acoustic event acquisition recorded. We repeated this process until the rate of acoustic events slowed and a minimum of 10 min passed between individual acoustic events. After data acquisition was complete, we removed the wood samples from the clamp and sensor assembly and placed them into a 70° oven for 24 h. The clamp and sensor were weighed and that value subtracted from sample measurements. We determined percent of cumulative ultrasonic acoustic emissions (%UAE) and RWD (based on equation 1 above) for each interval during acquisition of acoustic events. We then used equation 2 above and the parameters in Table 2.2 to calculate mean water potential (MPa) values for each RWD value. Multiple examples in the literature show a strongly similar response between percent loss of conductivity (PLC: air injection pressure method) and percent cumulative ultrasonic acoustic emissions (%UAE) in which a 50% loss of conductivity correlates with 50% cumulative acoustic emissions (Cochard 1992, Hacke and Sauter 1996, Rosner et al. 2006, 2008 and 2009). To ensure accuracy of this method we compared the traditional, pressure sleeve, injection method with

the acoustic emissions method. Additionally, we tested the differences in the shape of the sigmoidal curves when cumulative energy was recorded as opposed to cumulative acoustic events (Figure 2.1). In order to further characterize species and site differences in response to drought-induced embolism we calculated the values of the air entry threshold ( $\Psi_{12}$ ), mean embolism value ( $\Psi_{50}$ ) and the full embolism point ( $\Psi_{88}$ ) (Domec *et al* 2002). To calculate  $\Psi_{12}$  and  $\Psi_{88}$  we fit a tangent line to the %UAE = 50 point of the sigmoidal curve. From the equation of this line we determined  $\Psi_{12}$  to be the water potential value where the line crossed the x-axis and  $\Psi_{88}$  to be the water potential where the regression line crossed the point at which %UAE equaled 100.

### *Statistical Analysis*

Site differences among populations were insignificant and thus all trees from the two sites per population (e.g. east-side Douglas-fir) were pooled together for analysis. This resulted in sample sites of  $n=12$  trees for physiological analyses and  $n=24$  trees for growth characteristics for each population. Physiological and growth characteristics were analyzed using one-way and two-way analysis of variance (ANOVA) with tree species and site as categorical variables. For multiple comparisons we used the Tukey adjustment for significance, otherwise confidence level for all tests was 95%. All statistical tests were done in S Plus (TIBICO Software, Palo Alto, California, USA)

## Results

### *Growth Characteristics*

The sapwood of ponderosa pine, as a function of total stem diameter, was three to four times thicker, than that of Douglas-fir ( $p<0.001$ ), but differences in sapwood thickness were only marginally significant between east and west-side populations ( $p=0.078$ , Table 2.3, Figure 2.2). On the other hand, the number of growth rings included in the sapwood, were significant between both species and sites ( $p>0.001$ ) as were growth rates ( $p<0.001$  for both comparisons Table 2.3), with west-side populations growing faster than east-side and ponderosa pine growing faster than Douglas-fir. Stem diameter at breast height was positively correlated with sapwood depth in linear regression ( $p=0.001$ ,  $r^2=0.25$ ) but not with tree age ( $p=0.40$ ).

### *Hydraulic parameters*

Differences in capacitance were not significant between species in east-side or west-side populations ( $p=0.25$ ), but were significantly higher in the east-side populations of both species ( $p<0.001$ , Table 2.4). There was no relationship between capacitance and earlywood latewood proportions ( $p=0.29$ ) but capacitance did decrease significantly, across species, with increasing wood density ( $p=0.001$ ). Outer- $k_{s\text{-}ax}$  values were not significantly different between

species ( $p=0.435$ , Table 2.5) but east-side populations of both species had significantly higher outer- $k_{s\text{-ax}}$  values than west-side populations ( $p<0.001$ ). East-side populations had significantly higher middle- $k_{s\text{-ax}}$  ( $p=0.009$ ) but showed no significant difference between species ( $p=0.78$ ). Inner- $k_{s\text{-ax}}$  was significantly lower in east-side populations as well ( $p=0.03$ ) but again, there was no difference between species ( $p=0.23$ ). Overall, there was a marked decrease in axial  $k_s$  with increasing distance from the cambium independent of species or population (Figure 2.5a). East-side populations of both species showed a greater decrease in  $k_s$ , with increasing distance from the cambium, than the west-side populations did (Table 2.5)

Overall,  $k_{s\text{-rad}}$  was higher in west-side populations of both species ( $p<0.001$ ) at all three sapwood depths. Furthermore, west-side Douglas-fir had significantly higher  $k_{s\text{-rad}}$  at all three sapwood depths than did ponderosa pine ( $p=0.009$ , Figure 2.5b). There was no significant difference between species in the east-side populations. ( $p=0.26$ ). Eastern and western populations of Douglas-fir showed peaked  $k_{s\text{-rad}}$  in middle sections of the sapwood while  $k_{s\text{-rad}}$  increased inward from the cambium in western ponderosa pine and decreased inward from the cambium in eastern ponderosa pine.

Air entry pressure ( $\Psi_{12}$ ) differed significantly among species and populations ( $p<0.001$ ) with west side ponderosa pine having the lowest and east side Douglas-fir having the highest (-2.1 MPa and -1.0 MPa respectively, Table

2.4). There was no significant difference between  $\Psi_{50}$  in east side populations of both species ( $p=0.23$ ) but west side populations had significantly lower  $\Psi_{50}$  in both species ( $p=0.002$ , Table 2.4, Figure 2.6a and b). Capacitance was significantly related to  $\Psi_{50}$  ( $p<0.001$ ,  $r^2=0.65$ , Figure 2.7a) and with  $k_{s\text{-ax}}$  ( $p<0.001$ ,  $r^2=0.40$ , Figure 2.7b).

## Discussion

This study aimed to investigate drought tolerance properties exhibited by species whose native ranges overlap as well as species with populations adapted to contrasting climates. East of the Cascade Crest, the native ranges of ponderosa pine and Douglas-fir overlap, and they can sometimes be found growing together, however ponderosa pine tends to grow and survive in drier conditions than Douglas-fir. A similar situation exists on the western side of the Cascade Crest in that Douglas-fir and ponderosa pine can be found growing together, yet ponderosa pine extends into areas that are too dry and too wet for Douglas-fir. The ability of ponderosa pine to extend into drier sites than Douglas-fir, on both the eastern and western side of the Cascade crest reflects a suite of physiological and anatomical adaptations for resisting drought conditions (Piñol and Sala 1999, Stout and Sala 2003). Ponderosa pine capitalizes on available water in a manner that allows for maximum carbon acquisition without reaching xylem tensions that promote

embolism formation and propagation by air-seeding, which has been hypothesized as a cause of tree mortality (McDowell *et al.* 2008).

Our first hypothesis for this study assumed that the greater sapwood thickness (assumed with identical leaf area and transpirational demand) in ponderosa pine would promote the transport of water under lesser tension promoting success in drier conditions by allowing for the development of sapwood that was less vulnerable to embolism that simultaneously transported water efficiently and maximized the use of stored water. Furthermore we hypothesized that the thicker sapwood of ponderosa pine would have greater radial conductivity than Douglas-fir to allow greater access to the entire sapwood profile.

Darcy's law states that for material with similar conductivities, as cross-sectional area decreases, the pressure gradient required to drive water movement must increase, on the other hand doubling the cross sectional area of water flow would lead to a 50% decrease in required pressure gradient to conduct the same amount of water. The sapwood of ponderosa pine was measured to be three to four times thicker than Douglas-fir and the difference in spatial utilization of sapwood in ponderosa pine suggests a strategy to reduce tension in the water column by spreading flow over a greater area. Unfortunately without direct measurements of total leaf area and *in situ* measurements of tension gradients, it is difficult to comment on the efficacy of this strategy. The number of growth rings in the sapwood did not differ significantly between east and west ponderosa pine

however eastern Douglas-fir had almost twice as many growth rings in the sapwood compared to the western population suggesting a potential age related, anatomical (number of cambial divisions) mechanism for sapwood maintenance as opposed to a physiological mechanism. In addition to large differences in precipitation and temperature ranges, soils on the east and west-side of the Cascades have different structures. The soils on the eastern side of the Cascade Crest tend to be extremely well drained, low in organic matter with a shallow profile and have low available water content (AWC) in the range of  $5\text{--}15 \text{ cm cm}^{-1}$ , while the soils on the western side tend to be less well drained, much deeper, have a higher organic matter content and characterized by higher AWC values around  $25\text{--}30 \text{ cm cm}^{-1}$ . In fact the higher AWC values may explain the higher productivity and growth rates of west-side sites when considering that less than 10% of annual precipitation falls during the growing season.

Our second hypothesis stated that thicker stem sapwood of eastern species would allow for a greater cross sectional area of water transport, and allow for the production of more vulnerable but less energy intensive wood. Eastern populations of Douglas-fir and ponderosa pine in this study, had similar sapwood water storage and vulnerability to embolism characteristics while western populations of ponderosa pine had significantly lower mean embolism pressure ( $\Psi_{50}$ ) than Douglas-fir, coupled with similar capacitance values. There was a strong relationship between  $\Psi_{50}$  and sapwood capacitance, among species and populations, suggesting that the release of stored water into the transpiration

stream buffers tensions in the water column contributing to the avoidance of embolism. By relying more on stored water to buffer water column tensions and spreading sap flow over a greater cross-sectional area, ponderosa pine may be able to avoid xylem tensions that would provoke catastrophic hydraulic failure, allowing it to continue assimilating carbon under conditions that would result in hydraulic constraints on carbon assimilation in Douglas-fir.

Eastside trees had about twice the sapwood capacitance as west-side populations of both species, but there was little difference between species at each site. Water storage in tree stems can occur in elastic storage compartments such as the vacuole of living parenchyma and in inelastic compartments such as intercellular spaces (Carlquist 1975). Water can also be stored extracellularly, i.e. water held by capillarity in the xylem (Holbrook 1995). Water storage in elastic living tissue may be responsible for substantial changes in volume over the course of a day or a season (Goldstein *et al.* 1984, Milne *et al* 1989, Herzog *et al* 1995) but have been shown to contribute less than 1% to daily water use (Čermák *et al* 2007). Water may also be released into the transpiration stream from the formation of embolism and can contribute a significant portion to total capacitance depending on species (Meinzer *et al.* 2008). By depending on embolism formation for the release of water however, the tree risks the onset of runaway embolism formation and potentially catastrophic xylem dysfunction (Sperry and Tyree 1988). However, embolism has been shown to buffer water column tensions without a significant loss of conductivity and an ability to avoid runaway

embolism formation (Holtta *et al.* 2009). In this study, more than half of the capacitive discharge of water occurred at water potentials above -1 MPa in both east and west side populations of both species (Figure 2.4). Furthermore the amount of water released from the air entry pressure to full embolism pressure was an insignificant portion of total water released (Figure 2.4, Table 2.4). These data suggest that the contribution of water from embolism formation is insignificant compared to water stored and released at water potentials above air entry pressure, held by other forces such as capillarity or in elastic compartments such as sapwood or vascular parenchyma. Never the less, there exists a need to rely on stored water and its capacity to buffer water column tensions. Stored water can contribute substantially to daily water use (Waring and Running 1978) and includes water released into the transpiration stream by the formation of embolisms in xylem tracheary cells (Zimmerman 1983, Tyree and Yang 1990). There have been several reports regarding the contribution of stored water to daily and seasonal transpiration. Phillips *et al.* (2003) showed an increased dependence on stored water with increasing tree size with stored water contributing 7 to 23% to daily water use in Douglas-fir and 3 to 10% in ponderosa pine. Domec *et al* (2005) showed similar capacitance values in trunk wood of old growth and young ponderosa pines. Čermák *et al.* (2007) showed a 20% contribution of capacitance to daily water use, in Douglas-fir, with the greatest amount coming from the lower stem, and claimed that whole tree water storage could sustain transpiration for an entire week, while upper crown water storage could only sustain transpiration for a

few hours. Furthermore, Waring and Running (1978) showed that 75% of tree water storage was in the stem sapwood and contributed about 6% to daily water use. Regardless of the absolute or relative amounts of water involved, the above mentioned studies that compare sap flux between bole and branch segments indicate a dynamic buffering capacity in the trunk that potentially regulates xylem water potential to avoid embolism inducing xylem tensions. Studies indicate a tradeoff between water storage capacity and other functional traits including vulnerability to embolism in Douglas-fir (Domec and Gartner 2002) select chaparral species of the Rhamnaceae (Pratt *et al.* 2007) and 11 tropical forest canopy trees (Meinzer *et al.* 2008), water transport efficiency in temperate conifers and tropical canopy trees (Meinzer *et al.* 2003 and 2006) and wood density (Meinzer *et al.* 2003, 2008b, Scholz *et al.* 2007). The results of our study follow the tradeoff seen between vulnerability to embolism and sapwood capacitance (Figure 2.7a) supporting the hypothesis that sapwood capacitance contributes to hydraulic safety by buffering water column tensions. Additionally, increased capacitive discharge of water seems to occur simultaneously with greater water transport efficiency (Figure 2.7a), suggesting that sapwood water storage contributes to overall specific conductivity.

The greater success of ponderosa pine extending into drier areas than Douglas-fir is most likely due to a suite of physiological and anatomical adaptations instead of a single characteristic, but the end result would be to constrain xylem tension thereby extending the period during which stomata are

able to remain open for photosynthetic gas exchange. The efficiency of water transport in eastern populations was higher, and formed a strong negative relationship with  $\Psi_{50}$ , epitomizing the trade-off between transport efficiency and safety. However, Waring and Running (1978) showed that following a precipitation event, trees are capable of recharging water stores almost as quickly as they are depleted. The soils found in stands of eastern populations of Douglas-fir and ponderosa pine are very well drained and low in organic content, simultaneously precipitation events, while rare, tend to be heavy and present the tree with an opportunity to recharge its water status. Increased transport efficiency would facilitate this recharge quickly and effectively.

