

Variation in xylem structure and function in stems and roots of trees to 20 m depth

Andrew J. McElrone¹, William T. Pockman², Jordi Martínez-Vilalta³ and Robert B. Jackson^{1,4}

¹Department of Biology, Duke University, Durham, NC 27708, USA; ²Department of Biology, University of New Mexico, Albuquerque, NM, USA;

³School of Earth, Environmental and Geographical Sciences, University of Edinburgh, Edinburgh, UK; ⁴Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA

Summary

Author for correspondence:

Andrew J. McElrone

Tel: +1 919 660 7289

Fax: +1 919 660 7293

Email: mcelrone@duke.edu

Received: 28 January 2004

Accepted: 16 April 2004

- To assess hydraulic architecture and limitations to water transport across whole trees, we compared xylem anatomy, vulnerability to cavitation (Ψ_{50}) and specific hydraulic conductivity (K_s) of stems, shallow roots and deep roots (from caves to 20 m depth) for four species: *Juniperus ashei*, *Bumelia lanuginosa*, *Quercus fusiformis* and *Quercus sinuata*.
- Mean, maximum and hydraulically weighted (D_h) conduit diameters and K_s were largest in deep roots, intermediate in shallow roots, and smallest in stems ($P < 0.05$ for each). Mean vessel diameters of deep roots were 2.1–4.2-fold greater than in stems, and K_s was seven to 38 times larger in the deep roots.
- Ψ_{50} also increased from stems to roots with depth, as much as 24-fold from stems to deep roots in *B. lanuginosa*. For all species together, Ψ_{50} was positively correlated with both D_h and K_s , suggesting a potential trade-off exists between conducting efficiency and safety.
- The anatomical and hydraulic differences documented here suggest that the structure of deep roots minimizes flow resistance and maximizes deep water uptake.

Key words: deep roots, xylem anatomy, vulnerability to cavitation, hydraulic conductivity, xylem conduits, caves, whole-tree hydraulic architecture.

New Phytologist (2004) **163**: 507–517

© *New Phytologist* (2004) doi: 10.1111/j.1469-8137.2004.01127.x

Introduction

Stems and roots perform many functions critical to plant growth and survival. In particular, water uptake and transport through the xylem are essential for replacing water lost during transpiration, preventing desiccation, and allowing continued photosynthesis (Kramer & Boyer, 1995). Many studies have examined water transport characteristics from the base to the top of a tree (e.g. Zimmermann, 1983), yet half or more of the flow path has remained unstudied in species and systems where deep roots are prevalent (Stone & Kalisz, 1991; Canadell *et al.*, 1996; Schenk & Jackson, 2002). Deep roots can contribute substantially to water uptake in some systems, accounting for > 75% of transpiration during the dry season (Nepstad *et al.*, 1994). Despite the importance of deep roots, there are many unanswered questions regarding their structural and functional characteristics relative to the entire water-flow path of trees (Jackson, 1999).

Variations in xylem anatomy and hydraulic properties occur at several levels: interspecific, intraspecific and intraplant (Zimmermann, 1983; Ewers, 1985; Tyree & Ewers, 1991; Sperry & Saliendra, 1994; Jackson *et al.*, 2000). At the intraplant level, conduits in shallow roots are often wider than those in stems of the same plant (Baas, 1982). Zimmermann (1983) recognized this as one of the basic organizing principles of tree hydraulic architecture. Variations in xylem conduit diameter can radically affect the functions of different portions of the conducting system because of the fourth-power relationship between radius and flow through a capillary tube, as described by the Hagen–Poiseuille law (Zimmermann, 1983; Tyree & Ewers, 1991). Thus for the same pressure difference across a segment, even a small increase in mean conduit diameter has exponential effects on specific hydraulic conductivity (K_s). Consistent with these predictions, numerous studies have shown that measured K_s is higher in shallow roots than in stems (Alder *et al.*, 1996; Kavanaugh *et al.*, 1999;

Martínez-Vilalta *et al.*, 2002). Interestingly, Pate *et al.* (1995, 1998) reported that sinker roots of *Banksia prionotes* extending to 2 m deep exhibited larger-diameter xylem conduits, greater conduit length, and higher area-specific hydraulic conductivity than lateral roots of the same individuals. Except for these two studies, we know of no data that relate xylem structure and function of deep roots to that of stems and shallow roots and the consequences of such differences for whole-tree water use.

Increased hydraulic efficiency of roots compared with shoots apparently comes at the expense of increased cavitation vulnerability. Vulnerability to drought-induced embolism tends to be larger in roots than in stems of a given species (Sperry & Saliendra, 1994; Sperry & Ikeda, 1997; Jackson *et al.*, 2000; Martínez-Vilalta *et al.*, 2002). Within a plant, wider vessels are also associated with higher vulnerabilities to drought-induced embolism (Hargrave *et al.*, 1994; Sperry & Saliendra, 1994), resulting in a trade-off between hydraulic efficiency and the xylem tensions that can occur without cavitation. This pattern in the hydraulic architecture of trees is consistent with two aspects of water transport through the plant: (i) water potentials (Ψ) become more negative, moving from the base to the apex of the tree; and (ii) embolisms may be more easily reversible in roots because of positive root pressures (Tyree & Sperry, 1989). The second aspect may explain why roots are not only more vulnerable to embolism, but also appear to operate at xylem tensions closer to their cavitation threshold than shoots (Tyree & Sperry, 1988; Alder *et al.*, 1996). For this reason, roots may be the best place to look for hydraulic differences within and among species, with differences potentially greater with depth.

Despite the importance of deep roots to overall plant water supply in many systems, little *in situ* research has been conducted because of the difficulty of accessing deep roots without excavation and disturbance of the rooting zone. Jackson *et al.* (1999) recently used limestone caves in the Edward's Plateau region of central Texas to access deep roots *in situ* and developed new molecular techniques to determine the identity of these roots to species or individual (Linder *et al.*, 2000; Linder and co-workers, unpublished data). In the current study we used these cave systems to evaluate structural and functional differences among stems, shallow roots and deep roots in four common tree species. We tested the hypothesis that xylem conduit diameter, specific hydraulic conductivity and vulnerability to cavitation across multiple tree species are smallest in stems, intermediate in shallow roots, and largest in deep roots.

Materials and Methods

Study area and plant material

Plant material was collected from the Edwards Plateau of central Texas, a karst region with characteristically shallow

(frequently < 20 cm depth) soils on top of fractured Cretaceous limestone (Elliot & Veni, 1994). Mean annual precipitation in this region ranges from 600 mm in the west to 800 mm in the east, and monthly mean temperature ranges from 7.9°C in January to 27.4°C in July. The vegetation is mainly savanna and woodland dominated by trees in the genera *Quercus*, *Juniperus*, *Ulmus*, *Celtis*, *Bumelia* and, further to the south and west, *Prosopis* (Auken *et al.*, 1980). We sampled four woody species, two at Cotterell cave in Austin, TX (30°22' N, 97°45' W) [*Juniperus ashei* Buchh. and *Quercus sinuata* (Torr.) C.H. Mull], and two from Powell's and Neel's caves in Menard, TX (30°55' N, 99°54' W) [*Quercus fusiformis* Small and *Bumelia lanuginosa* (Michx.) Pers.]. Powell's and Neel's caves are on adjacent properties and are connected by a perennial subterranean stream 18–20 m deep. All four study species persist on the shallow soils overlying limestone bedrock throughout the Edward's Plateau, and are considered slow-growing and drought-tolerant (Auken *et al.*, 1980).

