

Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees

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ABSTRACT

We examined functional coordination among stem and root vulnerability to xylem cavitation, plant water transport characteristics and leaf traits in 14 co-occurring temperate tree species. Relationships were evaluated using both traditional cross-species correlations and phylogenetically independent contrast (PIC) correlations. For stems, the xylem tension at which 50% of hydraulic conductivity was lost (Ψ_{50}) was positively associated ($P < 0.001$) with specific conductivity (K_S) and with mean hydraulically weighted xylem conduit diameter (D_{h-w}), but was only marginally ($P = 0.06$) associated with leaf specific conductivity (K_L). The PIC correlation for each of these relationships, however, was not statistically significant. There was also no relationship between root Ψ_{50} and root K_S in either cross-species or PIC analysis. Photosynthetic rate (A) and stomatal conductance (g_s) were strongly and positively correlated with root Ψ_{50} in the cross-species analysis ($P < 0.001$), a relationship that was robust to phylogenetic correction ($P < 0.01$). A and g_s were also positively correlated with stem Ψ_{50} in the cross-species analysis ($P = 0.02$ and 0.10 , respectively). However, only A was associated with stem Ψ_{50} in the PIC analysis ($P = 0.04$). Although the relationship between vulnerability to cavitation and xylem conductivity traits within specific organs (i.e. stems and roots) was weak, the strong correlation between g_s and root Ψ_{50} across species suggests that there is a trade-off between vulnerability to cavitation and water transport capacity at the whole-plant level. Our results were therefore consistent with the expectation of coordination between vulnerability to xylem cavitation and the regulation of stomatal conductance, and highlight the potential physiological and evolutionary significance of root hydraulic properties in controlling interspecific variation in leaf function.

Key-words: hydraulic architecture; leaf and root coordination; photosynthesis; stomatal conductance.

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INTRODUCTION

Water supply to leaves depends on maintaining an intact water column in the xylem from roots to shoots. Because this hydraulic pathway is under increasing tension during transpiration, it is vulnerable to cavitation through air seeding, which occurs when air bubbles are aspirated into water-filled conduits (Zimmermann 1983; Tyree & Sperry 1989). Each time cavitation occurs, the resulting vapour-filled conduit no longer carries water, causing a decrease in xylem hydraulic conductivity (Tyree & Ewers 1991). The vulnerability of plants to xylem cavitation is often correlated with variation in moisture availability, and recent evidence indicates that this relationship is adaptive across a broad range of taxonomic groups (Maherali, Pockman & Jackson 2004).

However, our understanding of how interspecific variation in the vulnerability of xylem to cavitation is associated with variation in other plant functional traits that control carbon and water balance is incomplete. For example, resistance to water stress-induced cavitation via air seeding in a large majority of angiosperms depends on the surface tension of the meniscus in each pore of the pit membrane that connects adjacent conduits (Jarbeau, Ewers & Davis 1995). Increased resistance to cavitation should therefore be accompanied by decreased pore hydraulic conductivity and, ultimately, stem hydraulic conductivity (Zimmermann 1983; Sperry & Hacke 2004). Nevertheless, evidence for a trade-off between stem cavitation resistance and xylem water transport across species is inconsistent (e.g. Maherali *et al.* 2004). The direct link between sustained water transport and leaf transpiration also suggests that there should be functional coordination between vulnerability to xylem cavitation and the regulation of stomatal conductance (g_s) across species (Sperry & Pockman 1993; Sparks & Black 1999; Brodrigg *et al.* 2003). In addition, the observation that tissue construction costs are associated with vulnerability to xylem cavitation across species (Hacke *et al.* 2001) suggests that xylem cavitation could be associated with traits that affect plant carbon uptake. Although there is evidence of functional coordination between hydraulic conductivity (K_H) and gas exchange across species (Brodrigg & Feild 2000; Meinzer 2002), few studies have explicitly considered the link between cavitation resistance and suites

of leaf physiological traits that control CO₂ and H₂O fluxes across species.

Many insights about the functional significance of xylem cavitation across species have been derived from studies on stems (Tyree & Ewers 1991; Pockman & Sperry 2000; Maherali *et al.* 2004). However, root xylem properties generally differ from stems and may be more tightly linked to the control of whole plant water transport (Jackson, Sperry & Dawson 2000). For example, roots typically have wider xylem conduits, and in consequence, higher segment hydraulic conductivity per unit cross-sectional area than stems (e.g. McElrone *et al.* 2004). In addition, roots are generally more vulnerable to xylem cavitation than stems, suggesting that they may be the weakest link along the soil-to-leaf water transport pathway (Kavanagh *et al.* 1999; Martínez-Vilalta *et al.* 2002; McElrone *et al.* 2004). Therefore, incorporating information on the hydraulic properties of roots in addition to that of stems in comparative studies may help identify adaptive relationships between xylem function and other physiological traits.

We examined the relationship between resistance to xylem cavitation and other physiological traits using a group of diverse, co-occurring tree species commonly found in the temperate forest of eastern North America. We determined: (1) if there was a trade-off between vulnerability to xylem cavitation and xylem water transport traits; (2) whether vulnerability to xylem cavitation was correlated with traits associated with leaf carbon and water vapour fluxes; and (3) if the strength of these relationships differed between stems and roots. Because a significant proportion of species at our Duke Forest study site were from the genus *Quercus*, we also took the opportunity to examine functional associations within this clade by incor-

porating additional oak species from a nearby temperate sand hill savannah ecosystem. To determine the influence of shared ancestry on the strength of trait relationships and to account for the statistical non-independence of species, we calculated correlations with and without phylogenetic information (e.g. Maherali *et al.* 2004).

MATERIALS AND METHODS

Study site and plant material

Our study was conducted in the Durham Division of Duke Forest, located near Durham, NC (36°01'N, 78°59'W, elevation 150 m). The climate is warm temperate, with a mean annual temperature of 15 °C and average monthly maxima and minima of 26 °C (July) and -2.3 °C (January), respectively (1971–2000 record; Southeast Regional Climate Center, <http://cirrus.dnr.state.sc.us>). The mean annual precipitation is 1220 mm, with approximately half this amount (627 mm) received during the growing season (April–September). The site was an 80–100-year-old second-growth mixed hardwood conifer forest upon well-drained loamy soils with a predominantly flat topography. We sampled 14 common canopy and subcanopy tree species from nine seed plant families and a variety of wood types (Table 1). Stems from two additional temperate oak species were sampled from a site typical of the sandhills ecosystem in the south-eastern USA located in the US Army's Fort Bragg military base (35°10'17"N, 79°22'56"W). This site is characterized by deep (≈ 4 m) sandy soils and experiences similar weather conditions to the Duke Forest site. The site is managed as a longleaf pine savannah with periodic burning every 3 years that maintains a *Pinus palus-*

Table 1. Species names, family, wood type and location for the 16 tree species from the Piedmont (Duke Forest) and Sandhills (Fort Bragg) of North Carolina used in the study

Species	Family	Wood type	Location
Angiosperms			
<i>Acer rubrum</i> L.	Aceraceae	DP	Duke Forest
<i>Cornus florida</i> L.	Cornaceae	DP	Duke Forest
<i>Oxydendrum arboreum</i> (L) DC	Ericaceae	DP	Duke Forest
<i>Quercus alba</i> L.	Fagaceae	RP	Duke Forest
<i>Quercus falcata</i> Michaux	Fagaceae	RP	Duke Forest
<i>Quercus laevis</i> Walter	Fagaceae	RP	Fort Bragg
<i>Quercus nigra</i> L.	Fagaceae	RP	Duke Forest
<i>Quercus phellos</i> L.	Fagaceae	RP	Duke Forest
<i>Quercus rubra</i> L.	Fagaceae	RP	Duke Forest
<i>Quercus stellata</i> Wang.	Fagaceae	RP	Fort Bragg
<i>Cercis canadensis</i> L.	Fabaceae	sRP	Duke Forest
<i>Liquidambar styraciflua</i> L.	Hamamelidaceae	DP	Duke Forest
<i>Nyssa sylvatica</i> Marshall	Nyssaceae	DP	Duke Forest
Conifers			
<i>Juniperus virginiana</i> L.	Cupressaceae	NP	Duke Forest
<i>Pinus taeda</i> L.	Pinaceae	NP	Duke Forest
<i>Pinus echinata</i> Miller	Pinaceae	NP	Duke Forest

All angiosperms are winter deciduous and all conifers are evergreen.

