Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem

JARMILA PITTERMANN[1], JOHN S. SPERRY[2], JAMES K. WHEELER[3], UWE G. HACKE[2] & ELZARD H. SIKKEMA[2]

[1]*Department of Integrative Biology, University of California, Berkeley, CA 94720,* [2]*Department of Biology, University of Utah, Salt Lake City, UT 84112, and* [3]*Biological Laboratories, Harvard University, Cambridge, MA 02138, USA*

ABSTRACT

Wood structure and function of juvenile wood from 18 conifer species from four conifer families (Araucariaceae, Cupressaceae, Pinaceae and Podocarpaceae) were examined for a trade-off between wood reinforcement and hydraulic efficiency. Wood density and tracheid 'thickness-to-span' ratio were used as anatomical proxies for mechanical properties. The thickness:span represented the ratio of tracheid double wall thickness to lumen diameter. Hydraulic resistivity (R) of tracheids on a cross-sectional area basis (R_{CA}) increased over 50-fold with increasing density and thickness:span, implying a strength versus efficiency conflict. The conflict arose because density and thickness:span were increased by narrowing tracheid diameter rather than by thickening walls, which may be developmentally difficult. In the Pinaceae and Cupressaceae species, density and thickness:span correlated strongly with protection from drought-induced embolism, suggesting that mechanical strength was required in part to withstand tracheid collapse by negative sap pressure. These species showed a corresponding trade-off between increasing R_{CA} and embolism protection. In contrast, species of Podocarpaceae and Araucariaceae were overbuilt for their embolism protection and were hydraulically inefficient, having greater density, thickness:span and R_{CA}, none of which were correlated with vulnerability to embolism.

Key-words: embolism; hydraulic resistivity; mechanical strength; plant water transport; wood density.

INTRODUCTION

Conifer wood, despite its homogenous tracheid-based structure, shows considerable variation in its hydraulic resistivity (R) across species and organs (Domec & Gartner 2002a; Tyree & Zimmermann 2002; Burgess, Pittermann & Dawson 2006; Pittermann *et al*. 2006). This variation can be attributed to a wide range of tracheid sizes, with lower resistivity found in wood composed of longer and wider tracheids (Pittermann *et al*. 2006). What explains the range of tracheid dimensions and the subsequent variation in

Correspondence: Jarmila Pittermann. Fax: +1 510 642 1054; e-mail: pittermann@berkeley.edu

hydraulic efficiency? Narrow tracheid diameters occur in conifers that are resistant to freezing-induced embolism (Pittermann & Sperry 2003, 2005), yet narrow and freeze-resistant tracheids can also be found in conifers belonging to the Araucariaceae and Podocarpaceae inhabiting rain forests that never experience frost (Pittermann *et al*. 2006). Protection from drought-induced embolism is presumably a function of the ability of pits to prevent air-seeding (Sperry & Tyree 1990; Jarbeau, Ewers & Davis 1995; Tyree & Zimmermann 2002), but there is no evidence that more air-tight tracheid pitting requires smaller tracheids (Pittermann *et al*. 2006). However, conifer tracheids function not only in water transport, but also in the mechanical support of the plant body so it may be that mechanical function is a factor in limiting tracheid size. In this paper, we explore the link between hydraulic efficiency, tracheid size and anatomical determinants of wood mechanical properties across four conifer families.

Mechanical strength is clearly required to resist wind or snow loading, as well as the pull of gravity (Vogel 2003). The need to resist bending and compressive stress is most important for trunk and branch wood, and less so for the wood of the smaller roots remote from the stem. We refer to stresses that arise from support of the plant body as 'support stress'. Mechanical strength is also required to support the xylem conduit network against implosion caused by negative sap pressures. Indeed, this may have been the original function of the first lignified secondary walls in vascular plants (Raven 1987). We refer to this hydraulically related mechanical demand as 'transport stress', and it is superimposed on support stress in the sapwood. The magnitude of transport stress will be a function of the range of negative pressure in the xylem conduits, which is in turn related to habitat and vulnerability to embolism.

Using the xylem pressure (P_x) required to reduce hydraulic conductance by 50% (the P_{50}) as a proxy for a species' negative pressure range, Hacke *et al*. (2001) showed that a more negative P_{50} was associated with greater wood density, a well-known correlate of wood strength (stress at failure) and elastic modulus (Panshin & de Zeeuw 1980; Niklas 1992; Evans & Ilic 2001; Lundgren 2004). Wood density is closely related to the ratio of cell wall thickness to lumen diameter ('thickness:span', see also Yasue *et al*. 2000), and this ratio was shown to increase significantly with more negative P_{50} (Hacke *et al*. 2001). Estimated safety factors

from conduit implosion averaged 1.8 for angiosperm vessels (Sperry & Hacke 2004) but were more than doubled at 4.8 for conifer stem tracheids (Hacke, Sperry & Pittermann 2004). The greater safety margin for stem tracheids presumably reflects their additional role in bearing support stress, a role played by the fibre matrix in angiosperm wood (Hacke & Sperry 2001; Baas *et al*. 2004). Safety margins from implosion were 2.4 for conifer root tracheids, half of the safety margins associated with stem tracheids (Hacke *et al*. 2004), which is consistent with less additional support stress in roots than stems. These results suggest that the range of negative sap pressure that must be sustained by the wood requires a minimum tracheid strength to avoid implosion. While the tracheids may be stronger and thus more reinforced than this minimum, the added carbon cost would seemingly select against additional fortification of the tracheid walls unless it was necessary for bearing support stress.

