

The stem xylem of Patagonian shrubs operates far from the point of catastrophic dysfunction and is additionally protected from drought-induced embolism by leaves and roots

SANDRA J. BUCCI^{1,2}, FABIAN G. SCHOLZ^{1,2}, MARIA LAURA PESCHIUTTA^{1,2}, NADIA S. ARIAS^{1,2},
FREDERICK C. MEINZER³ & GUILLERMO GOLDSTEIN^{1,4,5}

¹Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), 1917 Buenos Aires, Argentina, ²Grupo de Estudios Biofísicos y Eco-fisiológicos (GEBEF), Departamento de Biología, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, 9000 Comodoro Rivadavia, Argentina, ³USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA, ⁴Laboratorio de Ecología Funcional (LEF), Departamento de Ecología, Genética y Evolución, FCEyN, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina and ⁵Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, USA

ABSTRACT

Hydraulic architecture was studied in shrub species differing in rooting depth in a cold desert in Southern Argentina. All species exhibited strong hydraulic segmentation between leaves, stems and roots with leaves being the most vulnerable part of the hydraulic pathway. Two types of safety margins describing the degree of conservation of the hydraulic integrity were used: the difference between minimum stem or leaf water potential (Ψ) and the Ψ at which stem or leaf hydraulic function was reduced by 50% ($\Psi - \Psi_{50}$), and the difference between leaf and stem Ψ_{50} . Leaf Ψ_{50} – stem Ψ_{50} increased with decreasing rooting depth. Large diurnal decreases in root-specific hydraulic conductivity suggested high root vulnerability to embolism across all species. Although stem Ψ_{50} became more negative with decreasing species-specific Ψ_{soil} and minimum stem Ψ , leaf Ψ_{50} was independent of Ψ and minimum leaf Ψ . Species with embolism-resistant stems also had higher maximum stem hydraulic conductivity. Safety margins for stems were >2.1 MPa, whereas those for leaves were negative or only slightly positive. Leaves acted as safety valves to protect the integrity of the upstream hydraulic pathway, whereas embolism in lateral roots may help to decouple portions of the plant from the impact of drier soil layers.

Key-words: hydraulic conductivity; hydraulic segmentation; leaf water potential; safety margin.

INTRODUCTION

The hydraulic architecture of the entire root-to-leaf pathway in shrubs is less well characterized than that of trees (but see Jacobsen *et al.* 2005; Pratt *et al.* 2007a,b and Hacke, Jacobsen & Pratt 2009). As trees increase in height, gravitational and path-length effects require xylem pressures in their uppermost branches and leaves to become increasingly

negative in order to sustain long-distance water transport (Zimmerman 1983). This vertical trend in stem xylem pressure is typically associated with a corresponding trend of increasing resistance to tension-induced embolism with increasing height (Ryan & Yoder 1997; Woodruff, Bond & Meinzer 2004; Domec *et al.* 2008). However, even if the length of the hydraulic pathway is relatively short as in shrubs, steepening hydrostatic pressure gradients from roots to leaves during periods of high transpiration or low soil water availability can present an increased risk of xylem embolism and reduced water transport capacity (Bucci *et al.* 2009; Scholz *et al.* 2012).

Plants exhibit a range of mechanisms for avoiding or reducing the risk of hydraulic dysfunction. Among these, stomatal regulation of transpiration constrains fluctuations in xylem pressure (Whitehead 1998; Sperry *et al.* 2002; McDowell *et al.* 2008), but at a concomitant cost of reduced CO₂ uptake and consequently reduced growth. An alternative mechanism that could be advantageous in an adaptive context would be to avoid strong stomatal restriction of gas exchange and thereby maximize carbon gain by relying on xylem structural features that prevent or reduce the risk of air-seeded embolism (Hacke, Sperry & Pittermann 2000; Domec *et al.* 2006, 2008). Resistance of xylem to drought-induced embolism varies between species, but also along the hydraulic continuum within the plant. In general, stems are less vulnerable to embolism than leaves and roots (Nardini, Salleo & Raimondo 2003; Choat *et al.* 2005; Pratt *et al.* 2007b; Bucci *et al.* 2008, 2012; Hao *et al.* 2008) resulting in varying degrees of hydraulic segmentation (Tyree & Ewers 1991) that may serve to prevent substantial loss of hydraulic function in woody stems with their high construction costs. Shedding of leaves during seasonal drought can constrain maximum xylem tension and loss of conductivity in stems (Brodribb & Holbrook 2003; Bucci *et al.* 2005). On a shorter timescale, diurnal loss and recovery of leaf hydraulic conductance in some species appears to be integral to a stomatal control mechanism that prevents excessive loss of stem

Correspondence: Sandra J. Bucci. E-mail: sj_bucci@unpata.edu.ar

hydraulic capacity under non-extreme conditions (Bucci *et al.* 2003; Brodribb & Holbrook 2004). Similarly, the degree of stomatal limitation of transpiration in some woody species is coordinated with diurnal and seasonal loss and recovery of root hydraulic conductance (Domec *et al.* 2004, 2006). However, extensive embolism-induced desiccation and death of roots during prolonged droughts, even though restricting reverse water loss into the soil may set woody plants on a trajectory to mortality despite eventual release from drought.

Comparisons of xylem vulnerability to embolism across species and within different parts of the same plant are often based on the xylem pressure corresponding to 50% loss of conductivity (Ψ_{50}). Some studies have reported that stem Ψ_{50} exhibits adaptive adjustments in relation to water availability across a wide range of species (Brodribb & Hill 1999; Pockman & Sperry 2000; Maherali, Pockman & Jackson 2004; Choat *et al.* 2012). However, xylem resistance to embolism in stems seems to be decoupled from precipitation gradients in some species such as riparian, groundwater-dependent (phreatophytic) and drought-deciduous trees in tropical dry forests (Choat *et al.* 2012), suggesting that these species rely principally on mechanisms to avoid embolism-inducing levels of xylem tension rather than on structural reinforcement of their xylem. Plants may also be at considerable risk of catastrophic hydraulic failure even when water availability is not severely restricted. Under these conditions, stomatal regulation of xylem tension plays a critical role in ensuring that hydraulic safety margins are adequate in the xylem pathway upstream (Meinzer *et al.* 2009). Hydraulic safety margins can be defined in terms of differences between typical minimum (most negative) values of xylem pressure experienced in a particular plant organ and critical points on the xylem vulnerability curve for that organ such as Ψ_{50} and Ψ_e , the embolism or air entry threshold (Meinzer *et al.* 2009). Hydraulic safety margins are typically positive, but can be negative, signifying loss of conductivity beyond the vulnerability curve reference point selected for defining the safety margin. Leaves often have negative safety margins, but when they do, they normally undergo daily cycles of loss and full recovery of their hydraulic conductance (Bucci *et al.* 2003; Brodribb & Holbrook 2004; Johnson *et al.* 2009). Alternatively, hydraulic safety margins can be defined in terms of differences between the same point on hydraulic vulnerability curves for adjacent organs such as leaves and stems (Johnson *et al.* 2012).