For each species we sampled stems, shallow roots and deep roots from adult trees. Samples for *J. ashei* and *Q. sinuata* anatomy were collected in February, June and July 1999 (six replicates per species and tissue), and those for hydraulic measurements in June and July 1999 (four replicates per species and tissue). The samples for *B. lanuginosa* and *Q. fusiformis* anatomy and hydraulic measurements were collected in March and June 2003. Deep roots were sampled at a depth of approx. 7 m in Cotterell cave and 18–20 m in Powell's/Neel's caves. The deep roots were either excavated from soil on the cave floor/walls and along the banks of the underground stream, or sampled while hanging aurally. All deep roots were identified to species using sequence variation within ribosomal DNA in the internal transcribed spacer region (Jackson *et al.*, 1999; Linder *et al.*, 2000). Shallow roots were excavated from 5–10 cm deep in the soil.

Anatomical measurements and electron micrographs

We cut transverse sections (approx. 50 μ m wide) of the collected segments with a rotary microtome (Spencer 820 Microtome, American Optical Co., Buffalo, NY, USA). The sections were stained with toluidine blue (0.5%) to increase contrast and mounted in glycerol. Slides were then viewed with a compound microscope (Zeiss Axioscop; Carl Zeiss, Inc., Germany) attached to a computer and monochrome video camera (MTI CCD-72; Dage MTI, MI, USA). The magnification was $\times 25$ for *Quercus* and *B. lanuginosa* sections, $\times 100$ for *J. ashei* roots, and $\times 200$ for *J. ashei* stems. We used the image analysis package IMAGE 1 (ver. 4, Imaging Corporation, West Chester, PA, USA) to analyze the sections.

For each transverse section we measured two representative sectors approx. 90° apart, reaching from the vascular cambium to the pith. For stems, vascular rays were used to establish the boundaries of each sector. Rays were not apparent in

B. lanuginosa and oak roots, so arbitrary radial boundaries through the midpoint of the axis were used. Unstained vessel lumens were easily distinguished by color from the rest of the tissue (blue stained). All vessels not filled with tyloses within each sector were measured. To eliminate artifacts such as tracheids in oaks, partially occluded vessels or gaps in the wood, all conduits with an area or diameter smaller than a lower limit were excluded. In each case these values were selected to maximize agreement between the visually identified vessels and those selected by computer. The handful of functional vessels or tracheids below the selected cut-off for each species would have a negligible effect on the conductive capacity of the xylem. For each selected vessel, the diameter (measured in two directions), total area and perimeter were measured. The number of vessels measured per section varied between 60 and 627. For each vessel diameter class, theoretical hydraulic conductivity (K_h) was calculated using the Hagen–Poiseuille law and the mean number of vessels within a given class (Zimmermann, 1983; Tyree & Ewers, 1991). For these calculations we used average conduit diameters and set the viscosity of xylem sap to 1.0 centipoise at room temperature (approx. 21°C). We then generated the cumulative theoretical K_h curves by totaling the K_h of each successive diameter class, moving from smallest to largest diameter classes.

For illustrative purposes one or two representative sections of each species and tissue were observed with a Phillips XL30 environmental scanning electron microscope (ESEM; $\times 44$ for all the oak sections, $\times 200$ for *B. lanuginosa* and $\times 352$ for *J. ashei*), and photographed with a Polaroid CU-5 land camera. The negatives were scanned (Hewlett Packard ScanJet 4c) and stored in tif format.

Hydraulic measurements

We followed Sperry *et al.* (1988) to measure hydraulic conductivity. Briefly, segments of length 13–14 cm (*J. ashei*) or 80–150 cm (*B. lanuginosa* and *Q. fusiformis*) were re-cut underwater, shaved at the ends with fresh razor blades, and attached to a tubing manifold filled with filtered (0.2 μm) HCl solution (pH ≈ 2) for *J. ashei* or with filtered ultrapure water for *B. lanuginosa* and *Q. fusiformis*. Segment lengths were larger than the longest vessel for each species. Segment diameters for all stems and roots ranged only from 4.5 to 10 mm to control for size and age effects. To remove any air emboli initially present in vessels, the segments were flushed with solution at high pressure (110–120 kPa) for 30 min. The initial maximum hydraulic conductivity (K_h^{max}) was then measured as the quotient of the mass flow rate of solution through the segment and the pressure gradient along it. Hydraulic conductivity was measured continuously at 10 s intervals, and calculated from a running mean of five sequential readings. Two background measurements without a pressure gradient were taken before and after the actual measurement, and were used to correct for any water flow not

driven by the imposed pressure gradient (for example, evaporation from the surface of the segment, or the balance and uptake of water by living cells in the segment). The pressure used during measurements was 4–10 kPa for stems and 1–8 kPa for roots. Specific hydraulic conductivity (K_s) was calculated as the quotient of K_h^{max} and the stele transverse area (cross-sectional area without bark and cortex).

Vulnerability curves: centrifuge and air-injection methods

We measured vulnerability to cavitation in stems, shallow roots and deep roots of each species using either the centrifuge method (Pockman *et al.*, 1995; Alder *et al.*, 1997) or the air-injection technique (Sperry & Saliendra, 1994). The centrifuge method was used for *J. ashei* only because maximum tracheid lengths are much shorter than the total segment length (13–14 cm). Immediately after the initial conductivity measurement, *J. ashei* segments were spun in a Sorvall RC-5C centrifuge (DuPont Instruments, Wilmington, DE, USA) with their ends immersed in water. Rotor design was as described by Alder *et al.* (1997), except that three segments could be spun at once. A standard segment length was used to ensure the cut ends of the segment were immersed in the water-filled Plexiglas reservoirs during spinning. Stems were spun for 15 min at the target velocity equivalent to a given Ψ . After spinning, segments were attached to the experimental apparatus and hydraulic conductivity was measured. This procedure was repeated with increasing centrifuge speeds, resulting in treatment pressures at 1.5 MPa increments until the segment lost > 99% of its initial conductivity, or the maximum centrifuge speed was reached (21 000 r.p.m., -9.5 MPa).

The longer vessel lengths of *B. lanuginosa* and *Q. fusiformis* required use of the air-injection technique to measure vulnerability to cavitation on much longer segments (Cochard *et al.*, 1992; Sperry & Saliendra, 1994). The centrifuge and air-injection methods have repeatedly been compared and shown to give similar results (Cochard *et al.*, 1992; Sperry & Saliendra, 1994; Pockman *et al.*, 1995). With this technique, a segment was inserted inside a pressure chamber with both ends protruding. Ends of the segment were attached to the hydraulic measurement apparatus as described above, and maximum K_s was measured after the segment was flushed with solution at high pressure. Pressure was raised inside the chamber to a designated value, depending on the tissue, and maintained at that pressure for 15 min. After pressurization the segment was allowed to equilibrate and K_s was measured again. This process was repeated at progressively higher pressures (P) to generate a vulnerability curve for each segment. The percentage loss of conductivity (PLC) after each pressure application was calculated relative to the maximum value of K_s :

$$\text{PLC} = 100 \times [1 - (K_{s,p}/\text{max } K_s)] \quad \text{Eqn 1}$$

Segments for *Q. sinuata* were not long enough to obtain accurate estimates of K_s and vulnerability to cavitation, and therefore are not presented.

