

Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa

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Summary

1 Climate change in South Africa may threaten the sclerophyllous evergreen shrubs of this region. Available data suggest that they are not as tolerant of water stress as chaparral shrubs occurring in climatically similar California, USA.

2 Seventeen species from nine angiosperm families, including both fynbos and succulent karoo species, were studied at a field site in Western Cape Province, South Africa. Minimum seasonal pressure potential (P_{\min}), xylem specific conductivity (K_s), stem strength against breakage (modulus of rupture, MOR), xylem density, theoretical vessel implosion resistance ($(tlb)_h^2$) and several fibre and vessel anatomical traits were measured.

3 Species displayed great variability in P_{\min} , similar to the range reported for chaparral and karoo shrub species, but in contrast to previous reports for fynbos shrubs.

4 More negative P_{\min} was associated with having greater xylem density, MOR and $(tlb)_h^2$. There was no relationship between P_{\min} and traits associated with increased water transport efficiency.

5 Xylem density integrates many xylem traits related to water stress tolerance, including P_{\min} , MOR and $(tlb)_h^2$, as well as percentage fibre wall, parenchyma, vessel area and fibre lumen diameter.

6 Xylem density may be an integral trait for predicting the impact of climate change on evergreen shrubs.

Key-words: climate change, fynbos, hydraulic conductivity, Mediterranean-type climate, phylogenetic independent contrasts, succulent karoo, water relations, xylem pressure potential

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Introduction

The Cape Floristic Region of South Africa is dominated by evergreen shrubs with tough, leathery leaves collectively referred to as fynbos. This region is classified as a Mediterranean-type climate region, referring to its climatic similarity with the Mediterranean Basin region. This climate type is exemplified by hot dry summers

and cool moist winters, with greater than 65% of annual precipitation occurring during the winter months (Aschmann 1973). In the more arid areas adjacent to fynbos, fynbos is replaced by more succulent vegetation called succulent karoo (Cowling & Holmes 1992). These biogeographical regions contain some of the highest levels of regional diversity in the world (Cowling *et al.* 1996) and are particularly threatened by global climate change (McCarthy *et al.* 2001; Lovett, Midgley & Barnard 2005). Predictions for future climate in the region point

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to significant changes in water availability (Shulze & Perks 2000; McCarthy *et al.* 2001), yet seasonal water relations (Miller *et al.* 1983; Moll & Sommerville 1985; van der Heyden & Lewis 1989; Richardson & Kruger 1990; Smith & Richardson 1990) and associated xylem traits (February & Manders 1999) have only been studied among very few species.

Minimum seasonal pressure potential (P_{\min}) is a measure of the maximum water stress that a plant experiences in the field and is often correlated with plant water stress tolerance (Hacke *et al.* 2000; Sperry & Hacke 2002; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Differential P_{\min} among co-occurring plants may indicate variation in water availability and differential susceptibility to depleted water resources. A less negative P_{\min} annually indicates greater access to soil moisture and suggests greater rooting depth (Moll & Sommerville 1985; Smith & Richardson 1990) but may be coupled with greater susceptibility to water stress. Conversely, a lower P_{\min} annually and associated greater plant water stress tolerance may be coupled to lower water availability, more extreme drought in dry years (Jacobsen *et al.* 2006), and greater risk of drought-induced dieback and mortality (Davis *et al.* 2002; Paddock 2006).

The evergreen shrubs that occur in the Mediterranean-type climate region of South Africa experience a predictable annual summer dry period (Cowling *et al.* 2005). Thus, the evergreen shrubs of this region must be able to both resist the negative pressures that develop within the xylem with water stress as well as maintain water transport to evergreen leaves and actively expanding shoots during the summer dry period (Agenbag 2006). We examined xylem traits associated with water transport efficiency and water stress tolerance as well as P_{\min} in 17 evergreen shrub species occurring in the south-western region of the Western Cape Province, South Africa.

Several xylem anatomical traits were measured to ascertain what xylem structural traits may be associated with decreased P_{\min} , including increased stem mechanical strength (modulus of rupture), increased vessel and fibre wall thickness, increased percentage fibre wall area per transverse area, and decreased fibre lumen diameter. These traits have been shown to be related to P_{\min} , perhaps due to the increased structural support needed to buttress xylem against negative pressure (Jacobsen *et al.* 2005). We also estimated the resistance of xylem vessels to implosion under negative pressure as an additional estimate of water stress tolerance, by calculating the square of the ratio of the intervessel wall thickness to vessel lumen diameter (i.e. $(t/b)_v^2$; Hacke *et al.* 2001). A greater $(t/b)_v^2$ is associated with greater vessel mechanical strength against implosion under negative pressure. Traits associated with greater hydraulic conductivity and capacitance, including increased vessel and fibre lumen diameter and vessel and parenchyma area per transverse xylem area, were also measured.