Differential access to soil water resources can also shape plant hydraulic architecture. Patagonian cold desert shrub species exhibit diverse rooting depths. Pre-dawn leaf water potentials (Ψ_{leaf}) during the peak of the dry season range from -4 MPa in shallow-rooted species to -1 MPa in deep-rooted species (Bucci *et al.* 2009). This range of pre-dawn Ψ_{leaf} suggests that shallow-rooted shrubs of the Patagonian steppe are adapted to utilizing soil water deposited mainly by small rainfall events, whereas the deep-rooted species have access to more abundant and stable water sources. The cost of having an extensive root system contributes to low, but relatively constant growth rates in the deeply rooted species. Because they rely on small rain pulses, the shallow-rooted

species grow relatively fast during a short period of time restricted to spring and early summer growing season (Bucci *et al.* 2009).

The objective of this study was to assess hydraulic architecture along the root-to-leaf pathway in seven dominant cold desert woody species in Southern Patagonia, Argentina. The species differed in rooting depth and therefore in access to soil water resources. We sought to determine the extent to which hydraulic segmentation exists in these small shrub species as manifested by differences in vulnerability to embolism between roots, stems and leaves and in relation to rooting depth. Consistent with the hydraulic segmentation paradigm, we hypothesized that leaves would be more vulnerable to drought-induced embolism compared to stems and roots. We also hypothesized that shallow-rooted species, relying mainly on short rainfall pulses during the summer, have a wider safety margin between leaf and stem Ψ_{50} (leaf Ψ_{50} – stem Ψ_{50}), and consequently are less vulnerable to drought-induced embolism, compared to deeply rooted species.

MATERIALS AND METHODS

Site and species description

The research was carried out at La Dora Ranch in northwest Santa Cruz, Argentina ($46^{\circ} 31' S$ and $71^{\circ} 03' W$), at an elevation of 400 to 420 m.a.s.l. The study sites are located in an area characterized by rolling small hills where the vertical distance between the top of the hills and the lower part of the topography is less than 10 m. Mean annual rainfall is 188 mm falling mostly in the fall and winter (April to September), sometimes in the form of snow, and the mean annual air temperature is $9.0^{\circ} C$. Average summer (December to February) and winter (June to August) temperatures are $14^{\circ} C$ and $3^{\circ} C$, respectively. Soils are generally either gravelly sandy loams or gravelly loamy sands.

The vegetation is typical of a Patagonian shrub steppe characterized by tussock grasses and shrubs. The dominant shrub species are *Mulinum spinosum* (Cav.) Pers, *Adesmia boronioides* J.D. Hooker, *Senecio flaginoides* De Candolle and *Colliguaja integerrima* Gilles et Hooker ex Hooker. Shrubs show a clear-cut seasonal pattern of growth; most of them decrease metabolic activity during winter and exhibit an active growth phase during the spring and summer. Seven dominant shrub species were selected ($n = 3$ to 5 plants per species): *A. boronioides*, *Berberis microphylla* Jussieu Lam; *C. integerrima*, *Schinus johnstonii* Barkley, *Lycium chilense* Miers ex Bertero, *M. spinosum* and *S. flaginoides*. Species were selected to encompass also a wide range of maximum rooting depth (Bucci *et al.* 2009). Table 1 shows rooting depth and height of the selected species. All measurements were done during the dry season (January and February).

Soil, root, stem and leaf water potentials

Pre-dawn and minimum leaf water potential (Ψ_{leaf}) were measured with a pressure chamber (PMS; Albany, OR, USA)

Table 1. Leaf phenology, maximum rooting depth, plant height, leaf area to stem active sapwood area ratio ($A_L:A_s$) and mean vessel lumen diameter in the stems of seven Patagonian shrub species. Values are mean \pm 1 SE ($n = 5$). Values within parenthesis are standard errors. Rooting depth data were extracted from Bucci *et al.* (2009)

Species	Leaf phenology	Rooting depth (m)	Height (cm)	$A_L:A_s$ ($m^2 cm^{-2}$)	Vessel lumen size (μm)
<i>Adesmia boronioides</i>	Evergreen	<2.0	90 (7)	0.080 (0.002)	8.6 (1.6)
<i>Berberis microphylla</i>	Evergreen	>2.0	170 (9)	0.057 (0.006)	9.9 (0.2)
<i>Colliguaja integerrima</i>	Evergreen	<2.0	105 (11)	0.110 (0.009)	9.3 (0.2)
<i>Lycium chilense</i>	Deciduous	<2.0	88 (5)	0.066 (0.003)	9.6 (1.8)
<i>Mulinum spinosum</i>	Deciduous	<1.0	40 (6)	0.0081 (0.009)	15.7 (2.2)
<i>Senecio filaginoides</i>	Evergreen	<1.0	55 (4)	0.074 (0.008)	11.5 (0.2)
<i>Schinus molle</i>	Evergreen	>2.0	210 (15)	0.050 (0.014)	7.6 (1.2)

during February 2011. Ten leafy twigs from different individuals per species were obtained before dawn and at 14 h. Pre-dawn Ψ_{leaf} represents the species-specific soil water potential of the layers explored by the roots (Ψ_{soil}) because previous studies revealed that leaves of these species had no nocturnal transpiration and that Ψ_{leaf} equilibrates nocturnally with Ψ_{soil} (Bucci *et al.* 2009, 2011). To estimate minimum Ψ_{root} and Ψ_{stem} , leaves were enclosed in plastic bags and aluminium foil to prevent transpiration the afternoon before measurements were completed the following day (Domec *et al.* 2006). The covered Ψ_{leaf} reflected Ψ_{stem} which was assumed to be close to Ψ_{root} because the tension gradient in the stem is expected to be of a smaller magnitude than the tension gradients in the leaves.

Root and stem hydraulic conductivity

To measure root- and stem-specific hydraulic conductivity (k_s), we used five large roots and terminal branches per species excised before dawn (0600 h) and in the early afternoon (1400 h). Roots were extracted between 10 and 40 cm soil depth. A small portion of the root and branch segments' cut end was immediately removed by re-cutting under water. The branches were then tightly covered with black plastic bags and transported to the laboratory with the cut ends of the branches under water. Immediately after arriving at the laboratory within 1 h of sample collection, roots and stems segments 10 to 30 cm long were rapidly re-cut under water and attached to a hydraulic conductivity apparatus filled with distilled, filtered (0.22 μm) and degassed water (Tyree & Sperry 1989). Maximum root vessel length was estimated using the method described by Zimmerman and Jeje (1981) and varied from 3.3 cm to 11.7 cm across species. For maximum stem vessel length, we used the information obtained by Bucci *et al.* (2009) for the same study species in the same site as used in the current study. Hydraulic conductivity ($kg m s^{-1} MPa^{-1}$) was calculated as $k_h = J_v / (\Delta P / \Delta X)$, where J_v is the flow rate through the root or stem segment ($kg s^{-1}$) and $\Delta P / \Delta X$ is the pressure gradient across the segment ($MPa m^{-1}$). Specific hydraulic conductivity was obtained by dividing k_h by the active xylem area (A_s). The active xylem area for water transport measured in the middle of stem segments was obtained by introducing indigo carmine dye to stem segments from one cut end.

