

AN ABSTRACT OF THE THESIS OF

Sonya M. Dunham for the degree of Master of Science in Forest Science and Wood Science presented on April 21, 2006.

Title: Douglas-fir Hydraulic Architecture and Relationships Among Xylem Properties at Multiple Scales via a Bayesian Analysis

Abstract approved:

Signature redacted for privacy.

Lisa M. Ganio Barbara L. Gartner

This study investigated variation in xylem anatomy, hydraulic properties, and the relationship between anatomy and properties within Douglas-fir trees at multiple scales. The hierarchical scales in the study included fertilization treatments (fertilized and unfertilized), trees within the treatments, and positions within the trees. Tracheid diameter, tracheid length, percent latewood, number of pits per cell, wood density, and specific conductivity (K_s) were measured on seven positions within each of 16 fertilized and unfertilized trees: the stem at cambial age 54 (breast height), 25, and 5; a branch at cambial age 20 and 7; and a root at cambial age 42 and 22. Vulnerability to embolism, sap velocity, and sap temperature were also measured on the oldest of the above-listed parts of each position. A Bayesian hierarchical regression model (HM) was developed that partitioned variation among scales. When compared to a simple linear regression model that assumed independence among observations, the HM consistently gave more precise estimates for regression coefficients and provided a more informative and precise estimate of variance. For any anatomical feature or xylem property there was little variation between treatments, however there was great variation among positions. Relationships between xylem anatomy and xylem properties were evident at larger scales (between treatments and among positions); however at a smaller scale usually only the branches and roots maintained the relationship. Tradeoffs among xylem properties were also different at each scale, often existing at larger scales but only within a few positions or positions within

treatments at smaller scales. The fact that the relationships analyzed were dependent upon scale suggests that factors driving the relationships are likewise dependent on scale and that wood structure is adapted not only for whole tree functioning but also for small scale, position specific functional demands.

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Douglas-fir Hydraulic Architecture and Relationships Among Xylem Properties at
Multiple Scales via a Bayesian Analysis

by
Sonya M. Dunham

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APPROVED:

Signature redacted for privacy.

Co-Major Professor, representing Wood Science and Engineering

Signature redacted for privacy.

Co-Major Professor, representing Forest Science

Signature redacted for privacy.

Head of the Department of Wood Science and Engineering

Signature redacted for privacy.

Head of the Department of Forest Science

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Dean of the Graduate School

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Introduction

The ability to transport water from the soil to its leaves is vital to a tree's survival. Mass water flow in trees is driven by the pressure differential between the water in the tree and the dry atmosphere created by the vapor pressure deficit, so the water column in the xylem, or wood is under tension. Water is supplied to the leaf from the roots via the xylem. Xylem in conifers is mostly comprised of elongated cells, called tracheids, which are hollow and dead upon maturity. Growth rings in wood are made up of earlywood and latewood; the earlywood is made towards the beginning of the growing season and is characterized by much wider tracheids than the latewood, which is put on later in the season and is much denser. Water moves from one cell up to another, primarily in the earlywood, through small mesh openings, or pits, between the tracheids. If the tension in the xylem becomes too great due to low soil water availability, or a severe vapor pressure deficit, air may be forced through pit membranes and expand to fill the tracheid or vessel with air, creating an embolized cell that is incapable of water transport. The conductivity (permeability) of xylem decreases as cells become embolized, so vulnerable xylem runs the risk of approaching devastating degrees of low conductivity if xylem tension is too high.

Xylem anatomy and xylem properties vary among different species (Martínez-Vilalta et al. 2002), environments (Bouffier et al. 2003), parts of a tree (Choat et al. 2005b), and ages of wood (Domec and Gartner 2002a). The research presented in this thesis investigated within-tree and among-tree variation in xylem anatomy and xylem properties and relationships between them at multiple scales in Douglas-fir trees using statistical hierarchical modeling. There is much interest in understanding how xylem structure relates to xylem function, and this study will add to existing knowledge by addressing commonly overlooked issues related to hierarchical scaling in tree physiology. The study will also provide information on properties and anatomy of roots and branches of different ages in relation to the stem to help us understand patterns of how whole trees are structured.

It is generally expected that variation in xylem anatomy can explain the variation seen in xylem properties such as wood density, conductivity, and xylem vulnerability to embolism. This notion underlies a great majority of research in tree physiology that aims to understand plant water relations and hydraulics. For example, xylem with a high percentage of latewood and thick tracheid walls is expected to have a high density (from the greater percentage of cell wall material), low conductivity (from the lower percentage of open conducting area), and low vulnerability to embolism (because of stiffer cell walls that can resist implosion, Hacke et al. 2001; Jacobsen et al. 2005).

Variation in xylem properties between species and populations is often used to explain plant distribution across climatic gradients in terms of drought tolerance (e.g. Kolb and Davis 1994; Mencuccini and Comstock 1997; Brodribb and Hill 1999; Kavanagh et al. 1999; Hacke et al. 2000b; Maherali and DeLucia 2000; Pockman and Sperry 2000; Stout and Sala 2003) and to differentiate between water transport strategies among species (Wagner et al. 1998; Hacke et al. 2000b). Another common topic in current research is the degree to which trees are built to optimize hydraulic and structural properties and whether there are limiting tradeoffs among the properties (e.g. Brodribb and Hill 1999; Kavanagh et al. 1999; Hacke et al. 2000b; Pockman and Sperry 2000; Domec and Gartner 2001; Martínez-Vilalta et al. 2002). Ideally xylem would be highly resistant to embolism, highly conductive, and mechanically stable but not overbuilt (in the sense of using more carbon and energy to make dense wood than necessary). Woody plants do not appear to optimize all these characteristics simultaneously (Gartner 1991; Wagner et al. 1998; Davis et al. 1999; Maherali et al. 2004) because there is a tradeoff between among these properties (Tyree and Zimmerman 2002). Knowledge of where the balance lies among these properties for different plant parts will give insight in to the importance of the three different roles in different parts of the same plant.

There are several underlying questions that may help our understanding of these larger issues of tree structure and function that seem to have either been overlooked in the background, assumed not to be important, deemed logistically problematic, or just have not yet been considered. One is the question of how the various positions along the xylem pathway are related. Studies of xylem conductivity and vulnerability to embolism of larger and older trees almost always are based on measurements of branch or root segments from 3 mm to 20 mm in diameter (e.g. Cochard 1992; Sperry and Saliendra 1994; Hacke and Sauter 1996; Maherali and DeLucia 2000; Martínez-Vilalta et al. 2002) and very rarely for segments from the main stem, structural roots, or older branch segments. Several theories predict how xylem should change along the water transport pathway to optimize whole tree hydraulic efficiency and safety against embolism (Comstock and Sperry 2000; McCulloh and Sperry 2005) but there are only a few physical studies that sample more than two sections along the pathway (such as Domec and Gartner 2002a; Choat et al. 2005b). Small branch and root segments are logistically easier to work with than large diameter samples. Measuring conductivity involves forcing water through a longitudinal sample and calculating the volume flow rate. Rubber tubing used in these measurements easily fits around the small diameter samples and the natural xylem boundary at the cambium and bark prevent leaks though the sides. Small branches and roots, however, may not be representative of the whole plant, and it is unknown whether the anatomy, density, and water transport properties of small branches and roots are correlated with those characteristics of other positions in the tree.

Spicer and Gartner (1998) developed a method to measure hydraulic properties on samples chiseled from larger sections. Samples cut from large stems and branches are enclosed in a latex sleeve within a slightly pressurized chamber (to hold the sleeve against the sides of the sample) during the measurement, preventing water from escaping the sample through cut tracheids along the sides. This method is more time consuming because of the sample preparation, but it allows for the comparison of hydraulic properties between large and small diameter parts of a tree which is

noticeably lacking in the current literature except for a few studies (including Spicer and Gartner 2001; Domec and Gartner 2002a; Domec et al. 2005). A more in depth knowledge of within-tree variation and how positions are related within the tree would provide a context for studies that only include one or two positions.

Another unaddressed issue is how the biological hierarchy in which trees exist should be dealt with an analysis and how it should inform inferences. Biological hierarchies may be found throughout nature and describe the various scales at which organisms, processes, and stimuli exist and interact. In tree physiology a large scale category is life strategy (e.g. gymnosperm vs. angiosperm). Smaller scales can include species, populations, stands, trees, positions, growth rings, and individual cells. Xylem structure and function are influenced by processes and stimuli operating at different scales so inferences about how they relate to each other may be different at each scale. In studies investigating how xylem properties relate to xylem anatomy and how properties are related, data are often collected across species, across environmental conditions within species, and across positions within trees. Reports may be given of results at each scale, but explicit descriptions of how multiple scales are dealt with in statistical analysis and how results from each scale are compared are generally lacking.

The multiple sources of variation within a biological hierarchy can be accounted for in statistical analyses via statistical hierarchical models that explicitly link information from multiple scales in a single analysis. Though not yet common in tree physiology, statistical hierarchical models are becoming widely used in many ecological fields such as population and landscape ecology (Link et al. 2002; Rivot and Prevost 2002; Wyatt 2002; Boone et al. 2005). Whenever research deals with more than one scale, there is dependence among observations because of the interrelated nature of hierarchical scales. Accounting for dependence among observations at multiple scales in statistical analysis should provide more precise results compared to an analysis that assumes independence among observations (Bryk and Raudenbush 1992; Wyatt

2002). Statistical hierarchical models facilitate analysis of hierarchical data and comparison among scales by including complex variance structures that partition variation among scales.

This study is an effort to begin to fill these knowledge gaps of how to take into account biological hierarchies in tree physiology and within-tree variation in xylem anatomy and properties. Various anatomical, hydraulic, and structural characteristics were measured on seven positions, including both young and old sections of stems, roots, and branches of Douglas-fir trees (*Pseudotsuga menziesii*) in fertilized and unfertilized treatments. The first goal was to develop a statistical hierarchical model that reflected the multiple sources of variation in the biological hierarchy. The biological hierarchy was comprised of positions within trees, trees within treatments, and treatments within species (Douglas-fir). Using this statistical model, the second goal was to determine how xylem characteristics differed among the seven positions and whether they were correlated within trees. The third goal was to investigate how wood anatomy influences xylem hydraulic and structural properties at each scale. Lastly, the fourth goal was to examine how xylem properties were related at each scale, which would give insights into tradeoffs among properties and the scale at which the tradeoffs affect xylem structure and function.

The next chapter, 'Partitioning variation among multiple scales via a Bayesian hierarchical model for xylem properties in Douglas-fir,' begins with a description of the role of hierarchies in ecological research, with special attention to how they are currently dealt with in tree physiology, and an introduction to statistical hierarchical models. A hierarchical regression model and a typical simple linear regression model are developed to address the example of tracheid diameter regressed on conductivity. Results from the two models are then compared and the merits of the hierarchical model are highlighted. In the third chapter, 'Bayesian analysis of Douglas-fir hydraulic architecture at multiple scales,' the second and third objectives of identifying anatomical and hydraulic differences among positions and examining how

xylem anatomy affects xylem properties are addressed using the hierarchical model developed in first chapter. Building on the position differences and relationships found in the second chapter, the fourth chapter, 'Structural and hydraulic tradeoffs at multiple scales in Douglas-fir,' focuses on whether tradeoffs exist between xylem properties in Douglas-fir and, if so, at what scale. A qualitative summary of the results are provided in the Conclusion, along with a discussion of the how this work is relevant in tree physiology and wood science.

Partitioning variation among multiple scales via a Bayesian hierarchical model for xylem properties in Douglas-fir

Sonya M. Dunham, Lisa M. Ganio, Alix I. Gitelman, Barbara L. Gartner

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Abstract

Hierarchical scales permeate research in tree physiology. Positions are nested within trees, trees are nested within species, and species may be within geographic regions or climate zones. We discuss the importance of matching the sampling and analysis scales to the biological scales naturally occurring in the data. Scales within biological hierarchies represent multiple sources of variation, so estimation of variation in hierarchical data should account for these scales. We demonstrate the utility of applying a statistical hierarchical model to a question in tree physiology, the relationship between specific conductivity and tracheid diameter, by comparing the structure and results of a simple linear regression (SLR) and a hierarchical model (HM) within a Bayesian context. The two models shared similar mean structures but differed in how variation was partitioned among multiple scales; the SLR model assumed independence among observations and that variation only came from a single scale and the HM had a complex variance structure that partitioned variation among all scales. Our data included hierarchical scales of position, tree, fertilization treatment, and species (Douglas-fir). We found that the HM consistently gave more precise estimates for slope and intercept parameters compared to the SLR, resulting in a higher number of differences between positions than in the SLR. The HM also provided a detailed description of covariances within the data at multiple scales, compared to the single estimate in the SLR, thus providing a better estimate of the variation.

Introduction

As anticipated by Wiens (1989), ‘scale’ has indeed become an ecological buzzword. Research involving multiple scales is now prevalent in ecological literature reflecting an increased awareness of the scaled structure of ecological systems (O'Neill et al. 1989; Kotliar and Wiens 1990; Bergstrom and Tweedie 1998; Dungan et al. 2002; Gotway and Young 2002; Wikle 2003). The field of tree physiology is no exception. Many recent studies have discussed ways to ‘scale up’ or extrapolate to the whole tree,

stand, or regional level based on lower level measurements (e.g. Ehleringer and Field 1993; Rayment et al. 2002) or have investigated allometric scaling in trees (e.g. Enquist 2002; Santiago et al. 2004). Another notion of scale that is present in nearly all research in tree physiology, though not often explicitly identified, is the hierarchical nature of the biological system and data sampling methods.

O'Neill et al. (1989) describe all biological systems as complex, multi-level systems in which scales range from the single cell to the entire biosphere. Biological scales can reflect ecological processes, biological structures, or other natural phenomena (Dungan et al. 2002). Trees are made up of unique positions (roots, trunk, branches, etc.) and are parts of larger organizational groupings (species, forest stands, geographic regions, etc.), and this hierarchical organization appears throughout tree physiology literature. Examples of recent studies are presented in Table 2.1 to illustrate typical biological scales present in research investigating hydraulic architecture.

Our objectives are to describe the importance of reflecting biological hierarchical scales in sampling and analytic methods and to apply a Bayesian hierarchical model to an example from tree physiology, specifically the relationship between specific conductivity and tracheid diameter in Douglas-fir (*Pseudotsuga menziesii*). We will illustrate and compare results from two statistical models; a simple linear regression and a hierarchical regression model, both analyzed by Bayesian methods. The biological scales in our data included the overall level of species, two fertilization treatments (fertilized and un-fertilized), trees within the treatments, and positions within trees. The hierarchy of the research design may be described graphically with the treatment scale indexed by j , followed by tree (indexed by i) and position (indexed by h , Figure 2.1).

Table 2.1 Examples of hierarchical data and analyses in recent tree physiology literature. Hierarchical scales are listed from smallest to largest scale. These scales were determined from reading the descriptions of methods and experimental design in each study and may not have been explicitly described as hierarchical scales by the authors. The third column, Statistical analysis, categorizes the studies according how the analyses in the paper deal with the multiple scales in the data: “Independence among scales” = statistical methods assume independence among samples, “Repeated measures” = statistical methods reflect correlations among the scale in parentheses, and “Analysis unclear” = statistical methods were not explicitly described in the paper.

Paper	Hierarchical scales	Statistical analysis
Choat et al. (2005a)	Branch, tree, species, functional group	Independence among scales
Hacke et al. (2000a)	Position (root and branch), tree, soil type	Independence among scales
Hacke et al. (2000b)	Position (root and branch), tree, species, functional group	Independence among scales
Kolb and Davis (1994)	Date, plant, species, irrigation treatment or site	Independence among scales
Mencuccini and Comstock (1997)	Position (root, stem, twig), seedling, treatment, population, species	Independence among scales
Pockman and Sperry (2000)	Time of day, date, year, plant, species, wood type or habitat	Independence among scales
Pratt et al. (2005)	Plant, age class, species, site	Independence among scales
Sparks and Black (1999)	Seedling, population, site	Independence among scales
Domec and Gartner (2002) (2002a)	Date, vertical and radial position in the trunk, tree, age class	Repeated measures (date)
Ewers et al. (2000)	Position (root and stem), tree, plot, fertilization and irrigation treatments	Repeated measures (position)
Martínez-Vilalta et al. (2002)	Date, position (root and stem), tree, species	Repeated measures (date)
Stout and Sala (2003)	Date, positions (root and branch), tree, species, site	Repeated measures (date)
Brodribb et al. (2003)	Position (leaf and branch), seedling, species	Analysis unclear
Kavanagh et al. (1999)	Position (root and stem), seedling, population	Analysis unclear
Sperry and Saliendra (1994)	Position (root, stem, and twig), tree, age class	Analysis unclear

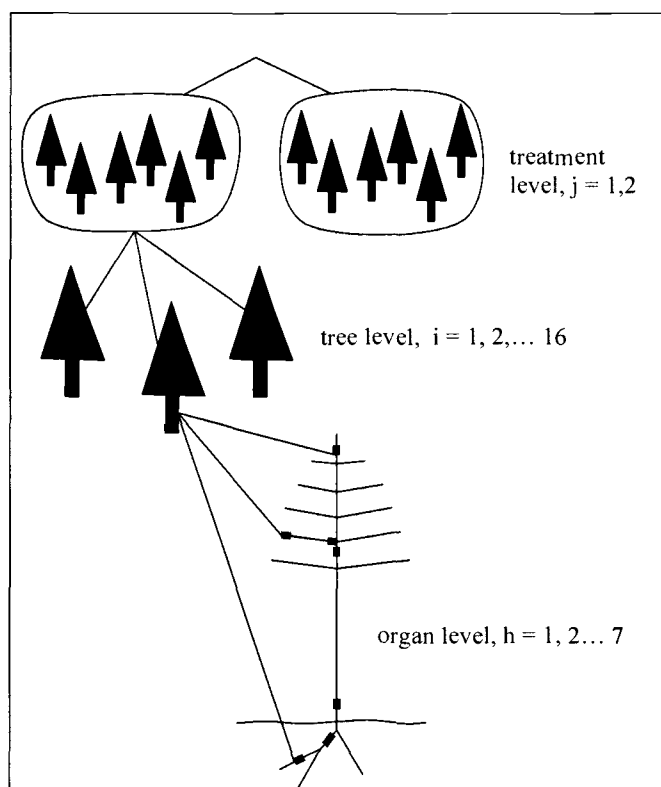


Figure 2.1 Graphical representation of the biological hierarchy. Position is indexed by h , tree by i , and treatment by j .

The role of hierarchies

Biological hierarchies represent sources of variation within an ecological system. Variation can come from any scale within the hierarchy; there may be variation among and within positions within a tree, among trees within a stand, and among groups of stands within a landscape. Within-tree variation in xylem anatomy and properties may be found among positions (Fegel 1941; Sperry and Saliendra 1994; Hacke and Sauter 1996; Mencuccini and Comstock 1997; Martínez-Vilalta et al. 2002), among cambial ages (Domec and Gartner 2001; Domec and Gartner 2002a; McElrone et al. 2004; Choat et al. 2005b), and along vertical and radial profiles (Domec and Gartner 2001; Domec and Gartner 2002a; Martínez-Vilalta et al. 2002; McElrone et al. 2004; Choat et al. 2005b). Variation among trees can be due to population (Kavanagh et al. 1999;

Bouffier et al. 2003; Stout and Sala 2003), microclimate, or any other factor that affects the development of an individual tree relative to others in the same group. Higher scales are any broader categorization that further partition variation, such as species (Mencuccini and Comstock 1997; Martínez-Vilalta et al. 2002; Stout and Sala 2003; Jacobsen et al. 2005; Pratt et al. 2005), and environmental conditions or treatment groups (Jozsa and Brix 1989; Ewers et al. 2000).

Anthropocentric scales (Wiens 1989), or scales that are created by researchers to facilitate data collection, include ‘sampling’ scales that define the size of the unit upon which measurements are made (Dungan et al. 2002), and the ‘analysis’ scales that define the size of the unit used for data analysis and inferences (Robinson and Ek 2000; Dungan et al. 2002; Wikle 2003). Sampling scales in tree physiology are typically based on geographical organization (plots, stands), biological organization (species, growth form, leaf phenology), or position within the tree (height, cambial age, type of organ; column 2 in Table 2.1). Sampling scales tend to reflect closely the biological scales and sources of variation relevant to the scope of the research.

