Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral

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Summary

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• Here, hypotheses about stem and root xylem structure and function were assessed by analyzing xylem in nine chaparral Rhamnaceae species.
• Traits characterizing xylem transport efficiency and safety, mechanical strength and storage were analyzed using linear regression, principal components analysis and phylogenetic independent contrasts (PICs).
• Stems showed a strong, positive correlation between xylem mechanical strength (xylem density and modulus of rupture) and xylem transport safety (resistance to cavitation and estimated vessel implosion resistance), and this was supported by PICs. Like stems, greater root cavitation resistance was correlated with greater vessel implosion resistance; however, unlike stems, root cavitation resistance was not correlated with xylem density and modulus of rupture. Also different from stems, roots displayed a trade-off between xylem transport safety from cavitation and xylem transport efficiency. Both stems and roots showed a trade-off between xylem transport safety and xylem storage of water and nutrients, respectively.
• Stems and roots differ in xylem structural and functional relationships, associated with differences in their local environment (air vs soil) and their primary functions.

Key words: anatomy, capacitance, cavitation, *Ceanothus*, evolution, *Rhamnus*, trade-offs, xylem.

Introduction

The xylem of angiosperms has three basic functions, namely long-distance water transport, mechanical support of the plant body, and storage of water and nutrients. Understanding how these xylem functions are integrated to maximize fitness is critical for testing ecological and evolutionary hypotheses concerning the diversity of structural, physiological and life-history types found among woody plant species.

Analyses of stem xylem transport show that a greater ability to conduct water safely (i.e. greater resistance to cavitation) is correlated with less efficient water transport (Hacke *et al.*, 2006). For freezing-induced cavitation, this relationship occurs because vessels with larger volumes (and diameters) are simultaneously more efficient at transport and more prone to freeze–thaw-induced cavitation (Davis *et al.*, 1999a; Pittermann & Sperry, 2003; Pratt *et al.*, 2005). For water stress-induced cavitation, the trade-off between safety and efficiency is explained by the pit area hypothesis. According to this model, taxa with more efficient transport are more susceptible to cavitation because they have a greater pitted area per vessel. More pit area leads to a greater probability of having a large pit membrane pore, and larger pores are more prone to cavitation by air seeding (Hargrave *et al.*, 1993; Choat *et al.*, 2003; Wheeler *et al.*, 2005; Hacke *et al.*, 2006). Alternatively, more pit area may lead to a greater probability that a larger pit pore is adjacent to a gas-filled space.

The mechanical and transport functions of xylem may be interdependent, even though the cells in angiosperms that provide most of the mechanical support (fibers) are not the same cells that provide most of the water transport (vessels). At the cellular level, vessel pairs are exposed to large hoop stresses that could lead to cell collapse if the adjoining vessel wall thickness (t) to lumen (b) ratio ((t/b)^2) is not high enough (Hacke *et al.*, 2001; Cochard *et al.*, 2004; Brodribb & Holbrook, 2005). Thus, in addition to avoiding cavitation by air-seeding, safe xylem transport is also characterized by resistance to vessel implosion. At the tissue level, the fiber matrix surrounding the vessels also appears to be important in resisting vessel wall collapse under negative pressures (Hacke *et al.*, 2001; Jacobsen *et al.*, 2005, in press). For stems, these patterns lead to a positive relationship between xylem safety from cavitation and mechanical strength (xylem density, modulus of rupture (MOR) and modulus of elasticity (MOE)).

Storage of both water and nutrients in stem xylem are important factors affecting plant fitness and life history. For example, stored water helps to buffer against severe seasonal water deficits (Waring & Running, 1978; Stratton *et al.*, 2000; Pratt & Black, 2006). Xylem is also an important storage tissue for nutrients, such as nitrogen and starch (Sauter & van Cleve, 1994; Kozlowski & Pallardy, 1996).

Although storage is important, much research on xylem function has focused on xylem transport and mechanics, and thus we currently lack information on how xylem storage impacts the water transport and mechanical support functions of xylem. Moreover, relatively little is known about how these relationships vary between root and stem xylem. It is possible that roots have different transport and mechanical constraints compared with stems because their mechanical environment is a soil matrix and roots generally experience less negative hydrostatic pressures than stems. Furthermore, roots uniquely function in below-ground anchorage, radial transport and storage.

The purpose of the study presented here was to examine relationships between xylem transport and the mechanical and storage traits of stems and roots of nine Rhamnaceae species. Xylem transport was estimated in the two categories of safety and efficiency. Xylem safety was quantified as resistance to water stress-induced xylem cavitation (Ψ_{50}; i.e. the water potential at 50% loss of hydraulic conductivity), estimated vessel pair implosion resistance ((t/b)$_h^2$) and vessel redundancy. Xylem efficiency was quantified as xylem-specific hydraulic conductivity (K_s) and hydraulic mean vessel diameter (d_h). Storage of water was quantified, for stems only, as capacitance, and potential nutrient storage was estimated as the percentage parenchyma in cross-section. Stem and root biomechanics were quantified as xylem density, MOR, MOE and cross-sectional fiber wall area. Relationships between traits were assessed using linear regressions and principal component (PC) analysis.

The taxa sampled in the present study are all in the family Rhamnaceae and form three clades (Fig. 1). Of the six *Ceanothus*
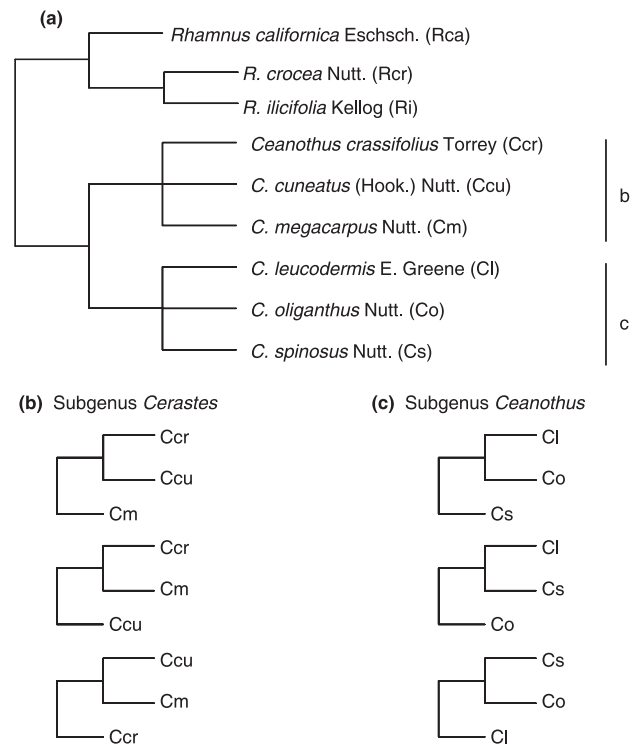

Fig. 1 Phylogeny used to determine phylogenetic independent contrasts among nine species, followed by abbreviations in parentheses (a). (b and c) The three alternative resolutions for each of the two polytomies existing in the phylogeny (Jacobsen *et al.*, in press). All of these alternative trees and combinations were considered in analyses, resulting in nine possible trees. The phylogeny was constructed using published results referenced in the text, and all branch lengths are assumed to be equal. Although we place *Rhamnus californica* in the genus *Rhamnus* here, recent molecular analyses have placed it in the genus *Frangula* (Bolmgren & Oxelman, 2004).