How does reinforcement against support and transport stresses constrain tracheid size and hydraulic efficiency? Increased wood strength clearly comes at an increased construction cost because it requires greater thickness-to-span ratios and wood density. However, it is not necessarily the case that hydraulic efficiency need decline if the increase in thickness:span and density is uncoupled from tracheid lumen size. We evaluated the trade-offs between strength and conducting efficiency by using the thickness:span and wood density as anatomical proxies for wood strength. We compared these strength parameters in the roots and stems of conifers belonging to four families, in order to determine: (1) whether a trade-off exists between strength parameters and hydraulic efficiency; (2) the anatomical basis for any trade-off; and (3) the relationship of strength parameters to transport stress as represented by the P_{50}.

MATERIALS AND METHODS

Plant material

Table 1 lists the study species and collection sites. We collected stems (and roots where possible) from 18 conifer species belonging to the Pinaceae, Cupressaceae, Podocarpaceae and Araucariaceae families. The species were selected to sample broadly across phylogeny and habitat because they represented important elements of their native forest flora. The Cupressaceae are globally distributed, but logistics constrained our study to the North American species. Similarly, members of the Podocarpaceae and Araucariaceae are widespread across Asia, Polynesia, and Central and South America (Enright & Hill 1995), but we collected from New Zealand and New Caledonia to more efficiently capture their diversity. North temperate species were gathered from environments where climates range from mild and moist to cold and dry. The Podocarpaceae and Araucariaceae were collected in regions where severe moisture deficit and freezing temperatures are a rare occurrence (Table 1; National Institute of Water & Atmospheric Research of New Zealand, Météo

France—Nouvelle Caledonie 2006; N.I.W.A. 2006; World Meteorological Organization 2006).

Whenever possible, lateral branches were sampled from the lower, sun-exposed peripheral canopy of mature trees. In *Agathis australis* where adults often reached heights in excess of 50 m, lateral branches were collected from younger trees whose trunk diameter ranged from 15 to 20 cm at breast height, and whose heights reached approximately 15–20 m. Branches of *Sequoia sempervirens* and *Taxodium distichum* were obtained by either climbing 30–50 m into the canopy or shooting the stems down, respectively. The north-temperate species inhabited forests or woodlands with open canopies, but the Araucariaceae and Podocarpaceae were gathered from shadier, closed-canopy forests with the exception of *Retrophyllum minor*, *Agathis ovata* and *Araucaria laubenfelsii*, which were grown as scattered individuals in open conditions (Table 1).

The branch and root segments were at least 18 cm long and straight, with stems measuring approximately 8–12 mm in diameter, while the roots were typically smaller than 6 mm in diameter. Care was taken to harvest roots whose function were purely absorptive rather than having a mechanical role in order to clearly differentiate between structural and hydraulic properties of stem and root wood. Branch material was selected to minimize the presence of significant amounts of compression wood because this may alter the hydraulic properties of the segment (Spicer & Gartner 1998).

All samples were wrapped in moist paper towels, wrapped in two plastic bags and transported to our laboratory in Salt Lake City, UT, USA. Although samples collected outside of the United States were in transit for at least 3 d because of customs inspection, we are confident that this did not affect the outcome of our experiments: we measured the hydraulic conductivity and vulnerability to embolism in *Pinus contorta* stems either on the day of the harvest, or a week later, and observed no significant differences in either conductivity or resistance to embolism in the two groups (Pittermann *et al*. 2006).

Tracheid area resistivity (R_{CA})

Our measure of hydraulic efficiency was the resistivity of a tracheid on a cross-sectional area basis, including the walls (R_{CA}). The R of a stem or root segment was measured according to the method of Sperry (1993), and was calculated as the pressure gradient/flow rate through a segment of known length. The segments were mounted on a tubing apparatus where R was measured gravimetrically under a pressure of 5 kPa using filtered water (0.22 μm, Catadyne filtration system). We determined the flow into the segments without a pressure head before and after each gravimetric flow measurement. These background flows were averaged and subtracted from the pressure-induced flow in order to improve accuracy.

Functional xylem area was obtained by perfusing the segments with 0.1% basic fuchsin for at least 24 h, sectioning the perfused wood and measuring the stained tracheid

Table 1. Conifer species, families, symbol abbreviations, collection site and regional climatic parameters