Though the biological and sampling scales are closely aligned in tree physiology, the analysis scales are often very different. Most tree physiology studies addressing multiple scales regard levels and observations within each level as independent (column 3 in Table 2.1). T-tests and ANOVA are often used to detect differences but they may incorrectly assume independence between the components being tested (e.g. Kolb and Davis 1994; Mencuccini and Comstock 1997; Sparks and Black 1999; Hacke et al. 2000a; Hacke et al. 2000b; Pockman and Sperry 2000; Choat et al. 2005a; Pratt et al. 2005). Repeated measures analyses are occasionally used to model two-level hierarchies, such as density measurements from several radial positions in the same disk from a tree or water potential measurements taken on the same tree at several times throughout the day or year (e.g. Ewers et al. 2000; Domec and Gartner 2002a; Martínez-Vilalta et al. 2002; Stout and Sala 2003). In other cases, the statistical methods are not explicitly described so it is difficult to discern if the

reported results reflect the interdependence and correlations present in the study's hierarchical structure (e.g. Sperry and Saliendra 1994; Kavanagh et al. 1999; Brodribb et al. 2003).

Failing to match the analysis scales with biological and sampling scales may result in misrepresentation of replication, incorrect estimates of standard error, and possibly inaccurate conclusions (Bryk and Raudenbush 1992). Correlations among units within a hierarchical scale may exist because units share characteristics of the scale in which they are nested. Positions sampled from the same tree are likely correlated because they share genetic material, exist in the same geographic location, and are part of the same tree. Trees within a stand may be correlated because they share similar environmental and climatic conditions that distinguish them from other stands. Analyses that assume independence among correlated units within a scale fail to partition variance among the biological scales present in the data.

By way of illustration, consider two analyses of a study testing for a difference between two treatments of four plots, each of which contains ten trees. Suppose there is low variation among trees within each plot but high variation among plots. The first analysis ignores the plots, treats the trees as replicates, and estimates a single value of the variance among the trees. The second partitions the variance into two parts; one part accounts for variation among plots and the other describes the remaining among-tree variance. The second analysis mirrors the biological and sampling scales and, after accounting for the relatively large between plot variance, better estimates the within-tree variance. The first method, treating all the data as coming from the same scale in the analysis, overestimates the variation among the trees by not separating from it the variation among the plots. Thus, this analysis for a completely randomized block design is an example of a hierarchical model.

Statistical hierarchical models (Bryk and Raudenbush 1992; Wikle 2003; Boone et al. 2005) resolve the issues noted above with hierarchical data by explicitly incorporating

different scales and modeling linkages among scales in a single analysis. Within a statistical hierarchical model the response is defined at the lowest level of the hierarchy and the explanatory data are modeled at various appropriate higher levels. Each level is represented by a sub-model. Dependence among observations within a level is specified in the sub-model for that scale. Sub-models of higher levels (or larger scales) define components of lower levels and thus represent interactions between scales. The mean and variance components are thus partitioned within and among the explanatory scales. This improves estimation at all scales as well as estimates of cross-level interactions by minimizing the potential confounding effects of scale and improving estimates of standard errors (Bryk and Raudenbush 1992).

Hierarchical models may be analyzed by either frequentist or Bayesian methods. We use Bayesian techniques because the Bayesian ‘posterior’ distribution for each parameter is more directly interpretable than the point estimates and p-value results of a frequentist analysis (Reckhow 1990; Crome et al. 1996; Ellison 1996). In the Bayesian perspective, parameters are modeled as random variables and inference is based on the posterior conditional distribution of a parameter. This is contrasted with frequentist inference which is based on theoretical ‘long-run’ sampling distributions of parameters. For a more detailed discussion of frequentist vs. Bayesian analysis in ecological research see the special section ‘Ecological Application of Bayesian Inference’ introduced by Dixon and Ellison (1996) in *Ecological Applications*.

Methods

Research design and data collection

Data were collected during 2004 in a 54-year-old naturally regenerated Douglas-fir stand in the western foothills of the Cascade Mountains (44° 25.6' N, 122° 35.7' W). Half of the stand was fertilized with nitrogen in 2000 by aerial application, and 16 trees were randomly selected from both the fertilized and unfertilized treatment. Wood samples were taken from seven positions in each tree. Three samples were taken from the trunk (at cambial ages 54, 25, and 5), two samples were taken from a

branch (cambial ages 20 and 7), and two samples were taken from a root (cambial ages of approximately 42 and 22, Table 2.2). Specific conductivity (K) and tracheid diameter (D) were measured for each of the seven positions on each of 16 trees in each of the two treatments, for a total of 224 samples. Specific conductivity was measured for each sample as per Spicer and Gartner (1998). Slides of microtome sections from each sample were viewed under a compound microscope using MetaVue[®] (copyright 1992-2004) and 100 tracheid diameters were measured in the radial direction on each sample. Further details may be found in the next chapter.

Table 2.2 Cambial age, tracheid diameter, and specific conductivity from 54-year old Douglas-fir trees in the western Cascade foothills. Values are means (95% BCI), n = 32 trees.

Position	Cambial age (years)	Tracheid diameter (μm)	K ($\text{m}^2/\text{s MPa}$)
Stem 52	51.6 (50.6, 52.7)	16.2 (15.1, 17.2)	0.0045 (0.0039, 0.0051)
Stem 25	24.4 (23.3, 25.5)	16.4 (15.4, 17.4)	0.0074 (0.0066, 0.0081)
Stem 5	5.3 (5.1, 5.4)	9.9 (9.2, 10.5)	0.0017 (0.0015, 0.0018)
Branch 20	20.2 (19.2, 21.3)	7.2 (6.6, 7.8)	0.0014 (0.0012, 0.0016)
Branch 7	7.0 (6.4, 7.6)	6.3 (5.4, 7.0)	0.0003 (0.0001, 0.0004)
Root 42	42.2 (40.2, 43.9)	15.8 (14.8, 16.8)	0.0036 (0.0028, 0.0044)
Root 22	22.3 (19.2, 25.2)	16.8 (14.7, 18.6)	0.0069 (0.0052, 0.0086)

Models for mean $\ln(K)$

Let $\ln(K_{hij})$ be the natural log of specific conductivity and let D_{hij} be tracheid diameter for position h , tree i , and treatment j . We used the natural logarithm of the response to facilitate a linear relationship between K_{hij} and D_{hij} . We first present four models for the mean of $\ln(K_{hij})$ that we use for both the simple linear regression model (SLR) and

the hierarchical model (HM). We then describe how the variance and covariance structures differ between the SLR and the HM.

The basic model for the mean of $\ln(K_{hij})$, μ_{hij} , is the same for the SLR and HM:

$$\mu_{hij} = \beta_{1hj} + \beta_{2hj} D_{hij}$$

where β_{1hj} and β_{2hj} are the intercept and slope coefficients, respectively. There are four variants of this model, depending on whether we assume the relationship between $\ln(K)$ and D is the same for all positions and both treatments, different for each position, different for each treatment, or different for each position in either treatment. First, if we assume the relationship is the same for all positions and treatments,

$$\mu_{hij} = \beta_1 + \beta_2 D_{hij},$$

then we get a common mean (CM) model.

Next, if we let the relationship vary by treatment but not by position then the result is a treatment mean (TM) model:

$$\mu_{hij} = \beta_{1j} + \beta_{2j} D_{hij}.$$

We could also let the relationship differ for each position but not treatment:

$$\mu_{hij} = \beta_{1h} + \beta_{2h} D_{hij},$$

which is an position mean (PM) model. The relationship may be different for each position in each of the two treatments, in which case β_1 and β_2 are unique for each position – treatment combination. This is the position and treatment mean (PTM) model:

$$\mu_{hij} = \beta_{1hj} + \beta_{2hj} D_{hij}.$$

We also considered a null version of each of each mean model to verify the importance of including a slope coefficient (β_2). The CM null model, for example, is simply

$$\mu_{hij} = \beta_1.$$

Models for the variance and covariance

The SLR and HM differ in how residual variation is partitioned. Let us first begin with the familiar linear regression model for comparison.

Simple linear regression model

The model for SLR is typically written as

$$\ln(K_{hij}) = \beta_{1hj} + \beta_{2hj}D_{hij} + \varepsilon_{hij}$$

$$\varepsilon_{hij} \sim N(0, \sigma^2)$$

where the ε_{hij} are the model error and are assumed to be independent and normally distributed with mean zero and variance σ^2 . Equivalently we will write

$$\ln(K_{hij}) \sim N(\mu_{hij}, \sigma^2),$$

where $\mu_{hij} = \beta_{1hj} + \beta_{2hj}D_{hij}$.

This allows direct comparison with the HM described next. Notice that in this model the variance parameter, σ^2 , describes the variation regardless of position, tree, or treatment and thus ignores the fact that variation may come from different scales or that there may be correlations between components.

Hierarchical model

The distinguishing feature of the HM is that it imposes a hierarchical variance structure on background variation. In the HM, $\ln(K)$ from the positions on the same tree are modeled as multivariate so that each response variable, $\ln(K_{ij})$, is a 1x7 vector of $\ln(K_{hij})$ values from the seven positions on each tree and

$$\ln(K_{ij}) \sim \text{MVN}_7(\mu_{ij}, \Sigma_j)$$

$$\mu_{ij} = \beta_{1hj} + \beta_{2hj}D_{ij}$$

where $\mu_{ij} = [\mu_{1ij}, \mu_{2ij}, \mu_{3ij}, \mu_{4ij}, \mu_{5ij}, \mu_{6ij}, \mu_{7ij}]$ is the vector of position means of $\ln(K_{ij})$ for tree i and treatment j , and Σ_j is the 7x7 matrix of covariances among $\ln(K)$ from different positions within a tree. The covariances are parameters that describe how much information is shared among observations from the same tree. In this model Σ is allowed to differ between fertilized and unfertilized trees ($j = 1, 2$).

Model fitting

Simple linear regression model priors

In the Bayesian context, the regression coefficients and σ^2 in the SLR are modeled as random variables and are given ‘prior distributions’ (or priors). Informative priors may be specified to contain previously known information about the parameter or they may be diffuse distributions representing little or no previous knowledge (‘non-informative’). We chose to specify the priors for the coefficients and σ^2 as non-informative normal and gamma distributions, respectively (Gelman et al. 2003):

$$\beta_{1hj} \sim \text{Normal}(0, 1000)$$

$$\beta_{2hj} \sim \text{Normal}(0, 1000)$$

$$\sigma^2 \sim \text{Gamma}(1000, 1000)$$

Hierarchical model priors

In the HR the value $[\beta_{1hj}, \beta_{2hj}]$ is given a multivariate normal prior:

$$\beta_{hj} = [\beta_{1hj}, \beta_{2hj}] \sim \text{MVN}_2(\theta, \mathbf{T})$$

where $\theta = [\theta_1, \theta_2]$ is the mean of β_{hj} , and \mathbf{T} is the covariance matrix for the regression coefficients. This is a general model that allows for correlations between β_{1hj} and β_{2hj} but does not require it: the \mathbf{T} matrix may be a simple diagonal matrix. Distributions for Σ_j , θ , and \mathbf{T} must also be specified. The joint prior distribution of the elements of a covariance matrix such as Σ_j is typically described by a Wishart distribution (Gelman et al. 2003):

$$\Sigma_j \sim \text{Wishart}(\mathbf{R}_7, \rho_1)$$

where \mathbf{R}_7 is the 7x7 identity covariance matrix and ρ_1 is the associated degrees of freedom. Analogously, the joint prior distribution of the elements in \mathbf{T} is given by:

$$\mathbf{T} \sim \text{Wishart}(\mathbf{S}_2, \rho_2)$$

where \mathbf{S}_2 is a 2x2 identity matrix and ρ_2 is the degrees of freedom. To represent vague prior information we chose ρ_1 and ρ_2 to be the rank of the covariance matrices, $\rho_1 = 7$

and $p_2 = 2$. To reflect vague prior information for the elements of θ they were given non-informative independent normal priors:

$$\theta_1 \sim N(0, 1000)$$

$$\theta_2 \sim N(0, 1000)$$

Posterior estimation and summarization

Models were fitted using a Markov Chain Monte Carlo (MCMC) algorithm with Gibbs sampling in WinBUGS, version 1.4 (Spiegelhalter et al. 2003). We ran four independent chains initialized at dispersed starting values for each model. Chain convergence was assessed using the Gelman-Rubin statistic, modified by Brooks and Gelman (1998) as calculated in WinBUGS®. The chains for the SLR converged almost immediately and the HM typically converged after 30,000 iterations. We used a sample of 2000 iterations from each of the four chains after they converged to describe the posterior distributions of each parameter. Posterior probability distributions for parameters were summarized by calculating the mean and the 95% posterior probability interval (2.5 and 97.5 percentiles) or Bayesian credibility interval (BCI, Ellison 1996).

The deviance information criterion (DIC) was calculated for each model. The DIC is a summary of the discrepancy between a model and the data in the Bayesian context (Spiegelhalter et al. 2002; Gelman et al. 2003) and is calculated as:

$$DIC = E\left[(-2 \times \ln p(y|\Phi))\right] - 2(pD)$$

where $E\left[(-2 \times \ln p(y|\Phi))\right]$ is the average deviance ($-2 \times \ln p(y|\Phi)$), $p(y|\Phi)$ is the model likelihood, and pD is the effective number of parameters. Discrepancy between the model and the data may be artificially decreased by including additional parameters so the DIC includes pD as a penalty for larger models. Models were compared by calculating ΔDIC_i , the difference between the criterion value for model i and the minimum criterion value within the group of models being compared

($\Delta DIC_i = DIC_i - DIC_{\min}$). The model with the lowest DIC, or $\Delta DIC_i = 0$, is deemed to be “best” supported by the data.

Frequentist and Bayesian methods differ in the way results are reported and interpreted. The p-value from a frequentist hypothesis test provides the probability that the test statistic is as extreme, or more extreme, than the one that was obtained from the sample that was collected, given that the null hypothesis is true. If a p-value supports the rejection of the null hypothesis then the parameter of interest is statistically significantly different from the value specified in the null hypothesis. Frequentist 95% confidence intervals are sets of values for the parameter of interest that might have been obtained from 95% of other hypothetical samples drawn from the sampling distribution, but that were not actually sampled. The Bayesian approach incorporates prior information along with the data that were collected to produce a posterior probability distribution for the parameter of interest. That is, Bayesian methods condition only on the sample that was collected, not on hypothetical samples. The Bayesian 95% credibility interval is more directly interpretable as the probability that the parameter has a value between the interval bounds is 95% (Ellison 1996). If a 95% BCI spans only positive or negative values, then the parameter is reported to be positive or negative, respectively. A 95% BCI that spans both positive and negative values and includes zero, is reported to be not different from zero, especially if zero is near the center of the interval. We will provide 95% BCIs for regression slopes and will report if they are positive, negative, or not different from zero. For a more detailed explanation of Bayesian inference see Ellison (1996) and Reckhow (1990).

Results

DIC – mean model selection

ΔDIC distinguished clearly among the eight SLR and HM models (Table 2.3). For each of the eight mean models the full model (including a slope) was better supported

than the null (only an intercept). The PM HM was best supported by the data, and for each of the four different mean models the HM was always better supported than the SLR. Even after penalizing the HM for its large number of parameters HM performed better than the SLR.

Table 2.3 Comparison of Δ DIC and number of parameters for each regression model. HM = hierarchical model, SLR = simple linear regression model, CM = common mean model, TM = treatment mean model, PM = position mean model, PTM = position and treatment mean model, null = intercept only, p = total number of parameters (includes regression coefficients, variance components, and prior parameters).

Variance model	Mean model	Δ DIC	p
HM	PM	0	130
HM	PTM	2	162
SLR	PTM	60	29
SLR	PM	65	15
HM	PM null	78	116
HM	PTM null	84	134
HM	TM	103	114
HM	TM null	111	106
HM	CM	123	106
HM	CM null	142	99
SLR	PM null	149	8
SLR	PTM null	158	15
SLR	CM	229	3
SLR	TM	232	5
SLR	CM null	419	2
SLR	TM null	420	3

The models that included a different relationship for each position (PM and PTM models) fit the data better than those that did not distinguish among positions (CM and TM). The PM model was better supported by the data than the PTM model for both the SLR and HM (Table 2.3). Though the PTM model included almost twice as many parameters and thus may have actually fit the data better than the PM model, the fact that the PTM model DIC was higher than the PM model DIC indicates that the increased fit of the PTM model was not great enough to justify the additional parameterization. The PM model was best supported by the data, meaning that of all

the mean models compared it best represented the variation in the data, and thus it will be the primary focus of our comparison of the HM and SLR below.

Posterior distributions of slope and intercept parameters

Overall the data showed a positive relationship between $\ln(K)$ and D (Figure 2.2). Under the PM HM, a positive slope between $\ln(K)$ and D was evident only for Branch 7, Root 42, and Root 22 (the 95% BCIs for the slope for these positions included only positive values, Figure 2.3, solid lines), however the 95% BCI for Stem 25 only included non-positive values near the lower limit of the BCI. The 95% BCIs indicated that the slope for Branch 7 was different from all other positions, and Root 22 was different from Stem 52, Stem 25 and Stem 5. Branch 7 had a considerably steeper slope than either of the roots. Branch 7 had the lowest K and D and Root 42 had the highest.

Results from the PM SLR showed similar trends. Only the slopes of Branch 7, Root 42, and Root 22 were positive (Figure 2.3, dashed lines). Based on 95% BCIs under the PM SLR, Branch 7 had a different slope from all other positions; however the slope of Root 22 was only different from the slope of Stem 52.

Posterior variance and covariance measures

The PM HM consistently estimated narrower (more precise) 95% BCIs than the PM SLR for both the slope and intercept (Figure 2.3). Compared to the PM HM, the PM SLR provided considerably less information about the variation in the data. In the PM SLR the posterior distribution for the variation in the data, σ^2 , had a mean value of 0.277, with a 95% BCI from 0.223 to 0.341.

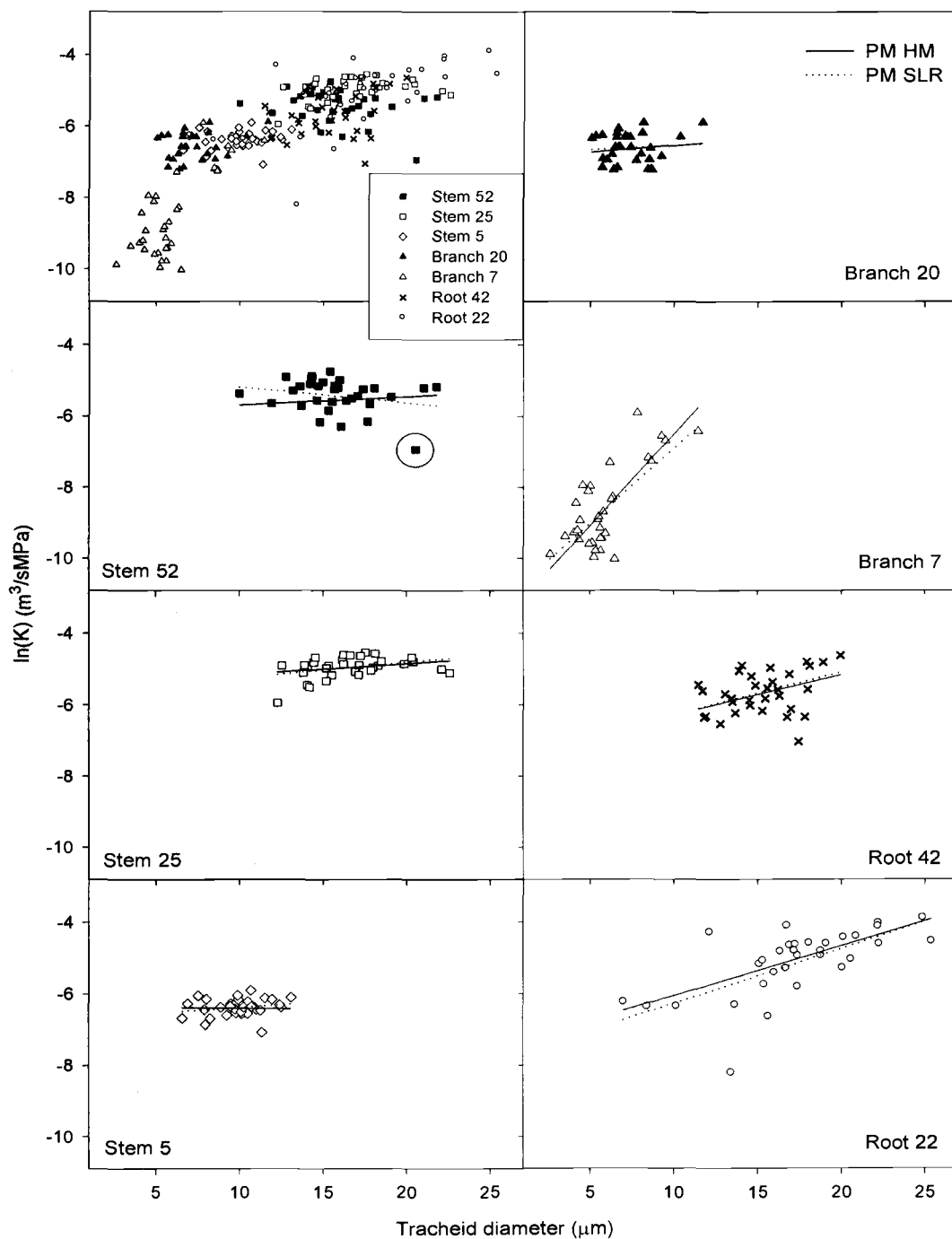


Figure 2.2 The natural log of K (specific conductivity) as a function of tracheid diameter for 32 Douglas-fir trees in the western Cascade foothills. Regression results are plotted from the PM HM (position mean hierarchical model; solid line) and PM SLR (position mean simple linear regression model; dotted line). The top left plot contains the entire dataset. The influential outlier for Stem 52 is circled.

