

Height-related trends in leaf xylem anatomy and shoot hydraulic characteristics in a tall conifer: safety versus efficiency in water transport

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Summary

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- Hydraulic vulnerability of Douglas-fir (*Pseudotsuga menziesii*) branchlets decreases with height, allowing shoots at greater height to maintain hydraulic conductance (K_{shoot}) at more negative leaf water potentials (Ψ_l).
- To determine the basis for this trend shoot hydraulic and tracheid anatomical properties of foliage from the tops of Douglas-fir trees were analysed along a height gradient from 5 to 55 m.
- Values of Ψ_l at which K_{shoot} was substantially reduced, declined with height by 0.012 Mpa m^{-1} . Maximum K_{shoot} was reduced by $0.082 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ for every 1 m 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 $18.4 \text{ } \mu\text{m}^2 \text{ m}^{-1}$, $0.029 \text{ } \mu\text{m m}^{-1}$, 0.42 m^{-1} and $5.3 \text{ } \mu\text{m m}^{-1}$, respectively. Tracheid thickness-to-span ratio (t_w/b)² increased with height by $1.04 \times 10^{-3} \text{ m}^{-1}$ 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: embolism, foliar anatomy, growth limitation, hydraulic conductance, *Pseudotsuga menziesii*, water stress.

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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 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 (Ψ) near the tops of tall trees. Frictional resistance during transpiration leads to an additional path-length dependent reduction in Ψ (Bauerle *et al.*, 1999). In the absence of osmotic adjustment, the turgor of leaf cells will decrease in direct proportion with Ψ 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 Ψ , 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_{shoot}) and stomatal opening at more negative values of Ψ . 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 intraspecific 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 analysed 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 that 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_n), 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 (*P. 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, USA. Samples were collected within 1–5 m of the tops of the trees at mean sampling heights of 5.0, 12.7, 18.3, 34.5 and 55.0 m. All samples were obtained from branches in fully sun-exposed locations. Access to treetops 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, Carson WA, USA). Tree tops in all other height classes were accessed by nonspur climbing.

The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is c. 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 (*c.* 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 *c.* 1 m throughout the summer dry period (Warren *et al.*, 2005; Meinzer *et al.*, 2007).

Leaf hydraulic conductance and vulnerability

The maximum hydraulic conductance of terminal shoots (K_{shoot}) and shoot hydraulic vulnerability to embolism were determined. 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_{shoot} was measured as a proxy for leaf hydraulic conductance (K_{leaf}) because of the difficulty in measuring hydraulic conductance on individual Douglas-fir needles. We believe K_{shoot} to be a reliable proxy for K_{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:

$$K_{\text{shoot}} = C \ln(\Psi_0/\Psi_f)/t, \quad \text{Eqn 1}$$

(K_{shoot} = shoot hydraulic conductance ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$); C = capacitance; Ψ_0 = leaf water potential before partial rehydration; Ψ_f = leaf water potential after partial rehydration; t = duration of rehydration). Branches *c.* 30–50 cm long were collected from trees early in the morning before 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 (*c.* 10–15 cm long) for initial values (Ψ_0), and for final values after a period of rehydration of t seconds (Ψ_f). Deionized water used for rehydration of K_{shoot} samples was partly degassed 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 Ψ_0 before K_{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°C and 23°C, and the photosynthetic photon flux density at the foliage was maintained at *c.* 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during K_{shoot} measurements. Exposure time of foliage to full irradiance was typically less than 5 min. Rehydration times during K_{shoot} measurements ranged between 20 s and 290 s. All K_{shoot} samples, regardless of height class, bore only 2 yr of foliage (current and previous year).

We subjected some K_{shoot} samples to variable periods of 'prehydration' before initial measurements of Ψ_1 in order to obtain a wider range of initial values of Ψ_1 and K_{shoot} .

Prehydration times ranged from one to 12 h. The methods described by Brodribb & Holbrook (2003) were used to estimate C . Briefly, the Ψ_1 corresponding to turgor loss was estimated as the inflection point of the graph of Ψ_1 vs relative water content (RWC). The slope of the curve before, and following turgor loss provided C in terms of RWC (C_{RWC}) for pre-turgor loss and post-turgor loss, respectively. C_{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 $\text{mol m}^{-2} \text{MPa}^{-1}$ 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 $\text{mol m}^{-2} \text{MPa}^{-1}$ 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_{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 10 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 (5 m and 55 m), foliage was collected and analysed on multiple occasions throughout the study and no seasonal trends were evident in K_{shoot} .

