AN ABSTRACT OF THE DISSERTATION OF

David R. Woodruff for the degree of Doctor of Philosophy in Forest Science
presented on July 16, 2008.

Title: Height-Related Trends in Structure and Function of Douglas-fir Foliage.

Abstract approved:________________________________________________

Frederick C. Meinzer        Barbara Lachenbruch

This dissertation investigated the impacts of tree height upon a range of
physiological and structural characteristics of Douglas-fir foliage; relationships
between structural and functional trends with height; and compensatory mechanisms
that mitigate height-related growth constraints. Height-related trends in foliar
physiological and anatomical characteristics were examined both within trees as well as
between trees of different heights. Emphasis was placed on discovering the mechanistic
nature of the observed growth constraints and adaptive strategies. This research was
conducted in the interest of enhancing our knowledge of the causes of age- and size-
related decline in forest productivity.

The research conducted for this dissertation explored several previously
uninvestigated mechanisms for size-related reductions in forest productivity that are
associated with tree foliar structure and function. Height-related trends in foliar turgor
associated with the gravitational hydrostatic pressure gradient were identified as well as
subsequent impacts on leaf and shoot morphology. Osmotic potential declined with height providing evidence of osmotic adjustment to offset the height-related decline in leaf turgor. This osmotic adjustment occurred only seasonally, and not during the spring when turgor maintenance is most important for leaf expansion. Gradients in leaf turgor were reflected in height-related trends in leaf and shoot morphology. Previous work has documented height-related trends in foliar morphological characteristics and earlier studies have examined osmotic adjustment as an adaptation to drought and salinity stress. Height-related trends in foliar morphological characteristics have typically been attributed to light gradients along vertical profiles. The work in this dissertation is the first to evaluate the effects of the gravitational component of water potential on the osmotic, morphological and growth characteristics of foliage along a height gradient in tall trees.

Trends in leaf hydraulic efficiency and safety along a height gradient were examined, and correlations between changes in leaf hydraulic conductance ($K_{\text{leaf}}$) and changes in stomatal conductance ($g_s$) were identified that provided evidence of a control mechanism for $g_s$. A consistent relationship was discovered between changes in $K_{\text{leaf}}$ and $g_s$ providing evidence that leaf tracheid embolism is associated with stomatal closure during periods of declining water availability. Hydraulic vulnerability curves of foliage collected at different heights indicated that increased height is correlated with reduced hydraulic efficiency and increased hydraulic safety. These opposing trends suggest the existence of a compensatory mechanism in foliage that functions to reduce hydraulic vulnerability at the expense of transport capacity. The research in this dissertation is the first to examine the dynamic relationships between leaf hydraulics
and stomatal behavior in temperate conifers, and the first to examine how these characteristics are affected by tree height.

Height-related trends in leaf xylem anatomical characteristics were examined and strong correlations were identified between these anatomical characteristics and the patterns of leaf hydraulic efficiency and safety that were observed in previous work for this dissertation. Theoretical estimates of leaf hydraulic efficiency ($K_{\text{leaf-theoretical}}$) based upon the Hagen-Poiseille equation and measured leaf tracheid anatomical characteristics were strongly correlated with laboratory measurements of $K_{\text{leaf}}$, providing further evidence of a causal relationship between height-related trends in both leaf tracheid anatomical properties and leaf hydraulic function. Earlier studies have documented connections between leaf anatomical characteristics and leaf physiological characteristics such as correlations between leaf architecture and gas exchange and leaf architecture and transport efficiency. The research in this dissertation however, is the first to examine the impact of tree height on leaf anatomical characteristics and associated shoot physiological properties.

Trends in gas-exchange along a height gradient were identified, independent of the immediate effects of path length and gravitational resistance. Photosynthesis under ambient [CO$_2$] declined with height in a manner that was consistent with height-related trends in mesophyll anatomy and independent of leaf nitrogen content. Analyses of mesophyll resistance from assimilation versus internal CO$_2$ concentration (A-C$_i$) curves indicated that height-related trends in mesophyll resistance were correlated with trends in both photosynthesis and mesophyll anatomy along a height gradient. Analyses of integrated and instantaneous water use efficiency indicated that height-related trends in
integrated water use efficiency are attributable to the effects of path length and gravity on stomatal behavior as opposed to a height-related trend in intrinsic foliar properties. The research in this dissertation is the first to isolate the influence of height-related trends in foliar structure on different gas exchange properties such as assimilation and mesophyll conductance, independent of the direct effects of vertical gradients in xylem tension on stomatal and photosynthetic physiology.
Height-Related Trends in Structure and Function of Douglas-fir Foliage

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David R. Woodruff, Author
CONTRIBUTION OF AUTHORS

Dr. Frederick Meinzer provided assistance in data collection for chapter 2 and financial support, assistance in conceptual creation, advice on data analysis and interpretation, and comments for chapters 2, 3, 4 and 5. Dr. Barbara Bond provided assistance in data collection, assistance in conceptual creation, advice on data analysis and interpretation, and comments for chapter 2. Dr. Barbara Lachenbruch provided use of microscopy facilities, advice on tissue preparation for microscopy analysis and comments for chapters 3, 4 and 5. Dr. Katherine McCulloh provided advice on tissue preparation for microscopy analysis, assistance in Cryo-SEM sample preparation, advice on data analysis and interpretation, analysis of Cryo-SEM images and comments for chapter 3. Dr. Jeffery Warren provided assistance in Cryo-SEM sample preparation, advice on data analysis and interpretation and comments for chapter 3. Dr. Daniel Johnson provided stomatal counts, advice on data analysis and interpretation, and comments for chapter 5.
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CHAPTER 1. INTRODUCTION

Carbon in Forest Ecosystems

Information about age- or size-related trends in forest ecological processes can lead to changes in forest management practices. Since continued atmospheric CO$_2$ enrichment is projected to have substantial impacts upon climate throughout the world (IPCC 2007), improving our understanding of growth dynamics of forest ecosystems has the potential for profound practical importance. Human activity is changing the chemical composition of trace gases in the earth’s atmosphere resulting in global changes in the earth’s climate (McCarthy et al. 2001; IPCC 2007). Climate change is no longer just an issue of interest for the scientific community. It has become an issue of widespread public concern and a key factor in policy formation for many bodies of government throughout the world (Burton et al. 2006). The feedback dynamics of carbon exchange with forest ecosystem responses to climatic change are critical to the understanding and prediction of global change. The world's vegetation absorbs approximately 60 billion tons of carbon by photosynthesis annually and releases a similar amount through respiration (Schlesinger 1997). Most of the terrestrial
photosynthesis and respiration is carried out by ecosystems that produce woody material, such as forests. Forest ecosystems account for more than 75% of the carbon stored in terrestrial ecosystems and approximately 40% of the carbon exchange between the atmosphere and the terrestrial biosphere each year (Schlesinger 1997). Carbon assimilation sets the upper limit on the amount of carbon that can be sequestered in the biomass of forest ecosystems. Given the magnitude of forest productivity, even a small difference in assimilation could represent a substantial impact upon terrestrial carbon storage.

**Size-Related Decline in Forest Productivity**

It would be difficult to overstate the importance of the role that foliage plays in forest ecosystems. As the principle photosynthetic tissue of plants, leaves have immense influence upon forest productivity. Foliar physiology is substantially influenced by its anatomy (Pachepsky *et al.* 1995) and variability in foliar structure can lead to a diversity of leaf functional characteristics, even within a single tree species (Norman & Jarvis 1974; Kira 1975; Tadaki 1977; Oker-Blom 1984; Leverenz & Hinckley 1990; Carter & Smith 1985; Guttschick 1999; Stenberg *et al.* 2001). Because of its relatively short lifespan, foliage has the potential to adjust to changing conditions relatively rapidly. Although fairly regular patterns of light and water potential strongly influence phenological events, each cohort of leaves is produced under potentially different circumstances as a result of year-to-year variation in climate conditions or due to the ongoing development of the tree. These changes may have substantial
influence upon foliar structural characteristics and thus each generation of foliage may face new growth constraints. Investigating height-related trends in foliar structure and function thus represents an opportunity to evaluate not only growth limitations that are imposed by changing circumstances, but a tree’s ability to acclimate to and overcome developmental limitations that are associated with growth and ontogeny.

Leaf water potential ($\Psi_l$) affects growth directly because of the role that one of its components, turgor, plays in a variety of growth-related processes, including cell formation, expansion and metabolism (Cleland 1967; Boyer 1968; Hsiao et al. 1976; Gould & Measures 1977; Cosgrove et al. 1984; Ray 1987). $\Psi_l$ also directly affects stomatal conductance ($g_s$) and thus photosynthesis and growth (Jarvis 1976). In the absence of transpiration, the gravitational component of water potential contributes 0.01 MPa to the xylem tension gradient for every one meter increase in height (Scholander et al. 1965; Hellkvist, Richards & Jarvis 1974; Zimmermann 1983; Bauerle et al. 1999; Koch et al. 2004; but see Zimmerman et al. 2007). During transpiration, there is an additional increase in the tension gradient due to frictional resistance to flow through the xylem (Richter 1973; Hellkvist et al. 1974; Connor, Legge & Turner 1977; Bauerle et al. 1999). Thus, in tall trees, the impact of height on physiological processes regulated by water availability can be substantial. At the top of a 60-m-tall tree, the gravitational component of water potential alone will represent a 0.6 MPa reduction in leaf water potential. Leaf turgor and $g_s$ are likely to be reduced in taller individuals of the same species growing under comparable conditions, unless compensatory adjustments in water relations or leaf hydraulic architecture occur.
A number of mechanisms have been proposed as potential mitigating factors for the frictional component of the xylem tension gradient. Among these are reductions in leaf area relative to sapwood area (McDowell et al. 2002b), an increase in specific conductivity of sapwood ($K_s$) (Domec & Gartner 2002; 2003) and increased reliance on stored water (capacitance) with increasing tree size and height (Phillips et al. 2003). Trade-offs exist however that limit the effectiveness of these adaptive strategies. The hydrostatic component of xylem tension gradient is independent of tree hydraulics and therefore inevitable. Other adaptive strategies to mitigate loss of productivity include epicormic shoot development (Ishii, Ford & Kennedy 2007), and redistribution of soil water during periods of drought (Brooks et al. 2002).

The issue of age- or size-related decline in forest productivity is a fundamental problem to forest biologists and foresters. The decline is a major factor in setting the rotational age of a commercial forest, yet conclusive evidence for all the mechanisms responsible for the decline has yet to be discovered. Growth and aboveground accumulation of biomass follows a common pattern as tree size increases. Initial growth is slow and increases as leaf area accumulates. Productivity peaks when leaf area reaches its maximum and then declines as the trees within the stand continue to age (Assman 1970; Turner & Long 1975; Tadaki et al. 1977; Grier et al. 1981; Gholz & Fisher 1982; Grier et al. 1989; Long & Smith 1992; Ryan & Waring 1992). Figure 1.1. illustrates the classic relationship between stand age and aboveground productivity. After the period of maximum above ground wood production, productivity will decline 20-80% (Gower et al. 1996), or 30- 90% (Ryan et al. 1997).
over a period of years to centuries. Evidence for a definitive cause of this decline remains elusive however, and there is ongoing debate among researchers about the causes for this phenomenon (Ryan et al. 1997; McDowell et al. 2002; Barnard and Ryan 2003; Ryan et al. 2004; Ryan et al. 2006). Although no definitive cause of age-related decline in productivity has been determined, evidence for some causes has been identified in some cases. Proposed causes for age-related reductions in productivity include an increase in respiration relative to photosynthesis (Yoda et al. 1965; Whittaker & Woodwell 1967), reduced leaf area due to reduced nutrient availability (Binkley et al. 1995), or due to abrasion between tree canopies (Rudnicki et al. 2003), increased below-ground carbon allocation (Gower et al. 1996), genetics-related reductions in photosynthetic capacity induced by a finite capacity for repeated meristematic divisions (Haffner et al. 1991), changes in stand level nutrient use efficiency (Binkley et al. 1992) and reduced photosynthesis due to increased hydraulic resistance with increased tree height (Ryan & Yoder 1997). None of these proposed mechanisms are mutually exclusive, nor do they preclude any other mechanisms for size-related decline in productivity.

An increase in tree size is typically associated with increased age. As such these two factors confound each other and there has been uncertainty as to whether observed reductions in productivity have been a function of size, age, or some combination of both. Recent experimental studies have begun to separate the effects of extrinsic factors such as size from those of intrinsic factors such as age and emerging evidence suggests that ontogenetic trends in growth are mainly a function of tree size
(height) rather than age (Mencuccini et al. 2005; Bond et al. 2007; Martinez-Vilalta et al. 2007). Mencuccini et al. (2005) conducted grafting experiments in which they compared growth and gas exchange of trees of different ages and sizes. For the four species that they studied, they found that size, not age, accounted for the observed reductions in relative growth rates and net assimilation rates. Bond et al. (2007) conducted a similar study on Douglas-fir in which cuttings from old growth trees grafted onto rootstock exhibited dramatic increases in diameter growth and stem elongation suggesting that size, not age drives developmental changes in height growth of Douglas-fir. Martinez-Vilalta et al. (2007) measured growth and seasonal water use of old and young Scots pine growing in stands with differing recent management histories. Initial correlations of age with decreased growth efficiency disappeared when trees were released from competition.

**Douglas-fir as a model species**

Douglas-fir forests represent ideal systems in which to address the issues of age- or size-related decline in forest productivity. On the basis of above ground volume, Douglas-fir is the dominant tree species in the United States with nearly twice the volume of the next most common species, loblolly pine (Smith et al. 2002). It is also one of the tallest tree species of trees on earth (Carder 1995; Van Pelt 2001). The ability to more accurately quantify the height-related trends in the structure and function of Douglas-fir foliage could improve modeling capabilities for net primary productivity and net ecosystem productivity of this important ecosystem type. More
reliable models of forest and ecosystem productivity could improve our understanding of the role that forests may play as sources or sinks for atmospheric or terrestrial carbon. They could also improve our ability to predict how changes in climate will affect the carbon balance of forest ecosystems.

**Objectives**

The objectives of this dissertation were: 1) to characterize height-dependent variation in leaf tissue water relations and leaf expansion in order investigate the existence of a causal link between height-related reduction in shoot growth and the influence of the gravitational water potential gradient on leaf turgor; 2) to evaluate the role of compensatory osmotic adjustment in mitigating the impact of height-related trends in $\Psi_l$ on leaf turgor; 3) to evaluate the role of changes in leaf hydraulic architecture as a mechanism involved in the regulation of stomatal control of transpiration along a gradient of increasing height; 4) to determine the extent to which height influences foliar xylem anatomical features related to leaf hydraulic efficiency and vulnerability to tension-induced embolism; and 5) to evaluate the extent to which height imposes developmental constraints upon foliage that lead to intrinsic limitations for gas exchange.
References


CHAPTER 2. DOES TURGOR LIMIT GROWTH IN TALL TREES?

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Abstract

The gravitational component of water potential contributes a standing 0.01 MPa m\(^{-1}\) to the xylem tension gradient in plants. In tall trees, this contribution can significantly reduce the water potential near the tree tops. The turgor of cells in buds and leaves is expected to decrease in direct proportion with leaf water potential along a height gradient unless osmotic adjustment occurs. We used the pressure-volume technique to characterize height-dependent variation in leaf tissue water relations and shoot growth characteristics in young and old Douglas-fir trees to determine the extent to which growth limitation with increasing height may be linked to the influence of the gravitational water potential gradient on leaf turgor. Values of leaf water potential (\(\Psi_l\)), bulk osmotic potential at full and zero turgor, and other key tissue water relations characteristics were estimated on foliage obtained at 13.5 m near the tops of young (c. 25-year-old) trees and at 34.7, 44.2 and 55.6 m in the crowns of old-growth (c. 450-year-old) trees during portions of three consecutive growing seasons. The sampling periods coincided with bud swelling, expansion and maturation of new foliage. Vertical gradients of \(\Psi_l\) and pressure-volume analyses indicated that turgor decreased with increasing height, particularly during the late spring when vegetative buds began to swell. Vertical trends in branch elongation, leaf dimensions and leaf mass per area were consistent with increasing turgor limitation on shoot growth with increasing height. During the late spring (May), no osmotic adjustment to compensate for the gravitational gradient of \(\Psi_l\) was observed. By July, osmotic adjustment had occurred,
but it was not sufficient to fully compensate for the vertical gradient of $\Psi_l$. In tall trees, the gravitational component of $\Psi_l$ is superimposed on phenologically-driven changes in leaf water relations characteristics, imposing potential constraints on turgor that may be indistinguishable from those associated with soil water deficits.

**Key-words:** Douglas-fir; gravitational component of water potential; height growth; osmotic adjustment; pressure-volume curve; *Pseudotsuga menziesii*; turgor maintenance

**Introduction**

A great deal of attention has recently been focused on the mechanisms responsible for reduced growth in trees as they age and increase in height (Friend 1993; Yoder *et al.* 1994; Ryan & Yoder 1997; Magnani, Mencuccini & Grace 2000; McDowell *et al.* 2002). Much of this research has addressed the hydraulic limitation hypothesis, which proposes that reduced growth in aging and tall trees may be linked to reductions in leaf-specific hydraulic conductance, which in turn lead to reductions in stomatal conductance and therefore photosynthesis (Ryan & Yoder 1997). In addition to strictly hydraulic constraints that may require stomatal limitation of transpiration to regulate leaf water potential ($\Psi_l$) within a relatively narrow range, the gravitational component of $\Psi$ influences $\Psi_l$ whether or not transpiration is occurring.

In the absence of transpiration, the gravitational component of water potential should result in a standing xylem tension gradient of 0.01 MPa per meter increase in height (Scholander *et al.* 1965; Hellkvist, Richards & Jarvis 1974; Zimmermann 1983;
Bauerle et al. 1999). Thus, at the top of a non-transpiring 60-m-tall tree, leaf water potential should be at least 0.6 MPa more negative than foliage at ground level. When transpiration occurs, frictional resistances will lower leaf water potential even further (Hellkvist et al. 1974; Connor, Legge & Turner 1977; Bauerle et al. 1999). The turgor of cells in leaves and buds is expected to decrease in direct proportion with the leaf water potential if the osmotic potential of these cells remains constant. A variety of growth-related processes, including cell formation, expansion and metabolism are dependent on turgor pressure and cell volume (Boyer 1968; Hsiao et al. 1976; Gould & Measures 1977; Ray 1987). However, osmotic adjustment, the active accumulation of symplastic solutes, could maintain turgor and cell volume and may serve to sustain growth and photosynthetic gas exchange with increasing tree height. Nevertheless, compensatory osmotic adjustment may only partially offset reductions in growth with increasing height if it results in a substantial amount of resources being diverted from leaf expansion towards production and accumulation of osmolytes for turgor and volume maintenance.

Osmotic adjustment has been well documented as an adaptation to drought and salinity stress (Hsiao et al. 1976; Osonubi & Davies 1978; Turner & Jones 1980; Morgan 1984; McNulty 1985; Heuer & Plaut 1989; Pereira & Pallardy 1989; Ranney, Bassuk & Whitlow 1991; Rieger 1995). However, we know of no studies that have attempted to evaluate the effects of the gravitational component of water potential on the osmotic, morphological and growth characteristics of foliage along a height gradient in tall trees. The objective of this study, therefore, was to characterize height-
dependent variation in leaf tissue water relations and morphological characteristics in young and old Douglas-fir trees to determine the extent to which shoot growth limitation with increasing height may be linked to the influence of the gravitational water potential gradient on leaf turgor. The pressure-volume technique was employed at various phenological stages during two consecutive growing seasons to assess water relations characteristics of foliage sampled along a height gradient from approximately 14 to 57 meters.

