

AN ABSTRACT OF THE THESIS OF

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Title: Water Movement in the Stem Xylem in Relation to Xylem Specific Conductivity in Four Hardwood Species

Abstract approved:

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In deciduous species, water exits stems mainly through leaf traces attached to the outer growth ring and yet we know that water ascends throughout the entire cross-section of the sapwood. There is an increasing amount of information on sap flow and sapwood hydraulic properties from separate studies, but little information exists on how flow and hydraulics vary radially along the sapwood within a tree. In this study, we determined axial sap flux (J_s) and axial specific conductivity (k_s) at five different radial locations within the sapwood, specifically at 1.2, 2.0, 3.5, 5.0 and 7.0 cm inward from the cambium, and used these values to estimate the tension gradient in the water column at those radial positions. Four hardwood species growing in the Pacific Northwest were used for this study: *Acer macrophyllum* Pursh (bigleaf maple, n=8), *Alnus rubra* Bong. (red alder, n=8), *Populus trichocarpa* Torr. & Gray x *Populus deltoides* Bartr. ex Marsh. (cottonwood, n=4) and *Arbutus menziesii* Pursh (Pacific madrone, n=7). Field and laboratory study were performed during 2003 and 2004, and values were pooled for these two years. Sap flux decreased from the outer to the inner part of the sapwood in all species. *Arbutus*, followed by *Acer*, showed the highest differences within all analyzed depths. *Alnus* had a

slight declining sap flux profile along the first three depths, with a marked decline from $10.3 \text{ g m}^{-2} \text{ s}^{-1}$ to $5.7 \text{ g m}^{-2} \text{ s}^{-1}$ in the two inner positions. *Populus* showed the highest sap flux values, averaging $32.1 \text{ g m}^{-2} \text{ s}^{-1}$ in the two outer depths and these values fluctuated as $23.7 \text{ g m}^{-2} \text{ s}^{-1}$, $26.3 \text{ g m}^{-2} \text{ s}^{-1}$ and $23.9 \text{ g m}^{-2} \text{ s}^{-1}$, along the radius. Specific conductivity showed a similar radial pattern in *Alnus*, *Populus* and *Arbutus*, declining from the outer sapwood inward. Of these species, *Alnus* had the most uniform profile, decreasing, on average, by 12% at each depth. In *Arbutus* and *Populus*, k_s had quite high variance, and exhibited the lowest values in the inner depths. In contrast, *Acer* k_s fluctuated from the outer to the inner sapwood. In general, there was a relationship between J_s and k_s measured at the same locations for *Alnus*, *Arbutus*, and *Populus*. *Arbutus* had the most significant linear relationship between J_s and k_s at all analyzed depths, with $r^2 = 0.93$, followed by *Alnus* and *Populus* ($r^2 = 0.79$). *Acer* showed a fluctuating pattern, with a random J_s/k_s relationship by depth ($r^2 < 0.001$). Axial tension gradient appeared to be constant across the sapwood radius in *Alnus* and *Arbutus*, but it was non-uniform in the inner positions in *Acer* and *Populus*. Gradients remained constant in *Acer* at the first two depths then declined toward the 3.5 cm position, where the highest tension values occurred at the 5.0 cm position. There was a marked increase in tension gradient toward the inner depths in *Populus*. The observed J_s and k_s patterns along the sapwood also demonstrated the resistance to water flux in the radial direction. This demonstrates, in the studied species, the existence of different resistances to water flux in the radial direction. This tension gradient along the sapwood demonstrates the presence of measurable resistance to water flow in the radial

direction. This resistance may also affect wood utilization and processing, especially when dealing with drying and treating wood. This study also showed the importance of accurately estimating J_s along the entire sapwood in order to estimate whole-tree water use.

Water Movement in the Stem Xylem in Relation to Xylem Specific
Conductivity in Four Hardwood Species

by
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DEDICATION

God has given me a unique and wonderful family; he also blesses me with outstanding friends. I dedicate this work to everyone in my family and all my friends. This work is testament of their presence in my life.

WATER MOVEMENT IN THE STEM XYLEM IN RELATION TO XYLEM SPECIFIC CONDUCTIVITY IN FOUR HARDWOOD SPECIES

CHAPTER ONE: INTRODUCTION

Trees have been studied from many different perspectives in an attempt to make mechanistic links between their physiology and anatomy in order to understand how they function. Trees can be considered as hydraulic systems, where the conductive elements (vessels or tracheids) form a complex and integrated network with two main axes, axial and radial, and with the driving force being the tension gradient generated by leaf transpiration. Considerable information exists regarding the axial movement of water in woody plants (Zimmermann 1983; Wullschleger et al. 1998; Cruiziat et al. 2002), but little is known about radial water movement along the sapwood and its contribution to the total axial water stream in a tree bole.

Hydraulic architecture links the geometry of the water transport system in a tree with its physiology (Tyree and Ewers 1991). It constitutes the study of the structure and properties of the water conducting system in a tree, which possesses both qualitative (the vascular system anatomical structure) and quantitative (percentages of the different anatomical components, flow rates, wood permeability and pressure gradients throughout the plant) elements.

The cohesion tension theory (CTT) (Dixon and Joly 1895) is the most accepted mechanism to explain how water moves from roots to leaves, and relies on the assumptions of:

- Continuous water columns within a tree.
- The existence of a driving force (a tension gradient) for water movement.
- Cohesive strength sufficiency among water molecules.

Thus, as long as transpiration occurs, a tension gradient between the soil and the atmosphere develops and pulls water up the stem of the plant (Zimmermann 1983).

The Hagen-Poiseuille equation allows modeling of the water transport system in a tree by linking its physiology and anatomy. Thus, the flow rate of a fluid through an ideal capillary is described by:

$$L_p = (\pi r^4) / 8\eta$$

where " L_p " is the hydraulic conductivity, " η " is the viscosity of the liquid (MPa s), which is temperature and solute concentration dependent; and " r " is the capillary

radius (m) (Zimmermann 1983). Therefore, this equation describes the relationship among flux, the capillary radius, and the pressure gradient, and it clarifies the impact of even small changes in capillary radius on the total flux (Cruziat et al. 2002). The Hagen-Poiseuille equation, however, carries several

disadvantages: first, it overestimates specific conductivity values because it considers xylem conduits as ideal capillaries (Ewers and Cruiziat 1991), whereas vessels have more elliptical shapes (Lewis and Boose 1995; Roth 1996). Second, the model of xylem transport through the Hagen-Poiseuille equation requires the flow path to be entirely under the liquid phase and only works when all capillaries are full of water. Furthermore, it does not consider the fact that xylem elements have end walls at some point and that some of the capillaries are full of air.

Several parameters are fundamental when studying the hydraulic architecture of plants. First, specific conductivity (k_s) describes how readily water moves across a membrane in terms of the volume of water moving through a cross-sectional sapwood area per unit time, under a given pressure gradient (Tyree and Sperry, 1989; Cruiziat et al. 2002). Specific conductivity can be calculated as:

$$k_s = (V * L) / (A * t * \Delta P)$$

where " k_s " is in $\text{m}^2 \text{s}^{-1} \text{MPa}^{-1}$, " V " represents the volume of liquid (dm^3); " L " is sample length (m); " A " is the stem cross-sectional area (m^2), " t " is time (s), and " ΔP " is the pressure difference (MPa) (Tyree and Sperry, 1989). Specific conductivity is influenced by the number and/or diameter of conduits per cross-sectional area (Tyree and Ewers, 1991). As wood ages, sapwood becomes heartwood, losing its conductivity. Structurally, this loss of

conductivity occurs because the heartwood conduits are encrusted with gums, resins, and extractives (Ewers and Cruziat 1991).

The anatomy of the wood varies across the stem radius from pith to bark, therefore, features such as ring number, tree radial growth rate (reflected in wood density), and cell age may cause different radial specific conductivity patterns among species and within the branches, roots, and boles of individuals (Gartner and Meinzer, 2005). Spicer and Gartner (1998) designed a sleeve apparatus that allows direct measurement of specific conductivity on excised pieces of wood, allowing measurement of k_s at different depths along the sapwood. Using this accurate technique, Domec and Gartner (2001) found a decreasing k_s profile from the outside of the sapwood toward the heartwood. Through different approaches, quantifying the significance of the outermost ring to the hydraulic conductivity of the stem through sap flow and pathways of flow measurements, and conductive element estimates through the Hagen-Poiseuille equation, Ellmore and Ewers (1985) reported the same decreasing trend from the outside inward in the hardwood species *Ulmus americana*. We do not know, however, of published information from direct measurements (using the sleeve apparatus) of specific conductivity in diffuse-porous deciduous species at this date.

Sap flux (J_s) is the second key parameter in the study of tree hydraulic architecture, because it refers to the amount of water that moves per unit

sapwood area per unit time in a living tree. Sap flux has been assessed using different measurement techniques such as acidic dyes (Edwards and Booker 1984) and isotopically labeled water (James et al. 2003). Nevertheless, due to technological improvements during the 20th century, more sophisticated methods have been developed including the use of heat for sap flux analysis, with Cohen et al. (1981); and Granier (1985, 1987) using different approaches, which present the advantage of giving more reliable values of sap flux.

Granier's thermal dissipation technique (1985, 1987) allows the measurement of water flow in a stem section of known dimensions. In this technique, two sensors, both containing a thermocouple, are inserted into a tree. The bottom sensor (reference sensor) is not heated, whereas the upper sensor is. The temperature at the heated sensor (upper sensor) is at its maximum if no water transport takes place, so the temperature difference between the two sensors is at its maximum. Conversely, if flow occurs, the heated sensor will be cooled down by the water flow, which is at average stem temperature (Figure 1) (Granier 1987; Lu et al. 2004). Consequently, the measured temperature difference between the sensors will decrease. Therefore, sap flow rate is proportional to the degree of cooling at the upper (heated) sensor. The temperature difference obtained from each pair of sensors is transformed to sap flux by using an empirically derived equation:

$$J_s = 119 k^{1.231} (\text{g m}^{-2} \text{s}^{-1})$$

where $k = (\Delta T_m - \Delta T) / \Delta T$ and ΔT_m it is the temperature difference when sap flux density equals zero (no water flow) (Granier 1985).