The phylogenetic relationship among species is shown in Fig. 1.

RP, ring-porous; sRP, semi-ring porous; DP, diffuse porous; NP, non-porous.

tris canopy with various oak species, of which *Quercus laevis* and *Quercus stellata* are the most abundant, in the subcanopy.

Leaf gas exchange

To facilitate access for gas exchange measurements and to ensure that all samples experienced similar light environments, we sampled saplings or young trees 2–4 m tall that occurred either in canopy gaps created by natural disturbance or near clearings associated with forest roads. We measured photosynthetic CO_2 assimilation (A) and stomatal conductance (g_s) to water vapour with an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) on 2–3 sun-exposed leaves of 9–12 individuals for each species on July 2000, after the leaves had expanded fully. To ensure that leaf gas exchange values reflected the maximum capacities of leaves, we attempted to make measurements under non-limiting conditions. Therefore, individuals were sampled before midday (930 and 1200 h EST). Red-blue light-emitting diodes maintained incident irradiance at saturating levels ($1800 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a Peltier cooling module maintained leaf temperature between 25 and 30 °C. Although the relative humidity was not controlled directly, vapour pressure deficit (VPD) varied between 1.5 and 2.0 kPa and approximated ambient conditions. To calculate g_s , we used a boundary layer conductance of $1.42 \text{ mol m}^{-2} \text{ s}^{-1}$, which was calculated on the basis of leaf area and fan speed using the energy balance algorithms of the LI-6400. Following enclosure in the leaf cuvette, leaves reached steady-state values (e.g. when the coefficients of variation of CO_2 and H_2O within the chamber were $< 0.25\%$) within 5 min. To assess the trade-off between CO_2 uptake and water loss, we calculated instantaneous water-use efficiency (WUE) as A/g_s . Gas exchange was expressed on a one-sided leaf area basis for all species. Angiosperm leaves were larger than the cuvette, so the default area of 6 cm^2 was used for all calculations. For *Pinus taeda*, we measured the widest diameter of each needle in the cuvette and calculated projected leaf area as the product of the length of the cuvette (3 cm) and the aggregate diameter of all needles. *Juniperus virginiana* leaf samples were excised after gas exchange data collection and projected leaf area was measured with a LI-3100A leaf area meter (Li-Cor, Inc.).

After performing gas exchange measurements, we measured shoot water potential (Ψ) with a pressure chamber (Plant Moisture Status Instrument Company, Corvallis, OR, USA). In addition, individual leaves used for gas exchange were excised and placed in plastic bags containing moist paper towels. For angiosperms, individual leaf size was subsequently measured with the LI-3100A. For *P. taeda*, individual fascicles were separated to allow needles to lay flat on the conveyor belt of the LI-3100A, facilitating projected area measurement. For *J. virginiana*, leaf size was defined as the projected area of scales on the most distal twig on a branch. Projected leaf area on these scales was measured as described above. All leaves were dried to constant mass in a forced convection oven at 65 °C for 48 h and

were weighed. Specific leaf area (SLA) was calculated by dividing leaf size by leaf mass. Leaves were subsequently ground to powder with a Crescent Wig-L-Bug (Crescent Dental, Lyons, IL, USA). Powder samples were assessed for C and N content (%) using a CE Instruments NC 2100 elemental analyser (ThermoQuest Italia, Milan, Italy). Integrated WUE was determined on six of these powdered samples per species by measuring carbon isotope discrimination ($\delta^{13}\text{C}$; Farquhar, Ehleringer & Hubick 1989) with a Finnigan MAT Delta Plus XL continuous flow mass spectrometer (Finnigan MAT GmbH, Bremen, Germany).

Vulnerability to xylem cavitation

We measured the vulnerability of xylem to cavitation on stem and root segments of 5–7 of the same individuals of each species previously sampled for gas exchange. We collected 0.5–1.0-m long sun-exposed shoots and 30–70-cm long roots from 20–50-cm soil depths during August and September of 2001 (stems) and 2003 (roots). Stem and root samples were typically 2–5 years old, as determined by growth ring counts of cross sections. To minimize dehydration, all tissues were immediately placed in plastic bags containing moist paper towels. In the laboratory, all stem samples were recut under water to a length of 14 cm, and the cut ends were trimmed with a razor blade. Root samples were recut to longer lengths (between 24 and 59 cm) in order to minimize the refilling of cavitated vessels during measurements, particularly for *Quercus*. Although we have no information on vessel length distributions for our species, previous studies on diffuse porous angiosperms indicated that the average vessel length for similar aged tissue was $< 4 \text{ cm}$ (Sperry, Hacke & Wheeler 2005). Average vessel length is typically longer in ring-porous species (e.g. $\approx 13 \text{ cm}$ in *Vitis vinifera*, Sperry *et al.* 2005), suggesting that some vessels may have been open in *Quercus* stems.

K_H was measured as described by Sperry, Donnelly & Tyree (1988) in an air-conditioned laboratory (20 °C). Segments were cleared of air emboli by perfusing them at high pressure with filtered ($0.2 \mu\text{m}$) distilled water for 15–20 min at 100 kPa. A hydrostatic pressure of 1.5–2 kPa was used to measure volume flow rate (Q , kg/s), which was calculated by collecting efflux continuously with a vial placed on a 0.0001 g balance connected to a computer. Hydraulic conductivity (K_H ; $\text{kg m MPa}^{-1} \text{ s}^{-1}$) was expressed as the volume flow rate divided by the pressure gradient [$Q / (dP / dx)$]. Specific conductivity (K_S ; $\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$) was calculated by dividing K_H by the segment's cross-sectional xylem area. Leaf specific conductivity (K_L ; $\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$) was calculated by dividing K_H by the leaf area distal to the measured segment. Leaf area distal to the measured stem segment was measured on fresh tissue using a LI-3100A leaf area meter (Li-Cor, Inc.).

Vulnerability to drought-induced xylem cavitation was measured as the reduction in K_H of a stem or root segment as a function of decreasing xylem tension (a vulnerability curve; Sperry *et al.* 1988). For stem segments of all species and root segments of *J. virginiana*, vulnerability curves

were constructed using the centrifuge technique (Alder *et al.* 1997). Segments were spun on an axis for 5 min in a custom designed rotor (Alder *et al.* 1997) at a specific rotational velocity to produce xylem tensions ranging from -0.5 to -10 MPa in 0.5 – 2 MPa increments. K_H was measured on each stem following each successive spin in the centrifuge. Preliminary experiments indicated that for many species (especially ring-porous *Quercus*), an initial spin at 1 MPa produced large (70 – 90%) losses in K_H . As a result, it was possible that flushing stems prior to constructing vulnerability curves refilled non-functional conduits (called cavitation fatigue; Hacke, Sperry & Pittermann 2000) or that this initial exposure to low negative pressures caused open vessels to cavitate. Regardless of the process operating, all stems were spun at low speed (-0.5 MPa) after flushing to allow functional conduits to remain filled while embolizing non-functional conduits and open vessels (Hacke *et al.* 2000). In addition, only water transport measurements made after this initial spin were used in calculations to prevent non-functional conduits and open vessels from contributing to estimates of K_H , K_S and K_L .

Although the use of pure water as a perfusing solution can underestimate K_H because of hydrogels in the pit membrane (Zwieniecki, Melcher & Holbrook 2001), we used pure water rather than a solution of KCl for our measurements. The effect of KCl on K_H varies considerably across angiosperms (Boyce *et al.* 2004) and even among individuals of a single species (Zwieniecki *et al.* 2001). This variable effect may be associated with differences in the ionic concentrations of xylem sap across individuals and species. As a result, the use of a constant KCl solution in our interspecific comparisons may not correct the bias associated with using pure water. Nevertheless, the use of pure water instead of KCl as our perfusing solution may have reduced K_H in our study species and may have altered patterns of interspecific variation in hydraulic traits. We note, however, that KCl and pure water have produced similar vulnerability curves in other angiosperm species (Davis *et al.* 2002). In addition, previous studies have detected trade-offs between vulnerability to cavitation and K_H despite using non-KCl solutions (e.g. Sperry & Pockman 1993; Hacke *et al.* 2000; Martínez-Vilalta *et al.* 2002).