Stem xylem vulnerability

Hydraulic vulnerability was determined for terminal stems from 3 to 5 plants per species by plotting the percent loss of hydraulic conductivity (PLC) against stem Ψ . Different PLC values were obtained by allowing large branches excised in the morning to dehydrate slowly in air for different time periods (Tyree & Sperry 1989). The stem lengths used were longer (from 5 to 20 cm long depending on the species) than the longest xylem vessels measured by Bucci *et al.* (2009) for these species. After allowing time for partial dehydration, measurements of Ψ_{leaf} on the same branch on leafy twigs adjacent to the stem segments and the corresponding hydraulic conductivity (k_h) were obtained for each stem. The Ψ_{stem} and Ψ_{leaf} were assumed to be in equilibrium because the branches were slowly dehydrated. Maximum hydraulic conductivity ($k_{h,max}$) was obtained by flushing the samples with filtered water at a pressure of 0.2 MPa for 15 min to remove air bubbles from embolized vessels. Percent loss of hydraulic conductivity was calculated as

$$PLC = 100(1 - k_h / k_{h,max})$$

Leaf hydraulic conductance and leaf vulnerability

Leaf hydraulic vulnerability curves were determined by measuring leaf hydraulic conductance (K_{leaf} ; $mmol m^{-2} s^{-1} MPa^{-1}$) using the partial rehydration method described by Brodribb & Holbrook (2003). The measurement is based on the analogy between rehydration of desiccated leaves and charging of a capacitor through a resistor as follows:

$$K_{leaf} = C \ln(\Psi_0 / \Psi_t) / t$$

where C is leaf capacitance, Ψ_0 is leaf water potential before rehydration, and Ψ_t is leaf water potential after rehydration for t seconds. A range of Ψ_{leaf} was attained through slow bench drying of branches collected from the field. Some small branches were previously hydrated for 1 or 2 h to obtain Ψ_{leaf} close to zero. Leaf water potentials for K_{leaf} calculations were then measured after 0.5 to 1 h equilibration inside black plastic bags with slightly humid paper towels. Leaf vulnerability curves were plotted as loss of K_{leaf} (%) against initial Ψ_{leaf} before rehydration.

Capacitance values both before and after turgor loss point (π^0) were calculated from leaf pressure-volume relations previously determined by Scholz *et al.* (2012) for these species in the same study area. Capacitance was expressed in absolute terms and normalized by leaf area ($\text{mmol m}^{-2} \text{MPa}^{-1}$) using the following equation:

$$C = \Delta RWC / \Delta \Psi_L \times (DM/LA) \times (WM/DM) / M$$

Where *RWC* is leaf relative water content, *DM* is leaf dry mass (g), *LA* is leaf area (m^2), *WM* (g) is mass of leaf water at 100% *RWC* ($WM = \text{fresh mass} - \text{dry mass}$) and *M* is molar mass of water (g mol^{-1}).

Wood density and vessel size

Wood density (ρ) was measured on ten terminal branches from each species. After removal of bark and pith, ρ was calculated as: $\rho = M V^{-1}$, where *M* is the dry mass of the sample (oven dried at 60 °C for 72 h) and *V* is the sample volume. Volume was estimated by submerging the sample in a container with distilled water resting on a digital balance with a 0.001 g precision.

Vessel lumen dimensions were measured for five terminal branches. Cross-sections of the stems were mounted in glycerine and images of them were taken using a camera mounted on a microscope. Images were analyzed using the freeware software ImageJ (NIH, Bethesda, MD, USA; <http://rsbweb.nih.gov/ij/>).

Safety margin

To describe the degree of conservation of the integrity of the hydraulic pathway across species, we used two different types of safety margins. One safety margin was defined as the difference between minimum Ψ_{stem} or Ψ_{leaf} and that at which leaf or stem hydraulic function was substantially reduced (stem or leaf Ψ_{50}). This safety margin can be positive or negative depending on whether operating ranges of Ψ_{leaf} and Ψ_{stem} are above or below their values of Ψ_{50} .

Another safety margin that considers the hydraulic traits of different organs connected in series within plant was quantified as the difference between water potential at 50% loss of leaf hydraulic conductance and water potential at 50% loss of stem hydraulic conductance (i.e. leaf $\Psi_{50} - \text{stem } \Psi_{50}$; Johnson *et al.* 2012). This safety margin was determined to evaluate whether the leaves were vulnerable enough to protect the stems from hydraulic dysfunction.

Statistical analysis

The SPSS 11.5 statistical package (SPSS, Chicago, IL, USA) was used for statistical analysis. A one-way analysis of variance (ANOVA) was used to test diurnal loss of hydraulic capacity for differences between roots, stems and leaves, and within each organ for difference among groups of rooting depth and vessel size for differences between species. To test for differences in average Ψ_{50} between stems and leaves across species

and in average root k_s and average root k_s , a Student's *t*-test was performed. Linear regressions were fitted using Sigma Plot 11 software (Systat Software, Inc., Chicago, IL, USA).

RESULTS

There were species-specific differences in stem vulnerability to embolism during the summer with the water potential at 50% loss of maximum stem hydraulic conductivity (stem Ψ_{50}) ranging from -3.6 MPa in *S. johnstonii* to -5.7 MPa in *M. spinosum* (Fig. 1). Leaf hydraulic vulnerability curves also differed among species (Fig. 2) with Ψ_{leaf} at 50% loss of K_{leaf} (leaf Ψ_{50}) ranging from -2.6 MPa in *S. filaginoides* to -3.2 MPa in *B. microphylla*. Species-specific values of Ψ_{50} also showed a smaller range of variation in leaves (0.6 MPa) than in stems (2.1 MPa). Estimated losses of K_{leaf} at minimum Ψ_{leaf} *in situ* ranged from 40% in *S. johnstonii* to 81% in *M. spinosum* (Fig. 2).

Average values of stem Ψ_{50} and leaf Ψ_{50} across all seven species differed significantly from each other (Student's *t*-test; $t = 6.73$; $P < 0.001$). Figure 3 shows that there was no correlation between species-specific stem Ψ_{50} and leaf Ψ_{50} ; however, all species had leaves more vulnerable to loss of hydraulic conductance than stems. Leaf Ψ_{50} was similar across species with an average value of about -3.0 MPa. The hydraulic safety margin between leaf and stem Ψ_{50} (leaf $\Psi_{50} - \text{stem } \Psi_{50}$) varied across species and with the species-specific soil water potential (Ψ_{soil}) estimated from the pre-dawn Ψ of leaves covered to prevent disequilibrium between leaf and soil Ψ induced by nocturnal transpiration (Fig. 4a). At one extreme, the more deeply rooted *S. johnstonii* (Table 1) had access to more readily available soil water sources and exhibited a narrow safety margin (0.8 MPa). At the other extreme, the wide safety margin of *M. spinosum* (2.7 MPa) was associated with its shallow roots being restricted to drier upper soil layers. The variation in leaf $\Psi_{50} - \text{stem } \Psi_{50}$ across species was mainly a consequence of interspecific variation in stem Ψ_{50} with variations in species-specific Ψ_{soil} because leaf Ψ_{50} was unrelated to Ψ_{soil} (Fig. 4b).