Materials and Methods

Field Sites

The two study sites were located within 5 km of each other in the Wind River Basin of southwestern Washington, U.S.A. The site with the tallest trees was a 450-yr-old Douglas-fir/western hemlock (*Pseudotsuga menziesii* (Mirb.) Franco/*Tsuga heterophylla* (Raf.) Sarg.) forest located at the Wind River Canopy Crane Research Facility (WRCCRF) within the T.T. Munger Research Natural Area in the Gifford Pinchot National Forest (Shaw *et al.* 2004). The stand has a mean density of about 437 trees ha$^{-1}$ with a mean height of 62 m. The WRCCRF contains a 75-m-tall construction tower crane with an 85-m jib that provided access to the crowns of the study trees via a suspended gondola. The second site, located in the Wind River Experimental Forest, was clear-cut in 1976 and planted with Douglas-fir seedlings in 1978 (McDowell *et al.* 2002; Phillips *et al.* 2002). During the study period, the mean density was about 1529
trees ha\(^{-1}\) with a mean height of 16 m. A 19.5-m-tall scaffold tower provided access to the crowns of the study trees.

The altitudes of the younger and older stands are 561 and 371 m, respectively. The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.5 m, over 70% of which falls as snow, with a dry season from June through September. Very low precipitation between June and September (~119 mm) typically leads to drought conditions. The mean annual temperature is 8.7°C with means of 0°C in January and 17.5°C in July. The soils are well-drained and of volcanic origin.

**Sampling and Measurements**

Branch sampling locations were established near the tops of five 14–17-m-tall Douglas-fir trees in the younger stand and at three heights within the crowns of five 54-59-m-tall Douglas-fir trees in the old-growth stand. Mean height for the sampling locations was 13.5 m at the tops of the young trees, and 34.7 m, 44.2 m and 55.6 m for the lower, middle and upper third of the old trees, respectively. Samples were collected during the spring (May 1), early summer (July 5), and late summer (August 21) of 2001, during the spring (May 2) and early summer (June 27) of 2002, and during the early summer (June 24) of 2003.

Bulk leaf water relations characteristics were determined from pressure-volume analysis (Scholander *et al.* 1965; Tyree & Hammel 1972). Twigs approximately 10-15 cm long were excised early in the morning prior to significant transpirational water
loss, sealed in plastic bags with moist paper to prevent desiccation, and then stored in a refrigerator until pressure-volume curve determinations began, within 2-6 h of excision. The twigs were not re-hydrated artificially to avoid rehydration-induced artifacts reported for a number of woody species (Bowman & Roberts 1985; Meinzer et al. 1986; Evans, Black & Link 1990). Pressure-volume curves were initiated by first determining the fresh weight of the twig, and then measuring its water potential ($\Psi_1$) with a pressure chamber (PMS Instrument Company, Corvallis, OR). Although early morning sampling of foliage was not always conducted prior to dawn due to logistical constraints associated with the use of the canopy crane, the initial value of $\Psi_1$ was considered to be equivalent to predawn $\Psi_1$ and is referred to hereafter as “predawn” for purposes of convention. Alternate determinations of fresh weight and $\Psi_1$ were repeated during slow dehydration on the laboratory bench until values of $\Psi_1$ exceeded the measuring range of the pressure chamber (-4.0 MPa). The inverse of water potential was plotted against relative water content to create a pressure-volume curve. A characteristic pressure-volume curve consists of an initial curvilinear and sharply declining series of points, followed by a more linear series with a less severe decline. The point of transition between these two portions of the graph represent turgor loss point for the sample (Tyree & Hammel 1972). The key tissue water relations parameters determined from pressure-volume analysis were osmotic potential at full and zero turgor, symplastic water fraction, and the moles of osmotically active solutes in the sample ($N_s$) calculated according to Tyree et al. (1978),

$$N_s = \psi_{fr}^{100}V_{100}/(RT)$$

(2.1)
where $\psi_{1100}$ is the osmotic potential at full hydration, $V_{100}$ is the osmotic (symplastic) water volume at full hydration, $R$ is the universal gas constant and $T$ is the Kelvin temperature at which the pressure-volume curve was determined. The saturated weight of each sample, necessary for calculating tissue relative water content, was estimated by fitting a linear regression to a plot of sample fresh weight against $\psi_1$ for points above the turgor loss point and extrapolating to zero $\psi_1$. On some sampling dates, twigs were excised from all sampling heights at midday for determination of midday $\psi_1$. Values of turgor at each sampling height were estimated from the difference between values of $\psi_1$ in situ and the value for osmotic potential derived from the pressure-volume curve.

During June 2003, pressure-volume analyses were performed on twig samples bearing only current year, expanding foliage to determine whether height-dependent trends in tissue water relations parameters were similar to those previously observed in old-growth trees using twig samples bearing multiple years of foliage. In addition, bulk foliage osmotic potential was determined with a vapor pressure osmometer (Vapro 5520 Wescor, Inc., Logan, Utah) to compare values obtained with this direct method to those derived from pressure-volume analysis. Sample twigs were initially frozen at –80° C to facilitate subsequent sap extraction. Current year foliage was removed from frozen sample twigs, placed in a sleeve of Tygon tubing, which was squeezed with a bench vise to express foliage sap. A 10 $\mu$l sample of sap was immediately obtained for determination of osmotic potential.
Leaf and branch growth and morphological characteristics were determined on foliage samples collected at the same locations as those used for the pressure-volume curves and osmometer samples. Needle linear dimensions and branch annual extension increments were determined with digital calipers. Leaf mass per area was obtained by determining needle areas with a scanner and ImageJ version 1.27 image analysis software (http://rsb.info.nih.gov/ij/), then weighing the needles after oven-drying.

Results

Both predawn and midday $\Psi_1$ decreased in a linear fashion with increasing height (Fig. 2.1). The vertical water potential gradient was substantially steeper than that expected from the gravitational component alone (Fig. 2.1, dashed line). Even though values of $\Psi_1$ for the lowest sampling height (13.5 m) were obtained from younger trees in a nearby stand, single regression lines adequately described the relationship between $\Psi_1$ and height.

Height-dependent trends in the characteristics of leaf water relations varied seasonally and from year to year. In May of 2001, osmotic potential at full turgor increased with height ($p = 0.02$; Fig 2.2), whereas in May of 2002 no vertical trend in osmotic potential was detected (Fig. 2.2). In both July and August 2001, osmotic potential decreased linearly with increasing height ($p \leq 0.01$) at a mean rate consistent
with the gravitational component of water potential (Fig. 2.2). A similar decrease in osmotic potential with increasing height was observed in June of 2002 (Fig. 2.2). As with the vertical trends in $\Psi_l$, single regression lines adequately described the dependence of osmotic potential on height when data for young and old trees were combined. Seasonal changes in vertical profiles of symplast solute content on a tissue dry weight basis indicated that seasonal changes in vertical profiles of osmotic potential were associated with true osmotic adjustment (Fig. 2.3). In May of 2001, symplast solute content decreased by 30% between 13.5 and 55.6 m, whereas by July solute content was greater at all heights and increased by 15% between 13.5 and 55.6 m.

Vertical trends in turgor varied seasonally in a manner consistent with those of osmotic potential. In May of 2001, when osmotic potential increased slightly with height, predawn turgor decreased with increasing height ($p < 0.02$) at a rate similar to that of the gravitational component of $\Psi_l$ (Fig. 2.4). At midday, the decline in turgor with height was even steeper than it was before dawn, with estimated turgor values near zero at the 55.6 m sampling height (Fig. 2.4). Similarly, in May of 2002, when osmotic potential was independent of height, the vertical decline in predawn turgor ($p = 0.03$) was c. 0.015 MPa m$^{-1}$ (Fig. 2.4). Although the vertical decline in predawn turgor was substantially smaller later in the season in both 2001 and 2002 (Fig. 2.4), it still amounted to about 0.25 MPa between 13.5 and 55.6 m MPa in July and August of 2001.
Height-dependent trends in osmotic potential and turgor for twigs bearing only current-year expanding foliage were similar to those of twigs bearing multiple years of foliage. In June 2003, osmotic potential at zero turgor decreased linearly with increasing height \((p<0.01)\) at a rate similar to that of the gravitational component of \(\Psi_l\) (Fig. 2.5a), whereas osmotic potential at full turgor was independent of height. Predawn turgor decreased with increasing height \((p=0.067; \text{ Fig. } 2.5b)\). Midday turgor showed the same trend with height, although not significantly. The values of foliage osmotic potential determined by osmometry (Fig. 2.5a, squares) were slightly, but not significantly more negative than values of osmotic potential at full turgor obtained from pressure-volume curves.

Leaf and branch growth and morphological characteristics also exhibited marked changes along a vertical gradient. In the old-growth trees, leaf mass/area increased sharply with height (Fig. 2.6). Strong vertical trends of decreasing needle length, needle width, and branch extension growth with increasing height were also observed (Fig. 2.7).

**Discussion**

Vertical gradients of \(\Psi_l\) and pressure-volume analyses of Douglas-fir twigs collected at different heights suggested that turgor decreased with increasing height, particularly during the late spring when vegetative buds began to swell. The decline in turgor with increasing height was evident both near dawn and at midday, although the rate of change with respect to height was greater at midday due to a steeper water
potential gradient. Vertical trends in leaf physical and morphological characteristics were consistent with the hypothesis that reduced turgor may limit leaf expansion at greater heights. During the late spring, no osmotic adjustment to compensate for the gravitational gradient of $\Psi_l$ was observed. In fact, symplast solute content actually decreased with increasing height in May. By July, osmotic adjustment had occurred, but it was not sufficient to fully compensate for the vertical gradient of $\Psi_l$, which was nearly identical to the gradient observed in an earlier study conducted in the old-growth stand (Bauerle et al. 1999). It should be noted that although some of our samples used to estimate osmotic potential, and therefore turgor, included both mature and expanding foliage, subsequent analyses on samples containing only current year expanding foliage showed similar results (Fig. 2.5) thus validating the use of twig samples containing multiple years of foliage. This is not surprising given the fact that in developing needles, which constituted a lesser fraction of the multi-year sample weight early in the season, solute concentration and turgor were likely to have been considerably lower than in older needles. By June and July, expanding foliage made up half or more of the foliage on twig samples.

Seasonal trends in leaf water relations characteristics, particularly osmotic potential, have been reported in previous studies (Tyree et al. 1978; Davies & Lakso 1979; Ritchie & Shula 1984; Kubiske & Abrams 1991) and seem to be related to stages of phenological development. In a previous study of Douglas-fir seedlings, leaf osmotic potential began to increase during late winter and early spring, reaching maximum (least negative) values between April and May, then rapidly decreasing to a
new stable minimum value by mid-June (Ritchie & Shula 1984). The timing of water stress in relation to phenological stage is important in terms of internal competition for water (Hale & Orcutt 1987). In tall trees, the gravitational component of $\Psi_l$ is superimposed on phenologically-driven changes in leaf water relations characteristics, imposing potential constraints on turgor that may be indistinguishable from those associated with soil water deficits. Even near dawn, the vertical $\Psi_l$ gradient was markedly steeper than the expected gravitational gradient in the absence of transpiration (Fig. 2.1), suggesting that nocturnal stomatal opening and transpiration may have prevented $\Psi_l$ from fully equilibrating with that of the soil during the night (Reed & Waring 1974; Meinzer 1982; Donovan, Richards & Linton 2003).

When buds swell in Douglas-fir, foliar sugars are metabolized more rapidly than they are produced and therefore tend to be limited (Krueger & Trappe 1967; Billow, Matson & Yoder 1994). The rapid decline in osmotic solute concentration of previous years’ foliage of Douglas-fir and other evergreen conifers observed during early spring may be a manifestation of this phenomenon and suggests that sugars and other soluble carbohydrates may comprise a significant fraction of cellular osmolytes. The decline in symplast solute content of mature foliage with increasing height observed during May 2001 (Fig. 2.3), a period when buds are normally swelling, was also consistent with low availability of solutes relative to the demands of developing foliage. By July, when needles are normally fully expanded, symplast solute content was greater at all heights and increased with height, resulting in a doubling of solute content at 55.6 m between May and July. Billow et al. (1994) found that sugar
concentrations of unfertilized Douglas-fir foliage nearly doubled between May and October.

Height-dependent variation in needle length, width, leaf mass per area and shoot extension was consistent with diminished capacity for cell expansion with increasing height (Figs. 2.6 and 2.7). Trends in leaf morphological and physical characteristics with canopy position have been well documented, but are typically attributed to variation in the light environment (Niinemets & Tenhumen 1997; Sprugel, Brooks & Hinckley 1996; Ellsworth & Reich 1993). However, a recent study investigating the impact of shading and leaf water status upon leaf morphology along a height gradient within tall conifers reported a lack of any strong shading effect with decreasing height; consistent with the hypothesis that the gravitational component of leaf water potential causes specific leaf area to decrease with increasing height (Marshall & Monserud 2003). Similarly, the sharp decline in leaf mass per area with decreasing height observed in old-growth trees in the present study did not appear to be associated with height-dependent variation in the light environment because the light transmittance fraction measured in an earlier study in the same stand (Parker, Davis & Chapotin 2002) did not change appreciably in the height interval over which our foliage samples were collected (Fig. 2.6). Moreover, the length and width size of needles from young trees at 13.5 m, near the fully-illuminated tops, was substantially greater than that of needles at 55.6 m height in the old-growth stand (Fig. 2.7). In a study of changes in needle anatomy with tree age, Apple et al. (2002) found that needles from the tops of Douglas-fir saplings had a significantly greater percentage of
photosynthetic mesophyll tissue than needles from the tops of old-growth trees. Higher astrosclereid abundance and larger vascular cylinders in needles from old-growth trees were identified as probable causes of reduced photosynthetic cell area in needles from tall trees. However, mesophyll cell size, which was not reported in their study, may also have contributed to the patterns observed.

If environmental factors such as relative humidity, wind speed and temperature vary with canopy height, they might have effects on leaf water balance that are additive to the effects of the hydrostatic gradient, and the impact on leaf water balance might also affect the physicochemical properties of leaves. However, gradients in leaf mass per area have been noted even in the absence of any vertical environmental variation. Marshall and Monserud (2003) measured vertical trends in leaf mass per area of similar magnitude to those reported here in stands with densities sufficiently low as to result in little variability with height in such environmental parameters. Taken together, the preceding observations suggest that the gravitational component of water potential plays a dominant role in governing morphological changes in Douglas-fir needles along a height gradient.

**Conclusions**

Physiological constraints on turgor imposed by the gravitational component of plant water potential imply a series of negative feedbacks that limit leaf expansion and branch extension growth in tall Douglas-fir trees. Increasingly small annual increments of photosynthetically active leaf area not only limit the overall potential for
photosynthate production, but also reduce the allocation of photosynthate to growth of other organs because of the greater investment of photosynthate required to produce a given amount of leaf area as height increases. The osmotic adjustment required to maintain turgor as height increases also implies an enhanced expenditure of energy to produce and maintain a given amount of photosynthetic surface area. Energy is expended and photosynthate diverted in the production of organic solutes, and in the uptake, accumulation and sequestering of inorganic solutes. Future research to identify and quantify the principal solutes involved in this osmotic adjustment would contribute to a more complete understanding of its energy requirements.

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References


Figure 2.1. Predawn (open symbols) and midday (filled symbols) leaf water potentials measured along a height gradient on young (triangles) and old (circles) trees in July 2001. Bars represent standard errors for measurements on three samples at each location from each of five trees ($n = 15$). The dashed lines represent the theoretical gravitational gradient in the absence of transpiration (-0.01 MPa m$^{-1}$).

slope = -0.017 MPa m$^{-1}$

slope = -0.018 MPa m$^{-1}$
Figure 2.2. Leaf osmotic potential at full (filled symbols) and zero (open symbols) turgor as a function of height on young (triangles) and old (circles) trees during the 2001 and 2002 growing seasons. Bars represent standard errors for measurements on one sample from each of five trees ($n = 5$).
Figure 2.3. Symplast solute content on a dry matter basis ($N_s/DM$) as a function of height on young (triangles) and old (circles) trees for May and July 2001. Bars represent standard errors for measurements on one sample from each of five trees ($n = 5$).
Figure 2.4. Predawn (filled symbols) and midday (open symbols) turgor as a function of height on young (triangles) and old (circles) trees during the 2001 and 2002 growing seasons. Midday values were not available for sampling sessions when midday water potentials were not able to be collected. Bars represent standard errors for measurements on one sample from each of five trees ($n = 5$).
Figure 2.5. Water relations characteristics of expanding foliage as a function of height on old-growth trees during the 2003 growing season. (a) Osmotic potential at full (filled circles) and zero (open circles) turgor estimated from pressure-volume curves and osmotic potential of sap expressed from frozen and thawed needles (squares) determined by vapor pressure osmometry. (b) Predawn (filled circles) and midday (open circles) turgor.
Figure 2.6. Leaf mass per area and light transmittance fraction in relation to height for five old-growth Douglas-fir trees accessible from the canopy crane. Light transmittance data are from Parker et al. 2002.
Figure 2.7. Needle length and width in relation to height for the 2002 cohort of foliage and branch extension growth in relation to height for the 2003 cohort of foliage on the five young and the five old-growth Douglas-fir trees accessible from the canopy crane. Bars represent standard errors for measurements on 10 needles from each sampling location in needle dimension plots and standard errors for measurements on five twigs for branch extension plot.
CHAPTER 3. IMPACTS OF TREE HEIGHT ON LEAF HYDRAULIC ARCHITECTURE AND STOMATAL CONTROL IN DOUGLAS-FIR

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Abstract

This study investigated the mechanisms involved in the regulation of stomatal closure in Douglas-fir and evaluated the potential impact of compensatory adjustments in response to increasing tree height upon these mechanisms. In the laboratory we measured leaf hydraulic conductance ($K_{\text{leaf}}$) as leaf water potential ($\Psi_l$) declined for comparison with in-situ diurnal patterns of stomatal conductance ($g_s$) and $\Psi_l$ in Douglas-fir across a height gradient, allowing us to infer linkages between diurnal changes in $K_{\text{leaf}}$ and $g_s$. A recently developed timed re-hydration technique was used in conjunction with data from pressure-volume curves to develop hydraulic vulnerability curves for needles attached to small twigs. Laboratory-measured $K_{\text{leaf}}$ declined with increasing leaf water stress and was substantially reduced at $\Psi_l$ values of -1.34, -1.45, -1.56 and -1.92 MPa for foliage sampled at mean heights of approximately 20, 35, 44 and 55 m, respectively. In situ $g_s$ measurements showed that stomatal closure was initiated at $\Psi_l$ values of -1.21, -1.36, -1.74 and -1.86 MPa along the height gradient, which was highly correlated with $\Psi_l$ values at loss of $K_{\text{leaf}}$. Cryogenic scanning electron microscopy images showed that relative abundances of embolized tracheids in the central vein increased with increasing leaf water stress. Leaf embolism appeared to be coupled to changes in $g_s$ and may perform a vital function in stomatal regulation of plant water status and water transport in conifers. The observed trends in $g_s$ and $K_{\text{leaf}}$ in response to changes in $\Psi_l$ along a height gradient suggest that the foliage at the tops of tall trees is capable of maintaining stomatal conductance at more negative $\Psi_l$. This
adaptation may allow taller trees to continue to photosynthesize during periods of greater water stress.

**Key-words:** leaf hydraulic conductance, stomatal conductance, growth limitation, water stress, *Pseudotsuga menziesii*

**Introduction**

Stomata play a critical role in regulation of plant water status by balancing transpiration with dynamic changes in the capacity of the soil-plant hydraulic system to supply water to the leaves. Failure of stomata to sense and adequately respond to changes in the balance between water supply and evaporative demand would rapidly trigger a cascade of deleterious consequences including lethal dehydration of leaves and massive xylem embolism upstream from the leaves leading to total hydraulic failure. Despite decades of intensive research on the physiology of stomata, the specific mechanisms that permit coordination of stomatal conductance ($g_s$) with plant water balance and hydraulic properties remain elusive (Meinzer 2002; Franks 2004; Buckley 2005). Regardless of the mechanism, there does seem to be general agreement that stomata sense the water potential ($\Psi$) somewhere within the leaf (Comstock & Mencuccini 1998; Cochard et al. 2002). Nevertheless, there is ample evidence that stomatal responses to leaf water status are often modulated by root-derived chemical signals arriving in the transpiration stream (Davies & Zhang 1991; Tardieu & Davies 1993). Plant capacitance and its ability to confer elasticity on the relationship between
changes in transpiration and leaf water potential (Waring & Running 1978; Goldstein et al. 1998; Meinzer, James & Goldstein 2004) adds further complexity to interpretation of the mechanisms that govern stomatal behavior.