The large size of conduits in roots (McElrone *et al.* 2004) prevented us from constructing vulnerability curves with the centrifuge method because attaching samples to the hydrostatic measuring apparatus refilled embolized conduits. Therefore, vulnerability curves for roots were constructed using the air-injection method (Cochard, Cruiziat & Tyree 1992; Sperry & Saliendra 1994) in which xylem cavitation was induced by successively increasing positive air pressures on a segment inside a double-ended pressure chamber. Previous studies indicate that the centrifuge and air-injection methods yield very similar vulnerability curves (Pockman, Sperry & O'Leary 1995). Each root segment was notched (0.5 – 1 mm deep) with a razor blade to provide an entry point for air, and the bark at the proximal and distal ends was removed. The root segment was then inserted into a pressure chamber with both ends protrud-

ing. The root ends were connected to the hydraulic measurement apparatus and K_H was measured as described previously. The pressure inside the chamber was then raised to 0.5 MPa and this pressure was maintained for 15 min. After pressurization, the segment was allowed to equilibrate for 10 min at low pressure (0.1 MPa) followed by 5 more minutes at 0 MPa, and K_H was measured again. This process was repeated at progressively higher pressures in 0.5 – 1.0 MPa increments to generate a vulnerability curve for each segment. To maintain consistency with vulnerability curves constructed with the centrifuge method, K_H after pressurization at 0.5 MPa was taken as the reference value.

Percent loss in conductivity (*PLC*) following each spin in the centrifuge or pressurization of the chamber was calculated as $PLC = 100 * [(K_{max} - K_H) / K_{max}]$, where K_H is the hydraulic conductivity of the segment measured after each chamber pressurization and K_{max} is the hydraulic conductivity after the initial 0.5 MPa spin or pressurization. Vulnerability curves were fit with an exponential sigmoid equation (Pammenter & Van Der Willigen 1998):

$$PLC = \frac{100}{[1 + \exp(a(\Psi - b))]} \quad (1)$$

where Ψ is the negative of the injection pressure, a is a measure of the degree that conductivity responds to injection pressure or tension (curve slope) and b represents the Ψ at which a 50% loss in conductivity occurs (Ψ_{50} or curve displacement along the x -axis). Coefficients a and b were estimated using the non-linear regression procedure in Systat 8.0 (SPSS, Evanston, IL, USA).

Xylem conduit measurements

Xylem conduit diameter was measured on stem segments used for hydraulic measurements. Stem cross sections ($20 \mu\text{m}$ thick) were made with a sliding microtome (American Optical Co., Buffalo, NY, USA) and stained with toluidine blue. The cross sections were viewed with a light microscope interfaced with a Nikon CoolPix 950 digital camera (Nikon Inc., Melville, NY, USA). The captured images were downloaded onto a computer for image analysis using NIH Image 1.58 (U.S. National Institutes of Health; <http://rsb.info.nih.gov/nih-image/>). We measured radial strips of cells on sectors spaced at 90° intervals in the outermost portion (2 – 5 growth rings) of each cross section. Up to 300 conduits were measured for each stem segment. Hydraulic diameter (D_h) of lumens was calculated as $D_h = 2xy / [x + y]$ for tracheids and $D_h = (2x^2y^2 / [x^2 + y^2])^{1/2}$ for vessels, where x and y are the short and long sides of the conduit, respectively (Lewis & Boose 1995). To determine the functional significance of conduit size distribution within each segment, we calculated the hydraulically weighted mean diameter (D_{h-w}) for each segment as $\Sigma D_h^5 / \Sigma D_h^4$ (Pockman & Sperry 2000).

Statistical analyses

Because our primary interest was in examining which traits are functionally integrated with xylem vulnerability to cav-

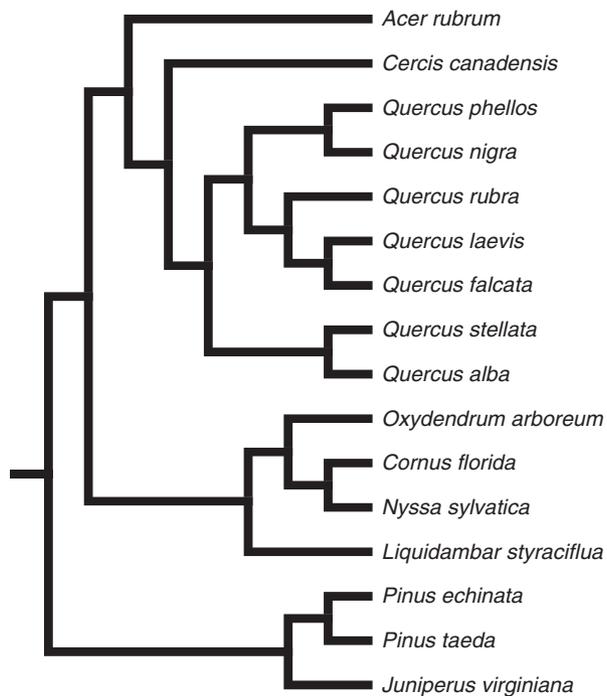


Figure 1. A phylogenetic tree showing the relationship among the species used in this study.

itation, correlations were examined with root and stem Ψ_{50} as the anchor traits (i.e. Schwilk & Ackerly 2001). One limitation of solely using cross-species comparisons to determine functional integration of physiological traits is that these correlations may be biased by the similarity of closely related species (Felsenstein 1985; Harvey & Pagel 1991). This shared evolutionary history prevents individual species from being statistically independent data points (Harvey & Pagel 1991). To account for shared evolutionary history, we calculated phylogenetically independent contrasts (PICs, Felsenstein 1985), which are determined for each speciation event in the phylogeny (e.g. as the difference in trait values between sister taxa along each branch; Felsenstein 1985; Garland, Harvey & Ives 1992). This method identifies adaptive relationships because the strength and sign of the contrast correlation indicates whether evolutionary shifts in a trait are associated with changes in another trait or ecological variable (Pagel 1993; Ackerly & Reich 1999).

A fully bifurcated phylogenetic tree (Fig. 1) incorporating taxa sampled at our study site was derived from published sources using methods and assumptions described in Maherali *et al.* (2004). Relationships between traits were evaluated using traditional cross-species correlations and correlations among PICs. Pearson correlations (R) for the cross-species analysis were calculated using SPSS 10.0 (SPSS, Evanston, IL, USA). PICs and Pearson correlations among PICs were calculated using the Phenotypic Diversity Analysis Programs (PDAP; <http://www.biology.ucr.edu/people/faculty/Garland/PDAP.html>; Garland, Midford & Ives 1999; Garland & Ives 2000) from within the Mesquite

system for phylogenetic computing (Maddison & Maddison 2004). All data were log-transformed prior to analyses. Because trees from various sources were combined to produce our phylogeny, it was impossible to incorporate into our analysis information on phylogenetic branch lengths, which indicate the number of evolutionary changes along each ancestor–descendant pathway (Harvey & Pagel 1991). In the absence of such information, we assumed that branch lengths are equal to calculate PIC correlations, a conservative assumption that minimizes Type I error rate (Ackerly 2000). We note that because our study species were sampled non-randomly and disjunctly across seed plant families, ancestral reconstructions based on parsimony are likely to be biased, and this bias will influence the strength and statistical significance of PIC correlations. However, this limitation must be balanced against inflated Type I error rates of non-phylogenetically corrected cross-species correlations (Garland *et al.* 1992; Ackerly 2000). To be conservative and to facilitate interpretation, we present both standard and PIC correlations.

RESULTS

The vulnerability of roots and stems to xylem cavitation varied widely across species (Figs 2 & 3). The most resistant species in the community, *J. virginiana*, reached 50% stem cavitation (Ψ_{50}) at a water potential that was nearly eight-fold more negative than that of the most vulnerable species, *Quercus falcata*. In general, stems of *Quercus* were more vulnerable to cavitation than those of other species. Even the *Quercus* species occurring on well-drained sandy soils at the Fort Bragg site were more vulnerable to cavitation than most of the non-*Quercus* Duke Forest species. With the exception of *Cercis canadensis*, *Quercus* species also had roots that were the most vulnerable to cavitation. In all species, roots were more vulnerable to xylem cavitation than stems (Figs 2 & 3). In addition, the K_S of roots was on average 5.6 times higher than that of stems (paired *t*-test, $P < 0.01$, Table 2).

Based on rank order, species with low stem Ψ_{50} also had low root Ψ_{50} (Fig. 4). However, we observed that differences between stem and root Ψ_{50} increased as overall vulnerability to xylem cavitation decreased. For example, species with vulnerable xylem, such as *Quercus* and *Nyssa sylvatica*, had similar stem and root Ψ_{50} , whereas species with resistant xylem, such as *Cornus florida* and *J. virginiana*, had stems with much more negative stem Ψ_{50} than root Ψ_{50} (Fig. 4).