Values were determined for the Ψ_1 at which 20, 50 and 80% loss of hydraulic conductance occurred (Ψ_{20} , Ψ_{50} and Ψ_{80} , respectively). These values of Ψ_1 were obtained from a sigmoid regression curve fitted to mean K_{shoot} versus Ψ data. For example, Ψ_{50} represents the leaf water potential at 50 percent of the regression curve's maximum value on the y -axis (Fig. 1). Following our method used in previous work (Woodruff *et al.*, 2007) to identify an objective and functionally relevant measure of shoot hydraulic vulnerability an analysis was used that was 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_{shoot} had declined to an initial minimum value (Ψ_1 at minimum K_{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_{shoot} values ($n = 2$ –28 branches per height class) over each Ψ range (Fig. 1). The value of K_{shoot} representing the midpoint between the y -intercept of the sigmoid function and 0 (dotted line in Fig. 1) was selected for estimating the slope of the portion of the sigmoid curve containing the relatively rapid and nearly linear decline in K_{shoot} . We took the derivative of the sigmoid function to calculate its instantaneous slope at the K_{shoot} midpoint and used the

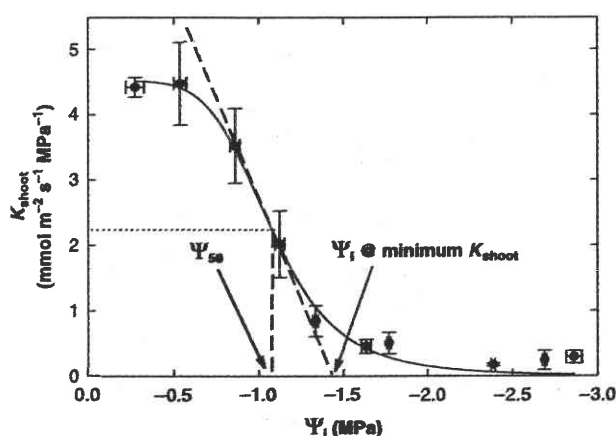


Fig. 1 Example of a typical relationship between mean hydraulic conductance (K_{shoot}) and leaf water potential (Ψ_l) illustrating the method used to estimate the Ψ_l at which K_{shoot} has declined to its initial minimum value. Data are from Douglas-fir (*Pseudotsuga menziesii*) 34.5 m height class. The horizontal dotted line represents the midpoint of the y-range of the sigmoid curve (50% loss of K_{shoot}). The instantaneous slope at this point yielded a tangent (dashed line) whose intercept with the x-axis was designated as the value of Ψ_l at which K_{shoot} had reached its initial minimum value. Bars, $\pm$ SE; $n = 2$ –28 branches per height class.

x-intercept of the resulting tangent (dashed line in Fig. 1) as an objective estimate of Ψ_l at minimum K_{shoot} . Maximum K_{shoot} ($K_{\text{shoot-max}}$) was estimated from the mean of the K_{shoot} values obtained from the most hydrated samples in each height class ($\Psi_l = 0$ to -0.5 MPa). $K_{\text{shoot-max}}$, Ψ_l at minimum K_{shoot} and Ψ_{50} data were analysed using regression analysis. Error bars for $K_{\text{shoot-max}}$ represent standard errors of branches ($n = 7$ –28).

Pressure–volume analyses (Tyree & Hammel, 1972) were conducted on branchlets c. 10-cm-long. These samples were excised from branch samples that were collected early in the morning before 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 Ψ_l with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Alternate determinations of fresh mass and Ψ_l were repeated during slow dehydration of the twig on the laboratory bench until values of Ψ_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, 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 analysed 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

(LA_t), hydraulic mean diameter of leaf tracheid lumens (D_h), number of tracheids per needle cross section ($T\#$), leaf tracheid length (T_l), pit number per tracheid (Pit $\#$) 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 samples were each submerged in c. 10 ml of a solution of 15 g sodium chlorite dissolved in 250 ml of distilled water. 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 analysed with an image analysis system consisting of a compound microscope and video camera. Anatomical characteristics obtained from macerations were obtained from between six and eight branches per height class, and anatomical characteristics obtained from hand sectioning represent between six and nine branches per height class. All anatomical measurements were obtained from foliage produced during 2006. Images were obtained using $\times 20$ or $\times 40$ objective lenses with total magnifications of $\times 200$ and $\times 400$. Data were pooled per height class and subjected to regression analysis.

