Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis

Alexandria L. Pivovaroff[1], Lawren Sack[2] and Louis S. Santiago[1]

[1]Department of Botany and Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA 92521, USA; [2]Department of Ecology and Evolutionary Biology, University of California, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606, USA

Author for correspondence:
Alexandria L. Pivovaroff
Tel: +1 562 881 4640
Email: alexandria.pivovaroff@email.ucr.edu

Summary

- Coordination of water movement among plant organs is important for understanding plant water use strategies. The hydraulic segmentation hypothesis (HSH) proposes that hydraulic conductance in shorter lived, 'expendable' organs such as leaves and longer lived, more 'expensive' organs such as stems may be decoupled, with resistance in leaves acting as a bottleneck or 'safety valve'.
- We tested the HSH in woody species from a Mediterranean-type ecosystem by measuring leaf hydraulic conductance (K_{leaf}) and stem hydraulic conductivity (K_S). We also investigated whether leaves function as safety valves by relating K_{leaf} and the hydraulic safety margin (stem water potential minus the water potential at which 50% of conductivity is lost ($\Psi_{stem} - \Psi_{50}$)). We also examined related plant traits including the operating range of water potentials, wood density, leaf mass per area, and leaf area to sapwood area ratio to provide insight into whole-plant water use strategies.
- For hydrated shoots, K_{leaf} was negatively correlated with K_S, supporting the HSH. Additionally, K_{leaf} was positively correlated with the hydraulic safety margin and negatively correlated with the leaf area to sapwood area ratio.
- Consistent with the HSH, our data indicate that leaves may act as control valves for species with high K_S, or a low safety margin. This critical role of leaves appears to contribute importantly to plant ecological specialization in a drought-prone environment.

Introduction

Hydraulic architecture refers to the arrangement of hydraulic resistances in the xylem of plants, and is a critical determinant of plant function (Zimmermann, 1978; McCulloh & Sperry, 2005). Hydraulic traits regulate the use and loss of water and influence other physiological processes, including gas exchange and growth (Zimmermann, 1978; Sperry, 2000; Santiago *et al.*, 2004a; McCulloh & Sperry, 2005; Brodribb *et al.*, 2010). The key parameter in describing hydraulic relationships is hydraulic conductance, a measure of the efficiency of water movement through a material such as plant stems or leaves (Sperry *et al.*, 1988; Choat & PrometheusWiki contributors, 2011). The definition of hydraulic conductance is based on an analogy with Ohm's law:

$$R = \Delta V / I \qquad \text{Eqn 1}$$

where R is resistance, ΔV is the difference in voltage (potential difference), and I is current (flow of electric charge). For plant water transport, we take plant hydraulic conductance as the

inverse of hydraulic resistance, pressure analogous to voltage, and flow rate analogous to current. Hence we can model plant hydraulic conductance as the flow of water for a given pressure or water potential difference:

$$k_h = F / \Delta P \qquad \text{Eqn 2}$$

where k_h is hydraulic conductance, F is flow rate, and ΔP is pressure difference.

Water moves through plants within the soil–plant–atmosphere continuum (SPAC) as a continuous column of liquid from areas of less negative water potential to more negative water potential, as described by the cohesion-tension theory (Dixon & Joly, 1895). However, studies have most often focused on hydraulic conductance in single plant organs, that is, within only leaves, stems, or roots. This typical focus on individual plant organs has led to a lack of understanding of how hydraulic conductance is *coordinated* between organs such as stems and leaves. One might assume that, because stems supply leaves with water, stem and leaf hydraulic conductances would increase in tandem. Conversely, discrete organs could vary in their hydraulic conductance.

For example, if distal organs such as leaves had lower hydraulic conductance than large, main stems, this decoupling would maintain the water status of longer lived and more 'expensive' organs that represent a significant carbon investment at the expense of shorter lived and 'cheaper' organs that can be more easily replaced (Zimmermann, 1983). This 'hydraulic segmentation' hypothesis (HSH), as first proposed by Zimmermann, is in fact one explanation for the distribution of relative hydraulic resistance in woody plants (Tyree & Zimmermann, 2002), because greater hydraulic resistance in distal organs may function as a 'bottleneck' to water flow through the whole plant (Zimmermann, 1978; Sack & Holbrook, 2006). Early research on some diffuse-porous trees indicated that leaves and small, terminal branches represented a 'distinct hydraulic constriction' in woody plants, with lower hydraulic conductance than main stems (Zimmermann, 1978). This segmentation principle was later separated into two distinct mechanisms: hydraulic segmentation and vulnerability segmentation (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). Vulnerability segmentation refers to distal organs being more vulnerable to cavitation than basal portions, and several studies have shown that leaves tend to be more vulnerable to hydraulic decline than stems (Tyree et al., 1993; Tsuda & Tyree, 1997; Choat et al., 2005; Hao et al., 2007; Chen et al., 2009; Johnson et al., 2011; Bucci et al., 2012). The putative purpose of segmentation, whether hydraulic or vulnerability, relates to the concept that 'leaves function as a safety valve' (Chen et al., 2009, 2010), and previous studies have demonstrated support for this 'safety valve' hypothesis (Chen et al., 2009, 2010; Johnson et al., 2009, 2011, 2012; McCulloh et al., 2014), although these data generally come from relatively humid environments. The aim of this study was to test the relationship between water movement in stems and leaves in the context of the HSH in a water-limited and highly seasonal environment.

Understanding coordination of water movement between the stem and leaf may be of particular importance, especially in ecosystems with seasonal water availability. In Mediterranean-type ecosystems, plants face the dual challenge of providing water to support rapid growth during the brief wet winter and conducting water across drastically different water potential gradients during the extended dry summer (Cowling et al., 2005). We first examined whether the relative resistance to water transport varied between stems and leaves, and investigated the ecological implications. We hypothesized that leaf hydraulic conductance would be negatively correlated with stem hydraulic conductivity, in support of the HSH. In addition, we investigated whether leaves function in a manner similar to a 'safety valve', hypothesizing that species with a lower hydraulic safety margin would also have a lower leaf hydraulic conductance (Meinzer et al., 2009; Delzon & Cochard, 2014). Finally, we quantified additional functional traits, including leaf mass per area (LMA), leaf area to sapwood area ratio (LA : SA), and wood density, along with operating stem and leaf water potentials, to evaluate the relationships among these traits, to characterize overall plant water use strategies in the context of hydraulic segmentation, and to explore new implications for the cross-species coordination of hydraulic conductance between stems and leaves.