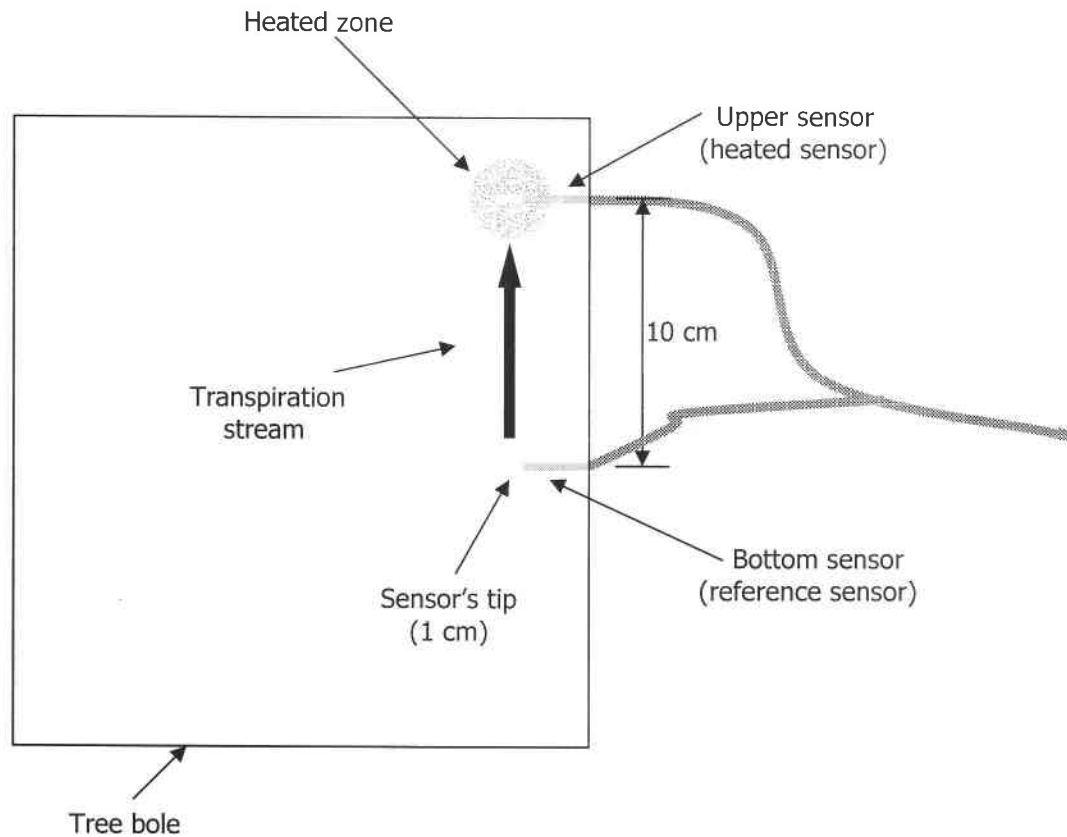


Figure 1.1. Diagram of the heat dissipation technique.

Other approaches and techniques to measure sap flux include the heat pulse velocity technique introduced by Huber and Schmidt (1937) and later used by Marshall (1958) and Cohen et al. (1981) among others, the heat-field deformation method by Cermak and Nadezhdina (1998), and a modification of

the thermal dissipation technique (Granier 1985, 1987) where the technique's principle is kept, but sensors of different custom lengths (James et al. 2002), allow measurement of sap flux at different sapwood depths. This has helped to generate important information in terms of radial sap flux trends along the sapwood, showing the existence of non-uniform sap flux patterns (James et al. 2003) along the sapwood zone. Among the published sap flux patterns are the constant profiles found in *Anacardium excelsum* (James et al. 2002) and *Laurus azorica* and *Ilex perado* (Jiménez et al. 2000) species. Interestingly, a sap flux profile that peaks near the cambium and later declines along the sapwood has been reported for both conifer species (*Picea excelsa*, Cermak et al. 1992) and the hardwood species (*Populus canescens*) (Nadezhdina et al. 2002) (Figure 2). This information constitutes an important step in understanding how crown biomass, as the amount of transpiring leaves, and the conducting system inside a tree are related in terms of water movement along the sapwood, and could be used to help to obtain better estimates of whole tree and stand water use.

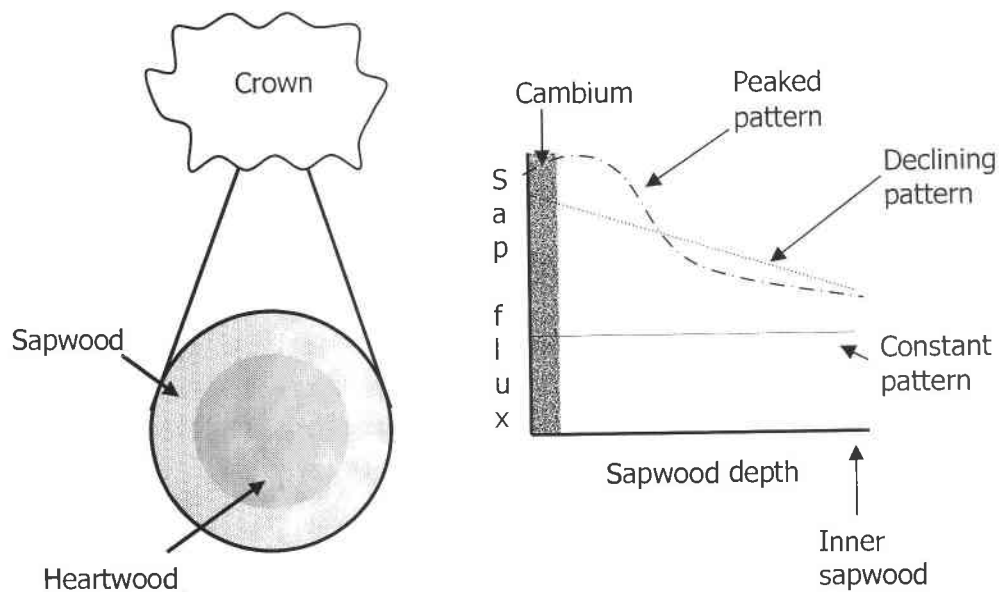


Figure 1.2. Sap flux profiles along the sapwood.

The relationship between the sap flux and the hydraulic architecture of a tree can be assessed through Darcy's law, which allows estimation of the pressure gradient (the water ascent driving force) by using sap flux (J_s) and specific conductivity (k_s) values, measured at the same location, through:

$$\Delta P/\Delta X = J_s/k_s \text{ (MPa m}^{-1}\text{)}$$

where $\Delta P/\Delta X$ is the pressure gradient. Thus, by piecing together numerous segments using sap flux and specific conductivity values through Darcy's law, one can begin to understand the relationship within these three components, axially and radially, along the sapwood, under steady state conditions (when transpiration and root absorption are constant and equal to each other).

James et al. (2003) estimated pressure gradients in hardwood trees growing

in Panama; however, theoretical J_s values based on the Hagen-Poiseuille equation were used. We know of one study reporting pressure gradients along the sapwood for *Pseudotsuga menziesii* that used J_s and k_s values obtained from measurements at the same locations (Domec et al. in review), but there are no studies using the same approach in hardwood species.

The radial variation in sap flux and specific conductivity patterns along the sapwood in hardwood species is not well known. Wood density and xylem functionality change from pith to bark in a tree. Typically, vessel diameter increases as one approach the cambium (Gartner et al. 1997). Thus, one could predict changes in both J_s and k_s radial patterns with increasing values from the pith outward.

The aim of the present study was to characterize the relationship between J_s and k_s over a range of radial positions, and to use these values through Darcy's law to estimate axial tension gradients. We used diffuse-porous and semi-ring porous hardwood species because of their constant anatomical structure along the growth ring. Thus, growth ring width was not expected to represent any effect on the water transport system of the species studied. We focused the present investigation on three diffuse-porous hardwood species (*Acer macrophyllum* Pursh, *Alnus rubra* Bong., *Arbutus menziesii* Pursh), and one semi-ring porous hardwood hybrid (*Populus trichocarpa* Torr. & Gray x *Populus deltoides* Bartr. ex Marsh.) A series of

sap flux measurements were performed in live trees using heat dissipation sensors of variable length (James et al. 2002), inserted at five radial positions (1.2, 2.0, 3.5, 5.0 and 7.0 cm) from the cambium inward. Specific conductivity was measured using excised stem samples taken from the same depths at which the sap flux sensors were inserted, and tension gradients were estimated using the obtained values for sap flux and specific conductivity in Darcy's law equation.

There have been few studies on radial water movement. From knowledge of anatomy, it is not apparent that wood typically has radial pathways for water flow. Therefore, we expect there to be resistance to water movement in the radial direction.

Because of the longevities of the leaves of these species (a life span of less than 14 months), we assumed that leaves have the most direct hydraulic connections in the outer sapwood. Therefore, two hypotheses were tested. First, assuming that leaves would have the most direct hydraulic connections in the outer sapwood, and that there is radial resistance to water movement, then outer sapwood would have higher sap flux and specific conductivity values than inner sapwood during transpiration in all species studied. Second, based on the same assumptions, outer sapwood would have higher tension gradients than inner sapwood during transpiration in all species studied.

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CHAPTER 2: WATER MOVEMENT IN THE STEM XYLEM IN RELATION TO XYLEM SPECIFIC CONDUCTIVITY IN FOUR HARDWOOD SPECIES

ABSTRACT

We know that water moves through a tree due to the pulling force that is generated by transpiration in the leaves, and that wood anatomical characteristics vary from pith to bark in a tree. Thus, assuming that leaves have the most direct hydraulic connections in the outer sapwood, we hypothesized that outer sapwood would have higher sap flux and specific conductivity values, and that outer sapwood would have higher tension gradients than inner sapwood during transpiration. In order to test these hypotheses, we used the four hardwood species: *Acer macrophyllum* Pursh (n=8), *Alnus rubra* Bong. (n=8), *Populus trichocarpa* Torr. & Gray x *Populus deltoides* Bartr. ex Marsh. hybrids (n=4), and *Arbutus menziesii* Pursh (n=7) from the Pacific Northwest. During the summers of 2003 and 2004, sap flux (J_s) was measured using heat dissipation sensors that measured a one-cm segment and that were inserted to five depths: 1.2, 2.0, 3.5, 5.0, and 7.0 cm. After trees were felled, we measured specific conductivity (k_s), wood density (D), and relative water content (RWC) on excised segments. Next, axial tension gradients within the sapwood were estimated from the obtained J_s and k_s values, and radial tension gradients were estimated from adjacent axial tension gradient differences. Sap flux was highest in the outer sapwood in all

species, with curves declining toward the inner sapwood. Specific conductivity declined from the outer sapwood inward in *Alnus*, *Populus*, and *Arbutus*. In *Acer*, however, k_s increased along the first three depths to then decline in the mid-sapwood and increased at the last point. *Acer* showed the highest tension gradient in the outer sapwood, *Alnus* and *Arbutus* had a relative constant pattern, with the highest tension gradients in the mid-points of the analyzed sapwood. *Populus* showed a sharp increment in tension gradients in the last two depths, which could be due to significant low specific conductivity values measured at the same depths, and also as a result of bacterial wet-wood infection present in all individuals. Radial tension gradient estimates were about 1000 times greater than the axial estimates, which show the high resistance to water movement in the radial direction. Because sapwood densities and relative water contents were relatively constant along the sapwood in all species, neither of these parameters suggests that neither juvenile wood, nor water content is responsible for the observed patterns. The presence of bacterial wet-wood infections in *Populus* and *Arbutus* could have influenced the RWC increment obtained at the two deepest points. All sensor locations showed significant diurnal flow, but surprisingly many samples from the innermost positions in *Populus* and *Arbutus* appeared to be heartwood, both on the basis of color and an indicator stain. Because J_s and K_s are non-uniform throughout the sapwood, more care should be taken when

extrapolating tree water transport properties base on external sapwood sap flux measurements, and more scrutiny should be given to the use of sapwood area/leaf area ratios for estimating tree productivity and vigor.