Results

Physiological trends with height

Timed rehydration measurements showed that K_{shoot} declined sigmoidally with Ψ_l . 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_{shoot} on Ψ_l (Fig. 2). $K_{\text{shoot-max}}$ was used as a measure of hydraulic efficiency and Ψ_l at minimum K_{shoot} and Ψ_{50} were used as measures of hydraulic vulnerability. $K_{\text{shoot-max}}$ decreased with increasing height ($P = 0.035$) from 7.2 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ at 5 m, to 3.2 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ at 55.0 m (Fig. 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. 3b). Values of Ψ_l at minimum K_{shoot} and Ψ_{50} decreased linearly with increasing height at a rate of 0.012 MPa m^{-1} ($P = 0.020$) and $-0.009 \text{ MPa m}^{-1}$ ($P = 0.0088$), respectively. Similar results were obtained for height-related trends in Ψ_{80} and Ψ_{20} ($P = 0.021$ and 0.0078, respectively). There was a positive correlation between $K_{\text{shoot-max}}$ and Ψ_l at minimum K_{shoot} ($P = 0.0047$) and between $K_{\text{shoot-max}}$ and Ψ_{50} ($P = 0.009$), showing that increased resistance to tension-induced loss of K_{shoot} was associated with reduced maximum water transport capacity (Fig. 4).

Anatomical trends with height

Quantitative analyses of leaf tracheid characteristics revealed height-related trends in anatomy (Fig. 5). Observed trends

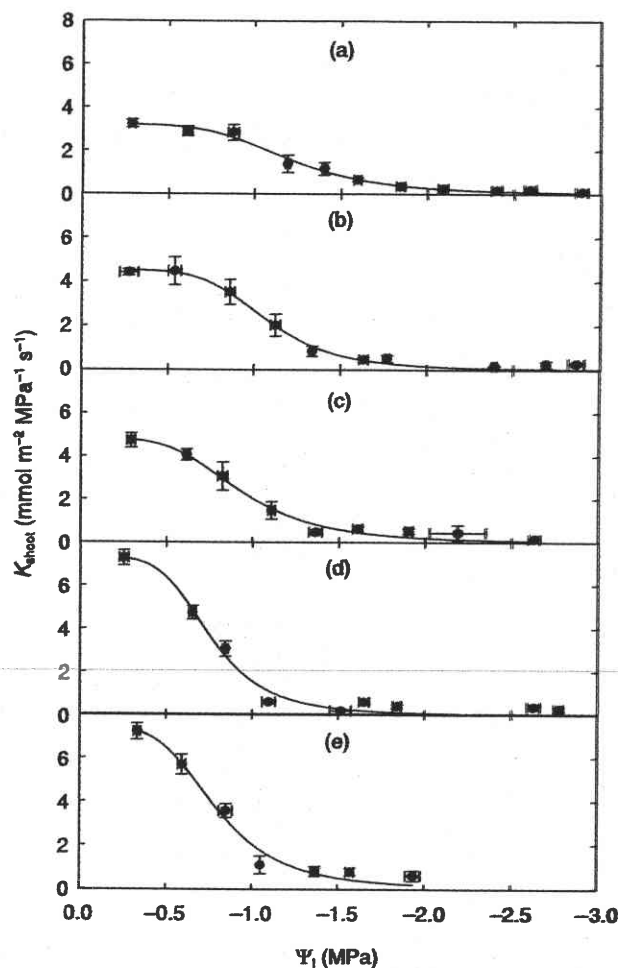


Fig. 2 Shoot hydraulic conductance (K_{shoot}) in relation to leaf water potential (Ψ_l) for Douglas-fir (*Pseudotsuga menziesii*) foliage samples obtained within 1–5 m of the tops of the trees at mean sampling heights of (a) 55.0 m, (b) 34.5 m, (c) 18.3 m, (d) 12.7 m and (e) 5.0 m. A logistic three-parameter sigmoid function ($y = a/(1 + (x/x_0)^b)$) was fit to K_{shoot} in relation to Ψ_l .