Leaf hydraulic architecture and its relationship to stomatal control of gas exchange have recently been investigated by a number of workers. Consistent with previous work documenting coordination of $g_s$ with overall plant hydraulic conductance (Meinzer & Grantz 1990; Meinzer et al. 1995; Hubbard et al. 2001), recent work has shown that maximum $g_s$ and leaf hydraulic conductance ($K_{leaf}$) are also tightly coordinated within and across species (Brodribb & Holbrook 2004a; Brodribb et al. 2005). The xylem of leaves appears to be more vulnerable to embolism than that of the stems to which they are attached even though leaves comprise the terminal portion of the liquid water transport pathway causing their xylem to be subjected to greater tension than in stems (Brodribb & Holbrook 2003; Brodribb et al. 2003; Bucci et al. 2003). The comparatively high vulnerability to embolism in relation to tension experienced in leaf xylem results in substantial loss of $K_{leaf}$ on a regular basis, even under non-extreme environmental conditions (Bucci et al. 2003; Brodribb & Holbrook 2004b; Meinzer et al. 2004). To compensate for daily loss of $K_{leaf}$, leaves apparently have vigorous embolism repair mechanisms that restore xylem conductivity over periods of minutes (Trifilo et al. 2003) to hours (Zwieniecki et al. 2000; Bucci et al. 2003), which suggests that embolism repair occurs even while substantial tension is present in conducting xylem adjacent to the non-conducting xylem elements. In addition, $g_s$ appears to be tightly coupled to shifts in the dynamic balance between leaf
embolism formation and repair throughout the day (Brodribb & Holbrook 2004b; Meinzer et al. 2004). Taken together, these observations imply that daily cycles of embolism in leaves may be an inherent component of the stomatal regulatory system rather than a phenomenon to be avoided at all costs.

In tall trees, the impact of height on physiological processes regulated by water availability must be taken into account. In the absence of transpiration, the gravitational component of water potential results in a xylem tension gradient of about 0.01 MPa per meter increase in height. When transpiration is occurring, frictional resistances come into play, increasing the vertical tension gradient as a function of the flow rate, path length and xylem characteristics. Compensatory adjustments in tree hydraulic architecture with increasing height can diminish the frictional component of the tension gradient (McDowell et al. 2002a, b; Phillips et al. 2003; McCulloh & Sperry 2005), but the hydrostatic component is independent of tree hydraulics and therefore unavoidable. In tall conifers, reduced shoot expansion with increasing height appears to be largely a consequence of reduced turgor resulting from insufficient osmotic adjustment to fully compensate for the vertical increase in tension (Koch et al. 2004; Woodruff et al. 2004). Steadily declining $\Psi_l$ along a tree height gradient also has implications for stomatal control of leaf gas exchange. Mean $g_s$ and therefore photosynthesis are likely to be lower in taller than in shorter individuals of the same species growing under similar conditions, unless compensatory adjustments in leaf hydraulic architecture and water relations occur.
Here we use Douglas-fir as a model species to study the impact of increasing tree height on leaf hydraulic architecture and its relationship to stomatal control of transpiration. Douglas-fir is well suited for this type of study because it can attain heights greater than 100 m and the crowns of old-growth specimens can span a vertical distance of over 30 m. Moreover, if $K_{\text{leaf}}$ is found to fluctuate diurnally as a result of embolism and reversal, it can be unambiguously concluded that tracheid refilling occurs in the presence of substantial xylem tension because of the heights involved. We know of no other studies on dynamic relationships between leaf hydraulics and stomatal behavior in temperate conifers, nor of how these characteristics are affected by tree height.

**Materials and Methods**

**Field sites and sampling heights**

The two study sites were located within 5 km of each other in the Wind River Basin of southwestern Washington, U.S.A. The site with the tallest trees was a 450-yr-old Douglas-fir /western hemlock (*Pseudotsuga menziesii* (Mirb.) Franco/*Tsuga heterophylla* (Raf.) Sarg.) forest located at the Wind River Canopy Crane Research Facility (WRCCRF) within the T.T. Munger Research Natural Area in the Gifford Pinchot National Forest (Shaw *et al.* 2004). The stand has a mean density of about 437 trees ha$^{-1}$ with a basal area of 82.9 m$^2$ ha$^{-2}$. The WRCCRF contains a 75-m-tall construction tower crane with an 85-m jib that provided access to the crowns of the study trees via a suspended gondola. The second site, located in the Wind River
Experimental Forest, was clear-cut in 1976 and planted with Douglas-fir seedlings in 1978 (Phillips et al. 2002). During the study period, the mean density was about 1529 trees ha\(^{-1}\) with a mean height of approximately 23 m. A 25-m-tall scaffold tower provided access to the crowns of the study trees. The elevations of the younger and older stands are 561 and 371 m, respectively.

The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.2 m, much of which falls as snow, with a dry season from June through September. Very low precipitation between June and September (~119 mm) typically leads to drought conditions in the upper portion of the soil profile, but readily available soil water remains accessible to Douglas-fir roots at depths greater than about 1 meter throughout the summer dry period (Warren et al. 2005; Meinzer, Warren & Brooks 2006). The mean annual temperature is 8.7°C with means of 0°C in January and 17.5°C in July. The soils are well-drained and of volcanic origin.

Branch measurement and sampling locations were established near the tops of three 22–25-m-tall Douglas-fir trees in the younger stand; and at bottom-, mid- and upper-canopy positions in three 57-59-m-tall Douglas-fir trees in the old-growth stand. Mean height for the sampling locations was 20.0 m near the tops of the young trees, and 33.6, 44.0 and 55.4 m in the old trees. Sampling locations were deliberately located within trees of different height classes (old-growth and young) in order to rule out the possibility of height-related trends in leaf hydraulic architecture and stomatal behaviour being driven by confounding factors such as light transmittance through the canopy.
Leaf water status and stomatal conductance

Measurements of $\Psi_1$ were conducted with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA), and stomatal conductance ($g_s$) with a steady-state porometer (LI-1600, Li-Cor Inc., Lincoln, NE, USA). Diurnal measurements of $\Psi_1$ and $g_s$ were conducted concurrently at least every 30 minutes on two to three separate trees per site. Measurements of $g_s$ were conducted repeatedly on the same foliage over the entire diurnal period and destructive measurements of $\Psi_1$ were conducted on nearby adjacent foliage. Measurements commenced prior to dawn and continued until after 1600 standard time at the young site and until wind conditions created safety concerns in the crane gondola at the old site; typically between 1400 and 1500 standard time. Measurements were conducted on July 20th and September 1st, 2005 at the old site and on July 23rd and September 23rd, 2002 at the young site.

Measurement of $K_{\text{leaf}}$

The methods used to determine leaf hydraulic conductance were adapted from Brodribb & Holbrook (2003) and they involve the use of the following equation derived from the relationship between re-hydrating a leaf and the recharging of a capacitor:

$$K_{\text{leaf}} = C \ln [\Psi_0 / \Psi_f]/t,$$  \hspace{1cm} (3.1)
where: $K_{\text{leaf}}$ = leaf hydraulic conductance, $C$ = capacitance, $\Psi_o$ = leaf water potential prior to re-hydration, $\Psi_f$ = leaf water potential after re-hydration, $t$ = duration of re-hydration.

Branches approximately 30-40cm long were collected from trees early in the morning prior to significant transpirational water loss and placed in plastic bags with moist paper towels, and stored in a refrigerator. Over the next three days, measurements of leaf water potential were conducted on excised twigs (~10-15cm long) initially ($\Psi_o$) and after a period of re-hydration of $t$ seconds ($\Psi_f$). $K_{\text{leaf}}$ measurement samples were collected from the three separate trees on July 22$^{\text{nd}}$, 28$^{\text{th}}$, and August 24$^{\text{th}}$, 2004. De-ionized water used for re-hydration of $K_{\text{leaf}}$ samples was de-gassed by vacuuming for a minimum of 2 hours. Water temperature was consistently between 21 and 23 degrees C.

The influence of light level during re-hydration was assessed by conducting a series of $K_{\text{leaf}}$ measurements at varying levels of light intensity and was found to be negligible. Some $K_{\text{leaf}}$ samples underwent periods of hydration prior to initial measurements of $\Psi_1$ in order to obtain a wider range of initial values of $\Psi_1$. Periods of re-hydration following initial $\Psi_1$ were variable and not recorded.

$C$ was determined on an individual tree basis from the slope of the relationship between relative water content and $\Psi_1$ obtained from pressure-volume curves (Table 3.1). Pressure-volume analyses (Scholander et al. 1965; Tyree & Hammel 1972) were conducted on branchlets approximately 10 cm long during August of 2001. These samples were excised early in the morning prior to significant transpirational water loss, sealed in plastic bags with moist paper to prevent desiccation, and then stored in a
refrigerator within 1-4 h of excision. Pressure-volume curves were initiated by first
determining the fresh weight of the twig, and then measuring \( \Psi_l \) with a pressure
chamber (PMS Instrument Company, Corvallis, OR). Alternate determinations of fresh
weight and \( \Psi_l \) were repeated during slow dehydration of the twig on the laboratory
bench until values of \( \Psi_l \) exceeded the measuring range of the pressure chamber (-4.0
MPa). The inverse of water potential was plotted against relative water content to create
a pressure-volume curve. For normalizing \( C \) on a leaf area basis, leaf areas of the
branchlets were obtained with a scanner and ImageJ version 1.27 image analysis
software (http://rsb.info.nih.gov/ij/).

**Cryo-scanning electron microscopy**

To visually determine if observed declines in \( K_{\text{leaf}} \) were associated with
increasing frequency of embolized tracheids, cryo-scanning electron microscopy (cryo-
SEM) was performed on needle samples of varying levels of dehydration. Branches
approximately 30-40cm long were collected from old growth tree 3013 on January 25th,
2006, placed in plastic bags with moist paper towels, and stored in a refrigerator. Over
the next three days, branchlets (~10-15cm long) were removed from the refrigerator,
placed in dark drawers, and allowed to dehydrate for varying amounts of time.
Branches were in the light for as short a time as possible before sampling. This ensured
all needles were equilibrated to the same water potential and prevented tension
gradients from developing within the branches, which has been shown to create
embolisms as samples are frozen in liquid nitrogen (Cochard et al. 2000). For branches
over a range of water potentials (well-hydrated to water-stressed), 2-3 needles were removed with a razor blade, inserted with forceps into a fracture rivet filled with Tissue-Tek (Electron Microscopy Sciences, Hatfield, PA, USA), and plunged into liquid nitrogen. Once the sample reached liquid nitrogen temperatures (i.e., when the boiling stopped), it was inserted quickly into a pre-cooled vial and returned to the liquid nitrogen. While the needles were being frozen, the water potential of the branch was determined with a pressure chamber (PMS Instrument Company, Corvallis, OR).

The frozen leaf samples were shipped at liquid nitrogen temperatures to the cryo-SEM facilities at the University of British Columbia. There the fracture rivets containing the needles were mounted in a cryo-prep unit (K1270, Emitech USA, Houston, USA) and the needles were fractured by touching them with a metal prong. They were then sublimed for about 10 min while being viewed in the cryo-SEM (S4700 FESEM, Hitachi High-Technologies Corp., Berkshire, UK). Various images of each needle were made at magnifications ranging from 90-5000x. Empty tracheids within leaf vascular bundles were counted for each cryo-SEM image in order to obtain an estimate of the relative amount of leaf tracheid cavitation associated with varying levels of Ψ_l.

**Sap flow and crown conductance**

Variable length heat dissipation sap flow probes with a heated and reference sensor measuring length of 10 mm at the probe tip (James et al. 2002) were used to estimate sap flux at a stem height of approximately 2.5 m in a 1.4-m-diameter, 58-m-
tall Douglas-fir tree. For probe installation, two 38-gauge (2.58-mm-diameter) holes separated axially by 10 cm were drilled into the sapwood to a depth of 1.5 cm. The sensors were coated with thermally conductive silicone heat sink compound prior to insertion. All probes were protected from potential sun flecks by reflective insulation. Signals from the sap flow probes were scanned every minute and 10-min means were recorded by a data logger (CR10X, Campbell Scientific Corp., Logan, UT) equipped with a 32-channel multiplexer (AM416; Campbell Scientific). Concurrent differential voltage measurements across the copper thermocouple leads were converted to a temperature difference between the heated and reference sensor ($\Delta T$), which was converted to sap flux ($\nu$; g m$^{-2}$ s$^{-1}$) using the empirical calibration of Granier (1985):

$$\nu = 119 \, k^{1.231},$$

(3.2)

where:

$$k = (\Delta T_m - \Delta T)/\Delta T,$$

(3.3)

and where $\Delta T_m$ is the temperature difference when sap flux is assumed to be zero. Data were obtained on the predominantly clear days of 31 July and 21 and 28 August, 2002.

In large trees, hydraulic capacitance typically results in substantial and variable time lags in the response of sap flux near the base of the stem to changes in transpiration in the crown (Goldstein et al. 1998; Phillips et al. 2003). Data collected with a new set of sap flow probes installed in August, 2005 in three upper branches at a mean height of 54 m and near the base of the trunk provided a means of estimating crown transpiration from the earlier measurements of sap flux near the base of the same tree by using crown sap flux as a proxy for transpiration. Mean daily courses of sap
flux for the upper branches and trunk base were determined for a period of five clear days (20-24 August). The mean time courses were then normalized with respect to the corresponding maximum sap flux at each location and the ratio of normalized crown and basal sap flux was plotted in relation to time of day between 0700 and 1430 h, which corresponded to the time interval over which concurrent measurements of basal sap flux and Ψ₁ were obtained earlier. The ratio of normalized crown and basal sap flux followed an exponential decay trajectory (r² = 0.94), approaching unity by 1200 h as capacitive discharge to the transpiration stream diminished. This variable ratio constituted a time-dependent multiplier that was used to calculate crown transpiration on a unit sapwood area basis from basal sap flux at the times Ψ₁ was measured. Crown conductance (gₓ) on a unit sapwood area basis (mol m⁻² s⁻¹) was calculated by dividing the resulting values of crown transpiration by the corresponding values of vapour pressure deficit measured by a meteorological station at a height of 60 m on the canopy crane tower.

Results

Leaf hydraulic conductance followed a sigmoidal trajectory with declining Ψ₁ (Fig. 3.1). A three-parameter sigmoid function (y = a/1+e⁻^(x-x₀/b)) yielded r² values ranging from 0.69 to 0.85 for the dependence of K_leaf on Ψ₁. In the interest of identifying a functionally relevant connection between changes in K_leaf, Ψ₁ and control of gₓ, an objective means was employed for determining a critical value of K_leaf in relation to Ψ₁. We used an analysis similar to that proposed for description of
vulnerability curves by Domec & Gartner (2001). The value of $K_{\text{leaf}}$ representing the midpoint between the y-intercept of the sigmoid function and zero ($K_{\text{leaf}}$ midpoint, Fig. 3.2, dotted line) was selected for estimating the slope of the portion of the sigmoid curve containing the relatively rapid and nearly linear decline in $K_{\text{leaf}}$. After taking the derivative of the sigmoid function to calculate its instantaneous slope at the $K_{\text{leaf}}$ midpoint, the x-intercept of the resulting tangent was used as an objective estimate for the value of $\Psi_1$ at which $K_{\text{leaf}}$ had declined to its initial minimum value (Fig. 3.2, dashed line). This point, which corresponds to the transition from an abrupt, nearly linear decline in $K_{\text{leaf}}$ to a more gradual asymptotic decline, is hereafter referred to as $\Psi_1$ at minimum $K_{\text{leaf}}$. Diurnal measurements of $g_s$ and $\Psi_1$ showed a typical pattern of $g_s$ increasing in the early morning once sunlight reached the foliage and then declining sharply later in the morning (Fig. 3.3A). There was no consistent relationship between $g_s$ and $\Psi_1$ because $\Psi_1$ declined continuously as $g_s$ rose to its maximum, then declined. However, a threshold value of $\Psi_1$ corresponding to the point at which $g_s$ began to decline was estimated using diurnal data for each of the four sampling heights (Fig. 3.3B). This point is hereafter referred to as $\Psi_1$ at $g_s$ decline. When values of $\Psi_1$ at minimum $K_{\text{leaf}}$ and $g_s$ decline were plotted as functions of sampling height, clear height-dependent linear relationships were apparent (Fig. 3.4). The value of $\Psi_1$ at minimum $K_{\text{leaf}}$ decreased from -1.34 MPa at 20 m near the tops of young trees to -1.92 MPa at 55 m near the tops of old-growth trees. Similarly, in situ values of $\Psi_1$ at $g_s$ decline ranged from -1.21 MPA at 20 m to -1.86 MPa at 55 m. Values of $\Psi_1$ at minimum $K_{\text{leaf}}$ and $g_s$ decline thus spanned a similar range across the height gradient such that when plotted
against each other they did not significantly depart from a one-to-one relationship (Fig. 3.4, inset).

Simultaneous measurements of sap flow in upper branches and near the base of the trunk of a 1.4-m-diameter, 58-m-tall tree provided a means of estimating crown transpiration and vapor phase conductance from earlier measurements of basal sap flow in the same tree (see methods). A plot of $g_c$ against concurrent values of $\Psi_l$ measured on upper branches revealed biphasic relationship in which $g_c$ initially increased to a maximum value as $\Psi_l$ declined, then decreased with further reductions in $\Psi_l$. The threshold value of $\Psi_l$ at which $g_c$ began to decline was about -1.68 MPa, which was similar to the independently measured mean value of -1.65 MPa for $\Psi_l$ at decline in $g_s$ for the three old growth crown heights shown in Figure 3.4B. Note that crown conductance is expressed on a sapwood rather than leaf area basis in Figure 3.5 because the total crown leaf area was not estimated. However, using a leaf area-to-sapwood area ratio of about 0.4 m$^2$ cm$^{-2}$ as estimated by McDowell et al. (2002b) for Douglas-fir trees at the same study site, the maximum values of $g_c$ in Figure 3.5 are about 80 to 90 mmol m$^{-2}$ s$^{-1}$ on a leaf area basis, which compare favorably with the independent porometric measurements of $g_s$ (Fig. 3.3a).

Cryo-SEM images of needles snap-frozen in liquid nitrogen indicated that the relative abundance of water-filled versus embolized tracheids in the central vein varied as a function of needle water status. In a sample frozen at $\Psi_l = -0.12$ MPa, nearly all of the non-transfusion tracheids were water-filled (Fig. 3.6a). The prevalence of water-filled and presumably functional tracheids in Figure 3.6a was consistent with near
maximal values of $K_{\text{leaf}}$ at $\Psi_l = -0.12 \text{ MPa}$ (Fig. 3.1). In contrast, an image of a sample frozen when $\Psi_l$ was -2.10 MPa revealed an abundance of embolized tracheids (Fig. 3.6b). Corresponding values of $K_{\text{leaf}}$ were near zero when $\Psi_l$ was -2.10 MPa (Fig. 3.1).