In the cross-species analysis, stem Ψ_{50} increased significantly with specific conductivity (K_S ; Fig. 5a, $P < 0.001$) and with mean hydraulically weighted conduit diameter (D_{h-w} ; Fig. 5c, $P < 0.001$), but was only marginally associated with leaf specific conductivity (K_L ; Fig. 5b, $P = 0.06$). However, the phylogenetically independent contrast (PIC, Table 3) correlation for each of these relationships was not statistically significant (Fig. 5a–c, insets). The absence of an evolutionary correlation across taxa is consistent with the observation that data points among more closely related

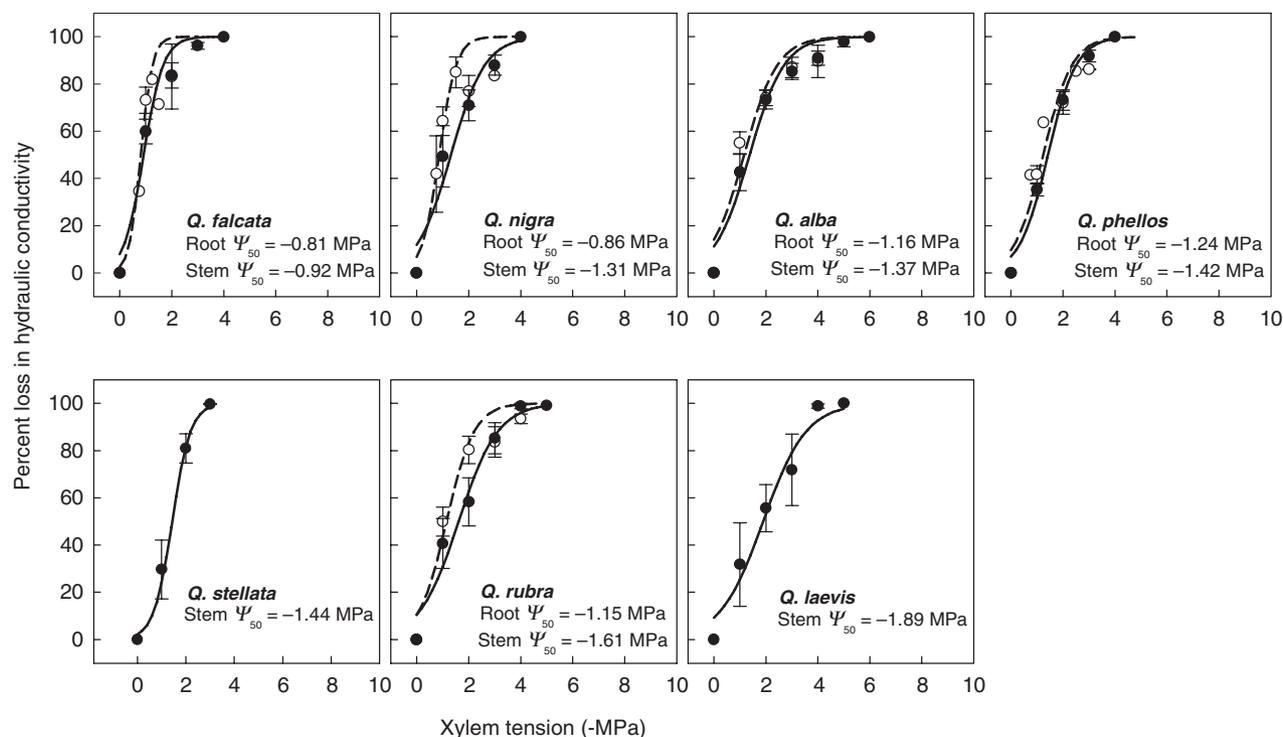


Figure 2. Curves of the vulnerability of xylem to cavitation for stems (filled circles and solid lines) and roots (empty circles and dashed lines) in seven oak species from Duke Forest and Fort Bragg, NC. Although means (± 1 SE) are presented, curves were fit using all data. The xylem tension causing 50% loss in hydraulic conductivity (Ψ_{50}) for each species is shown at the bottom of each panel.

species tended to be clustered on all three plots of the relationship between hydraulic efficiency and Ψ_{50} (Fig. 5a–c). In addition, there was no relationship between root Ψ_{50} and root K_S for both cross-species and PIC correlations (Table 3). Within *Quercus*, Ψ_{50} increased significantly with

increasing K_L (Fig. 6b, $P = 0.05$) but was not associated with either K_S (Fig. 6a) or D_{h-w} (Fig. 6c). Although significant PIC correlations suggested that there were evolutionary correlations between Ψ_{50} and K_S (Fig. 6a, inset, $P = 0.05$) and Ψ_{50} and K_L (Fig. 6b, inset, $P = 0.01$), these relationships

Table 2. Shoot water potential (MPa) during gas exchange measurements, and stem K_S ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$), stem K_L ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) and root K_S ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) for each species in the study

Species	Water potential (± 1 SE)	Stem K_S	Stem K_L ($\times 10^{-4}$)	Root K_S
Angiosperms				
<i>Acer rubrum</i>	-1.30 ± 0.11	0.55 ± 0.09	1.20 ± 0.21	0.90 ± 0.23
<i>Cornus florida</i>	-0.81 ± 0.07	0.15 ± 0.02	0.22 ± 0.02	1.62 ± 0.52
<i>Oxydendrum arboreum</i>	-1.24 ± 0.11	0.32 ± 0.06	0.56 ± 0.11	1.48 ± 0.66
<i>Quercus alba</i>	-1.14 ± 0.19	1.37 ± 0.18	2.02 ± 0.25	15.4 ± 5.98
<i>Quercus falcata</i>	-1.92 ± 0.07	1.49 ± 0.58	2.78 ± 0.79	4.82 ± 1.86
<i>Quercus laevis</i>	–	0.49 ± 0.27	0.84 ± 0.12	–
<i>Quercus nigra</i>	-1.54 ± 0.26	1.19 ± 0.20	2.56 ± 0.48	2.66 ± 0.89
<i>Quercus phellos</i>	-1.97 ± 0.16	1.35 ± 0.36	2.44 ± 0.58	11.1 ± 8.23
<i>Quercus rubra</i>	-1.30 ± 0.09	1.33 ± 0.30	2.26 ± 0.47	2.42 ± 0.82
<i>Quercus stellata</i>	–	0.81 ± 0.25	1.83 ± 0.49	–
<i>Cercis canadensis</i>	-1.41 ± 0.09	0.25 ± 0.07	0.38 ± 0.11	3.23 ± 0.87
<i>Liquidambar styraciflua</i>	-1.17 ± 0.10	0.70 ± 0.11	1.45 ± 0.29	2.07 ± 0.94
<i>Nyssa sylvatica</i>	-1.01 ± 0.09	0.18 ± 0.02	0.29 ± 0.04	2.14 ± 0.62
Conifers				
<i>Juniperus virginiana</i>	-1.10 ± 0.07	0.25 ± 0.04	1.38 ± 0.15	1.69 ± 1.21
<i>Pinus taeda</i>	-1.57 ± 0.04	0.32 ± 0.02	0.99 ± 0.15	3.82 ± 1.17
<i>Pinus echinata</i>	–	0.26 ± 0.04	2.12 ± 0.43	0.91 ± 0.32

No water potential data were collected for *Q. laevis*, *Q. stellata* and *P. echinata*.

No root data were collected for *Q. laevis* and *Q. stellata*.