Species	Family	Location, elevation (m a.s.l.), habitat	Mean annual temperature; (mean temp. of coldest month) (°C)	Mean annual precipitation (mm)	Organ	Figure symbol
Agathis australis (D. Don) Loudon	Araucariaceae	Waikano, North I., New Zealand S 37°25.6′-E 175°43.2, 100 m, dense forest	14.5 (9.7)	1198	R S	aa AA
Agathis ovata (C. Moore ex. Veill) Warb.	Araucariaceae	New Caledonia, Provence Sud, S 22°02′E 166°28′,750 m, open shrubland	19.9 (17.2)	1072	S	AO
Araucaria laubenfelsii Corbasson	Araucariaceae	New Caledonia, Provence Sud S 22°05′-E 166°26.8′, 120, open maquis	19.9 (17.2)	1071.5	S	AL
Juniperus osteosperma (Torrey) Little	Cupressaceae	St. George, UT, USA, N 37°104′ W 113°58′, 840 m, pinyon-juniper woodland	16 (5.3)	500	R S	co CO
Juniperus scopulorum Sargent	Cupressaceae	Uinta Mountains, UT, USA, N 40°3′ W 111°37′, 2500 m, juniper woodland	3 (−6.8)	925	S	CS
Sequoia sempervirens (D. Don) Endlicher	Cupressaceae	Sonoma, CA, USA, N 38°53′ W 123°25′, 355 m, coastal forest	13.6 (7.5)	1605	R S	cq CQ
Taxodium distichum (Linnaeus) Richard	Cupressaceae	Durham, NC, USA, N 35°52′ W 79°59′, 130 m, swamp forest	15.6 (3.6)	1490	R S	ct CT
Abies lasiocarpa (Hooker) Nuttall	Pinaceae	Brighton, UT, USA, N 40°46′ W 111°35′, 2825 m, subalpine forest	4 (−5.8)	1250	R S	pa PA
Picea engelmanii Parry ex Englemann	Pinaceae	Uinta Mountains, UT, USA, N 40°4′ W 111°2′, 2850 m; subalpine forest	2 (−8.7)	1200	R S	pe PE
Picea mariana (Miller) Britton Sterns, & Poggenburg	Pinaceae	Fairbanks, AK, USA, N 64°49′ W 147°45′, 200 m; open woodland, Bettles, AK, N 66°90 W151°58′, boreal forest	−2.7 (−23) −5.4 (−24.4)	1045	R S	pr PR
Pinus caribaea Morelet	Pinaceae	Andros I., Bahamas, N 24°43′ W 77°47′, 10 m, pine forest	25.8 (17)	2550	R S	pc PC
Pinus contorta Dougl. ex Loudon	Pinaceae	Uinta Mountains, UT, USA, N 40°3′ W 111°37′, 2500 m, mid-montane forest	3 (−6.8)	1675	R S	pt PT
Pinus monophylla Torrey & Fremont	Pinaceae	St. George, UT, USA, N 37°104′ W 113°58′, 840 m, pinyon-juniper woodland	16 (5.3)	480	R S	pm PM
Dacrydium cupressinum Solander ex G. Forst	Podocarpaceae	Te Puke, North I., New Zealand S 37°53.7′ E 176°12.5′; 100 m, dense forest	14.5 (9.7)	1198	R S	od OD
Phyllocladus trichomanoides D. Don in Lambert	Podocarpaceae	Waikatere, North I., New Zealand S 37°25.6′ E 175°43.9′, 300 m, dense forest	15.1 (10.8)	1240	S	OH
Podocarpus cunninghamii Colenso	Podocarpaceae	Wellington, New Zealand S 38°53.9′ E 175°25′, 50 m, open woodland	12.8 (8.8)	1249	S	OC
Prumnopytis ferruginea (G. Benn ex D. Don) de Laub.	Podocarpaceae	Waikatere, North I., New Zealand S 37°25.6′ E 175°43.9′, 300 m, dense forest	15.1 (10.8)	1240	R S	op OP
Retrophyllum minor Carriere	Podocarpaceae	New Caledonia, Provence Sud S 22°18.6′ E 166°58′, 150 m, open woodland	19.9 (17.2)	1072	S	OR

Mean annual temperature and mean temperature for the coldest month (in parentheses) and mean annual precipitation are indicated for closest stations at similar elevations to collecting sites (North American data from the National Weather Service [N.W.S.], New Zealand from the National Institute of Water and Atmospheric Research [N.I.W.A.], New Caledonia from Météo France and the World Meteorological Organization).

The first letter of the species abbreviation denotes family: A, Araucariaceae; C, Cupressaceae; P, Pinaceae; O, Podocarpaceae. Roots (R) and stems (S) are indicated by lower and upper case abbreviations, respectively.

area under a microscope or dissecting scope. The average R_{CA} for the segment was computed by multiplying the resistivity by the area of stained and conducting tracheids. The species mean R_{CA} was the mean of $n \geq 4$ segments.

Tracheid diameter (D), thickness-to-span ratio (C) and tracheid length (L)

The average tracheid diameter (D) and thickness-to-span ratio (C) reported in this paper are two variables from a larger data set that was structured according to a model for how wood anatomy determines R_{CA}. This model is described in detail elsewhere (Pittermann *et al.* 2006), and is summarized here only as it relates to how we measured D and C. The average R_{CA} of a xylem sample is assumed to be the sum of the average lumen (R_L) and end-wall (R_W) resistivities in series, multiplied by the average area of a tracheid (A_T):

$$R_{CA} = (R_L + R_W)A_T \tag{1}$$

The tracheid diameter is assumed to influence R_L according to the Hagen–Poiseuille equation:

$$R_L = 128 \, \eta/(\pi D^4) \tag{2}$$

where η is the dynamic viscosity (all resistivities were normalized to 20 °C). Because R_L is a sample average, the corresponding average D is calculated as the diameter of a tracheid with the average R_L. Although tracheids are square in cross section, the R_L of a square tube of side D is the same as that of a cylinder with diameter D (Lewis 1992), so the Hagen–Poiseuille equation applies. R_W values ranged from 0.0139 to 29 MPa·s mm^{-4} in *Pinus caribea* roots and *Juniperus osteosperma* stems, respectively, while R_L values varied from 0.0136 to 4.54 MPa·s mm^{-4} in *T. distichum* roots and *J. osteosperma*. The complete data set is presented in Pittermann *et al.* (2006).

For a square tracheid of side D, the A_T will be related to D and C:

$$A_t = D^2(1 + C)^2 \tag{3}$$

The average C for a sample was calculated from this equation from D and the arithmetic mean A_T for the sample. Errors were propagated from measurements of R_L and A_T to calculations of D and C using standard formulae.