Sensitivity to water stress varied greatly among fynbos species exposed to an experimental reduction in

Table 1 The species of evergreen shrubs located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa, along with the elevation at which the species was sampled (rounded to the nearest 10 m). Nomenclature follows Germishuizen & Meyer (2003) except for *Searsia undulata*, which follows Yi *et al.* (2004)

Species	Elevation (m)
<i>Brunia noduliflora</i> (E. Mey.) Kuntze	1160
<i>Nebelia laevis</i> O. Kuntze	1020
<i>Aspalathus pachyloba</i> R. Dahlgren	920
<i>Cliffortia ruscifolia</i> L.	920
<i>Erica cerinthoides</i> L.	920
<i>Leucadendron laureolum</i> (Lam.) Fourc.	920
<i>Leucadendron salignum</i> P.J. Bergius	920
<i>Metasia densa</i> (Lam.) Karis	920
<i>Protea laurifolia</i> Thunb.	920
<i>Protea repens</i> (L.) L.	920
<i>Aspalathus hirta</i> E. Mey.	850
<i>Erica plukenetti</i> L.	850
<i>Passerina obtusifolia</i> Thoday	660
<i>Euclea</i> sp.	540
<i>Pteronia fasciculata</i> L.f.	540
<i>Pteronia paniculata</i> Thunb.	540
<i>Searsia undulata</i> (Jacq.) T.S. Yi, A.J. Miller & J. Wen*	540

*Formerly *Rhus undulata*.

rainfall (Agenbag 2006). Xylem traits may be useful in explaining this variation. Increased knowledge of xylem traits associated with water stress tolerance in fynbos species may improve our ability to predict how plants will respond to changes in temperature and precipitation in the face of regional climate change.

Materials and methods

Xylem traits were measured in 17 species of evergreen sclerophyllous shrubs from nine angiosperm families located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa (Table 1, Fig. 1). This site falls within the Mediterranean-type climate region of South Africa. Individuals were sampled from the north-facing slope of the mountain at approximately 33°56.08' S, 19°31.26' E. Eight species were sampled at 920 m elevation (approximately 410 mm annual precipitation), two species were collected from as high as 1160 m elevation, and the remaining seven species were collected from between 845 and 540 m elevation (Table 1). The steep elevational gradient at this site corresponds to considerable variation in precipitation (approximately 315–600 mm annual precipitation) and temperature (see Agenbag 2006 for a complete description of the study site).

Species were identified by the Compton Herbarium of the South African National Biodiversity Institute and specimens are housed at the University of Stellenbosch. Identification of one species was not possible, although it was identified as being within the genus *Euclea*.

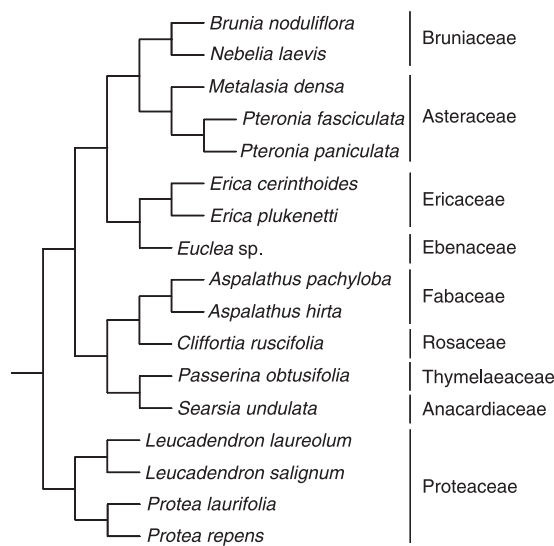


Fig. 1 Phylogeny of 17 species of evergreen shrubs sampled from Jonaskop, in the south-western Cape of South Africa. The phylogeny was constructed using published results referenced in the text and all branch lengths are assumed to be equal for determination of phylogenetic independent contrasts. Family divisions are indicated by the broken line on the right side of the figure. We were unable to identify one species (*Euclea* sp.) beyond the level of genus (see Methods for details).

Twelve individuals of each species were tagged at the site and the same individuals were used for each of the measured parameters. Two of the studied species are dioecious (*Leucadendron lauroleum* and *L. salignum* Proteaceae). For these two species, 12 female and 12 male individuals of each were tagged and measured.

Xylem pressure potentials were measured at pre-dawn and midday on 19 February and at midday on 26 February and 24 March 2004 using a pressure chamber (Model 1001, PMS Instrument Company, Corvallis, Oregon, USA). At each sampling time, we measured xylem pressure potentials (P_{\min}) on branchlets on a minimum of five plants per species with the pressure chamber technique (Scholander *et al.* 1965). The last rainfall event prior to the onset of the summer dry period occurred on 27 September 2003 (*c.* 17 mm). There were several small rain events over the course of the summer (less than 10 mm each), although xylem pressure potentials for most species declined over the measurement period. The summer drought ended on 1 April 2004 with a rainfall event of 21 mm, 1 week after the 24 March sample date. Thus, the duration of the seasonal drought period was about 6 months.