Materials and Methods

Study site and species

The study was conducted from 2011 to 2013 at the Santa Margarita Ecological Reserve (SMER; 33°29′N, 117°09′W), located at the Riverside-San Diego county line, California, USA. The reserve size is 1790 ha, with elevation ranging from 150 to 700 m. SMER has a Mediterranean-type climate and receives 36 cm of mean annual precipitation, mostly during the winter months (November–March), resulting in an annual seasonal drought between April and October. SMER has a mean annual temperature of 16.4°C (Vourlitis & Pasquini, 2009) and last burned > 35 yr ago. This study focused on the 17 most commonly occurring woody species at SMER (Table 1), with reported maximum vessel lengths varying from 0.29 m in *Adenostoma fasciculatum* to 1.96 m in *Quercus agrifolia* (Jacobsen et al., 2012). All samples were collected from the field during morning hours (08:00–12:00 h).

Water potentials

Predawn and midday water potentials (Ψ; MPa) were measured to establish seasonal variation in the water potential gradient driving flow. Measurements were taken on bagged and unbagged branches during the 2012 wet season (February; 15.1 cm of precipitation from November 2011 to February 2012; National Oceanic and Atmospheric Administration (NOAA) Fallbrook 5 and 6.5 NE CA US stations) and dry season (September; 0.5 cm of precipitation from June to September 2012; NOAA Fallbrook 5 and 6.5 NE CA US stations) with a pressure chamber (Model 1000; Plant Moisture Stress Instruments, Albany, OR, USA) to determine ranges of plant water status. The day before measurements, two branches on each of five individuals per species were

Table 1 Dominant woody study species, with symbols used to represent species in figures, and native or nonnative (NN) designation, at Santa Margarita Ecological Reserve, California, USA

Species	Family	Symbol	Native or NN
Adenostoma fasciculatum	Rosaceae	○	Native
Ceanothus tomentosus	Rhamnaceae	▽	Native
Heteromeles arbutifolia	Rosaceae	□	Native
Keckiella antirrhinoides	Plantaginaceae	●	Native
Malosma laurina	Anacardiaceae	◇	Native
Mimulus aurantiacus	Phrymaceae	■	Native
Nicotiana glauca	Solanaceae	⊟	NN
Olea europaea	Oleaceae	◆	NN
Quercus agrifolia	Fagaceae	⊕	Native
Quercus berberidifolia	Fagaceae	⊞	Native
Rhamnus ilicifolia	Rhamnaceae	⊕	Native
Rhus ovata	Anacardiaceae	△	Native
Sambucus nigra	Adoxaceae	◆	Native
Salvia mellifera	Lamiaceae	▲	Native
Schinus molle	Anacardiaceae	▣	NN
Senna artemisioides	Fabaceae	◐	NN
Xylococcus bicolor	Ericaceae	△	Native

covered with plastic bags and aluminum foil to stop those selected samples from transpiring and allow leaf and stem water potentials to equilibrate. Immediately following collection, branches were placed in plastic bags before being sealed and placed in a dark cooler. For predawn Ψ, one transpiring and one nontranspiring sample pair were measured from each individual. This was repeated for midday Ψ measurements. The nontranspiring Ψ measurements were taken to represent stem water potential (Ψ_{stem}) and the transpiring Ψ measurements were taken to represent leaf water potential (Ψ_{leaf}). We did not measure dry season water potentials for species that did not have leaves at the time of sampling, that is, *Keckiella antirrhinoides*, *Mimulus aurantiacus*, *Sambucus nigra*, and *Salvia mellifera*. For each species within each season, we calculated the diurnal range in Ψ_{stem} ($\Delta\Psi_{stem}$) as the difference between midday and predawn Ψ_{stem}, and that for Ψ_{leaf} ($\Delta\Psi_{leaf}$) as the difference between midday and predawn Ψ_{leaf}. We also calculated $\Delta\Psi_{stem-leaf}$ as the difference between midday Ψ_{stem} and Ψ_{leaf}. Finally, we calculated seasonal differences in both Ψ_{stem} and Ψ_{leaf}.

Leaf hydraulic conductance

Leaf hydraulic conductance (K_{leaf}) was measured during the spring (March–June) on newly mature leaves from at least four individuals of each species, using the evaporative flux method (Sack *et al.*, 2002; Sack & Scoffoni, 2013). In the laboratory, leaves from the same branches that were used for stem hydraulic conductivity (see below; 'Stem hydraulic conductivity and xylem vulnerability') were cut from the stem under water with a fresh razor blade. Leaves were immediately placed in beakers with filtered (0.2 μm), degassed water that covered the petiole, but did not touch the lamina. Samples were covered with plastic and allowed to hydrate overnight for *c.* 8 h.

Following rehydration, leaves were connected to tubing containing filtered (0.2 μm), degassed water that ran from a reservoir on a balance ($\pm$ 0.1 mg; Denver Instrument P-214; Sartorius, Bohemia, NY, USA) to the leaf. Leaves were held in place with a wood frame strung with fishing line, and placed over a fan to reduce the boundary layer around the leaf. Light sources arranged above the sample, who light was diffused through a plexiglass container filled with water that acted as a heat trap, produced a photon flux density of > 1200 μmol m^{-2} s^{-1} at the leaf level to induce stomatal opening.

The transpiration-driven flow of water into the leaf was measured from the balance interfaced to a computer, logging data every 60 s. Transpirational flow increased for *c.* 30 min at the beginning of the measurements before steady-state transpiration was achieved (coefficient of variance < 5%) for 10 min. Leaves were then placed in a Whirlpak bag (Nasco, Fort Atkinson, WI, USA) and rapidly removed from the tubing to measure leaf water potential with a pressure chamber (Model 1000; Plant Moisture Stress Instruments). Finally, leaf area was measured with a leaf area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). The leaf hydraulic conductance (K_{leaf}; mmol m^{-2} s^{-1} MPa^{-1}) was calculated as:

$$K_{leaf} = E/\Delta\Psi_{leaf} \qquad \text{Eqn 3}$$

normalized by leaf area (m^2), where E is the steady-state transpiration flow rate (mmol s^{-1}) and $\Delta\Psi_{leaf}$ is the water potential driving force ($-\Psi_{leaf}$; MPa).

Stem hydraulic conductivity and xylem vulnerability

Large branch samples *c.* 1 m in length were collected from at least four individuals of each species in the field. Cut ends were covered with parafilm and samples were placed in opaque plastic bags with wet paper towels until transported to the laboratory. Once in the laboratory, stem samples were cut under water to a length of *c.* 16 cm. Emboli were removed from stems by vacuum infiltration under filtered (0.2 μm) water for 8 h. Stem ends were then re-cut under water and ends cleanly shaved with a razor blade for a final sample length of 14.2 cm.