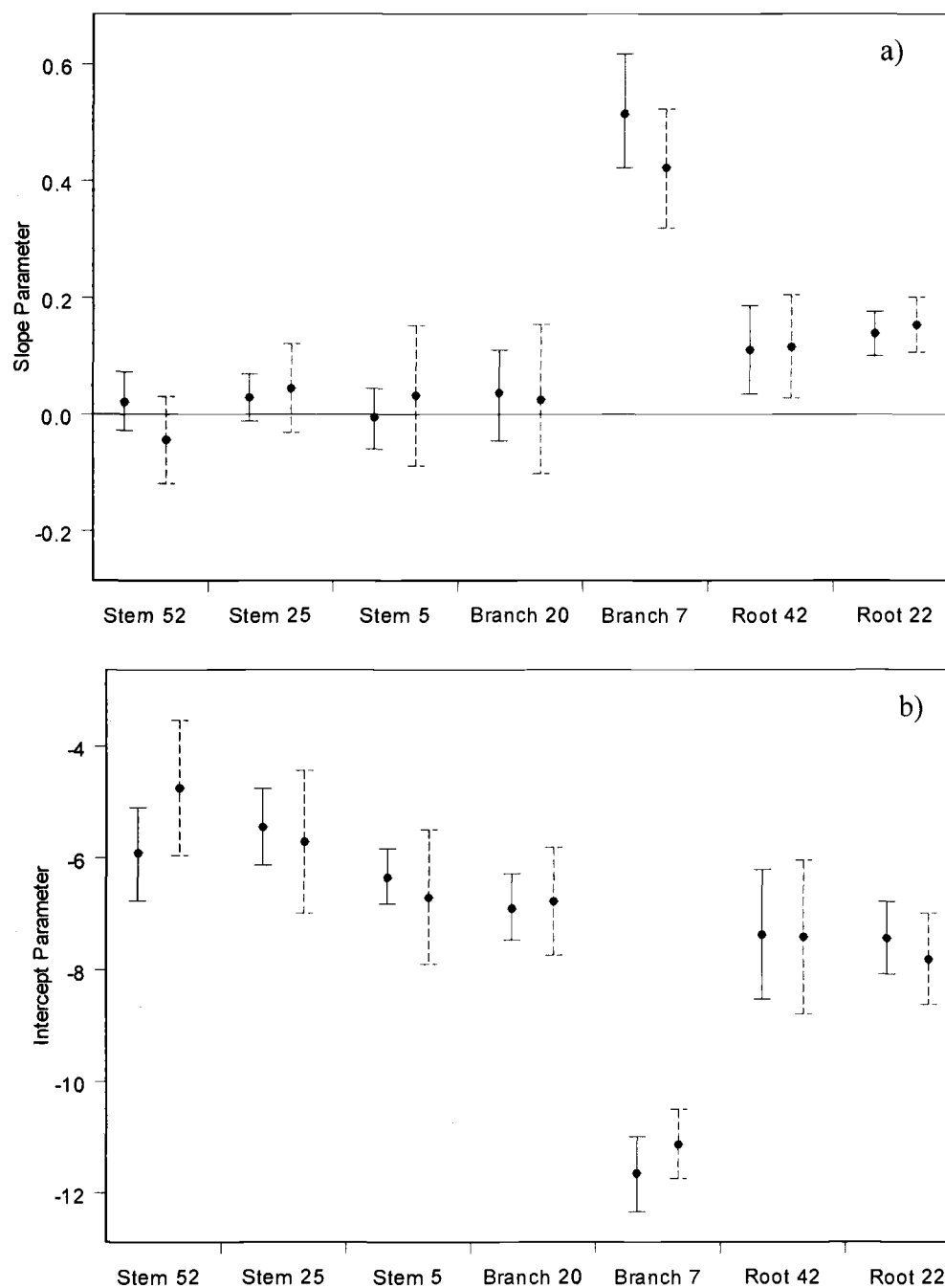


Figure 2.3 Comparison of a) 95% BCIs for slopes and b) 95% BCIs for intercepts estimated from the PM HM (position mean hierarchical model) and PM SLR (position mean simple linear regression model). Solid lines are from the PM HM, dotted lines are from the PM SLR, solid circles are the means of each distribution, and the top and bottom horizontal lines indicate the 97.5 and 2.5 percentiles, respectively.

In the PM HM model the covariance matrices, Σ_j , were rescaled to correlation matrices and provided evidence of correlations among positions (Table 2.4). We explored whether differences between the treatments warranted specifying a separate covariance matrix for each treatment (Σ_j), versus a single covariance matrix (Σ). The hierarchical model with a single correlation matrix and 81 parameters was 22 DIC units worse than the PM HM with Σ_j . The PM HM with Σ only showed correlations between Branch 7 and Stem 25, and between Branch 20 and Stem 5 (data not shown).

Table 2.4 Correlation matrix from the PM HM (position mean hierarchical model) from the regression of specific conductivity on tracheid diameter. Values are posterior means for correlations. The italicized top half of each matrix is from the unfertilized treatment, and the lower half in regular font is from the fertilized treatment. A value is underlined and in bold if its 95% BCI does not include zero.

	Stem 52	Stem 25	Stem 5	Branch 20	Branch 7	Root 42	Root 22
Stem 52		<i>0.07</i>	<i>-0.32</i>	<i>0.08</i>	<i>-0.28</i>	<i>0.19</i>	<i>0.45</i>
Stem 25	0.22		<i>0.06</i>	<i>-0.24</i>	<u>0.66</u>	<i>0.01</i>	<i>0.23</i>
Stem 5	<u>0.50</u>	-0.26		0.25	0.05	-0.18	-0.21
Branch 20	<u>0.49</u>	-0.14	<u>0.70</u>		-0.14	0.15	0.26
Branch 7	-0.43	<u>0.56</u>	-0.48	-0.39		-0.28	-0.06
Root 42	0.39	-0.11	0.16	0.33	-0.17		<u>0.58</u>
Root 22	<u>-0.55</u>	0.10	-0.14	-0.14	0.36	-0.09	

The PM HM with treatment-specific covariance, Σ_j revealed correlations between positions and differences in correlation patterns between treatments. Correlations were evident when the 95% BCI included only positive (indicating a positive correlation) or only negative (indicating a negative correlation) values. In the unfertilized treatment there were only two instances of correlations among positions compared to ten instances in the fertilized treatment (Table 2.4). Branch 7 and Stem 25 were correlated in both treatments, but Root 42 and Root 22 were only correlated in the unfertilized treatment. Four other correlations (Stem 5 and Stem 52, Branch 20 and Stem 52, Root 22 and Stem 52, and Branch 20 and Stem 5) were only present in the fertilized treatment. All of the correlations among positions were positive

correlations except for in Root 22 and Stem 52 in the fertilized treatment which was negative.

Discussion

Differences between the hierarchical model and simple linear regression model

The best supported SLR model was able to attribute variation in the mean to differences among positions (PM model) and it included a single variance parameter at one scale, among all samples from all positions regardless of tree or treatment. The PM HM with Σ , a single covariance matrix for both treatments, only described variation at two of the three levels in the hierarchy: within positions and among positions within trees but not between treatments. In contrast the model best supported by the data, the PM HM with Σ_j , partitioned means among positions within trees and variances and covariances among positions within trees, among trees, and between treatments. If responses from different positions within a tree or trees within treatments were assumed to be independent, then the only non-zero values in the variance-covariance matrix would be along the main diagonal, and the matrix would be the same as assumed in the SLR (i.e. Stem 52 would be highly correlated to itself but not to Branch 7). However, as seen in the correlation matrices in Table 2.4, there are several off-diagonal non-zero values indicating that the biological variability is more complex than can be captured in a single value.

The complex variance structures in the HM allow for what is often called ‘shrinkage towards the mean’ in hierarchical models (Bryk and Raudenbush 1992). Parameter estimates for lower level data in hierarchical models borrow support from the data as a whole. The estimates are thus ‘shrunk’ toward the overall mean solution. This shrinkage tends to be greatest when lower level data are relatively weak (e.g. small sample size) or when the data are extreme or unusual compared to the overall data (e.g. low sensitivity to outliers). Similar to Wyatt (2002) we found that the shrinkage towards the mean within the HM resulted in minimizing the influence of extreme

points and reducing variance in parameter estimates. The estimated slopes for Stem 52 demonstrate the effect of influential outliers in the PM SLR and PM HM (Figure 2.2, note the circled point for Stem 52). The PM SLR, relying solely on the data from Stem 52 samples, estimated a negative mean value for slope, influenced heavily by the one point with a relatively very low K value. The notion of a negative correlation between K and D is in contradiction to the great majority of previous research, theory, and intuitive thought (Tyree and Zimmerman 2002), as well as to the data from all the other six positions in this study. The PM HM, for the same data, estimated a positive mean value. We ran the analyses again with the outlier omitted to see if the estimated regressions changed. Under the PM SLR the slope flattened considerably while under the PM HM model there was essentially no change in the estimated line, indicating that the PM HM model is much less affected by influential outliers.

Hierarchical modeling improves inferences

We assessed the merits of using a hierarchical model to infer the relationship between K and D. By partitioning background variation among relevant biological and sampling scales the PM HM provided narrower 95% BCIs for the slope and thus revealed more differences between positions than the PM SLR. In the PM HM model the young root was different from all cambial ages sampled in the stem but the PM SLR detected a difference only between the young root and the oldest part of the stem (Figure 2.3). Correlations among positions and between treatments were also evident in the PM HM correlation matrix that could not be recognized with the single PM SLR variance (Table 2.4).

For logistic reasons, most research in tree physiology is conducted on small branches or roots less than 1 cm in diameter (Sperry and Saliendra 1994; Mencuccini and Comstock 1997; Brodribb and Hill 1999; Kavanagh et al. 1999; Nardini and Pitt 1999; Sparks and Black 1999; Matzner et al. 2001). In both fertilized and unfertilized treatments Branch 7 was only correlated with Stem 25 (Table 2.4), the section of the trunk directly adjacent to the branch, suggesting that the young branch tip may not be

representative of the whole tree. Similarly, Root 22 was only correlated to Root 42 in the unfertilized treatment and Stem 52 in the fertilized treatment (Table 2.4). In considering whole-tree strategies for water movement, the fact that different parts of a tree differ not only in anatomy and conductivity but also in how anatomy affects conductivity suggests that the form and structure of each position may be specialized to the part's function within the tree. A more detailed discussion of the biological implications of within-tree variation in specific conductivity and tracheid diameter may be found in the next chapter.

Hierarchal modeling in tree physiology

In tree physiology research, hierarchical statistical modeling provides an analytical method to represent hierarchical biological scales from positions up to the stand or region level. Our HM allowed variation to come from three levels: within an position, between positions within a tree, and between treatments. This accommodation came at the cost of requiring more parameters, but the HM was better supported by the data and improved inferences at all scales (Table 2.3). Even if a research question only pertains to one particular scale within the hierarchy, information from other scales can inform and refine inferences at that scale to provide a more precise and comprehensive answer.

We return to the idea of matching the biological, sampling, and analytical scales. Natural variation occurs within and between biological scales, so, as we have shown, the sampling and analytical scales must mirror the biological scales to best account for the variation present in the study. Commonly employed statistical analyses accommodate variation due to covariates and random variation from only one or two scales (Table 2.1), so the analyst runs the risk of misestimating variances and even the parameters themselves if multiple scales are present in the data (as in the slope estimates of Stem 52 in Figure 2.2). Hierarchical models can incorporate these complex variance structures into the data analysis. In the field of tree physiology, which is dominated by biological hierarchies, hierarchical modeling has the potential

to improve inferences by better estimating variation and increasing precision in parameter estimation

Bayesian analysis of Douglas-fir hydraulic architecture at multiple scales

Sonya M. Dunham, Barbara L. Gartner, Lisa M. Ganio

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Abstract

We used a Bayesian hierarchical model to analyze the variation in xylem anatomy, hydraulic properties, and the relationship between anatomy and properties within Douglas-fir trees. The hierarchical scales in our study included fertilization treatments (fertilized and unfertilized), trees within the treatments, and positions within the trees. We measured tracheid diameter, tracheid length, percent latewood, number of pits per cell, density, and specific conductivity (K_s) on seven positions in each of 16 fertilized and unfertilized trees: the stem at cambial age 54 (breast height), 25, and 5; a branch at cambial age 20 and 7; and a root at cambial age 42 and 22. Vulnerability to embolism was also measured on the oldest stem, branch, and root positions. For any measurement there was little variation between treatments, however there was great variation among positions. Tracheid diameter, tracheid length, number of pits per cell, K_s , and vulnerability to embolism decreased vertically from the roots to the branches. Correlations were evident between some positions for tracheid diameter, percent earlywood, pits per cell, and vulnerability to embolism, mostly in the fertilized treatment. We found evidence for large scale relationships (among all observations from all trees) between density and tracheid diameter, K_s and diameter, vulnerability and diameter, K_s and pits per cell, and vulnerability and pits per cell. At a smaller scale, however, usually only the branches and roots maintained the relationship.

Introduction

We now have considerable documentation of variation in xylem anatomy and hydraulic properties among trees and across species. It is generally expected that most if not all variation seen in xylem hydraulic and structural properties can be explained by variation in xylem anatomy. The great majority of the work in this area has been conducted on small diameter branches, small diameter roots, or seedlings, so inferences about within tree variation and whole tree hydraulic strategies are limited. Detailed awareness of within-tree trends is important for providing a context for studies in which only a single position in the tree or cambial age is considered,

understanding trends along the xylem pathway, and understanding how the effect of anatomy on xylem properties varies among scales. Recognition of both within- and among-tree variation is also fundamental to partitioning the variation when analyzing hierarchical data in tree physiology.

Variation in wood structure and properties may be partitioned among scales that comprise the biological hierarchy in which trees exist. Variation can come from within organs or cambial ages, among organs or within trees, among trees, among species, etc. At a small scale, within-tree variation may be explained by differences in xylem structure among organs or positions within the tree (Alder et al. 1996; Spicer and Gartner 1998; Hacke et al. 2000b; Domec and Gartner 2002a) or age-related tissue differences (Spicer and Gartner 2001; Domec and Gartner 2002a; McElrone et al. 2004; Choat et al. 2005b). At higher levels of the hierarchy variation may be due to climate and geographical location (Kavanagh et al. 1999; Bouffier et al. 2003; Stout and Sala 2003), nutrient or water availability (Mitchell and Hinckley 1993; Ewers et al. 2001; Hubbard et al. 2004), differences in life strategies (Lechowicz 1984; Sperry et al. 1994; Vogt 2001; Meinzer 2003) or species (Brodribb and Hill 1999; Stout and Sala 2003). Accounting for the multiple sources of variation when analyzing hierarchical data improves estimation of effects at all scales (Bryk and Raudenbush 1992). Also, relationships among xylem characteristics may not be uniform across the whole tree, so partitioning variation among different scales may lead to different inferences than when only one scale is considered (Kavanagh et al. 1999; Hacke et al. 2000b). We studied within and among tree variation at multiple scales in one species, Douglas-fir (*Pseudotsuga menziesii*). The scales in the biological hierarchy relevant to our study include the lower level of positions (stem, branch, and root samples at multiple cambial ages), a middle level of trees within treatments, and the higher level of fertilized versus unfertilized treatments (Figure 2.1).

Within-tree variation in anatomy and hydraulic properties may occur because different parts of the tree fulfill different mechanical and hydraulic roles. The lower stem,

branch sections close to the stem and structural roots bear most of the mechanical stress from self-weight of the tree. The tree top experiences a different wind environment than the base. In contrast to the main stem, the branches must provide structural support at an angle from vertical, which produces a lever arm and consequentially experience a torque due to gravity. Branches usually have narrower growth rings than the trunk and a high percentage of compression wood, and McAlister et al. (2000) found branches to have less bending strength but higher compression strength than stems. Roots are much more variable in shape than the stem (Nicolle and Ray 1996) and anchor the tree in the soil. Roots experience very different types of mechanical stress than the stem due to their orientation (angled away from the stem) and confinement in the soil.

Hydraulically all parts of a tree are connected along a single water transport pathway, however the hydraulic characteristics change from the roots to the branches (McCulloh and Sperry 2005). It may be that changes in xylem anatomy and hydraulic properties along the xylem flow pathway maximize overall hydraulic efficiency and minimize energy expenditure (West et al. 1999; McCulloh and Sperry 2005). The segmentation hypothesis predicts that distal parts of the xylem flow pathway are designed to embolize at less negative water potentials than more vital or costly parts of the tree, such as the main stem (Tyree and Zimmerman 2002). This is achieved by localizing areas of sudden increase in hydraulic resistance (e.g. by a sharp decrease in tracheid diameter) at petioles, branch junctions, and other points separating a more vital part from a more expendable one. Comstock and Sperry (2000) predicted that tracheid length should decrease ‘substantially’ and ‘continuously’ from the proximal to distal ends of the xylem flow path, which would also result in increased hydraulic resistance in distal branches. This trend is advantageous to whole-tree hydraulic safety because shorter cells, which provide better embolism containment, would be located in the upper parts of the tree that experience the greatest tension or lowest water potential. Water storage and temperature can also influence the hydraulic functioning of different positions within a tree. Roots affect water storage and

hydraulic redistribution in the soil (Burgess et al. 2001; Meinzer et al. 2004) and stems may store relatively large volumes of water (Phillips et al. 2003) and but branches, though they may experience the largest and earliest change in water potential during the day, are considerably smaller in size and thus may not contribute significantly to the amount of stored water used daily by the tree. Temperature has a great effect on water viscosity, with viscosity decreasing by 14% from 16 to 22 °C (CRC 1989/90). Viscosity has been shown to have a great effect on root conductance (Cochard et al. 2000). Branches and the upper stem are more exposed to variable temperatures than the roots and so branch conductance may be influenced to a greater degree by viscosity than roots.