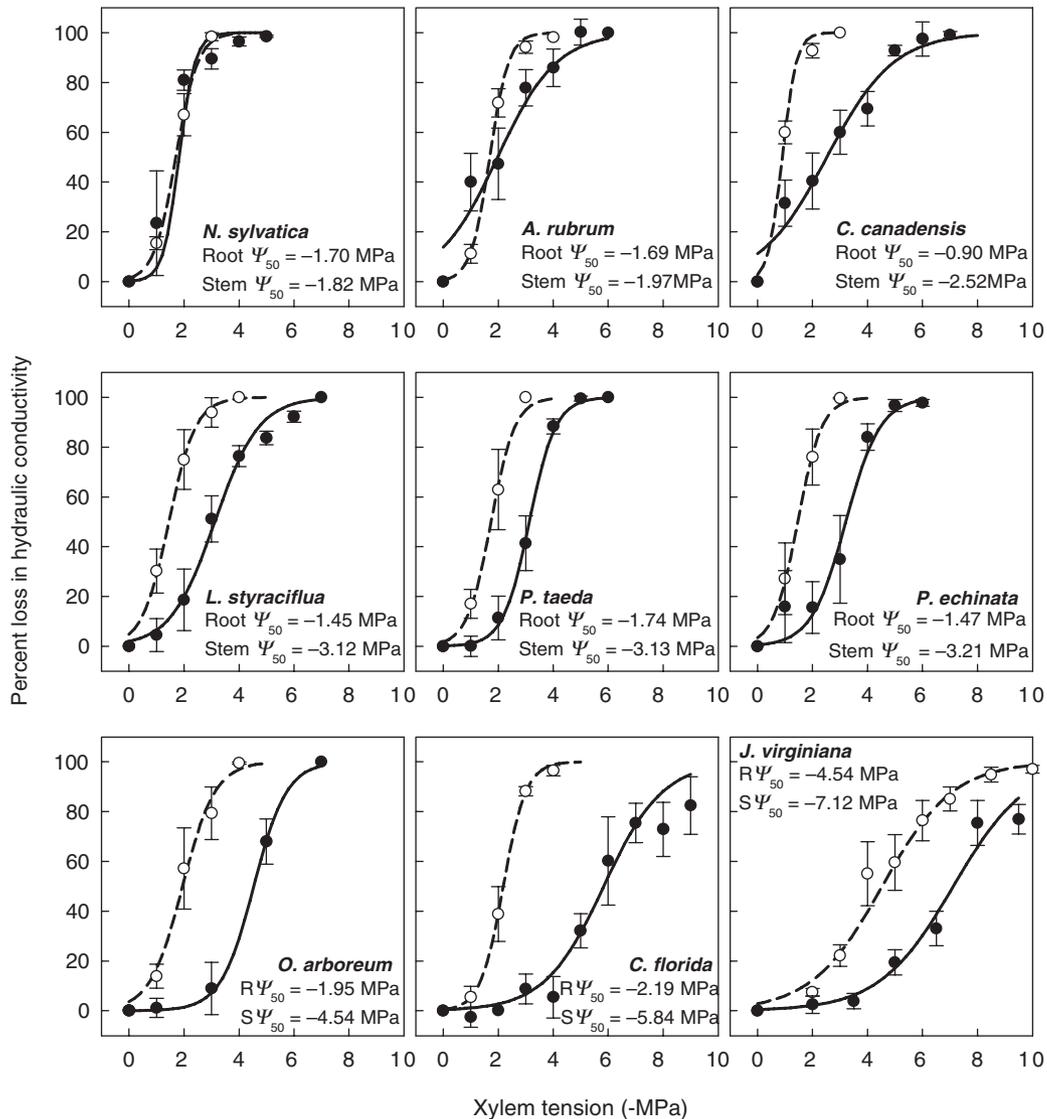


Figure 3. Curves of the vulnerability of xylem to cavitation for stems (filled circles and solid lines) and roots (empty circles and dashed lines) in nine deciduous and conifer species from Duke Forest, NC. Although means (± 1 SE) are presented, curves were fit using all data. The xylem tension causing 50% loss in hydraulic conductivity (Ψ_{50}) for each species is shown at the bottom of each panel.

were driven by a single contrast between *Q. laevis* and *Q. falcata* and therefore may not be biologically meaningful within the genus.

There were important associations between Ψ_{50} and leaf level traits, particularly for roots. In the cross-species analysis, both A and g_s increased with increasing stem Ψ_{50} (Fig. 7a & b, $P = 0.02$ and 0.10 , respectively). However, in the PIC analysis, there was only an association between A and stem Ψ_{50} ($P = 0.04$; Fig. 7a inset) while there was no relationship between g_s and stem Ψ_{50} (Fig. 7b inset). Both the cross-species and PIC correlations for these relationships were sensitive to the inclusion of conifers in the analysis. For example, when only angiosperms were included, A was weakly correlated with stem Ψ_{50} ($R = -0.47$, $P = 0.15$; $\text{PIC} = -0.18$, $P = 0.30$). In contrast, A and g_s increased strongly with increasing root Ψ_{50} in both the cross-species

($P < 0.001$; Fig. 7c and d) and PIC analyses ($P < 0.01$; Fig. 7c and d, inset). These relationships were also strong within the angiosperms (for A versus root Ψ_{50} : $R = 0.82$, $P = 0.002$, $\text{PIC} = -0.79$, $P = 0.002$; for g_s versus root Ψ_{50} : $R = -0.64$, $P = 0.04$, $\text{PIC} = -0.58$, $P = 0.03$).

Instantaneous WUE, calculated as A/g_s , was not correlated with Ψ_{50} in either stems or roots. In addition, A and g_s were not associated with measures of K_H (K_S and K_L) in stems (data not shown, $R = 0.35$, $P = 0.25$). Leaf size was not associated with stem Ψ_{50} but was positively correlated with root Ψ_{50} ($P < 0.01$, Table 3), a relationship that was robust to phylogenetic correction ($P = 0.01$; Table 3) but not statistically significant when conifers were removed from the analysis ($R = -0.31$, $P = 0.35$, $\text{PIC} = -0.23$, $P = 0.25$). Other leaf traits of functional significance such as integrated WUE (measured as $\delta^{13}\text{C}$), leaf nitrogen or SLA

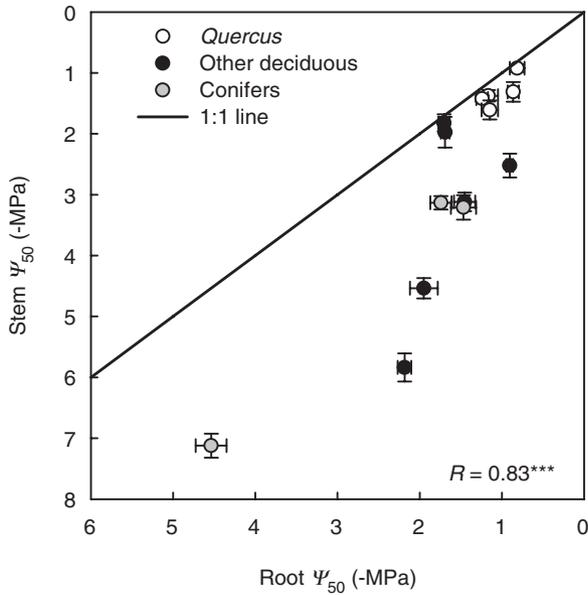


Figure 4. The xylem tension causing 50% loss in hydraulic conductivity (Ψ_{50}) for stems plotted against the same parameter for roots for 14 co-occurring species in Duke Forest.

were either marginally associated (e.g. $\delta^{13}\text{C}$) or not associated with stem or root Ψ_{50} (Table 3).

DISCUSSION

Vulnerability to xylem cavitation and water transport

Studies within single communities have provided evidence that there is a cross-species correlation, and thus a trade-off, between vulnerability to xylem cavitation and xylem transport capacity (Pockman & Sperry 2000; Martínez-Vilalta *et al.* 2002). On the surface, our results are consistent with these previous studies. We found considerable interspecific variation on how vulnerable stems and roots were to xylem cavitation for a group of co-occurring woody species (Table 2). There were also large differences across species in the efficiency of xylem transport, as measured by K_S and K_L . However, even though all three measures of hydraulic efficiency (K_S , K_L and mean D_{h-w}) increased with Ψ_{50} across 14 co-occurring species, there was no relationship between these parameters when phylogenetic information was included (Fig. 5a–c, insets). These results are consistent with a broader analysis of stems of 167 woody plant species across a variety of biomes (Maherali *et al.* 2004). Similarly, there was no relationship between the vulnerability of roots to cavitation and root K_S for these species for both cross-species and PIC correlations (Table 3). We conclude therefore that there was weak evidence for a trade-off between Ψ_{50} and the efficiency of xylem transport within stems or roots for the species in this temperate forest community.