Xylem density was determined as the dry mass per fresh volume of mature stems from at least six individuals per species or sex (Wagner *et al.* 1998; Hacke *et al.* 2001). The bark and pith were removed from stems prior to determination of xylem density. The stems were saturated in degassed HCl solution adjusted to a pH of 2 until they reached their maximum wet weight. Water-saturated volume was determined by water displacement in a graduated cylinder. The stems were dried at 60 °C to a constant weight and dry mass was determined.

Stem xylem modulus of rupture (MOR) was measured using a four-point bending test on an Instron Universal Testing Machine (model 4202, Instron Corporation, Canton, MA, USA) at Michigan State University (East Lansing, MI, USA) following the methods of Jacobsen *et al.* (2005). Twelve stems per species or per sex, approximately 0.3 m in length and 6–8 mm in diameter, were collected in the field, wrapped in moist paper towels, and placed in plastic bags. Stems were then express shipped, in a chilled cooler, to Michigan State University for mechanical analysis. All stems were measured within 4 days of field collection. A four-point bending test with a compression load cell of 500 N was conducted following the methods of Jacobsen *et al.* (2005). We were unable to collect stems from four species for mechanical analysis due to difficulty in obtaining straight stems of the required dimensions for measures: *Erica cerinthoides*, *Euclea* sp., *Cliffortia ruscifolia* and *Metalasia densa*.

Xylem specific hydraulic conductivity (K_s) was calculated following removal of air embolism by high-pressure perfusion (Sperry *et al.* 1988). One branch from a minimum of six different individuals per species was collected from the field. The collected branches were approximately 0.3 m in length and 6–8 mm in diameter. They were wrapped in wet paper towels, sealed in plastic bags, and placed in a cooler for transport back to the laboratory. All stems were measured in less than 24 hours from the time they were collected. Once in the laboratory, branches were submerged in water and trimmed from alternate ends until a final stem segment, 0.1 m in length, was obtained. Stems were connected to a tubing system and flushed with low pH degassed water (pH 2 HCl) that had been passed through a 0.1 µm filter. The stems were flushed at a pressure of 100 kPa for 1 hour to remove gas emboli from the xylem vessels. The K_b ($\text{kg m MPa}^{-1} \text{s}^{-1}$) was then measured gravimetrically on each stem segment (Sperry *et al.* 1988) using an analytical balance (AE260, Delta Range Balance, Mettler-Toledo, Columbus, Ohio, USA). The segments were then attached to a tubing system that allowed uptake of a 0.1% (mass/volume) filtered (0.1 µm filter) dye solution of crystal violet under a suction of 4–6 kPa for 20 min (Hargrave *et al.* 1994). The midpoint of each segment was then sectioned to a thickness of 30–40 µm using a sliding microtome. The stained xylem area was then measured using a digital camera (C-730 Ultra Zoom Camedia, Olympus America Inc., New York, USA) and image analysis software (Image v.1.61, National Institute of Health, Bethesda, Maryland, USA). The K_s value ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was calculated as $K_b/\text{active xylem area}$.

The same sections used for determination of active xylem area were also used for xylem anatomical measures. For each stem, images were taken of several wedge-shaped sectors to sample for vessel and fibre features using a digital camera (JVC TK-C1381 Colour Digital Video Camera, Wayne, New Jersey, USA) attached to a light microscope (DM LB, Leica Microsystems, Solms, Germany). Vessel lumen diameter (d) and wall thickness, fibre lumen

diameter and wall thickness, and percentage transverse fibre, vessel and parenchyma area were measured. All of the vessels and fibres in sectors were measured until 100 vessels and 100 fibres had been measured in a given stem. The hydraulic vessel diameter (d_h) was calculated from the formula $d_h = (\Sigma d^5)/(\Sigma d^4)$, based upon all the sampled vessels in a stem. This parameter weights the vessel diameters by their hydraulic contribution, which is a function of the diameter to the fourth power (Tyree & Zimmermann 2002). This was used in our analyses instead of mean d because it is more directly related to xylem water transport. The vessel implosion resistance ($(t/b)_h^2$) (Hacke *et al.* 2001) was determined for those vessels, within the sampled 100 vessels per stem, that formed pairs in which one or both vessels fell within $\pm 5 \mu\text{m}$ of the calculated d_h , with t as the combined wall thickness of adjoining vessels and b as the lumen diameter of the selected vessel. Percentage transverse area was estimated through measurements of vessel, fibre and parenchyma area in four randomly chosen cross-sectional sectors per stem.

We calculated phylogenetic independent contrasts (PICs) to test for patterns of correlated evolutionary change between traits (Felsenstein 1985) using COMPARE (v 4.6, E. P. Martins, Department of Biology, Indiana University, Indiana, USA). A correlation among PICs for two traits indicates that these traits have undergone changes of similar direction and magnitude across the phylogeny and supports a possible functional link between these traits. The phylogeny used for these analyses was constructed by hand using published phylogenetic data (Fig. 1). Relationships among families, genera and species were based on recent molecular phylogenies (Morton *et al.* 1996; Soltis *et al.* 1997; Bayer & Starr 1998; Soltis *et al.* 2000; Miller *et al.* 2001; Hilu *et al.* 2003; Barker *et al.* 2004; Yi *et al.* 2004; McGuire & Fron 2005). All analyses were run assuming equal branch lengths (Ackerly 2000). This is a conservative approach, thus there is less certainty associated with lack of correlation among contrasts but high confidence when there is a significant correlation among contrasts.