Maximum stem hydraulic conductivity (K_{max}) was determined by connecting stems to tubing filled with filtered (0.2 μm), de-gassed water flowing from an elevated source, through the stem, and into a reservoir on a balance ($\pm$ 0.1 mg; Denver Instrument P-214; Sartorius) that was interfaced with a computer to record the flow rate, allowing the calculation of conductivity (Sperry *et al.*, 1988). Following Torres-Ruiz *et al.* (2012), we corrected for stem passive water uptake by beginning and ending each conductivity measurement with a 'background' measurement. Stem hydraulic conductivity (K_h; kg m s^{-1} MPa^{-1}) was calculated as:

$$K_h = F \times L/\Delta P \qquad \text{Eqn 4}$$

where F is the flow rate (kg s^{-1}), L is the stem length (m), and ΔP is the driving force (MPa). Stem hydraulic conductivity was also normalized by sapwood area (A_{SW}; m^2) to determine stem sapwood-specific hydraulic conductivity (K_S; kg m^{-1} s^{-1} MPa^{-1}), and distal leaf area (A_L; m^2) to determine leaf specific hydraulic conductivity (K_L; kg m^{-1} s^{-1} MPa^{-1}).

Stem xylem vulnerability to cavitation, as determined by vulnerability curves, was measured using the 'static' centrifugation method (Alder *et al.*, 1997; Jacobsen & Pratt, 2012; Sperry *et al.*, 2012) to determine the water potential at which 50% of conductivity is lost (Ψ_{50}) and calculate the stem hydraulic safety margin (Meinzer *et al.*, 2009; Delzon & Cochard, 2014). The static centrifugation method differs from the cavitron, or flow centrifugation, method in that hydraulic conductivity is measured between spinning, whereas conductivity is measured during spinning with the cavitron (Cochard *et al.*, 2005).

Following the determination of K_{max}, stems were spun using a custom-built, 14.2-cm-diameter rotor in a refrigerated centrifuge (Sorvall RC-5Cplus; Thermo Scientific, Waltham, MA, USA) at progressively more negative stem water potentials to cause embolisms. Foam cosmetic sponges soaked with water were added to the vertical end of the plexiglass reservoirs to keep the ends of the stems wet, even when the rotor was not spinning (Jacobsen & Pratt, 2012; Tobin *et al.*, 2012). ΔP was always kept below 2 kPa to prevent possible flushing of

embolisms induced by centrifugation in wide and/or open vessels. Hydraulic conductivity was repeatedly measured on each stem after spinning at increasing speeds, and K_h declined with more negative water potentials and increasing levels of xylem cavitation. This was repeated until the stem lost > 90% conductivity. Per cent loss of conductivity (PLC) was calculated at each water potential step as:

$$PLC = 100 \times (1 - (K_h/K_{max})) \qquad \text{Eqn 5}$$

Vulnerability curves were constructed by plotting water potential versus PLC and fitting a Weibull model (Pammenter & Willigen, 1998):

$$PLC = 100/(1 + \exp(a(\Psi - b))) \qquad \text{Eqn 6}$$

from which stem Ψ_{50} was determined for each species. Stem hydraulic safety margin as calculated as midday $\Psi_{stem} - \Psi_{50}$ (Meinzer et al., 2009; Delzon & Cochard, 2014).

Functional traits: leaf area to sapwood area ratio, leaf mass per area, and wood density

Branch-level measurements of LA : SA were made during the spring (March–June) to determine the relationship between total transpiring leaf area and the area of sapwood supplying water to those leaves (Shinozaki et al., 1964; Martínez-Vilalta & PrometheusWiki contributors, 2011). The cross-sectional sapwood area of excised branch samples was determined by removing bark and measuring the diameter of the sapwood with digital calipers to calculate the cross-sectional area. If pith was present, its cross-sectional area was subtracted from the total sapwood area. All distal leaves were removed from the stem segment, and their total area was measured using a leaf area meter (Li-3100; Li-Cor Biosciences).

LMA was determined for individual leaves by measuring leaf area using a leaf area meter (Li-3100), drying leaves for 48 h at 65°C before measuring dry mass, and dividing dry mass (g) by fresh leaf area (m^2).

Wood density was measured by removing the bark and pith from stem subsamples and determining the fresh volume with the water displacement method (Osazuwa-Peters et al.; Williamson & Wiemann, 2010), then drying the xylem for 48 h at 65°C before measuring dry mass. Woody density (g cm^{-3}) was calculated as the ratio of xylem dry mass (g) to fresh volume (cm^3).

Statistics

We used Pearson's product-moment correlation coefficients to evaluate interspecific bivariate relationships among physiological variables using SAS ver. 9.3 (SAS Institute, Cary, NC, USA). Before testing correlations, when trait values were not normally distributed, they were log$_{10}$-transformed, which resulted in normal distributions. Negative values were converted to positive values to enable log$_{10}$-transformation when necessary (e.g. Ψ).

The overall difference between $\Delta\Psi_{stem}$ and $\Delta\Psi_{leaf}$ was analyzed using a paired t-test. *Rhamnus ilicifolia* Ψ_{50} was removed from hydraulic safety margin analyses because its calculated Ψ_{50} was beyond the range of collected data. We also evaluated the impact of phylogenetic relationships for our analyzed trade-offs using phylogenetically independent contrasts (PICs) with the R 'ape' package (ver. 3.0-11; Paradis et al., 2004). PHYLOMATIC (ver. 3) was used to build the tree and PHYLOCOM (ver. 4.2) to add branch lengths (Webb et al., 2008).

Results

Measurement of Ψ_{leaf} and Ψ_{stem} indicated that the study species experienced a wide range of tissue water status on an annual basis (Fig. 1). Midday Ψ_{leaf} (Fig. 1c) ranged from -1.19 MPa in *Schinus molle* during the wet season to -9.39 MPa in *Olea europaea* during the dry season. Midday Ψ_{stem} (Fig. 1d) ranged from a maximum of -0.95 MPa in *Schinus molle* during the wet season to a minimum of -8.66 MPa in *Xylococcus bicolor* during the dry season. Seasonal differences (Fig. 1) of midday Ψ between the wet and dry seasons also varied, with *Malosma laurina* exhibiting the smallest difference at 0.05 MPa for leaves and 0.15 MPa for stems and *X. bicolor* exhibiting the largest difference at 6.64 MPa for leaves and 7.08 MPa for stems. The diurnal range in Ψ_{leaf} ($\Delta\Psi_{leaf}$) was always greater in magnitude than that of the stem ($\Delta\Psi_{stem}$) for both the wet ($P < 0.0001$) and dry seasons ($P = 0.0025$; Fig. 2). $\Delta\Psi_{stem-leaf}$ was not correlated with any measured traits.