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

Relationships between anatomy and physiology

Correlations of $K_{\text{shoot-max}}$, Ψ_l at minimum K_{shoot} and Ψ_{50} with leaf tracheid anatomical characteristics were evaluated in order to elucidate potential causal relationships between leaf structural and shoot hydraulic attributes. Many of the leaf

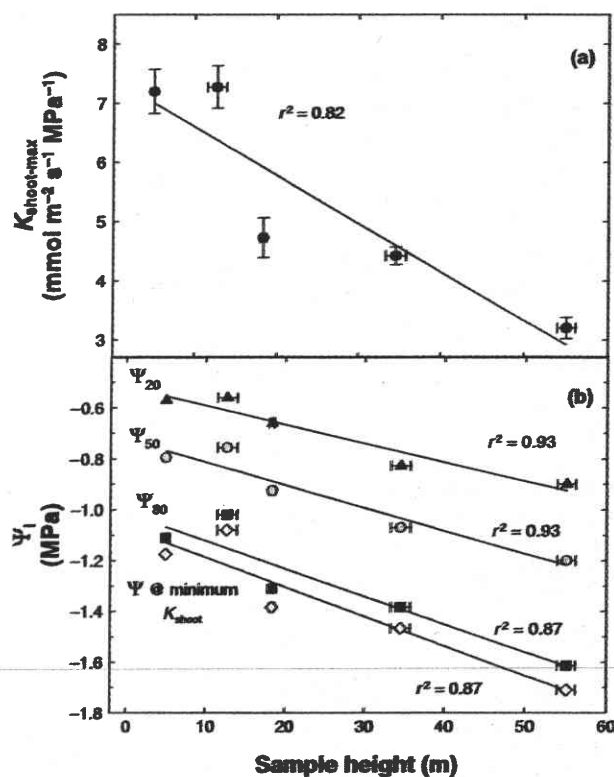


Fig. 3 (a) Maximum hydraulic conductance ($K_{\text{shoot-max}}$) (bars, $\pm$ SE; $n = 7$ –28 branches per height class) and (b) different measures of shoot hydraulic vulnerability versus height in Douglas-fir (*Pseudotsuga menziesii*). Values of Ψ_{20} , Ψ_{50} , Ψ_{80} and Ψ_l at minimum K_{shoot} were obtained from vulnerability curves as illustrated in Fig. 1.

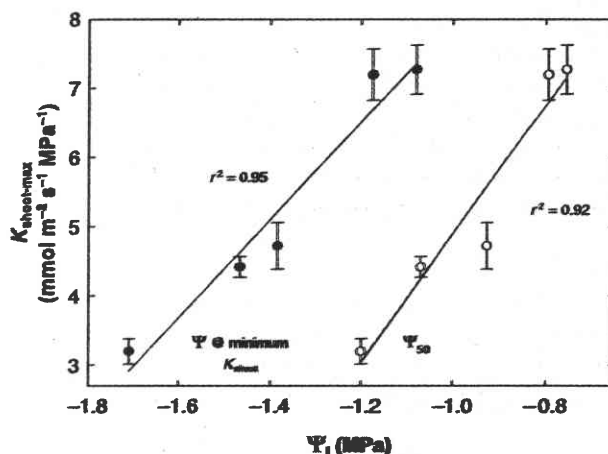


Fig. 4 Maximum hydraulic conductance ($K_{\text{shoot-max}}$) in relation to shoot hydraulic vulnerability measured as leaf water potential (Ψ_l) at minimum K_{shoot} and Ψ_{50} in Douglas-fir (*Pseudotsuga menziesii*).

tracheid anatomical properties analysed were significantly correlated with either $K_{\text{shoot-max}}$, Ψ_l at minimum K_{shoot} , Ψ_{50} , or all three (Table 1). LA_t was correlated with hydraulic efficiency ($P = 0.018$), with Ψ_l at minimum K_{shoot} ($P = 0.025$)

Fig. 5 Leaf tracheid anatomical characteristics along a height gradient sampled from the upper 1–5 m of Douglas fir (*Pseudotsuga menziesii*) trees of different height classes. (a) Total lumen area, (b) hydraulic mean diameter, (c) tracheid number, (d) tracheid length, (e) pit number and (f) thickness to span ratio (t_w/b)². Bars, $\pm$ SE; $n = 6$ –9 branches for (a–c, f); $n = 6$ –8 branches for (d, e).