Stem Ψ_{50} was positively related to the minimum Ψ_{stem} estimated from covered leaves in the early afternoon (Fig. 5a), whereas leaf Ψ_{50} was not significantly correlated with minimum Ψ_{leaf} (Fig. 5b). For all species, minimum Ψ_{stem} (Fig. 5a) was higher (less negative) than minimum Ψ_{leaf} (Fig. 5b). The safety margin defined as $\Psi_{\text{stem}} - \text{stem } \Psi_{50}$ was relatively large and similar among species (the horizontal distance between the linear regression and the 1:1 line in Fig. 5a). In contrast, the analogous safety margin assessed for leaves (minimum $\Psi_{\text{leaf}} - \text{leaf } \Psi_{50}$) was relatively small and varied depending on species and Ψ_{soil} (Fig. 6). Three species, *C. integerrima* and *S. johnstonii* and *B. microphylla* had very small but positive leaf hydraulic safety margins (< 0.3 MPa), whereas other species such as *M. spinosum* and *S. filaginoides* had negative safety margins (Figs 5b & 6).

Vessel lumen size varied significantly among species (one-way ANOVA, $F = 10.33$; $P < 0.001$) and ranged from $7.6 \mu\text{m}$ in *S. johnstonii* to $15.7 \mu\text{m}$ in *M. spinosum* (Table 1) and was negatively correlated with wood density ($R^2 = 0.63$,

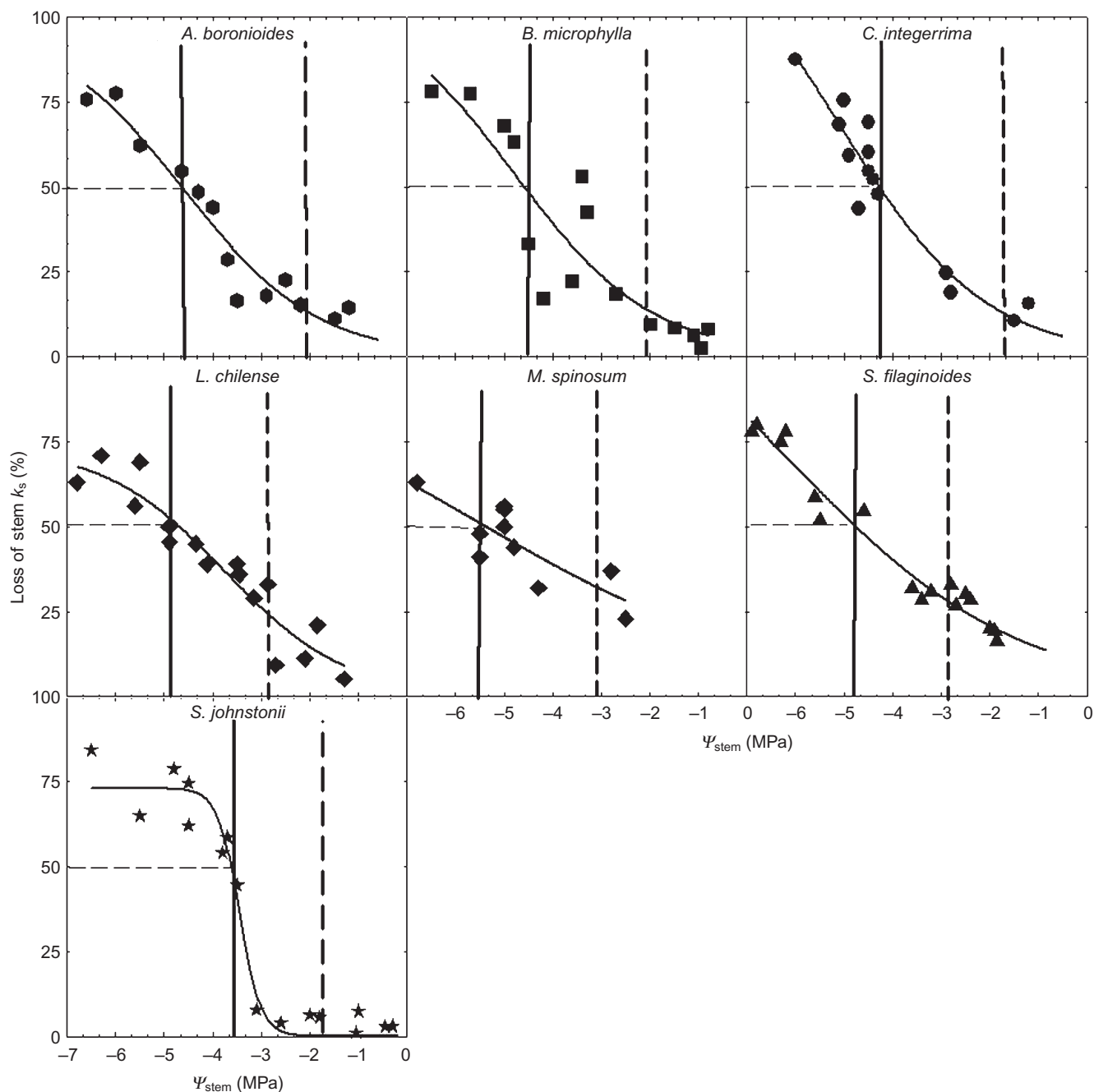


Figure 1. Stem hydraulic vulnerability curves expressed as percentage loss of stem-specific hydraulic conductivity (k_s) as a function of xylem water potential of seven shrub species during the summer. Sigmoid functions were fitted to the data ($R^2 > 0.9$; $P < 0.05$). In each panel, the water potential at 50% loss of maximum stem k_s (stem Ψ_{50} ; solid line) and minimum Ψ_{stem} , estimated from leaf water potential of covered leaves (dashed line) are indicated as vertical lines intersecting the x -axis.

$P < 0.001$). There was a negative relationship between stem Ψ_{50} and pre-dawn stem k_s (Fig. 7a) and a positive correlation between stem Ψ_{50} and wood density (Fig. 7b). The species with the lowest stem k_s (*S. johnstonii*) had the least negative stem Ψ_{50} (-3.6 MPa) and the densest wood (0.91 g cm $^{-3}$), whereas *M. spinosum* with the highest k_s exhibited the lightest wood (0.36 g cm $^{-3}$) and the lowest stem Ψ_{50} (-5.7 MPa). Stem k_s was positively correlated with vessel lumen diameter across species ($R^2 = 0.67$; $P < 0.05$).

Pre-dawn K_{leaf} increased linearly with species-specific Ψ_{soil} and therefore with increasing rooting depth (Fig. 8a). Pre-dawn stem k_s exhibited an opposite trend, decreasing linearly with increasing rooting depth as estimated from species-specific Ψ_{soil} (Fig. 8b). No significant relationship between pre-dawn root k_s and species-specific Ψ_{soil} was observed (Fig. 8c). Significant differences were found in average values of stem k_s and root k_s across all species between stems and roots (Student t -test; $t = -3.14$; $P < 0.05$).