Unpaired t -tests were used to compare xylem traits of male and female individuals of dioecious species and linear regression analyses were used to examine interspecific trait correlations (Minitab v.14.12, Minitab Inc., State College, Pennsylvania, USA and Statview v.5.0.1, SAS Institute Inc., Cary, North Carolina, USA). Regressions or differences were considered to be significant if $\alpha \leq 0.05$. Where females were found to differ from males, they were included as separate data points in analyses. A power fit was used to compare d_h and K_s because according to the Hagen-Poiseuille equation K_s is a function of diameter raised to the fourth power (Tyree & Zimmermann 2002). Principle component analysis was used to determine correlation patterns among multiple traits. This analysis is useful in summarizing the relationships between multiple traits into a single two-dimensional figure that may be easier to interpret than many separate regressions. Principle component analysis was used to examine both raw data as well as

PICs. Data were transformed as necessary to meet the assumptions of statistical models.

Results

INTRASPECIFIC COMPARISON OF SEXES

Two of the studied species, *Leucadendron lauroleum* and *L. salignum*, are dioecious. Females and males of *L. lauroleum* did not significantly differ in any of the xylem traits measured in this study (Fig. 2; $P > 0.05$ for all comparisons). Data from both sexes of *L. lauroleum* were therefore pooled for regression analyses. In contrast, females and males of *L. salignum* were different in their xylem traits related to conductive efficiency, such as xylem specific hydraulic conductivity (K_s), mean hydraulic vessel diameter (d_h), and percentage vessel area per transverse xylem area. Males of *L. salignum* had higher K_s (Fig. 2a; $P = 0.046$), greater d_h (Fig. 2b; $P = 0.003$), and greater percentage vessel area per transverse xylem area (Fig. 2c; $P = 0.020$) relative to females. In addition to these hydraulic traits, males also had a lower percentage fibre wall area per transverse xylem area compared with females (Fig. 2d; $P = 0.001$). Female and male *L. salignum* are thus included as separate data points in regressions involving these traits in which they differ significantly. Female and male *L. salignum* did not differ in the other xylem traits measured, including xylem density, modulus of rupture (MOR), $(t/b)_h^2$, minimum seasonal pressure potential (P_{\min}), fibre lumen diameter, fibre wall thickness, and percentage parenchyma per transverse xylem area ($P > 0.05$ for all).

MINIMUM SEASONAL PRESSURE POTENTIAL

Branchlet pressure potentials (P_x) were measured over three sample dates towards the end of the summer dry period. Over the course of the sample period pressure potentials of most species did not change or declined, indicating steady or declining water status. Pressure potential increased on the sampling date 24 March 2004 for a single species, *Searsia undulata* ($P = 0.016$). Pressure potentials for the two *Pteronia* spp. were approximately -8.5 MPa on 26 February 2004, and declined to below -10 MPa (exceeding the limits of the pressure chamber) on 24 March 2004. Thus, for these two species minimum seasonal pressure potential (P_{\min}) is unknown, but is lower than -10 MPa. For the remaining species, the lowest measured pressure potential was used to estimate P_{\min} .

Minimum seasonal pressure potentials (P_{\min}) varied greatly among the 17 species examined, ranging from a high of -1.89 MPa (*Leucadendron salignum*) to a low of less than -10 MPa (*Pteronia fasciculata* and *P. paniculata*) (Fig. 3). The mean P_{\min} across species, excluding the two *Pteronia* species for which P_{\min} values are lacking, was -3.4 ± 0.34 MPa. The mean P_{\min} across species, assuming that the two *Pteronia* spp. had P_{\min} of