Statistical analyses

Statistical analyses were performed using SAS ver. 8.0 (SAS Institute Inc., Cary, NC, USA). Comparisons of parameters between or within species were made with two-way ANOVA testing for xylem sampling position (stem, shallow root, deep root), species and xylem sampling position \times species effects. The distribution data for cumulative theoretical K_h were analyzed using the Atchison log-ratio method (McElrone *et al.*, 2003). Data from the vulnerability curve were fitted to the following function (Pammenter & Vander Willigen, 1998):

$$\text{PLC} = 100 / \{1 + e^{[a \times (\Psi - b)]}\} \quad \text{Eqn 2}$$

where a is related to the slope of the curve, and b determines the position of the curve on the abscissa and gives the water potential corresponding to PLC = 50 (Ψ_{50}) for each curve.

Results

Xylem conduit diameters and distributions

Conduit diameter was significantly related to depth in all four species studied (Figs 1, 2; $P < 0.0001$). In every case conduit diameters were smallest in stems, intermediate in shallow roots, and largest in deep roots. This trend was consistent for all three conduit size parameters (Fig. 1a–c), for all growth habits (evergreen vs deciduous), and for both the conifer and the angiosperms (Figs 1, 2). Deep roots had mean vessel diameters that were two and four times larger than in stems for *Q. fusiformis* and *J. ashei*, respectively. Across species, conduit diameters increased from the tracheid-bearing *J. ashei* to the vessel-bearing angiosperms (*B. lanuginosa*, *Q. fusiformis* and *Q. sinuata*), with oaks having the largest conduit diameters (Figs 1, 2).

Conduit diameter distributions for all four species and all three xylem sampling positions contained a small proportion of large conduits (Fig. 3a–d), but these larger conduits accounted for the bulk of water flow for all xylem sampling positions, as illustrated by the sharp increases in cumulative theoretical K_h at larger conduit classes (Fig. 3e–h). For example, only 24% of the conduits in *B. lanuginosa* deep roots were $\geq 100 \mu\text{m}$ (Fig. 3d), yet they accounted for 88% of the cumulative theoretical K_h (Fig. 3h). As sampling depth increased, the greater proportion of large conduits in each distribution and the concurrent increased mean conduit diameter resulted in greater flow capacity (Fig. 3e–h). This greater flow capacity is illustrated by the divergence of final cumulative theoretical K_h between xylem sampling positions. The conduit diameter distributions and cumulative theoretical K_h varied significantly

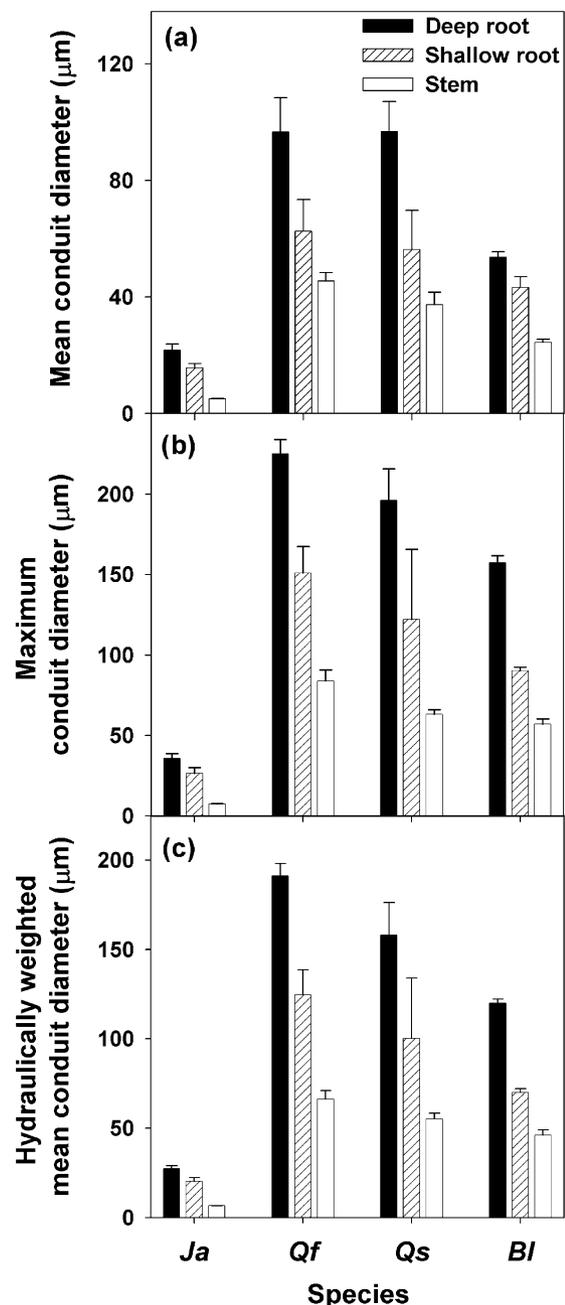


Fig. 1 Xylem conduit diameters in deep roots, shallow roots and stems of four tree species from the Edward's Plateau region of central Texas, USA: *Juniperus ashei* (*Ja*), *Quercus fusiformis* (*Qf*), *Quercus sinuata* (*Qs*), and *Bumelia lanuginosa* (*BI*). Conduits are tracheids for *J. ashei* and vessels for the other three species. Mean conduit diameter (a), maximum conduit diameter (b), and hydraulically weighted mean conduit diameter (c) data are presented as mean \pm SE ($n = 3$ –8). Hydraulically weighted mean conduit diameter weights the importance of the radii in proportion to the estimated hydraulic conductance of the conduits (Sperry *et al.*, 1994). Deep roots were collected at approx. 7 m depth for *J. ashei* and *Q. sinuata* from Cotterell cave in Austin, TX and at 18–20 m depth for *B. lanuginosa* and *Q. fusiformis* from Powell's and Neel's caves in Menard, TX. ANOVA results: species effect, $F = 30.14$, $P < 0.0001$; xylem sampling position effect, $F = 26.74$, $P < 0.0001$; species \times segment, $F = 1.85$, $P = 0.114$.