species sampled, three fall in each of two well-supported subgenera that diverged between 18 and 39 million years ago and subsequently spread throughout the California Floristic Province and beyond (Mason, 1942; Hardig *et al.*, 2000). *Ceanothus* spp., in the subgenus *Cerastes*, generally lack the ability to resprout following fire, have small thick leaves with stomatal crypts, have relatively shallow roots (Hellmers *et al.*, 1955) and occupy relatively arid microsites (McMinn, 1942; Davis *et al.*, 1999b; Ackerly, 2003). By contrast, *Ceanothus* spp., of the subgenus *Ceanothus*, often sprout following fire, have larger and thinner leaves lacking stomatal crypts and occupy more mesic microsites (Meentemeyer & Moody, 2002). The divergence of the two subgenera resulted in basal divergence in a suite of adaptive traits related to stress tolerance and life-history type, many of which have been conserved across extant taxa. Sampled species within the *Rhamnus* clade are all resprouters following fire, but other than that they tend to be more diverse in their range of leaf traits and apparent microsite aridity preference than species within either the *Cerastes* or *Ceanothus* clades. Because of the known

phylogenetic structure of numerous traits related to life history and stress tolerance among the sampled taxa, we calculated phylogenetic independent contrasts (PICs) for sampled traits. This approach assesses the influence of phylogeny on trait correlations and accounts for the statistical nonindependence of species (Felsenstein, 1985). However, because a fully resolved phylogeny is unavailable for the species sampled here, as well as other uncertainties when applying PICs in the present study (see the Materials and Methods), we report correlations with and without phylogenetic correction. Reporting both also has the benefit of allowing examination of ecological as well as evolutionary relationships in xylem traits.

Materials and Methods

Naturally occurring populations of nine Rhamnaceae shrub species were sampled in the Santa Monica Mountains in Los Angeles County, California (see Pratt *et al.*, 2007 for a description of study sites). These nine species fall into three well-supported monophyletic clades (Fig. 1) (McMinn, 1942; Hardig *et al.*, 2000). Nomenclature for species follows Hickman (1993).

Seasonal water status, hydraulic conductivity and vulnerability of the xylem to cavitation were all measured as described in Pratt *et al.* (2007). Xylem transverse areas of stems and roots were measured to calculate K_s, which is one measure of xylem hydraulic efficiency. Stems were thin-sectioned, mounted in glycerol, digitally photographed and the xylem area was measured with SCIONIMAGE (SCIONIMAGE version Beta 3b; Scion Corporation, Frederick, MD, USA). The pith was measured and subtracted from the xylem area.

Xylem anatomical traits were measured on the same stems and roots in which vulnerability to cavitation was measured. For each organ, images were taken of several wedge-shaped cross-sectional sectors, to sample for vessel and fiber features, using a digital camera (Spot RT color camera; Diagnostic Instruments, Sterling Heights, MI, USA) attached to a light microscope (Nikon Microphot-FX; Nikon, Garden City, NY, USA). ScionImage was used for all image analyses and was performed at California State University, Bakersfield (ScionImage version 4.03.2; Scion Corporation). Vessel lumen diameter (*d*) and wall thickness, fiber lumen diameter and wall thickness, and percentage transverse fiber, vessel and parenchyma area, were measured. All of the vessels and fibers in sectors were measured until 100 vessels and 100 fibers had been measured in a given organ. The d_h value was calculated from the formula $d_h = (\sum d^5)/(\sum d^4)$, based upon all the sampled vessels in an organ. This parameter weights the vessel diameters by their hydraulic contribution, which is a function of the diameter to the fourth power. The $(t/b)_h^2$ (Hacke *et al.*, 2001) was determined for those vessels, within the 100 vessels sampled per organ that formed pairs in which one or both vessels had diameters ± 5 μm of the calculated d_h, with *t* as the combined wall thickness of adjoining vessels and *b* as

the lumen diameter of the selected vessel. The percentage transverse area of cell types was estimated through measurements of vessel, fiber and parenchyma area in four randomly chosen cross-sectional sectors per organ at ×200 magnification. Fiber wall area in a cross-section was calculated using the average (*n* = 100) fiber wall to lumen ratio for each stem or root and the cross-sectional area of fibers for that same organ.

Xylem density was measured on stems used for vulnerability to cavitation measurements, following the methods of Hacke *et al.* (2000). Segments 3 cm long were soaked in water under vacuum until gas bubbles no longer appeared (*c.* 24 h). Bark and pith were removed from segments and the density was measured by water displacement on an analytical balance (AE163; Mettler-Toledo, Columbus, OH, USA). Samples were dried in an oven at 70°C, for at least 48 h, and the dry weight of the xylem was measured for calculation of densities: dry weight (kg)/fresh volume (m³).

The mechanical properties of stems and roots were sampled on organs similar in size to those used for vulnerability curves (Pratt *et al.*, 2007). Stems were sampled in October 2005 and roots were sampled in July 2002. Samples were shipped overnight on ice to Michigan State University where they were measured using an Instron Universal Machine (Model 4202; Instron Corporation, Canton, MA, USA). Stem and root segments were kept at approx. 10°C until MOE and MOR were measured. A four-point bending test, with a compression load cell of 500 N, was conducted as described by Woodrum *et al.* (2003) and Jacobsen *et al.* (2005) on stem segments cut to 0.275 m long.

Xylem capacitance was measured to estimate the water storage capacity of the xylem. This was performed by generating pressure–volume curves for xylem disks, with pith and bark removed, that measured *c.* 8 mm in diameter and 4.5 mm long. These dimensions were used because they were the largest that would fit in our C-52 psychrometer chambers (Wescor Inc., Logan, UT, USA). Plant stems for this procedure were removed in the early morning from plants growing at our field sites during the rainy winter months (28 October 2004 to 7 March 2005). The ends of cut stems (*c.* 0.5 m long) were immediately submerged in water to hydrate them and the leaves were sealed in a large plastic bag to minimize water loss. In the laboratory, stems were hydrated for *c.* 2 h. Branchlet water potential was measured with a pressure chamber before removing xylem disks, and ranged from −0.16 to −1.01 MPa. These values were similar to the predawn branchlet water potential measured in the field during the winter rainy season (Pratt *et al.*, 2007).

To construct pressure–volume curves, the xylem was allowed to dehydrate over time and the xylem water potential (Ψ_x) and mass were measured during drying. To prepare the xylem disks, xylem disks were cut in half under water, the pith was removed with forceps under a dissecting microscope and the bark was removed. Following this, the disks were blotted, weighed on an analytical balance (AE163; Mettler-Toledo),

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and then sampled for Ψ_x in psychrometers connected to a datalogger (Model CR-7; Campbell Scientific, Logan, UT, USA). This first mass was taken as the saturated mass (i.e. the mass near saturated water content). Psychrometers were calibrated with salt solutions of known water potential. Data were generated by taking measurements of tissue mass and Ψ_x over time (*c.* 7 d) as the disks dried down. Xylem mass was weighed before and after insertion into psychrometric chambers. Xylem dried rapidly when left in open air, but maintained a relatively constant mass while in chambers. Relative water content (RWC) was calculated as ((fresh mass − dry mass)/(saturated mass − dry mass) × 100). Capacitance was calculated as the change in percentage RWC per unit change in Ψ_x. The range in Ψ_x, used in the calculation for each species, was the difference in branchlet Ψ_x measured between the dry and wet seasons for plants growing at each field site (Pratt *et al.*, 2007).