Values for K_{leaf} were negatively correlated with K_S ($r = -0.54$; $P = 0.02$; PIC $r = -0.51$; $P = 0.04$; Fig. 3), with species having greater K_S exhibiting lower K_{leaf}, supporting the HSH. The operating hydraulic safety margin (February midday $\Psi_{stem} -$ stem Ψ_{50}) and K_{leaf} were positively correlated (Fig. 4), with species having stems that operate within a smaller safety margin also having lower K_{leaf}, consistent with leaves acting as a safety valve. We also found a negative relationship between K_{leaf} and LA : SA ($r = -0.56$; $P = 0.02$; PIC $r = -0.66$; $P = 0.004$; Fig. 5a) and a positive relationship between K_S and LA : SA ($r = 0.62$; $P = 0.008$; Fig. 5b). Additional significant relationships included K_S and stem Ψ_{50} ($-$MPa) which were negatively related ($r = -0.48$; $P = 0.06$; PIC $r = -0.61$; $P = 0.009$) and K_{leaf} and stem Ψ_{50} ($-$MPa) which were positively related ($r = 0.52$; $P = 0.04$; PIC $r = 0.55$; $P = 0.02$). K_L was not correlated with any other measured traits.

There was a positive correlation between LMA and wood density ($r = 0.66$; $P = 0.004$; PIC $r = 0.66$; $P = 0.003$; Fig. 6). LMA was negatively correlated with wet season predawn Ψ_{stem} and Ψ_{leaf} ($r = -0.65$; $P = 0.005$, and $r = -0.58$; $P = 0.01$, respectively) and midday Ψ_{stem} ($r = -0.56$; $P = 0.02$; Table 2), but not with any dry season Ψ measurements. Wood density was negatively correlated with wet season predawn and midday Ψ_{stem} ($r = -0.70$; $P = 0.002$, and $r = -0.61$; $P = 0.009$, respectively) and Ψ_{leaf} ($r = -0.71$; $P = 0.001$, and $r = -0.58$; $P = 0.01$, respectively), and dry season midday Ψ_{stem} ($r = -0.60$; $P = 0.03$; Table 2), but not with dry season predawn Ψ_{stem} or Ψ_{leaf}, or midday Ψ_{leaf}.
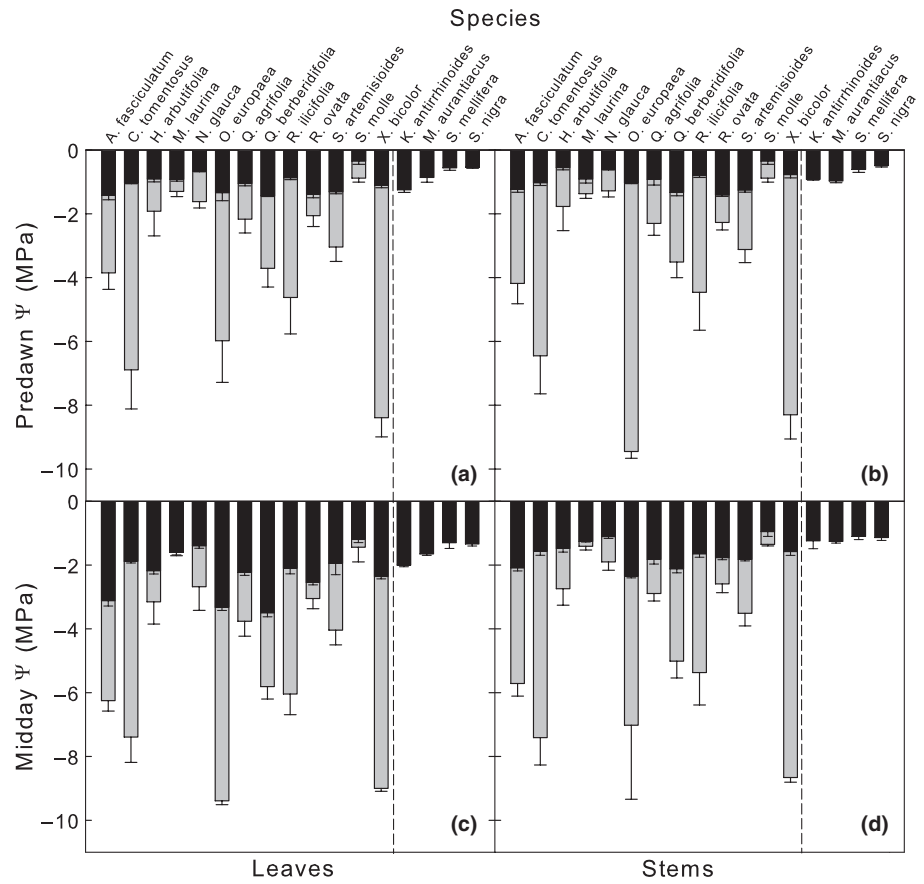
Fig. 1 Predawn and midday stem and leaf water potentials (Ψ) measured for dominant woody species in a California Mediterranean-type climate region during the wet (February; black bars) and dry (September; gray bars) seasons. (a) Predawn Ψ_{leaf}; (b) predawn Ψ_{stem}; (c) midday Ψ_{leaf}; (d) midday Ψ_{stem}. Mean values are reported $\pm$ SE. Water potentials were not measured on deciduous species during the dry season as they had no leaves.
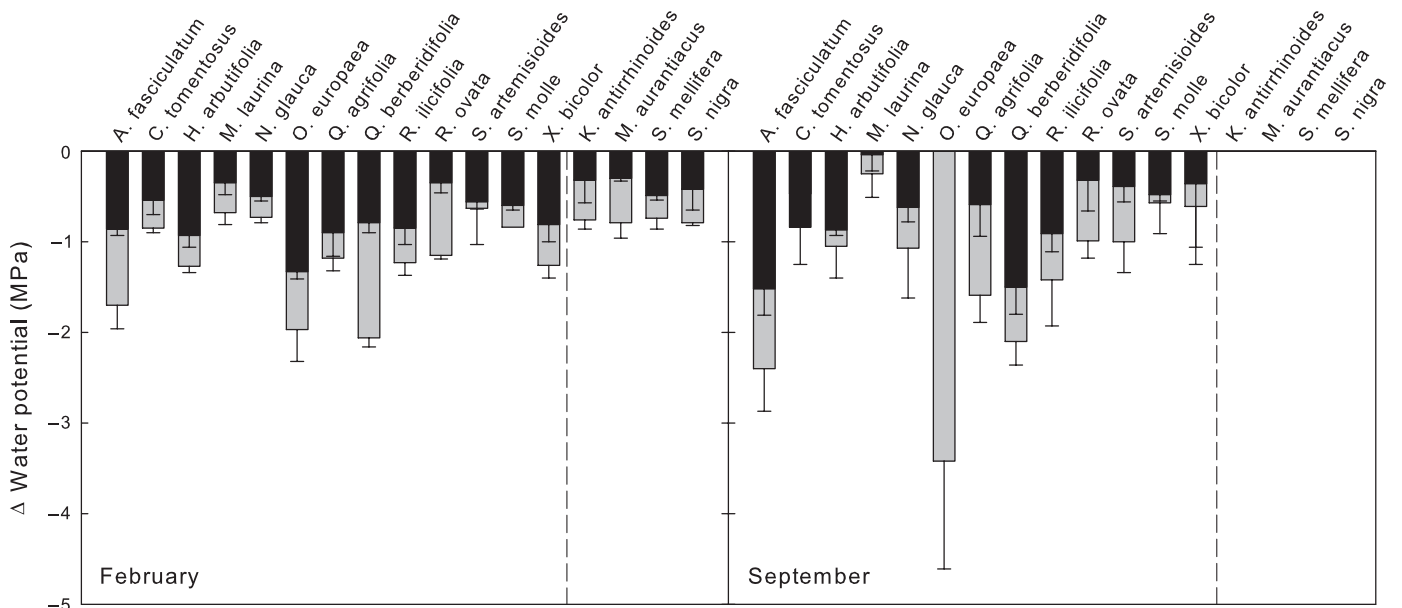