With the exception of mean embolism pressure in the west-side trees, overall hydraulic properties were similar between species. However, upon deeper consideration of whole tree water use compared with hydraulic architecture and anatomy these similarities dissolve. For example, capacitance values for both species were not statistically different between species at each site; however when the much larger volume of sapwood in ponderosa pine is taken into account the absolute amount of water storage ability is most likely significantly higher than Douglas-fir. Greater capacitive discharge of water combined with a greater cross-sectional area would play an enormous role in regulating xylem tension. Radial conductivity was higher throughout the sapwood profile in Douglas-fir (Figure 2.5) indicating less resistance to water flow radially and a simultaneous extraction of water from the inner sapwood under lower tension gradients. In ponderosa pine

however, the lower radial conductivity may serve as a regulator for utilization of stored water in outer and inner portions of sapwood, that are spatially removed from the major pathway of sap flow. The greater resistance to water flow indicates that a greater tension gradient is required to ‘pull’ the water from inner sapwood stores and may serve as a source of stored water that is released at higher tensions.

The greater reliance on sapwood stored water in eastern populations coupled with an increased transport efficiency and simultaneously increased vulnerability to embolism suggests a strategy to maximize the uptake of carbon in an environment and under conditions that would not be considered ideal, by avoiding high water column tensions as much as possible. Interestingly, it appears the western populations have a different strategy of tolerating drought stress and the high tensions in the water column that arise as a result.

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**Table 2.1:** Coordinates and characteristics of sample sites. Mean annual precipitation and maximum and minimum temperatures are from nearest city.

	Species Sampled	Number of Stands	Nearest City	Latitude/Longitude	Elevation (m)	General Site Characteristics	Annual Precipitation (mm)	Mean Summer/Winter Temperatures Temp (C°)
<b><i>Eastern Sites</i></b>								
Ochoco National Forest	Douglas-fir	2	Mitchell	43.584, -121.218	1185	(1) 20° slope and (2) flat area, both with mixed Douglas-fir and ponderosa pine	290	30 / -4
Pine Mountain	Ponderosa pine	1	Milican	43.767, -120.963	1400	Mixed ponderosa and lodgepole pine at desert fringe, flat area	300	27 / -4
Deschutes National Forest	Ponderosa pine	1	La Pine	43.584, -121.218	1680	Pure ponderosa pine stand, flat area	260	29 / -4.5
<b><i>Western Sites</i></b>								
Cascade Timber Consulting	Douglas-fir	1	Sweet Home	44.368, -122.828	285	Mixed stand of grand-fir big leaf maple and Douglas-fir, gentle slope	1390	27 / 0.5
MacDonald-Dunn	Douglas-fir	1	Corvallis	44.616, -123.293	200	Mixed stand of Douglas-fir, big leaf maple and grand fir, flat area	1110	28 / 1
Cascade Timber Consulting	Ponderosa pine	2	Sweet Home	44.368, -122.828	285	(1) Pure ponderosa pine stand and (2) Mixed ponderosa pine and Douglas-fir	1390	27 / 0.5

**Table 2.2:** Parameters of best fit curve fit to moisture release data fits the equation for a hyperbolic function  $\Psi_{\text{mean}} = (a * \text{RWD}) / (1 + b * \text{RWD})$ . These data were used to calculate water potential values for a given RWD.

		Coefficient	Coefficient Value	SE	F	p	r <sup>2</sup>
Douglas-fir	East	a	-1.186	0.178	-6.67	<0.0001	0.79
		b	-1.000	0.070	-14.25	<0.0001	
	West	a	-0.778	0.100	-7.72	<0.0001	0.52
		b	-1.006	0.035	-29.01	<0.0001	
Ponderosa pine	East	a	-0.683	0.1088	-6.27	<0.0001	0.85
		b	-0.981	0.0483	-20.31	<0.0001	
	West	a	-1.917	0.15	-12.78	<0.0001	0.85
		b	-0.881	0.0424	-20.77	<0.0001	

**Table 2.3:** General growth characteristics for sample populations including values of sapwood depth and tree diameter and total sapwood depth as a percentage of diameter at breast height (% SW Depth). Tree age and number of growth rings included in sapwood and growth rings of sapwood as a function of total growth rings (% Rings of SW) and average annual basal growth increment at breast height (Growth Rate), and average sapwood density across three different sapwood depths. Different letters within a column indicates a significant difference at p<0.05. Mean ± SE (n=24)

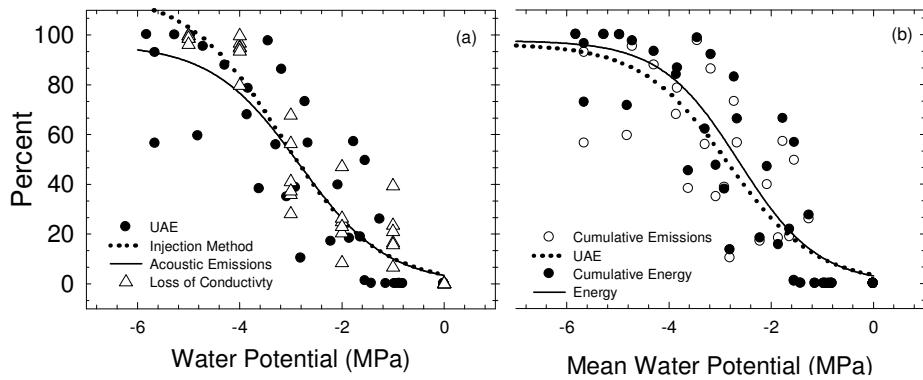
		Sapwood Depth (cm)	Diameter (cm)	% SW Depth	Age	Rings of Sapwood	% Rings of SW	Growth Rate (mm year <sup>-1</sup> )	Average Sapwood Density (g/cm <sup>3</sup> )
Douglas-fir	East	3.5 ± 0.1 a	37.4 ± 0.5 a	18.4 ± 0.6 a	99 ± 2 a	32. ± 1 a	32.3 ± 1.2 a	8.3 ± 0.01 a	0.39 ± 0.00 a
	West	2.7 ± 0.2 b	33.4 ± 0.6 b	15.9 ± 0.9 a	62 ± 1 b	10 ± 1 b	17.6 ± 1.1 b	13.1 ± 0.02 b	0.45 ± 0.01 b
Ponderosa pine	East	12.9 ± 0.3 c	39.0 ± 0.6 c	66.3 ± 1.4 b	80 ± 3 c	66 ± 2 c	84.0 ± 2.0 c	11.9 ± 0.02 c	0.47 ± 0.01 c
	West	11.8 ± 0.5 d	37.2 ± 0.3 d	63.6 ± 2.3 b	51 ± 1 d	42 ± 2 d	83.5 ± 2.3 d	17.7 ± 0.03 d	0.49 ± 0.01 d

**Table 2.4:** Values of capacitance ( $\text{kg m}^{-3} \text{ MPa}^{-1}$ ) and air entry threshold ( $\Psi_{12}$ , MPa) mean embolism pressure ( $\Psi_{50}$ ) and total embolism pressure ( $\Psi_{88}$ ) in sapwood of east-side and west-side populations of Douglas-fir and ponderosa pine. Mean  $\pm$  SE ( $n=12$ ). Values sharing the same letter within a column are not significantly different at  $p<0.05$

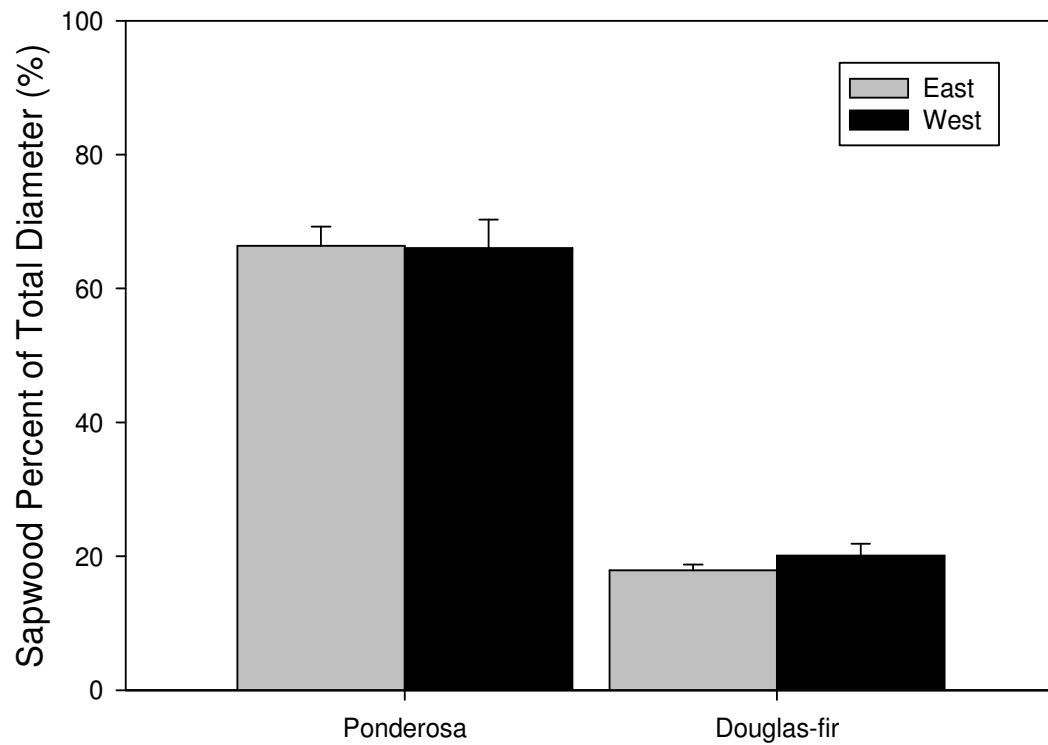
		Capacitance	$\Psi_{12}$	$\Psi_{50}$	$\Psi_{88}$
Douglas-fir	east side	$572 \pm 46\text{a}$	$-1.0 \pm 0.2\text{a}$	$-2.0 \pm 0.1\text{a}$	$-3.0 \pm 0.3\text{a}$
	west side	$308 \pm 19\text{b}$	$-1.6 \pm 0.1\text{b}$	$-2.4 \pm 0.1\text{b}$	$-3.7 \pm 0.2\text{b}$
Ponderosa pine	east side	$586 \pm 42\text{a}$	$-1.5 \pm 0.2\text{c}$	$-2.0 \pm 0.2\text{a}$	$-2.6 \pm 0.2\text{c}$
	west side	$240 \pm 24\text{b}$	$-2.1 \pm 0.1\text{d}$	$-3.3 \pm 0.1\text{c}$	$-4.4 \pm 0.2\text{d}$

**Table 2.5:** Values of axial and radial specific conductivity at outer, middle and inner trunk sapwood sections of east and west-side populations of Douglas-fir and ponderosa pine. Mean  $\pm$  SE ( $n=12$ )

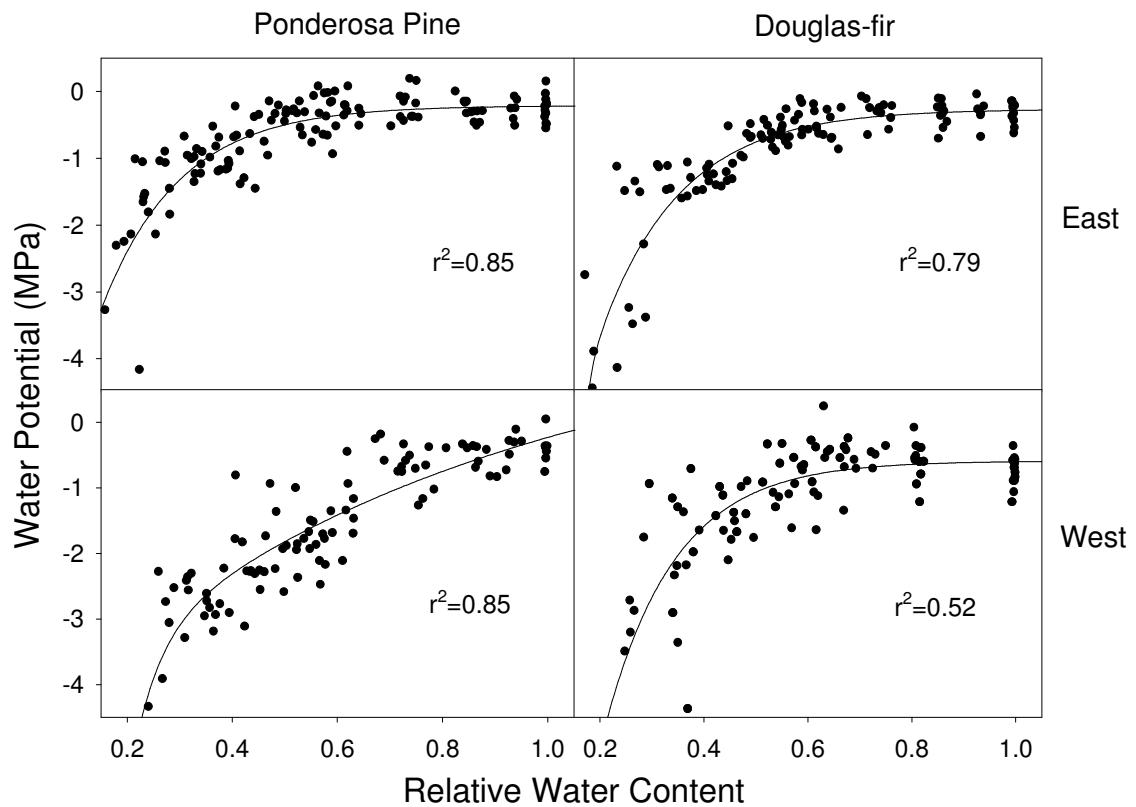
		$k_{s\text{-ax}}$ ( $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ )			$k_{s\text{-rad}}$ ( $10^{-3} \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ )		
		Outer	Middle	Inner	Outer	Middle	Inner
Douglas-fir	East	$5.30 \pm 0.2$	$4.40 \pm 0.2$	$2.44 \pm 0.3$	$2.25 \pm 0.2$	$2.43 \pm 0.1$	$2.19 \pm 0.2$
	West	$4.71 \pm 0.2$	$4.38 \pm 0.3$	$3.42 \pm 0.2$	$3.46 \pm 0.23$	$4.63 \pm 0.5$	$4.52 \pm 0.4$
Ponderosa pine	East	$5.46 \pm 0.1$	$4.96 \pm 0.2$	$2.55 \pm 0.3$	$2.40 \pm 0.1$	$2.02 \pm 0.1$	$2.23 \pm 0.0$
	West	$3.78 \pm 0.2$	$3.68 \pm 0.2$	$2.70 \pm 0.2$	$2.94 \pm 0.3$	$3.41 \pm 0.3$	$3.62 \pm 0.6$