To measure D, hand-cut, transverse sections were made of the xylem located in the middle region of the segment used in the R_{CA} measurements described earlier. These sections were stained in toluidine blue for 3 min, rinsed in distilled water and mounted on a glass slide with glycerin. We photographed the outer three to four growth rings of the sections under 200× magnification with a Nikon digital camera mounted on a Nikon Eclipse E600 microscope (model RT KE, Diagnostic Instruments, Salt Lake City, UT, USA). Tracheid lumen areas were measured using image analysis software (Image-Pro, Media Cybernetics, Carlsbad, CA, USA). A minimum of three to four radial files of tracheids were measured in three to six different sectors of the cross section, depending on the diameter of the root or

stem segment. The area containing the measured tracheids, including wall area, was also determined. On average, between 400 and 800 tracheids were measured per root or stem segment. We included both earlywood and latewood in our measurements because dye perfusions showed both types of wood to be conductive (but see Domec & Gartner 2002b), and growth rings were at times difficult to distinguish in the southern conifers. We avoided compression wood because of its minimal contribution to water transport and unusual hydraulic properties (Spicer & Gartner 1998; Mayr & Cochard 2003).

The tracheid lumen areas were converted to diameters (D) and the individual lumen resistivity estimated according to Eqn 2. The sum of the lumen resistivities was divided by the number of tracheids measured to yield the average R_L of the sector. The segment R_L was the mean of all sectors per segment. The average tracheid diameter (D) for the segment was obtained by solving Eqn 2 for D from the segment mean R_L. The species mean D was obtained from a minimum of four segments.

To determine thickness:span (C) for a segment, we measured the average A_T on the same 3–4 radial sectors of growth rings measured to obtain D on each segment, and average segment C was solved from Eqn 3. The average double wall thickness (T) for the segment was represented by T = CD. As for D, each species was represented by the mean C and T from at least four segments.

We also measured C directly for tracheids of 'hydraulic mean diameter' according to the methods of Hacke *et al.* (2001). The hydraulic mean diameter was calculated from tracheid diameter (d) distributions as $d_h = \Sigma d^5/\Sigma d^4$. The d_h is the mean of the distribution of d^5, where d^4 is assumed to be proportional to the contribution of each conduit of diameter d to total axis conductivity according to the Hagen–Poiseuille equation (Kolb & Sperry 1999). The d_h was determined for the same diameter distributions used to obtain D. The thickness-to-span ratio was measured directly on $n \geq 30$ tracheids that were within 10% of d_h to give the mean C_h ratio for the sample. The double wall thickness was measured on tangential walls to avoid the pitted radial walls.

Tracheid length was measured on individual tracheids obtained from wood macerations. Small samples of root and stem xylem were cleaned of their periderm and pith, and digested in a 1:1 solution of (80%+) glacial acetic acid and 30% hydrogen peroxide overnight at 60 °C (Mauseth & Fujii 1994). The samples were subsequently rinsed in distilled water, stained with toluidine blue for 3–5 min and mounted on a glass slide with glycerin. Digital photos of whole tracheids were taken at 20× magnification, and tracheid lengths were measured on at least 50 tracheids using the Image-Pro software (as described previously).

Wood density (W)

W was measured using the methods of (Hacke, Sperry & Pittermann 2000). A xylem sample (~ 1 cm long) was cleaned of any pith and periderm material, and then

submersed in water in order to measure its volume displacement. The displacement weight was converted to sample volume by multiplying it with 0.998 g cm^{-3}, which is the density of water at 20 °C. The wood samples were subsequently dried at 60 °C for 2–3 d, weighed and W was calculated as dry weight to fresh volume (g cm^{-3}). W was determined on no fewer than four samples per species root or stem organs and when possible, on the same samples used to determine embolism pressure.

## Vulnerability curves and embolism pressure

We used the centrifuge method to measure the species' vulnerability to water stress-induced embolism at progressively more negative P_x values (Pockman, Sperry & O'Leary 1995; Alder *et al.* 1997). The same root and stem segments from which R_{CA} was obtained were used to generate the vulnerability curves.

Hydraulic conductivity (K = 1/R) of the segments at each P_x was measured in the laboratory using the Sperry method (Sperry 1993) as described earlier. We did not flush the stems to remove native emboli to obtain maximum K, because in preliminary measurements the conductivity consistently decreased slightly following the flush, meaning there was no detectable native embolism (Mayr, Wolfschwenger & Bauer 2002; Pittermann & Sperry 2003).

Following the native conductivity measurement (K_{native}), the segments were spun in a custom-built rotor to increasingly negative P_x values (Pockman *et al.* 1995; Alder *et al.* 1997). At each P_x, the K was measured to assess the loss in conductivity as a result of embolism, and the percent loss of conductivity (PLC) was calculated using the formula PLC = 100*(1 − K/K$_{native}$). The PLC versus P_x data were fit with a Weibull function for each stem in order to calculate the P_x at which the segment showed a P_{50}, and the average P_{50} for each species was calculated from a minimum of four stems. The P_{50} is a convenient representation of the range of embolism pressure for a sample and has been used extensively to compare embolism resistance across species (Hacke & Sperry 2001; Hacke *et al.* 2001; Wheeler *et al.* 2005). For the usually sigmoidal vulnerability curves of conifer material, the P_{50} is very similar to the mean embolism pressure used to represent embolism vulnerability in other studies (Hacke *et al.* 2006).