Relationships between physiological and anatomical traits were analyzed as linear regressions (Minitab 14.12; State College, State College, PA, USA). The *a priori* goal was to determine associations between traits that characterize xylem transport, mechanics and storage. We quantified 12 traits associated with these three xylem functions. Because of the relatively small sample size, α was set at 0.10 to reduce the chance of committing type II errors. A sequential Bonferroni correction was used to hold the experiment-wise type I error rate at 0.10 (Sokal & Rohlf, 1995). Differences between stem and root traits were analyzed using analysis of variance (ANOVA), with species and organ (stem and root) as independent variables (α = 0.05). Data were transformed to satisfy assumptions of parametric models, and percentage data were arcsine transformed.

Because closely related species tend to have traits that are more similar than is the case for distantly related species, we also analyzed the data taking into account the evolutionary relationships of the sampled species (Preston & Ackerly, 2003; Pratt *et al.*, 2007). To do this we used PICs that allowed us to test for correlated evolutionary change between two traits (Felsenstein, 1985). Contrasts were calculated using COMPARE software (Martins, 2004).

PIC analyses should be interpreted in light of several limitations in relation to the analyses of the present study (see also Ackerly, 2004). First, the phylogeny of the sampled taxa is not fully resolved, resulting in nine possible phylogenies (Fig. 1). Contrasts were calculated for all nine possible phylogenies (Fig. 1). Correcting for multiple comparisons is customary in such a case, but this would have severely limited our power and inflated our type II error, especially with the relatively low sample size in the present study. Thus, for each regression between two traits, the average *P*-value for all nine phylogenies was calculated. We felt justified in taking the average because when the average correlation between two traits was significant, it was generally significant across all nine phylogenies. The few occurrences when this was not the case are highlighted in the Discussion. Second, information was

not available on the phylogenetic branch lengths, which is proportional to the degree of trait change between an ancestor and a descendent under a Brownian motion model of evolution (Felsenstein, 1985). Contrast calculations were based on equal branch lengths, a reasonable assumption in the absence of branch length information, and one that minimizes type I error rates (Ackerly, 2000). Finally, the objective of the present study was to sample nine species intensively, and thus the sample size in the present study is relatively small. On account of this, we set α at 0.10 to reduce the chance of committing type II errors. Because of the small sample size, poor phylogeny resolution and lack of branch length information, the PIC analyses should be viewed with caution. However, it should be noted that the raw data analyses should also be viewed with caution because testing the hypothesis that two traits are functionally related using phylogenetically uncorrected data leads to an inflation of type I error (Martins & Garland, 1991). Because of the different limitations of raw data and PICs we present both.

Principal components analysis (PCA) was used to examine relationships among traits simultaneously. Trait values were standardized by calculating *z*-scores before analysis (McGarigal *et al.*, 2000).

Results

The degree of water stress experienced by plants growing in the field during the dry season was correlated to cavitation resistance of stems and roots (Fig. 2). The slope and standard error of the regression between Ψ_{50} and minimum predawn dry-season water potential (Ψ_{min}) was 1.29 ± 0.25 for stems and 1.14 ± 0.37 for roots, with neither of the slopes being different from 1 (Fig. 2). The intercept of the stems and roots were significantly different, indicating that stems were more resistant to cavitation than roots ($P < 0.01$; Fig. 2; Table 1). PICs for these values were significantly correlated for stems, but not for roots (Fig. 2).

Greater resistance to water stress-induced cavitation (Ψ_{50}) was correlated with lower values of K_s for roots (Fig. 3), and this was true for raw trait values (Fig. 3) as well as for PIC values (Fig. 3). By contrast, stem Ψ_{50} was not correlated with K_s for raw data (Fig. 3) or PICs (Fig. 3). Root K_s was greater than stem K_s (Table 1).

Resistance to Ψ_{50} was correlated with several mechanical traits in stems but not in roots (Fig. 4). In stems, increasing xylem density, MOR and $(t/b)_h^2$ were correlated with increased cavitation resistance (Fig. 4a–c). Like stems, more negative Ψ_{50} values in roots were correlated with increasing $(t/b)_h^2$ (Fig. 4c); however, root xylem density (Fig. 4a) and MOR were not correlated with Ψ_{50} (Fig. 4b). Root $(t/b)_h^2$ was generally greater than or equal to stem $(t/b)_h^2$ (Fig. 4c) and, when analyzed across all species, $(t/b)_h^2$ was significantly greater for roots compared with stems (Table 1).

Correlations among stem PICs for Ψ_{50} with xylem density and MOR were significant (Fig. 4a,b), whereas Ψ_{50} and $(t/b)_h^2$

Fig. 2 The water potential at 50% loss of hydraulic conductivity (Ψ_{50}) for stems (black text, closed symbols and solid line) and roots (gray text, open symbols and dotted line), plotted as a function of dry season predawn water potential (Ψ_{min}). Phylogenetic independent contrasts for one of nine phylogenies (see the Materials and Methods) for Ψ_{50} are plotted as a function of predawn Ψ_{min} contrasts (inset). Abbreviations for species are given in Fig. 1. Noncontrast data points are means ± 1 standard error ($n = 6$), and lines are linear regressions. Regression lines are only shown that are statistically significant ($P = 0.001$ for stems and 0.02 for roots, and average $P = 0.03$ for stems in inset). The dashed line indicates a 1 : 1 line.
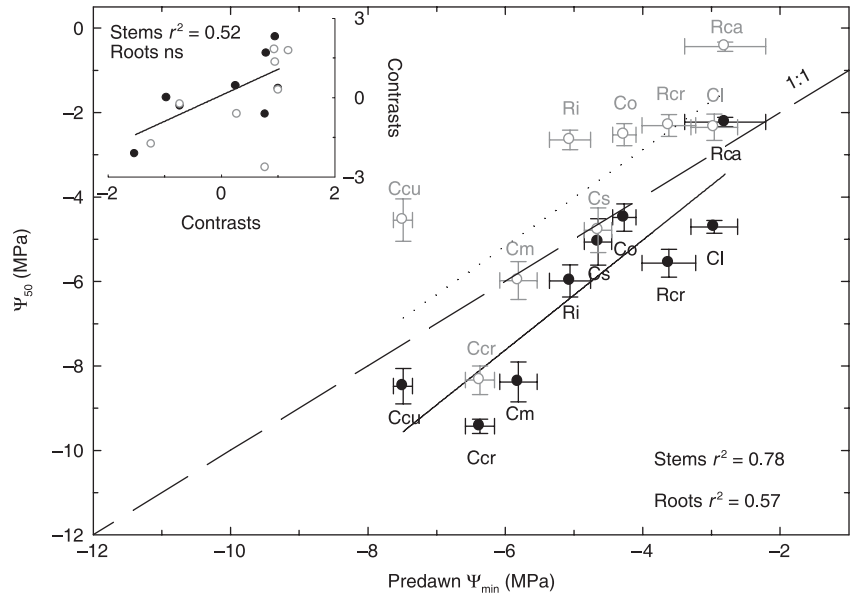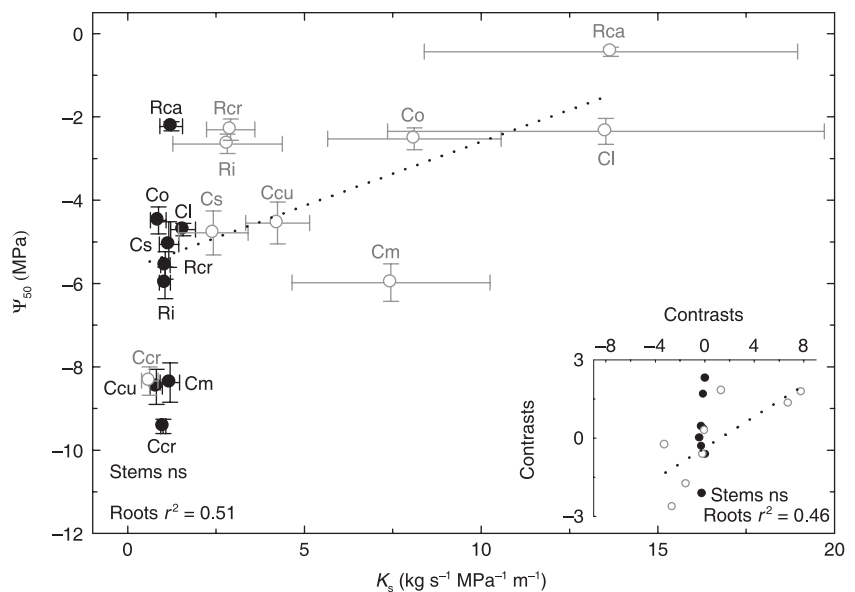
Table 1 Stem and root xylem traits compared via analysis of variance (ANOVA)