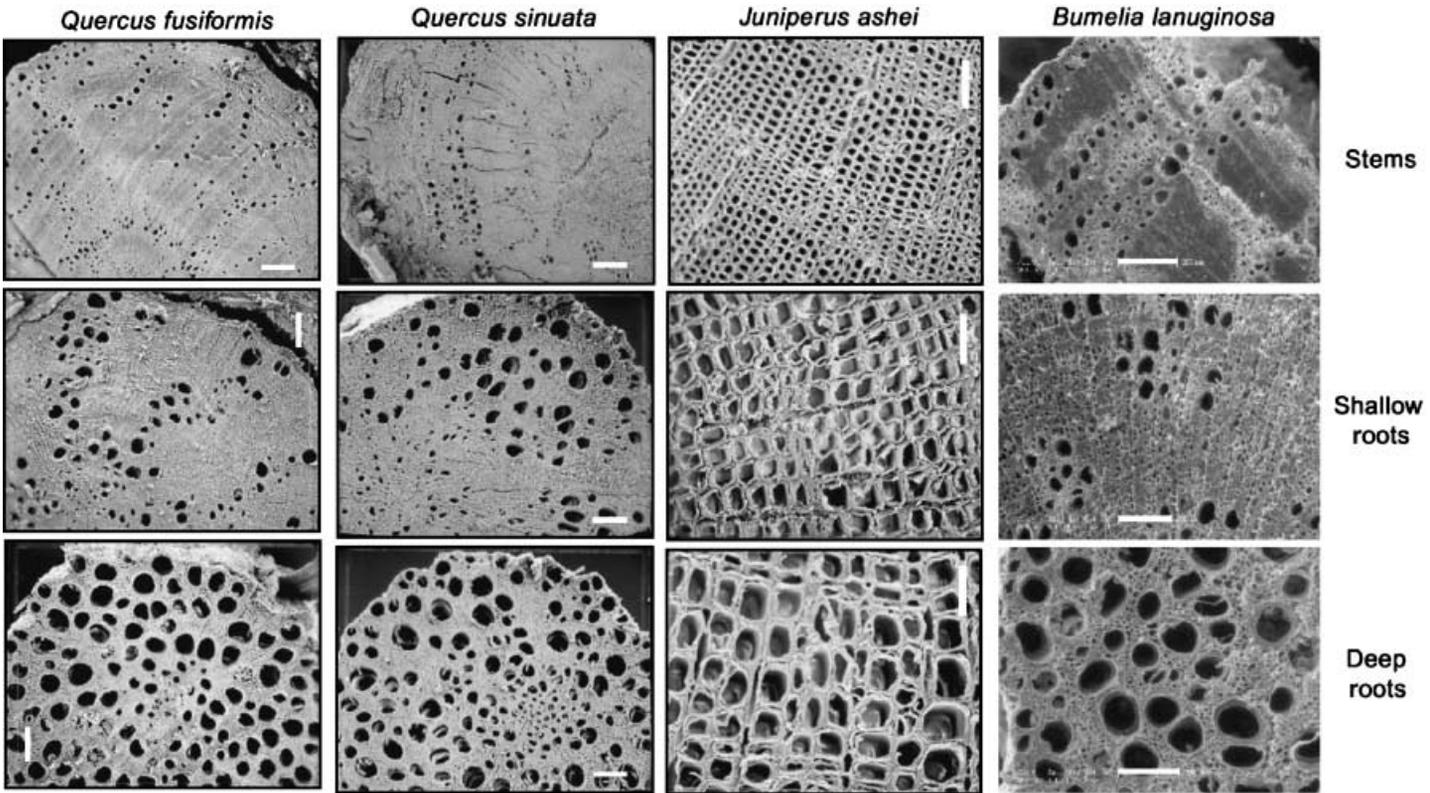


Fig. 2 Scanning electron micrographs of stems (top row), shallow roots (middle row) and deep roots (bottom row) for four tree species from the Edward's Plateau region of central Texas. Scale bars are different for each species: *Juniperus ashei* images were taken at the highest magnification so tracheids appear larger than vessels of other species. White bars represent 50 μm for *J. ashei*; 300 μm for *Quercus sinuata* and *Quercus fusiformis*; 200 μm for *Bumelia lanuginosa*.

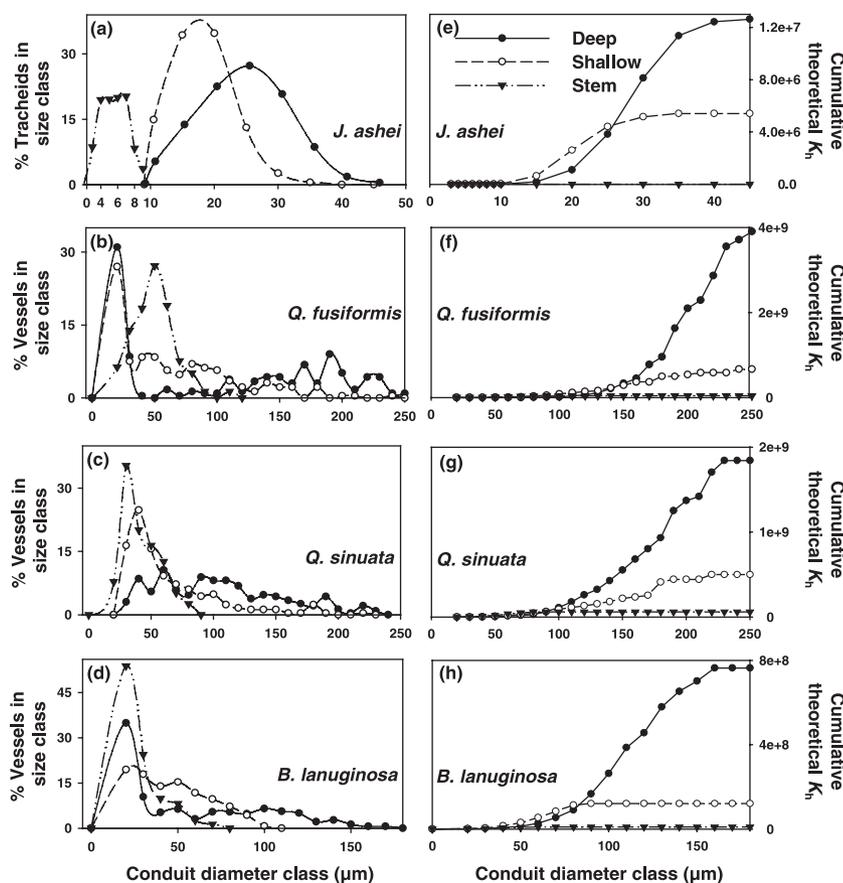


Fig. 3 Conduit diameter distributions (stacked panels a–d in left column) and cumulative percentage of total theoretical hydraulic conductivity (K_t) contributed by each conduit size class (stacked panels e–h in right column) for deep roots, shallow roots and stems of four species. Data from conduit diameter distributions were used to calculate cumulative theoretical K_t ($\mu\text{m}^4 \text{centipoise}^{-1}$) for each species using the Hagen–Poiseuille law (Zimmerman, 1983) (see Materials and Methods). Panels represent *Juniperus ashei* (a,e); *Quercus fusiformis* (b,f); *Quercus sinuata* (c,g); *Bumelia lanuginosa* (d,h). Atchison log-ratio model results: species effect, $F = 13.69$, $P < 0.0001$; xylem sampling position effect, $F = 4.29$, $P < 0.02$; species \times segment, $F = 5.47$, $P < 0.0003$.

among species and between xylem from different positions in a given species ($P = 0.02$ for all comparisons).

Specific hydraulic conductivity

Small increases in xylem conduit diameters can dramatically increase hydraulic conductivity because flow rate in capillaries is proportional to the fourth power of the capillary radius. Consistent with this expected relationship, measured K_s increased significantly with depth of the xylem sampling position and vessel diameter in all four species ($P < 0.0001$; Fig. 4). Comparing stems with deep roots, mean vessel diameters of deep roots in *B. lanuginosa* and *Q. fusiformis* were 2.2 and 2.1 times larger, but their respective K_s values were 38 and 25 times larger ($P < 0.05$). Although mean conduit diameters of deep roots in *J. ashei* were 4.2 times larger than in stems, K_s increased only sevenfold, probably because the shorter conifer tracheids increased flow resistance. K_s also increased significantly across species from *J. ashei* to *B. lanuginosa* to *Q. fusiformis*, a trend consistent with the varying conduit size of these species ($P < 0.0001$; Fig. 4).