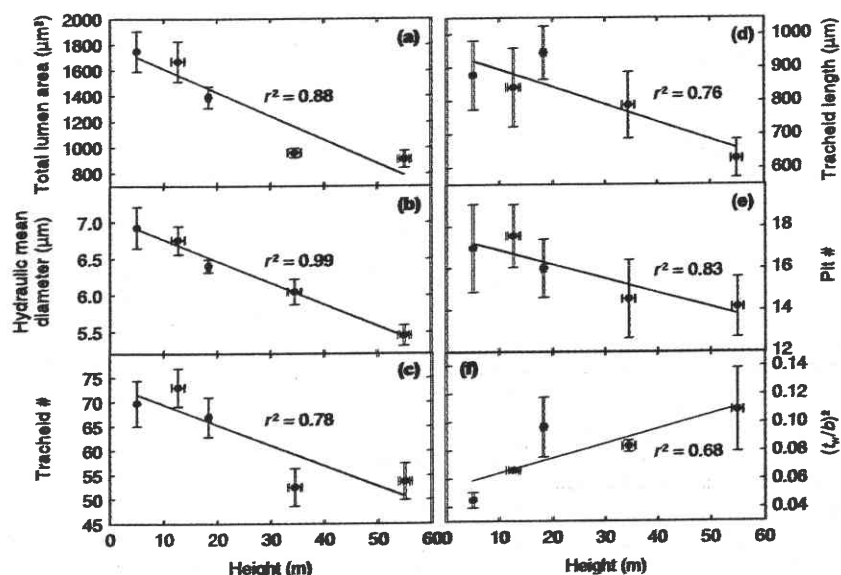


Table 1 Per cent of variance (r^2) and significance values for shoot hydraulic conductance (K_{shoot}) and vulnerability and leaf xylem anatomical characteristics of Douglas-fir (*Pseudotsuga menziesii*)

Leaf xylem anatomical characteristic	Hydraulic efficiency ($K_{\text{shoot-max}}$)	Hydraulic vulnerability	
		(Ψ_{50})	(Ψ_l at minimum K_{shoot})
LA_t (μm^2)	0.88* (+)	0.94** (+)	0.85* (+)
D_h (μm)	0.90* (+)	0.96** (+)	0.93** (+)
$T\#$	0.75 (+)	0.89* (+)	0.79* (+)
T_l (μm)	0.35 (+)	0.58 (+)	0.50 (+)
Pit#	0.90* (+)	0.97** (+)	0.92** (+)
$(t_w/b)^2$	0.84* (-)	0.66 (-)	0.72 (-)

LA_t , total lumen area per leaf cross section; D_h , hydraulic mean diameter of leaf tracheid lumens; $T\#$, total number of tracheids per leaf cross section; T_l , leaf tracheid length; Pit#, number of pits per tracheid; $(t_w/b)^2$, ratio of thickness of tracheid wall to span of tracheid lumen; Ψ_l , leaf water potential; Ψ_{50} , 50% loss of hydraulic conductance.

(+), Positive correlation; (-), negative correlation.

*, **, $P \leq 0.05$ and $P \leq 0.01$, respectively.

and Ψ_{50} ($P = 0.0066$). For every $100 \mu\text{m}^2$ increase in LA_t , there was an increase in hydraulic efficiency such that $K_{\text{shoot-max}}$ increased by $0.43 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$, and an increase in hydraulic vulnerability such that Ψ_l at minimum K_{shoot} increased by 0.06 MPa , and Ψ_{50} increased by 0.05 MPa . $K_{\text{shoot-max}}$, Ψ_l at minimum K_{shoot} and Ψ_{50} were also strongly correlated with changes in D_h ($P = 0.014$, $P = 0.0082$ and $P = 0.004$, respectively). On average, a $1 \mu\text{m}$ increase in D_h was correlated with an increase in $K_{\text{shoot-max}}$ of $2.9 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$, an increase in Ψ_l at minimum K_{shoot} of 0.40 MPa and an increase in Ψ_{50} of 0.31 MPa . $T\#$ was significantly correlated with Ψ_l at minimum K_{shoot} ($P = 0.044$) and with Ψ_{50} ($P = 0.015$), but marginally so with hydraulic efficiency ($P = 0.057$). T_l had no significant correlation with either hydraulic efficiency or vulnerability ($P = 0.29$ for $K_{\text{shoot-max}}$,