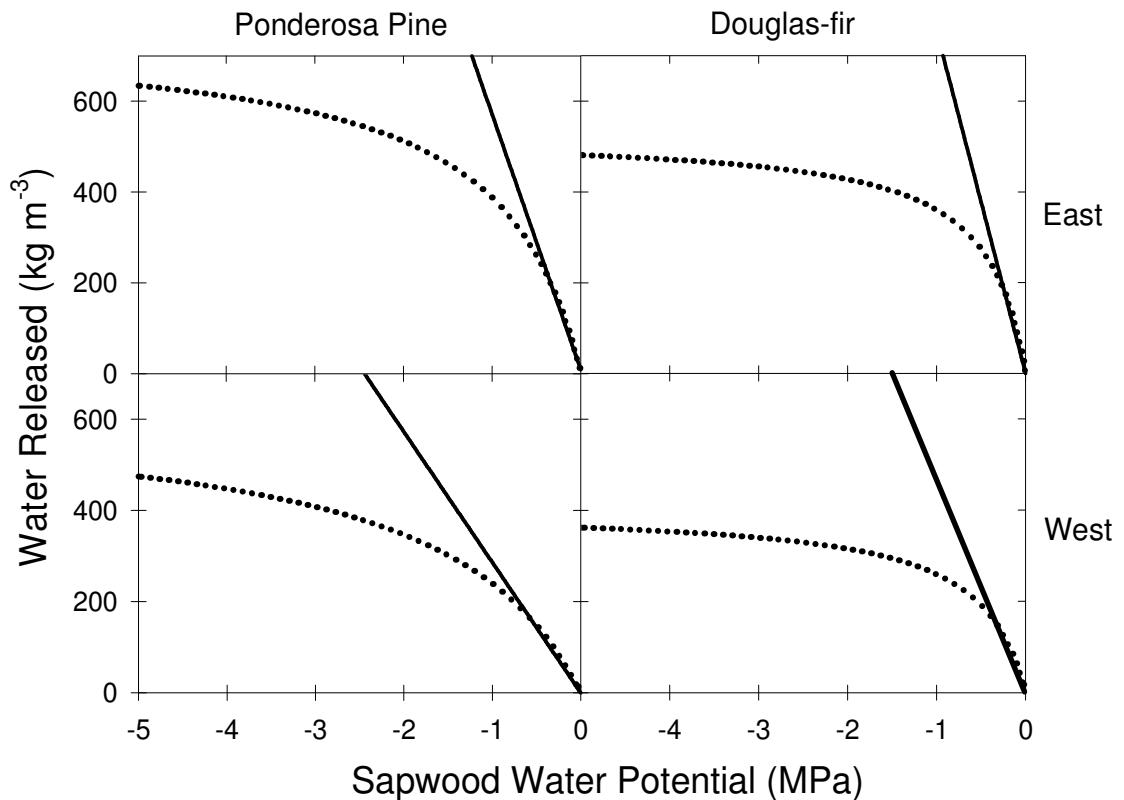
**Figure 2.1:** Comparison of injection method and acoustic emissions methods (a) and cumulative acoustic events (UAE) and cumulative acoustic energy (b) methods for vulnerability to embolism. Note: water potential in (a) corresponds to negative of applied pressure for injection method and mean water potential values derived from moisture release data for acoustic emissions.



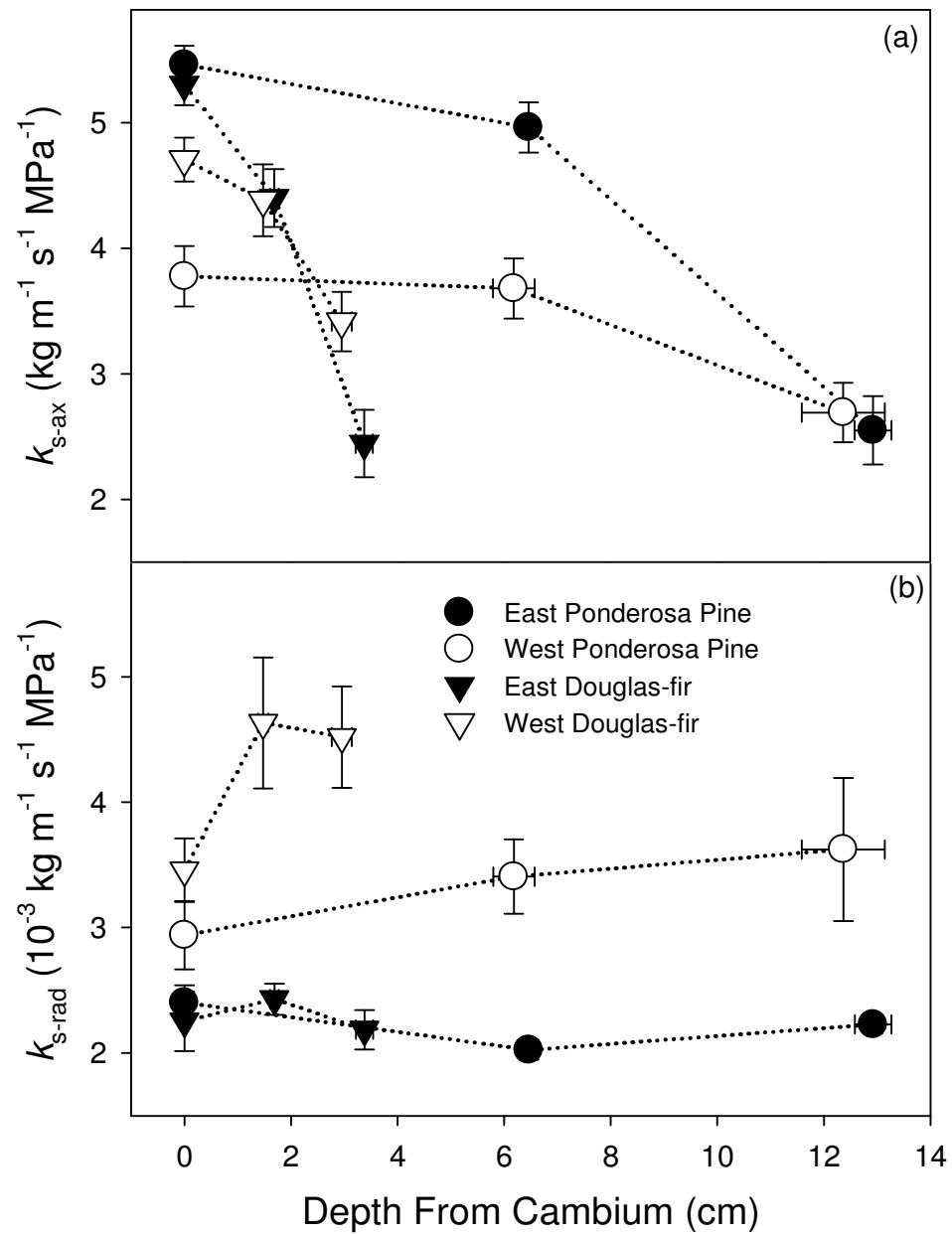
**Figure 2.2:** Sapwood thickness as a percentage of total stem diameter at breast height (1.3m) for east and west site populations of ponderosa pine and Douglas-fir.



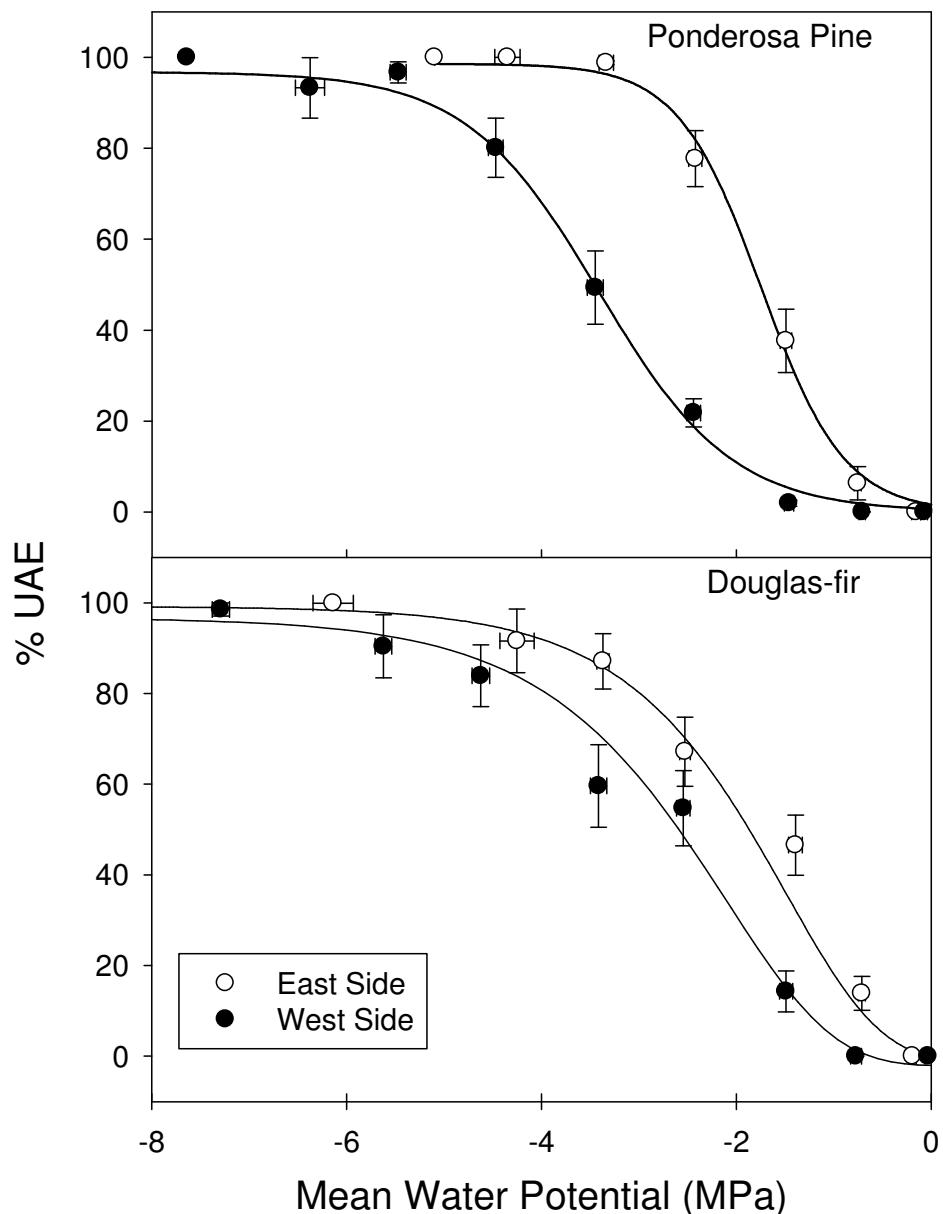
**Figure 2.3:** Moisture release curves of east and west populations of ponderosa pine and Douglas-fir show the relationship between sapwood water potential and relative water content