RESULTS

The average thickness-to-span ratio across all tracheid sizes of a species (C) was strongly correlated with the thickness-to-span ratio for tracheids of hydraulic mean diameter (C_h) (Fig. 1a, $r^2 = 0.7$). The scatter in the C:C_h relationship may have resulted in part from variation in tracheid diameter distributions among species. The average thickness-to-span ratio (C) was also well correlated with wood density (Fig. 1b, $r^2 = 0.54$), an expected result given that over 95% of the conifer wood volume is composed of tracheids. Variation in this relationship may be attributed to a number of factors, including the fact that the two measures were made
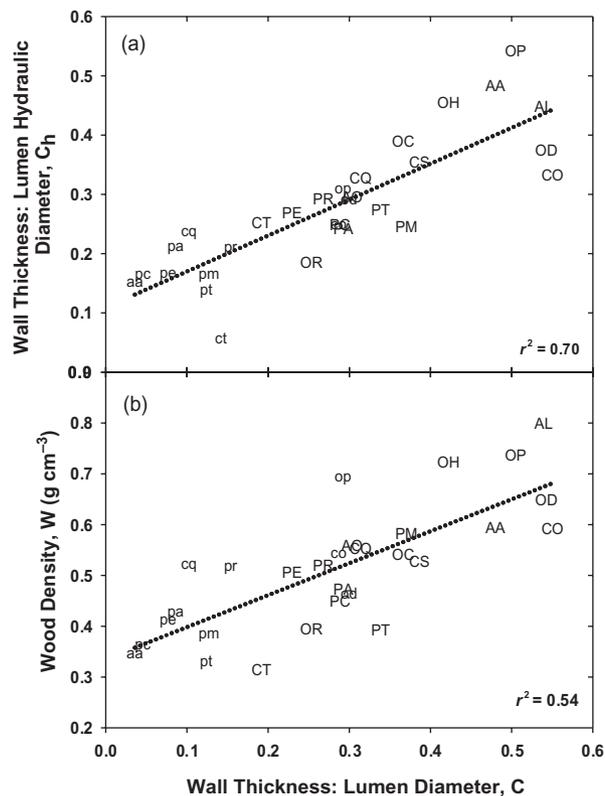

Figure 1. (a) Relationship between tissue-averaged wall thickness-to-lumen diameter ratio (C) versus the directly measured value for tracheids of hydraulically weighted diameter (C_h). Means of $n \geq 4$ samples per species, SE averaged 9.5% of the mean C and 14% of the mean C_h. (b) Wood density (W) versus tissue-averaged thickness-to-span ratio (C). The SE averaged 5.4% of the mean W. Stems and roots are represented by upper and lower case symbols, respectively. Species abbreviations are listed in Table 1.

on different portions of the xylem sample, the potential presence of compression wood in the wood density samples, which was avoided in the anatomical measurements, and variation in ratios of earlywood to latewood.

The R_{CA} increased with greater thickness:span or density (Fig. 2a), implying a major strength versus hydraulic efficiency trade-off. The R_{CA} also increased with narrower tracheid diameter (Fig. 2b), suggesting that narrow diameters and strength are coupled. This is illustrated more directly in Fig. 3, which shows that the increase in thickness:span across the data set resulted from a 2.5-fold reduction in tracheid diameter, rather than a major increase in double wall thickness. In our samples, conifers increased their thickness:span, and by implication wood density and strength, primarily by reducing their tracheid diameter rather than increasing their wall thickness.

An increase in wall reinforcement (C) was also associated with a decrease in tracheid length (Fig. 4), implying that stronger tracheids tend to be shorter. Tracheid lengths ranged from 5700 μm in the roots of *P. caribea* to 770 μm in the stems of *J. osteosperma*. In general, the data reflect organ-specific trends whereby tracheids were shorter in
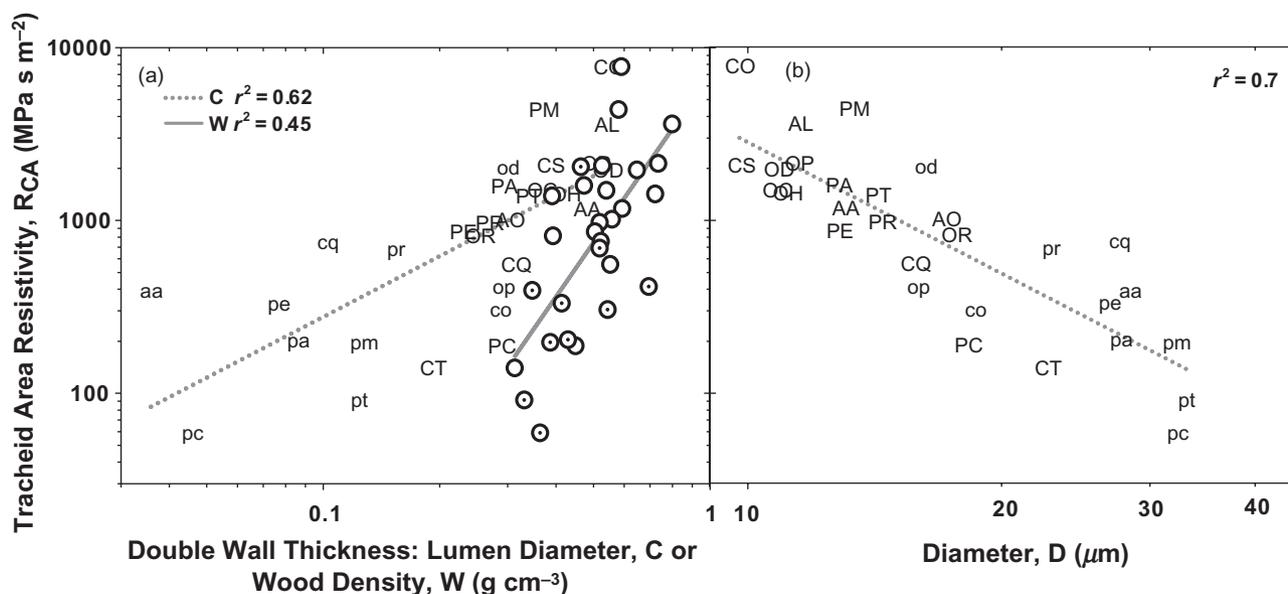
Figure 2. (a) Tracheid area resistivity (R_{CA}) versus thickness-to-span ratio (C, abbreviations are listed in Table 1) and wood density (W, circles). Means of $n \geq 4$ samples per species, SE averaged 24% of R_{CA}. Open circles represent wood density of stems, dotted circles represent roots. (b) R_{CA} as a function of average lumen diameter (D). Stems and roots are represented by upper and lower case symbols, respectively. Means of $n \geq 4$ samples per species, SE averaged 7% of D.
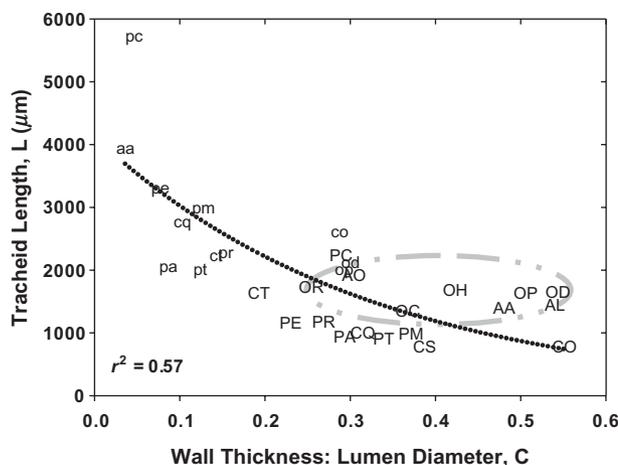
stems (upper case symbols) than roots (lower case symbols). However, the Podocarpaceae and Araucariaceae species (dashed oval) exhibited longer tracheids for a given thickness:span than their northern hemisphere counterparts.