Well hydrated needles frozen at $\Psi_l$ between 0 and -0.5 MPa had a mean number of empty tracheids of $3.4 \pm 1.1 \text{ SE}$ (Fig. 3.7). Needles frozen at $\Psi_l$ lower than -2.0 MPa had a substantially larger mean number of empty tracheids of $23.6 \pm 8.5 \text{ SE}$ ($p = 0.056, n = 16$). The vascular bundles of needles frozen at intermediate $\Psi_l$ between -0.5 and -1.25 MPa, and between -1.25 and -2.0 MPa had mean numbers of empty tracheids of $16.4 \pm 6.6 \text{ SE}$, and $26.6 \pm 7.6 \text{ SE}$, respectively. The nature of the relatively high error associated with empty tracheid numbers at low $\Psi_l$ can be attributed to the fact that needles frozen at $\Psi_l$ between -1.25 and -2.0, and below -2.0 MPa, fell into two distinct groups; one with relatively high numbers of empty tracheids, and one with relatively low numbers of empty tracheids (Fig. 3.7).

**Discussion**

Our results are consistent with the idea that increasing height above the ground imposes multiple constraints on the physiological functioning of leaves and other organs. In particular, features of hydraulic architecture and stomatal regulation of transpiration in Douglas-fir trees were finely tuned to vertical gradients in xylem tension. The slopes of vertical changes in the $\Psi_l$ at which critical minimum values of $K_{\text{leaf}}$ were attained and at which mid-morning stomatal closure began to occur were
indistinguishable from each other and from the vertical gradient of $\Psi_1$ previously measured in the same stand of trees (Bauerle et al. 1999; Woodruff et al. 2004). Furthermore, our data provide evidence for the mechanistic significance of height-dependent threshold values of $\Psi_1$ associated with partial stomatal closure to limit transpiration thereby dampening the diurnal decline in $\Psi_1$. Finally, cryo-SEM images suggested that tracheids in Douglas-fir needles are likely to embolize and refill on a daily basis during the growing season even under non-extreme environmental conditions.

**Compensatory adjustments to height**

The vertical gradient in $\Psi_1$ consists of a gravitational or hydrostatic component (-0.01 MPa m$^{-1}$) and a dynamic or frictional component influenced by a combination of xylem specific conductivity ($K_s$), sap velocity and path length. The hydrostatic component is unalterable and persists in the absence of transpiration, but a number of compensatory mechanisms can potentially mitigate the frictional component. These include increased $K_s$ (Domec & Gartner 2002; 2003), reductions in leaf area relative to sapwood area (McDowell et al. 2002b), and increased reliance on stored water (capacitance) with increasing tree size and height (Phillips et al. 2003). Nevertheless, trade-offs exist that constrain compensatory adjustments that could reduce the vertical gradient in xylem tension. For example, $K_s$ of sapwood near the base of the trunk increases with tree size in Douglas-fir (Domec & Gartner 2002), but $K_s$ of branch wood decreases with increasing height largely due to increases in tracheid pit membrane
resistance associated with decreasing membrane pore size necessary to diminish the risk of air-seeding in the presence of larger tensions in terminal branches near the top of the tree (Domec et al. 2006).

During the growing season, the vertical gradient in midday $\Psi_l$ of old-growth Douglas-fir trees at the study site is about -0.017 to -0.020 MPa m$^{-1}$ (Bauerle et al. 1999; Woodruff et al. 2004), which corresponds with vertical gradients in critical values of $\Psi_l$ associated with leaf hydraulic and stomatal function in the current study (-0.016 to -0.02 MPa m$^{-1}$, Fig. 3.4). $\Psi_l$ at minimum $K_{\text{leaf}}$ at an additional sampling height of 5 m was -1.05 MPa (data not shown as no concurrent data of $g_s$ and $\Psi_l$ were collected for this height). The inclusion of this point in the relationship between height and $\Psi_l$ at minimum $K_{\text{leaf}}$ left the slope of the relationship in Figure 4a unchanged at -0.016 MPa m$^{-1}$ ($r^2 = 0.95$). This convergence of vertical trends implies that there are functional links among these features that contribute to homeostasis in the operation of stomatal regulatory systems as tree height increases. The mechanisms responsible for increasing resistance to water stress-induced loss of $K_{\text{leaf}}$ with increasing height were not investigated, but may include changes in tracheid anatomical properties that could affect the tendency for tracheids to bend or collapse, as well as tracheid or pit anatomical properties which could confer greater resistance to air seeding.

$\Psi_l$ threshold for stomatal closure

Despite considerable empirical evidence that stomatal control of transpiration is mediated by some measure of plant water status, the specific locations at which the
signals are sensed, the exact nature of the signals and the means by which they are sensed remain somewhat elusive. The lack of a clear monotonic relationship between $g_s$ and $\Psi_l$ under field conditions (e.g. Fig. 3.3) has contributed to the problem. Recent efforts to explain the dynamic behavior of stomata have focused on models that include the interaction between fluctuating plant hydraulic conductance and water status, or gradients in water status within leaves, mediated by metabolic responses of guard cells (e.g. Buckley 2005; Franks 2005; Franks, Drake & Froend 2006).

It is well documented that $g_s$ and gas exchange are tightly coordinated with overall plant hydraulic conductance within and across species (Meinzer & Grantz 1990; Meinzer et al. 1995; Hubbard et al. 2001; Meinzer 2002; Mencuccini 2003). Recent work focused on leaves points to similar coordination between $g_s$ and $K_{\text{leaf}}$ (Sack et al. 2003; Brodribb & Holbrook 2004a, Brodribb et al. 2005) that includes a dynamic component as $K_{\text{leaf}}$ varies diurnally (Brodribb & Holbrook 2004b; Meinzer et al. 2004; Lo Gullo et al. 2005).

Although dynamic variations in $g_s$ and $K_{\text{leaf}}$ appear to be broadly coordinated on a diurnal basis, it has been difficult to link dynamic stomatal behavior with consistent values of $\Psi_l$ clearly associated with specific features of the dependence of $K_{\text{leaf}}$ on $\Psi_l$ (Brodribb & Holbrook 2003; 2004b). In the present study, the onset of diurnal stomatal closure was consistently associated with height-dependent threshold values of $\Psi_l$ that coincided with $K_{\text{leaf}}$ attaining an initial minimum value. Furthermore, independent measurements in a 58-m-tall old-growth tree indicated that the threshold value of $\Psi_l$ at which whole-crown vapor phase conductance began to decline (-1.68 MPa; Fig. 3.5)
was consistent with mean $\Psi_l$ thresholds for diurnal stomatal closure (-1.65 MPa) and for $K_{leaf}$ approaching an initial minimum value (-1.64 MPa) in the crowns of the old-growth trees (Fig. 3.4). Interestingly, both $g_s$ and $\Psi_l$ continued to decline even though further changes in $K_{leaf}$ were apparently small (cf. Figs. 3.1 & 3.3). Measurements of whole-tree sap flux suggested that this pattern of stomatal behavior caused transpiration to remain nearly constant once threshold values of $\Psi_l$ had been attained (data not shown). However, the continued decline in $\Psi_l$ once sap flux reached its maximum value implies that either tree hydraulic conductance underwent a significant decline or that internal water storage reservoirs were being exhausted resulting in an apparent decline in hydraulic conductance (Andrade et al. 1998; Philips et al. 2003a). The pattern of coordination between $g_s$ and $K_{leaf}$ observed in Douglas-fir suggests that the dynamic gradient in $\Psi$ within the leaf may play a role in coordination of stomatal and leaf hydraulic properties.

**Nature of changes in $K_{leaf}$**

Maximum values of $K_{leaf}$ measured in Douglas-fir were similar to those reported for other coniferous species (Brodribb & Holbrook 2005; Brodribb et al. 2005). Vulnerability of Douglas-fir needles to water stress-induced loss of conductance in this study was similar to that found by others in a number of tropical angiosperm species (Brodribb & Holbrook 2003; Bucci et al. 2003), but substantially greater than that observed in leaves of the tropical conifer *Podocarpus grayii* (Brodribb & Holbrook 2005) and four temperate pine species (Cochard et al. 2004).
Cryo-SEM images of needles suggested that increasing xylem embolism, rather than changes in the hydraulic properties of the extra-xylary pathway, was the primary cause of the water stress-induced decline in $K_{\text{leaf}}$. Nevertheless, concurrent water stress-dependent changes in the conductance of other structures such as the endodermal sheath surrounding the central vein cannot be ruled out. Other studies have shown close relationships between $K_{\text{leaf}}$ and the fraction of embolized or collapsed xylem elements using cryo-SEM (Cochard et al. 2004; Brodribb & Holbrook 2005) and other techniques (Bucci et al. 2003). Our results imply that xylem in Douglas-fir needles undergoes daily cycles of embolism and refilling. We did not assess the timing of $K_{\text{leaf}}$ recovery, but studies carried out on tropical species suggest that may occur rapidly during the afternoon (Bucci et al. 2003; Brodribb & Holbrook 2004b).

It is apparent that tracheid refilling in Douglas-fir needles must occur when substantial tension is present in non-embolized tracheid and capillary spaces because $\Psi_l$ never rises above -0.3 to -0.6 MPa in the crowns of the old-growth trees studied. Bucci et al. (2003) proposed that afternoon refilling of xylem in leaf petioles of two tropical savanna tree species is facilitated by transient pressure imbalances that drive water radially from living cells into embolized conduits. They found that the mechanical integrity of the petiole cortex was a requirement for embolism reversal, implying that it served as a pressure-confining barrier. In Douglas-fir needles, the endodermal sheath surrounding the central vein may serve as a pressure-confining barrier that allows transient pressure imbalances to be generated between the tracheids and surrounding
tissues. Daily osmotic changes in living cells may drive the water movement necessary to generate localized pressure imbalances (Canny 1997, 1998; Bucci et al. 2003).

In the present study, cryo-SEM images of needles at \( \Psi_l \) levels below those associated with decline in \( K_{\text{leaf}} \) fell into two distinct categories: one with relatively high numbers of empty tracheids, and one with numbers of empty tracheids comparable to needles at \( \Psi_l \) levels linked to maintenance of high \( K_{\text{leaf}} \). Non-embolized needles attached to shoot segments at low \( \Psi_l \), and the well-defined dichotomy between high and low numbers of embolized tracheids could be explained by the re-filling of embolized tracheids following a period of cavitation. Since re-filling must occur under conditions of tension within the water conducting pathway, it is reasonable to consider the existence of a mechanism which functions to dissociate different compartments of the overall water conducting pathway. The refilling of tracheids at the most distal portion of the water conducting pathway (the leaves) prior to the re-establishment of continuity within the overall pathway is likely to result in a condition of non-embolized leaf tracheids on shoot segments at low \( \Psi_l \). As more proximal cells continue to be refilled, the overall pathway becomes hydraulically reconnected and shoot water potential increases.

**Environmental effects on \( K_{\text{leaf}} \)**

In addition to xylem tension, environmental variables such as light and temperature may influence \( K_{\text{leaf}} \). As expected, \( K_{\text{leaf}} \) is greater in sun than in shade leaves (Sack et al. 2003) and in sun- versus shade-adapted species (Brodribb & Holbrook
Temperature responses of $K_{\text{leaf}}$ exceed those expected solely from changes in viscosity, consistent with a regulatory role for symplastic components of the leaf hydraulic network (Sack, Streeter & Holbrook 2004). Diel fluctuations of $K_{\text{leaf}}$ are likely to be superimposed on dynamic responses to environmental variables (Nardini, Salleo & Andri 2005). In the present study, the influence of xylem tension on $K_{\text{leaf}}$ was characterized in excised shoots allowed to dehydrate slowly. Absolute values of $K_{\text{leaf}}$ in intact Douglas-fir leaves experiencing similar magnitudes of xylem tension may differ depending on environmental conditions and the time of day. Nevertheless, daily courses of stomatal conductance and sap flow of intact Douglas-fir trees were tightly coordinated with critical values of $\Psi_1$ identified on leaf hydraulic vulnerability curves. Vertical trends in these values of $\Psi_1$ corresponded to combined hydrostatic and frictional tension gradients observed in intact trees, indicating that these relationships were robust and highly conserved. Although seasonal variability is to be expected in leaf pressure-volume characteristics, inter-annual variability in these characteristics is likely to be low due to the consistent seasonal drought during the growing seasons in pacific northwest forests (Waring & Franklin 1979), as well as to the relatively low inter-annual variability in soil moisture conditions, particularly below depths of one meter (Warren et al. 2005; Meinzer et al. 2006).

**Implications**

Douglas-fir leaves lost 50% of their hydraulic conductance at values of $\Psi_1$ between -0.75 and -1.25 MPa, depending on their height above the ground. In contrast,
terminal branches to which the leaves are attached do not lose 50% of their conductivity until stem $\Psi$ falls below -4 to -6 MPa, depending on tree age, size and site (Kavanagh et al. 1999; Domec & Gartner 2002). Daily minimum values of $\Psi_l$ during the growing season typically range between -2 and -3 MPa in mature trees. Therefore, leaf xylem should experience daily cycles of embolism and refilling, whereas little or no embolism is expected to occur in stem xylem. Rapidly reversible embolism in leaves may constitute part of an essential hydraulic signal that enables stomata to maintain stem and leaf $\Psi$ at set points that insure the integrity of the water transport system upstream. Failure of stomata to quickly respond to rapid increases in transpiration could result in sharp increases in stem xylem tension and loss of conductivity, especially if water from sapwood storage has already been exhausted. Moreover, reliance on temporary leaf cavitation to regulate the water status and degree of embolism in stems could be advantageous if embolism repair processes in woody stems are not as vigorous as those observed in leaves.

**Acknowledgements**

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work assistance, to Derrick Horne at UBC for his assistance with cryo-imaging and J.C. Domec for discussion.
References


Table 3.1. $\Psi_l$ at turgor loss point, leaf water capacitance ($C$) prior to and following turgor loss point, and osmotic potential at full turgor for foliage at different heights determined by pressure-volume analysis.

<table>
<thead>
<tr>
<th>height class</th>
<th>height (m)</th>
<th>$\Psi_l$ at turgor loss (MPa)</th>
<th>$C$ pre turgor loss (mol m$^{-2}$ MPa$^{-1}$)</th>
<th>$C$ post turgor loss (mol m$^{-2}$ MPa$^{-1}$)</th>
<th>osmotic potential @ full turgor (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>old top</td>
<td>55.4 ± 1.37</td>
<td>3.52 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>1.24 ± 0.13</td>
<td>-2.77 ± 0.03</td>
</tr>
<tr>
<td>old mid</td>
<td>44.0 ± 0.15</td>
<td>3.34 ± 0.12</td>
<td>0.45 ± 0.03</td>
<td>1.22 ± 0.05</td>
<td>-2.64 ± 0.06</td>
</tr>
<tr>
<td>old low</td>
<td>33.6 ± 1.18</td>
<td>3.13 ± 0.07</td>
<td>0.37 ± 0.02</td>
<td>1.07 ± 0.12</td>
<td>-2.48 ± 0.09</td>
</tr>
<tr>
<td>young top</td>
<td>15.5 ± 0.87</td>
<td>2.85 ± 0.03</td>
<td>0.45 ± 0.03</td>
<td>2.37 ± 0.22</td>
<td>-2.42 ± 0.04</td>
</tr>
</tbody>
</table>
Figure 3.1. Leaf hydraulic conductance ($K_{\text{leaf}}$) in relation to leaf water potential ($\Psi_l$) for foliage samples obtained at three mean heights in the crowns of old-growth trees, and from the upper crowns of young trees growing in a nearby stand. A three-parameter sigmoid function ($y = a/1+e^{-\left(x-x_0\right)/b}$) was fit to $K_{\text{leaf}}$ in relation to $\Psi_l$. Arrows indicate $\Psi_l$ at loss of turgor. Different symbols indicate distinct individual trees within each height class.
Figure 3.2. Example of a typical relationship between $K_{\text{leaf}}$ and $\Psi_l$ illustrating the method used to estimate the $\Psi_l$ at which $K_{\text{leaf}}$ declines to its initial minimum value. The horizontal dotted line represents the midpoint of the y-range of the sigmoid curve. The instantaneous slope at this point yielded a tangent (dashed line) whose intercept with the x-axis was considered to be the value of $\Psi_l$ at which $K_{\text{leaf}}$ had reached its initial minimum value.
Figure 3.3. Time courses of $g_s$ (A) and $\Psi_l$ (B) from two trees at a mean measurement height of ~55 m on September 1st, 2005. The $\Psi_l$ value at which $g_s$ begins to decline is indicated by the intersection of the dashed lines. A four-parameter log-normal function was fit to diurnal courses of $g_s$ and a cubic polynomial function was fit to diurnal courses of $\Psi_l$ (B). $\Psi_l$ values at the point where $g_s$ declined were assessed for all four sampling heights from diurnal measurements of $\Psi_l$ and $g_s$. Each data point represents a mean of three measurements and error bars are standard errors.
Figure 3.4. Height-dependence of critical values of $\Psi_l$. (A) $\Psi_l$ at which $K_{leaf}$ has declined to its initial minimum value. (B) $\Psi_l$ at which $g_s$ begins to decline from its daily maximum value. Measurements were conducted on July 20th 2005 (35m, 44m and 55m heights), September 1st, 2005 (55m height only) and on July 23rd and September 23rd 2002 (20m height only). Errors bars are from mean values of two separate diurnal sessions at the 20m and 55m locations. Only a single sampling session was conducted at the 35m and 44m height locations. Inset: $\Psi_l$ at which $K_{leaf}$ has declined to its initial minimum value in relation to $\Psi_l$ at which $g_s$ declines from its daily maximum for the heights indicated in Fig. 3.4. The dashed line represents a 1:1 relationship.
Figure 3.5. Crown vapor phase conductance in relation to $\Psi_1$ in a 1.4-m-diameter, 58-m-tall Douglas-fir tree. Data were obtained on 31 July (♦), 21 August (●) and 28 August (■) 2002.
Figure 3.6. Images observed with the cryo-SEM technique of vascular bundles in Douglas-fir needles at $\Psi_1 = -0.125$ MPa (A), and -2.1 MPa (B). The white bars represent 100\,\mu m.
Figure 3.7. Number of empty tracheids per needle counted from images observed with the cryo-SEM in relation to leaf water potential ($\Psi_l$) for foliage samples obtained from tree 3013. Data are grouped into four classes of $\Psi_l$: > -0.5 MPa; -0.5 to -1.25 MPa; -1.25 to -2.0 MPa; and < -2.0 MPa. Data for the two most negative classes of $\Psi_l$ (-1.25 to -2.0 MPa and < -2.0 MPa) are divided into two categories: needles with relatively high numbers of empty tracheids (●) and needles with relatively low numbers of empty tracheids (Δ).
CHAPTER 4. HEIGHT-RELATED TRENDS IN LEAF XYLEM ANATOMY AND SHOOT HYDRAULIC CHARACTERISTICS IN A TALL CONIFER: SAFETY VERSUS EFFICIENCY IN WATER TRANSPORT

David R. Woodruff, Frederick C. Meinzer, Barbara Lachenbruch
Summary

- Hydraulic vulnerability of Douglas-fir (*Pseudotsuga menziesii*) branchlets decreases with height, allowing shoots at greater height to maintain hydraulic conductance ($K_{\text{shoot}}$) at more negative leaf water potentials ($\Psi_1$).

- To determine the basis for this trend we analyzed shoot hydraulic and tracheid anatomical properties of foliage from the tops of Douglas-fir trees along a height gradient from 5 to 55 m.

- Values of $\Psi_1$ at which $K_{\text{shoot}}$ was substantially reduced, declined with height by 0.012 MPa m$^{-1}$. Maximum $K_{\text{shoot}}$ was reduced by 0.082 mmol m$^{-2}$ MPa$^{-1}$ s$^{-1}$ for every one-meter increase in height. Total tracheid lumen area per needle cross section, hydraulic mean diameter of leaf tracheid lumens, total number of tracheids per needle cross section and leaf tracheid length decreased with height by 18.4 $\mu$m$^2$ m$^{-1}$, 0.029 $\mu$m m$^{-1}$, 0.42 m$^{-1}$, and 5.3 $\mu$m m$^{-1}$ respectively. Tracheid thickness-to-span ratio ($t_w/b$)$^2$ increased with height by $1.04 \times 10^{-3}$ m$^{-1}$ and pit number per tracheid decreased with height by 0.07 m$^{-1}$.