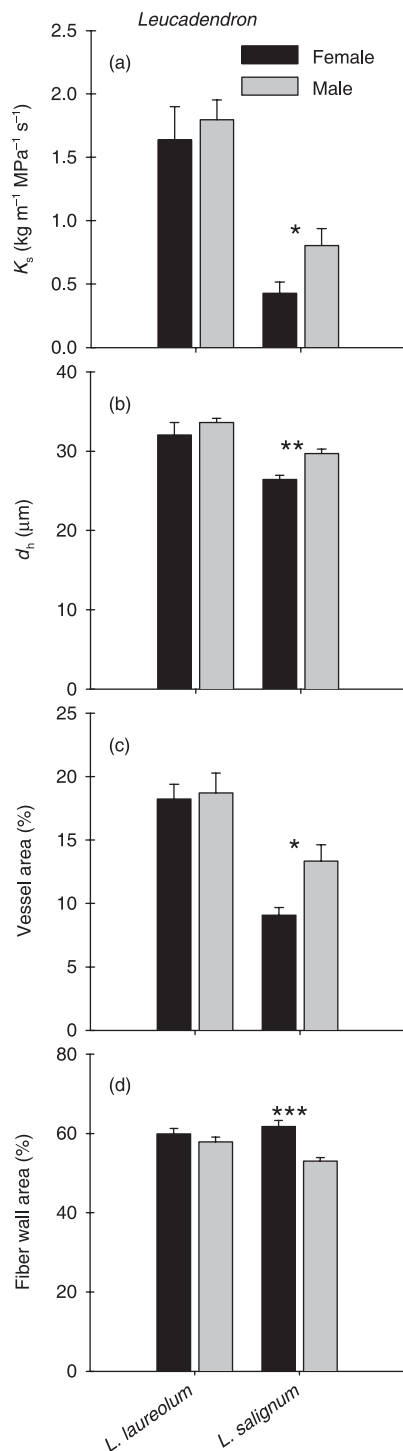


Fig. 2 Xylem specific hydraulic conductivity (K_s) (a), mean hydraulic vessel diameter (d_h) (b), percentage vessel area per transverse xylem area (c), and percentage fibre wall area per transverse xylem area (d) of females (black bars ± 1 SE; $n = 6$) and males (grey bars ± 1 SE; $n = 6$) of two dioecious species, *Leucadendron laureolum* and *L. salignum*. Data were analysed using unpaired t -tests and asterisks indicate significance (* $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$, no asterisk = not significant).

–10 MPa, was -4.34 ± 0.61 MPa. Because specific P_{\min} values for the *Pteronia* spp. are lacking and they were presumably lower than –10 MPa, the mean P_{\min} across all species may actually be more negative.

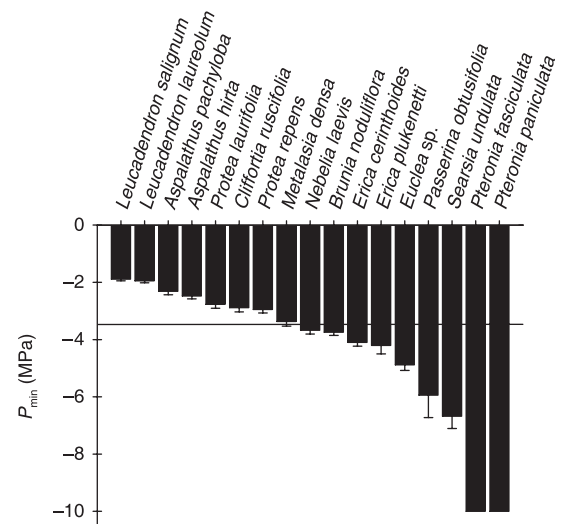


Fig. 3 Minimum seasonal pressure potentials (P_{\min}) measured in 2004 on 17 evergreen shrub species (± 1 SE; $n = 5-12$). Pressure potentials for two species (*Pteronia fasciculata* and *P. paniculata*) exceeded the range of the pressure chamber (less than –10 MPa) and therefore their P_{\min} are unknown and error bars are lacking (see Results for details). The horizontal line indicates the mean P_{\min} for 15 species (–3.4 MPa). The two *Pteronia* species have been excluded from this mean because P_{\min} for these species exceeded the measurable range (less than –10 MPa) and are therefore lacking.

CORRELATIONS OF RAW TRAIT VALUES

Lower P_{\min} was correlated with greater xylem density (Fig. 4a, Table 2), greater stem mechanical strength against breakage (Fig. 4b, modulus of rupture, MOR; Table 2), and increased ratio of vessel wall thickness to lumen diameter (Fig. 4c, Table 2). Minimum seasonal pressure potential was not correlated with other stem xylem properties, including fibre lumen diameter and wall thickness, percentage vessel, parenchyma or fibre wall area per transverse xylem area, K_s , or d_h (Table 2).

Xylem density was correlated with stem mechanical and cellular properties. At the stem level, increased xylem density was correlated with increased MOR (Fig. 5a, Table 2), increased percentage fibre wall area per transverse xylem area (Fig. 5b, Table 2), and decreased percentage parenchyma area and percentage vessel area per transverse xylem area (Table 2). At the cellular level, greater xylem density was correlated with smaller fibre lumen diameters (Table 2) and increased $(t/b)_n^2$ (Fig. 5c; $r^2 = 0.53$, $P = 0.003$ for 14 species). Three species, *Passerina obtusifolia*, *Pteronia fasciculata* and *Pteronia paniculata*, had $(t/b)_n^2$ values outside the range previously reported for angiosperms, and which significantly impacted the slope of the regression (Fig. 5c; $P = 0.004$). Because of the significant impact of these few points on the regression, values for these species were excluded from the regression analysis, although the regression was still significant when they were included ($r^2 = 0.65$, $P < 0.001$ for all 17 species). Xylem density was not correlated with estimates of hydraulic efficiency, including K_s and d_h , nor with fibre wall thickness (Table 2).