INTRODUCTION

The secondary xylem of trees has three main functions: mechanical support for the trunk and crown biomass, water transport throughout the tree, and water storage (Ewers and Cruiziat 1991). In terms of water transport, the cohesion tension theory (CTT) (Dixon and Joly 1895) has been proposed to explain the mechanism involved in the movement of water from roots to leaves through the xylem. It states that evaporation in the leaves generates a tension gradient in the soil-plant-atmosphere continuum that drives water movement along a tree (Scholander et al. 1965; Zimmermann 1978, 1983). The continuous increase in tension within a section of the tree can be quantified using Ohm's law, which associates the ascent of sap with electrical analogies (Tyree and Sperry 1988, 1989), allowing one to model the steady-state transport of water through the soil-plant-atmosphere continuum (Tyree and Ewers 1991).

A plant's hydraulic architecture is a map of the anatomical and physiological properties that govern its water movement (Zimmermann 1978). In terms of anatomical features, the hydraulic architecture considers a tree as a whole network in which many pathways exist that allows axial and radial flow

through an interconnected vascular system (Comstock and Sperry 2000; Cruziat et al. 2002). A key physiological component of hydraulic architecture is the specific conductivity (k_s ; units $\text{m}^2 \text{s}^{-1} \text{MPa}^{-1}$) of xylem, a measure of the permeability of the sapwood in terms of the volume of water moving through a cross-sectional area per unit time, under a given pressure gradient (Tyree and Sperry 1989; Tyree and Ewers 1991). Specific conductivity is also influenced by the number and/or diameter of conduits per cross-sectional area (Tyree and Ewers 1991), because more conducting area and/or more large-diameter conducting elements may transport more water (Zimmermann 1983). The Hagen-Poiseuille equation (Zimmermann 1983; Tyree and Ewers 1991) can be used to estimate theoretical conductivity, by using anatomical characteristics (number of transporting conduits (tracheids/vessels) per cross section and their diameter). This equation also accounts for the viscosity of the fluid. The use of this equation helps to describe general hydraulic trends, like variations due to stem diameter increments and wood properties (Lovisol and Schubert 1998; Domec and Gartner 2002b). However, the problem is that this equation does not account for the fact that vessel lumens are not cylindrical, but oval in shape, that the pathway is not always full of water, so it is not conducting, and that xylem elements have end walls at some point.

Several techniques have been developed to assess k_s and other aspects of hydraulic architecture *in vivo* and *in vitro*, to know and understand radial

patterns of specific conductivity within a tree. Spicer and Gartner (1998) developed an *in vitro* technique using excised wood segments to assess conductivity of pieces of sapwood from within larger stem segments. The technique solved the problem of how to measure k_s in stem and branch segments that were too large to be fully enclosed in a tube for water transport studies. It also allowed direct k_s measurements, where samples can be sealed to allow uni-directional flow in the sample.

Studies have found variations in k_s across the depth of secondary xylem in *Pseudotsuga menziesii* and *Pinus ponderosa* trees, with k_s decreasing in trunk from the outside in, along with wood density (Spicer and Gartner 2001; Domec and Gartner 2002, 2003). To our knowledge, however, there have been no studies on the radial trends in k_s for hardwoods. The wood produced immediately next to the pith and for the next several growth rings (8-30) is often called the juvenile wood, and typically it has shorter, narrower tracheids and vessels than wood produced beyond this zone (Panshin and De Zeeuw 1980). Because of the anatomy alone (and the Hagen-Poiseuille law) one would expect lower k_s in samples that were near the pith than ones that were farther out (Spicer and Gartner 2001). However, if all the sampling occurs in the mature wood, one still sees a decrease in k_s from the outer sapwood to the inner sapwood (Domec and Gartner 2002b, 2003), which can not be attributed to the wood's original anatomy. This effect may be attributed to accumulated blockages

from substances like gums, resins, and gases, and may be related to a functionality decrease of the inner sapwood parenchyma, as a consequence of cell aging (Gartner et al. 2000).

Water movement in intact trees has been characterized by a number of methods that employ heat as a tracer. For example, the heat dissipation technique developed by Granier (1985, 1987) estimates sap flux (J_s ; units $\text{g m}^{-2} \text{s}^{-1}$), the mass (or volume) of water moving through a given cross section per unit time. With this method, sap flux values are obtained from an empirically-derived equation that relates flux to the temperature difference between a heated sensor and an unheated reference sensor (Granier 1987; Lu et al. 2004). There are numerous publications reporting sap flux for a diverse group of plants (e.g., Andrade et al. 1998; Wullschlegel et al. 1998). However, most of them report fluxes for only the outer part of the sapwood. Newer techniques, like the heat field deformation method by Cermak and Nadezhdina (1998), and a modification of Granier's method by James et al. (2002), permit a more detailed characterization of radial patterns in the axial sap flux values. Available literature suggests the existence of quite variable sap flux along the sapwood radius. In different cases, sap flux remains relatively constant throughout the sapwood, decreases sharply towards the pith, or peaks near the outer depths of the sapwood radius (Phillips et al. 1996; Jimenez et al. 2000; Ford et al. 2004; Gartner and Meinzer 2005).

Radial patterns of hydrostatic pressure gradients at different times of the day can be deduced from specific conductivity and sap flux values using Darcy's law:

$$J_s/k_s = \Delta P/\Delta X$$

where $\Delta P/\Delta X$ (MPa m⁻¹) is the hydrostatic pressure gradient. However, this procedure has been carried out with only a few species: the hardwoods *Anacardium excelsum*, *Cordia alliodora*, *Ficus insipida*, and *Schefflera morototoni* (James et al. 2003), and the softwood *Pseudotsuga menziesii* (Domec et al. in review). James et al. (2003) calculated k_s based on xylem anatomical characteristics using the Hagen-Poiseuille equation rather than direct measurements.

The driving force for water movement in the xylem is the tension gradient generated by transpiration from the leaf surfaces. In species in which the foliage lives one year or less, the driving force must initially be in the outer ring of the sapwood because the foliage can mainly be attached to the outer growth ring. If there is no resistance for radial water transport, then that driving force will be spread over the entire sapwood, and $\Delta P/\Delta X$ will be the same at all radial positions within one level in the tree. On the other extreme, if there is no radial water movement (complete resistance to radial water transport), then the outer growth ring will have a large $\Delta P/\Delta X$, and there will be no hydrostatic potential gradient caused by transpiration in other interior

locations of the sapwood. In this case, the only location of J_s will be the outer ring of sapwood because there is no driving force in the other locations. If there is some radial resistance to water transport, then there will be some $\Delta P/\Delta X$ in the interior sapwood, but it should always be lower than the value at the outermost growth ring.

Among hardwoods, diffuse-porous species exhibit the most radially uniform anatomical patterns along the sapwood (Zimmermann 1978; Gartner et al. 1997). Thus, in the present study, three Pacific Northwest diffuse-porous hardwood species and one semi-ring porous species were chosen to characterize the relationships between J_s and k_s over a range of radial positions. The secondary objective was to estimate axial tension gradients *in situ* using the measured values of J_s and k_s . We measured sap flux using heat dissipation probes at different radial positions from one height in the trees, then felled them and measured specific conductivity on samples excised from near the locations in which the sap flux had been estimated. Because we assumed that leaves would have the most direct hydraulic connections in the outer sapwood, we hypothesized that outer sapwood would have higher sap flux and specific conductivity values, and that outer sapwood would have higher tension gradients than inner sapwood during transpiration in all species studied.

MATERIALS AND METHODS

Study site and plant material

Four hardwood species growing in the Pacific Northwest of United States of America were selected for this investigation (Table 1). Three of the species are deciduous: *Alnus rubra* Bong. (red alder), *Acer macrophyllum* Pursh (bigleaf maple) and *Populus trichocarpa* Torr. & Gray x *Populus deltoides* Bartr. ex Marsh. hybrids (cottonwood), and one is evergreen *Arbutus menziesii* Pursh (Pacific madrone), with foliage lasting up to about 14 months (<http://students.washington.edu/melliott/arbutus/leaves.html>). The cottonwood has semi-ring porous wood structure, and the other three species have diffuse-porous wood.

This study was undertaken at the McDonald-Dunn Research Forest (44° 36' N, 123° 20' W), near Corvallis, Oregon. This forest ranges in elevation from 27 to 460 m and receives 2600 to 3861 mm of precipitation per year (Pater et al. 1997, Walker et al. 2001). The majority of stands in the McDonald-Dunn Forest are either coniferous or a coniferous/hardwood mixture, with a median stand age of 60 years. Four different sites were used based on the need to have sufficient individuals of each species that were close enough together to use one data logger for groups of four trees. During 2003, *Alnus* and *Acer* were studied in a site located in the northwest part of the research forest at

about 410 m elevation. *Arbutus* was studied in a site that was approximately 1.6 km away from the *Alnus* and *Acers* site, at about 260 m elevation. The trees were locally abundant but were in a *Pseudotsuga menziesii*-dominated landscape. *Populus* was added to this study in 2004. Trees were located in a private plantation approximately 15 km southwest from the Research Forest, elevation 75 m.

Table 1. Characteristics of trees studied. Numbers in height, DBH and age columns represent mean and range values.