Many studies have compared structural and hydraulic characteristics of young, small diameter roots and branches, and a few have considered the effect of cambial age and height in the stem. Comparisons of young roots and branches (usually < 1 cm in diameter) have found roots to be more vulnerable to embolism, to have a wider tracheid diameter, to have larger pit membranes, and to have a higher specific conductivity than branches (Alder et al. 1996; Mencuccini and Comstock 1997; Sperry and Ikeda 1997; Kavanagh et al. 1999; Matzner et al. 2001; Martínez-Vilalta et al. 2002; Stout and Sala 2003). Within an organ, smaller diameter (< 5 mm) branches and roots embolize at less negative pressures than larger (> 8 mm) branches and roots (Sperry and Ikeda 1997). Domec and Gartner (2002a) found that older branches (> 5 cm in diameter) were less vulnerable than stem wood of several cambial ages, but there is a noticeable lack of similar data for large, older, structural roots near the base of the stem. In one survey of eight conifer species, branches typically had much shorter, narrow tracheids and higher wood density than stems, with roots having lower density and longer, wider tracheids than stems (Fegel 1941). The effect of cambial age on xylem characteristics has been assessed almost entirely just within stems (Spicer and Gartner 2001; Domec and Gartner 2002a; Gartner et al. 2002). Juvenile wood (wood made by young cambium in which xylem properties change dramatically year to year) tends to have narrower tracheids, lower conductivity, and less vulnerable

xylem. Peterson et al. (Submitted) identified similar patterns of increasing tracheid diameter and length with cambial age in roots and branches, but noted the lack of vulnerability data for large, structural roots. Choat et al. (2005b) found that vulnerability to embolism and vessel diameter decreased along the xylem pathway in sugar maple from looking at small roots, the trunk, several ages of branches, petioles and leaves, but a similarly comprehensive investigation, including multiple ages of roots, bole and branches, has not been done along the xylem pathway in conifers. Not only are studies of whole tree variation in anatomy and hydraulic properties generally lacking, but there is also very little information on how xylem properties of organs and cambial ages are correlated within trees – a measure that would further inform us of how the parts of a tree relate to one another.

Nitrogen fertilization, a large scale source of among tree variation in our study, has become a common silvicultural practice in the Pacific Northwest to increase growth rates. The addition of nitrogen can increase leaf area and water demand (Ewers et al. 1999; Hubbard et al. 2004) but has also been shown to reduce transpiration and root mass relative to leaf area (Axelsson and Axelsson 1986; Ewers et al. 2001). A reduction in fine root mass relative to leaf area may result in a higher sensitivity to drought unless the hydraulic architecture is also altered to be more resistant to embolism (Ewers et al. 2000). Several changes in xylem anatomy have been observed in conifers during the first five to ten years following fertilization. Earlywood production increases but the amount of latewood remains constant, shorter tracheids are produced, and there is often a mild to severe decrease in wood density (Zobel and van Buijtenen 1989). Ewers et al. (2000) found that though root tracheid diameter and distribution did not differ between fertilized and unfertilized trees, small roots (1.6 to 4.1 mm in diameter) from fertilized trees were 28% less conductive and 50% more vulnerable to embolism than roots from the other treatments. Small diameter branch vulnerability to embolism did not differ between treatments; however no measurements of tracheid diameter, conductivity, and vulnerability were made on the stem.

In addition to including positions that are not typically included in studies of xylem hydraulics this study is unique because we used a Bayesian statistical hierarchical model to reflect the biological hierarchy in the study (positions within trees, trees within treatments, Figure 2.1) and to partition variation in the data among each level (within positions, within trees or among positions, among trees, within treatments, and between treatments). As shown in the previous chapter, partitioning variation among scales in a hierarchical regression model increases precision of regression coefficient estimates and provides a more informative description of variation than a commonly used simple linear regression model. Our first objective in this study was to describe the variation in xylem anatomy and xylem properties in Douglas-fir in two environments (fertilized and unfertilized) and in different positions within those trees (stems, branches, and roots of different cambial ages). We expected that roots, stems, and branches at various cambial ages would be anatomically and functionally unique and that most of the variation in the data would come from among positions instead of from between treatments.

Our second objective was to explore how relationships between xylem anatomy and hydraulic properties change at different scales. A relationship between variables among all positions and both treatments would be evidence for a large-scale trend influenced by factors that act equally within and among trees. A unique relationship for each treatment would suggest that the trend is influenced by environmental factors. If there are no general relationships among positions but only position-specific trends, then the relationship is governed more by small-scale factors at the within-position scale. We expected that there would be both large-scale and small-scale relationships between xylem anatomy and hydraulic properties, but that the relationships would be different at each scale.

Methods and materials

Site and tree descriptions

Data were collected in 2004 on 32 Douglas-fir (*Pseudotsuga menziesii*) trees in the western foothills of the Cascade Mountains (44° 25.6' N, 122° 35.7' W). The trees were part of a 54-year-old naturally regenerated stand. Half of the trees were fertilized with nitrogen (urea) in 2000 by aerial application with a target rate of about 224.2 kg nitrogen per hectare (or 200 lbs per acre). Four 15 by 15 m² plots in each fertilization treatment (fertilized, unfertilized) were randomly selected, and then four trees were randomly selected within each plot for a total of 16 trees from fertilized plots and 16 trees from unfertilized plots. Among the plots the slope ranged from flat to 22° and the aspect varied from southwest to north-northeast (all but one aspect was northerly). The understory contained vine maple and sword fern, and ranged from dense to very open between the trees. For the sampled trees, average DBH was 53.6 cm (ranging from 42.5 to 68.5 cm), and average tree age at breast height was 52 (Table 3.1). In measurements of four points in each of the treatments, we found that both treatments had similar basal area density (32.1 m²hectare⁻¹ for unfertilized, 33.4 m²hectare⁻¹ for fertilized) and overstory closure (70.7% for unfertilized, 74.2% for fertilized).

Table 3.1 Characteristics of samples from 54-year-old Douglas-fir trees in the western Cascade foothills. Values are means (95% BCI), n = 32 trees, height values in italics are estimates based on sampling procedures, and n.d. indicates no data).

Position	Cambial age (years)	Height from ground (m)	Tracheid diameter (μm)	Tracheid length (mm)	Percent latewood	Number of pits/cell	K_s ($\text{m}^2\text{s}^{-1}\text{MPa}^{-1}$)	Density (g cm^{-3})	Ψ_{50} (MPa)
Stem 52	51.6 (50.6, 52.7)	<i>1.4</i>	16.2 (15.1, 17.2)	3.2 (3.0, 3.3)	48.2 (45.7, 50.7)	67.4 (61.8, 73.1)	0.0045 (0.0039, 0.0051)	0.48 (0.44, 0.51)	-3.4 (-3.6, -3.2)
Stem 25	24.4 (23.3, 25.5)	26.2 (24.9, 27.6)	16.4 (15.4, 17.4)	3.5 (3.3, 3.6)	57.6 (55.0, 60.2)	90.2 (83.1, 96.9)	0.0074 (0.0066, 0.0081)	0.41 (0.37, 0.44)	n.d.
Stem 5	5.3 (5.1, 5.4)	41.6 (40.4, 42.9)	9.9 (9.2, 10.5)	1.4 (1.3, 1.5)	33.7 (30.1, 37.1)	41.9 (38.9, 45.2)	0.0017 (0.0015, 0.0018)	0.40 (0.37, 0.44)	n.d.
Branch 20	20.2 (19.2, 21.3)	26.2 (24.9, 27.6)	7.2 (6.6, 7.8)	1.6 (1.5, 1.7)	52.4 (45.0, 55.0)	50.9 (46.7, 55.3)	0.0014 (0.0012, 0.0016)	0.47 (0.43, 0.50)	-5.6 (-5.8, -5.4)
Branch 7	7.0 (6.4, 7.6)	26.2 (24.9, 27.6)	6.3 (5.4, 7.0)	1.2 (1.1, 1.3)	63.0 (60.2, 65.9)	38.2 (35.1, 41.2)	0.0003 (0.0001, 0.0004)	0.49 (0.46, 0.53)	n.d.
Root 42	42.2 (40.2, 43.9)	<i>-0.5</i>	15.8 (14.8, 16.8)	3.0 (2.8, 3.3)	58.1 (55.6, 60.2)	73.4 (65.1, 81.3)	0.0036 (0.0028, 0.0044)	0.44 (0.39, 0.48)	-3.3 (-3.6, -3.0)
Root 22	22.3 (19.2, 25.2)	<i>-1</i>	16.8 (14.7, 18.6)	3.8 (3.5, 4.1)	55.1 (51.6, 58.7)	123.5 (105.8, 140.6)	0.0069 (0.0052, 0.0086)	0.39 (0.34, 0.44)	n.d.

Sampling methods

Seven sampling points were selected on each tree (lowest level in Figure 2.1). Bole sections were taken at about cambial age 52 (breast height, called Stem 52), cambial age 25 (Stem 25), and cambial age 5 (the leader, Stem 5). Branches were accessed and selected by tree climbing. The ideal branch was on the north side of the tree (to minimize variation due to ordinal direction), was live and horizontal, was not immediately under another branch, and had a large part of its tip that extended beyond the majority of the canopy profile. Branches were located in the mid- to lower crown directly in the branch whorl immediately above the Stem 25 section. Cambial age 20 branch sections (Branch 20) were taken approximately 0.5 m out from the branch junction and cambial age 7 branch sections (Branch 7) were taken from the near the tips of the same branch. Roots were selected as close to the north side of the tree as possible. Two sections were taken from each root; one from the large structural portion approximately 0.5 m from where the root went below the soil (Root 42) and one from a smaller, younger, non-structural, approximately 3 cm diameter portion further along the root (Root 22). The ages of the old branches and roots were initially estimated based on appearance and location on the tree due to difficulty in coring the tree while tree climbing and through thick and dirty bark, respectively. Therefore, ages were estimated by growth ring count after the samples were cut (Table 3.1).

The trees were felled during summer 2004 and a disk at least 20 cm tall was excised from each of the seven sampling points and taken back to the lab. Disks were either immediately cut into samples for use in measurements of conductivity, anatomy, and density or kept in damp conditions at 3 °C for up to four days before being separated into three samples. For samples with large enough diameter (Stem 52, Stem 25, Root 42, and usually Stem 5), the conductivity, anatomy, and density samples were from radially-adjacent portions of the disk. For Branch 22, Branch 7, and Root 22, samples were taken sequentially along the axis.

Measurements and data collection

Anatomy

Small 1 cm by 1 cm by 2 cm-long anatomy samples were cut from the outermost growth rings of each disk (1 anatomy sample per disk, 7 disks per tree, 16 trees per fertilization treatment, 2 fertilization treatments). They were stored in a solution of 50% ethanol and 50% water at room temperature until slides were made. Anatomy measurements were made using a compound microscope (tracheid diameter, percent earlywood, and pits/mm) or dissecting microscope (tracheid length), a camera, and MetaVue imaging software (version 6.2r6, copyright 1992-2004)

Tracheid diameter. Transverse microtome sections were cut from each anatomy sample and stained with safranin-O. A radial transect was randomly chosen on each slide and the earlywood lumen diameters, excluding cell walls, were measured in the radial direction. Only earlywood tracheids were measured because Domec and Gartner (2002b) demonstrated that up to 90% of flow through stems occurred in through the earlywood. A total of 100 diameters were measured for each sample, with a second randomly selected transect used if necessary.

Percent latewood. Two radial transects across the cross-sections of each sample were randomly selected for measurement of the width of the earlywood and latewood rings. Only complete growth rings were measured. The demarcation between earlywood and latewood was taken as the point along the transect where lumen diameter was twice the width of the cell walls. Percent latewood (%LW) was calculated as

$$\%LW = \left(\frac{lw}{ew + lw} \right) \times 100,$$

where ew is the total combined width of the earlywood rings in each sample and lw is the total combined width of the latewood rings.

Tracheid length and pits per cell. After the transverse sections had been made, matchstick-sized pieces were cut from the remainder of the anatomy samples for maceration. They were placed in individual test tubes. We made a solution of 15 g sodium chloride dissolved in 250 ml of distilled water and then submerged each sample with approximately 5 ml of the solution. Five drops of acetic acid were added to each test tube and then the pieces were boiled for about three hours. The macerated cells were rinsed, stained with safranin-O, and mounted on slides. One hundred tracheid lengths were measured for each sample. The number of pits per cell were measured for ten tracheids per sample.

Density

Green volume and dry mass were measured for small blocks (about 2 x 2 x 2 cm) from the outermost wood of each disk. Basic density (D) was calculated as

$$D = \frac{M_d}{V_g},$$

where M_d is the dry mass of the block (g), and V_g is the green volume of the block (cm^3).

Specific conductivity

Specific conductivity (K_s , $\text{m}^2\text{s}^{-1}\text{MPa}^{-1}$) is an expression of how permeable a segment of xylem is for a given length and surface area, and is calculated according to Darcy's law as

$$K_s = \frac{Ql}{A\Delta P},$$

where Q is the volume flow rate (m^3s^{-1}), l is the length of the segment (m), A is the cross-sectional area of the segment (m^2), and ΔP is the pressure gradient across the segment (MPa). Longitudinal samples about 1 cm by 1 cm by 10 cm were chiseled from the outer-most growth rings from each section, except for the Branch 7 sections that were simply cut underwater to a length of 10 cm. Samples were then submerged in water and put under a vacuum to refill any embolized cells. All samples were kept

submerged at 3 °C for up to a week before being measured. Immediately before conductivity was measured the ends of each sample were re-cut under water and the dimensions of the sample were measured with a ruler. The samples were fit with tubing and sealed in a pressure-sleeve apparatus as per Spicer and Gartner (1998) to prevent solution from being pushed out the sides of the sample. A dilute solution of hydrochloric acid (pH 2, filtered to 22 µm) was fed through the tubing with a pressure head of approximately 0.006 MPa. The solution entered a 1 mL pipette after passing through the sample. The time it took the fluid to pass by the tick marks on the pipette was timed with a stopwatch. Volume flow rate was calculated as the average of at least six timed intervals. The temperature of the solution remained constant at about 20 °C as measured by a thermometer and thus had a viscosity of $\eta_{20} = 0.001002 \text{ Pa s}$ (CRC 1989/90).

We also calculated a corrected K_s ($K_{s \text{ cor}}$) using the in situ sap temperature from each tree. From mid-July to early October 2004 we measured sap temperature (T) in Stem 54, Branch 20, and Root 42 of each study tree before they were felled for sectioning. Thermocouples were installed into the xylem on eight trees at a time, four fertilized and four unfertilized, 1.5 cm beneath the cambium. Sap temperature was recorded for at least three sunny days for each tree. Signals from the probes were monitored every minute and 10-minute means were recorded by a data logger (CR 10x; Campbell Scientific) equipped with a 32-channel multiplexer (AM416; Campbell Scientific). Maximum sap viscosity (η_s) was calculated using the maximum average temperature (T_{max}) for three days using the empirical relationships described in the CRC Handbook of Chemistry and Physics (CRC 1989/90):

$$\eta_s = \begin{cases} T_{\text{max}} < 20^\circ & 0.001 \times 10 \exp \left(\frac{1301}{998.333 + 8.186(T - 20) + 0.006(T - 20)^2} - 1.302 \right) \\ T_{\text{max}} \geq 20^\circ & 0.001 \times 10 \exp \left(\frac{1.327(20 - T) - 0.001(T - 20)^2}{T + 105} - 1.302 \right) \end{cases}$$

We calculated $K_{s\text{ cor}}$ using the ratio of η_{20} to η_s :

$$K_{s\text{ cor}} = K_s \times \frac{\eta_{20}}{\eta_s}.$$

Vulnerability to embolism

Vulnerability curves (VCs) were constructed using the conductivity samples.

Vulnerability to embolism was measured by alternately measuring K_s and inducing increasing degrees of embolism using the air-injection technique (Sperry and Saliendra 1994; Domec and Gartner 2001). Initial K_s was measured for each sample, and then embolisms were induced by putting positive pressure on the lateral faces of the sample in a small pressure chamber, with the sample ends exposed to normal atmospheric conditions. The samples were submerged in water for 15-30 minutes to allow air bubbles to diffuse throughout the wood, and then K_s was measured again. A larger pressure was then imposed; the samples were submerged in water, and K_s was measured again. These steps were repeated until the sample reached close to total loss in conductivity. Percent loss in conductivity (PLC) at a given pressure was calculated as

$$\text{PLC} = \left(\frac{K_{s(i)} - K_{s(\Psi)}}{K_{s(i)}} \right) \times 100,$$

where $K_{s(i)}$ is the initial specific conductivity and $K_{s(\Psi)}$ is the specific conductivity of the sample after being exposed to a positive pressure, Ψ . Vulnerability curves were constructed by plotting PLC versus the applied pressure. The negative of the pressure at which a sample has reached 50% loss in conductivity (Ψ_{50}) is used to compare vulnerability to embolism between samples. A unique third-degree polynomial was fit to each VC and solved to find Ψ_{50} for each sample.

Statistical methods

Data were analyzed using Bayesian hierarchical models as described in detail in the previous chapter. The statistical hierarchical model included complex variance-covariance matrices that reflected the natural hierarchical partitioning of variation

positions within a tree, and between treatments. Correlations among positions suggest that the positions are not independent of each other, in other words, that when the variable of interest has a high value in one position, it also has a high value in the other position. Biologically these correlations are important because they imply that positions react similarly to stimuli (e.g., the wood plays very similar roles in different positions) or else the function is canalized (the wood performs the same role, even though it may not be optimal). The former idea bears on adaptation, and the later suggests genetic constraints. The lack of correlation would suggest that the positions are adapted to different functions and would suggest that the genes allow different expression in different parts of the plant.

We regressed xylem properties on xylem anatomy at different scales to explore how the relationships change depending on the scale considered. For each regression analysis we compared four models for the mean of the response variable; a common mean model (CM, one line fitted to the entire data, reflects large scale trends), a treatment mean model (TM, one line fitted to each treatment, medium scale trends), an position mean model (PM, one line fitted to each position, small scale), and an position + treatment mean model (PTM, one line for each position-treatment combination, smallest scale).

The models were fit to the data using WinBUGS version 1.4 (Spiegelhalter et al. 2003) and compared using the Deviance Information Criterion, or DIC (Spiegelhalter et al. 2002; Gelman et al. 2003). We calculated ΔDIC_i , the difference between the DIC for model i and the minimum criterion value within the group of models being compared ($\Delta DIC_i = DIC_i - DIC_{\min}$). The model with the lowest criterion value, or $\Delta DIC_i = 0$, is best supported by the data. Posterior probability distributions were summarized by calculating the mean value and 95% credibility interval (95% BCIs).

Frequentist and Bayesian methods differ in the way results are reported and interpreted. The p-value from a frequentist hypothesis test provides the probability

that the test statistic is as extreme, or more extreme, than the one that was obtained from the sample that was collected, given that the null hypothesis is true. If a p-value supports the rejection of the null hypothesis then the parameter of interest is statistically significantly different from the value specified in the null hypothesis. Frequentist 95% confidence intervals are sets of values for the parameter of interest that might have been obtained from 95% of other hypothetical samples drawn from the sampling distribution, but that were not actually sampled. The Bayesian approach incorporates prior information along with the data that were collected to produce a posterior probability distribution for the parameter of interest. That is, Bayesian methods condition only on the sample that was collected, not on hypothetical samples. The Bayesian 95% credibility interval is more directly interpretable as the probability that the parameter has a value between the interval bounds is 95% (Ellison 1996). If a 95% BCI spans only positive or negative values, then the parameter is reported to take on positive or negative values, respectively. A 95% BCI that spans both positive and negative values and includes zero, is reported to be not different from zero, especially if zero is near the center of the interval. We will provide 95% BCIs for regression slopes and will report if they are positive, negative, or not different from zero. For a more detailed explanation of Bayesian inference see Ellison (1996) and Reckhow (1990).

Results

Within tree variation in anatomy and xylem properties

There were no differences between fertilized and unfertilized samples for any of the response variables within any position, e.g. Stem 5 K_s was the same in both treatments (the 95% BCIs from each treatment overlapped for each position, data not shown). There were some instances of two positions having similar characteristics in the unfertilized treatment but dissimilar characteristics in the fertilized treatment; for example, unfertilized Stem 5 and Branch 20 had similar K_s in the unfertilized treatment however fertilized Stem 5 and Branch 2 had slightly different values from each other (based on 95% BCIs, data not shown).