	K_s (kg s^{-1} MPa^{-1} m^{-1})	d_h (µm)	$(t/b)_h^2$	Ψ_{50} (MPa)	Density (kg m^{-3})	MOR (N mm^{-2})	MOE (N mm^{-2})	FWA (%)	Parenchyma (%)
Stems	1.10 (0.07)	32.9 (1.1)	0.029 (0.001)	−6.03 (0.77)	616 (23)	207 (9)	7018 (531)	58.5 (1.5)	13.0 (1.0)
Roots	6.19 (1.60)	61.5 (4.4)	0.033 (0.003)	−3.77 (0.79)	582 (16)	202 (20)	8334 (897)	49.3 (1.2)	17.5 (1.2)
P	< 0.001	< 0.001	0.01	< 0.001	0.002	0.95	0.19	< 0.001	< 0.001

Data are means ($n = 9$) and 1 standard error in parentheses. Capacitance was not included because it was only measured on stems. See Fig. 1 for trait abbreviations and see the Materials and Methods for their corresponding xylem functions. Density, refers to xylem density; d_h, hydraulic mean vessel diameter; FWA, fiber wall area; K_s, xylem-specific hydraulic conductivity; MOE, modulus of elasticity; MOR, modulus of rupture; $(t/b)_h^2$, vessel pair implosion resistance; Ψ_{50}, water potential at 50% loss of hydraulic conductivity.

Fig. 3 The water potential at 50% loss of hydraulic conductivity (Ψ_{50}) for stems (black text, closed symbols and solid line) and roots (gray text, open symbols and dotted line), plotted as a function of xylem-specific hydraulic conductivity (K_s). Phylogenetic independent contrasts for one of nine phylogenies (see the Materials and Methods) for Ψ_{50} are plotted as a function of K_s contrasts (inset). Abbreviations for species are given in Fig. 1. Data points are means ± 1 standard error ($n = 6$), and lines are linear regressions. Regression lines are only shown when they are statistically significant ($P = 0.03$ for root data (P-value and r^2 are for semilog transformed root data), and average $P = 0.07$ in inset); ns, nonsignificant regression.
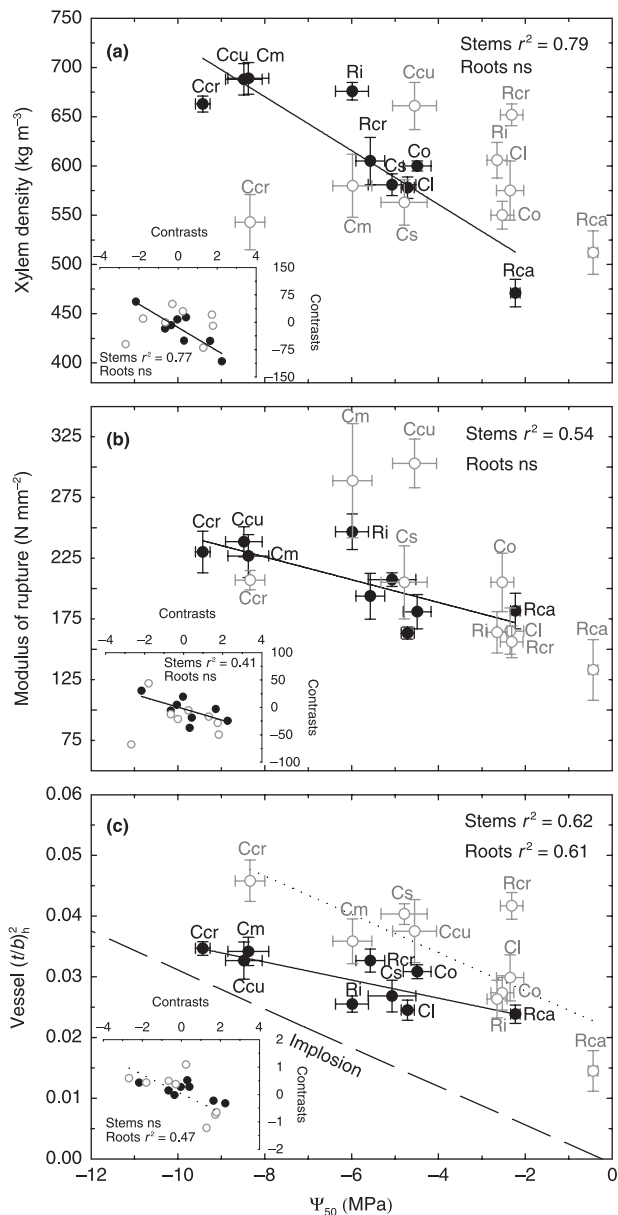
Fig. 4 Stem (black text, closed symbols and solid line) and root (gray text, open symbols and dotted line) xylem density (a), modulus of rupture (MOR) (b) and vessel thickness to span ratio $(t/b)_h^2$ (c), plotted as a function of the water potential at 50% loss in hydraulic conductivity (Ψ_{50}). The dashed line in (c) represents the line of theoretical implosion for vessels, below which vessels are in danger of collapsing (Hacke *et al.*, 2001). MOR and modulus of elasticity (MOE) were correlated for stems and roots ($r^2 = 0.55$, $P = 0.02$), but only MOR is shown, for brevity. Phylogenetic independent contrasts for xylem density (inset in a), MOR (inset in b) and $(t/b)_h^2 \times 100$ (inset in c), are plotted as a function of Ψ_{50} contrasts. Abbreviations for species are in Fig. 1. Noncontrast data points are means ± 1 standard error ($n = 6$), and lines are linear regressions. Regression lines are only shown that are statistically significant and others are noted as ns, for not significant.
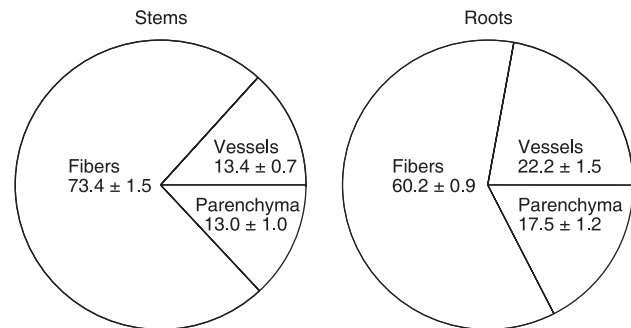

Fig. 5 Stem and root percentage of cell types in xylem cross-sections. Data are averages ± 1 standard error ($n = 9$). Vessel and fiber areas refer to wall plus lumen area.

were not correlated (Fig. 4c). In contrast to stems, root PICs for Ψ_{50} were not correlated with xylem density or MOR (Fig. 4a,b), but were correlated with $(t/b)_h^2$ (Fig. 4c).