Scientific name	Common name	trees (n)	Height (m)	DBH (cm)	Age at DBH(years)
<i>Acer macrophyllum</i> Pursh	bigleaf maple	8	24.1 (22.4 – 29.)	24.1 (18.7 - 29.)	51 (39 – 70)
<i>Alnus rubra</i> Bong.	red alder	8	25.8 (19.8 – 28.)	27.2 (21.1 - 32.)	43 (40 – 45)
<i>Populus trichocarpa</i> Torr. & Gray x <i>P. deltoides</i> Bartr. ex Marsh. hybrids	cottonwood	4	26.6 (25.4 – 27.)	30.5 (26.5 – 36.)	11
<i>Arbutus menziesii</i> Pursh	Pacific madrone	7	23.2 (17.5 – 32.)	29.1 (21.0 - 41.)	41 (36 – 47)

We studied four trees of each species each year, except that *Populus* was only studied in the second year. Also, only three *Arbutus* trees were studied the second year due to tree availability. Within a species, we chose trees that appeared to be similar in height, age, and sun exposure, and that had no obvious signs of major damage or disease. All four trees were located within a 15 m radius so that we could use one data logger for each species sap flux measurements. Tree height and diameter at breast height (DBH) were measured, and tree age was estimated for each individual (Table 1). The trees ranged from 17.5 – 32.1 m in height, 18.7 – 41.4 cm DBH, and 11 - 70 years old.

Sap flux measurements

Granier (1985) designed a technique to measure axial sap flux in trees. This technique is based on a probe made of two sensors. There is a known distance of 10-15 cm between the sensors, a continuously heated downstream sensor, and an unheated upstream sensor. The sensors are connected by two constantan wires that connect two copper-constantan thermocouples in series, providing a measure of the temperature difference between the two sensors. The bottom sensor (un-heated sensor) detects the wood temperature (reference sensor); while the upper one (heated sensor) is cooled by the upstream

movement of water developed by the transpiration stream. All the information that comes out of the probes is recorded with a data logger unit powered with an external power supply.

Using the Granier probes' principle, we constructed heat dissipation sap flux probes of different length following the methodology of James et al. (2002). These probes allowed us to access different sapwood depths in the same individual, thus, several radial sap flux values could be recorded at the same time. Specifically, the probes had 1 cm sensing zones that were inserted into trees at depths of 0.2-1.2 cm, 1.0-2.0 cm, 2.5-3.5, 4.0-5.0, and 6.0-7.0 inward from the cambium.

Sap flux was measured concurrently in all four trees of one species; these were sampled sequentially in a different order each year. In order to ensure the mechanical stability of the outer part of each sensor, we removed a plug of bark 30 mm diameter at each insertion point, and after sensors were inserted, we fixed them with a 30 mm diameter rubber stopper that we placed in the removed plug of bark. Next, we drilled a 2.6 mm diameter hole for each sensor. Probes were separated from one another by 10 cm both vertically and horizontally, in a spiral stair-step pattern, where the shallowest probe (1.2 cm sensor) was located in the bottom and the deepest probe (7.0 cm sensor) was located at the top (Figure 1). This pattern is used to avoid heat interaction between probes. Then

the 30 cm longitudinal section of the bole with its inserted probes was covered with a reflective insulation material to avoid external thermal influences.

Sap flux data were collected continuously using a data logger (CR10x; Campbell Scientific) and a storage module (SM192; Campbell Scientific). Data from the probes were scanned every minute. Averages every ten minutes were recorded continuously until we had recorded data for at least three clear (non-cloudy) days with the heating unit supply on. This was carried on from one to three days of data collection with the heat supply off to account for temperature differences due to the basal temperature of the wood.

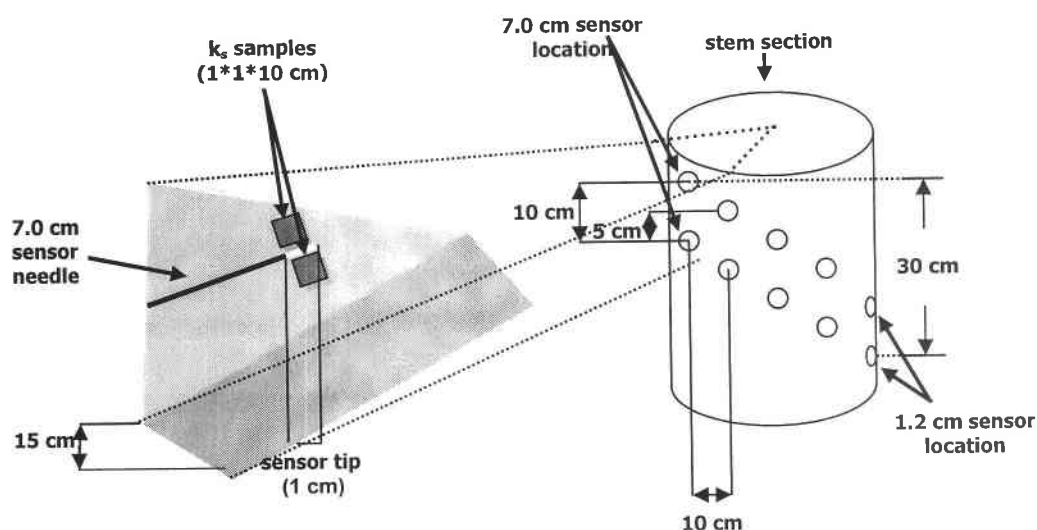


Figure 2.1. Sap flux density and specific conductivity sampling diagram.

The sap flux measurements were conducted from July to September 2003, and June to August 2004. In this mediterranean-climate site, the

weather is usually precipitation-free during this period. Lapsed time between species data collection was dependent on weather conditions as well as sap flux equipment operation. Specifically, 2003 data were collected on the following dates: *Alnus* (July 12–30), *Acer* (August 4–15), and *Arbutus* (September 11–27). In 2004, species and dates were: *Arbutus* (June 16–30), *Populus* (July 1–12), *Acer* (July 13–26), and *Alnus* (July 27–August 9).

We constructed diurnal plots of sap flux (J_s ; $\text{g m}^{-2} \text{ s}^{-1}$) for each probe using the following sequence. First, the temperature difference (ΔT) between the two sensors was converted to sap flux using Granier's calibration (1985), which was revalidated by James et al. (2002) with:

$$J_s = 119 k^{1.231}$$

where $k = (\Delta T_m - \Delta T) / \Delta T$ and ΔT_m is the temperature difference when sap flux density equals zero. Secondly, we took the values for the time of highest evaporative demand during the day (10:00–16:00 hours) and used averages for each sap flux depth for data analyses. In the third step, to account for temperature differences due to the basal temperature of the wood, data from the un-heated probes were used for the final sap flux calculations, thus, sap flux values calculated with this un-heated system were subtracted from the values obtained with the heat on. After doing this analysis for all individuals during all the days of sap flux measurements, we picked a day based on sensors operation and from there made graphs and the statistical analysis for all species.

Material collection

After sap flux measurements were completed, but before felling the trees, we took two cores (150 to 200 mm length, and 10 mm diameter) per tree from about breast height; close to where the sap flux sensors were inserted, to determine field moisture content for each sensor depth. Each individual core was wrapped immediately in plastic wrap, and then placed in a self sealing bag.

All trees were felled after sap flux measurements were completed. The harvesting of trees was always done between 7:00 and 8:30 am. Then, to get material for the conductivity measurements, two disks (at least 15 cm tall) were taken from each individual. These disks were cut from the exact zone where the sap flux probes had been inserted.

All disks were transported back to the lab (less than one hour away) in plastic bags to maintain the field moisture content. Samples to be processed the same day were kept in the bags in the cold room until processing. Samples to be processed the next day were placed in a plastic container filled with water in a cold room (5°C) until processing. If more than a day was needed to process the samples, water in the plastic container was changed every 24 hours. Samples were usually processed in less than two days from the time of harvest.

Moisture content

Once in the laboratory, moisture content cores were unwrapped. One-cm long samples were excised then weighed (fresh mass) from each one of the five sapwood depths at where sensors had been located: 0.2-1.2 cm, 1.0-2.0 cm, 2.5-3.5, 4.0-5.0, and 6.0-7.0. Two consecutive pieces were cut for the first two depths, this was from 0.2-1.2 and 1.2-2.0 due to overlapped sections. After oven-drying for 4-5 days at 103 °C, samples were re-weighed (oven-dry mass).

Moisture content (MC) was calculated as:

$$\% \text{ MC} = 100 * (\text{fresh mass} - \text{oven-dry mass}) / \text{oven-dry mass}$$

The two replicate values for each depth per tree were averaged for data analyses.

Wood density determination

Two estimates of wood density were obtained on each segment of the cores that were collected for moisture content. Re-hydrated ("green") volume was estimated by first placing core segments in a container filled with tap water and placing them under a vacuum for 24 hours. Next, we measured volume displacement of water to estimate sample volume. A water-filled container was placed on an electronic balance, and core segments were submerged in it to

estimate the volume of the sample as the mass of the water displaced by submersion (Archimedes' principle).

Density (D) of each sample was calculated as:

$$D = \text{dry mass} / \text{re-hydrated volume.}$$

Replicate values for each depth, one from each of the two cores, were averaged for data analyses.

Relative water content

Relative water content, the proportion of void wood volume filled with water, was calculated based in the fresh and dry mass, and the re-hydrated volume for each core segment used for the wood density analyses. Relative water content was calculated as:

% RWC = $[(\text{fresh mass} - \text{oven-dry mass}) / (\text{re-hydrated volume} - (\text{oven-dry mass} / 1.53))]$ (Domec and Gartner 2003). Here, 1.53 has units of g cm^{-3} and represents the density of dry cell material (Siau 1984). Replicate values for each depth were averaged for data analyses.

Conductivity measurements

In the sample preparation room, one 15 cm wood disk was processed at a time. Pieces from that disk were kept under water at all times. Each specific conductivity disk was dissected and inspected sufficiently to learn the exact

depth (not growth ring) to which each sap flux probe was inserted. Thus, each specific conductivity sample was taken from the point where the tip of the sensor was inserted in the sapwood zone; two samples per depth were taken (Figure 1). Then, we excised a wood sample corresponding to each depth that was about 1 cm x 1 cm (radial x tangential) and about 10 cm tall (longitudinal). This was accomplished, initially with an electric chain saw, then a band saw, but for the final shaping we used a chisel, so that wood would break along the grain. In addition, in the final stages both ends of these pieces were re-cut with a new razor blade to clear the cell lumens.

The shaped conductivity samples were placed in a vacuum with tap water for 24 hours to remove gas from the wood. During these 24 hours, samples were kept in the cold room to avoid fungal growth, and water was changed approximately every 3 to 4 hours.