How is the variation in strength related to variation in transport stress across species as represented by their P_{50}? The Pinaceae and Cupressaceae species showed a strong relationship between increasing resistance to embolism and increasing thickness:span and wood density (Fig. 5a & b). For a given embolism pressure, stems (upper case symbols)

showed a greater thickness:span and wood density than roots (lower case symbols) – indicating an additional reinforcement consistent with their greater role in resisting support stress of the canopy. Interestingly, the Podocarpaceae and Araucariaceae species exhibited thickness:span and density values that were approximately 30% greater on average for a given P_{50} than the Pinaceae/Cupressaceae species and showed no relationship between strength parameters and P_{50} (Fig. 5a & b, dashed line). Root data were only available for three of the Podocarpaceae/Araucariaceae, but were not clearly distinct, and therefore stems and roots were pooled.
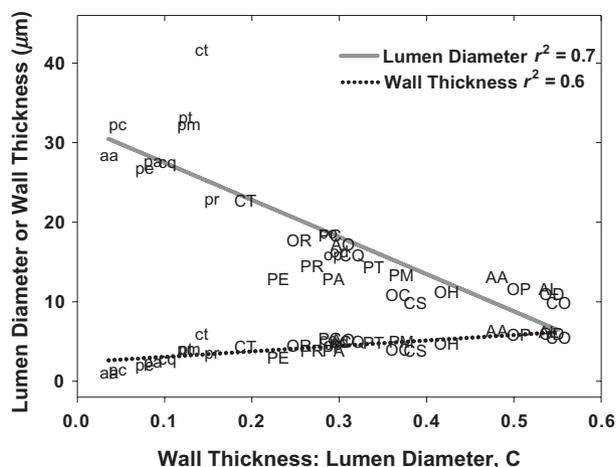
Figure 3. Lumen diameter (D) and tracheid double wall thickness (T) versus T/D thickness-to-span ratio (C). Means of $n \geq 4$ samples per species, SE averaged 1.2% of mean T. Stems and roots are represented by upper and lower case symbols, respectively. Species abbreviations are listed in Table 1.

Figure 4. Tracheid length (L) versus thickness-to-span ratio (C). Means of $n \geq 4$ samples per species, SE averaged 3% of mean L. Stems and roots are represented by upper case and lower case symbols, respectively. Species abbreviations are listed in Table 1.

Figure 5. (a) Wood density (W) versus embolism pressure (P_{50}). (b) Thickness-to-span ratio (C) versus P_{50}. In both panels, separate regressions are shown for root (lower case) and stem (upper case) data of Pinaceae/Cupressaceae (P + C) species. Root data were limited for Podocarpaceae/Araucariaceae (O + A) species and data were pooled for the two organs (dashed regressions). Means of $n \geq 4$ samples per species, SE averaged 12% of P_{50}. Species abbreviations are listed in Table 1.

ns, not significant.

In the Pinaceae/Cupressaceae species, the significant increase in thickness:span with more negative P_{50} (Fig. 5b) translated into decreasing tracheid diameters with more negative P_{50} (Fig. 6). This trend is attributed to the greater contribution of tracheid diameter rather than wall thickness (T) to the variation in the thickness:span (Fig. 3). Roots had much larger diameter for the same P_{50} than stems, consistent with thickness:span being much lower for roots (Fig. 6). A similar but less significant ($r^2 = 0.39$, $P = 0.04$) result was observed in the pooled root and stem data of the Podocarpaceae/Araucariaceae sample (Fig. 6). This result is consistent with the lack of a strong relationship between thickness:span and P_{50} in these species (Fig. 5b).

The roots and stems of the Pinaceae/Cupressaceae exhibited a significant increase in R_{CA} across the range of embolism pressures, with the stems showing R_{CA} values that are on average 6.5-fold greater than those in the roots (Fig. 7). No relationship existed between R_{CA} and P_{50} in the Araucariaceae and Podocarpaceae, and the R_{CA} values averaged 25% higher than those of the Pinaceae and Cupressaceae.