In contrast, the mean anatomical characteristics did vary among positions (Table 3.1). In general Root 22, Root 42, Stem 52, and Stem 25 behaved similarly and Branch 20, Branch 7, and Bole 5 tended to be alike. Roots and the two older trunk locations tended to have much wider, longer tracheids, more pits per cell, more vulnerable xylem and higher K_s than did the branches and the top of the tree. For samples from the more distal positions (Root 22, Branch 7, Stem 5), tracheid diameter and length decreased from root to stem tip to branch (Table 3.1). In the roots tracheid diameter, tracheid length, number of pits per cell, and K_s decreased with cambial age, but in the bole and branches these characteristics increased with cambial age. Density was higher in both branch samples and in Stem 52 than in all other locations. Percent latewood was lowest at the top of the tree (Stem 5) and highest in the branch tips (Branch 7).

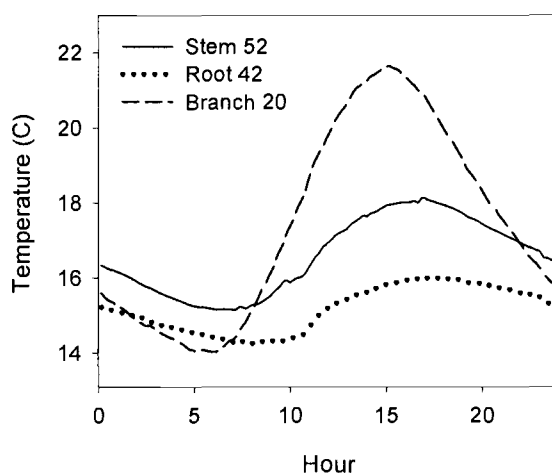


Figure 3.1 Diurnal temperature variation for Stem 52, Root 42, and Branch 20 measured on 32 Douglas-fir trees in the western Cascade foothills during summer 2004, $n = 32$ trees over averaged over three days.

The diurnal pattern in sap temperature varied greatly among Stem 53, Root 42, and Branch 20 (Figure 3.1). Branch 20 experienced the greatest daily variation in T and Stem 54 and Root 42 had similar diurnal variation. Branch 20 reached a substantially

higher maximum T each day compared to Stem 54 and Root 42 (Table 3.2), which were essentially equal (the 95% BCIs for Stem 54 and Root 42 overlapped substantially). All three positions had similar minimum daily T (Table 3.2). The 95% BCIs for average temperature (over three days) overlapped for all three positions, though the BCIs for Branch 20 and Root 42 overlapped by only 0.2 °C. Temperature had a great affect on sap viscosity, but the differences in viscosity only caused noticeable changes in K_s for Stem 52 and Root 42 (compare K_s and $K_{s\text{ cor}}$, Table 3.2). The difference between K_s and $K_{s\text{ cor}}$ was not large enough to significantly change any of the relationships we report below involving K_s .

Table 3.2 Xylem temperatures for Stem 52, Branch 20, and Root 42 (average, maximum, and minimum). Values are posterior means (95% credibility interval) for 32 trees from three days of measurements.

Position	Average T (°C)	Max daily T (°C)	Min daily T (°C)	Max viscosity (10 ⁻² Pa s)	K_s (m ² s ⁻¹ MPa ⁻¹)	$K_{s\text{ cor}}$ (m ² s ⁻¹ MPa ⁻¹)
Stem 52	15.4 (14.2, 16.6)	16.3 (15.2, 17.7)	14.3 (13.3, 15.4)	1.09 (1.05, 1.14)	0.0043 (0.0037, 0.0049)	0.0045 (0.0039, 0.0051)
Branch 20	16.9 (15.4, 18.3)	21.3 (19.5, 23.2)	13.6 (12.7, 14.6)	0.97 (0.92, 1.01)	0.0014 (0.0012, 0.0016)	0.0014 (0.0012, 0.0016)
Root 42	14.8 (13.9, 15.6)	15.8 (14.9, 16.8)	13.8 (13.1, 14.6)	1.11 (1.07, 1.14)	0.0034 (0.0027, 0.0041)	0.0036 (0.0028, 0.0044)

Vertical trends within trees were evident in tracheid diameter, tracheid length (not shown), number of pits per cell, K_s , and Ψ_{50} (Figure 3.3). Tracheid diameter, number of pits per cell, and K_s decreased along the xylem pathway from roots to tree top and along the pathway from trunk to branch tip. Vulnerability to embolism decreased vertically from Root 42 to Branch 20.

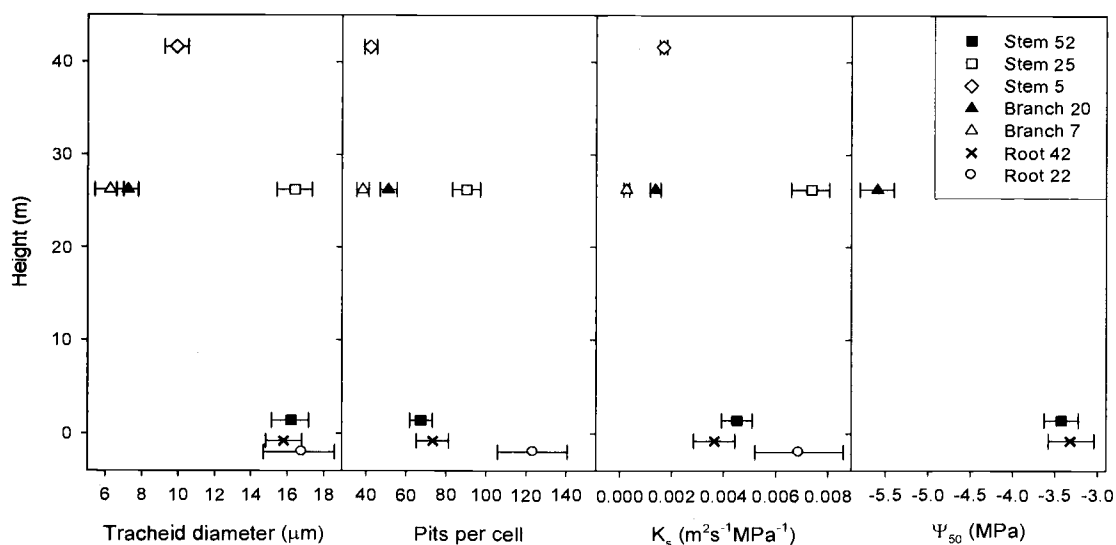


Figure 3.2 Vertical profiles of a) tracheid diameter, b) pits per cell, c) specific conductivity, and d) Ψ_{50} in 32 54-year-old Douglas-fir trees. Error bars are 95% BCIs.

Not only did the mean values vary between positions, but the variance did as well. Variability (as measured by the width of the 95% BCIs, Table 3.1, and standard deviation) in tracheid diameter followed a vertical trend: the Root 22 had the widest and most variable tracheid diameter, $16.8 \mu\text{m} \pm 1.0$ (1 SD), while Branch 20 and Branch 7 had smaller and less variable tracheid diameters ($7.2 \mu\text{m} \pm 0.3$ and $6.3 \mu\text{m} \pm 0.4$ respectively). Tracheid length followed a similar vertical trend in variability. Density and K_s were considerably more variable in roots than in the other positions.

There was a positive relationship between tracheid diameter and tracheid length within the whole tree (tracheid length = $1.27 + 0.058 \times \text{tracheid diameter}$, where length is in mm and diameter is in μm). This relationship was not as strong or absent when each position was considered individually. Branch 20, Branch 7, and Root 22 all showed a positive correlation between tracheid length and diameter, however the other positions, when considered individually, did not (data not shown).

Correlations among positions

The variance-covariance matrices were rescaled to correlation matrices for ease of interpretation. Correlations were evident among the positions for tracheid diameter, percent earlywood, pits per cell, and vulnerability to embolism (Table 3.3).

Correlations for which the 95% credibility interval included only positive or negative values were visually assessed to confirm that the correlation was not due to one or two influential outliers. There were more correlations in the fertilized trees than within the unfertilized trees, and the magnitude of correlations tended to be larger in fertilized trees. The tracheid diameters of three pairs of positions were correlated in unfertilized trees, whereas correlations were evident in eight pairs of positions in trees from the fertilized treatment. Percent latewood was only correlated between two positions in the unfertilized treatment, though there were five pairs of positions correlated in the fertilized treatment. Number of pits per cell was correlated in two pairs of fertilized positions and the specific conductivity of four pairs of fertilized positions. For Ψ_{50} there were no correlations in the unfertilized trees; however Stem 52 and Branch 20 were positively correlated in the fertilized treatment. Density and tracheid length were not correlated within a tree.

Correlations between anatomy and xylem properties

The Δ DIC values for each set of regression analyses are presented in Table 3.4. For each regression considered the Δ DIC indicated that either the PM or the PTM model was best supported by the data. The positions were different enough to warrant the additional parameters required to fit a different line to each position instead of a single line for the entire data (CM model) or a line for each treatment (TM model). DIC values within 5 units are considered to be equivalent. For cases in which the PM and PTM model DICs are equivalent (K_s and tracheid diameter, Ψ_{50} and tracheid diameter), the PM model is preferred because the additional parameters required to fit the PTM model do not significantly improve support for the model.

Table 3.3 Correlation matrices for tracheid diameter, percent earlywood, K_s , and Ψ_{50} . The italicized top half of each matrix is from the unfertilized treatment and the lower half in regular font is from the fertilized treatment. Values underlined and in bold indicate correlations for which the 95% credibility interval did not include zero.

Tracheid diameter							
	Stem 52	Stem 25	Stem 5	Branch 20	Branch 7	Root 42	Root 22
Stem 52		<i>0.50</i>	<u>0.54</u>	<i>0.45</i>	<i>0.52</i>	<i>0.48</i>	<i>0.23</i>
Stem 25	<u>0.83</u>		<i>0.30</i>	<i>0.35</i>	<i>0.09</i>	<i>0.25</i>	<i>-0.08</i>
Stem 5	<i>0.14</i>	<i>0.25</i>		<i>0.38</i>	<i>0.42</i>	<u>0.53</u>	<i>0.39</i>
Branch 20	<u>0.69</u>	<u>0.75</u>	<u>0.52</u>		<i>0.32</i>	<i>0.29</i>	<i>0.31</i>
Branch 7	<u>0.76</u>	<u>0.80</u>	<u>0.48</u>	<u>0.89</u>		<u>0.61</u>	<i>0.11</i>
Root 42	<i>0.14</i>	<i>0.34</i>	<i>0.25</i>	<i>0.31</i>	<i>0.42</i>		<i>0.13</i>
Root 22	<i>0.13</i>	<i>-0.05</i>	<i>0.23</i>	<i>0.15</i>	<i>0.17</i>	<i>0.08</i>	

Percent latewood							
	Stem 52	Stem 25	Stem 5	Branch 20	Branch 7	Root 42	Root 22
Stem 52		<i>0.38</i>	<i>-0.08</i>	<i>-0.18</i>	<i>-0.36</i>	<i>-0.03</i>	<i>0.03</i>
Stem 25	<i>0.41</i>		<i>-0.34</i>	<i>0.23</i>	<i>0.13</i>	<i>0.06</i>	<i>-0.23</i>
Stem 5	<i>-0.18</i>	<u>-0.64</u>		<i>-0.18</i>	<i>-0.29</i>	<i>-0.25</i>	<i>0.44</i>
Branch 20	<i>0.26</i>	<i>0.16</i>	<i>0.01</i>		<i>0.14</i>	<u>-0.53</u>	<i>-0.24</i>
Branch 7	<i>-0.18</i>	<u>-0.48</u>	<u>0.51</u>	<i>0.02</i>		<i>0.03</i>	<i>-0.25</i>
Root 42	<u>-0.61</u>	<i>-0.45</i>	<i>0.24</i>	<i>-0.17</i>	<i>0.43</i>		<i>-0.12</i>
Root 22	<i>-0.07</i>	<i>0.25</i>	<i>-0.29</i>	<u>-0.52</u>	<i>-0.29</i>	<i>-0.11</i>	

Pits per cell							
	Stem 52	Stem 25	Stem 5	Branch 20	Branch 7	Root 42	Root 22
Stem 52		<i>0.29</i>	<i>0.08</i>	<i>0.35</i>	<i>0.10</i>	<i>0.36</i>	<i>0.31</i>
Stem 25	<i>0.39</i>		<i>0.16</i>	<i>0.26</i>	<i>0.35</i>	<u>0.63</u>	<i>0.12</i>
Stem 5	<i>0.37</i>	<i>0.07</i>		<i>0.08</i>	<i>0.17</i>	<i>0.22</i>	<i>-0.08</i>
Branch 20	<i>-0.05</i>	<i>0.07</i>	<i>0.04</i>		<i>0.42</i>	<i>0.25</i>	<i>-0.09</i>
Branch 7	<i>-0.04</i>	<i>0.17</i>	<i>-0.14</i>	<u>0.62</u>		<i>0.23</i>	<i>-0.10</i>
Root 42	<i>-0.37</i>	<i>-0.28</i>	<i>-0.09</i>	<i>-0.25</i>	<i>-0.14</i>		<i>-0.16</i>
Root 22	<i>-0.04</i>	<i>0.36</i>	<i>-0.38</i>	<i>-0.10</i>	<u>-0.51</u>	<i>0.02</i>	

K_s							
	Stem 52	Stem 25	Stem 5	Branch 20	Branch 7	Root 42	Root 22
Stem 52		<i>-0.15</i>	<i>-0.32</i>	<i>0.22</i>	<i>-0.01</i>	<i>0.09</i>	<i>0.02</i>
Stem 25	<i>0.14</i>		<i>0.00</i>	<i>-0.21</i>	<i>0.20</i>	<i>0.01</i>	<i>0.03</i>
Stem 5	<u>0.59</u>	<i>-0.31</i>		<i>0.28</i>	<i>-0.32</i>	<i>-0.42</i>	<i>-0.20</i>
Branch 20	<u>0.52</u>	<i>-0.14</i>	<u>0.73</u>		<i>-0.35</i>	<i>-0.22</i>	<i>0.02</i>
Branch 7	<i>-0.03</i>	<i>0.19</i>	<i>0.07</i>	<i>0.31</i>		<i>0.17</i>	<i>-0.15</i>
Root 42	<i>0.28</i>	<i>-0.12</i>	<i>0.35</i>	<u>0.57</u>	<i>0.32</i>		<i>0.26</i>
Root 22	<i>-0.57</i>	<i>-0.32</i>	<i>-0.07</i>	<i>0.18</i>	<i>0.25</i>	<i>0.26</i>	

Ψ_{50}			
	Stem 52	Branch 20	Root 42
Stem 52		<i>0.27</i>	<i>-0.31</i>
Branch 20	<u>0.82</u>		<i>-0.10</i>
Root 42	<i>0.07</i>	<i>-0.002</i>	

Table 3.4 Δ DIC values for each regression model fit to data from 16 fertilized and 16 unfertilized 54-year-old Douglas-fir trees. Regressions analyzed only for Stem 52, Branch 20, and Root 42 are indicated with (3 positions) in the Variables column, p = number of parameters, and Non-zero slopes are those for which the 95% credibility interval does not include zero.

Variables	Mean model	Δ DIC	p	Non-zero slopes
Density ~ tracheid diameter	CM	7	106	Common
	TM	10	114	Unfertilized
	PM	0	130	Root 42, Root 22
	PTM	27	162	Fertilized and unfertilized Root 22
$\ln(K_s)$ ~ tracheid diameter	CM	123	106	Common
	TM	103	114	Unfertilized, fertilized
	PM	0	130	Branch 20, Branch 7, Root 42, Root 22
	PTM	2	162	
Ψ_{50} ~ tracheid diameter (3 positions)	CM	20	26	Common
	TM	23	34	Unfertilized, fertilized
	PM	1	50	
	PTM	0	82	Unfertilized Branch 20, fertilized Root 42
Density ~ percent latewood	CM	3	106	
	TM	6	114	
	PM	0	130	
	PTM	27	162	Fertilized Root 42
$\ln(K_s)$ ~ pits/cell	CM	68	106	Common
	TM	39	114	Unfertilized, fertilized
	PM	0	130	Stem 25, Root 42, Root 22
	PTM	6	162	Unfertilized Branch 7, fertilized Stem 25, Root 42
Ψ_{50} ~ pits/cell (3 positions)	CM	82	26	Common
	TM	76	34	Fertilized
	PM	0	50	Root 42
	PTM	4	82	Unfertilized Root 42, fertilized Stem 52

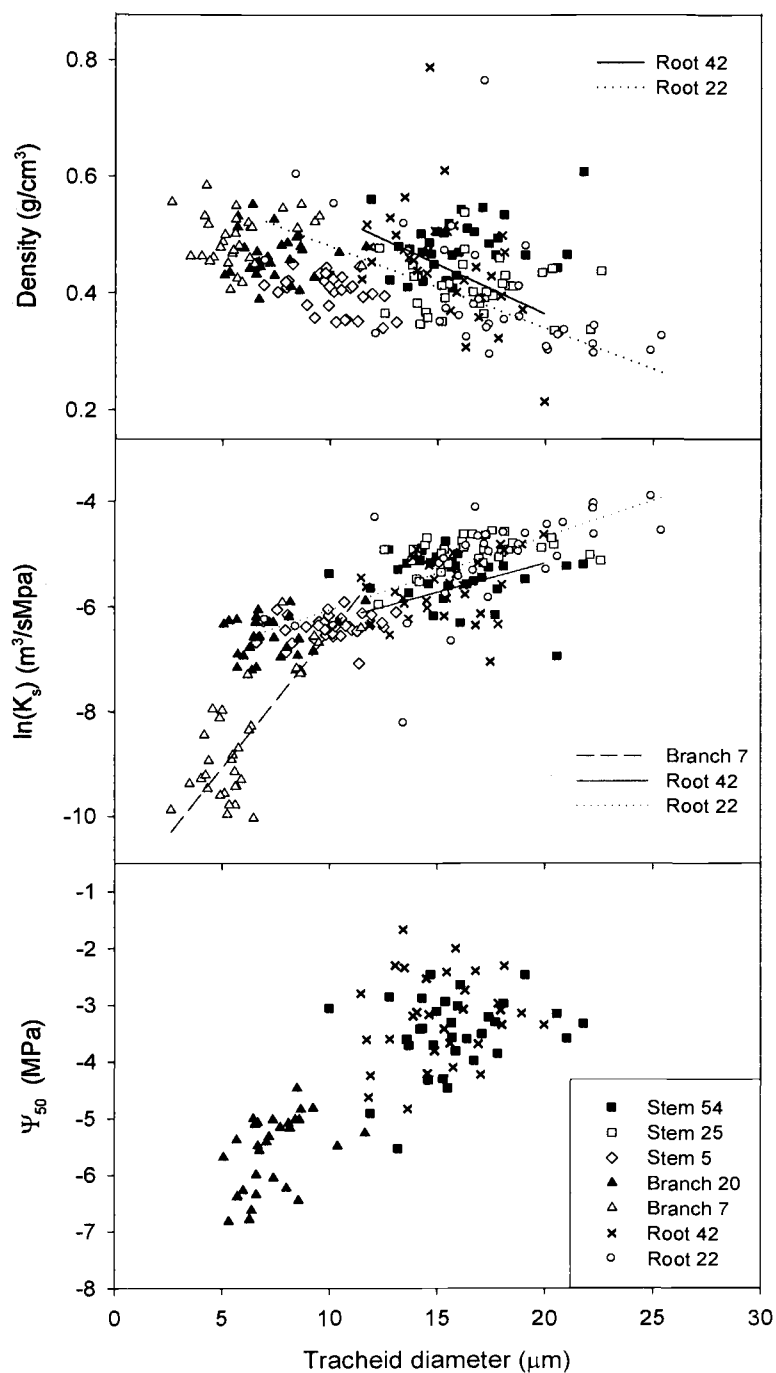


Figure 3.3 a) Density, b) K_s , and c) Ψ_{50} as a function of tracheid diameter. Regression lines are plotted only for positions that had non-zero slopes under the position mean model (Table 3.4).

Density and tracheid diameter

There was an overall negative correlation between density and tracheid diameter (Figure 3.3a). Under the PM model ($\Delta\text{DIC} = 0$, Table 3.3) only two of the seven positions (Root 42 and Root 22) had negative slopes but were not different from each other; respective mean slopes and 95% BCIs were -0.017 (-0.034,-0.001) and -0.014 (-0.024,0.005).