On the day of the measurements, samples were equilibrated at room temperature for one hour, to avoid changes in liquid viscosity during the process. Next, samples were placed in a pressure sleeve apparatus and ends of the 10 cm samples were fitted with tubing that extended beyond the ends of the pressure sleeve. The pressure sleeve used low air pressure (about 0.14-0.17 MPa) to press a latex membrane to the sample sides to prevent fluid leakage along the sample's sides (Spicer and Gartner 1998). The tube on the upstream end of the sample was attached to a reservoir of distilled water acidified with HCl to pH 2;

this reservoir functioned as the pressure provider. The tube emerging from the downstream end of the sample was attached to an empty 1.0 ml pipette.

At the time of the measurements, volume flow was recorded by timing how long it took the meniscus to pass five sequential 0.1 ml marks on the pipette. Using a measuring tape we recorded the vertical distance between the top of the acidified solution and the sample. Then with a caliper, we measured sample length, and sample width and sample depth for calculation of sample cross-sectional area.

Specific conductivity (k_s) was calculated as:

$$k_s = (V * L) / (A * t * \Delta P)$$

with units $\text{m}^2 \text{s}^{-1} \text{MPa}^{-1}$ where "V" represents volume of liquid, "L" is the sample length, "A" is sample cross-sectional area, "t" is time, and " ΔP " is the difference in pressure head on the sample (calculated from the height of the HCl solution reservoir above the sample). The pressure gradient across the samples ranged from 0.007 to 0.0035 MPa m^{-1} (depending on exact sample length), but was usually about 0.005 MPa m^{-1} . The k_s values for one individual of *Acer* were considered outliers, so that tree was eliminated from further study of sap flux, conductivity, and tension gradients. For each tree, means of the two samples per sapwood depth were used for further analyses.

Tension gradient

Axial tension gradients at each of the five radial positions were estimated as: $\Delta P/\Delta X = J_s/k_s$. We used the same J_s and k_s values per depth per individual per species as before, and averages of the two tension gradient estimates per depth were used for further analyses.

We then estimated radial tension gradients from the axial tension gradients for the four zones between the sensors: 1.2-2.0 cm, 2.0-3.5 cm, 3.5-5.0 cm, and 5.0-7.0 cm. Radial tension gradients were estimated by taking the difference between adjacent axial tension gradient values, divided by the distance between the two depths, they were 0.8, 1.5, 1.5 and 2 cm.

Data analyses

For sap flux, maximum transpiration hours (10:00- 16:00) were used to take averages per depth per tree. Specific conductivity was analyzed using averages of the two samples per depth per individual. Tension gradients were analyzed using the J_s and k_s paired average values per depth per individual per species. Mean sap flux, specific conductivity and tension gradient averages with their standard errors were plotted against sapwood depth for inspection of species trends. Furthermore, J_s was then plotted against k_s . In these plots, the maximum values per depth per species were used for J_s , rather than the 8-hour mean value used above.

Averages of the two years of data for wood density and relative water content per individual per species were plotted against sapwood depth for analyses. Also total averages of axial tension gradients were plotted against averages of radial axial tension differences between adjacent sapwood depths; this axial-radial tension analysis was followed by a graphical inspection of total radial axial tension averages against sapwood depth.

Differences of radial sap flux and specific conductivity values, and tension gradient estimates per species were made using one-way ANOVA analysis (Zar, 1999), followed by the least significant difference test (Zar, 1999) to test for differences in this parameters between outer and inner sapwood depths.

RESULTS

Radial patterns of sap flux profiles (J_s)

At the five analyzed depths (1.2, 2.0, 3.5, 5.0 and 7.0 cm inward from the cambium), sap flux (J_s) was highest in the outer sapwood (1.2 to 2.0 cm) in three of the four species (Figure 2). The J_s profile decreased from the outer to the inner part of the sapwood with significant differences in *Acer* ($F=12.6$, $p<0.001$), *Alnus* ($F=9.81$, $p<0.001$), and *Arbutus* ($F=6.63$, $p<0.001$). Of these three species, *Arbutus*, followed by *Acer*, showed the highest difference within all analyzed depths. *Alnus* had a slow declining sap flux profile along the first

three examined depths (1.2, 2.0, and 3.5 cm), with an observable decline from $10.3 \text{ g m}^{-2} \text{ s}^{-1}$ to $5.7 \text{ g m}^{-2} \text{ s}^{-1}$ in the inner two analyzed depths.

Populus had higher sap flux than the other species, with high variance among and within individuals at all positions, and there were no significant differences in sap flux by radial position ($F=0.66$, $p=0.62$) (Figure 2). The indicator stain showed that the inner positions were heartwood (Figure 5). The lowest average sap flux value for *Populus* was recorded at 7.0 cm ($23.9 \text{ g m}^{-2} \text{ s}^{-1}$); this value was still higher than any other showed by the other species.

In contrast to *Populus*, maximum average sap flux density in *Acer* was $13.6 \text{ g m}^{-2} \text{ s}^{-1}$, then steadily declining along the next four depths. In *Alnus*, sap flux density declined from an average of $13.4 \text{ g m}^{-2} \text{ s}^{-1}$ in the outer point to $4.6 \text{ g m}^{-2} \text{ s}^{-1}$ at 7.0 cm depth. *Arbutus* had the lowest variation in sap flux values in its innermost positions (5.7, 3.7, and $2.1 \text{ g m}^{-2} \text{ s}^{-1}$ on average), all of which the indicator stain identified as apparent heartwood (Figure 5).

Radial profiles of specific conductivity (k_s)

Specific conductivity (k_s) showed a declining trend from the outer part of the sapwood inward in three of the species. There were not, however, significant differences among the analyzed depths in *Alnus*, *Populus*, and *Arbutus* ($F<1.0$, $p>0.5$) (Figure 2). *Alnus* showed the most uniform profile of k_s , decreasing uniformly by almost 12% from the outside toward the interior of

the sapwood. The inner depths had quite high variance for k_s and showed the lowest k_s values in *Arbutus* and *Populus*. These were the two species that exhibited the most apparent heartwood in those locations (Figure 5). There was significant difference between the k_s in the outer sapwood and the values obtained at 5.0 cm depth ($q=5.04$) in *Acer*. The radial pattern of k_s in this species increased from the 1.2 to the 2.0 and to the 3.5 cm positions by almost 20% at each depth, and then declined by about 73% at 5.0 cm to increase by 60% at the 7.0 cm position.

Axial tension gradient profiles

We estimated the tension gradient that would be required to give the measured J_s (averaged from 10:00 – 16:00 hours) with the measured k_s . In *Alnus* and *Arbutus* the tension gradient was uniform by radial position (Figure 2) ($F<1.0$, $p>0.5$). There were significant differences along the analyzed depths in *Acer* ($F=11.3$, $p<0.001$).

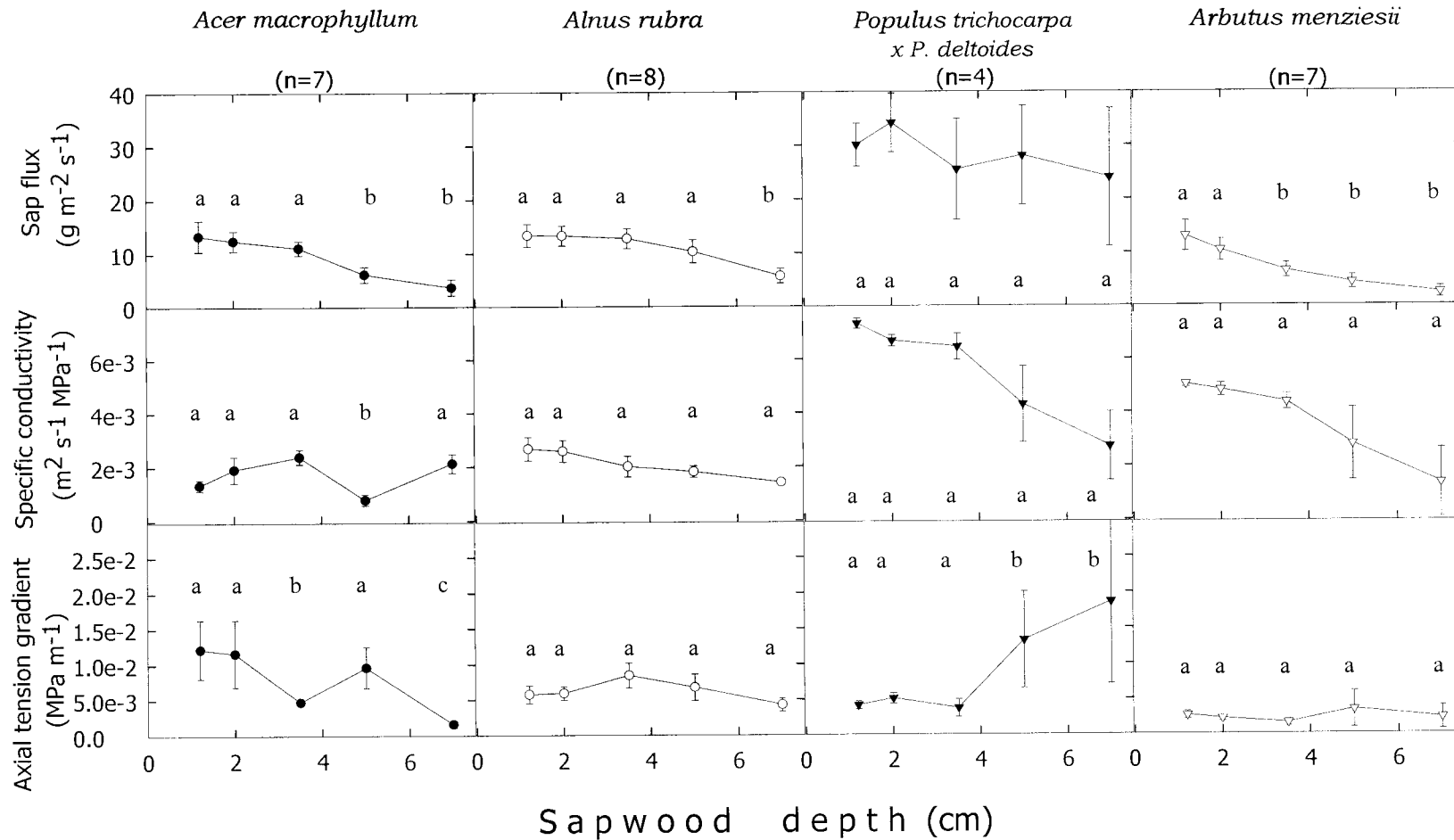


Figure 2.2. Radial gradients of axial sap flux, specific conductivity and tension gradient for the four studied species (Mean \pm S.E.). Same letters represent non-statistical differences, different letters indicate significant differences at $p > 0.05$.

and *Populus* ($F=9.57$, $p<0.001$), with the highest tension gradients at 5.0 cm depth in *Acer*, and at the two inner depths in *Populus*. In both species, the calculated increase in tension gradient at those depths could be due to the values obtained for either k_s or J_s at these locations, because the tension gradient is simply the ratio between these two parameters (See methods) (Figure 2). Of the four species analyzed, *Arbutus* showed the most uniform pattern and the lowest tension gradient values.