Figure 6. Tracheid diameter (D) versus embolism pressure (P_{50}). Separate regressions shown for root (lower case, dotted line) and stem (upper case, solid line) data for Pinaceae/Cupressaceae (P + C) species. Root data were limited for Podocarpaceae/Araucariaceae (O + A) species, and data were pooled across organs (dashed regression). Means of $n \geq 4$ samples, errors quoted in preceding figures. Species abbreviations are listed in Table 1.

DISCUSSION

The results are consistent with a significant trade-off in conifer wood structure between attaining greater mechanical reinforcement (i.e. higher thickness:span, or wood density) at the expense of reduced hydraulic efficiency (i.e. greater R_{CA}; Fig. 2a). The conflict arises because variation in thickness:span and wood density was achieved by the narrowing of tracheid diameter rather than increasing tracheid wall

Figure 7. Tracheid area resistivity (R_{CA}) versus embolism pressure (P_{50}). Separate regressions shown for root (lower case, dotted line) and stem (upper case, solid line) data for Pinaceae/Cupressaceae (P + C) species. Root data was limited for Podocarpaceae/Araucariaceae (O + A) species, and data was pooled across organs (dashed regression). Means of $n \geq 4$ samples, errors quoted in preceding figures. Species abbreviations are listed in Table 1.

ns, not significant.

thickness (Figs 2b & 3). Although restricted to juvenile wood for ease of sampling and measurement, the results are nevertheless based on a wide range of tracheid size because of the inclusion of root xylem and diverse species.

The observed trade-off is not inevitable. It would be less costly in terms of hydraulic efficiency and equally beneficial in terms of mechanics, if conifers increased tracheid thickness:span by making thicker walls rather than narrowing their lumens. According to Eqs 1 and 3, increasing thickness:span from 0.1 to 0.5 solely by increasing wall thickness only causes a 1.9-fold rise in R_{CA} – much less than observed (Fig. 2a). In fact, R_{CA} need not increase at all if lumen diameter increases along with thickness:span. In contrast, achieving the same increase in thickness:span solely by decreasing diameter causes a 46-fold increase in R_{CA}, which is similar to what was observed (Fig. 2a).

Why do conifers not strengthen their wood by thickening their tracheid walls rather than shrinking lumen diameter, thereby avoiding such a severe trade-off with hydraulic efficiency? We suspect that there is a developmental limit on how thick a tracheid wall can grow during the time allowed for differentiation during the growth season. This implies that a constraint on maximum wall thickness causes the observed strength versus hydraulic efficiency trade-off.

Roots and stems make for an interesting contrast in this regard. According to our interpretation, tracheids of absorptive roots can have larger diameters than stem tracheids (Figs 2b, 4 & 6) because being relatively free of support stress, they do not need to be as strong as stem tracheids (Fig. 5a & b). Hence, for a similar wall thickness, root tracheids grow to larger diameters with greater hydraulic efficiency. Furthermore, roots in general exhibit lesser degrees of 'transport stress' (less negative P_{50}) relative to the stems (Fig. 5), which reduces their need for reinforcement against potential implosion (thickness:span) attributed to high negative P_x values. Interestingly, tracheids from the primary xylem of ferns can be much wider (to 100 μm, Veres 1990) than in conifer wood, consistent with their having to bear minimal support stress because they do not function in supporting the plant.

If strength requirements constrain tracheid diameter, do they also limit length (L)? In a related paper on the same data set, we found that tracheid diameter (D) was nearly optimized to achieve the greatest hydraulic efficiency for a given tracheid length (Pittermann *et al*. 2006). This implies a constraint on length. Although tracheid size is proximally limited by the dimensions of fusiform initials (Siau 1971), what ultimately drives the variation in fusiform initial length? It is possible that length and diameter scaling might influence mechanical strength independently of the thickness-to-span ratio. Too long a tracheid for a given D might weaken the wood. This would be consistent with fairly constant L/D ratios, which averaged 104 ± 5.9 for the data set. Although tracheid length was identified as an important parameter for influencing wood mechanical properties (Panshin & de Zeeuw 1980), it is rarely analysed independently of anatomical correlates such as tracheid diameter, and so the influence of length per se is difficult

to assess. Nevertheless, our data indicate that strength requirements impact both tracheid diameter and length: mechanically strong tracheids (high thickness:span and density) had narrow lumens but were also short (Figs 3 & 4). Once again, the tracheids of primary xylem in ferns can be quite long (to 2 cm, Veres 1990) with L/D ratios up to 200, consistent with a minimal role of these tracheids in bearing canopy support stress.

Our results suggest that achieving greater wood strength comes with two disadvantages: lower hydraulic efficiency *and* greater construction cost per wood volume. To the extent that this double disadvantage reduces fitness through reduced growth rate and reproductive allocation, wood that is stronger than necessary to withstand its combined transport and support stress should be selected against. If support stress requires a fixed amount of reinforcement that is independent of transport stress, then wood strength should vary with the degree of transport stress (Hacke *et al*. 2001; Domec & Gartner 2002a). This summarizes the pattern we observed in the roots and stems of the Pinaceae and Cupressaceae species where thickness:span and wood density increased with embolism resistance and stems were more reinforced than roots (Fig. 5), as seen previously (Hacke *et al*. 2001, 2004).