K_s and tracheid diameter

K_s generally increased logarithmically with tracheid diameter; however the relationship varied considerably among positions (Figure 3.3b). Results from the PM model, which had $\Delta\text{DIC} = 0$ (Table 3.3), suggested negative slopes only for Branch 7, Root 42, and Root 22. The slope for Branch 7 was different from the slopes of Root 42 and Root 22 (the 95% BCIs did not overlap), but the two roots were not different from each other. The respective mean slopes and 95% BCIs for Branch 7, Root 42 and Root 22 were -11.6 (-12.3,-11.0), -7.38 (-8.5,-6.2), and -7.44 (-8.1,-6.8).

Ψ_{50} and tracheid diameter

There was an overall positive correlation between Ψ_{50} and tracheid diameter for Stem 52, Branch 20, and Root 42 (Figure 3.3c, Table 3.4). When a different line was fit to each position under the PM model none of the positions showed a relationship between Ψ_{50} and tracheid diameter, though the 95% slope BCI for Root 42 only barely included zero. The 95% slope BCIs for the slopes of Stem 52, Branch 20, and Root 42 were (-0.1,0.1), (-0.1,0.2), and -0.01,0.2) respectively)

Density and percent latewood

There was neither a general trend in the data between density and percent latewood nor a relationship within any of the positions individually (Figure 3.4, Table 3.4). All 95% BCIs for the slope of each position were centered on zero.

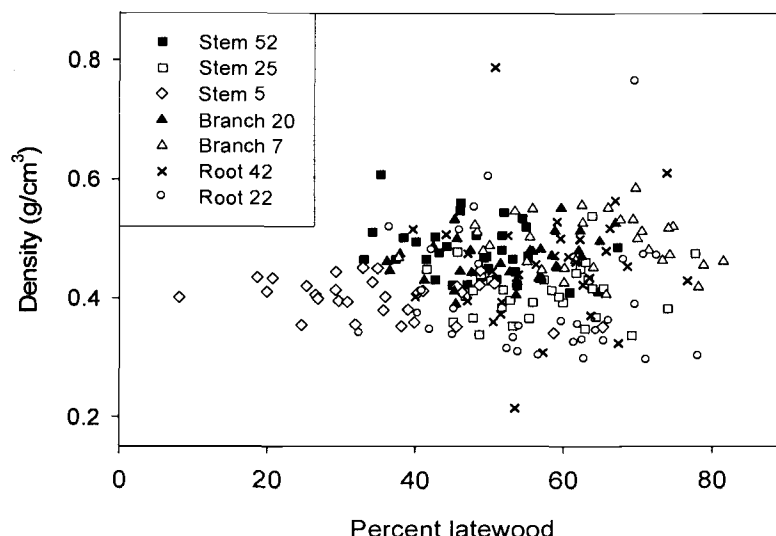


Figure 3.4 Density as a function of percent latewood for seven positions in 32 Douglas-fir trees in the western Cascade foothills.

Ks and pits per cell

Ks increased logarithmically with pits per cell (Figure 3.5a). Under the position mean model three positions had positive slopes for $\ln(Ks)$ (Table 3.4): Stem 25 (mean slope and the 95% BCI is 0.006 and 0.001,0.012), Root 42 (0.01 and 0.003,0.017), and Root 22 (0.006 and 0.001,0.011).

Ψ_{50} and pits per cell

There was an overall positive correlation between Ψ_{50} number of pits per cell (Figure 3.5b), however when a slope was fitted to each position it could be seen that Root 42 seemed to be driving the relationship. Of the three positions considered, only the slope for Root 42 was positive (Table 3.4, the 95% BCI was 0.008, 0.031 with a mean of 0.02).

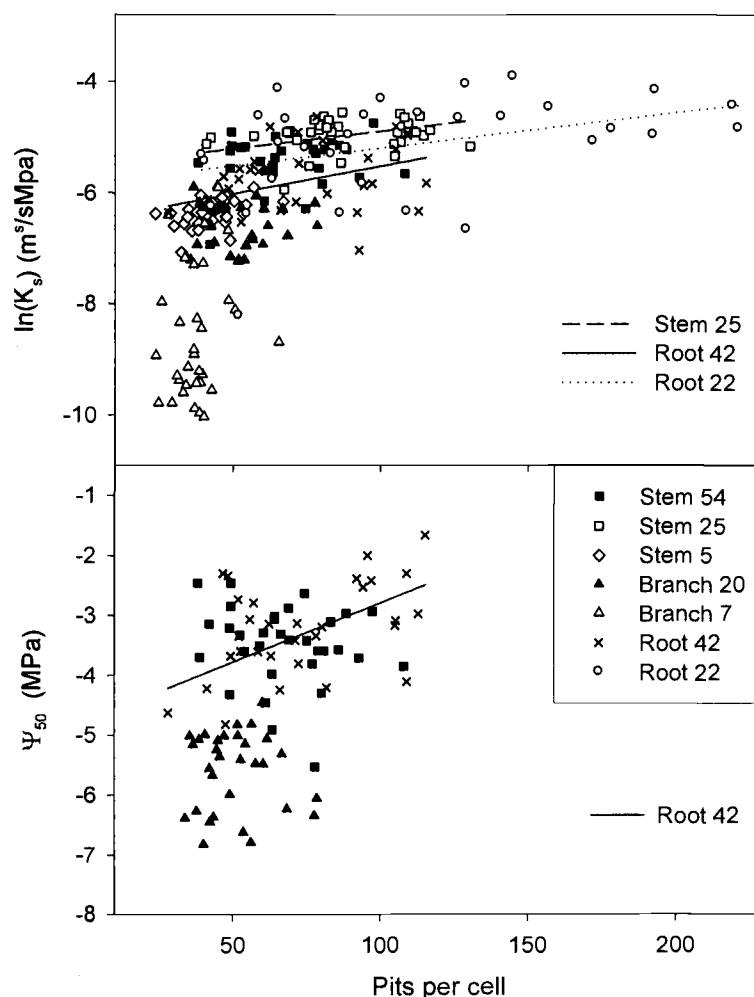


Figure 3.5 a) Specific conductivity and b) Ψ_{50} as a function number of pits per cell. Regression lines are plotted only for positions that had non-zero slopes under the position mean model (Table 3.4).

Discussion

The stem, branches, and roots of a tree fulfill different functional roles so it is not surprising that we found anatomical and hydraulic differences among them. While there have been numerous studies comparing anatomy and/or hydraulic architecture of small, young roots and small diameter branches or stems (Alder et al. 1996; Kavanagh et al. 1999; Ewers et al. 2000; Martínez-Vilalta et al. 2002; Stout and Sala 2003; McElrone et al. 2004; Choat et al. 2005b), there is very little such research comparing

large structural roots to large diameter stem sections or branches (Peterson et al. Submitted). We found Root 42 to be very similar to Stem 52 in anatomy and hydraulic properties. Branch 20 and Branch 7 were much more similar to xylem at the very top of the tree, Stem 5, than xylem closely adjacent to the branch, Stem 25. These findings are consistent with the segmentation hypothesis. In our data tracheid diameter, tracheid length, specific conductivity and vulnerability to embolism decrease distally along the flow pathway. In some aspects branches are hydraulically safer than the stem or roots because of their low vulnerability to embolism and smaller cell dimensions that would best contain embolisms from spreading when they do occur. It is expected from the height differences among the positions and the negative pressures required to overcome the hydrostatic gradient in the xylem that Ψ_{50} would decrease from the roots to the tree top (Tyree and Zimmerman 2002). However, the transition in tracheid length, tracheid diameter, density, and number of pits/cell from Stem 25 to Branch 20 samples was rather sharp considering the short distance between the samples. This suggests a sharp increase in hydraulic resistance at branch junctions that would localize the potential for embolism occurrence in the branches (Comstock and Sperry 2000). Branches and branch junctions in conifers thus protect the stem from embolism yet are also built to resist and contain embolism the best of all positions measured in our study. Roots likewise seem to be built to protect the main stem, but with a different strategy. The anatomical transitions from root to stem are rather gradual, but tracheid dimensions, number of pits/cell, and K_s increased considerably from Root 42 near the bole to the smaller Root 22 samples. Previous work has shown that small Douglas-fir roots to embolize around -1.0 MPa (Domec et al. 2004), meaning young roots would embolize much more readily than the large structural roots close to the trunk, thus protecting the larger parts of the tree that may be more difficult to repair or replace.

Our results suggest that properties of Branch 7, the part of the tree most often used in research, are not always correlated with properties of the other positions (Table 3.3), so inferences made from data only including small diameter branches may not

accurately represent the other parts of the tree. If only one or two parts of a tree are included in a study, it would be advantageous to know how those parts relate to the entire tree. We found that only a few positions were correlated for the variables we considered, and these correlations mostly occurred in the fertilized treatment. This result implies that positions may respond differently to the various stimuli that govern each of the variables we considered. For example, in the unfertilized treatment the stimulus that causes large tracheid diameters in branches does not in the same way affect stems. In fertilized trees, however, tracheid diameter in branches was highly correlated with tracheid diameter in the stem sections (Table 3.3), so it may be that without a nitrogen limitation stems and branches respond similarly to the stimulus that governs tracheid diameter.

We found that relationships between anatomy and properties were different at each scale. At the largest scale, among positions and treatments, we found that K_s and Ψ_{50} increased with increasing tracheid diameter and number of pits per cell. Also, wood density decreased with increasing tracheid diameter. When treatments were considered separately the relationships were very similar to the common mean model. However, at the smaller scale of within each position these relationships either disappeared or were only evident for a few of the positions, usually the roots (Table 3.4). This pattern may occur because the range of values spanned by each position for any given variable is much smaller than the range for the entire data. It also may occur because the relationships we considered between xylem anatomy and hydraulic properties are affected by large-scale factors that influence the entire xylem network but that are over-ridden by small-scale factors at the tissue level. Roots experience very different mechanical stresses and we found them to have much more variable xylem anatomy and properties than other positions. Just because a trend does or does not exist within a single position does not imply that the relationship between two variables is the similar within other positions or at a higher scale.

Trees are complex structures that exist within a biological hierarchy with scales from species and forest stand to position and cellular levels. Hierarchical modeling allowed us to account for the multiple sources of variation inherent in the biological hierarchy represented in the data. The previous chapter demonstrated that statistical hierarchical models resulted in more precise estimates of regression parameters than statistical methods that assume independence among samples and that do not partition variation among scales. Variation in our data could have come from treatment differences, tree differences, or position differences. In our data most of the variation came from position differences, as evidenced by the fact that regression models were best supported when a separate line was fit to each position (the position mean models, Table 3.4). This research shows that it is beneficial to consider the effects of scale and within tree variation when investigating the relationship between xylem anatomy and properties.

Structural and hydraulic tradeoffs at multiple scales in Douglas-fir

Sonya M. Dunham, Barbara L. Gartner, Lisa M. Ganio

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Abstract

Because the secondary xylem of trees has multiple roles in mechanical support and water transport, one would expect that fulfillment of some of the roles occurs to the detriment of fulfillment of other roles. We investigated the existence of several such hydraulic and structural tradeoffs in Douglas-fir xylem at several hierarchical scales among positions within trees, among trees within treatments (fertilized and unfertilized), within positions, and within positions in each treatment. The positions studied were stem (53, 25, and 5 years old), branch (20 and 7 years old), and root (42 and 22 years old) for specific conductivity (K_s) and basic wood density. We also measured vulnerability to embolism on the oldest age classes of the stem, branch, and root. Results from a Bayesian hierarchical model indicate that tradeoffs in Douglas-fir xylem were different at each scale. At the largest scale, among all trees, treatments, and positions, tradeoffs existed between K_s and density and between vulnerability to embolism and K_s . Vulnerability to embolism and density were only correlated within the unfertilized treatment at the next largest scale (within treatments), and all other tradeoffs looked the same within each treatments as at the large scale. At the smallest scales, within positions and within treatments, tradeoffs existed only within a few positions or positions in each treatment for K_s and density and vulnerability to embolism and K_s . We did not find evidence of a correlation between sap velocity and density. This research broadens the scope of understanding of xylem hydraulic and structural tradeoffs by explicitly linking multiple scales into a single analysis.

Introduction

Leaves require sunlight, water, and CO_2 to photosynthesize. In woody plants xylem provides both structural and hydraulic support for the leaves, holding them up to capture light and transporting water to meet evaporative and photosynthetic demands. Ideally xylem should be built in such a way to maximize hydraulic efficiency (conductivity), hydraulic safety (resistance to embolism), and structural safety (density or other strength property), but this has not been seen to be the case (Gartner 1991;

Wagner et al. 1998; Davis et al. 1999; Maherali et al. 2004). The wide variation seen in these xylem properties and the fact that these properties are not always maximized suggests that a tradeoff exists among these properties and that they cannot be optimized simultaneously (Gartner 1995; Tyree and Zimmerman 2002; Maherali et al. 2004). We focus on four tradeoffs discussed in the literature: a tradeoff between hydraulic safety and structural safety, between hydraulic efficiency and structural safety, between hydraulic safety and hydraulic efficiency, and between sap velocity and structural safety.

A tradeoff between hydraulic safety and structural safety exists when the anatomical features contributing to structural stability also influence vulnerability to embolism and vice versa. Wood density is strongly related to wood strength properties (Wagner et al. 1998; Jacobsen et al. 2005) and at large scales, across species, is positively correlated with hydraulic safety (Hacke et al. 2000b; Hacke et al. 2001; Jacobsen et al. 2005). Denser wood is stronger and thus able to withstand higher implosion stresses that are generated by negative water pressure and embolized xylem cells (Hacke et al. 2001). On one hand high density and high resistance to embolism are both positive qualities, especially for large trees requiring substantial mechanical support. However, high density requires high xylem construction cost in terms of carbon allocation, and then high 'maintenance cost' to keep the distal structures supported. If wood density is high and provides embolism resistance but is greater than needed for mechanical demands, the plant pays for the hydraulic safety by diverting carbon and energy away from other potential uses of the photosynthate.

High wood density has also been correlated to a reduction in growth rate (Roderick 2000; Johnson and Gartner in press), most likely because it influences xylem conductivity (Wagner et al. 1998; Bucci et al. 2004). A tradeoff between hydraulic efficiency and structural safety is expected because in softwoods, dense wood is usually associated with small earlywood lumen diameters as well as a high percentage of latewood (Wagner et al. 1998; Spicer and Gartner 2001; Jacobsen et al. 2005),

factors that can greatly reduce permeability (Domec and Gartner 2002b; Tyree and Zimmerman 2002; Jacobsen et al. 2005).

If density (or conduit diameter, pit pore diameter, or other anatomical feature) affects both hydraulic safety (positively) and efficiency (negatively) then we would expect a tradeoff between vulnerability to embolism and conductivity. Embolism-resistant xylem has the advantage of providing the water for photosynthesis in periods of low water availability, whereas high conductivity has the advantage of providing the water for photosynthesis in periods of higher water availability.

The wood density/sap velocity tradeoff is based on predictions from a model of wood function (Roderick and Berry (2001). Sap velocity is often closely associated with transpiration after leaf and sapwood area is accounted for (Phillips et al. 2002; Chuang et al. 2006). Wood density is related to the amount of solid material versus space available for water transport as well as how much the xylem structure can shrink and swell in response to the negative tension gradient and thus affects the velocity of sap as it travels up the tree (Roderick and Berry 2001). In summary, safe xylem in conifers may be built at the cost of having to construct wood with high density, and increasing wood density may decrease, among other factors, xylem conductivity and sap velocity.

At what scale do these costs influence xylem structure and properties? There is increasing evidence that large scale tradeoffs exist across species (Hacke et al. 2000b; Hacke et al. 2001; Meinzer 2003; Oliveras et al. 2003; Jacobsen et al. 2005), suggesting that all woody plants experience similar costs of optimizing xylem properties and that species differ in how much they are willing to physiologically 'pay' for xylem and structural safety. In this paper we will focus on smaller scales within a single species, Douglas-fir, including two different environments (fertilized and unfertilized treatments) and different positions and cambial ages (multiple ages within stems, roots, and branches). When a tradeoff is seen within a species across

environments and positions it suggests that xylem is designed according to the limitations of these tradeoffs regardless of position type or environmental conditions. In this case all trees, treatments, positions have the same costs associated with optimizing properties.

If there is no evidence for a whole-tree tradeoff within a species, then the relationship may dependent upon the growing environment. Water- or nutrient-limited trees may be on a tighter ‘budget’ than those with ample supplies and thus may not be able optimize xylem properties in the same way (Ewers et al. 1999; Ewers et al. 2000). It may also be that organs and xylem of different cambial ages are designed to different specifications depending on their function and position within the tree (Spicer and Gartner 2001; Domec and Gartner 2002a; Gartner 2006). A tradeoff may be evident for only one or some positions, or the tradeoffs may look very different for each position. The stem and branches may be constrained to the limitations of a tradeoff involving structural constraints, but in roots, especially roots far from the stem, structural integrity and strength would not be a constraint so costs involving density would be different. Even between the stem and branches structural demands and function within the tree are quite different (branches directly support leaves horizontally and stems vertically support the entire crown), so they too may have different strategies for managing the costs of optimizing xylem properties. There is also the possibility of an interaction between the environment and each position, which would suggest that positions, and their respective hydraulic management strategies, respond differently to environmental changes. A lack of a tradeoff at any scale would imply that the costs associated with optimizing hydraulic and structural properties are not as predicted within the scope of inference in the data.

In this study we examine the scale-dependence of tradeoffs between hydraulic and structural properties in Douglas-fir. The actual values for structural and hydraulic properties for the various positions have already been reported (previous chapter). We expect to see overall trends linking vulnerability to embolism, conductivity, density

and sap velocity when considering multiple positions and both fertilized and unfertilized trees. We also expect that there will be differences among the positions due to position function and location within the tree, and differences between the treatments due to the influence of the environment on tree growth and allocation patterns, and the associated xylem properties. Furthermore, we anticipate that the differences among positions and cambial ages within a tree are designed to maximize safety and efficiency along the xylem flow pathway by accounting for changes in tissue function and height.

Although studies have investigated how tradeoffs depend on environmental conditions (Mencuccini and Comstock 1997; Kavanagh et al. 1999; Bouffier et al. 2003) and organ type (Kavanagh et al. 1999; Hacke et al. 2000b; Martínez-Vilalta et al. 2002; Choat et al. 2005b) our research is unique for two reasons. First, most work in tree physiology is conducted on small diameter roots and branches (e.g Alder et al. 1996; Sperry and Ikeda 1997; Kavanagh et al. 1999; Ewers et al. 2000; Martínez-Vilalta et al. 2002; Stout and Sala 2003; McElrone et al. 2004; Choat et al. 2005b). In addition to these sampling locations we also include parts of the tree not as commonly studied: large diameter (> 2 cm) branch sections located close to the stem, the stem at breast height, the stem at the top of the tree, and older structural root sections near the stem. Second, we use a Bayesian hierarchical model to account for multiple sources of variation present in the biological hierarchy in our data (positions within trees and trees within treatments). Hierarchical statistical models explicitly link data from multiple scales in a single analysis and thus provide more accurate estimates of variance and more precise estimates of model parameters, such as regression coefficients (as shown in the second chapter).

Methods and materials

Site characteristics and plant materials

A detailed description of the research site and sampling methods may be found in the previous chapter. We measured 32 randomly selected Douglas-fir (*Pseudotsuga*

menziesii) trees growing in a 54-year-old natural regeneration stand (44° 25.6' N, 122° 35.7' W) in 2004. For the sampled trees, average DBH was 53.6 cm, and average tree age at breast height was 52. Half of the trees had been fertilized with urea in 2000. Seven sampling points were selected on each tree. Three bole sections were located at about cambial age 52 (breast height, called Stem 52), cambial age 25 (Stem 25), and cambial age 5 (the leader, Stem 5). Branches were located in the mid- to lower crown, about 28 meters high, in the branch whorl immediately above Stem 25. Old branch sections (Branch 20) were approximately 0.5 m out from the branch junction and the young branch sections (Branch 7) were near the tip of the same branch. The two root sections were located on the same root – one section was the large structural portion approximately 0.5 m from where the root went below the soil (Root 42) and the other was a smaller, younger, non-structural portion further along the root (never deeper than 1 m, Root 22). Measurements of tree height, branch height, stand density, and overstory (leaf) density are reported in the previous chapter.