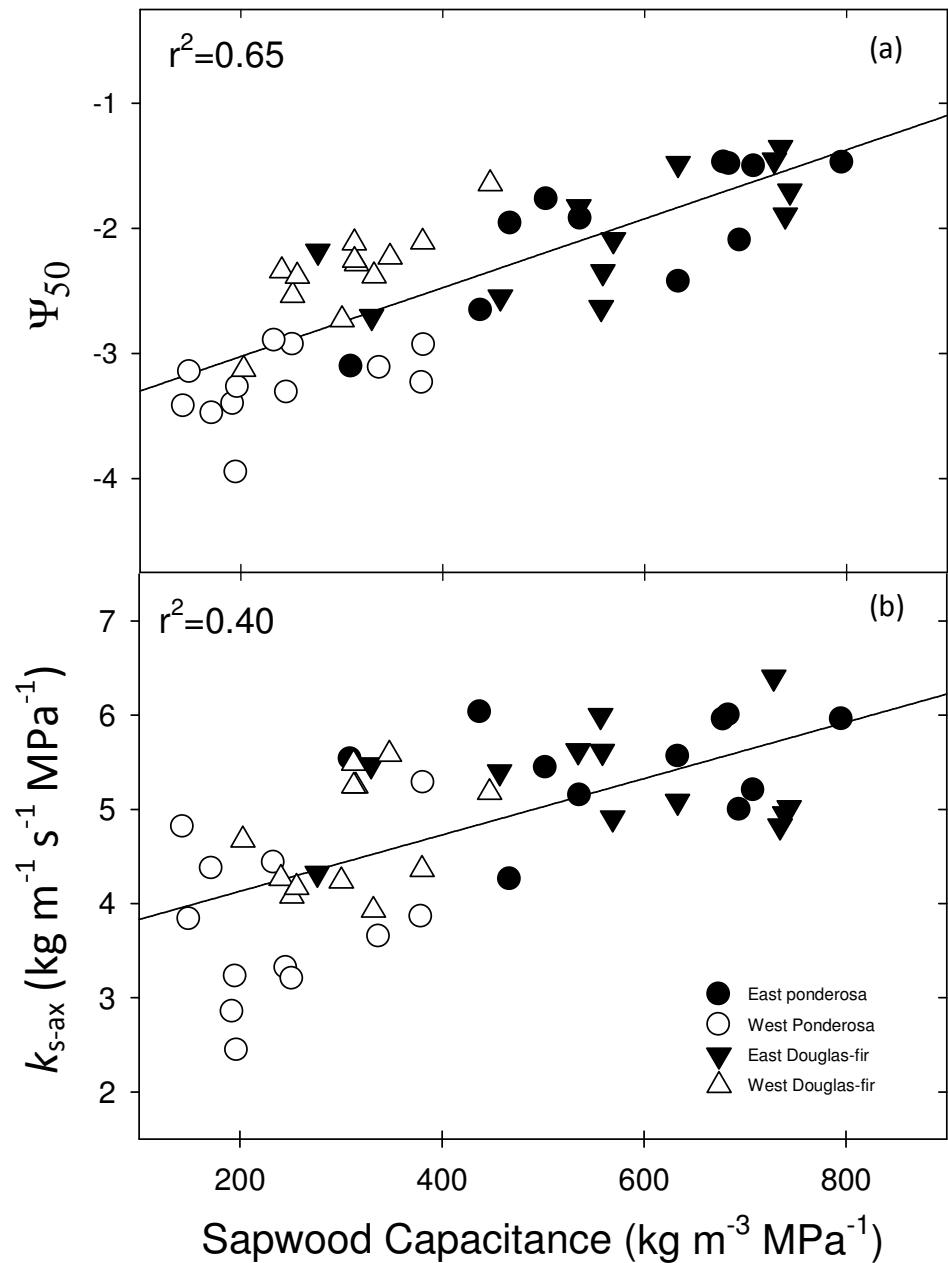
**Figure 2.4:** Representative graphs of capacitance show the relationship between the cumulative amount of water released ( $\text{kg m}^{-3}$ ) and water potential in east-side and west-side populations of Douglas-fir and ponderosa pine. Capacitance values are calculated as the slope of the initial, nearly linear portion of the curve.



**Figure 2.5:** Radial profiles of axial ( $k_{s\text{-ax}}$ ) and radial ( $k_{s\text{-rad}}$ ) specific conductivities ( $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) in east-side and west-side populations of ponderosa pine and Douglas-fir. Bars represent one standard error.



**Figure 2.6:** Vulnerability curves show the relationship between percent cumulative ultrasonic acoustic emissions (UAE) and mean water potential derived from moisture release data for ponderosa pine and Douglas-fir from east-side and west-side sites. 3-parameter, Sigmoidal curve fit to observational data. Bars represent one standard error.



**Figure 2.7:** Relationship between sapwood capacitance and hydraulic parameters. Upper panel (a) shows the relationship between sapwood capacitance and mean embolism pressure ( $\Psi_{50}$ ) and (b) axial specific conductivity ( $k_{s-\text{ax}}$ ).

**Xylem characteristics and radial conductivity in three species of  
conifers with populations adapted to contrasting climates**

David M. Barnard, Barbara Lachenbruch, Frederick C. Meinzer, Katherine A. McCulloh

## Chapter 3 - Xylem Characteristics and radial conductivity in three species of conifers with populations adapted to contrasting climates

### Abstract

This study investigated potential links between sapwood radial conductivity ( $k_{s\text{-rad}}$ ,  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) and xylem ray characteristics in an attempt to define the pathway of radial water movement in populations of *Pseudotsuga menziesii*, *Pinus ponderosa* and *Pinus contorta* adapted to the contrasting climates of the eastern and western sides of the Cascade Mountains in Oregon. The sapwood thickness of these three conifers varies widely and as do the percentages of ray parenchyma (RP) and ray tracheids (RT), offering an opportunity to investigate radial water transport strategies across a broad range of sapwood anatomy. A high pressure flow meter was used to determine  $k_{s\text{-rad}}$  and fluorescent microscopy was used for anatomical analyses. Radial conductivity was highest in the western population of *Pseudotsuga menziesii* ( $3.46 \times 10^{-3} \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) and lowest in the eastern population of *Pseudotsuga menziesii* ( $2.25 \times 10^{-3} \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ). RT lumen areas were larger and accounted for a greater proportion of total ray area in east-side populations. Total ray frequency was also higher in east-side populations but no trend was evident with RP cell areas or proportions. *Pinus contorta* had the largest RT and RP lumen areas, while *Pseudotsuga meinzesii* had the smallest RT lumen areas. The negative relationship between  $k_{s\text{-rad}}$  and the number of growth ring boundaries in a sample coupled with a

lack of relationships between ray anatomical properties and  $k_{s\text{-rad}}$ , did not support the hypothesis of RT or RP-facilitated radial water movement.

## Introduction

Transpiration generates a hydrostatic pressure gradient in the xylem that drives axial movement of water from roots to leaves. However, the sapwood of tree trunks typically contains multiple rings of functional xylem and tree architecture requires that water move toward more recently produced outer growth rings that have the most direct connections with transpiring leaves (Maton and Gartner 2005). Axial sap flow recorded in middle sections of sapwood that have no direct connection with leaves indicates that a driving force for radial water movement exists (Phillips et al. 1996; Ford *et al.* 2004a and b; Domec et al. 2006). However, the pathway for radial water flow in conifers has not been clarified. In general, there are no pits on tangential walls of axial tracheids with the exception of the last rows of the latewood (Lamin and Welle 1971, Koran 1977 and Kitin *et al.* 2009). Radial flow cannot occur through axial tracheids except where tracheids are not perfectly aligned tangentially, and may have semi-tangential pitting that actually transports water in an obliquely radial direction. Ray tissues, either ray tracheids (RT) or ray parenchyma (RP), constitute the more likely low resistance radial pathways spanning successive growth rings. Rays in conifers always contain RP cells, but certain species (mostly in the Pinaceae) have RTs as well (Panshin and de Zeeuw 1970, Esau 1977, Hoadley 1990). Due to

their anatomical similarity to axial tracheids, it has been assumed that RTs facilitate the radial movement of water within the stem (Tyree and Zimmerman 2002) but this has not been investigated. Physiological studies have assessed the importance of radial water movement (Domec *et al.* 2005a, James *et al.* 2003) and the magnitude of the tension gradients driving this movement (Domec *et al.* 2006), but we have seen no publications that relate ray anatomical characteristics and radial specific conductivity ( $k_{s\text{-rad}}$ ,  $\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ ). In this study we examine the relationships between ray anatomy and frequency, sapwood depth and  $k_{s\text{-rad}}$  in three coniferous species with populations adapted to the contrasting climates found on the eastern and western side of the crest of the Cascade Mountain range in Oregon. Our goal was to determine whether ray characteristics and sapwood depth are related to  $k_{s\text{-rad}}$ . This information will be helpful to increase physiological understanding of tree sapwood utilization for water transport and to increase understanding of potential pathways for sapwood colonization by pathogens. This research will also help wood technologists to better understand penetration of preservatives and fire retardants.

Radial profiles of axial sap flow vary widely among tree species (Phillips *et al.* 1996, Gartner and Meinzer 2005). In some tree species such as Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) the highest rates of sap flow occur in a narrow zone almost immediately adjacent to the vascular cambium (Domec *et al.* 2006). In other species such as pines the majority of axial water transport occurs at a distance about one third of the way inward from the cambium to the sapwood/heartwood boundary (Mark and Crews 1974, Ford *et al.* 2004a, b, Meinzer unpublished data). In

addition to conducting the bulk of sap flow in the middle sapwood, ponderosa pine also has extremely thick sapwood (up to about 80% of total stem diameter in mature trees) while Douglas-fir maintains 15-20% of total stem diameter as sapwood (Smith *et al.* 1956, Lassen and Okkonen 1969). Furthermore, most conifers have long-lived needles, which in many species can withdraw water from inner growth rings at least up to three years old (Maton *et al.* 2005), yet the portion of trunk sapwood conducting water may be upwards of 100 years old in ponderosa pine. These sap flow patterns and corresponding sapwood thickness require appropriate pathways for radial water movement.

Among the two cell types in rays of conifers, RP may or may not have lignified cell walls, but RTs always have lignified walls. RP consists of living cells in the sapwood connected to axial tracheids through half-bordered pit pairs, also called cross field pits (Panshin and de Zeeuw 1971, Coté and Day 1969). For water to move from an axial tracheid into a RP cell it has to traverse the pit membrane and then traverse the cell membrane into the RP. Once inside the RP it would presumably be able to move from cell to cell in the radial direction through plasmodesmata. The function of RP has been linked to heartwood formation (Bamber 1975, Spicer *et al.* 2005) storage of assimilates (Sauter and van Cleve 1994, Gartner *et al.* 2000) and a variety of processes in response to wounding and mechanical padding (reviewed in Gartner 1995).

RTs, much like their axial counterparts, are hollow and dead at maturity and are connected to other ray and axial tracheids by bordered pit pairs (Gordon 1912, Coté and Day 1969). For water to move from an axial tracheid into an RT cell, it has to traverse the pit membrane. Once inside an RT, the water would move radially through the pit membrane into the adjacent RT. Transmission electron microscopy of the RTs of southern yellow pine (*Pinus spp.*) showed that the bordered pits of RTs have a torus-margo pit membrane complex similar, but substantially smaller than those in axial tracheids (Coté and Day 1969). RT cell walls have also been shown to be more highly lignified than those of the RP in *Pinus radiata* (Singh *et al.* 2006). Due to their similarities to axial tracheids, it has been assumed that RTs function in water transport (Tyree and Zimmerman 2003). RTs are found almost exclusively in conifers (although they have been reported in the angiosperm genus *Banksia*, Chattaway 1948) and mostly in the Pinaceae, and are most prolific (in terms of their proportional composition of the rays) in the genus *Pinus* (Denne and Turner 2009), and also occur in certain members of the Cupressaceae and Taxodiaceae, in which they are often associated with traumatic injury (Gordon 1912). The large number of tree species lacking RTs and the phylogenetic occurrence of RTs raises the question of whether they contribute substantially to radial water transport or whether they are simply vestigial cells that have no evolved structure with an important role.

This study was carried out at sites throughout central and western Oregon spanning the widely contrasting climates found on the eastern and western side of the crest of the Cascade Mountain range. The region west of the Cascade Crest

experiences much higher precipitation (1000-3000 mm year<sup>-1</sup>) and a relatively narrow temperature range given the latitude. The region east of the Cascade Crest lies in the rain shadow of the Cascades (100-300 mm year<sup>-1</sup>) and experiences a more continental climate characterized by cold winters with frozen precipitation and warm, dry summers. The contrasting climates also give rise to different soil properties. While the soils on both the eastern and western side of the Cascade Crest are volcanic in origin, western soils tend to have higher organic content and higher water holding capacity than those found on the eastern side, which means they may release water more slowly to tree roots. Despite the contrasting climatic and edaphic conditions west and east of the Cascade Crest, several coniferous species have distribution ranges that encompass both regions. Douglas-fir is a dominant canopy species in the western and Coastal Range forests but the western or coastal population (*Pseudotsuga meintziesii* (Mirb.) Franco var. *meintziesii*) is genetically distinct from the eastern, or interior, population (*Pseudotsuga meintziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco). Shore pine (*Pinus contorta* Douglas ex Loudon var. *contorta*) found along the Pacific coast from Northern California to Alaska is a genetically distinct subspecies of the more common lodgepole pine found in the eastern Cascades (*Pinus contorta* Douglas ex Loudon var. *murrayana* (Balf) Engelm.). Ponderosa pine is the most widely distributed pine in North America (Burns and Honkala 1990) and can be found north into Canada, south into Mexico and from the Pacific Ocean east to the high plains of the mid-west. A unique population of ponderosa pine is found in the Willamette Valley of western Oregon (S. Meyers, personal communication), and

grows in the flatter portions of the valley and has gained some notoriety as a timber species due to its ability to grow well in situations that are either too wet or too dry for Douglas-fir. The difference in climatic and edaphic factors and the presence of genetically distinct populations of tree species presents a unique opportunity to study variation in structural and functional features of sapwood that may be adaptive for maintaining water transport under contrasting conditions.

The aim of the current study was to investigate differences in sapwood  $k_{s\text{-rad}}$  and their potential relationship to xylem ray anatomical characteristics in an attempt to infer radial flow pathways. We hypothesized that there would be a positive correlation between sapwood thickness and  $k_{s\text{-rad}}$  in individuals, populations and species because access to water in deeper portions of sapwood should require greater  $k_{s\text{-rad}}$ . Secondly, we hypothesized that populations growing east of the Cascade crest would have thicker sapwood and higher  $k_{s\text{-rad}}$  than western populations because individuals growing in the eastern sites were expected to experience steeper xylem tension gradients (other factors being equal) due to higher vapor pressure deficits and lower soil moisture availability. Our third hypothesis was that RTs, and not RP, serve as a pathway for radial water movement and that species and populations with higher RT frequencies or proportions would have higher  $k_{s\text{-rad}}$  values.