Fig. 2 Change in water potential from predawn to midday for stems ($\Delta\Psi_{stem}$; black bars) and leaves ($\Delta\Psi_{leaves}$; gray bars) during the wet season (February; left panel) and dry season (September; right panel) in a California Mediterranean-type climate region. Mean values are reported $\pm$ SE.

Discussion

We found a negative relationship between K_S and K_{leaf} (Fig. 3), indicating that, for woody shrubs in the California Mediterranean-type ecosystem, species with higher stem conductivity rely on low leaf hydraulic conductance to place limits on water loss during maximum gas exchange and thus act to maintain the water status of basal organs at the expense of distal
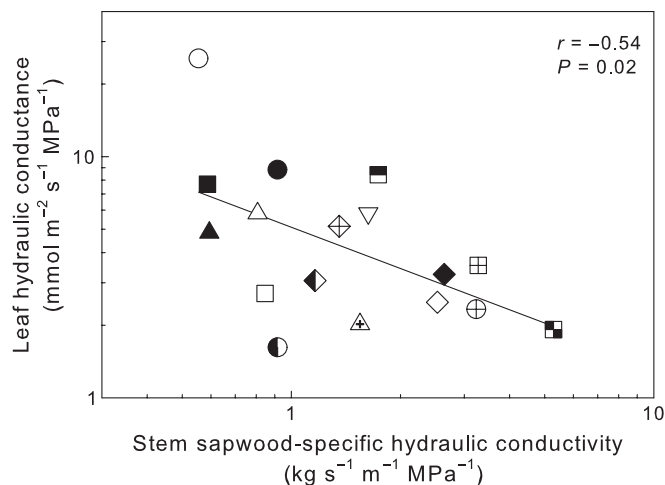
Fig. 3 Relationship between stem sapwood-specific hydraulic conductivity and leaf hydraulic conductance ($n = 17$) in a California Mediterranean-type climate region. Each symbol represents a different species mean. Corresponding species and symbols can be found in Table 1.
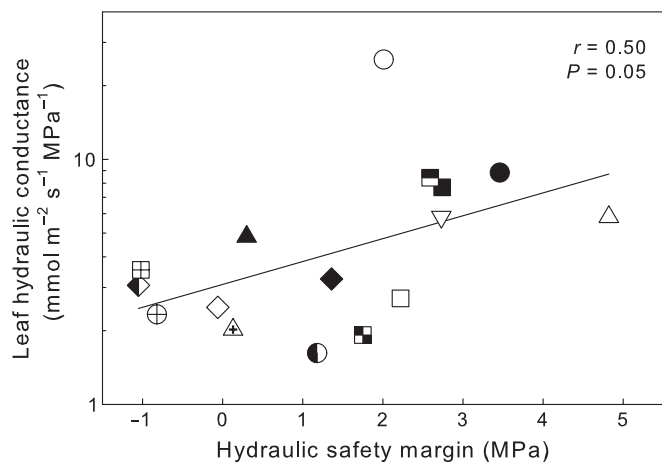
Fig. 4 Relationship between hydraulic safety margin and leaf hydraulic conductance ($n = 16$) in a California Mediterranean-type climate region. Each symbol represents a different species mean. Corresponding species and symbols can be found in Table 1.

organs. The role of leaves as safety valves was further clarified by the positive relationship between the hydraulic safety margin and K_{leaf} (Fig. 4), as species with stems that operate in a more narrow safety range may rely on hydraulic resistance in the leaves to act as a bottleneck to water flow, whereas species that operate within a wider safety range have higher K_{leaf}, consistent with less need of protection. Thus, the resistance to water transport at the leaf level has the potential to protect longer lived and more costly woody stems farther down the soil–plant–atmosphere continuum from reaching critical xylem water potentials that could produce catastrophic hydraulic failure, as hypothesized by Zimmermann (1983) in the original formulation of the HSH.

The inverse arrangement of hydraulic conductances of stems and leaves was related to shoot allometry. We found a negative relationship between K_{leaf} and LA : SA (Fig. 5a), demonstrating a balance between water movement in individual leaves and overall shoot transpirational demand. Plants with overall more leaf area

for a given sapwood area exhibited lower maximum K_{leaf} on an individual leaf area basis, while conversely species with lower LA : SA had higher K_{leaf} as there was less leaf area on a shoot competing for water from the transpirational stream. This finding is consistent with our sampling across species within a community that are subject to approximately the same precipitation and water availability. The negative relationship points to a constraint on total leaf area by the capacity of stems to support leaf water loss among these co-existing species. By contrast, a previous study of six *Nothofagus* species along five sites spanning a precipitation and elevation gradient reported a positive correlation between K_{leaf} and LA : SA (Bucci *et al.*, 2012). Yet, the *Nothofagus* system can receive more than four times the precipitation of the current study and is arranged along an altitudinal gradient, suggesting that altitudinal constraints limit both LA : SA and K_{leaf}. We also found a positive relationship between K_S and LA : SA (Fig. 5b), as previously reported for different species sets (Choat *et al.*, 2011; Gleason *et al.*, 2012). LA : SA is a major determinant of plant hydraulic architecture (Martínez-Vilalta & PrometheusWiki contributors, 2011), consistent with the pipe model theory (Shinozaki *et al.*, 1964), and previous studies have shown similar cases of balancing transpiring leaf area with stem conductive supply through the plant (Meinzer & Grantz, 1990).