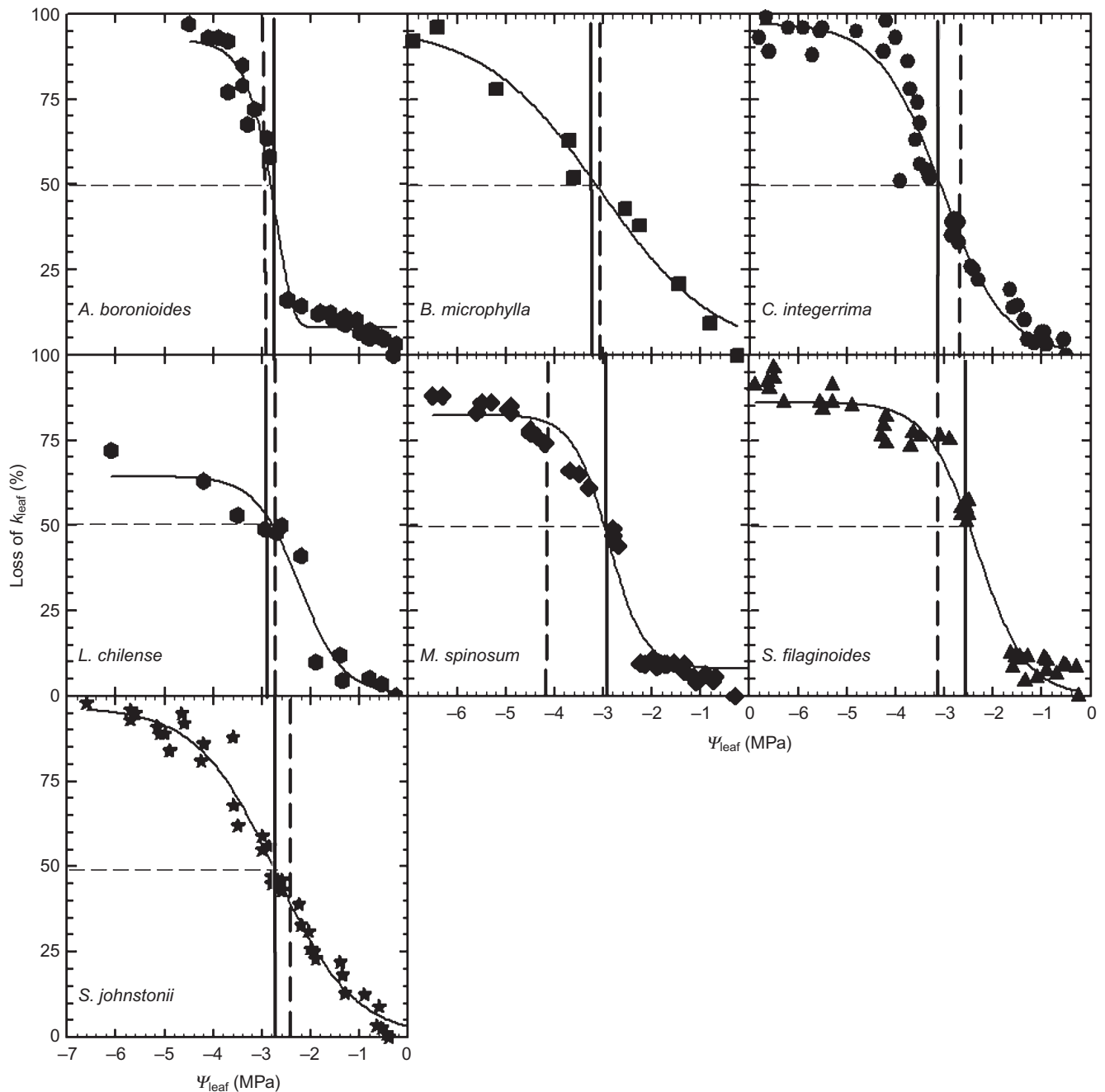


Figure 2. Leaf hydraulic vulnerability curves expressed as percentage loss of leaf hydraulic conductance (K_{leaf}) as a function of leaf water potential of seven shrubs species during summer. Sigmoid function curves were fitted to the data ($R^2 > 0.80$; $P < 0.05$). In each panel, the water potential at 50% loss of maximum leaf hydraulic conductance (solid line) and minimum Ψ_{leaf} (dashed line) are indicated as vertical lines intersecting the x -axis.

Hydraulic conductivity declined diurnally in roots, stems and leaves (Fig. 9a,b). Stem k_s decreased by 8 to 21% from pre-dawn to the early afternoon across species (Fig. 9a), whereas root k_s and K_{leaf} exhibited diurnal declines up to 69 and 85%, respectively, across species (Fig. 9b). There was no significant relationship between diurnal decline in K_{leaf} and leaf Ψ_{50} (Fig. 9a). In contrast, to leaves, the diurnal decline in stem k_s was negatively correlated with stem Ψ_{50} (Fig. 9a).

Diurnal declines in root k_s , stems k_s and K_{leaf} across species increased with decreasing minimum Ψ_{root} , Ψ_{branch} and Ψ_{leaf} , respectively (Fig. 9b).

There were significant correlations between diurnal declines in stem k_s and K_{leaf} with species-specific Ψ_{soil} across species (for stems $R^2 = 0.74$; $P < 0.01$; for leaves $R^2 = 0.76$; $P < 0.01$), with species exploring wettest soil layers showing smaller diurnal declines in stem k_s and K_{leaf} , and species with

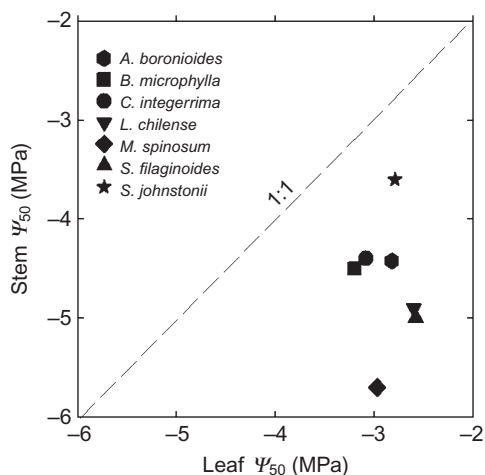


Figure 3. Leaf water potential at 50% loss of maximum leaf hydraulic conductance (leaf Ψ_{50}) in relation to stem water potential at 50% loss of maximum stem hydraulic conductivity (stem Ψ_{50}). The dashed line represents the 1:1 relationship between the two variables.

roots growing in soil layers with more negative Ψ_{soil} exhibiting larger diurnal losses in k_s (one-way ANOVA; $F = 18.9$; $P < 0.05$) and K_{leaf} (one-way ANOVA; $F = 19.9$; $P < 0.001$). A significant relationship between diurnal decline in k_s and species-specific Ψ_{soil} was not observed for roots (data not shown) but there was a significant effect of rooting depth on the loss of root k_s with shallow-rooted species exhibiting higher loss of k_s than deep-rooted species (one-way ANOVA; $F = 7.59$; $P < 0.05$).

DISCUSSION

Differences in vulnerability to embolism between leaves, stems and roots

Xylem hydraulic safety margins in stems of the Patagonian shrubs studied here were generally larger than those reported in a survey of angiosperm trees (Choat *et al.* 2012). Although the hydraulic architecture of shrubs is not as well studied as that of trees, the range of vulnerability to embolism in stems of the species studied here (-3.6 to -5.7 MPa) was similar to that found in woody species of other desert ecosystems (Hacke *et al.* 2000; Maherali *et al.* 2004; Jacobsen *et al.* 2007).