- Leaf anatomical adjustments that enhanced the ability to cope with vertical gradients of increasing xylem tension were attained at the expense of reduced water transport capacity and efficiency, possibly contributing to height-related decline in growth of Douglas-fir.

*Key-words:* hydraulic conductance, foliar anatomy, growth limitation, water stress, embolism, *Pseudotsuga menziesii*
Introduction

Growth and aboveground biomass accumulation follow a common pattern as tree size increases, with productivity peaking when leaf area reaches its maximum and then declining as tree age and size increase (Ryan & Waring 1992). Age- and size-related declines in forest productivity are major considerations in setting the rotational age of commercial forests, and also relate to issues of carbon storage, since changes in forest structure can influence large-scale biomass accumulation. Despite the ecological and practical significance of the ontogenetic decline in tree growth, the mechanisms responsible for it are not well understood (Ryan et al. 2006). However, available evidence suggests that ontogenetic trends in growth are mainly a function of tree size (height) rather than age (Koch et al. 2004; Woodruff et al. 2004; Bond et al. 2007; Mencuccini et al. 2007).

Height-related changes in leaf function may have an impact on tree growth and forest productivity because leaf stomata are responsible for maximizing photosynthetic carbon gain while simultaneously dealing with the antagonistic task of constraining transpirational water loss to avoid damaging levels of dehydration. The gravitational component of water potential leads to a 0.01 MPa increase in xylem sap tension per meter increase in height, which substantially reduces leaf water potential ($\Psi_l$) near the tops of tall trees. Frictional resistance during transpiration leads to an additional path length-dependent reduction in $\Psi_l$ (Bauerle et al. 1999). In the absence of osmotic adjustment, the turgor of leaf cells will decrease in direct proportion with $\Psi_l$ along
height and path length gradients. Cell volume increase during growth can be tightly coupled with cell turgor pressure (Lockhart 1965). In addition to cell expansion, a range of growth-related processes including cell division are sensitive to turgor pressure (Boyer 1968; Kirkham et al. 1972; Hsiao et al. 1976; Gould & Measures 1977). Given the relationship between height and Ψl, and the dependence of cell division and expansion upon turgor, a causal relationship between tree height and anatomical properties that influence foliar physiology seems likely. Leaves typically comprise a substantially smaller portion of the path-length in plant vascular systems than stems, yet they represent a disproportionately large fraction of the whole-plant hydraulic resistance (Yang & Tyree 1994; Nardini & Salleo 2000). Height-related changes in leaf anatomy that affect foliar water transport efficiency may thus help to explain observed size- or age-dependent reductions in forest productivity.

Although leaves comprise the terminal portion of the vascular system and are thus likely to experience more negative water potentials than other plant organs, they are typically more vulnerable to embolism than stems (Brodribb & Holbrook 2003; Bucci et al. 2003; Woodruff et al. 2007). In some cases, however, leaves have been found to be less vulnerable to embolism than stems (Sack & Holbrook 2006). Given their critical functions in gas exchange and their high vulnerability to loss of hydraulic conductance, leaves may exhibit adaptive morphological characteristics that maximize efficiency, minimize vulnerability, or both. Recent work has shown a correlation between tree height and hydraulic safety of Douglas-fir (Pseudotsuga menziesii) shoots (Woodruff et al. 2007), allowing shoots at greater height to maintain hydraulic
conductance \( K_{\text{shoot}} \) and stomatal opening at more negative values of \( \Psi_l \). This height-related trend may allow taller trees to continue to photosynthesize during periods of greater water stress, but previous work on stems and roots of Douglas-fir (Domec et al. 2006) implies that adaptations to minimize hydraulic vulnerability may also involve trade-offs that reduce shoot water transport capacity.

Substantial intra-specific variation has been found in leaf form and function (Sprugel et al. 1996; Grassi & Bagnaresi 2001) and even within individuals along height or light gradients (Niinemets et al. 1999; Koike et al. 2001; Woodruff et al. 2004). Despite the great diversity in leaf structural and physiological traits, leaf and shoot hydraulic properties appear to be coordinated with physiological function in a consistent manner across species. Recent research has begun to more fully describe the connections between leaf anatomical characteristics and leaf physiological function such as correlations between leaf architecture and transport efficiency (Aasamaa et al. 2005; Sack & Frole 2006); as well as leaf architecture and gas exchange (Salleo et al. 2001; Sack et al. 2003; Brodribb et al. 2005; 2007). Xylem conduit properties such as length, diameter, wall thickness and pit abundance have been analyzed as determinants of hydraulic conductivity and resistance to embolism of wood (Hacke et al. 2001; Pitterman et al. 2006; Sack & Holbrook 2006; Sperry et al. 2006). However, much less is known about the influence of these anatomical characteristics upon hydraulic efficiency and safety of leaf xylem. Specifically, we know of no other research which has investigated the impact of tree height upon these leaf anatomical characteristics and associated shoot physiological attributes.
The goal of this study was to determine the extent to which height may influence key anatomical features related to leaf hydraulic efficiency and tension-induced vulnerability in a coniferous tree, and to examine the extent to which the observed anatomical patterns are associated with adaptive physiological advantages. We examined leaf xylem anatomical properties likely to be associated with height-related trends in hydraulic function, including characteristics related to cell expansion such as hydraulic mean diameter of leaf tracheid lumens ($D_h$), leaf tracheid length ($T_l$) and tracheid wall thickness-to-span ratio ($t_w/b^2$). Analyses also included number of tracheids per needle cross section ($T_{#}$), which is dependent upon cell division; plus total lumen area per needle cross section ($LA_t$), a characteristic influenced by both cell expansion and cell division. Sampling was conducted exclusively from fully sun-exposed branches near the tops of trees of different height classes in order to rule out the potentially confounding influence of factors such as irradiance, relative humidity and branch length upon height-related trends in leaf hydraulic architecture and anatomy.

Materials and Methods

Site

Five separate stands, each containing Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees of a different height class, were located within 3.1 km of each other in the Wind River Basin of southwestern Washington, U.S.A. Samples were collected within one to five meters of the tops of the trees at mean sampling heights of
5.0, 12.7, 18.3, 34.5 and 55.0 m. All samples were obtained from branches in fully sun-exposed locations. Access to tree tops in the 55-m sampling height class was facilitated by a 75-m-tall construction tower crane at the Wind River Canopy Crane Research Facility (WRCCRF). Tree tops in all other height classes were accessed by non-spur climbing.

The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.2 m, much of which falls as snow, with a dry season from June through September. The mean annual temperature is 8.7°C with means of 0°C in January and 17.5°C in July. The soils are well-drained and of volcanic origin (Shaw et al. 2004). Very low precipitation between June and September (~119 mm) typically leads to drought conditions in the upper portion of the soil profile. Soil water however, remains accessible to Douglas-fir roots at depths greater than about 1 m throughout the summer dry period (Warren et al. 2005; Meinzer et al. 2007).

Leaf hydraulic conductance and vulnerability

We determined maximum hydraulic conductance of terminal shoots ($K_{\text{shoot}}$) and shoot hydraulic vulnerability to embolism. These characteristics represent the capacity of foliated shoots to transport water and their ability to avoid loss of hydraulic function as water stress increases. $K_{\text{shoot}}$ was measured as a proxy for leaf hydraulic conductance ($K_{\text{leaf}}$) because of the difficulty in measuring hydraulic conductance on individual Douglas-fir needles. We believe $K_{\text{shoot}}$ to be a reliable proxy for $K_{\text{leaf}}$ given that leaves
represent the majority of the hydraulic resistance to water flow in shoots and a considerable fraction of the entire hydraulic resistance within whole plants (Yang & Tyree 1994; Nardini & Salleo 2000; Sack & Holbrook 2006). The methods used were adapted from Brodribb & Holbrook (2003), and involve the use of the following equation based on an analogy between rehydrating a shoot and recharging a capacitor:

\[ K_{\text{shoot}} = C \ln(\Psi_o / \Psi_f) / t, \]

where \( K_{\text{shoot}} \) = shoot hydraulic conductance (mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\)), \( C \) = capacitance, \( \Psi_o \) = leaf water potential prior to partial rehydration, \( \Psi_f \) = leaf water potential after partial rehydration and \( t \) = duration of rehydration. Branches about 30–50 cm long were collected from trees early in the morning prior to significant transpirational water loss and were placed in plastic bags with moist paper towels and stored in the dark in a refrigerator. Measurements of leaf water potential were conducted over the next 3 d on excised twigs (~ 10–15 cm long) for initial values (\( \Psi_o \)), and for final values after a period of rehydration of \( t \) seconds (\( \Psi_f \)). Deionized water used for rehydration of \( K_{\text{shoot}} \) samples was partially de-gassed by subjecting it to a vacuum for a minimum of 2 h before transferring it into a separate container. Samples were dehydrated to a range of \( \Psi_o \) prior to \( K_{\text{shoot}} \) measurements in order to obtain full vulnerability curves. Branches were left to equilibrate in the dark in sealed plastic bags for at least 30 min following periods of dehydration. Water temperature was between 21 and 23 °C, and the photosynthetic photon flux density at the foliage was maintained at approximately 1000
μmol m$^{-2}$ s$^{-1}$ during $K_{\text{shoot}}$ measurements. Exposure time of foliage to full irradiance was typically less than 5 min. Rehydration times during $K_{\text{shoot}}$ measurements ranged between 20 and 290 s. All $K_{\text{shoot}}$ samples bore only foliage produced during the years 2006 and 2007.

We subjected some $K_{\text{shoot}}$ samples to variable periods of “pre-hydration” prior to initial measurements of $\Psi_1$ in order to obtain a wider range of initial values of $\Psi_1$ and $K_{\text{shoot}}$. Pre-hydration times ranged from one to 12 h. We estimated $C$ using the methods described by Brodribb & Holbrook (2003). Briefly, the $\Psi_1$ corresponding to turgor loss was estimated as the inflection point of the graph of $\Psi_1$ vs. relative water content (RWC). The slope of the curve prior to, and following turgor-loss provided $C$ in terms of RWC ($C_{\text{rwc}}$) for pre-turgor loss and post-turgor loss respectively. $C_{\text{rwc}}$ was then multiplied by the saturated mass of water in the shoots and then divided by leaf area in order to provide a value of $C$ expressed in absolute terms and normalized by leaf area. Mean pre-turgor loss $C$ values were 0.67, 0.52, 0.51, 0.55 and 0.47 mol m$^{-2}$ MPa$^{-1}$ for foliage from the 5.0, 12.7, 18.3, 34.5 and 55.0 m sampling heights, respectively. Post-turgor loss $C$ values were 2.65, 1.18, 1.31, 1.75, and 1.61 mol m$^{-2}$ MPa$^{-1}$ and turgor loss points were -2.22, -3.25, -2.94, -2.96, and -3.05 MPa for the same sampling heights, respectively. Samples for $K_{\text{shoot}}$ and pressure-volume curve measurements were collected from three separate trees for each sample height (except 34.5 m, for which there were two trees; and 55.0 m, for which there were four trees) at ten dates: 13 July, 21 July, 21 October 2005; 28 June, 1 July, 23 August, 21 September, 25 September, and 5 December 2006; and 24 January 2007. All foliage was sampled after hardening of
current year foliage to minimize confounding effects from intra-annual variability. For two of the heights (5m and 55m), foliage was collected and analyzed on multiple occasions throughout the study and no seasonal trends were evident in $K_{\text{shoot}}$.

We determined values of $\Psi_1$ at which 20, 50 and 80 percent loss of hydraulic conductance occurred ($\Psi_{20}$, $\Psi_{50}$ and $\Psi_{80}$, respectively). These values of $\Psi_1$ were obtained from a sigmoid regression curve fitted to mean $K_{\text{shoot}}$ versus $\Psi$ data. For example, $\Psi_{50}$ represents the leaf water potential at 50 percent of the regression curve’s maximum value on the y-axis (Fig. 4.1). Following our method used in previous work (Woodruff et al. 2007) to identify an objective and functionally relevant measure of shoot hydraulic vulnerability we used an analysis similar to that originally proposed for description of vulnerability curves for wood by Domec & Gartner (2001). This method provided a means for designating the point at which $K_{\text{shoot}}$ had declined to an initial minimum value ($\Psi_1$ at minimum $K_{\text{shoot}}$). For each height class, data were grouped by water potential ranges (0 to -0.5 MPa, < -0.5 to -0.75 MPa, < -0.75 to -1.0 MPa, < -1.0 to -1.25 MPa, < -1.25 to -1.5 MPa, < -1.5 to -1.75 MPa, < -1.75 to -2.0 MPa, < -2.0 to -2.25 MPa, < -2.25 to -2.5 MPa, < -2.5 to -2.75 MPa and < -2.75 to -3.0 MPa) to compute the corresponding mean $K_{\text{shoot}}$ values (n = 2 to 28 branches per height class) over each $\Psi$ range (Fig. 4.1). The value of $K_{\text{shoot}}$ representing the midpoint between the Y-intercept of the sigmoid function and 0 (dotted line in Fig. 4.1) was selected for estimating the slope of the portion of the sigmoid curve containing the relatively rapid and nearly linear decline in $K_{\text{shoot}}$. We took the derivative of the sigmoid function to calculate its instantaneous slope at the $K_{\text{shoot}}$ midpoint and used the x-intercept of the
resulting tangent (dashed line in Fig. 4.1) as an objective estimate of $\Psi_1$ at minimum $K_{\text{shoot}}$. Maximum $K_{\text{shoot}}$ ($K_{\text{shoot-max}}$) was estimated from the mean of the $K_{\text{shoot}}$ values obtained from the most hydrated samples in each height class ($\Psi_1 = 0$ to $-0.5$ MPa). $K_{\text{shoot-max}}$, $\Psi_1$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$ data were analyzed using regression and analysis of variance. Error bars for $K_{\text{shoot-max}}$ represent standard errors of branches ($n = 7$ to 28).

Pressure–volume analyses (Tyree & Hammel 1972) were conducted on branchlets approximately 10-cm-long. These samples were excised from branch samples that were collected early in the morning prior to significant transpirational water loss, sealed in plastic bags with moist paper to prevent desiccation and then stored in a refrigerator within 1–4 h of excision. Pressure–volume curves were initiated by first determining the fresh mass of the twig, and then measuring $\Psi_1$ with a pressure chamber (PMS Instrument Company, Corvallis, OR). Alternate determinations of fresh mass and $\Psi_1$ were repeated during slow dehydration of the twig on the laboratory bench until values of $\Psi_1$ exceeded the measuring range of the pressure chamber (-4.0 MPa). The inverse of water potential was plotted against relative water content to create a pressure–volume curve. For normalizing $C$ on a leaf area basis, one-sided leaf areas of the branchlets were obtained with a scanner and ImageJ version 1.27 image analysis software (Abramoff et al. 2004).
Leaf xylem anatomical characteristics

We analyzed leaf xylem anatomical properties likely to be associated with height-related trends in hydraulic function. These included total tracheid lumen area per needle cross section \( (L_A_t) \), hydraulic mean diameter of leaf tracheid lumens \( (D_h) \), number of tracheids per needle cross section \( (T_{n}) \), leaf tracheid length \( (T_l) \), pit number per tracheid \( (Pit_{n}) \) and tracheid wall thickness-to-span ratio \( (t_w/b)^2 \). Macerations of isolated needle xylem were prepared for analyses of tracheid length and pit number per tracheid. Cross sections of needles were made by hand sectioning of fresh tissue for analyses of all other anatomical characteristics. For macerations we made a solution of 15 g sodium chlorite dissolved in 250 ml of distilled water and then submerged each sample with approximately 10 ml of the solution. Ten drops of acetic acid were added to each test tube and the solutions were heated at 90 °C for at least 24 h. The macerated cells were rinsed, stained with toluidine blue, and mounted on slides. Each needle cross section was analyzed with an image analysis system consisting of a compound microscope and video camera. Anatomical characteristics obtained from macerations were obtained from between 6 to 8 branches per height class, and anatomical characteristics obtained from hand sectioning represent between 6 and 9 branches per height class. All anatomical measurements were obtained from foliage produced during 2006. Images were obtained using x20 or x40 objectives with total magnifications of x200 and x400. Data were pooled per height class and analyzed using regression analysis.
Results

Physiological trends with height

Timed rehydration measurements showed that $K_{\text{shoot}}$ declined sigmoidally with $\Psi_1$. A logistic three-parameter sigmoid function ($y = a/(1+(x/x_0)^b)$) yielded $r^2$ values ranging from 0.98 to 0.99 for the dependence of mean $K_{\text{shoot}}$ on $\Psi_1$ (Fig. 4.2). We used $K_{\text{shoot-max}}$ as a measure of hydraulic efficiency and $\Psi_1$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$ as measures of hydraulic vulnerability. $K_{\text{shoot-max}}$ decreased with increasing height ($P = 0.035$) from 7.2 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ at 5 m, to 3.2 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ at 55.0 m (Fig. 4.3a). Foliage from the tops of taller trees was more resistant to loss of hydraulic conductance than foliage from shorter trees regardless of the index of hydraulic vulnerability employed (Fig. 4.3b). Values of $\Psi_1$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$ decreased linearly with increasing height at a rate of 0.012 MPa m$^{-1}$ ($P = 0.020$) and -0.009 MPa m$^{-1}$ ($P = 0.0088$), respectively. Similar results were obtained for height-related trends in $\Psi_{80}$ and $\Psi_{20}$ ($P = 0.021$ and 0.0078, respectively). There was a positive correlation between $K_{\text{shoot-max}}$ and $\Psi_1$ at minimum $K_{\text{shoot}}$ ($P = 0.0047$); and between $K_{\text{shoot-max}}$ and $\Psi_{50}$ ($P = 0.009$), showing that increased resistance to tension-induced loss of $K_{\text{shoot}}$ was associated with reduced maximum water transport capacity (Fig. 4.4).

Anatomical trends with height

Quantitative analyses of leaf tracheid characteristics revealed height-related trends in anatomy (Fig. 4.5). Observed trends were consistent with the pronounced decline in $K_{\text{shoot-max}}$ with increasing height. $LA_t$ declined with height by 19 $\mu$m$^2$ m$^{-1}$ ($P =$
0.019). $D_h$ declined with increasing height by 0.031 μm m$^{-1}$ ($P = 0.0016$). $T\#$ decreased with height by 0.43 m$^{-1}$ ($P = 0.05$). $T_l$ declined with increasing height by 5.2 μm m$^{-1}$, although the trend was marginally significant ($P = 0.053$). $Pit\#$ decreased with height by 0.07 m$^{-1}$ ($P = 0.03$) and $(t_w/b)^2$ increased with increasing height by 0.001 m$^{-1}$ but the trend was not significant ($P = 0.085$).