$P = 0.18$ for Ψ_l at minimum K_{shoot} and $P = 0.13$ for Ψ_{50}). Pit# was significantly correlated with $K_{\text{shoot-max}}$ ($P = 0.014$), with Ψ_l at minimum K_{shoot} ($P = 0.0096$), and with Ψ_{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 Ψ_l at minimum K_{shoot} or Ψ_{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_l 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_l represents a combination of both a decline in D_h and $T\#$. Aside from 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 nongrowing 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_{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).

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) tracheid length was not a significant determinant of shoot hydraulic conductance. The strongest anatomical correlates of $K_{shoot-max}$ were Pit#, LA_l , and D_h . The observed significant relationship between D_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–Poiseuille equation:

$$K_t = \pi \rho D_h^4 / 128 \eta \quad \text{Eqn 2}$$

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

$$K_{leaf-theoretical} = \pi \rho D_h^4 T\# / 128 \eta A_l \quad \text{Eqn 3}$$

Leaf area measurements were conducted on foliage at each sampling height and these values were used to normalize $K_{leaf-theoretical}$ by leaf area. Leaf area was found to scale inversely with height by $0.24 \text{ mm}^2 \text{m}^{-1}$. Despite the trend in leaf area with height, $K_{shoot-max}$ correlated strongly with leaf-specific values of $K_{leaf-theoretical}$ ($P = 0.006$; Fig. 6). Note that although $K_{shoot-max}$ and $K_{leaf-theoretical}$ are both conductance values, they are not directly comparable because they have different units. The comparison between $K_{shoot-max}$ and $K_{leaf-theoretical}$ is still

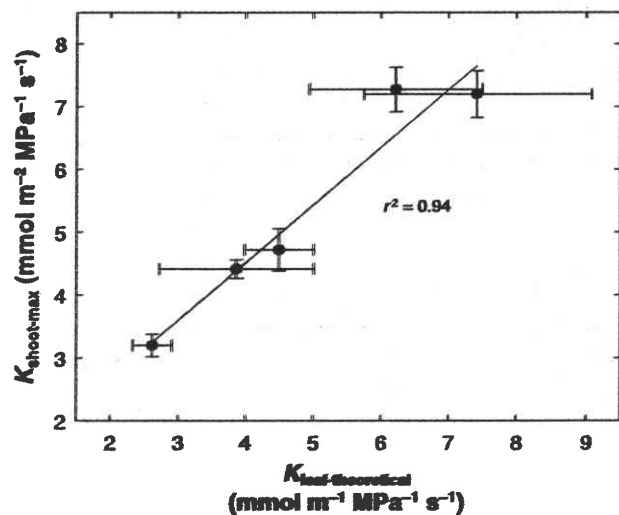


Fig. 6 The relationship between theoretical leaf area-specific conductance ($K_{leaf-theoretical}$) and measured maximum hydraulic conductance ($K_{shoot-max}$) in Douglas fir (*Pseudotsuga menziesii*). Bars, $\pm$ SE; $n = 6$ –9 branches for $K_{leaf-theoretical}$; $n = 7$ –28 branches for $K_{shoot-max}$.

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 $K_{shoot-max}$ would likely be substantially lower than $K_{leaf-theoretical}$ 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 (Fig. 3). It is important to note that although the 55-m 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) 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 Pit#, D_h and LA_t . Loss of hydraulic conductivity owing to embolism is believed to be caused by 'air seeding' (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 nonhydraulically 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 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 (Succoff, 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 as a result of 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 analysed 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-scanning electron microscopy (SEM) analyses of leaf tracheids at varying levels of dehydration, it was concluded that implosion of leaf tracheids in Douglas fir is an uncommon event and that short-term variation in K_{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 owing 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 ($-0.012 \text{ MPa m}^{-1}$ for Ψ_l at minimum K_{shoot} , Fig. 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 nonvascular leaf anatomical factors could be at play in the relationship between tree height and decline in productivity such as increased extravascular resistance to water transport (Brodribb *et al.*, 2007) or increased occurrence of nonphotosynthetic 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).

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