Relationships between J_s and k_s

Overall, when we look at all species J_s/k_s plot, there is a correlation ($r^2=0.66$, $p=0.002$) between these two parameters. Specifically, there is a significant relationship between J_s (maximum values per sapwood depth -see methods-) and k_s (maximum values per sapwood depth -see methods-), measured at the same locations for *Alnus*, *Arbutus*, and *Populus* (Figure 3). *Arbutus* had the most significant linear relationship between all analyzed depths, with $r^2= 0.93$, $p<0.01$, followed by *Alnus* and *Populus* ($r^2= 0.79$ for both regressions, $p<0.01$). Of all analyzed species, *Populus* showed the highest sap flux and larger specific conductivity values.

Relationship between sapwood density and RWC (%)

Density was relatively constant along the sapwood in all species (Figure 4), with no significant differences by depth ($F < 1.0$). *Populus* had the less dense wood, followed by *Alnus*, *Arbutus* and *Acer*. Relative water content also appeared to be constant in the first four analyzed depths in all species. *Alnus* had the lowest RWC, followed by *Acer*, *Populus* and *Arbutus*. There was an increase in RWC toward the innermost point (7.0 cm depth) in *Populus* and *Arbutus*, the two species that had wet-wood infection (Figure 3), with RWC increasing by about 10% in *Arbutus* and 23% in *Populus*. This increase in RWC at the inner depths was not significant in *Arbutus* individuals ($q = 4.1$), but was significant within the *Populus* trees ($q = 8.2$). This same situation, in which moisture levels are elevated in the zone with wet-wood, has been reported in other *Populus* species (Mackay 1975, Jeremic et al. 2004) and it is one of the characteristics, as mentioned earlier, that defines wet-wood.

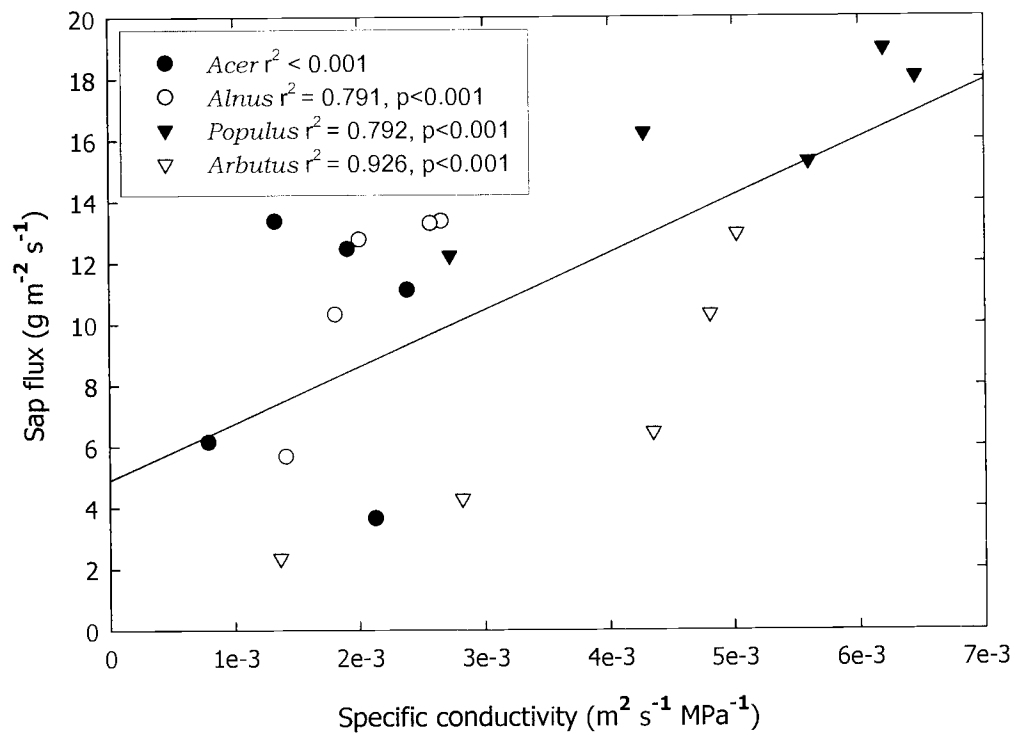


Figure 2.3. Sap flux and specific conductivity relationships by species. Data represent maximum values of all individuals per depth per species.

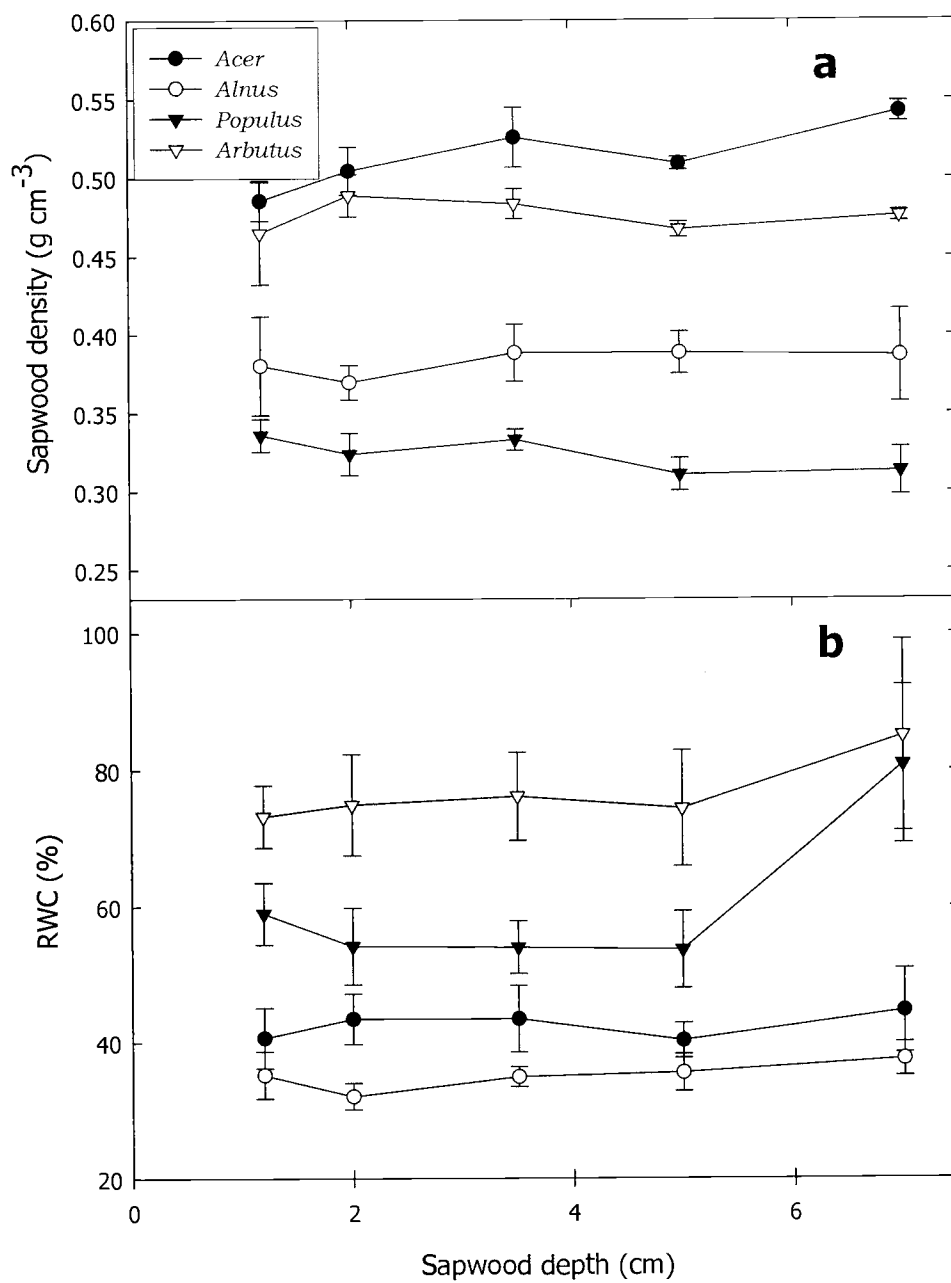


Figure 2.4. Sapwood density and relative water content relationships by species. Points represent maximum values per species per depth (\pm SE).

Sapwood-heartwood boundaries

Indicator stain showed that some of the sample locations in some of the species were actually heartwood (Figure 5). However, there was detectable sap flux at all locations for all individuals. In *Acer*, only one individual had one 7.0 cm probe that the indicator stain showed to be heartwood, but in *Populus* and *Arbutus*, all individuals had apparent heartwood in locations deeper than 3.5 cm inward from the cambium. Furthermore, in *Arbutus* and *Populus*, the innermost sapwood not only tested as heartwood by the indicator stain (Figure 5), but also had higher moisture content (Figure 4), had a darker color, and had a strong rotten smell, suggesting that bacterial wet-wood was present.

Tension gradient changes by sapwood depth

When looking at the radial tension gradient variations along the sapwood, *Acer*, *Alnus* and *Arbutus* showed a relative constant pattern with no significant differences ($F < 1.0$) from the outside in (Figure 6). *Populus*, however, presented a constant pattern in the first two depth intervals, and then it significantly ($q = 11.6$, $p < 0.001$) increased toward the inner-middle part of the sapwood, indicating higher resistance to radial flow at these locations.

Species	Distance inward from the cambium (cm)				
	1.2	2.0	3.5	5.0	7.0
<i>Acer</i>					■
<i>Alnus</i>					
<i>Populus</i>				■	■
				■	■
				■	■
				■	■
<i>Arbutus</i>			■	■	■
			■	■	■
			■	■	■
			■	■	■
			■	■	■
			■	■	■

Figure 2.5. Zones in which the indicator stain (Phloroglucinol) revealed the apparent presence of heartwood. Each row represents one individual, each cell is a sapwood depth location, from the cambium inward, and shaded cells represent stained zones.