## Methods and Materials

### *Sample Material*

For this study we chose *Pinus contorta* (*PICO*), *Pinus ponderosa* (*PIPO*) and *Pseudotsuga menziesii* (*PSME*) because they have RTs, they have distinct populations adapted to the east and west-sides of the Cascade Mountains, and they differ in their sapwood thicknesses. For the duration of this paper, when referring to location of a specific population, eastern denotes east of the crest of the Cascade Mountains while west refers to those populations from the western side of the Cascade crest. We sampled a total of 12 stands from central Oregon westward to the coast, spanning a range of precipitation and temperature regimes (Table 3.1). At each stand 12 trees were selected with a breast-height (1.3 m) diameter of 20-40 cm, similar crown sizes and an overall healthy appearance (i.e. lacking visible damage or insect or disease infestation). To characterize individual tree growth (i.e. sapwood depth, age), we took two 5-mm-diameter increment cores from breast height to a depth that included the pith, for all trees. For trees without visible trunk curvature or lean, and that grew on relatively flat ground, the cores were taken at 90° angles to one another. For trees that had trunk curvature, were leaning or growing on a slope, cores were taken at 180 ° to one another and 90 ° from the axis of lean to avoid both compression wood and opposite wood. For determination of  $k_{s\text{-rad}}$ , we then selected six of those 12 trees and took 12 mm increment cores from breast height. These cores were also taken at positions to limit the presence of compression or opposite wood. The number of 12

mm cores per tree depended on sapwood depth determined from the 5 mm cores. If sapwood depth was 3 cm or less, we took three cores to ensure sufficient sample material; otherwise only two cores were extracted. After removing the cores from the tree, we marked the heartwood/sapwood boundary (visible as the marked change in translucency when held up to light) with a pencil. All cores were then sealed into two nested plastic bags and placed in an ice-filled cooler for transport to the lab.

### *Radial Conductivity*

Each 12 mm diameter increment core was cut into 10 mm sections (extending in the radial direction) corresponding to outer, middle and inner sapwood. Outer sections began two growth rings inward from the vascular cambium to safeguard against including immature cells from the cambial zone. The middle sections were centered one-half the distance from the vascular cambium to the heartwood-sapwood boundary. Inner sapwood sections terminated two growth rings outward from the heartwood-sapwood boundary to ensure no heartwood was included in the sample. Fresh mass and then volume, determined by the immersion method, were recorded and we then placed the samples into a perfusion solution containing 0.22  $\mu\text{m}$  filtered and degassed, distilled water, adjusted to pH 2 with HCl to retard bacterial and fungal growth. The samples were infiltrated under partial vacuum overnight to remove any air embolism. Radial conductivity ( $k_{\text{s-rad}}$ ) was determined using the high pressure flow meter (HPFM) method described by Tyree (1993) and Yang and Tyree (1994).

Specifically, conductivity is calculated from the pressure drop across a capillary tube of known resistance placed between the sample and a pressurized tank. Resistance of capillary tubes was previously calculated by recording pressure differential (MPa) and flow rate ( $\text{kg s}^{-1}$ ). Calibration curves were derived from these data and used to determine flow rate ( $f$ ) through the sample ( $\text{kg s}^{-1}$ ) at a given pressure differential (MPa). The pressure gradient ( $\Delta P$ ) (MPa m<sup>-1</sup>) was calculated as

$$\Delta P = \frac{p_{ds}}{l}$$

where  $p_{ds}$  is the pressure (MPa) at the down-stream end of the capillary tube and  $l$  is the length (m) of the sample. Pressure gradient and flow rate were then used to calculate hydraulic conductivity ( $k_h$ ,  $\text{kg m s}^{-1} \text{ MPa}^{-1}$ ) as

$$k_h = \frac{f}{\Delta P}$$

Hydraulic conductivity was then normalized by the sample cross-sectional area ( $A$ , estimated as  $\pi r^2$  for the 12 mm-diameter increment core), resulting in specific conductivity ( $k_s$ ,  $\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ ), by the equation

$$k_s = \frac{k_h}{A}$$

Pilot work indicated that  $k_{s,\text{rad}}$  decreased with increasing pressure until the pressure differential reached around 0.12 MPa and then leveled off and did not decline with time, so samples were perfused for a period of 10-15 minutes (or until the pressure differential stabilized) at an applied pressure of 0.15 MPa.

### *Growth Ring Properties*

To determine the effect of growth ring boundaries and wood properties on  $k_{s\text{-rad}}$ , we counted the number of growth ring boundaries (the latewood from a previous year to the earlywood of the next year) and measured the earlywood and latewood widths of all included growth rings. These measurements were made under a dissecting microscope using a tree-ring measuring device. (Metronics QC10-V, Metronics Bedford, N.H., USA) on saturated samples. We then oven-dried the samples overnight at 70° C and recorded dry mass the following morning for determination of sample density (dry mass per saturated volume, g cm<sup>-3</sup>).

### *Ray Proportion and Anatomy*

We made 30-μm-thick radial sections of outer sapwood using a sliding microtome. Sections were not stained. They were mounted in glycerin and analyzed with a fluorescence microscope (Nikon Eclipse E400, Nikon Instruments, Melville, NY, USA). Utilizing the UV-2A filter, the lignified walls of the RTs fluoresced as much brighter blue, whereas the unlignified walls of the RP cells fluoresced as a darker blue. We recorded one 10x image (about 1.25mm<sup>2</sup>) for each sample using a microscope mounted digital camera (Micropublisher 5.0 RTU, QImaging, Surrey, B.C., Canada) and Q Capture Pro imaging software (QImaging Canada). We then used ImageJ image analysis software (NIH, Washington D.C. USA) for measurement of RT and RP lumen areas. ImageJ is equipped with an ellipse tool, which creates a smooth elliptical shape analogous to the shape of ray lumens, however the lumens of

certain cells are not elliptical (particularly the pyramidal RTs in Douglas-fir), for these cells we used the freehand pen tool (allows creation on non-geometric shapes) to more accurately outline the cell lumen. Fusiform rays were measured similarly to uniseriate rays, except, we did not measure lumen areas for epithelial cells or the actual resin canal. Additionally we counted total number of rays in the field of view (not including rays that extended outside of the field of view). From our microscopy analysis we were able to derive RT and RP lumen area frequency distribution, the proportion of total area comprised of each cell type ( $\mu\text{m mm}^{-2}$ ), and the frequency (no.  $\text{mm}^{-2}$ ) of cells per total area. We also calculated the proportion of total tangential area that was comprised of ray lumen area (total ray proportion,  $\mu\text{m mm}^{-2}$ ) as well as the number of whole rays in a specific tangential area (total ray frequency, no.  $\text{mm}^{-2}$ ).

### *Data Analysis*

Site differences among populations of each species were not significant (data not shown) so data from the two sites for each population of each species were pooled, resulting in  $n=24$  individuals per species per population for growth characteristics and  $n=12$  individuals for water transport and anatomical characteristics. We used one-way and two-way analysis of variance (ANOVA) for comparisons between groups and sub groups and linear regression for analysis of correlation. Multiple comparisons among sub-groups were completed using a Tukey adjustment for significance; otherwise, results were considered significant at the 95% confidence level. All statistical tests were done in S Plus (TIBICO Software, Palo Alto, California, USA)

## Results

### *General growth characteristics*

Sapwood thickness was greatest in both populations of *PIPO* with no significant difference between east and west-side populations ( $p=0.25$ , Table 3.2). East *PICO* had significantly thicker sapwood than the west *PICO* ( $p<0.0001$ ), and east *PSME* had significantly thicker sapwood than west *PSME* ( $p=0.013$ ). Number of growth rings in the sapwood of eastern populations was significantly higher in all three species ( $p<0.001$  for all). Sapwood density was significantly higher in western populations of *PIPO* ( $p<0.001$ , Table 3. 2) but did not differ between east-side and west-side populations of *PICO* ( $p=0.36$ ) or *PSME* ( $p=0.69$ ).

### *Radial conductivity*

$k_{s\text{-rad}}$  did not vary significantly among samples taken at different distances from the cambium in any species or population ( $p>0.10$  for all analyses, one-way ANOVA, Figure 3.1, Table 3.1).  $k_{s\text{-rad}}$  ranged from  $2.25 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$  in eastern *PSME* to  $3.46 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$  in western *PSME* and varied significantly among species in both east-side and west-side populations ( $p=0.0027$ ), with west *PSME* having the highest  $k_{s\text{-rad}}$ , followed by west *PICO*, then east *PICO*, then west *PIPO*, then east *PIPO* and finally east *PSME*. There was a significant negative relationship between the number of growth ring boundaries in the sample and  $k_{s\text{-rad}}$ , but the magnitude of the effect was very small ( $p=0.001$ ,  $r^2=0.14$ , Figure 3.2).  $k_{s\text{-rad}}$  was not significantly

correlated with wood density ( $p=0.43$ ) or sapwood depth ( $p=0.10$ ) but was significantly correlated with stem diameter ( $p=0.029$ ) and average annual growth increment ( $p=0.032$ ).  $k_{s\text{-rad}}$  was significantly correlated with total ray frequency ( $p=0.021$ ), but showed no significant correlation with total ray lumen proportion of total tangential area ( $p=0.86$ ), RT density or proportion ( $p=0.36$  and  $p=0.71$  respectively) or RP frequency or proportion ( $p=0.38$  and  $p=0.97$  respectively).

#### *Anatomical measurements*

Average RT lumen area ranged from  $0.05\mu\text{m}^2$  in west *PSME* up to  $0.2\mu\text{m}^2$  in west *PICO* (Figure 3.3). Differences were not significant between east-side and west-side populations of *PSME* ( $p=0.64$ ) or *PICO* ( $p=0.79$ ), but average RT lumen areas of east *PIPO* were significantly larger than west *PIPO* ( $p=0.004$ ). There were no significant differences in average RP cell areas ( $p=0.24$ ) between east-side and west-side populations (Figure 3.4). *PICO* had the largest RP lumen areas of east-side and west-side populations. East-side *PICO* was significantly larger than *PIPO* ( $p=0.007$ ) which were significantly larger than *PSME* ( $p=0.028$ ). West-side *PICO* RP cell areas were significantly larger than *PSME* ( $p=0.007$ ) but not *PIPO* ( $p=0.078$ ) and *PIPO* RP lumen areas were not significantly larger than *PSME* ( $p=0.16$ ). The proportion of total ray area that was RT (RT proportion) ( $\mu\text{m}^2 \text{ mm}^{-2}$ ) was significantly higher in east-side populations ( $p=0.001$ , figure 3.5a) and ranged from  $26\pm2\%$  in *PICO* down to  $4.0 \pm 0.5$  in *PSME* (Table 3.3). RP proportion was significantly higher in east-side *PSME* than *PIPO* and *PICO* ( $p=0.001$ , Figure 3.5b) but *PIPO* and *PICO* were not

significantly different ( $p=0.15$ ). RP proportion of west-side populations were not statistically different from one another ( $p=0.37$ , Table 3.3). Difference in RP proportion was not statistically different between east and west populations ( $p=0.69$ ) RT density (no.  $\text{mm}^{-2}$ ) was significantly higher in the east-side populations of all three species ( $p=0.0001$ , Table 3.3, Figure 3.6a). RT density did not differ significantly between *PICO* and *PIPO* in either east-side ( $p=0.35$ ) or west-side ( $p=0.24$ ) populations, but *PSME* was significantly lower in east-side and west-side varieties ( $p=0.001$  for both, Table 3.3). RP frequency was significantly higher in the east-side population of *PSME* ( $p<0.001$ ) than the west-side population, whereas west-side RP density was higher in *PIPO* ( $p=0.002$ ), there was no statistical difference between east-side and west-side populations of *PICO* ( $p=0.157$ , Table 3.3, Figure 3.6b). RP frequency did not differ statistically among the species of west-side populations ( $p=0.35$ ), however in east-side varieties, *PSME* had significantly higher RP frequency than both *PIPO* and *PICO* ( $p=0.001$  for both comparisons).

Total ray frequency was significantly higher in east-side populations of all species ( $p<0.001$ ) and was highest in east-side *PSME* and lowest in west *PICO* and varied significantly among species for ( $p<0.0001$ , Table 3.3, Figure 3.7a). The proportion of total area that was ray (the sum of RT proportion and RP proportion, called ray proportion) ( $\mu\text{m}^2 \text{ mm}^{-2}$ ) was significantly higher in the east-side population of *PSME* ( $p=0.046$ ) but differences were statistically indistinguishable between populations of *PIPO* ( $p=0.84$ ) and *PICO* ( $p=0.27$ , Table 3.3, Figure 3.7a). Overall,

*PICO* had significantly higher ( $p<0.001$ ) total ray proportion than *PSME* and *PIPO*, which did not differ significantly ( $p=0.34$ ).

## Discussion

Multiple pathways of radial water movement have been suggested in the literature and it is likely that water moving across growth ring boundaries does so through more than one pathway. Radial tension gradients can be up to 100 times greater than axial tension gradients (Domec *et al.* 2006) indicating that water moving radially encounters significantly greater resistance than water moving axially. The significant relationship between  $k_{s\text{-rad}}$  and number of growth ring boundaries suggests an increased resistance to water flow at growth ring boundaries and does not support the idea of RTs or RP facilitating water movement. This is further supported by lack of relationship between ray anatomical parameters and  $k_{s\text{-rad}}$ . The relationship of  $k_{s\text{-rad}}$  with stem diameter and radial growth rate suggests the vascular cambium may be, to some degree, dependent on a supply of water from the sapwood. It does not appear that greater  $k_{s\text{-rad}}$  is needed to facilitate movement of water from inner to outer sapwood in the species with thicker sapwood (*PIPO* and *PICO*) as shown by lower  $k_{s\text{-rad}}$  values. Furthermore, any benefit of greater RT or RP lumen area in increasing  $k_{s\text{-rad}}$  may have been confounded by sampling methods or hidden by other characteristics not addressed in this study.