Sap velocity

Heat dissipation sap flow probes were installed 1.5 cm beneath the cambium on the Branch 20, Stem 52, and Root 42 sections of each tree. Sap flow probes were constructed and installed following James et al. (2002) except only one sensor length, 1.5 cm, was used. All probes were shielded from sunlight with reflective insulation. Root 42 probes were installed approximately 0.5 m below the ground on a large structural root as close to the north side as possible. The small pits providing access to the roots were covered with reflective insulation once the probes were installed. Stem 52 probes were installed at breast height (approximately 1.4 m above ground) on the north side of the tree. Branch 20 probes were installed about 0.5 m out along the branch away from the bole and on the upper side of the branch to avoid compression wood. Compression wood and heartwood have much lower conductivity than regular sapwood (Spicer and Gartner 1998), so we wanted to avoid placing sap flow probes in these areas. Probes were installed on eight trees at a time, four fertilized and four unfertilized, and data were used for periods ranging from mid-July to early October

2004. After sap flow was measured on the first four fertilized and unfertilized trees the branches were collected and the heartwood/sapwood boundary was visually determined. The heartwood/sapwood boundary was at least as deep as the probes, so no changes were made in probe positions on the branches.

Sap flow was recorded for at least three sunny days for each tree. Signals from the probes were monitored every minute and 10-minute means were recorded by a data logger (CR 10x; Campbell Scientific) equipped with a 32-channel multiplexer (AM416; Campbell Scientific). Sap velocity (V_s , m s^{-1}) was calculated using the empirical calibration of Granier (1987), revalidated by Clearwater et al. (1999):

$$V_s = 0.119k^{1.231}$$

where $k = (\Delta t_m - \Delta t_s) / \Delta t_s$, Δt_s is the temperature difference between the two sap flow sensors, and Δt_m is the temperature difference with V_s is assumed to be zero (the highest temperature recorded in a 24 hour period).

Sap velocities are presented in several ways. Sap velocity profiles were normalized with respect to their maximum values to allow comparisons of when sap flow commenced and peaked between the positions and treatments. Diurnal sap velocity profiles were plotted either for each position using average values (between 0900 and 1600 Pacific Standard time) of 32 trees for three days or for each position by fertilization treatment, averaged over 16 trees in each treatment for three days. Maximum V_s was the highest sap velocity recorded within a 24 hour period.

The tradeoff between sap velocity and density predicted by Roderick and Berry (2001) assumes constant temperature and that the volume fraction of available space occupied by tracheids is equal among samples. Tracheid diameter and percent latewood, and thus number of tracheids per area, and sap temperature varied greatly among the three positions in which sap flow probes were installed (as shown in the previous chapter). We thus limited our investigation of a tradeoff between sap velocity and wood density to within each position separately.

Xylem properties

Eight trees were harvested at a time throughout the summer after their sap flow and T had been measured. Sections were cut from all seven positions (described above in *Site Characteristics and Plant Materials*) were kept damp and were taken back to the lab. We collected data on basic density and specific conductivity (K_s) for all seven positions, and vulnerability to embolism for Stem 53, Root 42, and Branch 20. The methods used to measure density and K_s , and to calculate Ψ_{50} are described in detail in the previous chapter, and are summarized here.

Basic density. Small blocks of about 2 x 2 x 2 cm were cut from the outermost wood of each of the seven sections from each tree. Green volume (V_g , cm^3) and dry mass (M_d , g) were measured and basic density (D) was calculated as

$$D = \frac{M_d}{V_g}.$$

Specific conductivity. Specific conductivity (K_s , $\text{m}^2\text{s}^{-1}\text{MPa}^{-1}$) is an expression of how permeable a segment of xylem is for a given length and surface area. Samples 1 cm by 1 cm by 10 cm (in the axial direction) were chiseled from the outermost growth rings of each section, except for the Branch 7 sections that were simply cut underwater to a length of 10 cm. Measurements were made using the pressure-sleeve apparatus described by Spicer and Gartner (1998) using a dilute solution of hydrochloric acid (pH 2, filtered to 22 μm) at 20°C. K_s is calculated according to Darcy's law as

$$K_s = \frac{Ql}{A\Delta P},$$

where Q is the volume flow rate (m^3s^{-1}), l is the length of the segment (m), A is the cross-sectional area of the segment (m^2), and ΔP is the pressure gradient across the segment (MPa).

Vulnerability to embolism. Vulnerability curves (VCs) were estimated for Stem 52, Root 42, and Branch 20 from all trees using the conductivity samples from these positions. Vulnerability to embolism was measured by alternately measuring K_s and inducing increasing degrees of embolism using the air-injection technique (Sperry and

Saliendra 1994; Domec and Gartner 2001). Percent loss of conductivity (PLC) at a given pressure was calculated as

$$PLC = \left(\frac{K_{s(i)} - K_{s(\Psi)}}{K_{s(i)}} \right) \times 100 ,$$

where $K_{s(i)}$ is the initial specific conductivity and $K_{s(\Psi)}$ is the specific conductivity of the sample after being exposed to a positive pressure, Ψ . Vulnerability curves were constructed by plotting PLC versus the applied pressure. The negative of the pressure at which a sample has reached 50% loss in conductivity (Ψ_{50}) was used to compare vulnerability to embolism between samples. A unique third-degree polynomial was fit to each VC and the equation of the polynomial was solved to find Ψ_{50} for each sample.

Analysis

Regressions were analyzed using the Bayesian hierarchical models described in the second chapter. The statistical hierarchical models included complex variance-covariance matrices that reflected the natural hierarchical partitioning of variation within positions, among positions, and between treatments and accounted for the dependence of observations within each scale. Regression coefficients were estimated for four types of mean models; a common mean model (CM, one line fitted to the entire data), a treatment mean model (TM, one line fitted to each treatment), an position mean model (PM, one line fitted to each position), and an position + treatment mean model (PTM, one line for each position-treatment combination). The CM model reflected large-scale relationships among the entire data. The TM and PM models reflected relationships within the middle levels of the hierarchy, and the PTM model represented the smallest scale, or finest resolution in the data.

The models were fit to the data using WinBUGS (Spiegelhalter et al. 2003) and compared using the Deviance Information Criterion, or DIC (Spiegelhalter et al. 2002). Models were compared by calculating ΔDIC_i , the difference between the DIC for model i and the minimum criterion value within the group of models being compared ($\Delta DIC_i = DIC_i - DIC_{\min}$). The model with the lowest criterion values, or

$\Delta\text{DIC}_i = 0$, is best supported by the data. Parameter probability distributions were summarized by calculating the mean value and 95% posterior probability interval (2.5 and 97.5 percentiles) or Bayesian credibility interval (BCI, Ellison 1996). Regression slopes are reported as positive or negative if their 95% BCI only includes positive or negative values, respectively, indicating that the value is non-zero. For further explanation of Bayesian interpretation see Ellison (1996) and Reckhow (1990).

Results

Sap velocity

Stem 52 and Branch 20 had similar high maximum V_s compared to Root 42 (Table 4.1). Stem 52 had the highest average V_s above Branch 20 and Root 42 which were similar (Table 4.1). Branch 20 had a more sharply peaked diurnal sap velocity profile than Stem 52 or Root 42 (Figure 4.1), which may explain why the maximum V_s for Branch 20 was similar to Stem 52 but the average V_s was much lower than that of Stem 52.

Fertilized trees consistently had higher and in some cases more variable maximum and average V_s than unfertilized trees for all three positions (Table 4.2). For average V_s the 95% BCIs for fertilized and unfertilized Stem 52 and Root 42 overlapped substantially, but the BCI's for Branch 20 only overlapped by 0.01 mm s⁻¹ (data not shown). Normalized diurnal sap velocity profiles show that sap flow began earlier in the day in the fertilized trees than the unfertilized trees (Figure 4.1).

Table 4.1 Cambial age, approximate height, maximum sap velocity (V_s), average V_s from 0900 to 1600 hours (Pacific Standard Time), density, Ψ_{50} , and K_s , for each of the seven positions sampled. Values are posterior means (95% BCI), $n = 32$, n.d. indicates no data, height values in italics are estimates based on sampling procedures, and values related to sap velocity are averages over three days of measurements.

Position	Cambial age (years)	Height from ground (m)	Max V_s (m s^{-1})	Average V_s (m s^{-1})	Density (g cm^{-3})	Ψ_{50} (MPa)	K_s ($10\text{m}^2\text{s}^{-1}\text{MPa}^{-1}$)
Stem 52	51.6 (50.6, 52.7)	<i>1.4</i>	0.17 (0.15, 0.19)	0.13 (0.12, 0.14)	0.48 (0.44, 0.51)	-3.4 (-3.6, -3.2)	0.0045 (0.0039, 0.0051)
Stem 25	24.4 (23.3, 25.5)	26.2 (24.9, 27.6)	n.d.	n.d.	0.41 (0.37, 0.44)	n.d.	0.0074 (0.0066, 0.0081)
Stem 5	5.3 (5.1, 5.4)	41.6 (40.4, 42.9)	n.d.	n.d.	0.40 (0.37, 0.44)	n.d.	0.0017 (0.0015, 0.0018)
Branch 20	20.2 (19.2, 21.3)	26.2 (24.9, 27.6)	0.17 (0.14, 0.19)	0.10 (0.09, 0.12)	0.47 (0.43, 0.50)	-5.6 (-5.8, -5.4)	0.0014 (0.0012, 0.0016)
Branch 7	7.0 (6.4, 7.6)	26.2 (24.9, 27.6)	n.d.	n.d.	0.49 (0.46, 0.53)	n.d.	0.0003 (0.0001, 0.0004)
Root 42	42.2 (40.2, 43.9)	-0.5	0.13 (0.10, 0.15)	0.10 (0.09, 0.12)	0.44 (0.39, 0.48)	-3.3 (-3.6, -3.0)	0.0036 (0.0028, 0.0044)
Root 22	22.3 (19.2, 25.2)	-1	n.d.	n.d.	0.39 (0.34, 0.44)	n.d.	0.0069 (0.0052, 0.0086)

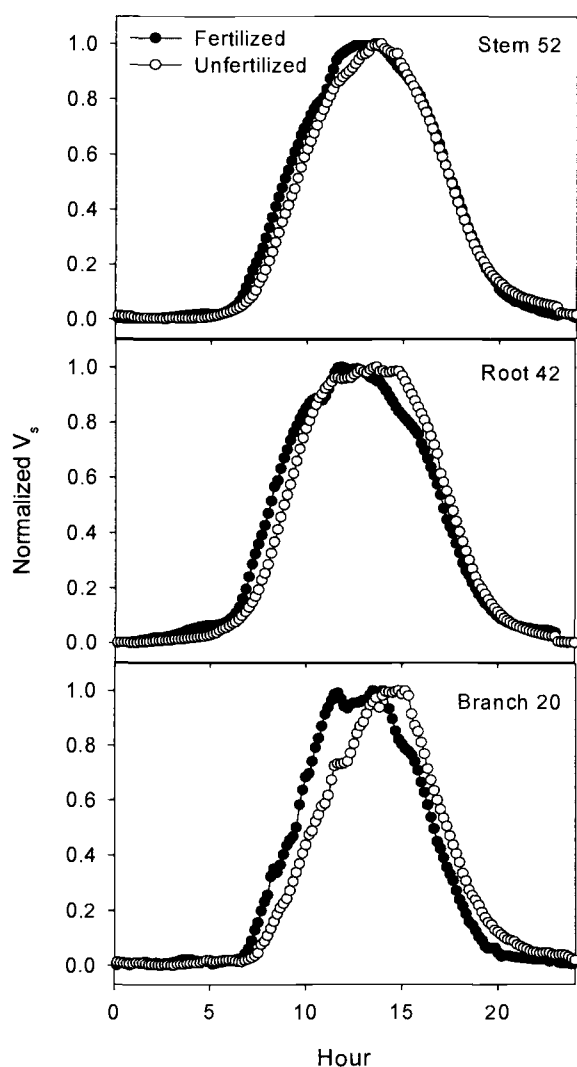


Figure 4.1 Normalized sap velocity in unfertilized vs. fertilized treatments of Douglas-fir trees in the western Cascade foothills averaged over three days for a) Stem 52, b) Branch 20, and c) Root 42, $n = 16$ trees per treatment.

Table 4.2 Maximum daily sap velocity and average sap velocity from 0900 to 1600 hours (Pacific Mean Time) for Stem 52, Branch 20, and Root 42 in the unfertilized and fertilized treatments. Values are posterior means \pm 1 SD for 16 trees from three days of measurements.

Position	Max V_s (m s^{-1})		Average V_s (m s^{-1})	
	Unfertilized	Fertilized	Unfertilized	Fertilized
Stem 52	0.17 (0.14, 0.19)	0.19 (0.16, 0.23)	0.13 (0.11, 0.15)	0.14 (0.12, 0.15)
Branch 20	0.16 (0.12, 0.19)	0.20 (0.14, 0.27)	0.10 (0.08, 0.11)	0.13 (0.10, 0.15)
Root 42	0.12 (0.09, 0.15)	0.14 (0.9, 0.18)	0.10 (0.08, 0.12)	0.11 (0.08, 0.13)

Table 4.3. Model results for relationships between variables at different scales with ΔDIC for each set of regression models. Non-zero slopes indicates those relationships for which the slope BCI did not include zero, the + or – following each non-zero slope indicates the sign of the correlation, and p = number of parameters. Sap velocity (V_s) refers to the average V_s from 0900 to 1600 hours Pacific Mean Time.

Variables	Model	ΔDIC	p	Non-zero slopes
$\Psi_{50} \sim \text{density}$	CM	74	26	
	TM	53	34	Unfertilized –
	PM	0	50	
	PTM	7	82	
$\ln(K_s) \sim \text{density}$	CM	90	106	Common –
	TM	53	114	Unfertilized –, fertilized –
	PM	12	130	Branch 20 –, Root 22 –
	PTM	0	162	Unfertilized Branch 20 –, Branch 7 –, Root 22 –, fertilized Stem 52 –, Stem 25 –, Branch 20 –, Branch 7 +, Root 22 –
$\Psi_{50} \sim \ln(K_s)$	CM	67	26	Common +
	TM	66	34	Fertilized +, unfertilized +
	PM	0	50	Root 42 +
	PTM	10	82	Fertilized Root 42 +
$V_s \sim \text{density}$	PM	0	50	
	PTM	10	82	Unfertilized Stem 52 +, fertilized Branch 20 –
$V_s \sim \% \text{ latewood}$	PM	0	50	
	PTM	8	82	

Vulnerability to embolism and wood density

The results from the CM model indicated that there was no overall relationship between Ψ_{50} and density (Table 4.3 and Figure 4.2). A relationship was also absent for each position under the PM model, the model that was best supported by the data ($\Delta\text{DIC} = 0$, Table 4.3). Although there was not an overall tradeoff or a tradeoff within positions between vulnerability and wood density, there was a negative within-tree correlation between Ψ_{50} and density just among the unfertilized trees under the TM model (mean slope and 95% BCI were -7.04 and -12.65, -1.75, regression not shown).

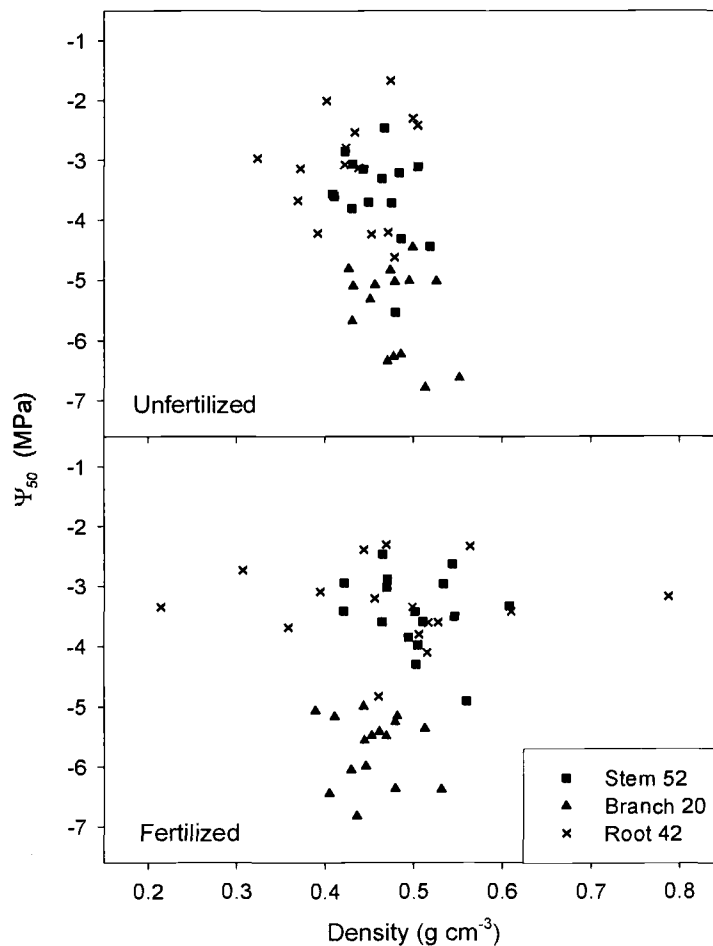


Figure 4.2 Relationship between Ψ_{50} and density in a) the unfertilized treatment and b) the fertilized treatment for Stem 52, Root 42, and Branch.

Specific conductivity and wood density

There was an overall negative correlation between $\ln(K_s)$ and wood density under the CM model (the mean and 95% BCI for the slope was -2.25 and -3.57, -1.03, Figure 4.3). Within each treatment there was also a negative correlation, however the correlation was much weaker in the fertilized treatment: the unfertilized treatment had a mean slope of -5.77 (95% BCI = -8.02, 3.16) and the fertilized treatment had a mean slope of -1.56 (95% BCI = -2.95, -0.29) from the TM model. Under the PM model only Branch 20 and Root 22 had negative slopes (slope means and 95% BCIs were -5.39 (-8.91, -2.24) and -5.18 (-7.47, -2.81), respectively).

The PTM model was best supported by the data (Table 4.3), indicating that treatment and position differences were large enough to warrant the additional parameters required by the PTM model over the simpler CM, TM, or PM models. A total of eight treatment + position combinations had non-zero slopes, five of which were in the fertilized treatment (Table 4.3). Branch 20, Branch 7, and Root 22 had non-zero slopes in both treatments, and Stem 52 and Stem 25 only showed correlations in the fertilized treatment. Of these relationships all but Branch 7 were negative correlations (Figure 4.3).

Vulnerability to embolism and specific conductivity

Overall, we found a positive logarithmic correlation between Ψ_{50} and K_s (slope and 95% BCI for $\ln(K_s)$ were 0.68 and 0.37, 1.00, Table 4.3). The PM model was best supported by the data, and under this model Root 42 had a positive slope (Figure 4.4, mean slope of 0.53, and 95% BCI for $\ln(K_s)$ of 0.15, 0.89). Under the PTM model the slope was positive only for fertilized Root 42 samples.