Resistance to water stress-induced cavitation via air seeding in a large majority of angiosperms depends on the

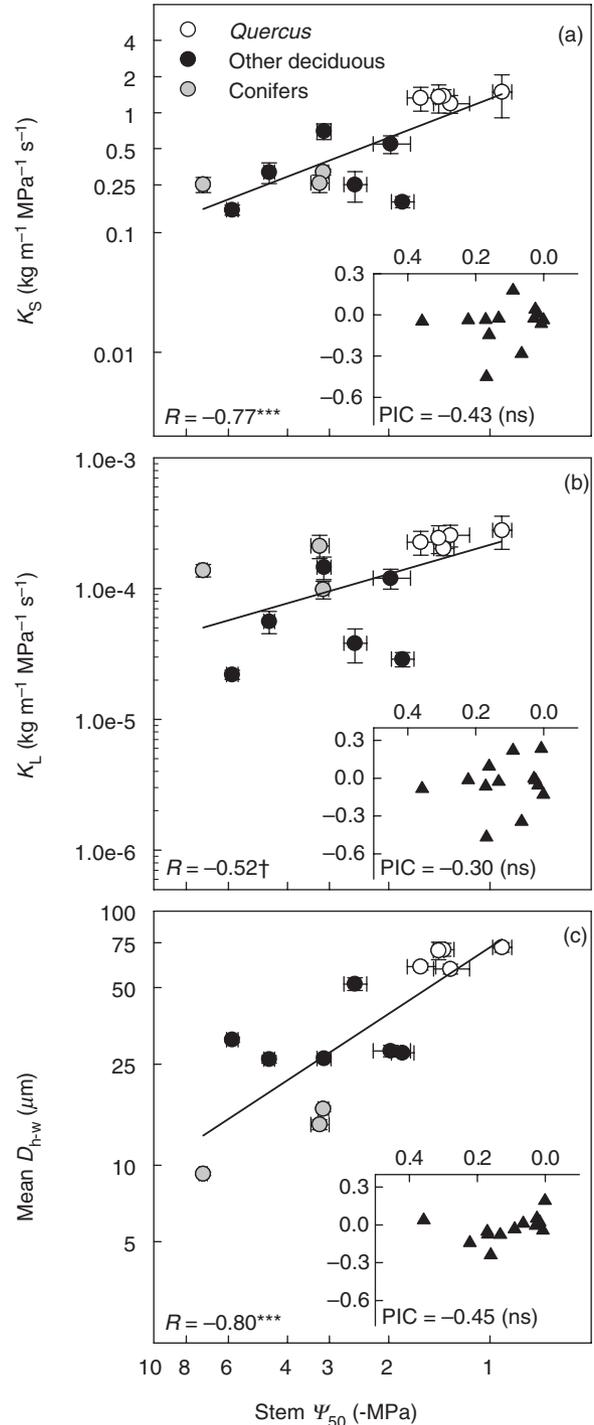


Figure 5. Specific conductivity (K_S ; a), leaf specific conductivity (K_L ; b), and hydraulically weighted conduit diameter (D_{h-w} ; c) expressed as functions of xylem tension causing 50% loss in hydraulic conductivity (Ψ_{50}) for stems of 14 co-occurring tree species from Duke Forest. Note that negative xylem tension data were converted to positive values for \log_{10} transformation. Therefore, the sign of the correlation coefficients are negative, even though they describe positive correlations. Plots of phylogenetically independent contrasts for each pair of traits and the corresponding phylogenetically independent contrast (PIC) correlation coefficients are shown in the insets. † $P = 0.10$, *** $P < 0.001$

Table 3. Magnitude and statistical significance of Pearson correlations on cross species and phylogenetically independent contrast (PIC) data sets comprising 14 co-occurring tree species in Duke Forest, Durham, NC

Trait or contrast	Cross species		PIC	
	Stem Ψ_{50}	Root Ψ_{50}	Stem Ψ_{50}	Root Ψ_{50}
Root Ψ_{50}	0.83****	–	0.62**	–
Root K_S	–	–0.43	–	–0.02
Stem K_S	–0.77****	–	–0.43	–
Stem K_L	–0.52*	–	–0.30	–
Stem D_{h-w}	–0.80****	–	–0.45	–
A_L/A_S	–0.36	–0.41	–0.09	–0.17
A	–0.65**	–0.90****	–0.51**	–0.92****
g_s	–0.47*	–0.80****	–0.11	–0.70***
$\delta^{13}C$	–0.31	–0.52*	0.002	–0.32
%N	–0.35	–0.33	0.06	0.10
Leaf size	–0.48	–0.72***	–0.17	–0.62**
SLA	–0.16	–0.44	0.04	–0.43

* $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$.

Negative xylem tension data were converted to positive values for \log_{10} transformation. Therefore, the sign of the correlation coefficients are negative, even though they describe positive correlations.

K_S , specific conductivity; K_L , leaf specific conductivity; D_{h-w} , hydraulically weighted mean conduit diameter; A_L/A_S , leaf: xylem area ratio; A , photosynthetic rate; g_s , stomatal conductance; SLA, specific leaf area.

surface tension of the meniscus in each of the pores of the pit membrane that connects adjacent conduits (Tyree & Sperry 1989). As a result, an increase in resistance to cavitation must be accompanied by a presumably costly decrease in pore K_H (Sperry & Hacke 2004). The absence of an evolutionary correlation between Ψ_{50} and xylem transport efficiency, despite a trade-off between air seeding pressure and pit conductivity, may be associated with differences in other xylem traits across our taxonomic sample (Fig. 1). For example, increased vessel length and diameter both increase K_H and may compensate for hydraulic limitations in the pit membrane (Comstock & Sperry 2000; Sperry & Hacke 2004).

Because the ability to detect trade-offs between vulnerability to cavitation and xylem water transport capacity may be confounded by large differences in xylem vessel length and diameter across taxa, comparative studies on closely related species with similar xylem anatomy may yield insights on the evolution of this physiological trade-off. However, our comparisons of Ψ_{50} and water transport capacity within a single genus, *Quercus*, were equivocal. Of the three indices of hydraulic efficiency, only K_L was correlated with vulnerability to cavitation in the cross-species analysis (Fig. 6). However, the statistical significance of this correlation was driven by the presence of a single species, *Q. laevis*. Similarly, the significant PIC correlations between K_H (both K_S and K_L) and Ψ_{50} were driven by a single contrast between *Q. laevis* and *Q. falcata*. The sensitivity of these relationships to a single data point suggests that there may not be an evolutionary trade-off between Ψ_{50} and xylem water transport capacity in *Quercus* stems.

Roots were more vulnerable to xylem cavitation than stems (Figs 2–4), a result that is consistent with previous studies (Kavanagh *et al.* 1999; Jackson *et al.* 2000; Martínez-Vilalta *et al.* 2002; McElrone *et al.* 2004). Interestingly, the

magnitude of the difference between stem Ψ_{50} and root Ψ_{50} appeared to be correlated with overall vulnerability to xylem cavitation. For example, species that were relatively vulnerable to cavitation, such as *Quercus*, had stem Ψ_{50} and root Ψ_{50} values that differed only modestly (an average of 25%), whereas resistant species such as *Oxydendrum arboreum* and *Cornus florida* had stem xylem that was more than twice as resistant to cavitation than root xylem. These differences may be species specific or reflect some level of acclimation by roots of all species to the relatively high soil moisture levels at the Duke Forest site (H. Maherali, personal observation). For example, it is possible that vulnerability to cavitation in roots is more closely calibrated to the less negative water potentials the roots experience relative to stems. Although there is some evidence that cavitation resistance in roots is phenotypically plastic (as reviewed in Sperry *et al.* 2002), little is known about the degree to which phenotypic plasticity influences vulnerability to cavitation in roots versus stems.

Root K_S was higher than stem K_S in all species (Table 2), a result that has been reported in other studies (e.g. Kavanagh *et al.* 1999; Martínez-Vilalta *et al.* 2002; McElrone *et al.* 2004). Higher K_S in roots also suggests that conduit diameter was greater in roots than in stems. Like differences in Ψ_{50} , systematically larger vessels in roots relative to stems may be associated with less negative water potential present in the root zone. If roots experience limited cavitation, then the maintenance of high K_S would decrease the overall hydraulic resistance of the root system, suggesting that root tissue may be specialized for water uptake and transport. It is also possible that roots are more specialized for water transport than stems because biomechanical stresses on xylem for canopy support and wind resistance would be less severe in roots than stems (McElrone *et al.* 2004).