The water potential at 50% of loss of hydraulic conductance in leaves was higher than -3.2 MPa for all of the Patagonian shrub species studied, confirming our hypothesis that their leaves would be more vulnerable to drought-induced loss of hydraulic function than their stems. Stems of deciduous species might be expected to show greater vulnerability to embolism than those of evergreen species because leaf shedding can mitigate the impact of drought on xylem tension (Brodribb *et al.* 2003). However, we found the opposite pattern, with stems being more vulnerable to hydraulic dysfunction in evergreen species. The leaves of all the study

species were equally vulnerable to loss of hydraulic conductance independent of leaf phenology, leaf life span and rooting depth. The safety margin between leaves and stems (leaf Ψ_{50} – stem Ψ_{50}) increased with decreasing rooting depth (decreasing soil water availability) from 0.8 to 2.7 MPa. This range is roughly similar to that found by Johnson *et al.* (2012) who studied 24 species of angiosperms, but the mean value across species in the present study was substantially higher (1.8 MPa compared with 0.8 MPa found by Johnson *et al.* 2012). The pattern of differences between leaf and stem hydraulic safety observed across Patagonian woody species indicates that the stems of species exploring more abundant and constant soil water sources were less protected from hydraulic dysfunction by leaves than stems of shallow-rooted species. The wider leaf-to-stem safety margin in shallow-rooted species is consistent with the need to conserve the stem hydraulic integrity to allow production of new leaves and fine roots during the driest periods when sporadic rainfall pulses transiently increase water availability in the shallowest soil layers. Additionally, shallow-rooted Patagonian

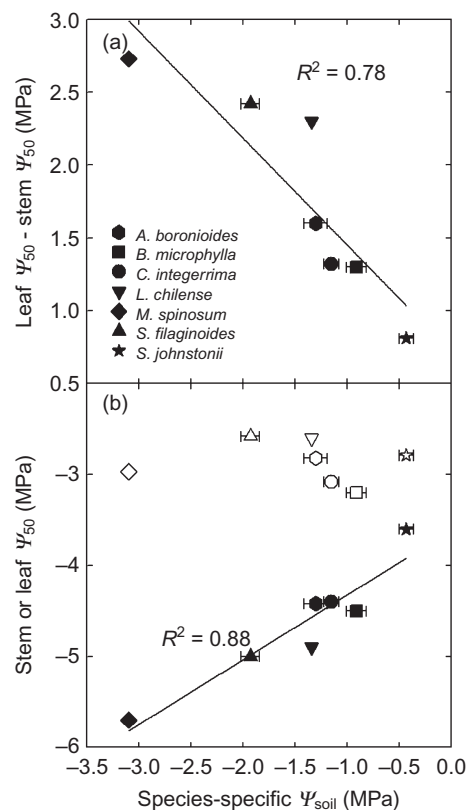


Figure 4. (a) Safety margin between leaf Ψ_{50} and stem Ψ_{50} (leaf Ψ_{50} – stem Ψ_{50}) in relation to species-specific soil water potential (Ψ_{soil}) estimated from the pre-dawn water potential of covered leaves. (b) Relationship between stem (closed symbols) or leaf (open symbols) water potential at 50% loss of maximum stem or leaf hydraulic conductance (stem Ψ_{50} and leaf Ψ_{50}) and species-specific Ψ_{soil} . The lines are linear regressions fitted to the data (a: $y = 0.65 - 0.74x$; $P < 0.005$; b: $y = -3.61 + 0.7x$; $P < 0.05$). No significant relationship was observed for leaves in (b).

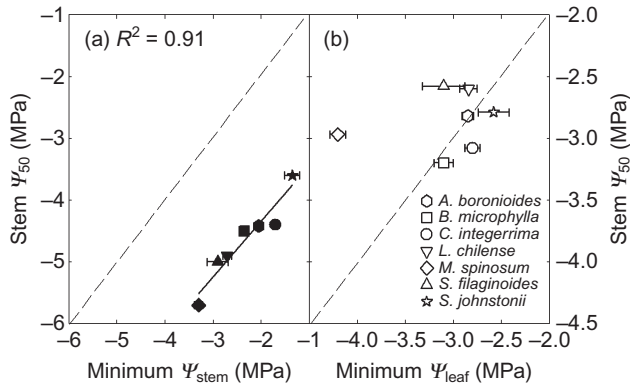


Figure 5. (a) Stem water potential at 50% loss of maximum stem hydraulic conductivity (stem Ψ_{50}) in relation to minimum stem water potential (Ψ_{stem}) observed in the field and (b) leaf water potential at 50% loss of maximum leaf hydraulic conductivity (leaf Ψ_{50}) in relation to minimum leaf water potential (Ψ_{leaf}) observed in the field. The solid line in (a) represents the linear regression fitted to the data ($y = 3.1 + 1.1x$; $P < 0.001$). No significant relationship was observed for leaves in (b). The dashed lines in both panels indicate the 1:1 relationship between variables.

shrubs are anisohydric (Bucci *et al.* 2009; Scholz *et al.* 2012) and consequently would require larger safety margins as an insurance against hydraulic failure in stems during periods of particularly severe drought.

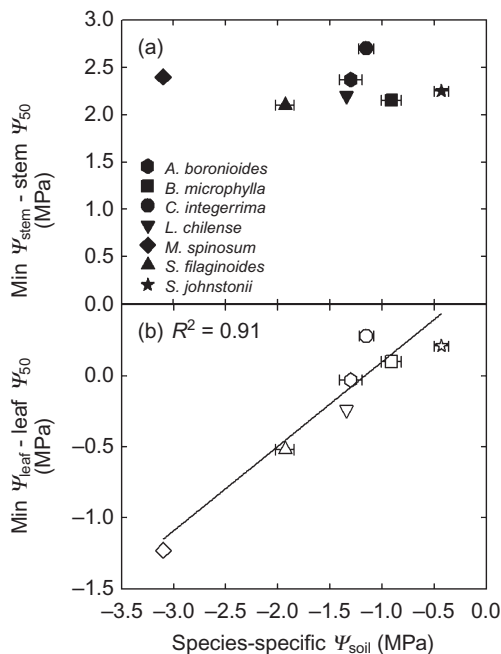


Figure 6. (a) Safety margin between minimum Ψ_{stem} and stem water potential at 50% loss of maximum hydraulic conductivity (min $\Psi_{\text{stem}} - \text{stem } \Psi_{50}$) in relation to species-specific soil water potential (Ψ_{soil}) estimated from the pre-dawn water potential of covered leaves. (b) Safety margin between minimum Ψ_{leaf} and leaf water potential at 50% loss of maximum hydraulic conductance (min $\Psi_{\text{leaf}} - \text{leaf } \Psi_{50}$) in relation to species-specific Ψ_{soil} . The line in (b) is the linear regression fitted to the data ($y = 0.49 + 0.49x$; $P < 0.05$).