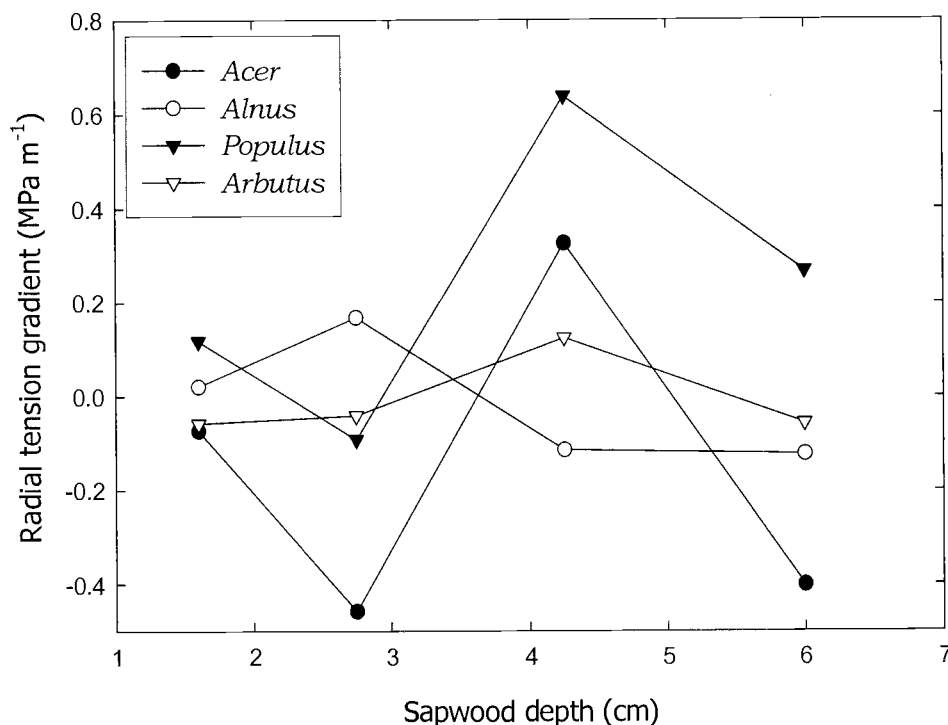


Figure 2.6. Radial axial tension estimates variation by sapwood depth. Points represent mean values of adjacent axial tension gradient estimated differences divided by the distance between the two depths.

DISCUSSION AND CONCLUSIONS

Sap flux and specific conductivity both tended to be highest in outer sapwood and declined with depth. The radial pattern of axial tension gradients appeared to be species-specific, with little variation along the outer points in all species, and more marked differences in the inner depths, especially in *Acer* and *Populus*. The pattern of wood density was relatively constant in the radial direction, suggesting that all measurements were made in the mature-wood

zone of the trees. The RWC was not higher in outer than inner sapwood. The combination of these factors suggests that the lower conductivity at the inner positions did not result from changes in either anatomy or air-blockages, but may have resulted from other accumulated blockages, such as the gums within the vessels, or as a result of the observed bacterial wet-wood infection in all *Populus* individuals and five of the seven *Arbutus* individuals. These two species registered significant sap flux in zones that we later identified using a stain, as heartwood.

Radial sap flux profiles

In three of the species (*Acer*, *Alnus*, and *Arbutus*) sap flux decreased from the outer part of the sapwood inward. In *Populus*, however, the sap flux profile presented big variation among the analyzed individual and showed no significant differences by radial position. The decreasing sap flux patterns in *Acer*, *Alnus*, and *Arbutus* may be due to leaves mostly connected to the outer sapwood through their leaf traces (Tison 1902; Maton and Gartner 2005), and so it is the outer sapwood that experiences the most driving force for water movement.

Lateral movement of water from storage compartments to the main zone of water movement as a consequence of transpiration in the leaves may also have influenced the observed fluxes, were significant flux is being moving axially

the inner sapwood. Cermak and Nadezhdina (1998) reported substantial amounts of water moving from storage compartments to the outer sapwood in *Populus interamericana* growing also in a temperate climate. Furthermore, the fact that *Acer* and *Alnus* have a wide functional sapwood area, with relative good vessel-to-vessel tangential connections, may explain the relatively low constant decrease in the observed sap flux values, where approximately a third of the amount measured at 1.2 cm depth, was still recorded at 7.0 cm depth inside the sapwood (Figure 2). The observed decrease in sap flux in the two most inner points in *Acer* and *Arbutus*, could be due to a high radial resistance to water movement and also to low pit density or clogged pits communicating vessels laterally.

Similar radial profiles (with higher values in the outer sapwood) in sap flux have been found in other hardwood species (*Olea europaea*, *Laurus azorica*, *Ilex perado*) growing in a similar climate (Cermak and Nadezhdina 1998; Jimenez et al. 2000), and in coniferous species, as well (e.g., *Pinus sylvestris*, Cermak and Nadezhdina 1998; *Pinus palustris*, Ford et al. 2004; and *Pseudotsuga menziesii*, Domec et al. in review). This non-uniform sap flux profile along the sapwood has also been reported for *Anacardium excelsum*, *Cordia alliodora*, *Ficus insipida*, and *Schefflera morototoni* trees growing in Panama (James et al. 2002, 2003), which indicates that different strategies for radial water movement could exist among a wide variety of woody plants.

The fact that all *Populus* individuals showed the highest sap flux values are consistent with the reported high water use (Edwards and Booker 1984), and wide functional sapwood (Cermak and Nadezhdina 1998) for this genus. What was surprising, however, was that even as a deciduous species, high sap flux values were recorded for all individuals at the innermost sapwood locations. This could be due to leaves attached to this zone; however, more research needs to be performed to elucidate these connections.

Radial specific conductivity profiles

Specific conductivity decreased in *Alnus*, *Populus*, and *Arbutus* from the outside inward. In *Alnus* and *Arbutus*, the decreasing k_s profile matched the observed sap flux patterns. Little axial tension gradient variation was observed, however, across the radius in these two particular species (Figure 2). Because anatomical characteristics such as vessel size, vessel number by cross-section area, and vessel-to-vessel axial and radial connectivity impact different physiological processes (e.g., leaf photosynthetic activity, Comstock and Sperry 2000), by allowing more or less water to be conducted radially along growth rings, the observed decreasing k_s values toward the inner sapwood could be due to air and/or gum blockages in the vessels as they approach the heartwood. This decline of k_s values from the outside in has been reported for a number of conifers (Edwards and Booker 1984; Domec and Gartner 2002, 2003; Gartner et

al. 2000) and hardwood species (Edwards and Booker 1984; James et al. 2003). Specifically, Edwards and Booker (1984) reported, based on water content and the use of stains, the outer sapwood to be more conductive than the inner zone in other *Populus* species growing in New Zealand. The decreasing k_s profile observed in *Alnus*, *Populus* and *Arbutus* in this study, has been also reported by James et al. (2003), who estimating k_s from anatomical data, concluded that the outer sapwood was more conductive than the inner sapwood in several species (*Anacardium excelsum*, *Schefflera morototoni*, *Cordia alliodora*, and *Ficus insipida*) of tropical trees growing in Panama, supporting the idea of a dependency of sap flux on specific conductivity. This brings the assumption that if leaves have the most direct hydraulic connections in the outer sapwood, where current year wood supplies the crown biomass with water, the observed k_s radial profile in *Acer*, with highest values at 2.0 cm and 3.5 cm depth, is intriguing, specially for being this a deciduous species. The increase in k_s values in the first three depths in *Acer* could be due to inter-vessel connections along the first 3.0 cm or so of the sapwood area. It could be also, that due to un-developed pit perforations near the cambium, k_s peaked at 3.5 cm depth in this species. It could be also that this network, with a curly grain pattern in the sampled individuals, could have been damaged for the outer (1.2 cm and 2.0 cm) depth samples, underestimating the real k_s values. As for the high *Acer* k_s values obtained at the deepest point, the statistical analyses support the hypothetical

underestimation of values at 5.0 cm depth. Otherwise, a constant trend could be obtained.

The higher k_s for *Populus* individuals, in the outer sapwood, could be as the result of the capillary radius and its effect on k_s values. This is because in all analyzed individuals vessel sizes were significantly larger in the three outer depths, a feature clearly observed by naked eye. Furthermore, because *Populus* has a semi-ring porous vessel distribution, in one individual, sensors were inserted at earlywood-latewood zones at the 2.0 cm depth, this same scenario happened in three individuals at 5.0 cm depth, and in all samples at 7.0 cm. Thus, the samples for the k_s measurements, as taken from the same zones, may have influenced the obtained low values at the deepest points. Also the enzymatic action of the bacteria on wet-wood cells (Ward and Pong 1980, Jeremic et al. 2004) that causes degradation of pit membranes (Verkasalo et al. 1993) may have influenced the decrease of k_s values at these inner points. Surprisingly, the observed decrease in k_s values was not reflected as much as we expected in the sap flux values.

Relationships between sapwood density and RWC (%)

All species showed a uniform sapwood density profile along the five sapwood examined depths. Relative water content (RWC) was also uniform along the five sapwood-examined depths in *Acer* and *Alnus*. *Populus* and

Arbutus, however, showed significant RWC increments in the deepest point. In the case of *Acer*, *Alnus* and *Arbutus* individuals, the zone in which the sensors were appeared to be all mature wood. Recall, juvenile wood is the wood formed during the first 25 years or so of a tree, and has intermediate (cell diameter, cell length) characteristics if compared with the cells of mature wood, that influence many wood properties, wood density among others (Haygreen and Bowyer 1996). As for their age, all *Populus* trees seemed to have only juvenile wood, a feature that may have influenced the highest k_s values at the outer sapwood locations.

In the present study, neither sapwood density, nor RWC appears to be linked to higher k_s , nor to the higher sap flux values (wetter wood) obtained at the outer sapwood points. Therefore, leaf attachment, crown size and geometry, as shown by the axial tension gradients, could be the major factors originating higher fluxes and conductivities in the outer sapwood. Furthermore, the apparent bacterial wet-wood infection in all *Populus* and *Arbutus* individuals may be producing the increase in RWC at the 7.0 cm depth, with the characteristic water-soaked wood with rancid odor (Schink and Ward 1984), due to the accumulation of fatty acids produced from the anaerobic bacterium that causes this infection (Jeremic et al. 2004).