In transverse xylem sections of stems and roots, stems and roots differed in their percentages of vessel, parenchyma and fiber area (Fig. 5; Table 1). The percentage fiber area was 13% greater in stems than in roots. In roots, the percentage area of parenchyma was *c.* 4% greater than in stems, and the percentage area of vessels was 9% greater than stems. The percentage of fiber wall area was also greater in transverse section for stems compared with roots (Table 1).

The percentages of vessel and parenchyma area were correlated with Ψ_{50} for roots, but not for stems, and only for raw values, not PICs (Fig. 6). Root percentage vessel and parenchyma areas were both correlated with Ψ_{50}, such that greater vessel and lower parenchyma areas were correlated with more negative Ψ_{50} (Fig. 6a,b). Thus, the roots with the most negative Ψ_{50} values tended to have a larger percentage of vessel area and a smaller percentage of parenchyma area in the xylem transverse section. This stands in contrast to stems where neither vessel nor parenchyma were correlated with Ψ_{50} (Fig. 6a,b).

Most xylem mechanical traits, such as xylem density, MOR and MOE, did not correlate with K_s for both stems and roots (data not shown). However, vessel $(t/b)_h^2$ was inversely correlated with K_s for roots, but not for stems (Fig. 7), and this was true for raw data and PICs (Fig. 7).

Xylem capacitance, measured only for stems, was strongly correlated with xylem density, Ψ_{50} and vessel $(t/b)_h^2$ (Fig. 8). Greater xylem capacitance was correlated with lower xylem density (Fig. 8a). Likewise, greater capacitance was correlated with a lower degree of cavitation resistance (less negative Ψ_{50}; Fig. 8b). Greater capacitance was also correlated with reduced $(t/b)_h^2$ (Fig. 8c). Lastly, greater capacitance was correlated with greater fiber lumen diameter ($r^2 = 0.60$; $P = 0.01$), and greater total fiber and vessel lumen in cross-section ($r^2 = 0.42$; $P = 0.05$; data not shown). PICs for stems were also correlated for capacitance and xylem density (Fig. 8a), and capacitance and
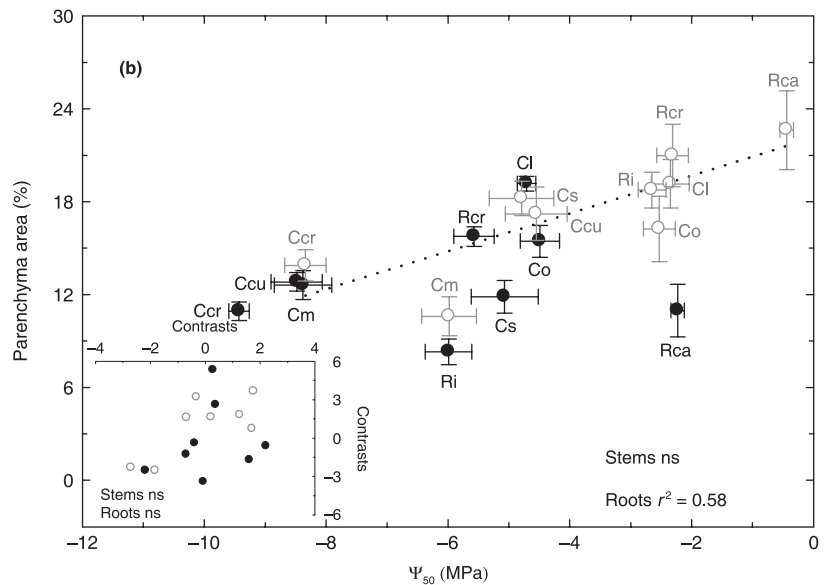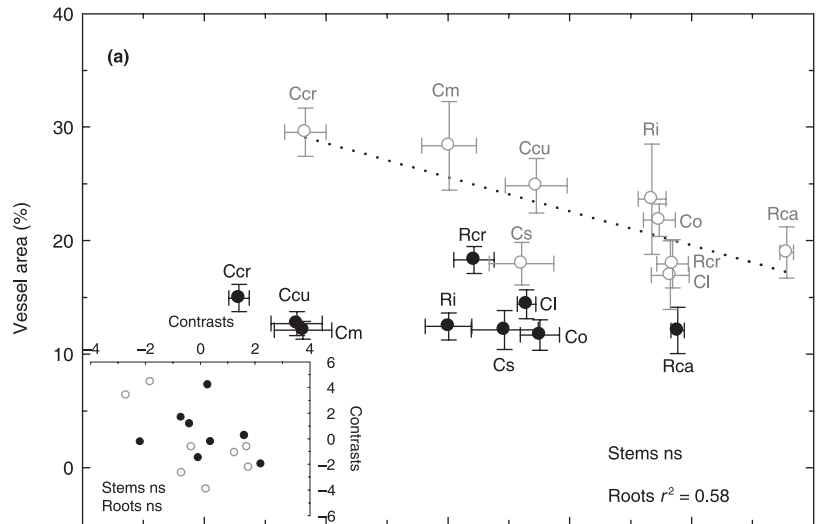
Fig. 6 Stem (black text, closed symbols and solid line) and root (gray text, open symbols and dotted line) cross-sectional area percentage of vessel (a) and parenchyma (b) areas plotted as a function of the water potential at 50% loss in hydraulic conductivity (Ψ_{50}). Phylogenetic independent contrasts for one of nine phylogenies for vessel (a) and parenchyma (b) areas are plotted as a function of Ψ_{50} contrasts in the insets. Abbreviations for species are in Fig. 1. Noncontrast data points are means ± 1 standard error ($N = 6$), and lines are linear regressions. Regression lines are only shown that are statistically significant and others are noted as ns, for not significant.
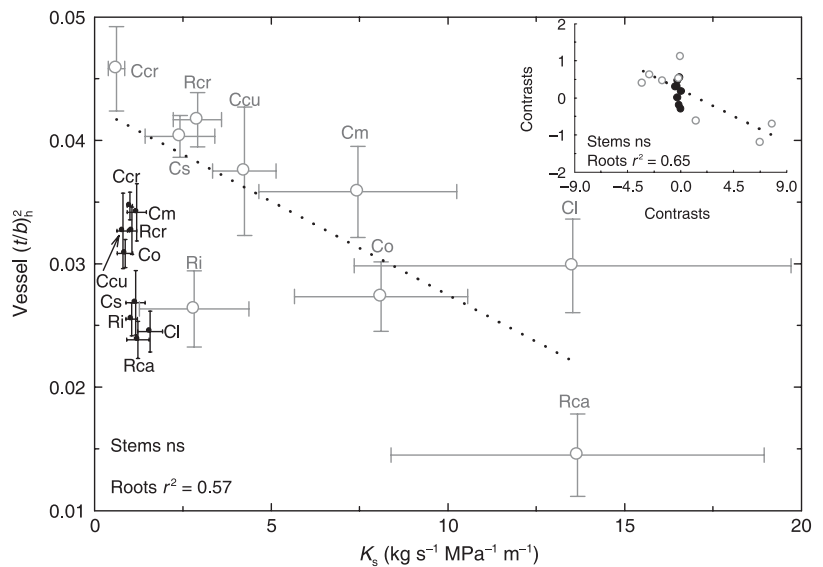
Fig. 7 Stem (black text, closed symbols and solid line) and root (gray text, open symbols and dotted line) vessel thickness to span ratio, $(t/b)_h^2$, plotted as a function of the xylem-specific hydraulic conductivity (K_s). Phylogenetic independent contrasts for one of nine phylogenies for $(t/b)_h^2 \times 100$ are plotted as a function of K_s contrasts (inset). Abbreviations for species are in Fig. 1. Noncontrast data points are means ± 1 standard error ($n = 6$), and lines are linear regressions. *P*-values for regressions are 0.01 for roots for both raw and contrast data, and stems are not significant (ns).
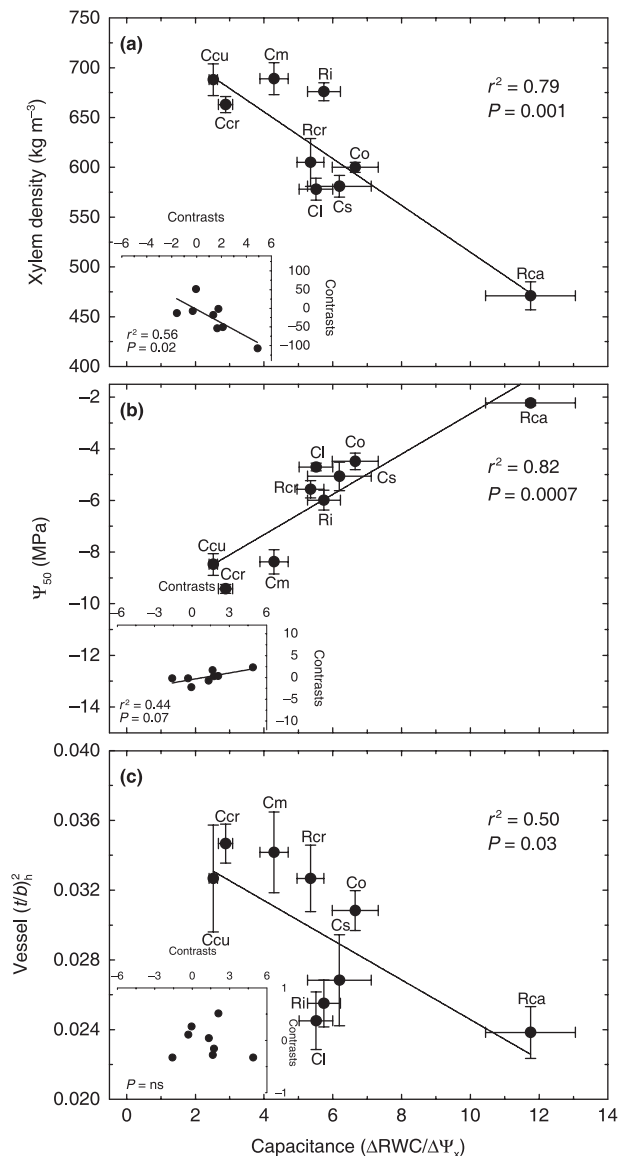
Fig. 8 Stem xylem density (a), water potential at 50% loss in hydraulic conductivity (Ψ_{50}; (b) and vessel resistance to implosion (c); $(t/b)^2_h$), plotted as a function of xylem water storage (capacitance). Phylogenetic independent contrasts for one of nine phylogenies for xylem density (a), Ψ_{50} (b) and $(t/b)^2_h$ (c), plotted as a function of capacitance contrasts, are shown as insets. Abbreviations for species are as detailed in Fig. 1. Noncontrast data points are means ± 1 standard error ($n = 6$), and lines are linear regressions. Regression lines are only shown that are statistically significant (ns, not significant).