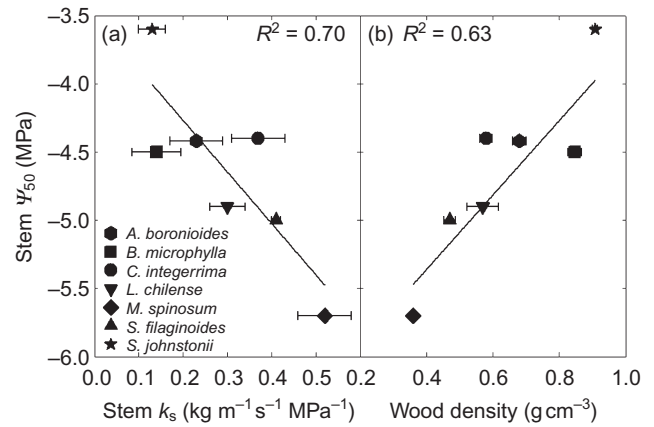


Figure 7. (a) Stem water potential at 50% loss of maximum stem hydraulic conductivity (stem Ψ_{50}) in relation to pre-dawn stem-specific hydraulic conductivity (stem k_s) and (b) stem Ψ_{50} in relation to wood density. The solid lines represent the linear regressions fitted to the data: (a) $y = -3.51 - 3.8x$; $P < 0.05$; (b) $y = -6.75 + 2.72x$; $P < 0.05$. Each point represents the mean value ± 1 SE of five individuals per species for stem k_s and wood density.

Vulnerability to embolism in roots was not measured in this study; however, the substantial diurnal loss of k_s observed in lateral roots suggests that roots had lower xylem resistance to loss of function than stems. This result may appear to contradict predictions of the hydraulic segmentation theory (Zimmerman 1983) that peripheral parts of the crown (i.e. leaves) should experience xylem cavitation first while the integrity of the rest of the hydraulic pathway is conserved (Tyree & Ewers 1991). Nevertheless, patterns of hydraulic vulnerability in Patagonian cold desert shrubs appear to be consistent with hydraulic segmentation in that partial loss of hydraulic function in shallow roots during periods of drought would enhance hydraulic isolation of the plant from drier soil layers (hydraulic rectification *sensu* Nobel & Sanderson 1984), thereby limiting reverse water flow via hydraulic redistribution from deeper roots to shallow lateral roots and into shallow soil layers. Studies evaluating the presence of hydraulic redistribution in these species are consistent with this explanation because reverse flow in roots was not detected with heat pulse methods even in species with dimorphic root systems that explored soil layers with different Ψ_{soil} (Scholz *et al.* unpublished observations). Alternatively, the high vulnerability of roots to loss of hydraulic function could be compensated by their high inherent water transport capacity (an order of magnitude higher than stem k_s) such that the fraction of root k_s remaining in the early afternoon is adequate to satisfy transpirational demands. Similar results comparing the vulnerability to embolism between stems and roots have been found by Hacke & Sauter (1996) in *Populus balsamifera* L and *Alnus glutinosa*, McElrone *et al.* (2004) in *Juniperus ashei*, *Bumelia lanuginosa*, *Quercus fusiformis* and *Q. sinuata* and by Pratt *et al.* (2007a) in Rhamnaceae species.

Overall, our results suggest that leaves and roots of Patagonian shrubs could act as hydraulic valves by preventing

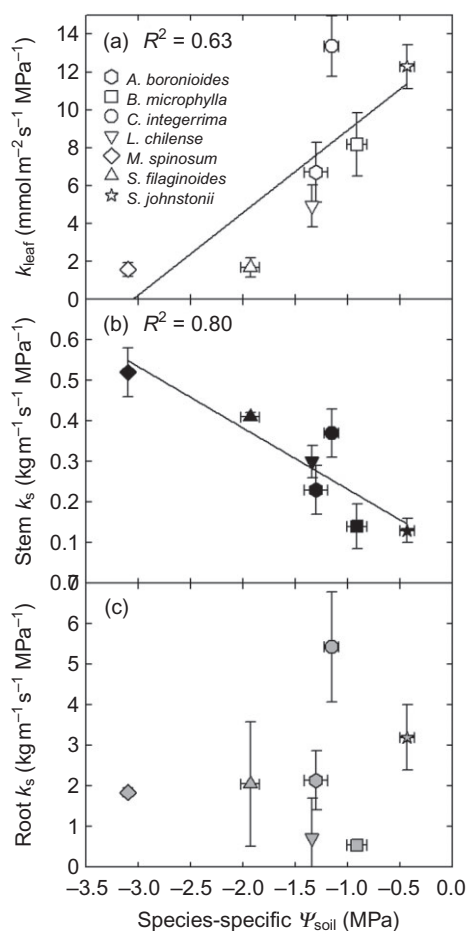


Figure 8. (a) Pre-dawn leaf hydraulic conductance (K_{leaf}), (b) Pre-dawn stem-specific hydraulic conductivity (stem k_s) and (c) Pre-dawn root-specific hydraulic conductivity (root k_s) in relation to species-specific soil water potential (Ψ_{soil}) estimated from the pre-dawn water potential of covered leaves. Values are means \pm 1 SE of five individuals per species.

stem hydraulic dysfunction. Loss of hydraulic function and consequent stomatal restriction of transpiration in leaves prevented stems from reaching levels of xylem tension that would cause substantial embolism, whereas loss of hydraulic function in roots partly decoupled the plants from the influence of drier shallow soil layers.

Trade-off between hydraulic safety and efficiency

Xylem that is both safe and efficient should be optimal for plants subjected to restricted soil water availability. However, traits that enhance resistance to embolism can reduce water transport capacity resulting in a potential conflict between safety and efficiency (Meinzer *et al.* 2010). Reports on the extent to which this compromise between hydraulic safety and efficiency exists vary in their conclusions (Kavanagh *et al.* 1999; Piñol & Sala 2000; Martínez-Vilalta *et al.* 2002; Wheeler *et al.* 2005; Bucci *et al.* 2012; Nardini, Pedà & La Rocca 2012). A compromise between safety and efficiency

was not observed in this study because the species with higher stem hydraulic safety (lower stem Ψ_{50}) had a more efficient stem water transport system (higher stem k_s). This finding suggests that resistance to loss of function in the water transport tissues of cold desert shrubs could be more associated with pit traits (e.g. porosity and thickness of pit membranes; Choat, Cobb & Jansen 2008; Lens *et al.* 2011) than to vessel size (Hacke *et al.* 2001). Vessel diameter, which influences the resistance to water flow, appears to be decoupled from pit characteristics, which hypothetically determine vulnerability to hydraulic failure (Hacke *et al.* 2009). The species with larger vessel diameters, such as *M. spinosum* and *S. filaginoides* had high resistance to embolism. Sperry & Hacke (2004) found that species with high cavitation resistance and thus less conductive pits do not necessarily have reduced overall xylem conductivity if conduit width and length increase to overcome the added pit resistance. Similarly to the stems, we did not find a trade-off across species at leaf level between K_{leaf} (on a area basis) and leaf Ψ_{50} , consistent with finding of Sack & Holbrook (2006); Chen *et al.* (2009); Blackman, Brodribb & Jordan (2010); and Nardini *et al.* (2012).