The scaling of strength with embolism resistance in the Pinaceae/Cupressaceae sample corresponds to the narrowing of tracheid diameter with greater embolism resistance (Fig. 6). Our interpretation is that tracheids do not have to be narrow to achieve embolism resistance, but they do have to be narrow to withstand implosion at more negative P_x. This explanation is consistent with the lack of a mechanistic or structural link between tracheid size and vulnerability to embolism by air-seeding at tracheid end walls as investigated on the same species (Pittermann *et al*. 2006).

Species of the Podocarpaceae/Araucariaceae were quite different from the Pinaceae/Cupressaceae group in showing little relationship between embolism resistance and strength parameters or R_{CA} (Fig. 5a & b; see also Brodribb & Hill 1999). Their wood tended to be much more reinforced, especially at modest P_{50} (Fig. 5a & b). On average, these species appear to be overbuilt for bearing transport stress and more costly in terms of construction. The consequently narrower tracheids (Fig. 6) also had high hydraulic resistivity (Fig. 7). Assuming that these traits apply to the adult as well as juvenile wood, trees of these Podocarpaceae/Araucariaceae species are supported by expensive and hydraulically inefficient wood. These traits support the reputation of these families even among conifers for being evolutionary relics that are slow growing but long-lived (Norton *et al*. 1987; Enright & Hill 1995; Toro & Gessel 1999). Dense wood is predicted to reduce growth rate (Roderick 2000), and high hydraulic resistivity is often correlated with low photosynthetic capacity (Brodribb & Feild 2000). In plantation settings, Podocarps tend to have a longer average rotation than the faster-growing Pinaceae (Bergin 2000), and southern hemisphere forestry relies heavily on alien Pinaceae (e.g. *Pinus radiata*; Lawery & Mead 1998; Toro & Gessel 1999). Species of *Pinus* can also

be aggressive competitors in some southern hemisphere ecosystems where they are frequently classified as 'invasive species' (Richardson 1998; Richardson & Rejmanek 2004).

While generally not species of choice for high-production forestry, Podocarpaceae and Araucariaceae appear to be stable elements in many of their native habitats (Enright & Hill 1995). What can be said about the adaptive significance of heavy and hydraulically inefficient wood in these species when it is not required for supporting extreme P_{50} values? We can dismiss the possibility that these families are bulking up their tracheid walls to compensate for a mechanically inferior cell wall chemistry. This is unlikely because Podocarpaceae/Araucariaceae species show similar density-specific stiffness as other conifers (Chudnoff 1979). There is also no obvious reason why their habitat or architecture requires exceptional wood strength.

Perhaps the pattern we observed in Podocarpaceae/Araucariaceae results from selection for longevity in wet conditions that are often combined with nutrient and light limitations (Brodribb & Hill 1997, 2004). Conifers of both hemispheres have a greater median lifespan than their angiosperm counterparts, and for a variety of reasons, this greater longevity helps create a stable niche despite angiosperm competition (Enright & Ogden 1995). Greater longevity may be particularly adaptive in nutrient-limited environments like many of our New Caledonia and New Zealand collecting sites (Jaffré 1995; Ogden & Stewart 1995; Coomes *et al*. 2005; Whitehead *et al*. 2005). Intuitively, denser and stronger wood ought to promote tree longevity by better withstanding prolonged exposure to mechanical and pathogenic threats. In reality, the relationship between wood density and longevity is complex because other wood traits aside from density can be important (e.g. decay resistance, resin content) and different habitats pose very different challenges to longer lifespan (Loehle 1988). The fact that wood density correlates with P_{50} in northern hemisphere data sets (Fig. 5; Hacke *et al*. 2001, 2004) implies a coincidence of longevity and drought adaptation in these species. A conspicuous example of the latter is the Methuselah of all conifers, *Pinus longaeva*, which grows in a stressful habitat. However, in the more consistently wet southern hemisphere habitats we sampled, long-lived conifers occur in very mesic conditions (Enright & Hill 1995). Hypothetically, this could result in species with wood that is dense for longevity but vulnerable to embolism, as observed (Fig. 5). The penalty of hydraulic inefficiency would be alleviated by the lower transpirational demand in the humid and low light conditions of wet evergreen forest. This hypothesis could be tested by broader taxon sampling designed to systematically uncouple longevity, phylogeny and embolism resistance.

While the adaptive significance of wood strength may be multifaceted and speculative, we conclude that mechanical constraints are more important for limiting tracheid size and conifer conducting efficiency than constraints that arise directly from the air-seeding mechanism of embolism or freezing-induced embolism. This is quite different from the emerging picture of angiosperm wood where protection against embolism by air-seeding and freezing does appear to limit vessel size (Wheeler *et al*. 2005; Hacke *et al*. 2006), and direct mechanical constraints are buffered by the presence of a supporting fibre matrix. While resistance to freezing-induced embolism appears to require a mean tracheid diameter below about 30 μm (Pittermann & Sperry 2003), this diameter threshold is well above the diameter range of rain forest conifers that we sampled from the southern hemisphere (e.g. Fig. 3) where freezing does not occur. This suggests that other factors, namely mechanical considerations, may be more important than resistance to drought or freezing stress in determining tracheid size.

Although the hydraulic efficiency of the individual conifer tracheid may be more constrained by mechanics than the angiosperm vessel, conifers gain efficiency at the sapwood level because nearly all of the sapwood is conducting. In angiosperms, the disadvantage of relieving vessels of a mechanical support function is that most of the sapwood space is taken up by non-conducting fibres. Conifers combine the efficiency of maximum sapwood conducting area with hydraulically superior torus-margo pitting, thereby minimizing the difference in sapwood area resistivity with angiosperms (Pittermann *et al*. 2005). Clearly, both wood types represent different solutions to the problem of integrating water transport and structural support, with each solution having its unique advantages.

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