The decrease in radial conductivity with increasing number of growth ring boundaries suggests that a significant amount of resistance to radial water flow occurs in the latewood portion of annual growth and supports the hypothesis of radial water movement through pitting on the tangential walls of axial tracheids instead of through ray cells or interstitial spaces in the rays. In fact, bordered pits on tangential walls were shown to comprise a fraction of total area similar to that of rays (Kitin *et al.* 2009). However, this pathway requires water transport through latewood cells. Latewood tracheids have thicker cell walls and smaller lumen areas which result in much lower axial conductivity than earlywood (Domec and Gartner 2002). However, if water crosses growth ring boundaries by this pathway, the increased resistance is unavoidable, but the radial water movement may be achieved by a slight diversion of axial water flow, through tangential pits, rather than through direct pith to cambium pathway.

Xylem rays, and their interstitial spaces, span multiple growth rings in the sapwood (Bannan 1937, Kitin *et al.* 2009) and could provide a pathway for radial water movement unaffected by the annual formation of the denser and more resistant latewood. RTs appear to provide an exceptionally efficient pathway for such movement; however the results of this study indicate that increased RT lumen areas, frequency and proportions do not affect  $k_{s\text{-rad}}$ . Previous studies investigating the radial pathway of preservative movements in conifer wood have yielded contrary results regarding the role of RTs as a gas or fluid pathway. Erickson and Balatinicz (1964) showed radial penetration of styrene through RTs and intercellular spaces in Douglas-

fir with the RP remaining impermeable in green wood. Comstock (1970) investigated directional gas permeability in several softwood species and discovered the highest radial gas permeability in pines, which would have had the highest RT counts and proportions (Hoadley 1990). Finally, Liese and Bauch (1967) emphasized the role RTs play in the radial penetration of preservatives in spruce and Douglas-fir from Oregon Forests. However, comparisons of gas and high molecular weight preservative movement in wood *ex vivo* with the radial movement of water *in vivo* are likely to be inappropriate.

RP may also serve as a pathway for radial water flow. The diffusion of water across the semi-permeable membrane, separating RP cells from one another and from axial tracheids, offers a resistance that is absent in tracheary cells, however the presence of membrane bound proteins (aquaporins) have been shown to facilitate and regulate water flow and may have potential implications for water storage and release. (Chrispeels and Maurel 1994, DeBoer and Volkov 2003 and Lovisolo and Schubert 2006). To date, the majority of research on RP has been aimed at metabolic function and storage (e.g., Gartner *et al.* 2000, Pruyn *et al.* 2005). Total ray density (no. mm<sup>-2</sup>) showed a weak relationship with  $k_{s\text{-rad}}$  which could be interpreted in one of two ways: either rays serve to facilitate water transport as a whole and the true effects are occluded in this study, or ray density (no. mm<sup>-2</sup>) may be indicative of the number of intercellular spaces between ray cells which have been proposed as a pathway of radial water movement (Kitin *et al.* 2009) and is supported by a lack of correlation between the proportion of area comprised of ray lumen and  $k_{s\text{-rad}}$ .

Differences in ray anatomy among species and between populations of species from contrasting habitats were substantial yet did not correlate strongly with growth characteristics. Rays originate at the vascular cambium from ray initials (Lev-Yadun 1995). Reports in the literature are conflicting regarding the effect that yearly radial growth increment (a proxy measurement for climate) has on the formation and characteristics of ray anatomy. Gartner *et al.* (2000) showed a decrease in ray frequency outward from the pith in Douglas-fir, but the decrease leveled off after about six years of growth, whereas the lumen areas remained relatively steady. Similarly, Yev-Ladun (1998) found no correlation between ray frequency and ring width in two species of pine. On the other hand Bannan (1937) showed a strong correlation between ring width and ray height in *Larix*, *Thuja* and *Tsuga*, while Gregory and Romberger (1975) showed a strong correlation between ring width (tracheids per ring) and both the total number of ray cells and ray height in *Picea glauca* and *Abies balsamea*. Based on the aforementioned research it appears that year to year variations in temperature and precipitation could affect the proportion and count of RTs and RP, thus making comparisons of ray anatomy from a single year's growth, as was done in this study, to conductivity measurements on a sample comprised of multiple years of growth unreliable. It is important to note that none of the above mentioned studies differentiated between RTs and RP which could prove to be particularly vexing in the pines assuming they have a larger proportion of RTs. (Hoadley 1990, Denne and Turner 2009). This presents a potential source of error in assuming homogeneity in ray anatomy parameters across multiple growth rings.

While more work is needed to fully understand the function of RTs, the results of the current study suggest a non-functional role, in water transport. RTs of several species, PIPO included, have convoluted wall thickenings that protrude into the lumen and should increase the resistance to water flow and further support the idea of limited functionality in water transport.

Although radial water movement is important in overall tree hydraulics, little is known about the pathways and thus factors regulating radial flow. More intensive work is needed to quantify sapwood  $k_{s\text{-rad}}$  from tree species spanning a variety of taxonomic and climatic variation. Real-time monitoring of water flow with dyes and/or isotopic tracers may prove beneficial for visualizing pathway and velocity of water movement in radial, tangential and axial planes. If such work could be done within individual growth rings as well as among several growth rings, one could also identify the role of within-ring anatomical changes (earlywood vs. latewood) as well as growth ring boundaries themselves in radial water movement. Flow studies in trees, in which dye was introduced to inner sapwood and later trees were cut, could show macroscopically the degree to which flow is radial vs. axial. Furthermore, a better understanding of ray anatomy is needed. Rays make up an estimated 10-20% of total wood mass (Panshin and de Zeeuw 1980), yet relatively little is known regarding their function and formation, or their impact on physiological functioning. A better understanding of the way in which trees utilize these basic tissues for the development of ecological strategies would certainly help to clarify how trees use their sapwood for water transport.

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**Table 3.1:** Stand locations and site characteristics from which samples were collected. Mean annual precipitation and maximum and minimum temperatures are data from nearest city.

	Species Sampled	No. of Stands	Nearest City	Latitude / Longitude	Elevation (m)	General Site Characteristics	Annual Precip. (mm)	Mean Summer Max and Winter Min. Temp (C°)
<b><i>Eastern Sites</i></b>								
Ochoco National Forest	<i>PSME</i>	2	Mitchell	43.584, -121.218	1185	(1) 20° slope and (2) flat area, both with mixed Douglas-fir and ponderosa pine	290	30 / -4
Sand Springs	<i>PICO</i>	1	Milican	43.627, -120.858	1645	Pure lodgepole pine stand, flat area adjacent to volcanic butte	285	29 / -4
Pine Mountain	<i>PIPO and PSME</i>	2	Milican	43.767, -120.963	1400	Mixed ponderosa and lodgepole pine at flat desert fringe area	300	27 / -4
Deschutes National Forest	<i>PIPO</i>	1	La Pine	43.584, -121.218	1680	Pure ponderosa pine stand, flat area	260	29 / -4.5
<b><i>Western Sites</i></b>								
MacDonald-Dunn	<i>PSME</i>	1	Corvallis	44.616, -123.293	200	Mixed stand of Douglas-fir, big leaf maple and grand fir, flat area	1110	28 / 1
Seal Rock	<i>PICO</i>	1	Waldport	44.496, -124.083	30	Mixed stand of shore pine and Sitka spruce.	2300	25 / 2.5
Gleneden	<i>PICO</i>	1	Depoe Bay	44.876, -124.036	10	Pure shore pine stand	2210	25 / 2.0
Cascade Timber Consulting	<i>PIPO and PSME</i>	3	Sweet Home	44.368, -122.828	285	(1) Pure ponderosa pine stand (2) Mixed ponderosa pine and Douglas-fir and (3) mixed Douglas-fir, big leaf maple and grand fir	1390	27 / 0.5

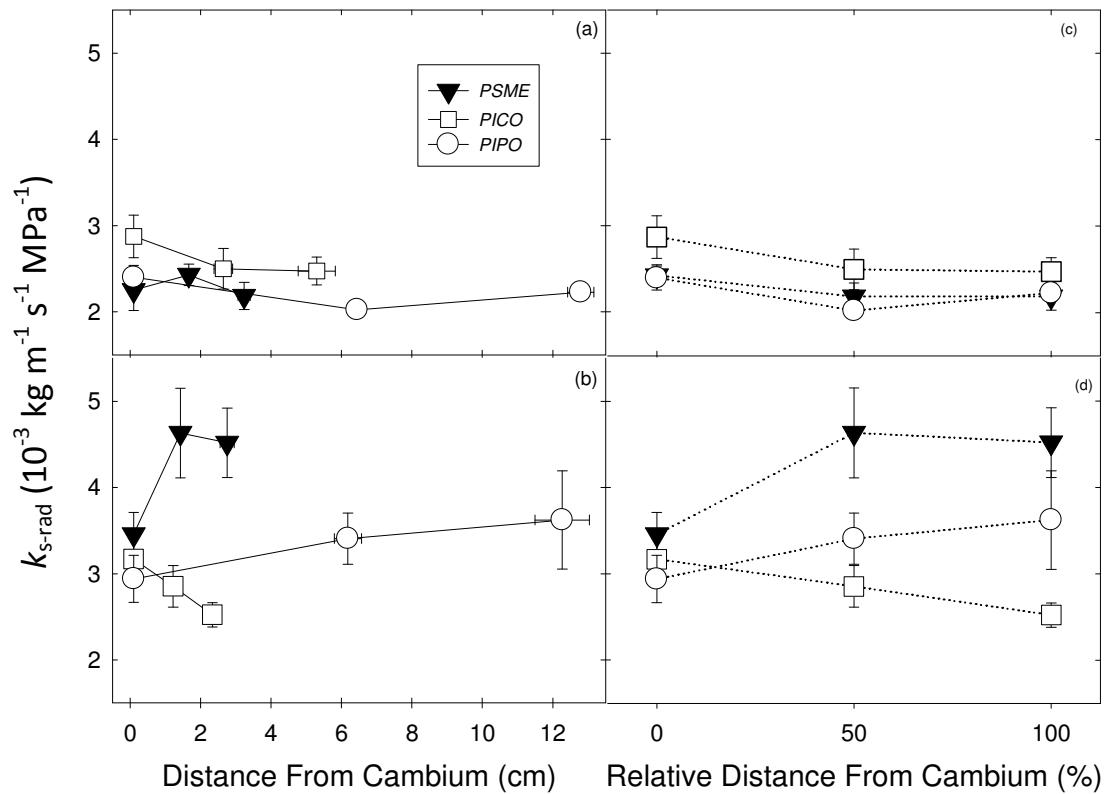
**Table 3.2:** General tree growth characteristics from east and west-side populations of the three species studied. Values indicate mean  $\pm$  SE ( $n=12$  for Sapwood Density and  $n=24$  for other characteristics). Differing letters in a column indicate statistical significance at ( $p=0.05$ )

	Sapwood Thickness (cm)	DBH (cm)	Age (years)	Rings of Sapwood (no.)	Growth Rate (cm year $^{-1}$ )	Sapwood Density (g cm $^{-3}$ )
<b>Eastern Populations</b>						
<i>Pseudotsuga menziesii</i>	3.3 $\pm$ 0.2a	37.7 $\pm$ 0.7a	101.4 $\pm$ 2.9a	31.5 $\pm$ 1.6a	0.37 $\pm$ 0.01a	0.49 $\pm$ 0.01a
<i>Pinus ponderosa</i>	12.9 $\pm$ 0.4b	39.4 $\pm$ 1.0a	80.5 $\pm$ 4.2b	68.6 $\pm$ 2.5b	0.50 $\pm$ 0.03b	0.39 $\pm$ 0.01b
<i>Pinus contorta</i>	5.2 $\pm$ 0.2c	31.6 $\pm$ 1.0b	85.0 $\pm$ 3.8b	41.2 $\pm$ 3.8c	0.38 $\pm$ 0.01c	0.39 $\pm$ 0.01b
<b>Western Populations</b>						
<i>Pseudotsuga menziesii</i>	2.5 $\pm$ 0.3a	33.7 $\pm$ 0.8b	61.1 $\pm$ 2.2c	9.6 $\pm$ 0.8d	0.56 $\pm$ 0.02d	0.50 $\pm$ 0.01a
<i>Pinus ponderosa</i>	12.1 $\pm$ 0.5b	37.4 $\pm$ 0.4a	51.2 $\pm$ 1.7d	42.6 $\pm$ 1.8e	0.74 $\pm$ 0.03e	0.48 $\pm$ 0.01c
<i>Pinus contorta</i>	2.4 $\pm$ 0.2c	27.0 $\pm$ 1.2c	41.5 $\pm$ 2.1e	13.4 $\pm$ 1.0f	0.66 $\pm$ 0.03f	0.41 $\pm$ 0.01b

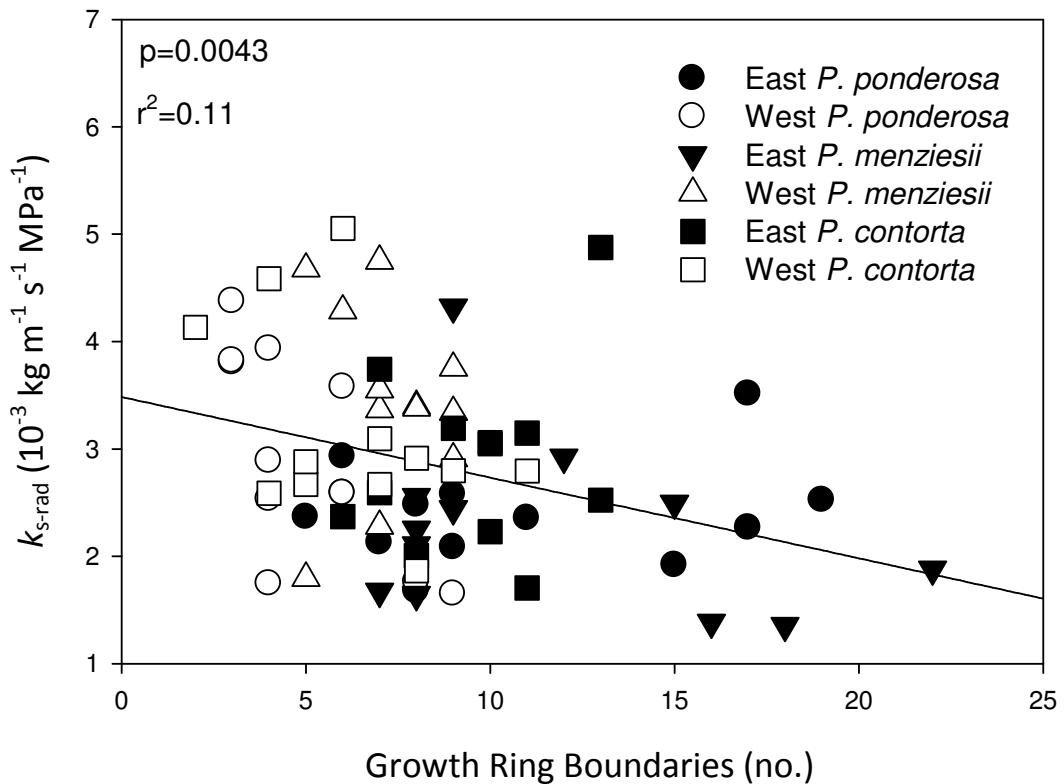


**Table 3.3:** Radial conductivity and ray properties. Values of  $k_{s\text{-rad}}$  for outer, middle and inner sapwood sections along with ray anatomy characteristics in eastern and western populations of three species. Differing letters in a column indicate statistical significance at  $p=0.05$ . Values represent mean $\pm$ SE ( $n=12$ ). Differing letters within a column indicate a significant difference at  $p=0.05$ .