**Relationships between anatomy and physiology**

We evaluated the correlations of $K_{\text{shoot-max}}$, $\Psi_l$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$ with leaf tracheid anatomical characteristics in order to elucidate potential causal relationships between leaf structural and shoot hydraulic attributes. Many of the leaf tracheid anatomical properties analyzed were significantly correlated with either $K_{\text{shoot-max}}$, $\Psi_l$ at minimum $K_{\text{shoot}}$, $\Psi_{50}$, or all three (Table 4.1). $L A_t$ was correlated with hydraulic efficiency ($P = 0.018$), with $\Psi_l$ at minimum $K_{\text{shoot}}$ ($P = 0.025$) and $\Psi_{50}$ ($P = 0.0066$). For every 100 μm$^2$ increase in $L A_t$, there was an increase in hydraulic efficiency such that $K_{\text{shoot-max}}$ increased by 0.43 mmol m$^{-2}$ MPa$^{-1}$ s$^{-1}$, and an increase in hydraulic vulnerability such that $\Psi_l$ at minimum $K_{\text{shoot}}$ increased by 0.06 MPa, and $\Psi_{50}$ increased by 0.05 MPa. $K_{\text{shoot-max}}$, $\Psi_l$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$ were also strongly correlated with changes in $D_h$ ($P = 0.014$, $P = 0.0082$ and $P = 0.004$, respectively). On average, a one μm increase in $D_h$ was correlated with an increase in $K_{\text{shoot-max}}$ of 2.9 mmol m$^{-2}$ MPa$^{-1}$ s$^{-1}$, an increase in $\Psi_l$ at minimum $K_{\text{shoot}}$ of 0.40 MPa and an increase in $\Psi_{50}$ of 0.31 MPa. $T\#$ was significantly correlated with $\Psi_l$ at minimum $K_{\text{shoot}}$ ($P = 0.044$).
and with $\Psi_{50}$ ($P = 0.015$), but marginally so with hydraulic efficiency ($P = 0.057$). $T_1$ had no significant correlation with either hydraulic efficiency or vulnerability ($P = 0.29$ for $K_{\text{shoot-max}}$, $P = 0.18$ for $\Psi_1$ at minimum $K_{\text{shoot}}$ and $P = 0.13$ for $\Psi_{50}$). $Pit_\#$ was significantly correlated with $K_{\text{shoot-max}}$ ($P = 0.014$), with $\Psi_1$ at minimum $K_{\text{shoot}}$ ($P = 0.0096$), and with $\Psi_{50}$ ($P = 0.0021$). $(t_w/b)^2$ was significantly correlated with $K_{\text{shoot-max}}$ ($P = 0.028$), but not significantly correlated with either $\Psi_1$ at minimum $K_{\text{shoot}}$ or $\Psi_{50}$ ($P = 0.071$ and $P = 0.094$, respectively).

**Discussion**

Our results indicate that there were opposing height-related trends in shoot hydraulic efficiency and safety in Douglas-fir, which were correlated with several leaf tracheid anatomical characteristics. The observed trends in leaf anatomy were consistent with likely impacts of reduced turgor on cell expansion and division with increased tree height (Koch *et al*. 2004; Woodruff *et al*. 2004). The decline in $D_h$ and $T_1$ with height is consistent with a reduction in the turgor-driven cell expansion associated with increased gravitational and path length resistance. Given that less cell expansion also implies a relative increase in available wall material per unit cross-sectional area, an increase in $(t_w/b)^2$ suggests both reduced lumen diameter and greater wall thickness, both resulting from a reduction in turgor-driven cell expansion. Reduced $T_\#$ with height-associated decline in turgor is consistent with previous work which has shown a causal link between turgor and cell division (Boyer 1968; Kirkham *et al*. 1972). The reduction in $LA_t$ represents a combination of both a decline in $D_h$ and $T_1$. Besides the
possibility of a correlation between overall tracheid size and the number of pits per tracheid, a direct connection between turgor during tracheid expansion and Pit# is unclear.

These results suggest that the effects of tree height upon leaf cell development lead to enhanced ability to avoid water stress-induced embolism at the expense of reduced water transport capacity and efficiency. Moreover, localized height-related hydraulic restrictions on water uptake by expanding cells in terminal shoots may have a negative synergistic impact on turgor-limited tissue expansion. The reduction in cell expansion limits hydraulic conductance due to the effect of reduced lumen diameter upon hydraulic resistance, and reduced hydraulic conductance may in turn limit potential cell expansion because it represents a hydraulic limitation imposed upon the system that supplies the water necessary for foliar cell expansion. During the relatively short period of shoot expansion in the late spring, osmotic adjustment in Douglas-fir is insufficient to compensate for the vertical gradient of increasing tension (Woodruff et al. 2004; Meinzer et al. 2008). Thus, if water entry into growing tissue does not keep pace with continuous cell wall relaxation, turgor will be further reduced, resulting in a substantial water potential disequilibrium between adjacent regions of growing and non-growing tissue (Boyer et al. 1985; Nonami & Boyer 1989; 1990).

Vulnerability to loss of hydraulic conductance in Douglas-fir shoots was substantially greater than that observed in shoots of the tropical conifer Podocarpus grayii (Brodribb & Holbrook 2005) and in the leaves of four temperate pine species (Cochard et al. 2004); but similar to that found in leaves for a number of tropical
angiosperm species (Brodribb & Holbrook 2003; Bucci et al. 2003), for several pteridophytes and gymnosperms (Brodribb & Holbrook 2006) and for Douglas-fir shoots along a vertical gradient within an individual forest canopy (Woodruff et al. 2007). Maximum values of $K_{\text{shoot}}$ measured in Douglas-fir were similar to those reported for other gymnosperm species (Brodribb & Holbrook 2005; 2006) and for Douglas-fir along a vertical gradient within a single forest canopy (Woodruff et al. 2007).

Tracheid length was not a significant determinant of shoot hydraulic conductance, contrary to studies indicating that conduit length accounts for a substantial portion of the variation in conducting efficiency of wood (Pothier et al. 1989; Domec et al. 2006; Sperry et al. 2006). The strongest anatomical correlates of $K_{\text{shoot-max}}$ were $Pit_{\text{th}}$, $LA_{\text{t}}$, and $D_{\text{h}}$. The observed significant relationship between $D_{\text{h}}$ and transport efficiency is not unexpected given that even small changes in conduit diameter lead to major changes in transport efficiency according to the Hagen-Poiseille equation:

$$K_t = \frac{\pi \rho D_h^4}{128 \eta}$$

(2)

where $K_t$ (m$^4$ MPa$^{-1}$ s$^{-1}$) is theoretical conductance, $\rho$ is the density of water (5.55 x 10$^7$ mmol m$^{-3}$) and $\eta$ is the viscosity of water (1.002 x 10$^{-9}$ MPa s at 20° C). An independent analysis of vertical trends in shoot hydraulic conductance was performed by calculating a theoretical leaf area-specific conductance ($K_{\text{leaf-theoretical}}$) using a
modified version of the Hagen-Pouseille equation, and using $T_\#$ (per needle) and leaf area of a single needle ($A_l$):

$$K_{\text{leaf-theoretical}} = \pi \rho D_h^4 T_\# / 128 \eta A_l$$  \hspace{1cm} (3)

Leaf area measurements were conducted upon foliage at each sampling height and these values were used to normalize $K_{\text{leaf-theoretical}}$ by leaf area. Leaf area was found to scale inversely with height by 0.24 mm$^2$ m$^{-1}$. Despite the trend in leaf area with height, $K_{\text{shoot-max}}$ correlated strongly with leaf-specific values of $K_{\text{leaf-theoretical}}$ ($P = 0.006$; Figure 4.6). Note that although $K_{\text{shoot-max}}$ and $K_{\text{leaf-theoretical}}$ are both conductance values, they are not directly comparable because they have different units. The comparison between $K_{\text{shoot-max}}$ and $K_{\text{leaf-theoretical}}$ is still noteworthy, however, because the strong positive correlation between the two provides additional support for a causal relationship between key leaf xylem anatomical properties (in this case $LA_t$ and $D_h$) and shoot hydraulic function. In the case of a direct comparison with identical units the values of $K_{\text{shoot-max}}$ would likely be substantially lower than $K_{\text{leaf-theoretical}}$ due to deviations in actual conduits from idealized tubes resulting from features in tracheid lumens that affect water flow such as warts and bordered pits (Domec et al. 2006).

Although the values of hydraulic efficiency and vulnerability were comparable to those found in Douglas-fir at the same site in a previous study (Woodruff et al. 2007), the trend in both of these with height was more consistent in the current study (Figure 4.3). It is important to note that although the 55m sampling locations in this
study and in Woodruff et al. (2007) were equivalent, all other sampling locations were different. In the previous study, samples were taken from three locations within a height gradient of a single old growth forest canopy, and at one location at the tops of smaller trees in a nearby stand. In the current study sampling was limited to the shorter branches at the tops of sun-exposed trees of different heights to eliminate any confounding effects from variable levels of irradiance, relative humidity or branch length upon height-related trends in leaf hydraulic architecture and anatomy. Branch length is a potentially critical factor because of the trend in branch length with depth within a forest canopy (K. Bible, unpublished data) and because of the substantially higher levels of resistance to water transport in branches compared to boles (Domec & Gartner 2002). The increased hydraulic resistance associated with the greater branch length found lower in the canopy is likely to limit water availability to the attached foliage. Branch length is thus a potentially confounding variable with height in its impact upon water availability to leaves.

The anatomical characteristics with the highest correlation to shoot vulnerability were \( \text{Pit}_{\text{opt}} \), \( Dh \) and \( LA_t \). Loss of hydraulic conductivity due to embolism is believed to be caused by “air seedling” (Zimmerman 1983; Sperry & Tyree 1988). Once the tension within a xylem conduit exceeds the capillary forces at the air-water interface in a pit membrane, an air bubble is pulled through a pore in the pit membrane from an adjacent air-filled conduit. The air bubble expands in the conduit resulting in an “embolized” or non-hydraulically functional conduit. The tension required to initiate air seeding is believed to be a function of the pit aperture diameter (Zimmerman 1983), pit membrane
and pit chamber characteristics (Domec et al. 2006), and the frequency of pits per conduit (McCully & Canny 1988). The very strong relationship between pit number and hydraulic vulnerability (Table 4.1) provides support for the theory of air seeding as a primary cause of hydraulic failure via embolism, and is consistent with previously observed relationships between xylem anatomy and loss of hydraulic conductivity in wood (Zimmerman 1983; Hacke et al. 2004; Domec et al. 2006).

Reduced conduit diameter has long been associated with increased resistance to embolism in wood (Sucoff 1969; Ewers 1985). The only mechanistic relationship between conduit size and hydraulic vulnerability that has been confirmed by experimentation is the association of freeze-thaw induced embolisms with conduit diameter (Davis et al. 1999). Studies of freezing-induced embolism in conifer wood have shown a substantially reduced susceptibility to this phenomenon than in angiosperm wood due to the smaller diameters of tracheids compared with vessels. Whether or not this trend holds true in conifer foliage is yet to be determined. Although shoot samples were not subjected to freeze-thaw treatments, repeated freeze-thaw cycles are common at the study site during the winter months. The potential for freeze-thaw-induced embolism is therefore potentially relevant for interpretation of the adaptive significance of trends in leaf tracheid anatomy in Douglas-fir.

The conduit thickness to span ratio is typically analyzed as a measure of vulnerability to implosion as opposed to vulnerability to embolism. It is not expected to have a direct influence upon resistance to embolism, since wall thickness is likely to be more relevant to cell wall implosion than to air seeding. However, Hacke et al. (2001)
have shown a constant safety factor between implosion and embolism in both
gymnosperms and angiosperms, so \((t_w/b)^2\) has been used as an index of vulnerability.
Based upon previous cryo-SEM analyses of leaf tracheids at varying levels of
dehydration, we concluded that implosion of leaf tracheids in Douglas-fir is an
uncommon event and that short-term variation in \(K_{\text{shoot}}\) are associated with reversible
embolism (Woodruff et al. 2007).

Reduced shoot hydraulic conductance is likely to limit stomatal conductance,
and therefore photosynthesis, consistent with age and height-related reductions in tree
growth. Height-related decline in shoot hydraulic conductance could thus be considered
complementary to other hydraulic factors which have been proposed as the basis for
height-related decline in productivity, such as increased stomatal closure due to
resistance associated with greater length of hydraulic path and the gravitational
potential gradient opposing the ascent of water in taller trees (Yoder et al. 1994; Ryan
& Yoder 1997). The slope of the relationship between tree height and shoot hydraulic
vulnerability (-0.012 MPa m\(^{-1}\) for \(\Psi_1\) at minimum \(K_{\text{shoot}}\), Fig. 4.3b) is slightly more
negative than the hydrostatic gradient, providing further evidence of gravity and path
length as the driving forces for height-related trends in hydraulic architecture. Other
non-vascular leaf anatomical factors could be at play in the relationship between tree
height and decline in productivity such as increased extra-vascular resistance to water
transport (Brodribb et al. 2007) or increased occurrence of non-photosynthetic foliar
biomass such as astrosclereids (Apple et al. 2002).
It is noteworthy that although both leaf xylem anatomy and shoot hydraulic properties were correlated with tree height, these were often more highly correlated with each other; providing further evidence of the influence of these foliar anatomical properties upon hydraulic function. The decline in shoot hydraulic vulnerability with increasing tree height partially mitigates the effects of increasing xylem tension associated with gravity and increased path-length resistance, and may allow taller trees to continue to photosynthesize during periods of greater water stress. This advantage could be derived from evolutionary forces selecting for leaf anatomical properties conferring greater resistance to loss of conductivity. The observed vertical trends in xylem anatomical traits governing hydraulic efficiency and safety could also be primarily driven by the height-related decline in turgor during periods of cell expansion and cell division (Kirkham et al. 1972; Woodruff et al. 2004; Meinzer et al. 2008).

Acknowledgements

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References


Table 4.1. Percent of variance ($r^2$) and significance values for shoot hydraulic conductance and vulnerability and leaf xylem anatomical characteristics.

<table>
<thead>
<tr>
<th>Leaf xylem anatomical characteristic</th>
<th>Hydraulic efficiency ($K_{\text{shoot-max}}$)</th>
<th>Hydraulic vulnerability ($\Psi_{50}$)</th>
<th>($\Psi_1$ @ minimum $K_{\text{shoot}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$LA_t$</td>
<td>0.88* (+)</td>
<td>0.94** (+)</td>
<td>0.85* (+)</td>
</tr>
<tr>
<td>Dh</td>
<td>0.90* (+)</td>
<td>0.96** (+)</td>
<td>0.93** (+)</td>
</tr>
<tr>
<td>Tracheid #</td>
<td>0.75 (+)</td>
<td>0.89* (+)</td>
<td>0.79* (+)</td>
</tr>
<tr>
<td>Tracheid length</td>
<td>0.35 (+)</td>
<td>0.58 (+)</td>
<td>0.50 (+)</td>
</tr>
<tr>
<td>Pit #</td>
<td>0.90* (+)</td>
<td>0.97** (+)</td>
<td>0.92** (+)</td>
</tr>
<tr>
<td>$(t_w/b)^2$</td>
<td>0.84* (-)</td>
<td>0.66 (-)</td>
<td>0.72 (-)</td>
</tr>
</tbody>
</table>

* $P \leq 0.05$, ** $P \leq 0.01$

(+) = positive correlation, and (-) = negative correlation

$LA_t$, total lumen area per leaf cross section ($\mu m^2$);

$D_h$, hydraulic mean diameter of leaf tracheid lumens ($\mu m$);

$T_s$, total number of tracheids per leaf cross section

$T_1$, leaf tracheid length ($\mu m$);

Pit #, number of pits per tracheid;

$(t_w/b)^2$, ratio of thickness of tracheid wall to span of tracheid lumen
Fig. 4.1. Example of a typical relationship between mean $K_{\text{shoot}}$ and $\Psi_1$ illustrating the method used to estimate the $\Psi_1$ at which $K_{\text{shoot}}$ has declined to its initial minimum value. Data are from the 34.5 m height class. The horizontal dotted line represents the midpoint of the y-range of the sigmoid curve (50% loss of $K_{\text{shoot}}$). The instantaneous slope at this point yielded a tangent (dashed line) whose intercept with the x-axis was designated as the value of $\Psi_1$ at which $K_{\text{shoot}}$ had reached its initial minimum value. Bars = ±SE, n = 2 to 28 branches per height class.
Fig. 4.2. Shoot hydraulic conductance ($K_{\text{shoot}}$) in relation to leaf water potential ($\Psi_l$) for foliage samples obtained within 1 to 5 m of the tops of the trees at mean sampling heights of 5.0, 12.7, 18.3, 34.5 and 55.0 m. A logistic three-parameter sigmoid function ($y = a/(1+(x/x_0)^b)$) was fit to $K_{\text{shoot}}$ in relation to $\Psi_l$. 
Fig. 4.3. a) $K_{\text{shoot-max}}$ (Bars = ±SE, n = 7 to 28 branches per height class), and b) different measures of shoot hydraulic vulnerability versus height. Values of $\Psi_{20}$, $\Psi_{50}$, $\Psi_{80}$ and $\Psi_1$ at minimum $K_{\text{shoot}}$ were obtained from vulnerability curves as illustrated in Fig 1.
Fig. 4.4. $K_{\text{shoot-max}}$ in relation to shoot hydraulic vulnerability measured as $\Psi_1$ at minimum $K_{\text{shoot}}$ and $\Psi_{50}$. 

$r^2 = 0.95$ 

$r^2 = 0.92$ 

$\Psi_{\text{at minimum}}$ 

$\Psi_{50}$
Fig. 4.5. Leaf tracheid anatomical characteristics along a height gradient sampled from the upper 1 to 5 m of trees of different height classes. Total lumen area (a), hydraulic mean diameter (b), tracheid number (c), tracheid length (d), pit number (e), and thickness to span ratio \((t_w/b)^2\) (f). Bars = ±SE, n = 6 to 9 branches for (a), (b), (c) and (f); n = 6 to 8 branches for (d) and (e).
Fig. 4.6. The relationship between theoretical leaf area-specific conductance ($K_{\text{leaf-theoretical}}$) and measured $K_{\text{shoot-max}}$. Bars = ±SE, n = 6 to 9 branches for $K_{\text{leaf-theoretical}}$ and 7 to 28 branches for $K_{\text{shoot max}}$. 

$r^2 = 0.94$
CHAPTER 5. COORDINATION OF LEAF STRUCTURE AND GAS EXCHANGE ALONG A HEIGHT GRADIENT IN A TALL CONIFER

David R. Woodruff, Frederick C. Meinzer, Barbara Lachenbruch, Daniel M. Johnson

In review
Summary

The gravitational component of water potential and frictional resistance during transpiration lead to substantial reductions in leaf water potential ($\Psi_l$) near the tops of tall trees, which can influence both leaf growth and physiology. We examined relationships between morphological features and gas exchange in foliage collected near the tops of Douglas-fir trees of different height classes ranging from 5 to 55 m. This sampling allowed us to investigate the impact of tree height on leaf structural characteristics in the absence of potentially confounding factors such as irradiance, temperature, relative humidity and branch length. The use of cut foliage for measurement of intrinsic gas exchange characteristics allowed us to identify height-related trends without the immediate influence of path length and gravity. Stomatal density ($D_s$), needle length ($L_n$), needle width ($W_n$) and needle area ($A_n$) declined with increasing tree height by 0.70 mm$^{-2}$ m$^{-1}$, 0.20 mm m$^{-1}$, 5.9$x$10$^{-3}$ mm m$^{-1}$ and 0.012 mm$^2$ m$^{-1}$, respectively. Needle thickness ($T_n$) and mesophyll thickness ($T_m$) increased with height by 4.8 x 10$^{-2}$ mm m$^{-1}$ and 0.74 $\mu$m m$^{-1}$, respectively. Mesophyll conductance ($g_m$) and assimilation under ambient [CO$_2$] ($A_{amb}$) decreased by 9.9$x$10$^{-4}$ mol m$^2$ s$^{-1}$ and 0.082 $\mu$mol m$^{-2}$ s$^{-1}$ per m increase in height, respectively. The observed trend in $A_{amb}$ was not associated with variation in foliar nitrogen content but was associated with $g_m$ and several leaf anatomical characteristics that are likely to be determined by the prevailing vertical tension gradient during foliar development. A linear increase in foliar $\delta^{13}$C values with height (0.042 ‰ m$^{-1}$) implied that relative stomatal limitation of photosynthesis in intact shoots also increased with height. These data suggest that
increasing height leads to both fixed structural constraints on leaf gas exchange and
dynamic constraints related to prevailing stomatal behavior.