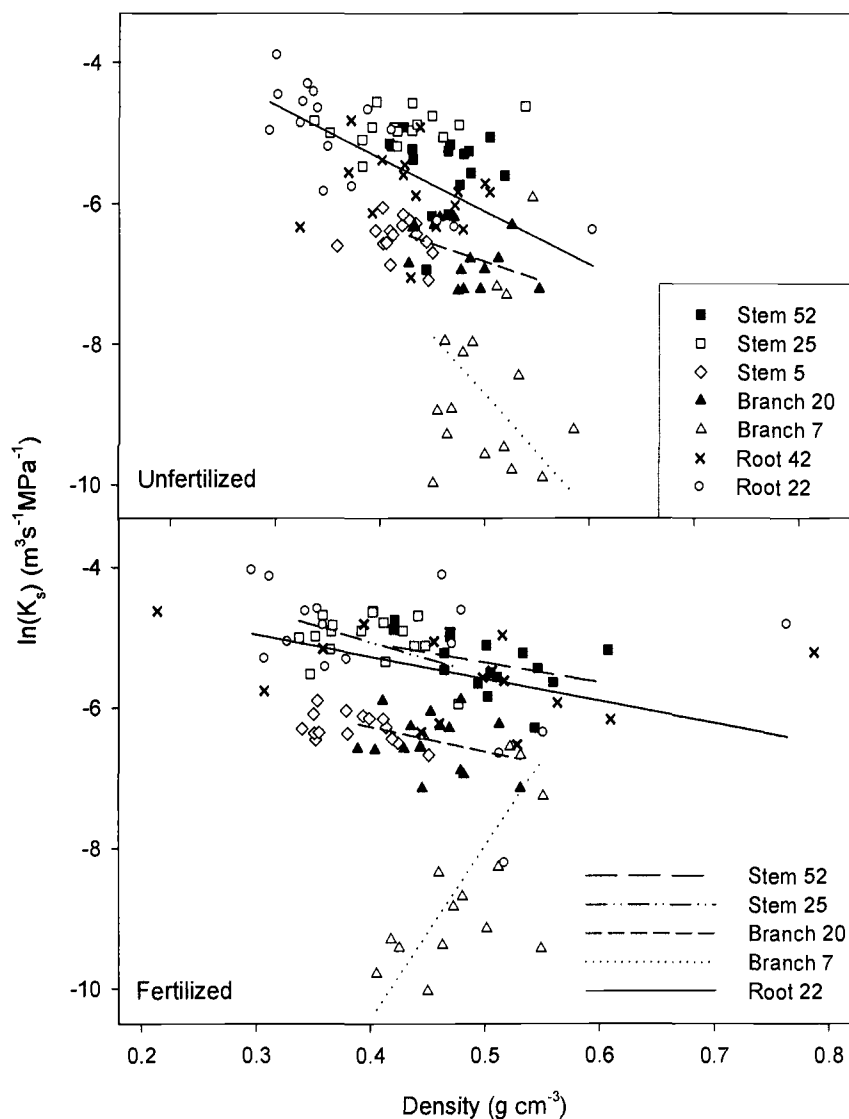


Figure 4.3 Relationship between $\ln(K_s)$ and density in a) the unfertilized treatment and b) the fertilized treatment. Regression lines are plotted only for positions that had non-zero slopes under the position mean model (Table 4.3).

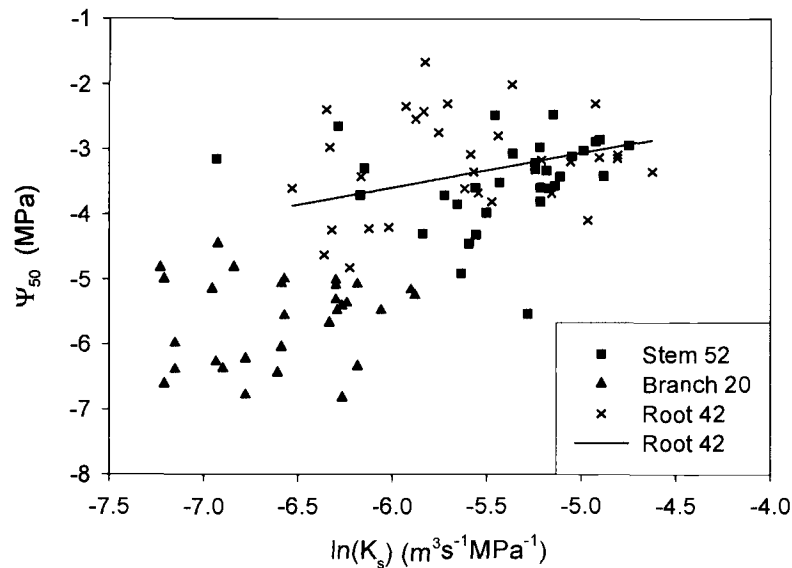


Figure 4.4 Relationship between Ψ_{50} and $\ln(K_s)$ for Stem 52, Root 42, and Branch 20. Regression lines are plotted only for positions that had non-zero slopes under the position mean model (Table 4.3).

Sap velocity and wood density

There was no evidence for a relationship between sap velocity and wood density within Stem 52, Branch 20, or Root 42 (Figure 4.5, left panels). The BCIs of all three slopes included both positive and negative values; the mean slopes (95% BCI) were 10.8 (-15, 37), -18.9 (-50.9, 11), and 7.7 (-11.3, 25.6) for Stem 52, Branch 20, and Root 42 respectively.

We also considered a relationship between sap velocity and percent latewood within each of the positions. Measurements of percent latewood, a quantification of the area in the cross section of a sample not used for water transport relative to the area available for sap flow in each sample, were taken from data collected as described in chapter 3. We found no evidence of a relationship between sap velocity and percent latewood (Figure 4.5, right panels). The mean slopes (95% BCI) for Stem 52, Branch 20, and Root 42 were -0.11 (-0.24, 0.02), -0.03 (-0.18, 0.10), and 0.03 (-0.15, 0.25)

respectively. Though all the 95% BCIs include zero, the interval for the slope of Stem 52 includes mostly negative values, indicating a possible weak relationship between sap velocity and percent latewood in Stem 52. The intervals for the other two positions are much more centered on zero.

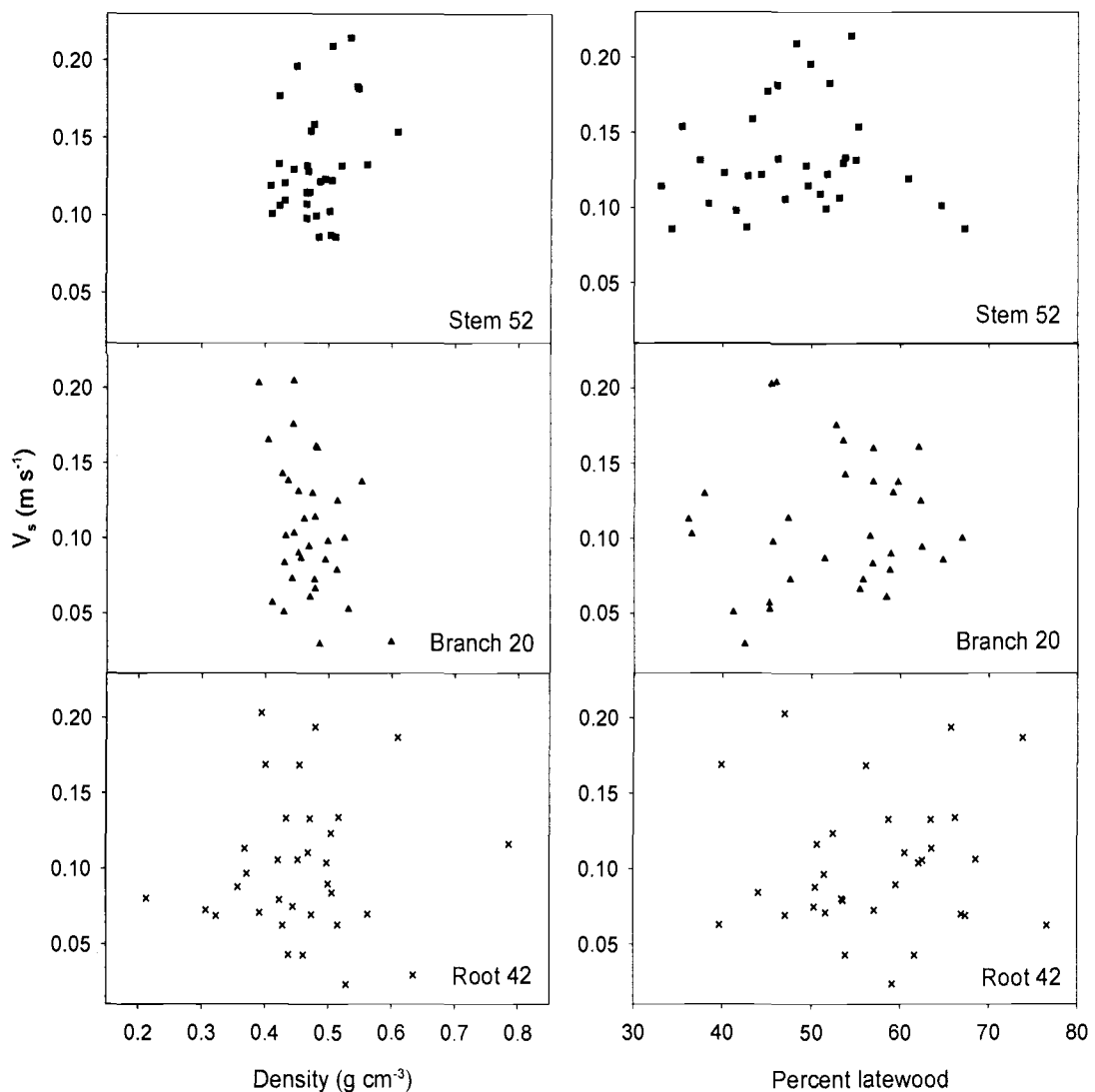


Figure 4.5 Sap velocity versus wood density (left panels) percent latewood (right panels) for Stem 52, Branch 20, and Root 42 in 32 Douglas-fir trees in the western Cascade foothills. All slopes were equal to horizontal.

Discussion

The notion of a tradeoff implies limitations, and specifically within this study it implies limitations in xylem structure and function. Optimizing beneficial xylem properties sometimes may come at the cost of reducing other beneficial properties because factors that positively influence one property negatively affect the other desirable characteristic. As we have found, these factors may be different at different scales; tradeoffs among hydraulic and structural properties depend not only on whole tree design criteria but also on environmental conditions and function of each position.

Hydraulic safety and structural safety

Contrary to the findings of several studies (Hacke et al. 2001; Oliveras et al. 2003; Jacobsen et al. 2005) we did not find an overall tradeoff between vulnerability to embolism and wood density. Curiously we did see a positive correlation between hydraulic safety and density among positions just in the unfertilized treatment (Figure 4.2, Table 4.3). This suggests that the factors limiting vulnerability to embolism and density are affected by the environment, in this case possibly of amount of nitrogen available to the tree and thus the lower water use and demand of the unfertilized trees compared to the fertilized trees (Table 4.2, Ewers et al. 1999; Hubbard et al. 2004). The unfertilized trees may be on a tighter carbon and energy budget and thus experience more restrictions in xylem construction than trees with a greater resource supply. The tradeoff may also still exist at a larger scale, such as among species (Hacke et al. 2001; Oliveras et al. 2003), or at a smaller scale, such as only in juvenile wood, or in a comparison of juvenile and mature wood of the same position (Domec and Gartner 2002a). Most research concerning hydraulic safety involves only young branches and roots (including Hacke et al. 2001; Oliveras et al. 2003), whereas we only considered mature wood in each position, supporting the latter possibility of the vulnerability to embolism and density tradeoff existing only in juvenile wood.

Hydraulic efficiency and structural safety

Similar to Oliveras et al. (2003) and Bouffier et al. (2003) we found evidence for a tradeoff between specific conductivity and wood density among positions and growing environments (Table 4.3, Figure 4.3). Our data are also consistent with the differences between juvenile and mature wood described by Domec and Gartner (2002a); K_s increases with cambial age in juvenile wood (Stem 5 to Stem 25 and from Branch 7 to Branch 20) but density stays the same, however in mature wood K_s is relatively constant with cambial age while density increases. This in part explains the lack of a relationship between specific conductivity and density within every position. Correlations were evident for more positions in the fertilized treatment than in the unfertilized treatment suggesting this tradeoff is also influenced by environmental conditions and specifically that after fertilization density is more affected by anatomical features that regulate conductivity.

Hydraulic safety and efficiency

Our results demonstrated a large scale tradeoff between vulnerability to embolism and specific conductivity among positions and fertilization treatments in Douglas-fir. However, a within-position tradeoff was only evident for Root 42 and within treatments and positions only fertilized Root 42 showed the expected relationship (Table 4.3). These findings are consistent with other studies that have found a tradeoff only when multiple positions are included in the analysis (Kavanagh et al. 1999; Oliveras et al. 2003; McElrone et al. 2004) and suggest that there is some factor that influences the relationship between vulnerability to embolism and conductivity which is shared among positions. Within each position, however, this factor is overridden by small scale factors. We previously found that specific conductivity and vulnerability to embolism were both correlated to tracheid diameter and number of pits per cell among positions and treatments; however at a lower scale they were only related to the number of pits per cell in Roots 42 but not in Stem 52 or Branch 20 (chapter 3). Also, xylem in stems and branches must not only balance hydraulic safety and efficiency but

also mechanical safety differently from roots, possibly confounding a direct correlation between vulnerability to embolism and conductivity.

Sap velocity and structural safety

Due to decreasing tracheid diameter and conductivity with height, sap velocity in conifers is expected to be higher in branches than in lower parts of the tree (Philips et al. 2003; McCulloh and Sperry 2005), however in our trees Branch 20 V_s was lower than Stem 54 V_s and similar to Root 42 (Table 4.2). Typically for conifers sap flow profiles peak just inside of the outermost growth rings and decrease towards the center of the stem (Philips et al. 1996; Nadezhkina et al. 2002; James et al. 2003). Our branches had about 2 cm of sap wood, often just enough that sensors were not touching the heartwood. Because the radial sap flow profile Branch 20 would be much narrower than in Stem 52 or Root 42, it may be that the sensors in the branches measured the zone of peak flow as well as part of the profile where sap flow was much less. The recorded sap flow measurement would then come from not just the peak flow, but also from areas with much less sap flow. Clearwater et al. (1999) found that in cases where the sap flow along the length of the sensor is not constant, the reading from the sensor is very difficult to interpret because it is not exactly an average of the flow along the sensor length.

This may be one reason why we did not see a relationship between density and sap velocity, at least in the branches. The complete lack of a tradeoff in our data is in sharp contrast to the very distinct relationship ($r^2 = 94$) found in stems of the conifer *Dacrydium cupressinum* by Barbour and Whitehead (2003). The model developed by Roderick and Berry (2001) related V_s to $(1 - \text{density})^2$, however our results did not change when this term was used as the explanatory variable instead of density. The variation in V_s was generally much greater than the variation in wood density within each position (Figure 4.5), and it may be that a greater range of density is needed for the tradeoff to be seen in Douglas-fir.

Conclusion

Though connected through a continuous hydraulic pathway, positions within a tree can be anatomically and hydraulically very different (chapter 3). Anatomical features and xylem characteristics are not all correlated among positions within a tree (chapters 2 and 3), making it difficult or impossible to extrapolate from one position to another, especially from the young branch tips which tend to behave very differently from all other parts of the tree we measured (Figure 4.3). Juvenile wood, as found in branch tips and the top of the tree, fulfills a different functional role in trees than mature wood (Domec and Gartner 2002a), apparently able to function with more negative water potentials, which makes sense for young trees and tree parts with inefficient or little access to stored water. Another reason that plant parts may differ in physiological strategy is that wood produced by at plant tips (root, branch, and bole) may be able to track the environment more closely than older parts because of their fewer active growth rings and the relatively greater influence of a new growth ring on the part's performance. Differences in the specific structural and hydraulic demands on stems, roots, and branches of different ages and positions appear to affect the degree to which these different plant parts experience tradeoffs exist among the different functions. Our research has shown that there are both large- and small-scale influences that govern the degree to which xylem properties are related. At a large scale the existence of a tradeoff between two properties means that wood from all positions and environments must follow the same structural limitations, however these limitations may be overridden at lower scales if environmental influences or position-specific factors impose a more critical restriction on xylem structure. Trees are complex structures and by studying trees from the perspective of multiple scales we may better understand intricacies of the design criteria that govern xylem structure and function.

Thesis Conclusions

In this research trees were studied within the context of a biological hierarchy that included scales of positions within trees, trees within fertilization treatments, and treatments nested within one species. A statistical hierarchical model that reflected the multiple sources of variation and organization in the biological hierarchy was better supported by the data compared to a typical simple linear regression model that treated all observations as though they were independent and within only one scale. The hierarchical model explicitly related multiple scales in a single analysis, supplied a more informative description of the variation in the data, and provided more precise results. Because the slope estimates were more precise from the hierarchical model, additional differences were seen among positions compared to the simple linear regression model. The complex variance structure of the hierarchical model indicated that correlations of observations among positions were dependent on position type and fertilization treatment. This suggests that different parts of a tree do not all respond similarly to stimuli and although no substantial difference was seen within positions from different treatments, correlations among anatomy and xylem properties of positions were affected by the environment.

For almost every comparison made of xylem anatomy and xylem properties, the greatest variation was found among positions. Relationships between xylem anatomy and xylem properties were usually evident at large scales but were not always present at smaller scales, indicating that factors governing the relationship were different at each scale. Similarly, tradeoffs between xylem properties at a large scale were often not present at small scales. The interpretation of why there are relationships between xylem anatomy and xylem properties and tradeoffs among properties depends on the scale at which the relationship is seen. Large scale relationships, among all trees, treatments and positions, imply that all xylem responds similarly to the stimuli that govern the relationship regardless of environmental conditions and position function.

When a relationship is found just within treatments, the implication is that factors governing the relationship are dependent on environmental conditions and thus the relationship may have adaptive flexibility. Small scale relationships unique to each position reflect how positions respond differently to stimuli and are constructed to fulfill their respective functional roles. Relationships at the smallest scale of within positions and treatments indicate how positions may respond differently to environmental stimuli.

There are several possible extensions of this study for future research. The Bayesian hierarchical model developed in chapter 2 may be applied with minimal, if any, alterations to many other research questions in tree physiology that span multiple scales. Including additional levels of scale, e.g. two Douglas-fir populations such as coastal versus inland, additional species, or different ages of trees, would broaden the scope of inference and provide further insight into xylem structure and function at different scales. Furthermore, future research could consider different environmental conditions, such as windy versus sheltered, or treatments. Because very few differences were seen between fertilization treatments it would have been interesting to have measured foliar nitrogen content to know how effective the fertilization application had been. Different types of fertilizer could be tested to determine how nutrients other than nitrogen may have an effect on wood structure. Also, though there is ample data in the literature of vulnerability to embolism for small diameter branches and roots of Douglas-fir which could be compared to findings in this study, it would have been interesting to include vulnerability data from all seven positions. Measurements of vulnerability to embolism of the young stem, root, and branch sections were not made in this study due to time constraints but would have enhanced our dataset and provided a more complete illustration of within-tree trends in vulnerability to embolism.

This research has implications in tree physiology and in tree breeding for wood improvement and utilization. In tree physiology this work provides a context for

studies that include only one or two positions, and provides a platform from which further discussion of the role of hierarchies may be initiated. Considering the variation found among positions, the lack of correlation between young branches and roots with most other positions, and the fact that relationships among xylem anatomy and xylem properties in the young branches tended to be considerably different than in other positions, small diameter branches and roots may not be representative of the entire tree or of differences among trees. The fact that relationships among properties were different at each scale suggests that although there are some large scale factors that influence xylem structure and function equally throughout the tree, xylem is also built to specifications unique to each position. It is therefore important to note the limited scope of inference of studies that just include young branches and roots and to consider how these positions relate to the rest of the tree. This study also brings to light the importance of accounting for biological hierarchies in data analysis and interpretation. Hierarchical scales represent multiple sources of variation as well as the different levels at which trends in xylem anatomy and properties may be affected. Explicitly discussing the scale or scales at which research is conducted and analyzed is essential so that data interpretation and inferences are made at the correct scale.

The goal of tree breeding programs is to select and reproduce trees of superior qualities as defined by wood utilization needs. This research has shown that xylem properties are related and that optimizing one property may result in detrimental changes in another. High wood density is a widely desired quality; however if trees with high density also have low conductivity, as found among trees, treatments, and positions in this study, then trees with dense wood may suffer from low hydraulic efficiency and thus have lower growth rates. When breeding trees for wood utilization purposes, it is necessary to not only discover how to select for the desired wood qualities but also how changing those properties affects hydraulic architecture and thus growth rates, drought resistance, and water demand.

Trees are an important natural resource that provide many ecosystem services. They take in carbon dioxide, give off oxygen, filter water and runoff, prevent soil erosion, and help cycle nutrients within their environment. Wood in particular is used for many applications including fuel and building material. As we strive to understand how trees function and grow and how to better manage forests, it is important to gather information that is as accurate and precise as possible. This research has demonstrated that by addressing questions of xylem structure and function at multiple scales and analytically accounting for these scales insight may be gained into how structure governs function and how trees manage water relations.

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