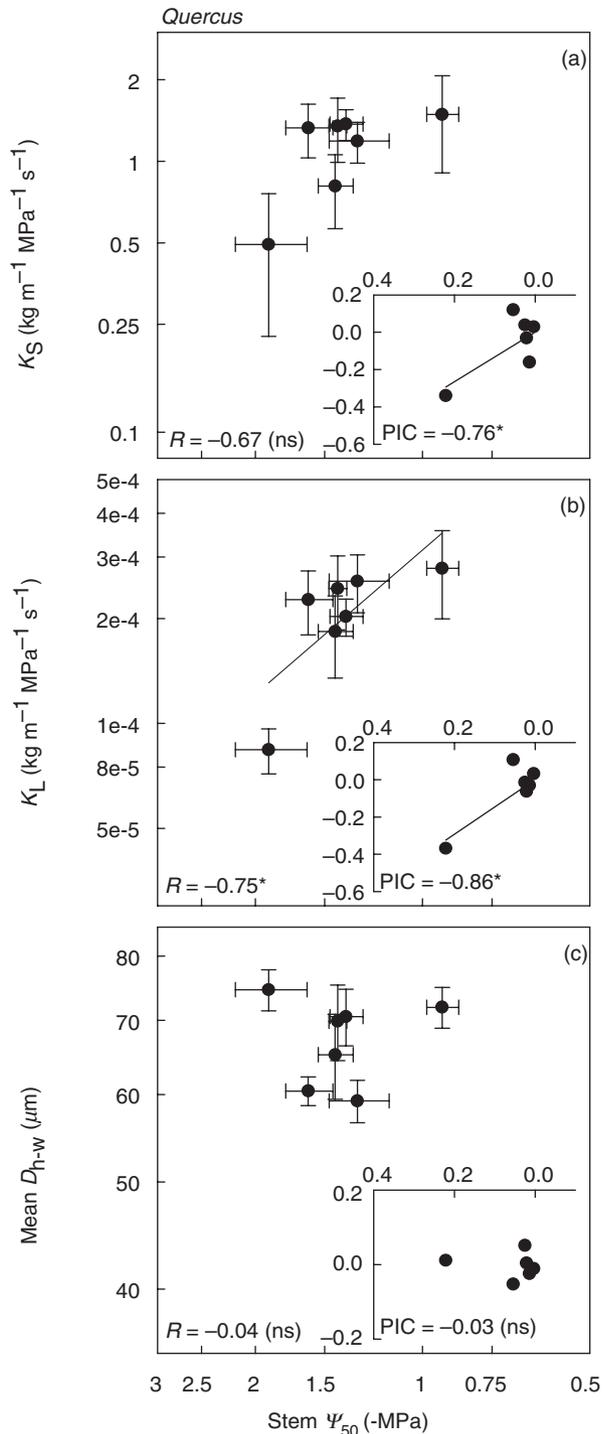


Figure 6. Specific conductivity (K_S ; a), leaf specific conductivity (K_L ; b), and hydraulically weighted conduit diameter (D_{h-w} ; c) expressed as functions of xylem tension at 50% cavitation (Ψ_{50}) for stems of seven *Quercus* species. Note that negative xylem tension data were converted to positive values for log₁₀ transformation. Therefore, the sign of the correlation coefficients are negative, even though they describe positive correlations. Plots of phylogenetically independent contrasts for each pair of traits and the corresponding phylogenetically independent contrast (PIC) correlation coefficients are shown in the insets. The statistically significant relationship (b) is driven by a single species (*Quercus laevis*). * $P \leq 0.05$

Vulnerability to xylem cavitation and leaf traits

Our results revealed a strong association between leaf gas exchange and the vulnerability of xylem to cavitation. For example, we observed that A and g_s both increased with increasing Ψ_{50} (Fig. 7a–d). Previous studies have identified roots as the most limiting component of the hydraulic pathway (as reviewed in Meinzer 2002; Sperry *et al.* 2002). Our results are consistent with this hypothesis; relationships between gas exchange and Ψ_{50} were stronger for roots than for stems. Moreover, the correlation between root Ψ_{50} and A or g_s was quite robust even with the inclusion of phylogenetic information, suggesting that the functional relationship among these traits is adaptive. In consequence, variation in the vulnerability of roots to cavitation across species may drive interspecific variation in leaf CO_2 and H_2O fluxes.

The observation that root Ψ_{50} increased with increasing maximum g_s is consistent with a more general trade-off between vulnerability to cavitation and water transport capacity (Fig. 7d). Because the magnitude of g_s depends on the hydraulic conductivity of the entire soil–leaf pathway (Sperry & Pockman 1993; Nardini & Salleo 2000; Meinzer 2002), our results suggest that the conducting efficiency versus xylem safety trade-off can be manifested at the level of the whole plant rather than just within specific organs. A relationship between root Ψ_{50} and maximum g_s is also consistent with the expectation of functional coordination between vulnerability to xylem cavitation and the regulation of stomatal conductance across species. For example, species with less negative root Ψ_{50} might behave as cavitation avoiders through tight regulation of stomatal opening, whereas those species with more negative root Ψ_{50} could behave as cavitation resisters with relatively weak stomatal regulation (Sparks & Black 1999; Martínez-Vilalta, Sala & Pinol 2004). If stomata act to maintain a constant leaf water potential through hydraulic regulation, stomatal sensitivity to VPD must be positively correlated with the magnitude of maximum g_s (Oren *et al.* 1999). Our results are consistent with this model because species in Duke Forest with high g_s , and therefore high stomatal sensitivity to water deficits, also have xylem that is more vulnerable to cavitation.

The correlation between photosynthetic rate and the vulnerability of roots to xylem cavitation may be an indirect effect of coordination between g_s and Ψ_{50} , rather than a direct influence of root hydraulic traits on photosynthetic capacity. Several lines of evidence support this conclusion. Firstly, photosynthesis and stomatal conductance are generally correlated both within populations and across species, suggesting that photosynthetic capacity is determined by stomatal limits to CO_2 diffusion (Wong, Cowan & Farquhar 1979). Secondly, we observed no relationship between vulnerability to cavitation and other traits associated with carbon uptake, such as N content and SLA (Table 3). Finally, although there was a weak association between root Ψ_{50} and integrated WUE (as measured by $\delta^{13}\text{C}$), variation in carbon isotope discrimination was correlated with g_s and not A (data not shown).