Whereas there was no trade-off between water transport efficiency and resistance to hydraulic disruption in stems across species, we found strong evidence for a trade-off between stem wood density and stem k_s across species. High wood density can have a greater relative impact on

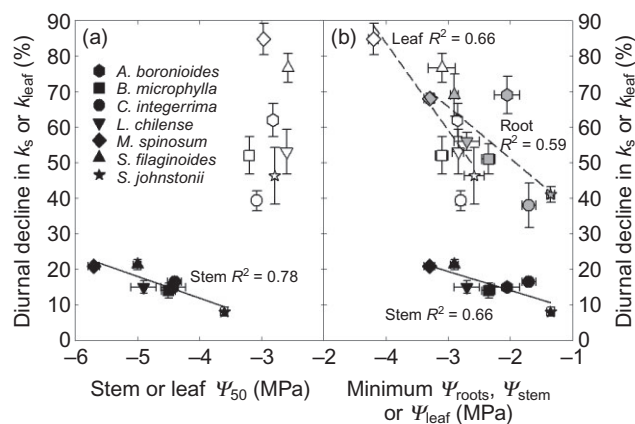


Figure 9. (a) Diurnal decline in stem-specific hydraulic conductivity (stem k_s , black symbols) and in leaf hydraulic conductance (K_{leaf} , open symbols) measured between pre-dawn and early afternoon in relation to stem or leaf water potential at 50% loss of maximum conductance (stem Ψ_{50} and leaf Ψ_{50} , respectively) and (b) diurnal decline in root-specific hydraulic conductivity (root k_s , grey symbols), stem-specific hydraulic conductivity (stem k_s , black symbols) and leaf hydraulic conductance (K_{leaf} , open symbols) measured between pre-dawn and early afternoon in relation to the minimum root (Ψ_{root}), stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials, respectively. Solid lines are the linear regressions fitted to the data: (a) $y = -9.7 - 5.3x$; $P < 0.05$; (b) stem: $y = 3.37 - 5.52x$; $P < 0.05$; root: $y = 22 - 14x$; $P = 0.06$; leaf: $y = 1.43 - 1.45x$; $P = 0.08$. There was no significant relationship between diurnal decline in K_{leaf} and leaf Ψ_{50} . Solid lines indicate significant regressions at $P \leq 0.05$ and dashed lines in (b) indicate marginally significant regressions at $P < 0.1$.

mechanical reinforcement than hydraulic safety in stems at the expense of reduced hydraulic conductivity (Pittermann *et al.* 2006; Wilson & Jackson 2006). Lower k_s and higher wood density found in taller, more deeply rooted species in this study (*S. johnstonii* and *B. microphylla*) possibly reflects a greater selective pressure for mechanical support to resist the frequent and strong winds in the Patagonian region, than for a highly efficient water transport system because these species have access to more stable and abundant soil water sources throughout the year. In addition, these species exhibited a larger soil-to-leaf water potential difference, which would partially compensate for their lower stem k_s (Bucci *et al.* 2009).

Are hydraulic vulnerability and diurnal variation in hydraulic conductance related to minimum water potential and rooting depth?

A key component in the drought resistance of woody species is their ability to limit embolism formation, which can be achieved by building a water transport pathway that is resistant to hydraulic failure, or by the avoidance of high xylem tensions. Rooting depth and thus the species-specific soil water availability was strongly correlated with stem Ψ_{50} across the Patagonian cold desert woody species studied here. The trend of stem vulnerability to hydraulic dysfunction across species in relation to rooting depth is consistent with the general trend observed in the literature where species in more xeric environments tend to have higher resistance to loss of hydraulic function compared to species from mesic sites. Stems were not only protected by leaves acting as hydraulic circuit breakers, but they also operated at minimum values of Ψ_{stem} far from thresholds for embolism formation across all species. Minimum Ψ_{stem} was correlated with stem Ψ_{50} as has been observed for other species (Pockman & Sperry 2000; Bhaskar, Valiente-Banuet & Ackerly 2007; Jacobsen *et al.* 2007; Pratt *et al.* 2007a; Choat *et al.* 2012), but was independent of species-specific Ψ_{soil} . Positive values of minimum Ψ_{stem} – stem Ψ_{50} likely reflect the role of stomatal regulation in preventing the excessive drop of xylem pressure (Meinzer *et al.* 2009). In a previous study (Scholz *et al.* 2012), we had determined that stomatal closure between morning and the time when minimum Ψ_{leaf} is reached varies between 20 and 60% of its maximum in these deep-rooted and shallow-rooted species, respectively. In addition to stomatal control, the leaf turgor loss observed in the species with shallow-rooted species (Scholz *et al.* 2012) could contribute to avoid large drop of Ψ_{stem} and thus to have high stem safety margins. We suggest that the relatively large safety margins observed in the present study may be related to unpredictable climate fluctuations, in the sense that these plants can be exposed to drought periods even more severe than those that were observed during the study. In addition, wide stem safety margins may ensure that CO_2 assimilation is maintained as long as possible during the dry period in species with limited access to soil water sources. For the deep-rooted species with high wood density ($>0.8 \text{ g cm}^{-3}$ for *B. microphylla* and *S. johnstonii*) and access to more

abundant soil water sources, a broad safety margin may be associated with the protection of a more costly carbon investment in their hydraulic system. A recent study (Choat *et al.* 2012) reported that stems of 70% of 226 forest species from 81 sites worldwide operate with narrow hydraulic safety margins ($<1 \text{ MPa}$) against catastrophic xylem dysfunction, suggesting that unless they have a high capacity for embolism reversal, those species could experience reductions in productivity and survival if temperature and aridity increase as predicted for many regions across the globe (IPCC 2007). Safety margins found in that study were largely independent of mean annual precipitation with all forest biomes being equally vulnerable to hydraulic failure regardless of their current rainfall regime. In contrast, the mean stem hydraulic safety margin of the seven shrub species studied here was 2.4 MPa, considerably higher than those reported in Choat *et al.*'s (2012) study.

The large diurnal loss of leaf and root hydraulic capacity observed in Patagonian shrubs (40 to 87% for leaves and 38 to 69% for roots) implies that these species are able to rapidly reverse loss of hydraulic function as has been reported for leaves (Bucci *et al.* 2003; Johnson *et al.* 2009) and roots (Domec *et al.* 2006) of several other species. Determination of leaf hydraulic vulnerability curves, particularly for deep-rooted species, indicated that at pre-dawn, the cumulative losses of K_{leaf} were small. It is noteworthy that although minimum Ψ_{leaf} ranged from -2.6 to -4.2 MPa , the range of leaf Ψ_{50} was much smaller (-2.6 to -3.2 MPa) resulting in positive leaf hydraulic safety margins for some species and negative safety margins for others. Thus, negative hydraulic safety margins for leaves may be more of an indicator of their tendency to function as hydraulic circuit breakers that can be reset overnight rather than an indicator of their susceptibility to catastrophic hydraulic failure.

In conclusion, leaves acted as safety valves to protect the integrity of the upstream hydraulic pathway, whereas embolism in lateral roots served to impede reverse water flow from the plant to dry soil, thereby decoupling portions of the plant from the impact of drier soil layers. Hydraulic segmentation in Patagonian shrubs appears to be the result of strong selective pressures to protect stems from hydraulic dysfunction during prolonged droughts.

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