Ψ_{50} (Fig. 8b), but PICs were not correlated for capacitance and $(t/b)^2_h$ (Fig. 8c).

PCs analyses summarize and confirm the linear regression analyses (Fig. 9). For stems, traits associated with mechanical strength (MOR, MOE, fiber wall area and xylem density) clustered with the xylem transport safety measures of Ψ_{50}, $(t/b)^2_h$ and Ψ_{min}, and loaded strongly along PC 1 (Fig. 9a).

Traits associated with xylem efficiency (K_s and d_h) and water storage (capacitance) were negatively associated with the mechanical and transport safety traits; however, the efficiency traits had relatively low loading values (< 0.3), indicating that the negative association was weak. The traits most strongly associated with PC 2 were percentage cell areas (vessel and parenchyma) and the biomechanical traits of $(t/b)^2_h$ and MOE. The clearest pattern among these traits is that high percentages of parenchyma were associated with lower values of MOE, indicating the importance of parenchyma in stem flexibility.

Like stems, root mechanical (MOE and MOR) and transport safety (Ψ_{50}, Ψ_{min} and $(t/b)^2_h$) traits clustered at one end of PC 1; however, unlike stems, the mechanical traits of xylem density and fiber wall area did not cluster with the other traits (Fig. 9b). Also, as in stems, transport efficiency traits (K_s and d_h) clustered and loaded most heavily along PC 1, opposite the transport safety and mechanical traits (Fig. 9b). In the case of roots, the negative loading scores for the efficiency traits were relatively strong (> 0.3), showing the trade-off between transport safety and efficiency present in roots. The strongest loading scores along PC 2 were for percentage cell areas (vessel and parenchyma) and the mechanical traits of xylem density and fiber wall area (Fig. 9b).