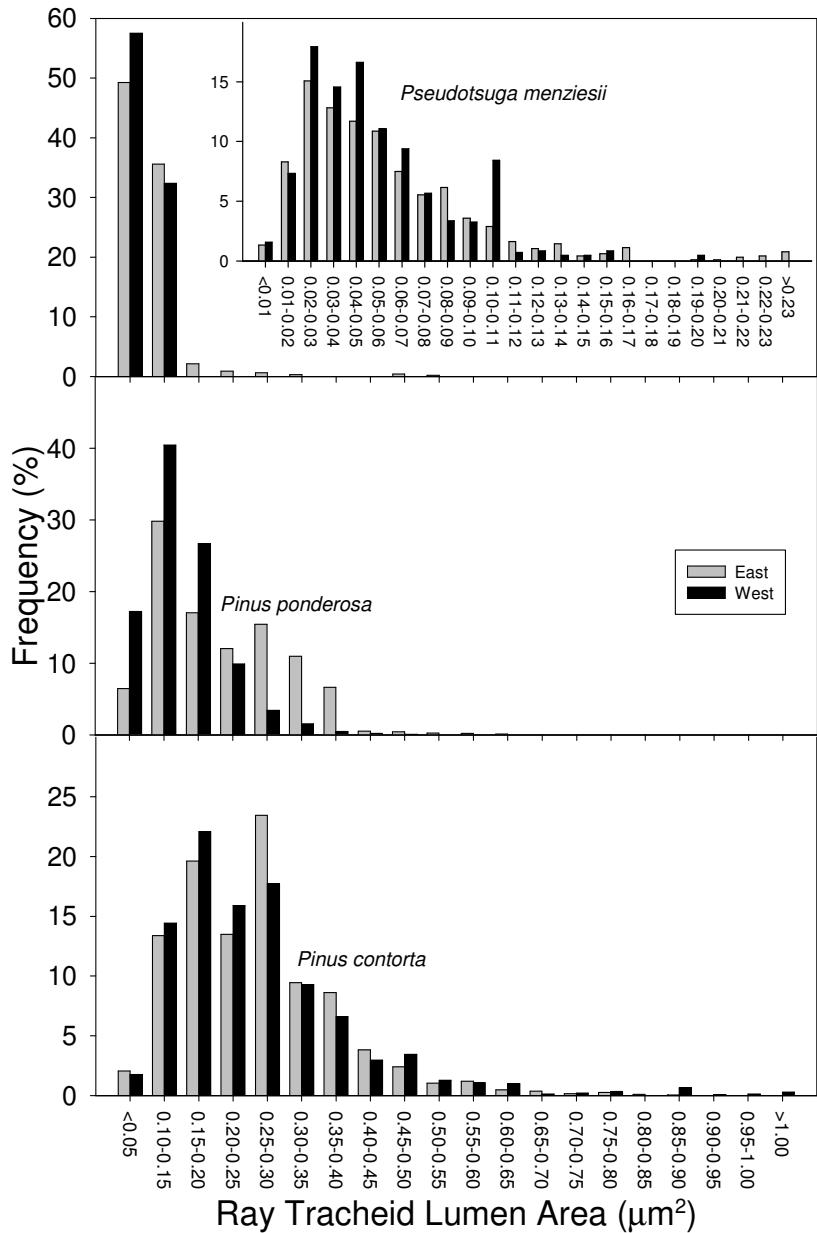
	$k_{s\text{-rad}}$ ( $10^{-3}$ kg m $^{-1}$ s $^{-1}$ MPa $^{-1}$ )			Ray Frequency (no. mm $^{-2}$ )	RT Proportion	RT Density	RP Proportion	RP Density
	Outer	Middle	Inner					
<b>Eastern Populations</b>								
<i>Pseudotsuga menziesii</i>	2.25 $\pm$ 0.2a	2.43 $\pm$ 0.1a	2.19 $\pm$ 0.2a	33.5 $\pm$ 0.6a	4.0 $\pm$ 0.5a	65.0 $\pm$ 1.8a	17.9 $\pm$ 1.1a	192 $\pm$ 10.2a
<i>Pinus ponderosa</i>	2.40 $\pm$ 0.1a	2.02 $\pm$ 0.1a	2.23 $\pm$ 0.1a	29.5 $\pm$ 0.8a	16.9 $\pm$ 1.5b	126 $\pm$ 6.4b	37.8 $\pm$ 4.4b	64.7 $\pm$ 3.3b
<i>Pinus contorta</i>	2.87 $\pm$ 0.3a	2.50 0.2a	2.47 $\pm$ 0.2a	26.1 $\pm$ 0.8a	25.7 $\pm$ 1.9c	127 $\pm$ 3.4b	23.1 $\pm$ 1.5c	63.8 $\pm$ 2.3b
<b>Western Populations</b>								
<i>Pseudotsuga menziesii</i>	3.46 $\pm$ 0.25b	4.63 $\pm$ 0.5b	4.52 $\pm$ 0.4b	28.1 $\pm$ 0.9b	3.0 $\pm$ 0.4a	57.5 $\pm$ 2.6a	22.2 $\pm$ 1.1d	131.7 $\pm$ 8.1c
<i>Pinus ponderosa</i>	2.94 $\pm$ 0.27b	3.41 $\pm$ 0.3c	3.62 $\pm$ 0.6c	25.4 $\pm$ 0.9b	9.3 $\pm$ 0.8d	94.9 $\pm$ 5.5c	29.9 $\pm$ 5.8e	83.3 $\pm$ 4.3d
<i>Pinus contorta</i>	3.17 $\pm$ 0.17b	2.85 $\pm$ 0.2d	2.52 $\pm$ 0.1d	23.1 $\pm$ 0.6d	20.2 $\pm$ 2.0e	97.3 $\pm$ 3.5c	31.8 $\pm$ 5.8f	69.3 $\pm$ 5.4e



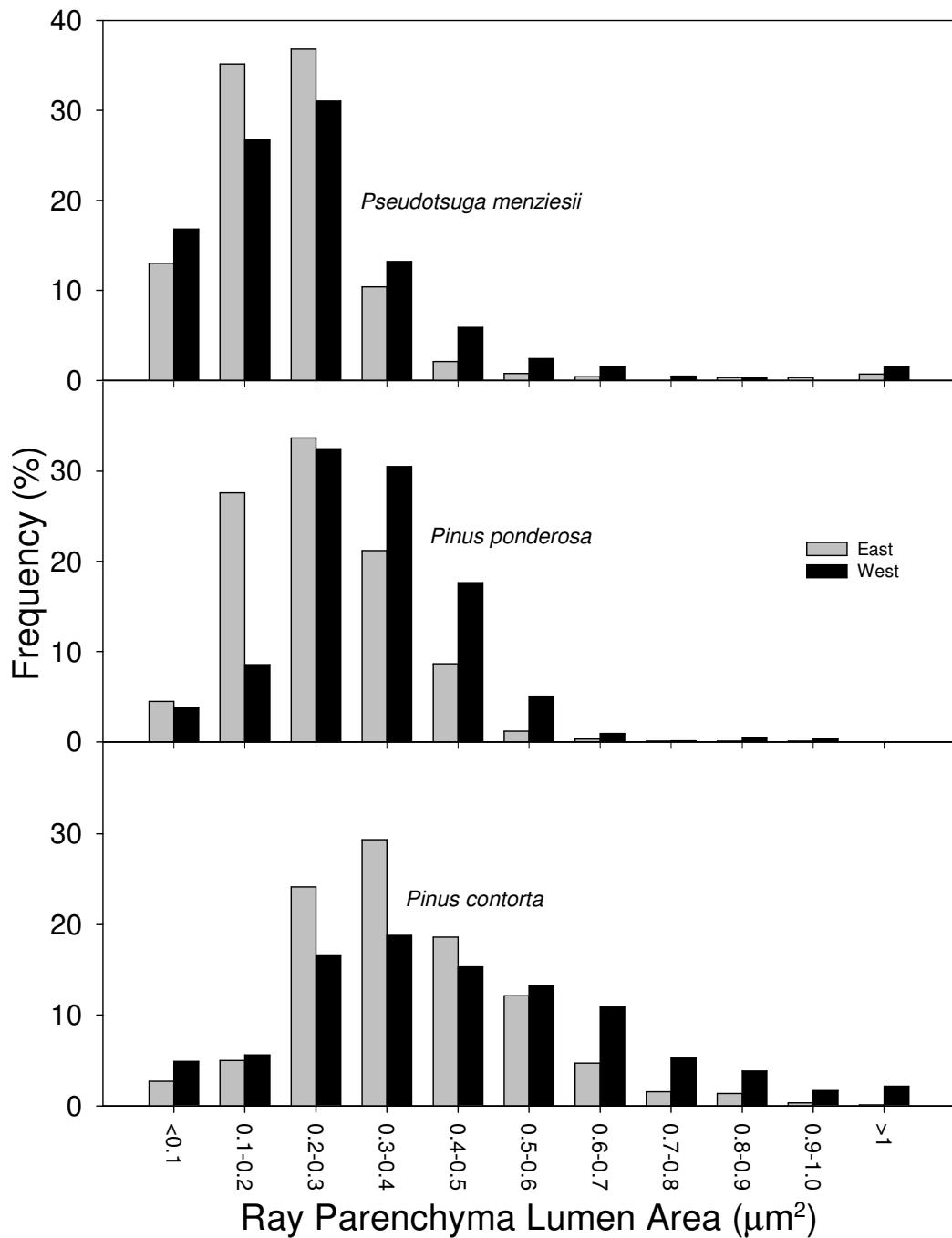
**Figure 3.1:** Sapwood radial conductivities of east side (upper panels) and west-side (lower panels) of *PSME*, *PIPO* and *PICO*. Panels (a) and (b) represent radial conductivities as absolute sapwood depths while panels (c) and (d) represent conductivities at relative sapwood depths. Error bars represent one standard error.



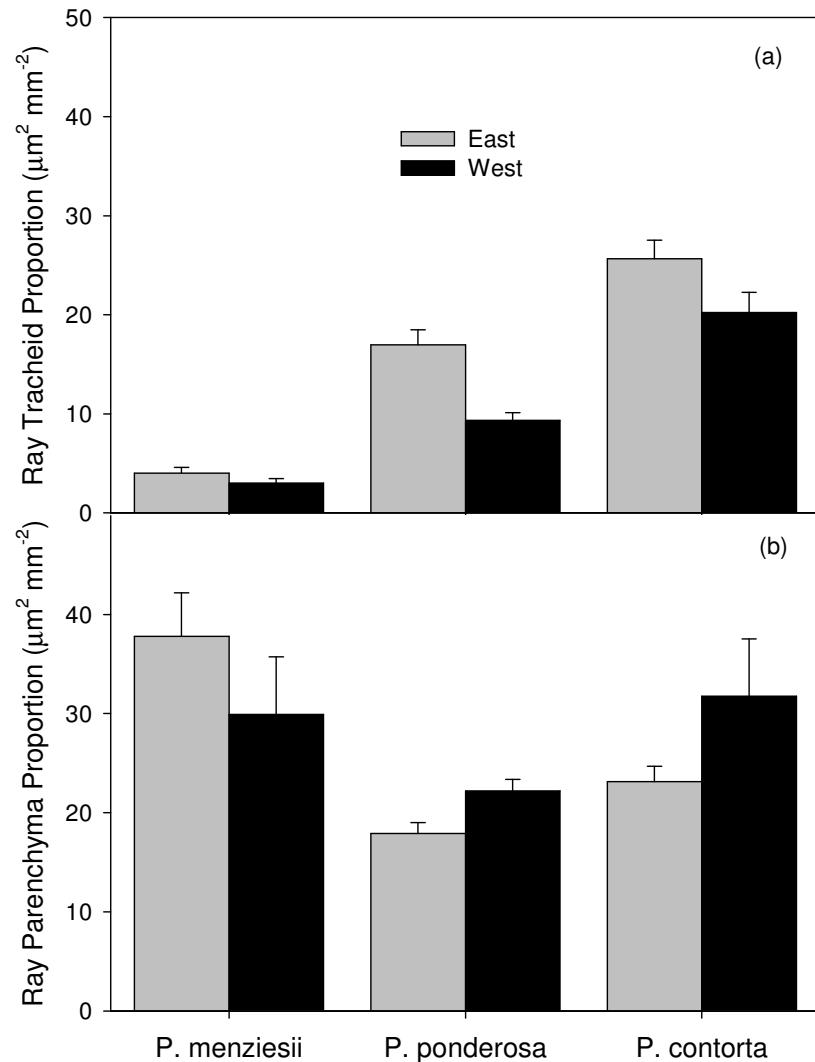
**Figure 3.2:** Relationship between total number of growth rings included in sample and radial conductivity ( $k_{s\text{-rad}}$ ) was collected in eastern and western populations of the three species studied. Regression line follows the equation  $f = 3.49 - 0.075x$ .