Vulnerability curves

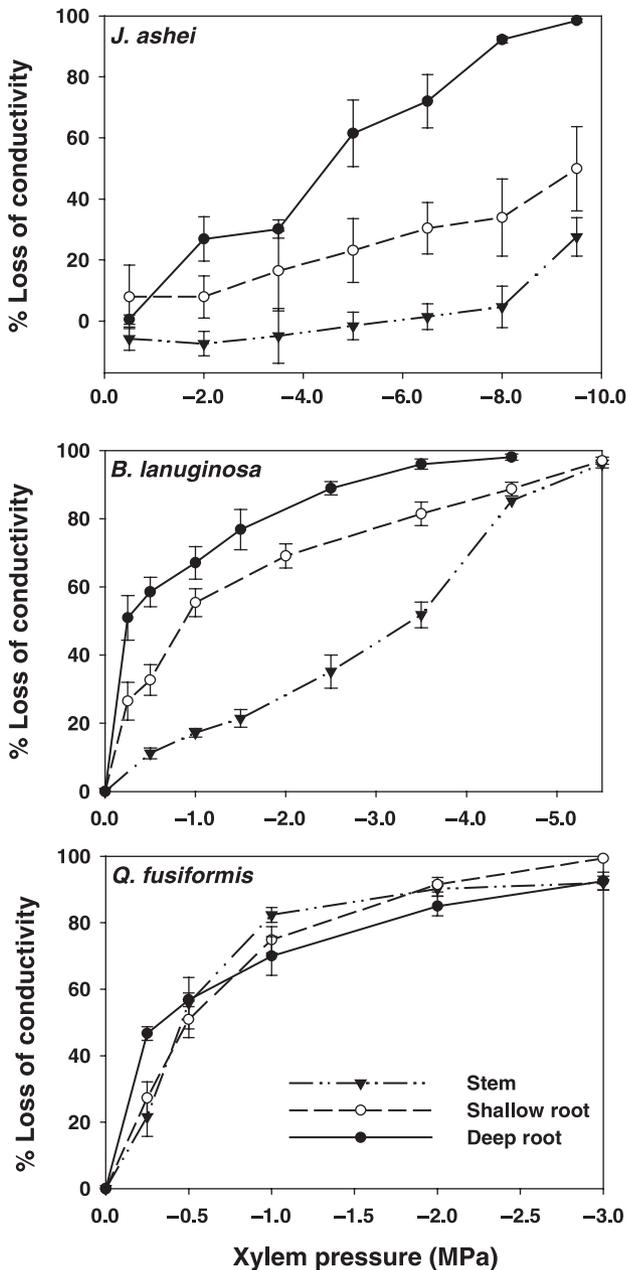
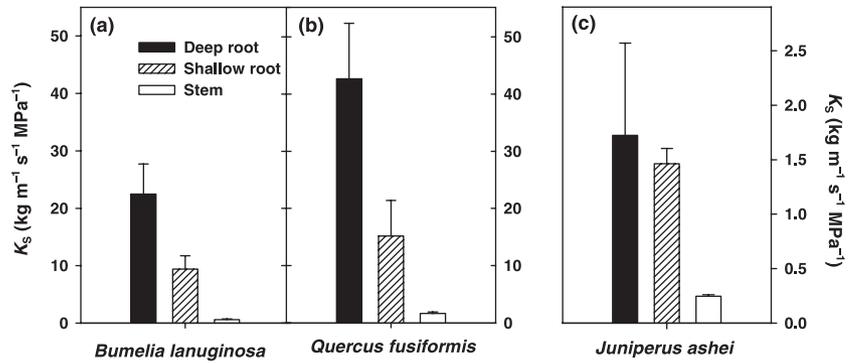
The increased theoretical and measured K_s of xylem sampling positions with depth corresponded with an increase in

vulnerability to cavitation in two of the three species (Fig. 5). Within *B. lanuginosa*, the slopes of the curves (coefficient a) were significantly different ($P < 0.03$), and Ψ_{50} (coefficient b) was significantly different between plant segments within both *B. lanuginosa* and *J. ashei* ($P < 0.008$). Ψ_{50} was 24 and three times more negative in stems relative to deep roots for *B. lanuginosa* and *J. ashei*, respectively. *Quercus fusiformis*, however, showed no change in vulnerability to cavitation with depth ($P > 0.05$ for slope and Ψ_{50}), and all segments were very susceptible to cavitation at low xylem tensions (Fig. 5, bottom panel). *Quercus fusiformis* segments were tested using both air-injection and centrifuge methods (data for centrifuge method not shown), and results were similar for both techniques.

Vulnerability vs conducting efficiency

Data for two of the three species in which we measured hydraulic parameters suggest that increased hydraulic efficiency with increased vessel diameter is achieved at a price of increased vulnerability to cavitation (Fig. 6a). Correlations of hydraulically weighted conduit diameters (D_h) and Ψ_{50} are significant for *B. lanuginosa* ($P = 0.0002$) and *J. ashei* ($P = 0.004$) (Fig. 6a), while a significant correlation between K_s and Ψ_{50} exists for *B. lanuginosa* only ($P < 0.0001$) (Fig. 6b). Both relationships are nonsignificant for *Q. fusiformis* ($P = 0.113$) (Fig. 6a,b).

Fig. 4 Specific hydraulic conductivity measured on deep roots, shallow roots and stems for *Bumelia lanuginosa* (a), *Quercus fusiformis* (b) and *Juniperus ashei* (c). Note the y-axis scaling change for *J. ashei*. Measurements were made using the Sperry technique (Sperry *et al.*, 1988). Data are mean \pm SE. ANOVA results: species effect, $F = 5.58$, $P < 0.0079$; xylem sampling position effect, $F = 9.90$, $P < 0.0004$; species \times segment, $F = 2.19$, $P = 0.090$.



Discussion

Xylem structure and water transport

Resistance to water flow in the xylem is determined in part by the diameter and length of conduits responsible for axial water transport. The differences in conduit diameter that we observed among stems, shallow roots and deep roots resulted in dramatic changes in K_s (Fig. 4). Because flow in capillary systems is proportional to the fourth power of conduit radius (Zimmermann, 1983; Tyree & Ewers, 1991), increases in overall conduit diameter, as observed here, result in nonlinear increases in K_s . The large differences in K_s between stems, shallow roots and deep roots are also likely influenced by changes in conduit length. Conduit length is often positively correlated with conduit diameter (Zimmermann & Jeje, 1981; Zimmermann & Potter, 1982; Ewers *et al.*, 1990). Although we did not measure it, we would expect that conduit length increases from stems to deep roots if the diameter-length correlation holds for the species studied here. Such changes in conduit length would contribute to the changes in conducting efficiency by reducing the number of times water moving between adjacent conduits must cross pit membranes, which