Our results indicate large variation in plant water use strategies among co-occurring species. This pattern indicates that withstanding drought in this semi-arid Mediterranean-type climate can be accomplished by adjustment in multiple traits. For example, *S. mellifera* and *K. antirrhinoides* are both deciduous species that drop their leaves during the dry season and physiologically 'shut down'. While they both 'avoid' drought through deciduous leaf phenology, they operate within different safety margins, with *K. antirrhinoides* having a much larger safety margin than *S. mellifera* (Fig. 4). Similarly, *M. laurina* and *X. bicolor* are both evergreen species that 'tolerate' drought by holding onto their leaves and continuing to function during the dry season, but *X. bicolor* operates in a much larger safety margin than *M. laurina* (Fig. 4). As such, our data are consistent with coordination of multiple traits, including water acquisition, stomatal control of water loss, and resistance to cavitation, to maintain hydraulic function (Meinzer & Grantz, 1991; Santiago *et al.*, 2004b; Meinzer *et al.*, 2009; Jones *et al.*, 2010). Thus, species with similarity in one trait can have large variation in other traits, allowing for complex combinations of hydraulic strategy variation that may promote species coexistence at this site (Wright, 2002; Sack *et al.*, 2005; Marks & Lechowicz, 2006; Angert *et al.*, 2009). Additionally, new attention has been focused on the hydraulic safety margin of woody plants (Delzon & Cochard, 2014), with differences in species' susceptibility to mortality found to be related to differences in their hydraulic safety margins (Plaut *et al.*, 2012), although drought-induced tree mortality is a complex process with multiple interacting factors (McDowell *et al.*, 2008; Anderegg *et al.*, 2012). These differences in hydraulic safety margin for co-occurring species at our site may have implications for shifts in community structure under climate change.

As water potential is the driving force behind hydraulic conductance as explained by Ohm's law, we also examined how
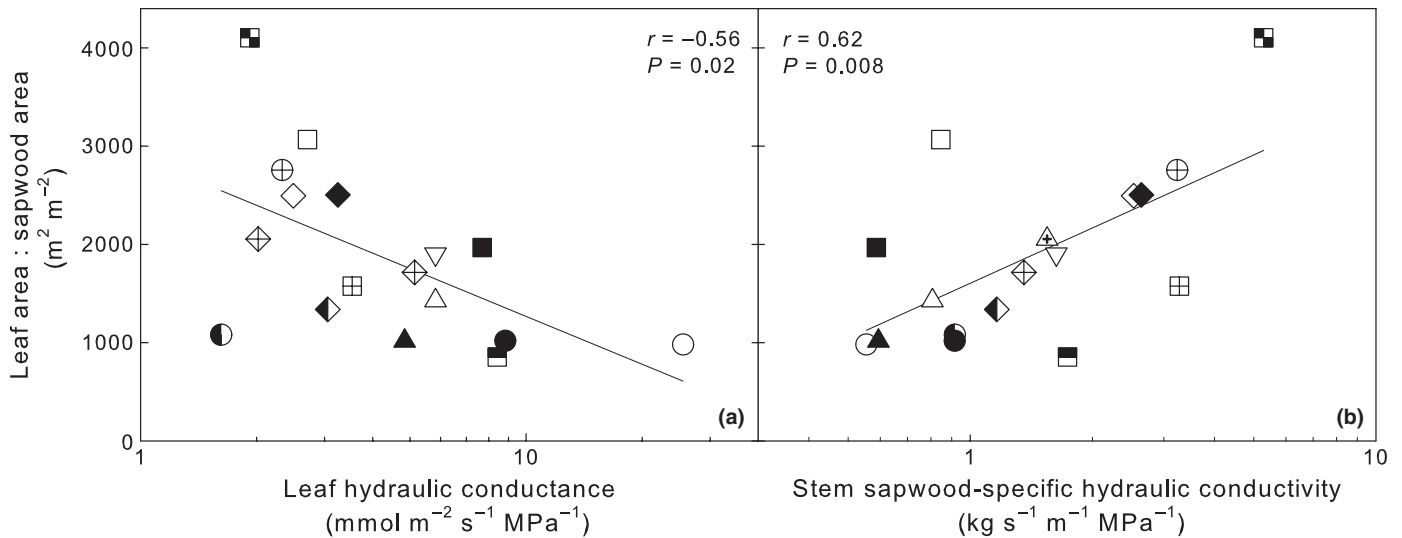
Fig. 5 Relationship between (a) leaf hydraulic conductance and leaf area to sapwood area ratio ($n = 17$), and (b) stem sapwood-specific hydraulic conductivity and leaf area to sapwood area ratio ($n = 17$) in a California Mediterranean-type climate region. Each symbol represents a different species mean. Corresponding species and symbols can be found in Table 1.
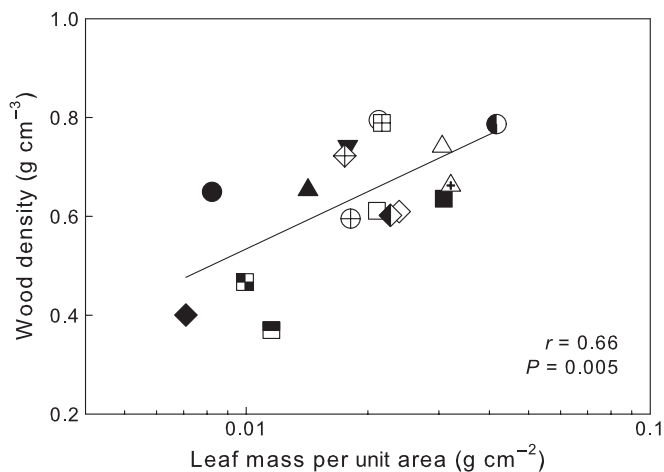
Fig. 6 Relationship between leaf mass per area and wood density ($n = 17$) in a California Mediterranean-type climate region. Each symbol represents a different species means. Corresponding species and symbols can be found in Table 1.