Introduction

A number of mechanisms have been studied as factors potentially responsible
for reduced growth in trees as they age and increase in height, none of which are
mutually exclusive. Proposed mechanisms include, but are not limited to reduced leaf
area due to reduced nutrient availability (Gower et al. 1996), genetics-related
reductions in photosynthetic capacity induced by a limited capacity for repeated
meristematic divisions (Haffner et al. 1991), reduced photosynthesis due to increased
hydraulic resistance with increased tree height (Mencuccini & Grace 1996; Ryan &
Yoder 1997; McDowell et al. 2002), reduced foliar expansion due to lower turgor
pressure (Koch et al. 2004; Woodruff et al. 2004) and reduced leaf hydraulic efficiency
due to height-related restrictions upon leaf expansion (Woodruff et al. 2008). Research
addressing how height affects gas exchange in trees has not yet parsed out the extrinsic
effects of path length and gravity from the intrinsic effects of height-related trends in
foliar structural characteristics upon gas exchange.

Leaf structural characteristics can influence gas exchange through their impact
on the efficiency or capacity of processes such as light absorption, carbon fixation or
control of water loss. Leaf structural characteristics that influence gas exchange can
vary in response to changes or gradients in environmental conditions (reviewed in
Abrams 1994; Gutschick 1999). Mesophyll cells comprise the bulk of foliar tissue and
represent the site of two types of resistance that can influence CO\textsubscript{2} assimilation ($A$): resistance to the transport of water from leaf veins and xylem conduits to stomata, and resistance to the movement of CO\textsubscript{2} from the intercellular air spaces to sites of carboxylation inside the chloroplasts. Recent research has begun to reveal the importance of internal leaf conductance for gas exchange. Across a range of 50 species, internal leaf resistance accounted for approximately 40% of the decrease in CO\textsubscript{2} concentration between the atmosphere and sites of carboxylation (Syvertsen et al. 1995; Hanba et al. 1999; Warren 2007, and references therein; Flexas et al. 2008). The determinants of internal leaf conductance are not yet fully understood, but are likely to include a combination of leaf anatomical and biochemical factors (Warren 2007). Leaf mesophyll structural characteristics that could affect the movement of either water or CO\textsubscript{2} are thus likely to have an impact upon $A$.

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the world’s tallest tree species (Carder 1995) and can attain heights greater than 100 m. On the basis of above ground volume, Douglas-fir is the dominant tree species in the United States with nearly twice the volume of the next most common species, loblolly pine (Smith et al. 2002). This study addresses the following questions: 1) what effect does tree height have upon the structural characteristics of Douglas-fir foliage, and 2) are there limitations upon gas exchange associated with intrinsic leaf structural characteristics that can be attributed to path length and gravity during the time of foliar development? The goal of this study was to determine the extent to which height imposes developmental constraints upon foliage that lead to intrinsic limitations for gas
exchange. We hypothesized that height-related trends in leaf structure impose constraints on leaf gas exchange that are independent of the direct effects of vertical gradients in xylem tension on stomatal and photosynthetic physiology.

We measured stomatal density ($D_s$), length ($L_n$), width ($W_n$) thickness ($T_n$), area ($L_A$) and mesophyll thickness ($T_m$) for Douglas-fir needles. Sampling was conducted exclusively from fully sun-exposed branches near the tops of trees of different height classes in order to rule out the potentially confounding influence of factors such as irradiance, relative humidity and branch length upon height-related trends in leaf structural characteristics. We also evaluated a number of parameters related to $A$ using photosynthetic CO$_2$ response curves, including $A$ at ambient [CO$_2$] ($A_{amb}$), maximum $A$ ($A_{max}$), maximum carboxylation rate allowed by rubisco ($V_{cmax}$), rate of photosynthetic electron transport ($J$), triose-phosphate use ($TPU$) and mesophyll conductance ($g_m$). These parameters were measured on branches sampled from the tops of trees of different height classes. We conducted measurements on detached shoots with their bases in water in order to eliminate the immediate effects of path length and gravity upon gas exchange, thereby enabling us to isolate the influence of any height-related trends in foliar structure upon different gas exchange parameters.

Materials and Methods

Field Site and Sampling

Four separate stands, each containing Douglas-fir trees of a different height class, were located within 3.1 km of each other in the Wind River Basin of
southwestern Washington, U.S.A. Access to tree tops in the 55-m sampling height class was facilitated by a 75-m-tall construction tower crane at the Wind River Canopy Crane Research Facility (WRCCRF). Tree tops in all other height classes were accessed by non-spur climbing. 30–50 cm long branch samples were collected within one to five meters of the tops of the trees at mean sampling heights of 5.0, 18.3, 33.5 and 55.0 m. All samples were obtained from fully sun-exposed locations. Branches were collected from trees early in the morning prior to significant transpirational water loss and were placed in plastic bags with moist paper towels and stored in the dark in a cooler and then at 5°C in a refrigerator.

The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.2 m, much of which falls as snow, with a dry season from June through September. The mean annual temperature is 8.7°C with means of 0°C in January and 17.5°C in July. The soils are well-drained and of volcanic origin (Shaw et al. 2004). Very low precipitation between June and September (~119 mm) typically leads to drought conditions in the upper portion of the soil profile. However, soil water remains accessible to Douglas-fir roots at depths greater than about 1 m throughout the summer dry period (Warren et al. 2005; Meinzer et al. 2007).

**Leaf Structural Characteristics**

Needle width, length and thickness were measured with digital calipers. Three needles were measured from three different branches for each tree, for a total of nine
measurements per tree. Leaf areas were measured on ten needles per branch, on three branches per tree and three trees per height class. One-sided leaf areas were obtained with a scanner and ImageJ version 1.27 image analysis software (Abramoff et al. 2004).

Mesophyll thickness ($T_m$) was used as a proxy for the distance necessary for water to move through mesophyll tissue inside the leaf and was estimated as the distance between the outside of the endodermis and the nearest inside edge of the leaf epidermis. Cross sections of needles were made by hand sectioning fresh tissue and mounting them on slides. Needle cross sections were analyzed for $T_m$ using an image analysis system consisting of a compound microscope and video camera. Three needles were analyzed from each of three branches, for a total of nine measurements per tree. Images were obtained using x10 objectives with total magnifications of x100. Data were pooled per tree and analyzed using regression analysis. Stomatal density was measured using a dissecting scope and a linear scale with mm increments. Stomata were counted over a 1 mm length near the center of the needles. Number of stomata per 1 mm length was divided by needle width to generate number of stomata per mm$^2$ leaf area. Three needles were counted from each of four to five branches for a total of 12 to 15 measurements per tree.

**Gas Exchange and Carbon Isotope Ratios**

Gas exchange measurements were conducted on detached foliage in the laboratory. Prior to starting the gas exchange measurement, we detached shoots of
about 15 to 20 cm length from the larger branch samples, taking care to submerge the shoot bases in de-gassed water as the cut was made. Our previous work on attached and detached Douglas-fir foliage showed that Douglas-fir shoots retain the same gas exchange characteristics for approximately four days after detachment. In this study, mean $A_{\text{max}}$ for 55 m samples at zero, one and two days following excision were 16.5, 14.6 and 19.7 $\mu$mol m$^{-2}$ s$^{-1}$, respectively ($P = 0.25$ for regression analysis of $A_{\text{max}}$ vs. days since excision).

We used a portable photosynthesis system equipped with a red/blue LED source and CO$_2$ injector (LI-6400; Li-Cor Inc.). The instrument was zeroed and the chemicals replaced prior to use each day. For determination of the dependence of $A$ on intercellular CO$_2$ concentration ($A$-$C_i$ curves), photosynthetic photon flux density was held at 1200 $\mu$mol m$^{-2}$ s$^{-1}$, vapor pressure deficit at 1.0 KPa and leaf temperature at 25º C. The cuvette CO$_2$ concentration was initially set near ambient, progressively lowered to 50 ppm, increased directly to ambient, and then progressively increased. The ambient CO$_2$ concentrations were set at the following levels and order: 400 ppm, 300 ppm, 200 ppm, 100 ppm, 50 ppm, 400 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, 1200 ppm, and increased at 200 ppm intervals until no further response of $A$ was observed. Leaves were allowed to equilibrate for at least two minutes following the increase in CO$_2$ concentration. Branch samples were measured within one to three days of excision.

The $A$-$C_i$ curves were used to estimate $g_m$, $V_{c_{\text{max}}}$, $J$ and $TPU$ using a utility developed by Sharkey et al. (2007) based upon the model developed by Farquhar et al. (1980). In this model, the biochemical reactions of photosynthesis are considered to be
either limited by the properties of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the regeneration of the substrate RuBP, or by triosephosphate use (TPU) limitation. When $A$ is Rubisco-limited, the response of $A$ to $[\text{CO}_2]$ can be described by the following equation:

$$A = V_{\text{max}} \left[ C_C - F^* / C_C + K_C (1+O/K_0) \right] - R_d$$

(1)

where $C_C$ and $O$ are the partial pressures of CO$_2$ and oxygen, respectively, at the sites of carboxylation, $K_C$ is the Michaelis constant of Rubisco for carbon dioxide, and $K_0$ is the inhibition constant of Rubisco for oxygen. When $A$ is limited by RuBP regeneration:

$$A = J \left[ C_C - F^*/4C_c + 8F^* \right] - R_d$$

(2)

where $J$ is the rate of electron transport. In the $A$-$C_i$ utility used to analyze photosynthesis, $C_C$ is replaced with $(C_i - A/g_m)$ and $g_m$ is estimated from the observed data by non-linear curve fitting and minimizing the sum of squared model deviations.

We determined the mean value of $C_i$ at standard ambient CO$_2$ concentration (385 ppm) for all $A$-$C_i$ curves in order to be able to characterize photosynthetic parameters under standard ambient [CO$_2$]. The mean leaf internal [CO$_2$] at 385 ppm sample [CO$_2$] was 235 ppm (se = 6.25) (Table 5.1). $A$ at ambient [CO$_2$] ($A_{\text{amb}}$) was determined by first establishing a mean $C_i$ value for ambient [CO$_2$] (385 ppm) and then
determining $A$ at that $C_i$ value for each $A$-$C_i$ curve. Instantaneous water use efficiency ($A/g_s$) was determined by dividing $A_{amb}$ by stomatal conductance ($g_s$) at $C_i = 235$ ppm. Stable carbon isotope analysis was conducted on leaf tissue in order to determine integrated intrinsic water use efficiency. Foliage on which gas exchange was measured was collected, dried and ground, and analyzed for $\delta^{13}C$ and nitrogen content ($N$) at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah. Samples were analyzed for $\delta^{13}C$ and $N$ using a Carla Erba 1110 elemental analyzer coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer through an open split interface. Integrated water use efficiency ($A/g_s$-integrated) was obtained from the following equation from Farquhar et al. (1989):

$$A/g_s\text{-integrated} = (C_A - C_i)/1.6 \quad (3)$$

An analysis of the vertical trend in integrated, in–situ photosynthesis ($A_{\text{integrated}}$) was performed by calculating $A$ from $\delta^{13}C$ values of leaf tissue. We determined discrimination ($\Delta$) against $^{13}C$ in leaf tissue using the following the following equation from Farquhar et al. (1982):

$$\Delta = \delta_{\text{source}} - \delta_{\text{product}} / 1+ (\delta_{\text{source}}/1000) \quad (4)$$

where $\delta_{\text{source}} = \delta^{13}C$ for atmospheric CO$_2$ (-8 $^{\circ}/_{oo}$), $\delta_{\text{product}} = \delta^{13}C$ for leaf tissue. We then used the following equation, also from Farquhar et al. (1982), to obtain $C_i$ at standard atmospheric [CO$_2$] ($C_A = 385$):
\[ \Delta = a + (b - a) \frac{C_i}{C_A} \] (5)

where \( a \) = discrimination against \( ^{13}C \) during diffusion (4.4 \( \text{‰} \)), \( b \) = discrimination against \( ^{13}C \) during the carboxylation reaction (27.0 \( \text{‰} \)). By rearranging this equation we were able to solve for \( C_i \):

\[ C_i = C_A \left( \Delta - \frac{a}{b} - a \right) \] (6)

We then used the 3-parameter exponential equation \( Y = Y_0 + a(1-e^{-bx}) \) describing the dependence of \( A \) on \( C_i \) obtained from gas exchange measurements in the lab to estimate \( A \) from the \( C_i \) values obtained from foliar \( \delta^{13}C \). \( g_s\text{-integrated} \) was determined from estimates of \( A/g_s\text{-integrated} \) and \( A\text{integrated} \). Maximum leaf hydraulic conductance (\( K_{\text{leaf-max}} \)) was measured using the methods described by Woodruff et al. (2008).

**Results**

**Leaf Structural Characteristics**

There were height-related trends in the examined leaf morphological characteristics (Fig. 5.1). A 0.2 mm decline in needle length per m increase in height was the single most significant trend in needle morphology (\( P < 0.0001 \)). One-sided needle area showed the next most significant height-related trend, declining by 0.21 mm\(^2\) for every meter increase in height (\( P = 0.0004 \)). Needle width declined with
increasing height by $5.9 \times 10^{-3}$ mm m$^{-1}$ ($P = 0.0025$), and needle thickness increased with height by $8.9 \times 10^{-4}$ mm m$^{-1}$ ($P = 0.0011$). Stomatal density showed a significant linear decline with increasing height (-0.7 stomata m$^{-1}$, $P = 0.0032$), but a three-parameter exponential decay function ($y = y_0 + ae^{-bx}$) yielded an improved regression fit with $r^2 = 0.77$ (data not shown) suggesting a possible limit to the reduction in stomatal density with increased height. Mesophyll thickness also showed a significant increase with increasing height (0.74 μm m$^{-1}$, $P = 0.0036$).

**Gas Exchange**

Gas exchange measurements showed that in the initial portion of the $A$-$C_i$ curves where $A$ is heavily influenced by $g_m$, $A$ declined with increasing height (Fig. 5.2). Mean $A_{amb}$ at 55m was 5.49 (se = 0.41) μmol m$^{-2}$ s$^{-1}$, and mean $A_{amb}$ at 5m was 9.52 (se = 0.42) μmol m$^{-2}$ s$^{-1}$, representing a 42% reduction in assimilation under ambient [CO$_2$] over a 50 m increase in height (Figs. 5.2 and 5.3). $A_{amb}$ and $g_m$ both declined significantly with height ($r^2 = 0.72$ and $P = 0.00051$; $r^2 = 0.57$ and $P = 0.0047$, respectively, data not shown). A three-parameter exponential decay function ($y = y_0 + ae^{-bx}$) yielded improved regression fits with $r^2 = 0.83$ and 0.70 for $A_{amb}$ and $g_m$ plotted against height, respectively (Fig. 5.3). Plotting $A_{amb}$ against $g_m$ revealed a more significant relationship than either variable plotted against height ($r^2 = 0.89$, $P < 0.0001$), consistent with $g_m$ as an important determinant of $A$ under non-saturating [CO$_2$] (Fig. 5.3, insert). The correlation between $A_{max}$ and height was also significant ($r^2 = 0.45$, $P = 0.017$, Table 5.1) although substantially less so than height vs. $A_{amb}$. Neither
$V_{\text{max}}$ nor leaf nitrogen per unit leaf area ($N_A$) were significantly correlated with height ($r^2 = 0.18$, $P = 0.17$, $r^2 = 0.32$, $P = 0.056$, respectively (Table 5.1). $J$ and TPU were also not significantly correlated with height ($r^2 = 0.01$, $P = 0.76$; $r^2 = 0.09$, $P = 0.34$, respectively). Foliar $\delta_{13}C$ increased significantly with height by 0.042 o/$^{\circ}$o m$^{-1}$ ($P = 0.0002$, data not shown). Integrated water use efficiency ($A/g_{s\text{-integrated}}$) increased significantly with height ($P = 0.00019$) by 0.45 µmol mol$^{-1}$ for every one m increase in height (Fig. 5.4a). In contrast to $A/g_{s\text{-integrated}}$, there was no significant increase in $A/g_s$ for detached twigs. Although not significant, there was in fact a slight decline in $A/g_s$ with increasing height ($P = 0.011$) (Fig. 5.4b). $T_m$ was negatively correlated with both maximum leaf hydraulic conductance ($K_{\text{leaf-max}}$) ($P = 0.00008$) (Fig. 5.5a) and $A_{\text{max}}$ ($P = 0.02$) (Fig. 5.5b). $A_{\text{max}}$ and $K_{\text{leaf-max}}$ were positively correlated with each other ($P = 0.02$, data not shown). $A_{\text{integrated}}$ was estimated from foliar $\delta_{13}C$ and thus reflects the photosynthetic history of the foliage when the shoot was still attached to the tree. We observed that $A_{\text{integrated}}$ decreased with increasing height ($r^2 = 0.89$, $P < 0.0001$), just as it had for $A_{\text{amb}}$, but that the decline was steeper. A three-parameter exponential decay function ($y = y_0 + ae^{bx}$) yielded an improved regression fit with $r^2 = 0.99$ for the correlation of $A_{\text{integrated}}$ with height (Fig. 5.6a). $g_{s\text{-integrated}}$ was also significantly correlated with height ($P = 0.0002$). A three-parameter exponential decay function ($y = y_0 + ae^{bx}$) yielded an improved regression fit with $r^2 = 0.98$ (Fig. 5.6b).
Discussion

We were able to isolate gravity and path length as causal factors for trends in gas exchange by collecting our samples exclusively from the tops of Douglas-fir trees of different heights, within a localized geographical area, thereby eliminating appreciable differences in irradiance, humidity, temperature and branch length as confounding variables in the development of foliar characteristics which could influence gas exchange. These size-related trends in gas exchange of Douglas-fir can thus be attributable to intrinsic properties of the shoot that arise during tissue development.

Leaf Structural Characteristics

Leaf structural characteristics can strongly impact processes involved in gas exchange such as light absorption, carbon fixation and water loss. Needle length, width and area each showed significant negative trends with increasing tree height, supporting earlier observations of reduced leaf expansion along height gradients within the crowns of individual Douglas-fir trees (Woodruff et al. 2004; Meinzer et al. 2008). Although one might assume that smaller leaves would have higher stomatal density due to the tighter packing of stomata amongst less expanded epidermal cells as has often been reported in sun vs. shade leaves (Givnish 1988; Osborn & Taylor 1990), we found a 19% reduction in mean stomatal density from the lowest to the tallest height class studied. Previous work has shown that cell division as well as expansion is sensitive to turgor pressure (Boyer 1968; Kirkham et al. 1972; Hsiao et al. 1976; Gould &
Measures 1977), which could have implications for the mechanisms that control the differentiation of epidermal cells to stomata. Beerling & Chaloner (1993) found a reduction in stomatal density in *Quercus* with increased temperature, and suggested that the observed response was a possible adaptation to reduce water loss. Although reduced stomatal density will tend to limit capacity for \( A \), particularly in leaves of higher density where lateral movement of CO\(_2\) through internal air spaces is limited (Parkhurst 1986), its primary consequence may be to reduce water loss, particularly in cases in which stomata do not completely close (Caird *et al.* 2002).

**Resistance to Transfer of CO\(_2\)**

There are two predominant resistances to the diffusion of CO\(_2\) as it moves from the atmosphere to the sites of carboxylation inside the leaf. Stomatal resistance occurs as CO\(_2\) diffuses through the stomata and into the substomatal cavities. The less-studied type of foliar resistance to CO\(_2\) uptake is mesophyll resistance (\( r_m \), the inverse of \( g_m \)) which involves the resistance to CO\(_2\) diffusion through the mesophyll cell walls and membranes, and through the liquid phase inside the mesophyll cells. At the mesophyll cell surface, CO\(_2\) enters the liquid phase and moves through the cell wall and through the chloroplast membrane to the site of carboxylation. Mesophyll resistance to CO\(_2\) transfer can account for a substantial proportion of the total resistance to CO\(_2\) transfer. Peña-Rojas *et al.* (2005) found \( g_m \) in *Quercus* to equal about one-half of \( g_s \). DeLucia *et al.* (2003) found \( g_m/g_s \) to be between 0.6 and 1.1 in conifers and between 1.0 and 4.2 in angiosperms, and in a survey of 50 species Warren (2007) found \( g_m/g_s \) to be 1.3 in
conifers and between 0.52 and 1.5 in angiosperms. In the current study, we found the ratio of $g_m$ from detached shoots to $g_s$-integrated from foliar $\delta^{13}C$ to range from between 0.47 to 0.71.