Axial tension gradients

Given the declining profiles of J_s and k_s along the sapwood, estimated axial tension gradients per species were non-uniform as well, with significant differences only in *Acer* and *Populus*. Three trees during 2004 highly influenced the high tension gradient observed at 5.0 cm depth in *Acer*. One of those individuals was considered an outlier; therefore, it was removed from the analyses. Thus, if one idealizes a less low average k_s value for the 5.0 cm depth, then a decreasing profile from the outside in could be drawn. The case of *Populus* is similar to this, where the marked low k_s values recorded at 5.0 cm and 7.0 cm depth are responsible for the high tension gradient estimates at the same depths, but where J_s and k_s had the lowest values.

In terms of the relationships between J_s and k_s , those of *Acer* and *Populus* are particularly interesting. In this study, *Acer* J_s values decreased from the outside inward, while those of k_s did not; instead, they increased from the 1.2 cm point toward 3.5 cm, to then decrease at 5.0 cm depth. These values, however, as mentioned earlier, may be due to errors during the k_s measurements in three of the four 2004 *Acer* individuals. Thus, if the decrease would be less pronounced, instead, a decreasing pattern from 3.5 cm toward the inner sapwood could have been obtained, and could have generated a decreasing tension gradient as observed in the other three species. In *Populus*,

the pronounced decrease in k_s values at the last two inner zones may have been a consequence of the modified physiological (wetter and weaker wood) and anatomical (deteriorated cell connections) micro environment created by the reported bacterial wet-wood infections found in all *Populus* individuals. Therefore, these high k_s differences originated tension gradients that rose exponentially at these same points, and then if the decline would not be that marked; a higher but less pronounced tension gradient profile could have been observed instead. If this mentioned scenario is not considered, the only possible explanation that could explain the observed pattern could be the presence of new leaves attached, somehow, to the inner sapwood, originating the estimated higher tension gradient in the inner points. This situation, however, seems unrealistic, motivating more detailed anatomical and physiological studies focused on this matter.

Radial tension gradients

The fact that radial tension gradients were about 1000 times higher than the axial gradients, reflects higher resistance to water movement in the radial direction, where instead of water moving through vessel perforations, it is moving through inter-vessel pits (smaller structures than the vessel perforations), and ray parenchyma. Furthermore, the size and number of the

inter-vessel pits connecting one element with its neighbor, also adds limitation for radial flow. Densely-pitted inter-vessel sections are reported to exist in regions where adjacent vessels make contact with each other in *Fraxinus lanuginosa* trees, and appeared to provide radial transport across growth-ring borders (Kitin et al. 2004).

Water transport in apparent heartwood

The study of sapwood locations that were apparently heartwood (Figure 3) and still registered some sap flux, and had substantial specific conductivity values, is a subject that needs more research, since this phenomenon goes against the heartwood definition. In this study, for *Populus*, the observed wet-wood condition does not appear to interfere with the sap flux, nor the conductivity of the sampled trees. The reasons for the observed J_s , k_s , and tension gradient patterns are unknown. For *Arbutus*, on the other hand, these J_s values were the lowest (almost zero, Figure 2) from all species and individuals studied, these low k_s values reflect the loss of the water transport functionality of the wood at these inner locations. This loss of functionality, however, could be due to blocked pit membranes as well as vessel elements, and to parenchymatic tissue becoming saturated with gums and other substances as cells age.

Further research

It would be useful to study the J_s , k_s , and tension gradients of more individuals and more species to see whether the patterns observed in these four species hold in other diffuse-porous species. It is unknown what structural features are responsible for the lower conductivity in inner than outer sapwood, so it could be useful to study the anatomy of vessels and pit membranes, and also the extractive contents across the radii. It has been reported to be difficult to detect wet-wood in living trees (Xu et al. 2000); therefore, more research is needed to understand this important feature, which has stimulated scientific as well as economical interest. Some questions that need to be answered in further research include: Are there visual signals, through leaves or bark, of the deterioration that interior infections (i.e. wet-wood) are causing to the wood? What is the anatomical impact of wet-wood in a tree and what is the deterioration rate? How can wet-wood transport water at all and also how/why can it have higher conductivity than normal wood? The question about the relationship between the magnitude of radial tension gradients and depths of sapwood in a given species requires more study. Likewise, we need more research and understanding how it is possible to have higher tension gradients in the inner than the outer sapwood.

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CHAPTER THREE: CONCLUSIONS, LIMITATIONS, IMPLICATIONS AND RECOMENDATIONS

In this chapter, overall conclusions of the present study, limitations and implications of the generated information are provided. Also, recommendations and further research questions are presented.

Sap flux (J_s) was typically higher in the outer than middle sapwood. This supports the idea that leaf connections are the driving force for water movement. Functional connections between leaf traces were investigated along the sapwood (Maton and Gartner 2005) and results showed the existence of different leaf traces connections that in turn could affect patterns of radial flux. The approach used by these authors could be implemented, in parallel, to the physiological measurements performed in this study. Thus, anatomical features would help to find differences among leaf longevities and functional connections among these. Also, a wider range of species, with different leaf longevities (evergreen species) and sapwood depths (wider sapwood areas) would also help to elucidate and understand radial patterns of sap flux among hardwood species. Would evergreen species, with older leaves, have deeper high sap flux? Would the pruning of different-aged leaves affect water transport?

We assume that the location within the sapwood of the leaf attachment is the major factor causing flux; therefore, leaves of deciduous

trees have the most direct hydraulic connections in the outer sapwood, with the current's year wood supplying the crown biomass. The observed decreasing pattern of specific conductivity (k_s) along the sapwood is not likely to be due to juvenile wood/mature wood geometric and physiological features, nor to RWC differences, but probably due to the existence of radial variability in the conductive element properties as cells age, where incrustations such as gums, air and extractives could affect the performance of the hydraulic system when moving towards the inner sapwood.

We used the data on J_s and k_s to calculate axial tension gradients, and then the differences between the tension gradients to estimate radial tension gradients. These radial resistances were higher in the inner than the outer sapwood in *Acer* and *Populus* individuals. In the case of *Populus*, which showed highest sap flux values even at the inner positions, while presenting also the highest incidence of bacterial wet-wood infections, the observed values could represent bias due to the age of the analyzed trees, which had merely juvenile wood with wide, but probably deteriorated connections due to the bacterial infection. Therefore, besides the high J_s values, *Populus* individuals, surprisingly, showed a marked k_s decrease in the inner depths, situation that needs more research, since it is unknown the extent to which the observed wet-

wood condition interferes in the normal functionality of this species conducting system. Interestingly, a different scenario was shown by *Arbutus* individuals, which had lower apparent infection, and presented the lowest flux and specific conductivity in the damaged inner depths, so that wet-wood may have affected J_s and k_s more evenly in this species, reflecting a different strategy to deal with the bacterial infection.

It is known that high resistance to water flow in the stem rises as a result of wood with low conductivity, individuals with little conducting tissue, and individuals with slow water movement from storage compartments (Zimmermann 1983; Tyree and Ewers 1991). Thus, the fact that radial tension gradient estimates were about 1000 times greater than the axial tension gradients, show the high resistance to water movement along the sapwood. This higher resistance to radial water movement shows the differences in the hydraulic system where water moving axially, primarily through the perforation plates, has less resistance to flow, than water moving radially through pits.

The relationship between J_s and k_s that resulted from using all J_s/k_s pairs demonstrates that the observed fluctuations by species could be due to inherent measurement and sample variability, especially in the cases of *Acer* and *Populus* individuals.

LIMITATIONS AND RECOMMENDATIONS

Due to the small number of replicates, the use of statistical tools was limited in this study. Therefore, more replicates per species are recommended for further studies addressing similar questions. This will open the possibility of use statistical tools that would contribute to better interpret the significance of radial J_s , k_s and tension gradient differences along the sapwood.

Because specific conductivity is influenced by the conductive element diameter (Zimmermann 1983), its length (Comstock and Sperry 2000), as well as by the number of embolised vessels (Sperry and Hacke 2004), the type of perforations plates and pit density (Utsumi et al. 1996), a parallel anatomical analysis would lead to a better interpretation of the anatomical and physiological interactions in a tree.

FURTHER RESEARCH

Within a tree, many possible anatomical pathways, with different resistances, link the hydraulic system along the plant-soil-atmosphere continuum (Zimmermann 1983; Wullschleger et al. 1998). The actual paths for the axial and radial water movement are still unknown. Therefore, to better understand the hydraulic connections in the water transport system in a tree, more radial and axial studies are needed,

specifically focused on leaf-sapwood connections, the pathways for radial water transport, and sapwood widths.

In the present study, the higher axial tension gradients in inner than outer sapwood of cottonwood suggest that the driving force is higher on the inner than the outer rings. Given that the leaves are one year old or younger, and thus are by necessity connected to the outer xylem at the stem and branch tips, this implies that the xylem from upper parts of the trees must be transferring the tension not to the outer growth ring but to the inner ones. This is an unexpected pattern and points the necessity to study, at more detail, the anatomical paths for water transport through dyes, and chemically labeled solutions analysis i.e. that may lead to a better understanding of the influence of the anatomical constituents, their proportion and distribution on J_s and k_s radial variations as well as their consequences along the hydrostatic system.

More attention must be focused on radial flow rate differences among and within species. Thus, further research expanding the number and diversity of woody species and considering the impact of the non-uniformity of the sapwood zone for the transport of water may help to get better physiological models of whole-tree water flux consider. Thus, corrections on leaf area/ sapwood area ratios may be evaluated for

existing models that considered the sapwood as a uniform area for water movement.

The influence of wet-wood infections on wood properties has been a subject of interest for several years; however, these studies have been limited to ring porous species, whereas *Populus*, a widely used genus in the wood and paper industry has been let behind. Regarding this genus, more research needs to address the role that genetics or chance play on the incidence of wet-wood infections because some individuals have it, others don't. The extent to which these wet-wood infections are affecting biomass productivity in these trees is still a subject of research, since it is unknown to which extent this disease affects the potential functionality of the wood. From the biological point of view is wet-wood always a bad thing? Is that a strategy of the tree to deal with other diseases? Is this a case of safety versus efficiency issue? To which extent inorganic and organic substances in the sap, as well as other chemical changes along the sapwood can have an effect on J_s , k_s and the generated tension gradient? Studies regarding the inspection of possible signs that would indicate the presence of these infections at early stages may help to get more yields from contaminated stands.

Finally, more research still needs to be done regarding the movement of water along the inner sapwood where little flow still existed?

This will help link sapwood width with its functionality. This information could be used to account for radial sap flux differences for water, which in turn could be used to modify whole-tree water use models that considered the sapwood as a uniform zone of axial flux. The generated information would be valuable for wood drying and treating.

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