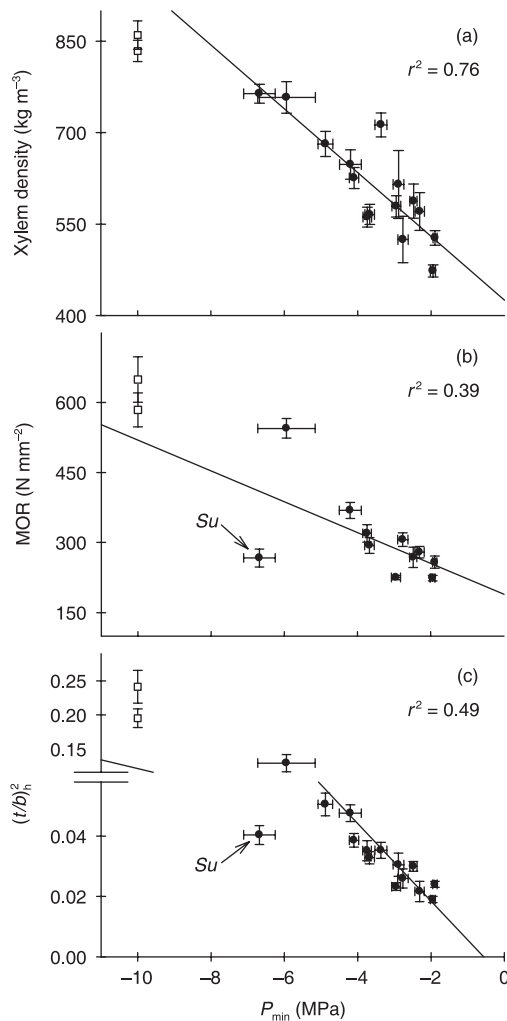


Fig. 4 Xylem density (a), mechanical strength against breakage (modulus of rupture, MOR) (b) and the ratio of vessel wall thickness to lumen diameter squared, an estimate of vessel implosion resistance ($(t/b)_h^2$) (c), as functions of minimum seasonal pressure potential (P_{\min}). Data points are means ± 1 SE. Specific P_{\min} values for two species (*Pteronia* spp.) are not known (see Results for details) and thus were not included in regression analyses although they are included in the graphs (open squares). There is a break in the y-axis in panel (c) and a change in scale above the break. In panels (b) and (c) *Su* = *Searsia undulata*. All regressions shown were significant ($P < 0.05$).

Modulus of rupture was negatively correlated with percentage vessel area and percentage parenchyma area per transverse xylem area, fibre lumen diameter, and d_h (Table 2). MOR was positively correlated with percentage fibre wall area (Fig. 5d) and $(t/b)_h^2$ (Fig. 5e, Table 2). MOR was not correlated with K_s or fibre wall thickness (Table 2).

Xylem specific hydraulic conductivity (K_s) was correlated with d_h using a power fit (Fig. 6; $r^2 = 0.71$, $P < 0.001$) and the exponent was not different from 4 (3.55 ± 0.63). K_s was not correlated with $(t/b)_h^2$, fibre lumen diameter, fibre wall thickness, or percentage parenchyma, vessel or fibre wall area per transverse xylem area (Table 2).

PHYLOGENETIC INDEPENDENT CONTRASTS CORRELATIONS

Phylogenetic independent contrasts (PICs) of P_{\min} and xylem density were correlated (Table 2), but PICs of P_{\min} were not correlated with any other contrasts of measured xylem traits, including fibre wall thickness, fibre lumen diameter, K_s , d_h , and percentage fibre wall, parenchyma or vessel area (Table 2).

Phylogenetic independent contrasts of xylem density were correlated with contrasts of MOR, fibre lumen diameter, $(t/b)_h^2$ and fibre wall thickness, but not with percentage parenchyma, vessel or fibre wall area per transverse xylem area contrasts (Table 2). Xylem density contrasts were also not correlated with contrasts of hydraulic traits (Table 2).

Phylogenetic independent contrasts of MOR were correlated with contrasts of several xylem parameters, including fibre lumen diameter, d_h , $(t/b)_h^2$, percentage vessel area, percentage fibre wall area, and percentage parenchyma area per transverse xylem area (Table 2). MOR contrasts were not correlated with K_s or fibre wall thickness contrasts (Table 2).

Phylogenetic independent contrasts of K_s were correlated with d_h , fibre wall thickness, percentage vessel area, and percentage fibre wall area per transverse xylem area contrasts, but not with $(t/b)_h^2$, fibre lumen diameter, or percentage parenchyma area contrasts (Table 2).

PRINCIPAL COMPONENTS ANALYSIS

Principal components analysis of the raw data, not taking phylogenetic relatedness into account, supported a division between pressure potential and structural traits compared with hydraulic traits (Fig. 7a). The first component described 61.4% of the variation among 11 traits and associated with P_{\min} , xylem density, MOR, $(t/b)_h^2$, fibre lumen diameter, and percentage fibre wall area and parenchyma area per transverse xylem area. The second component described 17.7% of the variation among 11 traits and was associated with hydraulic efficiency traits, including, K_s , d_h , and percentage vessel area per transverse xylem area. A third component described 9.4% of the variation and was associated with fibre wall thickness. Xylem density, $(t/b)_h^2$, MOR and percentage fibre wall area are all positively related to one another and are negatively related to P_{\min} , fibre lumen diameter and percentage parenchyma. Measures of transport efficiency, K_s , d_h and percentage vessel area, are positively related. This analysis helps elucidate the relationships among many traits simultaneously and summarizes them on a single graph. In this case, this analysis illustrates the relationship among the many xylem structural traits associated with water stress vs. the relationships among the vessel traits associated with increased hydraulic efficiency.