Table 2 Pearson's correlation coefficients between functional traits, including leaf area to sapwood area ratio (LA : SA), wood density, and leaf mass per area (LMA), with measures of leaf and stem water potentials (Ψ_{leaf} and Ψ_{stem}, respectively) for all dominant woody species growing at Santa Margarita Ecological Reserve, California, USA

Trait	LA : SA ($m^2\,m^{-2}$)	Wood density ($g\,cm^{-3}$)	LMA ($g\,cm^{-2}$)
Wet season			
Predawn Ψ_{leaf} (MPa)	$r = 0.48$, $P = 0.05$	$r = -0.71$, $P = 0.001$	$r = -0.58$, $P = 0.01$
Predawn Ψ_{stem} (MPa)	–	$r = -0.70$, $P = 0.002$	$r = -0.65$, $P = 0.005$
Midday Ψ_{leaf} (MPa)	–	$r = -0.58$, $P = 0.01$	–
Midday Ψ_{stem} (MPa)	–	$r = -0.61$, $P = 0.009$	$r = -0.56$, $P = 0.02$
Dry season			
Predawn Ψ_{leaf} (MPa)	–	–	–
Predawn Ψ_{stem} (MPa)	–	–	–
Midday Ψ_{leaf} (MPa)	–	–	–
Midday Ψ_{stem} (MPa)	–	$r = -0.60$, $P = 0.03$	–

water status and water transport capacity were related to LMA and wood density. LMA and wood density are relatively easy-to-measure functional traits that offer a potentially rapid way to characterize physiological tendencies for many species. Yet, how well these traits perform for predicting physiological processes for a particular species varies greatly across sites and species (Wright *et al.*, 2006). Species with high LMA tend to exhibit longer leaf lifespans, relatively low leaf nitrogen concentration, and low maximum photosynthetic rates (Wright *et al.*, 2004), whereas species with high wood density tend to exhibit low maximum hydraulic conductivity (Santiago *et al.*, 2004b) and greater stem xylem resistance to cavitation (Hacke *et al.*, 2001; Delzon *et al.*, 2010). Therefore, our data indicating that LMA and wood density were correlated with each other (Fig. 6) and with various measures of

water stress (Table 2) are consistent with previous findings in the literature (Westoby & Wright, 2006; Wright *et al.*, 2006). Species with higher wood density had higher LMA, showing greater investment in structure of stems and leaves, while species with lower wood density had lower LMA, possibly to maximize resource acquisition at the cost of safe structure. Overall, wood density and LMA were more correlated with wet season than dry season measures of water potential (Table 2), suggesting that wood density and LMA are optimized for resource capture during seasonally brief environmentally favorable conditions. Furthermore, species with more negative wet season water potentials also had greater wood density (Table 2), which is consistent

with previous findings implicating wood density in drought tolerance (Hacke *et al.*, 2001; Delzon *et al.*, 2010; Bucci *et al.*, 2012).

Our result of a negative correlation between water transport in stems and leaves, which supports the HSH, has important implications for plant hydraulic strategies. Suites of traits, including resistance to cavitation, leaf phenology, and rooting depth (Hellmers *et al.*, 1955; Ackerly, 2004), promote tolerance of periods of low water availability, whether on a seasonal basis or during prolonged events. Our findings highlight the role of leaf hydraulics in overall plant drought adaptation. This is especially important in the wake of environmental change, with warmer temperatures leading to global change-type drought and vegetation dieback (Allen, 2009). It should be mentioned that, while we measured *maximum* stem and leaf hydraulic conductance, which may not represent performance achieved in the field, especially as plant water supply declines, such maximum capacity measurements generally scale with integrated exposure to stress and resource availability (Field, 1991). Studying how stem and leaf hydraulic conductivities vary *in vivo* diurnally and seasonally would provide additional understanding of coordination of hydraulic and functional traits, as well as plant ecological strategy variation with respect to balancing water use to maximize growth and maintenance of water potential in the face of seasonal water deficit. Future studies may focus on additional plant growth forms and biomes to address the ecosystem dependence of established site-based patterns (Reich, 1993).

Acknowledgements

References

Ackerly DD. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* 74: 25–44.

Alder N, Pockman WT, Sperry JS, Nuismer S. 1997. Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* 48: 665–674.

Allen CD. 2009. Climate-induced forest dieback: an escalating global phenomenon. *Unasylva* 231: 60.

Anderegg WRL, Berry JA, Smirth DD, Sperry JS, Anderegg LDL, Field CB. 2012. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences, USA* 109: 233–237.

Angert AL, Huxman TE, Chesson P, Venable DL. 2009. Functional tradeoffs determine species coexistence via the storage effect. *Proceedings of the National Academy of Sciences, USA* 106: 11641–11645.

Brodribb TJ, Bowman DJMS, Nichols S, Delzon S, Burlett R. 2010. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytologist* 188: 533–542.

Bucci SJ, Scholz FG, Campanello PI, Montti L, Jimenez-Castillo M, Rockwell FA, Manna LL, Guerra P, Bernal PL, Troncoso O *et al.* 2012. Hydraulic differences along the water transport system of South American *Nothofagus* species: do leaves protect the stem functionality? *Tree Physiology* 32: 880–893.

Chen J-W, Zhang Q, Li X-S, Cao K-F. 2009. Independence of stem and leaf hydraulic traits in six Euphorbiaceae tree species with contrasting leaf phenology. *Planta* 230: 459–468.

Chen J-W, Zhang Q, Li X-S, Cao K-F. 2010. Gas exchange and hydraulics in seedlings of *Hevea brasiliensis* during water stress and recovery. *Tree Physiology* 30: 876–885.

Choat B, PrometheusWiki contributors. 2011. *Hydraulic conductance and conductivity.* [WWW document] URL http://PrometheusWiki [accessed 3 May 2011].

Choat B, Lahr EC, Melcher PJ, Zwieniecki MA, Holbrook NM. 2005. The spatial pattern of air seeding thresholds in mature sugar maple trees. *Plant, Cell & Environment* 28: 1082–1089.

Choat B, Medek DE, Stuart SA, Pasquet-Kok J, Egerton JJG, Salari H, Sack L, Ball MC. 2011. Xylem traits mediate a trade-off between resistance to freeze-thaw-induced embolism and photosynthetic capacity in overwintering evergreens. *New Phytologist* 191: 996–1005.

Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T. 2005. Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia Plantarum* 124: 410–418.

Cowling RM, Ojeda F, Lamont BB, Rundel PW, Lechmere-Oertel R. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeography* 14: 509–519.

Delzon S, Cochard H. 2014. Recent advances in tree hydraulics highlight the ecological significance of the hydraulic safety margin. *New Phytologist*. doi: 10.1111/nph.12798.

Delzon S, Douthe C, Sala A, Cochard H. 2010. Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. *Plant, Cell & Environment* 33: 2101–2111.

Dixon HH, Joly J. 1895. On the ascent of sap. *Philosophical Transactions of the Royal Society of London* 186: 563–576.

Field CB. 1991. Ecological scaling of carbon gain to stress and resource availability. In: Mooney H, Winner WE, Pell EJ, eds. *Response of plants to multiple stresses.* New York, NY, USA: Academic Press, 35–65.