Discussion

The species most resistant to cavitation (more negative Ψ_{50}) experienced the most negative predawn water potentials during the dry season (Ψ_{min}). The slope of this relationship was not different from 1.0 for stems and roots, which is similar to that reported in a study across 26 chaparral species sampled in the Santa Monica Mountains (Jacobsen *et al.*, in press) and among shrubs in the great basin (Hacke *et al.*, 2000). For stems of all species, data points were not different from, or fell below, the 1 : 1 line, indicating that they were predicted to have ≤ 50% loss in hydraulic conductivity during the dry season, assuming that the xylem water potential at midday was not significantly different from Ψ_{min}. This agrees with the recent stem measurements of dry season native embolism of 10 chaparral species (including four *Ceanothus* spp.) in the Santa Monica Mountains, which found a mean and standard error percentage loss of conductivity of $42 \pm 8\%$, a value not different from 50 (A. Jacobsen, unpublished). This supports our assumption that Ψ_{min} is a robust estimate of minimum xylem water potential. The fact that Ψ_{50} strongly correlates to Ψ_{min} shows that Ψ_{50} is a precise index of the maximum seasonal water stress experienced by the xylem of chaparral shrubs under natural field conditions. For roots, many species fell above the 1 : 1 line, indicating that they were predicted to have > 50% loss in hydraulic conductivity, which is caused by the greater vulnerability of roots to cavitation than stems. Differences between stem and root cavitation resistance is fully discussed in a separate study (Pratt *et al.*, 2007).
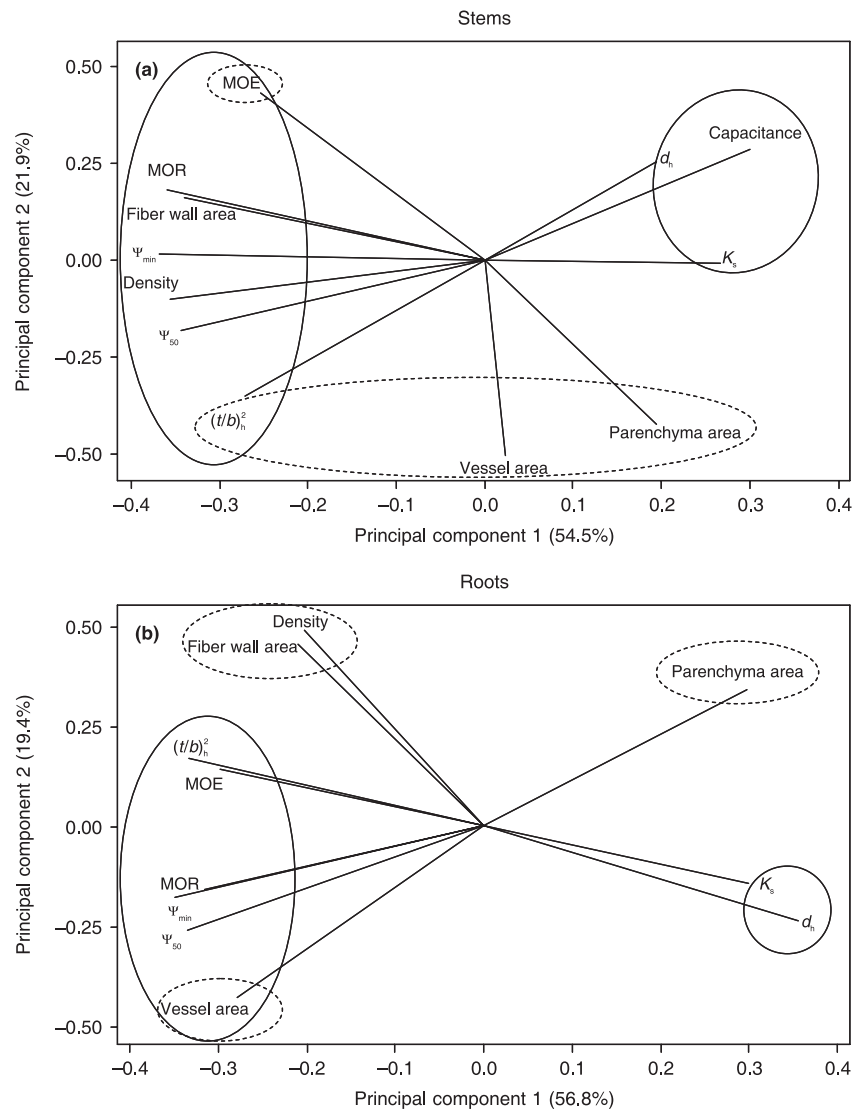
Stems

Roots

Fig. 9 Principal components analyses for stems (a) and roots (b). Trait abbreviations follow the previous figures, but also include MOE (modulus of elasticity), d_h (hydraulic mean vessel diameter) and density (xylem density). Solid circles surround traits that loaded most strongly on principal component (PC) 1, and traits circled with dashed lines loaded most strongly on PC 2. Overlap between circles denotes traits that loaded most strongly on PC 2, but also showed relatively strong loading on PC 1. Absolute values for water potential at 50% loss of hydraulic conductivity (Ψ_{50}) and dry season predawn water potential (Ψ_{min}) were used.

A greater degree of mechanical strength was correlated with greater resistance to cavitation for stems. This was true at the cellular level ($(t/b)^2_h$), tissue level (xylem density) and organ level (modulus of rupture). This probably reflects the need for xylem cells to resist the stresses created by negative hydrostatic pressures in the xylem (Hacke *et al.*, 2001; Jacobsen *et al.*, 2005). The correlations of PICs held for xylem density and MOR, indicating that the relationships were not significantly influenced by sampled taxa phylogeny. Estimated vessel implosion resistance ($(t/b)^2_h$) and cavitation resistance for stems were not correlated using PICs. This finding is similar to that of Jacobsen *et al.* (in press), who found that vessel implosion resistance and cavitation resistance were not evolutionarily correlated among 26 species of chaparral shrubs. It appears that the evolution of increased cavitation resistance was not concurrent with the evolution of increased $(t/b)^2_h$ in these species. This is surprising because increased cavitation resistance is expected to lead to greater negative hydrostatic

pressures and hoop stresses on vessel walls, necessitating higher $(t/b)^2_h$ to resist collapse. On the other hand, developing excessively high $(t/b)^2_h$ is presumably selected against as inefficient resource allocation, and it may reduce transport efficiency in roots (Fig. 7). Vessel implosion is a threshold-type response, and as long as $(t/b)^2_h$ is above the implosion threshold (dashed line in Fig. 4c), vessels should be safe from implosion. Thus, the evolution of increased cavitation resistance need not necessitate an increase in $(t/b)^2_h$ as long as the vessel pairs remain above the implosion threshold (Jacobsen *et al.*, in press). The role of fibers in supporting vessels against implosion further complicates the $(t/b)^2_h$ implosion resistance model, and more work is needed to more fully understand this relationship, a fact highlighted by the lack of correlation with PICs in both the present study and that of Jacobsen *et al.* (in press).

For roots, the cavitation resistance and mechanical trends were different from stems. There was no correlation between Ψ_{50} and xylem density among roots. This correlation is nearly

universal among woody stems, and may represent a fundamental difference between root and stem xylem (Hacke & Sperry, 2001; Jacobsen *et al.*, in press). Moreover, root resistance to cavitation and resistance to mechanical breakage were not correlated, as has been shown for stems (Jacobsen *et al.*, 2005, in press). On the other hand, greater cavitation resistance was correlated with greater estimated vessel implosion resistance for raw data and PICs for roots. We also note that the measured estimates of vessel implosion resistance were generally greater or equal for roots compared with stems. This is unexpected because stems will typically experience more severe negative hydrostatic pressures than roots. These data suggest that in roots, vessel structure may be more important to resisting implosion than the surrounding fiber matrix as compared with stems. Roots are surrounded by the soil matrix and thus, for mechanical support of the plant body, may not require as robust a fiber matrix as found in stems. In stems, the fiber matrix performs the dual function of supporting the plant body against gravity and supporting vessels from imploding under stresses caused by negative hydrostatic pressures (however, see Jacobsen *et al.*, 2007).

The MOR and MOE values were not significantly greater for stems than for roots, although stems have more fibers than roots. In addition, stems have more cell wall area (fiber and vessel) in cross-section than roots (R. B. Pratt, unpublished). The reason for comparable mechanical strength in roots may relate to bark differences in stems and roots. Roots generally have a thicker bark than stems (Esau, 1965), which may have contributed significantly to root mechanics, as has been shown in stems of other species (Niklas, 1999). In addition, parenchyma can significantly contribute to organ mechanical strength (Beery *et al.*, 1983; Woodrum *et al.*, 2003). Further work is needed to understand the structural basis for the relatively high root mechanical strength against bending stress.