Principal component analysis of PICs suggests that the traits that are correlated when raw data are analysed are also evolutionarily correlated (Fig. 7b). The first

Table 2 The coefficients of determination (r^2) and probability values (P) for regressions of raw trait values and phylogenetic independent contrast (PIC) values of several xylem structural and functional traits as measured on 17 evergreen shrub species. Bold text indicates a significant correlation. See Methods for abbreviations and trait descriptions

Traits		Correlations of raw trait values		PIC correlations	
Independent	Dependent	r^2	P	r^2	P
P_{\min}	Xylem density	0.76	< 0.001	0.50	0.005
	MOR	0.39	0.023	0.07	0.45
	Fibre wall thickness	0.00	0.97	0.22	0.09
	Fibre lumen diameter	0.23	0.06	0.05	0.44
	$(tlb)_h^2$	0.49	0.002	0.13	0.20
	% Fibre wall area	0.19	0.08	0.00	0.99
	% Parenchyma area	0.03	0.51	0.00	0.88
	% Vessel area	0.15	0.13	0.00	0.86
	d_h	0.01	0.73	0.03	0.54
K_s	0.19	0.08	0.20	0.11	
Xylem density	MOR	0.72	< 0.001	0.32	0.034
	Fibre wall thickness	0.03	0.46	0.26	0.043
	Fibre lumen diameter	0.58	< 0.001	0.31	0.026
	$(tlb)_h^2$	0.65	< 0.001	0.38	0.011
	% Fibre wall area	0.52	< 0.001	0.14	0.16
	% Parenchyma area	0.28	0.021	0.13	0.16
	% Vessel area	0.26	0.026	0.03	0.55
	d_h	0.08	0.24	0.00	0.94
	K_s	0.01	0.72	0.06	0.35
MOR	Fibre wall thickness	0.01	0.73	0.02	0.68
	Fibre lumen diameter	0.67	< 0.001	0.50	0.011
	$(tlb)_h^2$	0.93	< 0.001	0.86	< 0.001
	% Fibre wall area	0.83	< 0.001	0.78	< 0.001
	% Parenchyma area	0.45	0.006	0.44	0.019
	% Vessel area	0.41	0.010	0.42	0.023
	d_h	0.43	0.008	0.44	0.018
	K_s	0.11	0.22	0.32	0.06
K_s	Fibre wall thickness	0.10	0.18	0.35	0.015
	Fibre lumen diameter	0.03	0.50	0.03	0.56
	$(tlb)_h^2$	0.04	0.39	0.08	0.30
	% Fibre wall area	0.16	0.09	0.38	0.011
	% Parenchyma area	0.04	0.43	0.09	0.25
	% Vessel area	0.17	0.08	0.33	0.019

component described 57.8% of the variability among 11 traits and was associated with P_{\min} , xylem density and MOR contrasts, as well as the xylem anatomical trait contrasts of percentage fibre wall and parenchyma area per transverse xylem area, $(tlb)_h^2$, fibre lumen diameter and d_h . The second component described 23.2% of trait variability and was associated with trait contrasts of xylem density, K_s and fibre wall thickness. A third component explained 10% of trait variance and was associated with percentage vessel area per transverse xylem area. The positions of traits along the x -axis (component 1) appear to separate traits associated with greater transport efficiency and capacitance (K_s , d_h , percentage vessel area, percentage parenchyma area, and fibre lumen diameter) from those associated with greater tissue density and increased drought tolerance (P_{\min} , xylem density, MOR, percentage fibre wall area and $(tlb)_h^2$).

Discussion

The wide range of P_{\min} found among species occurring within close proximity (-1.9 to less than -10 MPa) suggests that these plant species differ in their utilization of water resources. Lower pressure potentials at midday were accompanied by lower pre-dawn pressure potentials. Thus, heterogeneity of P_{\min} indicates variable access to soil moisture and water utilization patterns among species, perhaps due to variable rooting depth, root phenology or soil characteristics among species. This variability may be an important factor in the great species richness of the area (Richardson *et al.* 2001).