Fig. 5 Vulnerability curves measured with the centrifuge method (Pockman *et al.*, 1995) for *Juniperus ashei* and with the air-injection method for *Bumelia lanuginosa* and *Quercus fusiformis* (mean \pm SE; $n = 3-7$). Note the different scales between panels. Vulnerability curves were fitted with the function from Pammenter & Vander Willigen (1998) as described in Materials and Methods. Coefficients a and b from Eqn 2 represent the slope of the curve and the water potential corresponding to 50% loss of conductivity (Ψ_{50}), respectively. Mean coefficient a values (listed in order of stems, shallow roots and deep roots) are 0.46, 0.27, 0.65 for *J. ashei*; 1.08, 1.02, 1.79 for *B. lanuginosa*; 4.27, 3.83, 2.41 for *Q. fusiformis*. Mean coefficient b values are -13.41 , -12.95 , -4.48 for *J. ashei*; -2.60 , -0.73 , -0.11 for *B. lanuginosa*; -0.50 , -0.57 , -0.43 for *Q. fusiformis*. ANOVA results for coefficient a : species effect, $F = 33.31$, $P < 0.0001$; xylem sampling position effect, $F = 5.54$, $P < 0.0127$; species \times segment, $F = 1.22$, $P > 0.05$. ANOVA results for coefficient b : species effect, $F = 37.03$, $P < 0.0001$; xylem sampling position effect, $F = 5.32$, $P < 0.0146$; species \times segment, $F = 2.14$, $P > 0.05$.

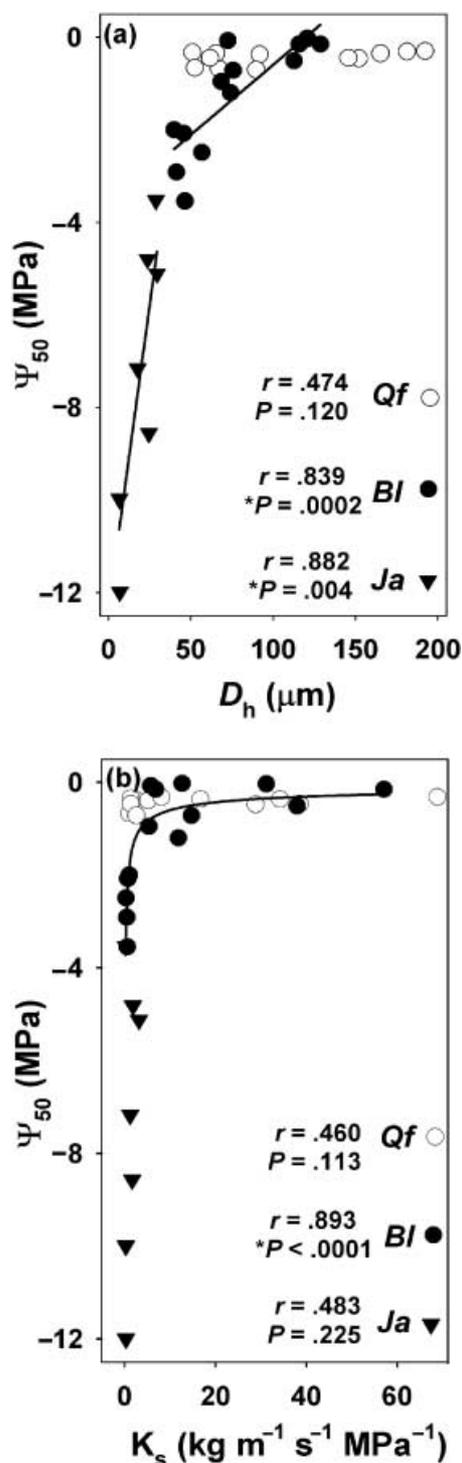


Fig. 6 Relationships between hydraulically weighted mean conduit diameter (D_h) and vulnerability to cavitation (Ψ_{50}) (a), and between specific hydraulic conductivity (K_s) and Ψ_{50} (b) for *Bumelia lanuginosa* (Bl), *Quercus fusiformis* (Qf) and *Juniperus ashei* (Ja). Relationships for all three species are included in each panel. Data within a species represent all values from deep roots, shallow roots and stems. Power functions and linear equations were used for regression analyses. Fitted lines represent significant regressions only; r and P values for all species are shown (*, significant regression).

are sites of high resistance to water flow (Zimmermann, 1983; Hacke *et al.*, 2004; Sperry & Hacke, 2004).

The large vertical changes in K_s that we observed should reduce or eliminate the effect of the long path length from deep roots on whole-plant hydraulic conductance. Water absorbed by deep roots must travel a greater distance to reach the canopy than water absorbed by shallow roots near the soil surface. Although the difference in path length will depend on the geometry of the root system, in the species studied here the path length is roughly doubled for water uptake from deep roots. If K_s remained constant from stems to deep roots, the increase in path length alone would have large effects on flow rate and xylem pressure that might limit leaf gas exchange. The increase in conduit diameter and K_s that we observed from stems to shallow roots to deep roots could overcome this limitation by reducing the flow resistance in deep roots. Sinker roots of *B. prionotes* extending to 2 m depth have been shown to exhibit a similar pattern, with larger diameter xylem conduits, greater conduit length, and higher area-specific hydraulic conductivity than lateral roots of the same individuals (Pate *et al.*, 1995, 1998). Interestingly, these differences were evident between deep roots 2 m below the root crown and shallow roots 2 m laterally from the root crown (Pate *et al.*, 1995). The persistence of structural differences between shallow and deep roots located the same distance from the root crown suggests that their xylem structure was influenced by differences in their orientation, either through internally detected signals or in response to differences in the environment (e.g. increased resistance because of gravity with depth) surrounding these roots. Our data cannot address this question because our sampling of deep and shallow roots was not structured to include constant path length in each category.

In most systems variation in temperature is greatest for stems and least for deep roots. In our system, deep soil temperature varies $< 1^\circ\text{C}$ from the monthly mean temperature (20.3°C), and deep roots never freeze. Shallow soil is responsive to seasonal changes in climate. Despite warm winter temperatures (January MAT is 7.9°C), shallow roots may occasionally experience brief episodes of freezing and freezing-induced xylem cavitation (Martínez-Vilalta & Pockman, 2002). Although larger xylem conduits are more vulnerable to freezing-induced cavitation (Davis *et al.*, 1999; Martínez-Vilalta & Pockman, 2002), it is unclear whether differences in freezing frequency could produce the gradient in conduit diameters that we observed.

The increased conduit diameter and K_s that we observed with depth are also important from the perspective of carbon allocation. Xylem dominated by large conduits requires less carbon than denser xylem with smaller diameter conduits (Tyree *et al.*, 1994). Moreover, the increased K_s in deep roots means that, for a given driving force, they can carry the same amount of water as a much larger shallow root or stem. However, radial hydraulic resistance limits water uptake by roots more than axial transport because most water must cross cell

membranes at the Casparian band (Steudle, 2001). Increased K_s of deep woody roots allows transport of water absorbed by a very large network of fine roots without large additional decreases in xylem water potential. In the underground stream in Powell's cave, a 1 cm diameter woody root of *Bumelia* or *Quercus* can support a network of absorbing fine roots that fills a volume roughly 5 m in length and 0.5 m in diameter (A.J.M. and W.T.P., unpublished observations). Thus the patterns that we have observed may reflect a balance between allocation to absorbing roots and woody roots that maximizes the water returned from the substantial carbon cost required to reach 20 m below the surface.