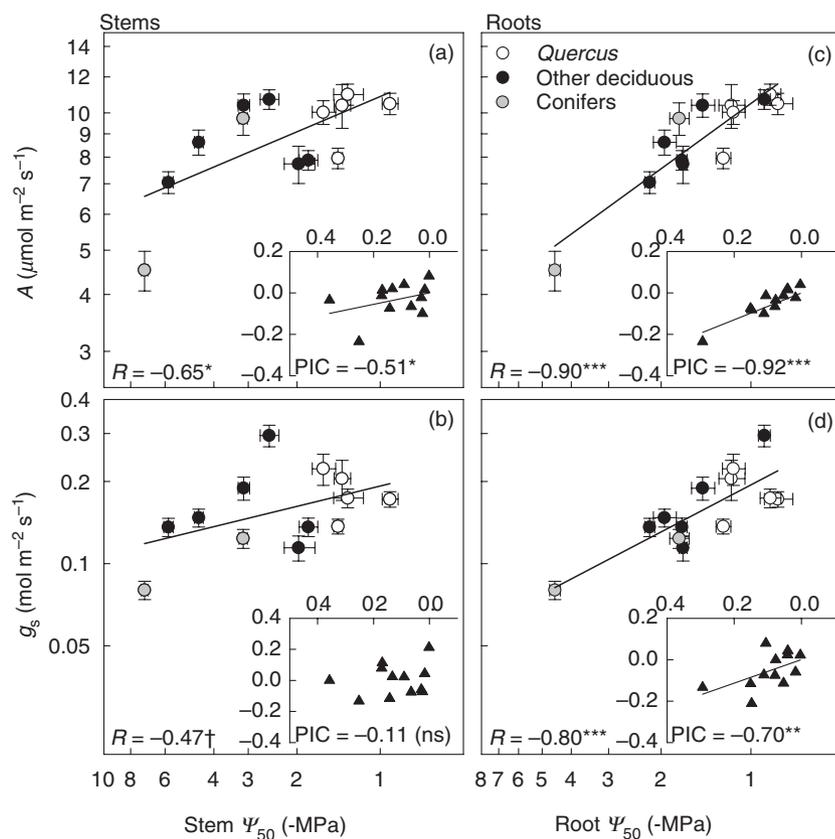


Figure 7. Photosynthetic rate (A), and stomatal conductance (g_s), expressed as functions of stem (a, b) and root (c, d) xylem tension at 50% cavitation for 13 co-occurring tree species in Duke Forest. Gas exchange was not measured on *P. echinata*. Note that negative xylem tension data were converted to positive values for log transformation. Therefore, the sign of the correlation coefficients are negative, even though they describe positive correlations. Plots of phylogenetically independent contrasts for each pair of traits and the corresponding phylogenetically independent contrast (PIC) correlation coefficients are shown in the insets. † $P = 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Unlike previous studies (as reviewed in Meinzer 2002), we did not observe a significant correlation between K_L and leaf gas exchange. It is possible that the relationship between K_L and leaf gas exchange was confounded by variation in leaf water potential across species (Table 2). For example, leaf water potential in some oaks (*Q. nigra*, *Q. phellos* and *Q. falcata*) was negative enough to exceed the point at which 50% of stem K_H would be lost (Fig. 2). As a result, stomatal closure likely occurred in these species, reducing gas exchange from its maximum. However, *Quercus* species generally had higher A and g_s than other species (Fig. 7a–d), suggesting that variation in leaf water potential may not have affected the relative ranking of gas exchange values among species. An alternate explanation for the absence of a correlation between K_L and leaf gas exchange is that stem xylem may have accounted for much less resistance of the hydraulic pathway than that provided by xylem in the petioles and leaf veins. This result has been observed in other studies of deciduous trees (e.g. Brodrribb, Holbrook & Gutiérrez 2002; Sack *et al.* 2003).

CONCLUSIONS

Our findings add to the recent body of evidence documenting an important role for roots in controlling plant water transport (Jackson *et al.* 2000; Sperry *et al.* 2002). In particular, we identified a strong link between the vulnerability of root xylem to cavitation and leaf gas exchange across species. As a result, physiological integration between these

different organs may represent a trade-off between vulnerability to xylem cavitation and water transport at the whole-plant level and may ultimately reflect correlated evolution between root and leaf traits. Moreover, the relationship between root vulnerability to cavitation and leaf CO_2 and H_2O fluxes represents a functional link between two major physiological strategies associated with plant performance (Westoby *et al.* 2002; Ackerly 2004). Further studies of links between hydraulic function and other major axes of plant performance (e.g. leaf life span, seed size, plant architecture) will increase our understanding of how organisms adapt to their environment and will provide a framework for predicting ecological responses to environmental change.

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REFERENCES

- Ackerly D.D. (2000) Taxon sampling, correlated evolution, and independent contrasts. *Evolution* **54**, 1480–1492.
- Ackerly D.D. (2004) Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* **74**, 25–44.
- Ackerly D.D. & Reich P.B. (1999) Convergence and correlations among leaf size and function in seed plants: a comparative test using independent contrasts. *American Journal of Botany* **86**, 1272–1281.
- Alder N.N., Pockman W.T., Sperry J.S. & Nuismer S. (1997) Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* **48**, 665–674.
- Boyce C.K., Zwieniecki M.A., Cody G.D., Jacobsen C., Wirick S., Knoll A.H. & Holbrook N.M. (2004) Evolution of xylem lignification and hydrogel transport regulation. *Proceedings of the National Academy of Sciences of the USA* **101**, 17555–17558.
- Brodribb T.J. & Feild T.S. (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell and Environment* **23**, 1381–1388.
- Brodribb T.J., Holbrook N.M. & Gutiérrez M.V. (2002) Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell and Environment* **25**, 1435–1444.
- Brodribb T.J., Holbrook N.M., Edwards E.J. & Gutiérrez M.V. (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell and Environment* **26**, 443–450.
- Cochard H., Cruziat P. & Tyree M.T. (1992) Use of positive pressures to establish vulnerability curves. *Plant Physiology* **100**, 205–209.
- Comstock J.P. & Sperry J.S. (2000) Some theoretical considerations of optimal conduit length for water transport in plants. *New Phytologist* **148**, 195–218.
- Davis S.D., Ewers F.W., Sperry J.S., Portwood K.A., Crocker M.C. & Adams G.C. (2002) Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *American Journal of Botany* **89**, 820–828.
- Farquhar G.D., Ehleringer J.R. & Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503–537.
- Felsenstein J. (1985) Phylogenies and the comparative method. *American Naturalist* **125**, 1–15.
- Garland T. & Ives A.R. (2000) Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist* **155**, 346–364.
- Garland T., Harvey P.H. & Ives A.R. (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* **41**, 18–32.
- Garland T., Midford P.E. & Ives A.R. (1999) An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral states. *American Zoologist* **39**, 374–388.
- Hacke U.G., Sperry J.S. & Pittermann J. (2000) Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. *Basic and Applied Ecology* **1**, 31–41.
- Hacke U.G., Sperry J.S., Pockman W.T., Davis S.D. & McCulloch K.A. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457–461.
- Harvey P.H. & Pagel M. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford, UK.
- Jackson R.B., Sperry J.S. & Dawson T.E. (2000) Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* **5**, 482–488.
- Jarbeau J.A., Ewers F.W. & Davis S.D. (1995) The mechanism of water stress induced embolism in two species of chaparral shrubs. *Plant, Cell and Environment* **18**, 189–196.
- Kavanagh K.L., Bond B.J., Aitken S.N., Gartner B.L. & Knowe S. (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology* **19**, 31–38.
- Lewis A.M. & Boose E.R. (1995) Estimating volume flow rates through xylem conduits. *American Journal of Botany* **82**, 1112–1116.
- McElrone A.J., Pockman W.T., Martínez-Vilalta J. & Jackson R.B. (2004) Variation in xylem structure and function in stems and roots of trees to 20 m depth. *New Phytologist* **163**, 507–517.
- Maddison W.P. & Maddison D.R. (2004) *Mesquite: A Modular System for Evolutionary Analysis*, Version 1.02 <http://mesquiteproject.org>
- Maherali H., Pockman W.T. & Jackson R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85**, 2184–2199.
- 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.
- Martínez-Vilalta J., Sala A. & Pinol J. (2004) The hydraulic architecture of Pinaceae – a review. *Plant Ecology* **171**, 3–13.
- Meinzer F.C. (2002) Coordination of vapour and liquid phase water transport properties in plants. *Plant, Cell and Environment* **25**, 265–274.
- Nardini A. & Salleo S. (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees* **15**, 14–24.
- Oren R., Sperry J.S., Katul G.G., Pataki D.E., Ewers B.E., Phillips N. & Schafer K.V.R. (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment* **22**, 1515–1526.
- Pagel M.D. (1993) Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. *Journal of Theoretical Biology* **164**, 191–205.
- Pammenter N.W. & Van Der Willigen C. (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* **18**, 589–593.
- Pockman W.P., Sperry J.S. & O'Leary J.W. (1995) Sustained and significant negative water pressure in xylem. *Nature* **378**, 715–716.
- Pockman W.T. & Sperry J.S. (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* **87**, 1287–1299.
- Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment* **26**, 1343–1356.
- Schwilk D.W. & Ackerly D.D. (2001) Flammability and serotiny as strategies: correlated evolution in pines. *Oikos* **94**, 326–336.
- Sparks J.P. & Black R.A. (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiology* **19**, 453–459.
- Sperry J.S. & Pockman W.T. (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16**, 279–287.

- Sperry J.S. & Saliendra N.Z. (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **17**, 1233–1241.
- Sperry J.S. & Hacke U.G. (2004) Analysis of circular bordered pit function. I. Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* **91**, 369–385.
- Sperry J.S., Donnelly J.R. & Tyree M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* **11**, 35–40.
- Sperry J.S., Hacke U.G., Oren R. & Comstock J.P. (2002) Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment* **25**, 251–263.
- Sperry J.S., Hacke U.G. & Wheeler J.K. (2005) Comparative analysis of end wall resistance in xylem conduits. *Plant, Cell and Environment* **28**, 456–465.
- Tyree M.T. & Sperry J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 19–38.
- Tyree M.T. & Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345–360.
- Westoby M., Falster D.S., Moles A.T., Vesk P.A. & Wright I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**, 125–159.
- Wong S.C., Cowan I.R. & Farquhar G.D. (1979) Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**, 424–426.
- Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer, New York, USA.
- Zwieniecki M.A., Melcher P.J. & Holbrook N.M. (2001) Hydrogel control of hydraulic resistance in plants. *Science* **291**, 1059–1062.

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