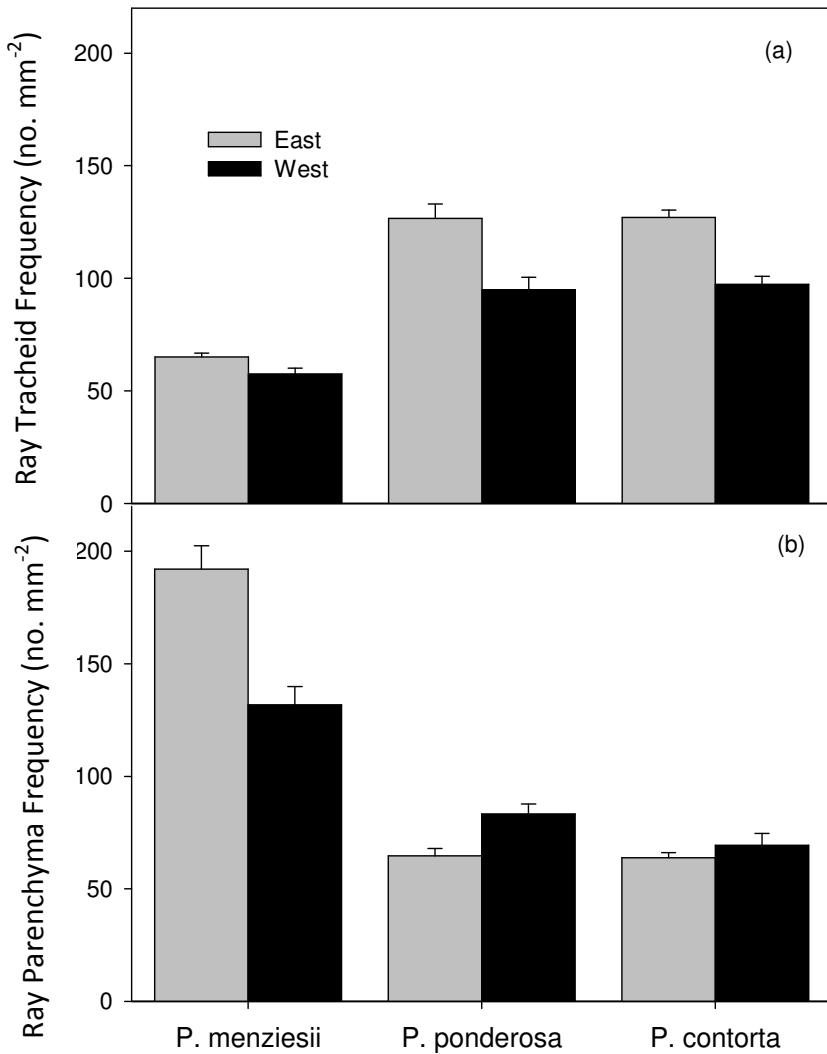
**Figure 3.3:** Frequency distribution of RT lumen areas of east and west-side populations of *PSME*, *PIPO* and *PICO*. Inset figure in top panel represents a higher resolution display of lumen distribution of *PSME*.



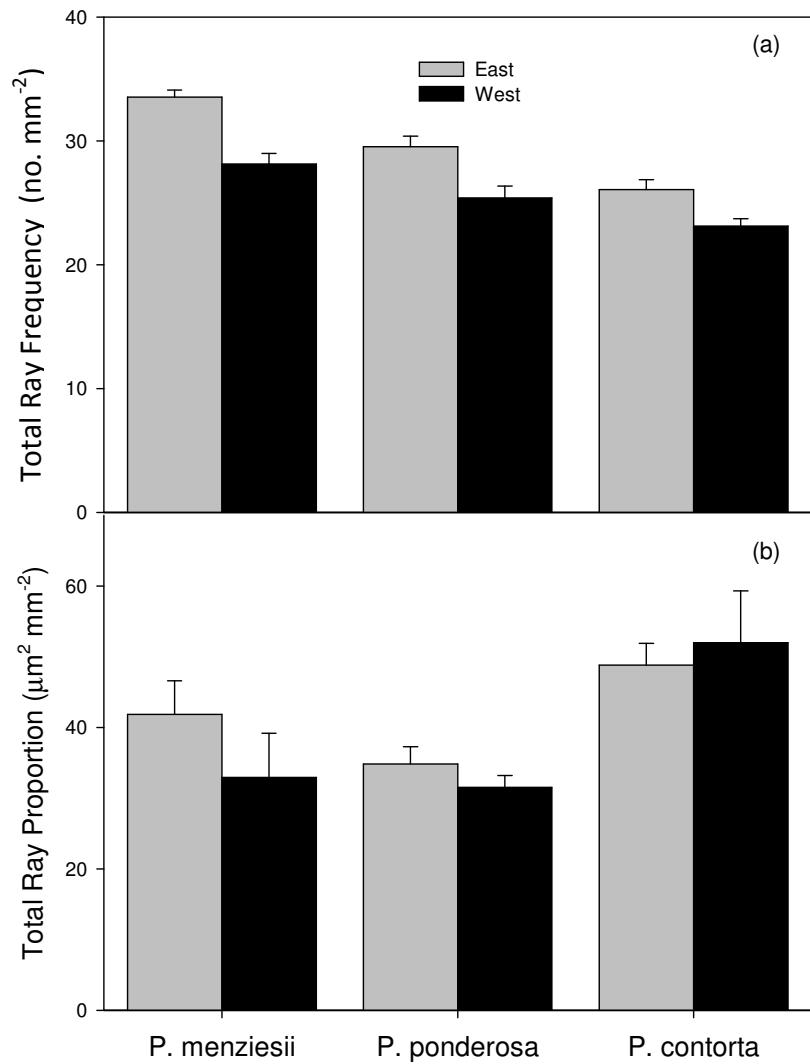
**Figure 3.4:** Frequency distribution of RP lumen areas of populations from the east-side and west-side of the crest of the Cascade Mountain range of Douglas-fir (*Pseudotsuga menziesii*) ponderosa pine (*Pinus ponderosa*) and the lodgepole and shore pine populations of *Pinus contorta*.



**Figure 3.5:** Ray tracheid and ray parenchyma proportions of populations from the east-side and west-side of the crest of the Cascade Mountain range of Douglas-fir (*Pseudotsuga menziesii*) ponderosa pine (*Pinus ponderosa*) and the lodgepole and shore pine populations of *Pinus contorta*. Bars represent one standard error.



**Figure 3.6:** RT and RP frequency expressed as number of cells per  $\text{mm}^2$  of populations from the east-side and west-side of the crest of the Cascade Mountain range of Douglas-fir (*Pseudotsuga menziesii*) ponderosa pine (*Pinus ponderosa*) and the lodgepole and shore pine populations of *Pinus contorta*. Bars represent one standard error.



**Figure 3.7:** Total ray frequency, reported as number of rays per  $\text{mm}^2$  (a) total area of tangential area analyzed comprised of ray cell lumens (b) of populations from the east-side and west-side of the crest of the Cascade Mountain range of Douglas-fir (*Pseudotsuga menziesii*) ponderosa pine (*Pinus ponderosa*) and the lodgepole and shore pine populations of *Pinus contorta*.

## Chapter 4 - Thesis Conclusions

Carbon acquisition is of paramount importance in tree survival and reproduction. Stomata must be open in order for CO<sub>2</sub> to diffuse to the sites of photosynthesis. However, open stomata also serve as the major pathway for transpirational water loss. As the rate of transpiration matches and exceeds the rate at which water may be absorbed from the soil the tension in the water column increases to point at which drought induced xylem dysfunction may occur and propagate (Tyree and Sperry 1988). The success of tree species in a variety of climatic and edaphic situations is due to a suite of physiological and anatomical adaptations aimed at allowing maximum carbon acquisition while minimizing or avoiding the impacts of high tensions in the xylem which could result in tree mortality (McDowell *et al.* 2008). The characteristics of water movement in trees generally are well understood with several types of tradeoffs between safety and efficiency of water transport having been identified (Saliendra *et al.* 1995, Tyree and Zimmerman 2002, Stout and Sala 2003, Sperry *et al.* 2008) as well as relationships between physiological and anatomical characteristics that serve to compensate one another (Meinzer 2003, 2006, 2008b, Scholz *et al.* 2007).

Stem sapwood serves several functions, but the two main functions are to provide structural support for the canopy and to act as a conduit for the transport of water from the soil to the canopy. A third function of sapwood can be as a storage tissue that can significantly contribute to daily transpirational water use (Waring and

Running 1978, Čermák *et al.* 2007) and may serve to buffer water column tensions to protect against xylem embolism. Because all water used by a tree must be transported through the trunk sapwood, understanding anatomical and physiological limitations and adaptations for maintaining water transport across ecological gradients is imperative in building a better understanding of tree structure and function. In this study we investigated the tradeoffs and relationships between factors regulating the safety ( $\Psi_{50}$ , capacitance) and efficiency ( $k_{s\text{-ax}}$ ,  $k_{s\text{-rad}}$ ) of water transport and storage in stem sapwood and found that many relationships and tradeoffs span contrasting climates.

### **Water transport and storage in contrasting climates**

The strong relationship between  $\Psi_{50}$  and capacitance across species and east and west-side populations suggests that water storage and release plays an important role in buffering water column tensions (Meinzer 2003, 2006, 2008a, James *et al.* 2006) which may result in the tree allocating fewer resources to producing wood that is more resistant to air seeding and spreading of embolism (Hacke *et al* 2003, Domec *et al.* 2006). This result also suggests that water released into the transpiration stream may be from embolized tracheids as opposed to elastic tissues (Holbrook 1995, Tyree and Zimmerman 2003), and that greater vulnerability to embolism may serve to buffer the water column. However, the release of water by embolism formation would require a mechanism for daily refilling of embolized tracheids, because otherwise the

additive effect of daily embolism would lead to formation of a negative feedback, reducing overall conductivity and potentially leading to runaway embolism and eventually tree mortality.

The strong relationship between capacitance and axial  $k_s$  suggests that high capacitance lends itself to high transport efficiency overall which may translate into greater carbon uptake (Hubbard et al. 2001). On the surface, this relationship does not appear to explain the greater success of ponderosa pine in drier habitats (Piñol and Sala 1999, Stout and Sala 2003), especially in east-side populations. However if the vastly different sapwood thickness between the two species combined with similar radial conductivities are scaled up to the whole tree and accessible sapwood *volume* is considered instead of sapwood thickness, the amount of stored water available to ponderosa pine for daily water use would be substantially greater than that available to Douglas-fir thus permitting the tree to maintain transpirational rates in micro-climatic conditions in which Douglas-fir would close its stomata and cease transpiration (Farquhar and Sharkey 1982).

Although there is a basic understanding of the fundamentals of water transport in the xylem of woody plants, the contribution of stored water to daily water use and the relationships between sapwood capacitance and transport properties begs for a better understanding. Douglas-fir and ponderosa pine, with their populations adapted to the contrasting climates found in Oregon, present a unique opportunity to

investigate strategies employed for maximum utilization of available water. However, the results of this study seem to suggest similar properties for the responses measured and thus future work would greatly benefit from investigations into leaf, branch and canopy level control of water use as well as supply-side studies investigating rooting patterns and soil properties that mediate water uptake.

### **Xylem ray anatomy and radial conductivity ( $k_s$ )**

The negative relationship between the number of growth ring boundaries in each sample and the resulting radial conductivity indicates that either the growth ring boundaries or the presence of latewood add resistance to radial water flow (Domec and Gartner 2002). Because earlywood to latewood proportions did not correlate with radial conductivity, it can be assumed that the resistance is encountered as water crosses from one growth ring into another. Kitin et al (2009) suggested the presence of bordered pits on tangential walls at growth ring boundaries may serve as a pathway for radial water movement and the results of this study support that hypothesis. If rays were responsible for the bulk of radial water transport, there should be little effect of growth rings on conductivity because rays span multiple growth rings, and water flowing radially through them would encounter no added resistance at growth ring boundaries. Somewhat contrary to this was the weakly significant relationship between radial conductivity and total ray density (no.  $\text{mm}^{-2}$ ). However multiple

reports in the literature conflict regarding the impact of inter-annual variations in climate on ray composition (Bannan 1937, Gregory and Romberger 1975, Yev-Ladun 1998, Gartner et al 2000). Because the samples used to collect radial conductivity contained as many as 20 growth rings, they also contained the variation of those growth rings from as many as 20 different years. No care was taken in orienting samples when mounting them for microtome sectioning (pith to bark conductivity may differ from bark to pith conductivity) and thus relating an overall physiological property determined from an anatomically heterogeneous sample, to a small subsection of that sample could be cause for a large source of error. Suggestions for future work would include better constraints on inter-annual variation in ray anatomy, by increasing samples size and accounting for annual variations in ray properties, and real time observation of radial fluid movement using fluorescent dyes and microscopy to visualize the pathway through which fluids move. Additional investigations in ray geometry, including cell lengths, pit aperture and chamber area, would also be beneficial to an understanding of their physiological role.

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