Finally, the biomechanical demands placed on wood, like wind loading and canopy support, may contribute to the anatomical differences that we observed. In above-ground tissues, wood density and hydraulic architecture have been shown to vary with biomechanical demands (Gartner, 1991); less dense, larger-diameter xylem is more efficient hydraulically but weaker mechanically than xylem dominated by smaller diameter conduits (Tyree *et al.*, 1994). However, biomechanical stresses experienced by stems, such as vertical support of the canopy, are unlikely to affect shallow and, especially, deep roots because they exist in a matrix of supporting rocks and soil. Therefore roots likely require less mechanical strength than the canopy, and without this need for structural support can construct xylem that is more efficient for water transport.

Vulnerability to cavitation

The increasing vulnerability to cavitation with depth observed in *B. lanuginosa* and *J. ashei* indicates that deep roots are potentially more limiting to water transport than shallow roots or stems. How frequently deep roots approach the pressures required to induce cavitation depends on the range of environmental conditions that they experience. Even in the absence of any flow through the plant, cavitation can occur as plants equilibrate with drying soil (North & Nobel, 1991). Deep roots like those in our cave systems frequently exist in an environment with high water availability, or where water content and the soil Ψ that determines plant Ψ change relatively slowly. Thus, despite their increased vulnerability, deep roots with access to relatively abundant water may be unlikely to experience soil Ψ sufficient to induce cavitation. Moreover, the presence of roots in these more slowly changing environments allows deep-rooted species to maintain higher Ψ than co-occurring, shallow-rooted species which exhibit Ψ that more closely tracks the larger variations observed in shallow soil (Pockman & Sperry, 2000).

Decreases in xylem pressure during transpiration can also induce cavitation. During transpiration xylem pressure decreases below soil Ψ in a manner determined by K_s and flow rate. As a result, Ψ is highest in the soil and decreases along the flow path to the point of evaporation in the leaves. Although vulnerability to cavitation in *B. lanuginosa* and

J. ashei increased with depth (Fig. 5), xylem sampling positions at depth may not operate any closer to critically low xylem tensions because xylem water potential in deep roots is typically higher than in stems. The safety margin between actual xylem pressure and the pressures required to induce xylem cavitation at each position in the plant are determined by the soil Ψ and the hydraulic conductance of the flow path to the point of interest. Because K_s is also greater in deep roots, any given flow rate will lead to a smaller decrease in xylem pressure in deep roots than would occur in shallow roots or stems. Thus the increased K_s associated with increased conduit diameter in deep roots may not necessarily come at a price of increased embolism, especially as embolism in roots may be easier to refill with positive root pressure (Alder *et al.*, 1996). Detailed evaluation of the behavior of deep roots during water uptake requires consideration of the vertical profiles of xylem hydraulic architecture, root-absorbing area, soil Ψ and soil texture (Jackson *et al.*, 2000; Sperry *et al.*, 2002).

Despite dramatic anatomical changes with depth, we found no difference in vulnerability to cavitation among stems, shallow roots and deep roots of *Q. fusiformis*. Although long vessels, such as those in *Q. fusiformis*, can interfere with air-injection measurements of vulnerability to cavitation (Martínez-Vilalta *et al.*, 2002), we used segments longer than the longest vessel. Furthermore, our air-injection results were consistent with measurements using the centrifuge technique. We were unable to use the dehydration method (Tyree & Sperry, 1989) because the large number of deep roots required would exceed the number that we can locate and harvest without depleting these resources for *in situ* physiological measurements. If the patterns we observed are supported by additional data, *Q. fusiformis* would be one of the few species where differences in vulnerability are not observed with changes in anatomy between roots and stems. This would require pit membrane pore diameter to remain relatively unchanged as vessel diameter increases in roots.

Conducting efficiency vs vulnerability to cavitation

Our results show a trade-off between conducting efficiency and vulnerability to cavitation across stems, shallow roots and deep roots of *B. lanuginosa* and *J. ashei* (Fig. 6). These results are consistent with the nonlinear relationships found in comparisons of the same organ across many species (Pockman & Sperry, 2000; Maherali *et al.*, 2004) and between stems and shallow roots of the same species (Martínez-Vilalta *et al.*, 2002).

Questions still remain about what drives this pattern of xylem anatomy and vulnerability to cavitation. Tsuda & Tyree (1997) suggested that increased K_s and vulnerability to cavitation in roots guarantees large water flow or water use when water is available, and was thought to be an adaptation to more mesic environments. However, our results show that

this pattern holds in woody roots and stems in drier habitats as well. The adjustment of xylem anatomy and K_s may be more affected by water availability to all or part of a tree's root system during tissue development. Root development and subsequent hydraulic characteristics within and between trees can be affected by microsite parameters, particularly water availability (Sperry & Ikeda, 1997; Kavanaugh *et al.*, 1999). Alder *et al.* (1996) found intraspecific differences in susceptibility to cavitation in root xylem between slope and riparian habitats, and suggested this was largely caused by environmental rather than genetic effects. The cave systems studied here provide an opportunity to address whether deep roots develop changes in xylem structure and function after tapping into a reliable deep water source, or whether these patterns are inherent characteristics of deep roots.

Conclusions

Quantifying hydraulic architecture and transport limitations in whole trees is challenging because of the inaccessibility of entire root systems, which constitute a large fraction of the total flow path. We were able to compare xylem structure and function across the entire flow path by using caves to collect roots up to 20 m deep. Our results show that vessel diameter, K_s , and vulnerability to cavitation for multiple tree species increased significantly with sampling depth (Figs 1–5). Although differences between stems and shallow roots have been observed before (Mencuccini & Comstock, 1997; Sperry & Ikeda, 1997; Kavanaugh *et al.*, 1999; Hacke *et al.*, 2000; Martínez-Vilalta *et al.*, 2002), our results both extend these observations and clearly show that deep roots differ in structure and function from their shallower counterparts.

Acknowledgements

This work was funded by National Science Foundation and Mellon Foundation grants to R.B.J., a USDA-NRI competitive research grant to R.B.J. and W.T.P. and a USDA-NRI grant to A.J.M. J.M. benefited from an FI grant and specific financial support for the stay at Duke University from the Catalan Government. Thanks to R. Linder for root identification, R.N. Addington for running initial air-injection vulnerability curves, T. Crocker for preparing and analyzing anatomical samples, and V. Engel, B.J. McElrone and C.J. Willson for collecting field samples in caves. Special thanks to J. Powell, G. Lundgren and M. Sanders for providing access to Powell's, Neel's and Cotterell caves, respectively, and T. Holsinger and the Texas Speleological Survey for information on the study caves. This manuscript was improved substantially by helpful comments from S. Berthrong, M. Brito Caldeira, C.W. Cook, T. Crocker, I.N. Forseth, K. Farley, M. Henn, K. Hofmockel, R. McCulley, M. Nuno Bugalho, C. Reid and three anonymous reviewers.