We found a 44% reduction in mean $g_m$ along a 50-m height gradient (Fig. 5.3b), which was strongly correlated with variation in $A_{amb}$ among height classes (Fig. 5.3, insert). Mesophyll conductance is considered to be a key determinant of $A$ at low values of $C_i$ and thus the initial slope of $A$-$C_i$ curves (Farquhar et al. 1980). Consistent with this relationship, the most pronounced differences in $A$ between height classes were observed under non-saturated [CO$_2$] ($A_{amb}$, Figs. 5.2, 5.3), whereas the trend in $A_{max}$ with increasing height was much less pronounced (Table 5.1). Given the importance of $g_m$ as a determinant of $A$ under non-saturating [CO$_2$], the greater height-related decline in $A_{amb}$ relative to that of $A_{max}$ provides further evidence of $g_m$ as a critical factor in the observed decline in $A_{amb}$ with increasing height in Douglas-fir.

Water stress has been shown to lead to reductions in $g_m$, even in cases where the water stress is only moderate (OToole et al. 1976; Loreto et al. 1997; Peña-Rojas et al. 2005). A proposed mechanism for the water stress-induced depression of $g_m$ is through reduced leaf turgor and its influence on mesophyll surface area. The height-related decline in $g_m$ in our study is thus likely to be associated with the effects of gravitational and transpirational xylem tension gradients upon leaf water potential (Bauerle et al. 1999) and turgor during leaf expansion (Woodruff et al. 2004). Warren et al. (2004) used simultaneous gas exchange and isotopic measurements to determine $g_m$ for Douglas-fir seedlings under different hydration regimes and found 73% higher $g_m$ in
well-watered Douglas-fir seedlings than in water-stressed seedlings (0.076 vs. 0.044 mol m$^{-2}$ s$^{-1}$, respectively). In the first study to report $g_m$ in a conifer, Warren et al. (2003) found mean $g_m$ was 0.16 mol m$^{-2}$ s$^{-1}$ in the lower canopy (17-20 whorls down from the top) and 0.20 mol m$^{-2}$ s$^{-1}$ in the upper canopy (4-8 whorls down from the top) within the same 34–m-tall Douglas-fir tree. Their observed height-related increase in $g_m$ suggests that light exposure within a tree crown may mask intrinsic height-related trends in $g_m$.

**Hydraulic Resistance**

In addition to resistance to the movement of CO$_2$ from the intercellular air spaces to sites of carboxylation inside the chloroplasts, the leaf mesophyll also presents a site of resistance to the transport of water from xylem conduits to the stomata. Water moves out of the leaf xylem and then through bundle sheath and mesophyll cells before evaporating in the intercellular air spaces. The resistance to water flow through living mesophyll tissue is substantially greater than through non-living vessels and tracheids (Boyer 1985) and thus mesophyll hydraulic resistance may represent a substantial limitation for gas exchange. Aasamaa et al. (2001) found that the area of mesophyll and epidermal cells per unit length of leaf cross-section was strongly and positively correlated with shoot hydraulic conductance across a range of deciduous trees. Brodribb et al. (2007) found that across 43 species, $A_{max}$ was correlated with the distance between veins and leaf surface and with leaf hydraulic conductance ($K_{leaf}$). They concluded that the relationship between vein location and photosynthetic rate was
determined by the conductance of the leaf mesophyll to water flow. In our study, the highly significant correlation between $T_m$ and $K_{\text{leaf-max}}$ (Fig. 5.5a) suggests that leaf structural characteristics that influence mesophyll resistance can have a substantial influence upon leaf hydraulic architecture, and subsequently photosynthesis.

**Nitrogen- and Water- Use Efficiency**

Nitrogen content per unit leaf area ($N_A$) was 17% lower at 55m than at 5m, although the correlation between height and $N_a$ was not significant ($P = 0.14$). The lack of a significant decline in $N_a$, despite the significant height-related decline in $A_{\text{amb}}$, indicates a reduction in photosynthetic $N$-use efficiency with increasing height.

The contrast between the strong increase in $A/g_s$-integrated with increasing height, and the slight decrease in $A/g_s$ with increasing height (Fig. 5.4) highlights the role of stomatal control of water loss and the resulting diffusional limitations on photosynthesis in attached shoots. The significant trend in $A/g_s$-integrated with height (Fig. 5.4) is consistent with height-related trends in foliar $\delta^{13}C$ obtained by others (Yoder *et al.* 1994; Koch *et al.* 2004). This height-related increase in intrinsic water use efficiency due to the impact of reduced $g_s$ on $A$ is one of the key components to the hydraulic limitation hypothesis (Ryan & Yoder 1997). The lack of a height-related increase in $A/g_s$ for shoots that were sampled along a height gradient, but which had their cut bases in water during photosynthesis measurements, provides further evidence that path length and gravity are responsible for the height-related trend in $A/g_s$-integrated. The slight decline in $A/g_s$ with increasing height for the cut shoots may reflect a height-
related trend in concentrations of osmotic solutes in stomatal guard cells and other leaf cells. Osmotic adjustment that partially maintains leaf cell turgor along a height gradient has been observed in conifers (Koch et al. 2004; Woodruff et al. 2004). Stomatal opening is activated and maintained by a solute-mediated influx of water into the guard cells. Thus, when the impacts of gravity and path-length on xylem tension are removed in detached shoots, they may exhibit greater \( g_s \) as a result of the height-related gradient in osmotic concentration.

Estimates of height-related variation of \( A \) in situ (\( A_{\text{integrated}} \), Fig. 5.6a) obtained from foliar \( \delta_{13}C \) values and \( A-C_i \) curves followed a pattern similar to that of \( A_{\text{amb}} \) (Fig. 5.3). \( A_{\text{integrated}} \) declined more steeply than \( A_{\text{amb}} \) with increasing height. The reductions in mean \( A_{\text{integrated}} \) and \( A_{\text{amb}} \) from the 5 m to the 55 m sampling heights were 5.64 and 4.03 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), respectively. Whereas \( A_{\text{integrated}} \) incorporates the stomatal component of limitation to \( A \) because it represents an estimate of integrated, in-situ \( A \); \( A_{\text{amb}} \) excludes the stomatal component because it is derived from the dependence of \( A \) upon substomatal [CO\(_2\)]. Mean integrated values of \( g_s \) (\( g_{s-\text{integrated}} \)) from the 5m to the 55 m sampling heights declined by 2.5 mmol m\(^{-2} \) s\(^{-1} \) for every 1 m increase in height (Fig. 5.6b). Although absolute values may not be directly comparable due to the uncertainties associated with estimating stomatal conductance from foliar \( \delta_{13}C \), the height-related trends in \( g_m \) and \( g_{s-\text{integrated}} \) suggest that \( g_s \) decreases more rapidly than \( g_m \) with increasing height.

Our data support the hypothesis that height-related trends in leaf structure impose constraints on leaf gas exchange that are independent of the direct effects of
vertical gradients in xylem tension on stomatal and photosynthetic physiology.

Nevertheless, gravity and path length, through their impact on xylem tension during leaf expansion, were likely to have been the principle determinants of the trends in foliar characteristics that were correlated with the observed height-related trends in gas exchange.

Acknowledgements

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References


Table 5.1. Foliage sample characteristics from within 1 to 5 m of the tops of the trees at mean sampling heights of 5.0, 18.3, 34.5 and 55.0 m (mean values with SE in parentheses): nitrogen content per unit leaf area ($N_A$) maximum photosynthesis ($A_{\text{max}}$), instantaneous water use efficiency ($A/g_s$), internal $\text{[CO}_2\text{]}$ under ambient $\text{[CO}_2\text{]}$ ($C_i$ at $C_a = 385$ ppm), maximum carboxylation rate allowed by rubisco ($V_{\text{cmax}}$), mesophyll conductance ($g_m$), rate of photosynthetic electron transport ($J$), and triose-phosphate use ($TPU$).

<table>
<thead>
<tr>
<th>Ht. (m)</th>
<th>$N_A$ (g m$^{-2}$)</th>
<th>$A_{\text{max}}$ (μmol m$^{-2}$ s$^{-1}$)</th>
<th>$A/g_s$ (μmol mol$^{-1}$)</th>
<th>$C_i$ at $C_a = 385$ ppm (ppm)</th>
<th>$V_{\text{cmax}}$ (μmol m$^{-2}$ s$^{-1}$)</th>
<th>$g_m$ (μmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$)</th>
<th>$J$ (μmol m$^{-2}$ s$^{-1}$)</th>
<th>$TPU$ (μmol m$^{-2}$ s$^{-1}$)</th>
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<td>99.04</td>
<td>0.10</td>
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<td>7.83</td>
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<td>0.086</td>
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<td>72.40</td>
<td>247.5</td>
<td>117.1</td>
<td>0.041</td>
<td>124.4</td>
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<td>251.6</td>
<td>78.62</td>
<td>0.056</td>
<td>107.4</td>
<td>7.19</td>
</tr>
</tbody>
</table>

Note: All values are mean ± SE.
Fig. 5.1. Leaf anatomical and structural characteristics in relation to tree height for foliage samples obtained within 1 to 5 m of the tops of Douglas-fir trees at mean sampling heights of 5.0, 18.3, 34.5 and 55.0 m. Needle length (a), needle width (b), needle thickness (c), leaf area per needle (d), stomatal density (e) and mesophyll thickness (f). Bars = ±SE, n = 3 branches per tree for (a), (b), (c), (d) and (f); n = 4 branches for (e).
Fig. 5.2. $A$ vs. intercellular CO$_2$ concentration ($A$-$C_i$ curves) for foliage samples obtained within 1 to 5 m of the tops of Douglas-fir trees at mean sampling heights of 5.0, 18.3, 34.5 and 55.0 m. The vertical dashed line represents 235 ppm, the mean value for $C_i$ under standard ambient [CO$_2$] (385 ppm).
Fig. 5.3. Assimilation at $C_i$ value of 235 ppm ($A_{\text{amb}}$) vs. height (a), and mesophyll conductance ($g_m$) vs. height (b). Insert shows $A_{\text{amb}}$ vs. $g_m$. Bars = ±SE, $n = 2$ branches.
Fig. 5.4. Integrated water use efficiency ($A/g_{s-integrated}$) vs. height (a), and instantaneous water use efficiency ($A/g_s$) vs. height (b). Bars = ±SE, n = 3 branches for (a) and n = 2 branches for (b).
Fig. 5.5. Maximum leaf hydraulic conductance ($K_{leaf-max}$) vs. mesophyll thickness ($T_m$) (a) and maximum photosynthesis ($A_{max}$) vs. $T_m$ (b). Horizontal bars = ±SE, $n = 3$ branches for $T_m$ (a and b). Vertical bars = ±SE, $n = 2$ to 8 branches for $K_{leaf-max}$ (a) and $n = 2$ branches for $A_{max}$ (b). 3 data points in (a) represent trees with only one branch with a $K_{leaf-max}$ value and thus lack vertical bars.
Fig. 5.6. Integrated assimilation ($A_{\text{integrated}}$) vs. height (a) and integrated stomatal conductance ($g_{s\text{-integrated}}$) vs. height (b). Bars = ±SE, $n = 3$ branches.
CHAPTER 6. CONCLUSIONS AND FUTURE WORK

Conclusions

Increased tree height leads to an increase in xylem tension (Fig 6.1a) due to both the gravitational hydrostatic gradient and frictional resistance to flow through the xylem. Increased xylem tension results in a reduction in leaf water potential and reduced leaf turgor (Fig 6.1b). In chapter two I investigated the physiological constraints on turgor that result from the gravitational component of plant water potential, and the subsequent reductions in shoot and leaf expansion (Fig 6.1c) in Douglas-fir trees. In tall trees such as Douglas-fir the gravitational component of $\Psi_l$ can lead to a substantial reduction in leaf turgor similar to that imposed by soil water deficits. Because leaf expansion is driven in part by turgor, an objective of this chapter was to determine the likelihood that height-related trends in leaf size were primarily associated with the gravitational component of $\Psi_l$. This research showed that leaf turgor decreased with increasing height, particularly during spring when vegetative buds were in the process of swelling. The observed height-related trends in leaf dimensions, leaf mass per area, and shoot expansion were consistent with a height-related decline in leaf turgor with increasing height. No osmotic adjustment to compensate for the gravitational gradient of $\Psi_l$ was observed during late spring (May). However, osmotic adjustment was observed after leaf expansion was completed (July). This osmotic adjustment largely mitigated but was not sufficient to completely offset the observed height-related decline in leaf turgor. This research provided evidence that
the impacts of height-associated reductions in turgor are several-fold because reduced
turgor leads to a greater amount of photosynthate needed to produce a given amount of
leaf area (6.1d, e); increasingly smaller annual increments in photosynthetically active
leaf area lead to a reduced capacity for assimilation, and osmotic adjustment to offset
turgor loss in leaves represents an expenditure of energy and diversion of resources in
the case of both organic solutes produced and inorganic solutes taken up and
sequestered by the tree (Fig 6.1f, g).

Stomatal conductance is linked directly to leaf hydraulic efficiency due to the
dependence of $g_s$ on the availability of water to leaf epidermal cells (Fig 6.1h), where
stomata react to reduced water supply by closing, which in turn limits the increase in
the xylem tension gradient in the tree (Fig. 6.1i). However, reduced $g_s$ also constrains
photosynthesis (Fig 6.1j) and growth (Fig 6.1k). In chapter three I investigated the
effect of height upon leaf hydraulic efficiency, leaf hydraulic vulnerability, and
stomatal regulation of transpiration in Douglas-fir. Stomatal regulation of transpiration
was tightly coordinated with vertical gradients in xylem tension. Values of $\Psi_1$ at which
morning stomatal closure was initiated were highly correlated with those at which
critical minimum values of $K_{leaf}$ were reached. Cryo-SEM images provided evidence
that the embolism and re-filling of leaf tracheids in Douglas-fir needles is likely to
occur on a daily basis during the growing season, even under non-extreme
environmental conditions. Height-related trends in stomatal regulation of transpiration
thus appeared to be related to reversible embolism in leaf tracheids. This reversible
embolism may represent a component of a hydraulic signal that allows stomata to
preserve the integrity of the tree’s water transporting system by maintaining stem Ψ above a critical minimum point beyond which the spread of embolism may become uncontrollable. Leaf embolism as a signal to initiate stomatal closure and to regulate the water status of woody stems may be beneficial if the capacity for embolism repair in leaves is greater than that in woody stems.

In chapter four I investigated height-related trends in leaf tracheid anatomical characteristics and their relationship to shoot hydraulic safety and efficiency in Douglas-fir. This research showed that several leaf tracheid anatomical characteristics were correlated with shoot hydraulic efficiency (Fig 6.1l), and/or hydraulic safety (Fig 6.1m). The results of this research also indicate that the observed vertical trends in hydraulic safety and efficiency were in opposition to each other. Several leaf anatomical characteristics varied with height in a manner that was consistent with the effects of reduced leaf turgor upon cell division and cell expansion (Fig 6.1n). Because height-related trends in tracheid anatomy were associated with reduced leaf water transport capacity, they were likely to impose an additional limitation on leaf expansion by constraining the hydraulic system that supplies the water necessary for cell expansion (Fig 6.1o). Height-related trends in both water transport capacity and turgor-limited cell expansion are thus likely to compound each other. Height-related trends in leaf cell development were also associated with reduced risk of water-stress induced embolisms in leaf tracheids. The effects of gravity and path length resistance upon xylem tension that are associated with increasing tree height were partially offset by the decline in shoot hydraulic vulnerability with increasing height. The observed height-
related increase in hydraulic safety may provide taller trees with the ability to maintain stomatal conductance (Fig. 6.1p) and thus photosynthesis during periods of greater water stress.

In chapter five I investigated the relationships between leaf structural characteristics and height-related trends in gas exchange in Douglas-fir. Height was associated with constraints on leaf gas exchange, independent of the direct impacts of vertical gradients in xylem tension on $g_s$ and photosynthesis. The observed trends in gas exchange with increasing height were correlated with height-related patterns in leaf structural characteristics, including gross leaf morphological characteristics such as leaf thickness, width and length (Fig. 6.1d), as well as internal leaf structure such as mesophyll thickness (Fig 6.1q). Leaf morphological characteristics influence leaf area and photosynthesis (Fig 6.1r), and mesophyll thickness influences leaf hydraulic efficiency because mesophyll represents a portion of the pathway for water transport to the leaf surface (Fig 6.1s). The greater the path length through mesophyll from the tracheids to the leaf surface, the greater the resistance to water transport to the leaf surface. Mesophyll tissue is particularly relevant to leaf hydraulic conductance because it exhibits substantially greater resistance to water flow than tracheids. Mesophyll characteristics that are likely to be tightly coupled to leaf turgor during leaf development can also impact photosynthesis through their influence on the diffusion of CO$_2$ through the mesophyll cell walls and membranes (Fig 6.1t and u). By collecting foliage exclusively from the tops of trees, within a localized geographical area, we were able to isolate gravity and path length as the causal factors for the observed trends in
leaf structural properties. Gas exchange measurements on detached foliage revealed height-related trends that were attributable to intrinsic foliar properties that develop during leaf expansion, as opposed to gradients in xylem tension related to path length and gravity at the time of the measurements. The direct connection between growth and height in the schematic (Fig 6.1v) illustrates the fact that if overall tree growth is sufficiently limited it will be reflected in a decrease or cessation in annual height increment, thus representing a feedback relationship between growth and height that is mediated in part by the structural and functional characteristics of foliage that have been studied for this dissertation.

The chapters presented here show that with increased height, Douglas-fir foliage is faced with a range of physiological constraints that impact expansion, hydraulic efficiency and gas exchange. This is noteworthy because it emphasizes the fact that there is no single cause of size-related growth decline that functions to the exclusion of other factors. The size-related decline in forest productivity is best considered to be a “syndrome” as opposed to the result of a single cause. The research conducted for this dissertation also identified adaptive compensatory strategies that mitigate some height-related constraints on growth. The size-related physiological constraints observed in Douglas-fir for this research are not necessarily experienced by all species in the same manner. Due to their substantially different structural and functional characteristics, species of *Eucalyptus*, for example may respond differently to height than Douglas-fir. It is possible that each species experiences a unique suite of size-induced structure-function relationships that limit growth and/or mitigate growth limitation.
Future work

Future work could involve investigating the observed physiological constraints, structure-function relationships and compensatory mechanisms across a range of other tree types. The extent to which these constraints, relationships and mechanisms are consistent across genera, families or divisions would provide insight into the universality of these phenomena. Similarly, conducting investigations over a range of environmental conditions, such as across a moisture gradient as opposed to a height gradient, could provide further insight into how productivity is affected by height and moisture availability, as well as how trees are able to benefit from structural and biochemical compensatory strategies to enhance safety or maintain growth under adverse conditions.

The relationship between leaf hydraulic conductance and leaf water potential and cryo-SEM images at different levels of leaf hydration provide evidence that Douglas-fir leaves embolize and refill on a daily basis. Future work in this area could involve investigating the mechanisms involved in refilling and how this occurs while the hydraulic pathway is under tension. Manipulation of aquaporins and/or concurrent measurements over a diurnal period of leaf water potential, sap flow and leaf hydraulic conductance, in conjunction with SEM images of cryogenically frozen tissues could provide information about daily refilling of embolized conduits. This re-filing process could involve the active movement of solutes to create a pressure gradient to refill cells.
that would otherwise be under tension. As such, this work could also involve efforts to identify and quantify the principal solutes involved.

Future research could also involve attempting to quantify the relative contributions of each of the observed physiological constraints to size-related reductions in forest productivity, as well as the extent to which adaptive strategies function to mitigate these constraints. Modeling could represent a useful approach to gaining a better understanding of the effect that each of these constraints and adaptive strategies has upon productivity.
Figure 6.1 Schematic illustrating some of the relationships between tree height, growth and leaf anatomical and functional characteristics.
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