Gleason SM, Butler DW, Ziemińska K, Waryszak P, Westoby M. 2012. Stem xylem conductivity is key to plant water balance across Australian angiosperm species. *Functional Ecology* 26: 343–352.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.

Hao G-Y, Hoffmann WA, Scholz FG, Bucci SJ, Meinzer FC, Franco AC, Cao K-F, Goldstein G. 2007. Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* 155: 405–415.

Hellmers H, Horton JS, Juhren G, O'Keefe J. 1955. Root systems of some chaparral plants in Southern California. *Ecology* 36: 667–678.

Jacobsen AL, Pratt RB. 2012. No evidence for an open vessel effect in centrifuge-based vulnerability curves of a long-vesselled liana (*Vitis vinifera*). *New Phytologist* 194: 982–990.

Jacobsen AL, Pratt RB, Tobin MF, Hacke UG, Ewers FW. 2012. A global analysis of xylem vessel length in woody plants. *American Journal of Botany* 99: 1583–1591.

Johnson DM, McCulloh KA, Meinzer FC, Woodruff DR, Eissenstat DM. 2011. Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. *Tree Physiology* 31: 659–668.

Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC. 2012. Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Science: An International Journal of Experimental Plant Biology* 195: 48–53.

Johnson DM, Woodruff DR, McCulloh KA, Meinzer FC. 2009. Leaf hydraulic conductance, measured *in situ*, declines and recovers daily: leaf hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. *Tree Physiology* 29: 879–887.

Jones TJ, Luton CD, Santiago LS, Goldstein G. 2010. Hydraulic constraints on photosynthesis in subtropical evergreen broad leaf forest and pine woodland trees of the Florida Everglades. *Trees* **24**: 471–478.

Marks CO, Lechowicz MJ. 2006. Alternative designs and the evolution of functional diversity. *The American Naturalist* **167**: 55–66.

Martínez-Vilalta J, PrometheusWiki contributors. 2011. *Water balance traits – leaf to sapwood area ratio.* [WWW document] URL http://PrometheusWiki [accessed 3 May 2011].

McCulloh KA, Johnson DM, Meinzer FC, Woodruff DR. 2014. The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. *Plant, Cell & Environment* **37**: 1171–1183.

McCulloh KA, Sperry JS. 2005. Patterns in hydraulic architecture and their implications for transport efficiency. *Tree Physiology* **25**: 257–267.

McDowell NG, Pockman WT, Allen CD, Breshears DD, Cobb NS, Kolb T, Plaut JA, Sperry JS, West AG, Williams DG *et al.* 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**: 719–739.

Meinzer FC, Grantz DA. 1990. Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant, Cell & Environment* **13**: 383–388.

Meinzer FC, Grantz DA. 1991. Coordination of stomatal, hydraulic, and canopy boundary layer properties: do stomata balance conductances by measuring transpiration? *Physiologia Plantarum* **83**: 324–329.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* **23**: 922–930.

Osazuwa-Peters O, Zanne AE, PrometheusWiki contributors. 2011. *Wood density.* [WWW document] URL http://PrometheusWiki [accessed 30 November 2012].

Pammenter N, Willigen C. 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* **18**: 589–593.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

Plaut JA, Yepez EA, Hill J, Pangle R, Sperry JS, Pockman WT, McDowell NG. 2012. Hydraulic limits preceding mortality in a piñon-juniper woodland under experimental drought. *Plant, Cell & Environment* **35**: 1601–1617.

Reich PB. 1993. Reconciling apparent discrepancies among studies relating life span, structure and function of leaves in contrasting plant life forms and climates: 'The blind men and the elephant retold'. *Functional Ecology* **77**: 721–725.

Sack L, Holbrook NM. 2006. Leaf hydraulics. *Annual Review of Plant Biology* **57**: 361–381.

Sack L, Melcher PJ, Zwieniecki MA, Holbrook NM. 2002. The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* **53**: 2177–2184.

Sack L, Scoffoni C. 2013. Measurement of leaf hydraulic conductance and stomatal conductance and their responses to irradiance and dehydration using the Evaporative Flux Method (EFM). *Journal of Visualized Experiments* **70**: e4179.

Sack L, Tyree MT, Holbrook NM. 2005. Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytologist* **167**: 403–413.

Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff DR, Jones TJ. 2004a. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* **140**: 543–550.

Santiago LS, Kitajima K, Wright SJ, Mulkey SS. 2004b. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. *Oecologia* **139**: 495–502.

Shinozaki K, Yoda K, Hozumi K, Kira T. 1964. A quantitative analysis of plant form – the pipe model theory. *Japanese Journal of Ecology* **14**: 97–105.

Sperry JS. 2000. Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* **104**: 13–23.

Sperry JS, Christman MA, Torres-Ruiz JM, Taneda H, Smirth DD. 2012. Vulnerability curves by centrifugation: is there an open vessel artifact, and are 'r' shaped curves necessarily invalid? *Plant, Cell & Environment* **35**: 601–610.

Sperry JS, Donnelly J, Tyree MT. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment* **11**: 35–40.

Tobin MF, Pratt RB, Jacobsen AL, De Guzman ME. 2012. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. *Plant Biology* **15**: 496–504.

Torres-Ruiz JM, Sperry JS, Fernández JE. 2012. Improving xylem hydraulic conductivity measurements by correcting the error caused by passive water uptake. *Physiologia Plantarum* **146**: 129–135.

Tsuda M, Tyree MT. 1997. Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum. Tree Physiology* **17**: 351–357.

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Ameglio T. 1993. Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell & Environment* **16**: 879–882.

Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345–360.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap.* Berlin, Heidelberg, Germany, New York, NY, USA: Springer.

Vourlitis GL, Pasquini SC. 2009. Experimental dry-season N deposition alters species composition in southern Californian mediterranean-type shrublands. *Ecology* **90**: 2183–2189.

Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098.

Westoby M, Wright IJ. 2006. Land-plant ecology on the basis of functional traits. *Trends in Ecology & Evolution* **21**: 261–268.

Williamson GB, Wiemann MC. 2010. Measuring wood specific gravity. . .correctly. *American Journal of Botany* **97**: 519–524.

Wright IJ, Falster DS, Pickup M, Westoby M. 2006. Cross-species patterns in the coordination between leaf and stem traits, and their implications for plant hydraulics. *Physiologia Plantarum* **127**: 445–456.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Wright SJ. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**: 1–14.

Zimmermann MH. 1978. Hydraulic architecture of some diffuse-porous trees. *Canadian Journal of Botany* **56**: 2286–2295.

Zimmermann MH. 1983. *Xylem structure and the ascent of sap.* Berlin, Germany: Springer-Verlag.