In roots there was a clear trade-off between xylem transport efficiency and transport safety, whereas stems exhibited no such trade-off (i.e. stem Ψ_{50} and K_s were not correlated, and nor were $(t/b)_h^2$ and K_s). The reason for this trade-off may relate to vessel resistance to implosion. Among roots, the fiber matrix does not provide as much support as in stems, and greater $(t/b)_h^2$ may be necessary to avoid vessel collapse under negative hydrostatic pressures. Increasing vessel wall thickness relative to the lumen diameter (i.e. $(t/b)_h^2$) may limit lumen diameter size. As hydraulic efficiency is proportional to the lumen radius to the fourth power, as described by the Hagen-Poiseuille relationship, even small decreases in lumen diameter could result in considerable efficiency declines. An interesting independent test of this pattern could be to assess the safety vs efficiency trade-off in mechanical parasites, such as lianas, which presumably do not have as robust a fiber matrix as free-standing plants. We also do not rule out the possibility that the pit area hypothesis could explain the observed trade-off (see the Introduction; Wheeler *et al.*, 2005; Hacke *et al.*, 2006), but a test of this will require further study.

In contrast to our findings in roots, a recent study found no evidence for correlated evolution between Ψ_{50} and K_s in roots sampled across a broad range of 16 temperate forest tree taxa, including three conifers (Maherali *et al.*, 2006). At the cellular level, K_s is influenced by conduit diameter and length, the pitted area of a conduit and the conductivity of pits. Cavitation resistance is determined by the largest diameter pore in a pit membrane adjacent to an air-filled area, the vessel pitted area and vessel implosion resistance. Large differences in these traits across sampled taxa may confound attempts to analyze trade-offs; thus, there is great value in comparing taxa with similar xylem anatomy (Maherali *et al.*, 2006). The taxa sampled here are from a common lineage and have similar xylem anatomy, and our results suggest a safety vs efficiency trade-off in this lineage. Because of the numerous anatomical and physiological traits influencing Ψ_{50} and K_s, a range of relationships between Ψ_{50} and K_s might be expected in different lineages and across broader taxonomic scales (Maherali *et al.*, 2004; Edwards, 2006).

Stems did not exhibit a correlation between percentage fiber wall area and K_s, suggesting no trade-off between efficiency and safety from mechanical stresses. In addition, there was no association between K_s and MOR here or in a previous study (Woodrum *et al.*, 2003). Thus, in stems we find no evidence for a trade-off between transport efficiency and mechanical support.

The correlations between the percentages of cell types in cross-section and resistance to cavitation yield further insight into differences between stems and roots. Previous studies of stems have found a correlation between fiber mechanical strength (cross-sectional fiber wall area) and resistance to water stress-induced cavitation (Jacobsen *et al.*, 2005, in press), suggesting a link between fiber properties and vessel resistance to negative hydrostatic pressures. We found that an increased percentage of fiber wall area in cross-section was not significantly correlated (after Bonferroni correction) with greater cavitation resistance ($P = 0.08$; data not shown). Roots also had no such correlation, suggesting a minimal role of fibers in vessel support. Therefore, in the present study there is no support for the hypothesis that increased fiber wall area is important in resisting negative hydrostatic pressures. It may not be necessary to increase the proportion of fiber wall area to resist negative pressure stress, and an alternative is that fibers may be differentially arranged to achieve adequate mechanical support.

The correlation between increased percentage vessel area and increased cavitation resistance in roots may suggest a functional relationship between these two traits; however, this is unlikely because of a lack of correlation with PICs. All things being equal, xylem transport efficiency increases with an increase in the area of vessels in cross-section. However, the chief advantage of increased vessel area for the species sampled here may be its correlation with more vessels per unit area (i.e. greater vessel redundancy) (R. B. Pratt, unpublished). Greater vessel redundancy helps to ensure sustained transport if

transport fails in some vessels because of cavitation or other damage (Carlquist, 1977; Zimmermann, 1983). Thus, root xylem that is more cavitation resistant appears to be more redundant, making it simultaneously more resistant and tolerant to hydraulic failure. The lack of correlation with PICs suggests that these two traits evolved independently of one another. It would be useful to evaluate samples from a broader range of taxa to establish if these traits are evolutionarily correlated across broader taxonomic scales.

Reduced percentage parenchyma area was correlated with greater resistance to cavitation in roots, but not in stems. This relationship suggests that root transport safety comes at the cost of reduced parenchyma area. Living parenchyma cells have the disadvantage of respiring and thus adding to maintenance respiration; however, root parenchyma cells are important for storing starch and nutrients, as well as for differentiating into lateral roots. Thus, a higher degree of water stress tolerance may reduce the carbohydrate and nutrient storage capacity of roots, and reduce the ability of roots to develop lateral roots to exploit resource pulses opportunistically or grow following injury. Consistent with this, species in the present study that are nonsprouters after fire have the lowest proportion of root parenchyma, and presumably rely less on root storage than postfire sprouters (Fig. 6b; lowest percentage parenchyma areas for roots are all nonsprouters = Ccr, Ccu, Cm, Co).

None of the percentages of cells in cross-section were correlated with cavitation resistance when analyzed using PICs. From the existing literature, it is unclear how evolutionarily labile are cell-type percentages (Preston *et al.*, 2006). A previous study of five maples also shows that the percentage of xylem cell types in stems is phylogenetically conserved (Woodrum *et al.*, 2003). It is worth noting, in the present study, that for correlations among cell percentages that were significant with raw data also showed several significant correlations among the nine phylogenies using PICs, even though the average *P*-value was not significant. This indicates that the percentage cell area analyses were sensitive to phylogeny, and a fully resolved phylogeny is needed to assess the relationships between cell type percentages and cavitation resistance in greater detail.

A greater ability of stem xylem to store water is strongly linked with reduced mechanical support and transport safety. For nonsucculent stems, greater water storage capacity is increased primarily by increasing fiber and vessel lumen diameters (Desch, 1973). For fibers, greater lumen volume should lead to reduced xylem density and MOR. For vessels, greater lumen diameter may lead to increased susceptibility to vessel implosion unless there is a concomitant increase in vessel wall thickness. The importance of xylem water storage in woody plant stems for most species is unclear. However, it has been estimated that stored water can contribute from 10 to 50% of the daily transpiration in certain tree species (Gartner & Meinzer, 2005). Furthermore, the strong inverse correlations, shown here, between transport safety and capacitance, and the correlations between water storage and seasonal changes in water status, are suggestive of an important role for stem water storage (Stratton *et al.*, 2000; Pratt & Black, 2006).

In summary, stems and roots have different xylem structural and functional relationships (summarized in Fig. 9). Stems are most strongly characterized by positive associations between mechanical strength and transport safety (i.e. resistance to water stress-induced cavitation). Stem mechanical strength vs safety relationships are found at the tissue (xylem density) and organ (MOE and MOR) levels, and are evolutionarily correlated. Stem mechanical strength appears to be important in maintaining xylem transport under negative hydrostatic pressure, probably as an aid to resist vessel implosion as well as resisting mechanical stresses caused by gravity. Roots show some important differences when compared with stems. Unlike stems, mechanical strength is not correlated with transport safety in roots. This difference between stems and roots probably relates to the contrasting mechanical environment of these two organs. This mechanical difference may also help to explain the relatively strong root transport safety vs efficiency trade-off because root vessels are not as well supported by a robust fiber matrix as are stems, and thus as root vessels become wider (more efficient) they become more susceptible to implosion (less safe).

Both stems and roots show a trade-off between transport safety and storage. For stems, this trade-off is solely related to water storage (capacitance) and not potential nutrient storage (percentage parenchyma). Roots show a trade-off between potential nutrient storage and transport safety, although this trade-off was not supported by PICs. Capacitance was not measured for roots. It would be valuable to study nutrient storage vs transport safety on a broader range of taxa to evaluate this potential trade-off in roots in greater detail.

Acknowledgements

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