References

- Alder NN, Sperry JS, Pockman WT. 1996. Root and stem xylem embolism, stomatal conductance and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* 105: 293–301.
- Alder NN, Pockman WT, Sperry JS, Nuissner S. 1997. Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* 48: 665–674.
- Auken OW, Ford AL, Stein A, Stein AG. 1980. Woody vegetation of upland communities in the southern Edwards Plateau. *Texas Journal of Science* 32: 23–35.
- Baas P. 1982. Systematic, phylogenetic, and ecological wood anatomy: history and perspectives. In: Baas P, ed. *New perspectives in wood anatomy*. The Hague: Nijhoff/Junk, 23–58.
- Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. Maximum rooting depth of vegetation types at the global scale. *Oecologia* 108: 583–595.
- Cochard H, Breda N, Granier G, Aussenac G. 1992. Vulnerability to air embolism of three European oak species (*Quercus petraea* (Matt) Liebl., *Q. pubescens* Willd., *Q. robur* L.). *Annals of Forest Science* 49: 225–233.
- Davis SD, Sperry JS, Hacke UG. 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany* 86: 1367–1372.
- Elliot WR, Veni G, eds. 1994. *The caves and karst of Texas*. Huntsville, AL, USA: National Speleological Society.
- Ewers FW. 1985. Xylem structure and water conduction in conifer trees, dicot trees, and lianas. *International Association of Wood Anatomists Bulletin* 6: 309–317.
- Ewers FW, Fisher JB, Chiu ST. 1990. A survey of vessel dimensions in stems of tropical lianas and other growth forms. *Oecologia* 84: 544–552.
- Gartner BL. 1991. Stem hydraulic properties of vines vs. shrubs of western poison oak, *Toxicodendron diversilobum*. *Oecologia* 87: 180–189.
- Hacke UG, Sperry JS, Pittermann J. 2000. Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. *Basic and Applied Ecology* 1: 31–41.
- Hacke UG, Sperry JS, Pittermann J. 2004. Analysis of circular bordered pit function II. Gymnosperm tracheids with torus–margo pit membranes. *American Journal of Botany* 91: 386–400.
- Hargrave KR, Kolb KJ, Ewers FW, Davis SD. 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist* 126: 695–705.
- Jackson RB. 1999. The importance of root distributions for hydrology, biogeochemistry, and ecosystem functioning. In: Tenhunen J, Kabat P, eds. *Dahlem Conference: integrating hydrology, ecosystem dynamics, and biogeochemistry in complex landscapes*. Chichester, UK: Wiley, 219–240.
- Jackson RB, Moore LA, Hoffmann WH, Pockman WT, Linder CR. 1999. Ecosystem rooting depth determined with caves and DNA. *Proceedings of the National Academy of Sciences, USA* 96: 11387–11392.
- Jackson RB, Sperry JS, Dawson TE. 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* 5: 482–488.
- Kavanaugh KL, Bond BJ, Aitken SN, Gartner BL, Knowe S. 1999. Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology* 19: 31–37.
- Kramer P, Boyer J. 1995. *Water relations of plants and soils*. San Diego, CA, USA: Academic Press.
- Linder CR, Moore LA, Jackson RB. 2000. A universal molecular method for identifying underground plant parts to species. *Molecular Ecology* 9: 1549–1559.
- Maherali H, Pockman WT, Jackson RB. 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* (In press.)
- Martínez-Vilalta J, Pockman WT. 2002. The vulnerability to freezing-induced xylem cavitation of *Larrea tridentata* (Zygophyllaceae) in the Chihuahuan desert. *American Journal of Botany* 89: 1916–1924.

- Martínez-Vilalta J, Prat E, Oliveras I, Pinol J. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* 133: 19–29.
- McElrone AJ, Sherald JL, Forseth IN. 2003. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. *Journal of Experimental Botany* 54: 419–430.
- Mencuccini M, Comstock J. 1997. Vulnerability to cavitation in populations of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions. *Journal of Experimental Botany* 311: 1323–1334.
- Nepstad DC, de Carvalho CR, Davidson EA, Jipp PH, Lefebvre PA, Negreiros GH, da Silva ED, Stone TA, Trumbore SE, Vieira S. 1994. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature* 372: 666–669.
- North GB, Nobel PS. 1991. Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of *Agave deserti* (Agavaceae). *American Journal of Botany* 78: 906–915.
- Pammenter NW, Vander Willigen C. 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* 18: 589–593.
- Pate JS, Jeschke WD, Aylward MJ. 1995. Hydraulic architecture and xylem structure of the dimorphic root systems of South West Australian species of the Proteaceae. *Journal of Experimental Botany* 46: 907–915.
- Pate J, Jeschke W, Dawson T, Raphael C, Hartung W, Bowen B. 1998. Growth and seasonal utilisation of water and nutrients by *Banksia prionotes*. *Australian Journal of Botany* 46: 511–532.
- Pockman WT, Sperry JS. 2000. Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. *American Journal of Botany* 87: 1287–1299.
- Pockman WT, Sperry JS, O'Leary JW. 1995. Sustained and significant negative water pressure in xylem. *Nature* 378: 715–716.
- Schenk HJ, Jackson RB. 2002. Rooting depths, lateral root spreads, and belowground/aboveground allometries of plants in water limited ecosystems. *Journal of Ecology* 90: 480–494.
- Sperry JS, Hacke UG. 2004. Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* 91: 369–385.
- Sperry J, Ikeda T. 1997. Xylem cavitation in roots and stems of Douglas fir and white fir. *Tree Physiology* 17: 275.
- Sperry JS, Nichols KL, Sollivan JEM, Eastlack SE. 1994. Xylem embolism in ring-porous, diffuse-porous and coniferous trees of northern Utah and interior Alaska. *Ecology* 75: 1736–1752.
- Sperry JS, Saliendra NZ. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell & Environment* 17: 1233–1241.
- Sperry JS, Donnelly JR, Tyree MT. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment* 11: 35–40.
- Sperry JS, Hacke UG, Oren R, Comstock JP. 2002. Water deficits and hydraulic limits to leaf water supply. *Plant, Cell & Environment* 25: 251–263.
- Steudle E. 2001. The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* 52: 847–875.
- Stone E, Kalisz P. 1991. On the maximum extent of tree roots. *Forest Ecology Management* 46: 59–102.
- Tsuda M, Tyree MT. 1997. Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. *Tree Physiology* 17: 351–357.
- Tyree MT, Ewers FW. 1991. Tansley Review 34. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345–360.
- Tyree MT, Sperry JS. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiology* 88: 574–580.
- Tyree MT, Sperry JS. 1989. Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Molecular Biology* 40: 19–38.
- Tyree MT, Davis SD, Cochard H. 1994. Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *International Association of Wood Anatomists Journal* 14: 335–360.
- Zimmermann MH. 1983. *Xylem structure and the ascent of sap*. New York, USA: Springer-Verlag.
- Zimmermann MH, Jeje AA. 1981. Vessel-length distribution of some American woody plants. *Canadian Journal of Botany* 59: 1882–1892.
- Zimmermann MH, Potter D. 1982. Vessel-length distribution in branches, stem, and roots of *Acer rubrum*. *International Association of Wood Anatomists (IAWA) Bulletin* 3: 103–109.



About New Phytologist

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org
- Regular papers, Letters, Research reviews, Rapid reports and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2003 average submission to decision time was just 35 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £108 in Europe/\$193 in the USA & Canada for the online edition (click on 'Subscribe' at the website)
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 592918) or, for a local contact in North America, the USA Office (newphytol@ornl.gov; tel 865 576 5261)