All of the species included in this study occur within the Mediterranean-type climate region of South Africa (Aschmann 1973). Because of the similarities in climate among the five Mediterranean-type climate regions of the world, species occurring in these regions have often

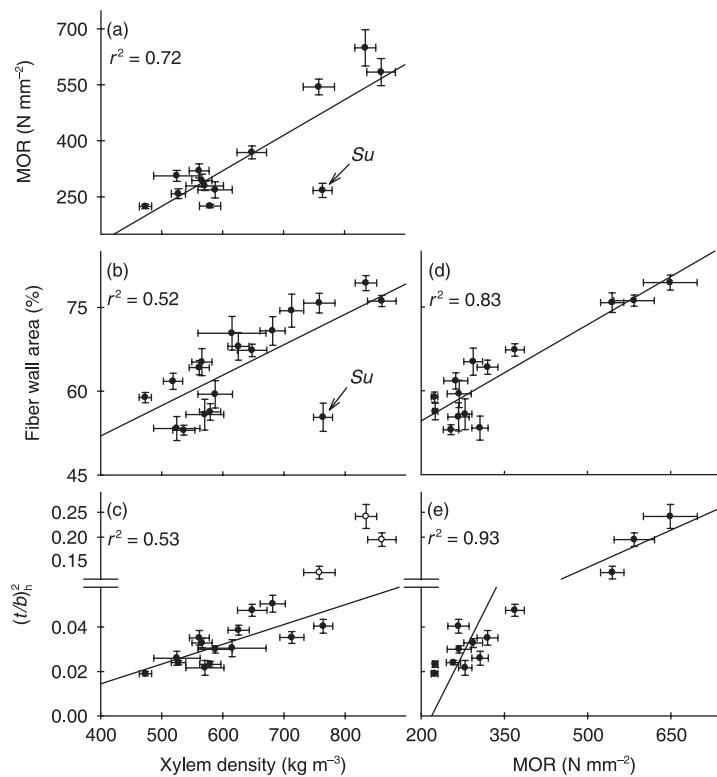


Fig. 5 Mechanical strength against breakage (modulus of rupture, MOR) (a), percentage fibre wall area per transverse xylem area (b and d), and the ratio of vessel wall thickness to lumen diameter squared, an estimate of vessel implosion resistance $((t/b)_h^2)$ (c and e) as functions of xylem density or MOR. Three data points significantly influenced the slope of the regression in panel (c) (open circles) and were excluded from the regression shown (see Results for details). In panels (a) and (b) *Su* = *Searsia undulata*. To accommodate elevated $(t/b)_h^2$ values in three species, there is a break in the y-axis in panels (c) and (e) and a change in scale above the breaks. All regressions were significant ($P < 0.05$).

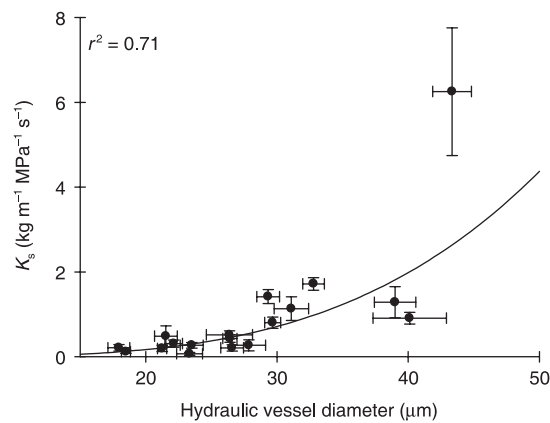


Fig. 6 Xylem specific conductivity (K_s) as a function of hydraulic vessel diameter (d_h). Data points are shown ± 1 SE. A power model was used to fit the data ($P < 0.001$; $y = 4.115^{-6} x^{3.547}$).

been compared (e.g. di Castri & Mooney 1973; Cody & Mooney 1978; Cowling & Campbell 1980; Kruger *et al.* 1983; Cowling *et al.* 1996). Studies that have compared minimum seasonal pressure potentials (P_{\min}) of species across regions, specifically with the California chaparral, have found that P_{\min} of South African fynbos are often much less negative (most were higher than -4 MPa; cf. Miller *et al.* 1983; Moll & Sommerville 1985; Davis & Midgley 1990; Smith *et al.* 1992) com-

pared with those found in California (cf. Burk 1978; Miller & Poole 1979; Poole & Miller 1981; Williams *et al.* 1997; Davis *et al.* 2002; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Other studies have found similarly high pressure potentials among South African fynbos species (van der Heyden & Lewis 1989; von Willert *et al.* 1989; Richardson & Kruger 1990; Smith & Richardson 1990).

In the present study, we found several species that had P_{\min} lower than -4 MPa and the mean P_{\min} across all species did not differ from the recently published mean P_{\min} of 26 species in California (-4.34 ± 0.61 for South Africa compared with -4.9 ± 0.42 MPa for California, $P = 0.50$; Jacobsen *et al.* 2006). The more negative P_{\min} values found in this study than in previous studies are likely to be due to an inclusion of different and a greater number of species, including some species associated with succulent karoo vegetation. When the two *Pteronia* species (characteristic of the arid, low elevation of the study gradient) are excluded from this analysis the P_{\min} for the 15 remaining species do differ from that of the California species ($P = 0.013$). For species that have been included in previous investigations and were part of the current study, our P_{\min} are consistent with published values, limiting the likelihood that site, sampling technique or sampling year significantly impacted results (Miller *et al.* 1983; van der Heyden &

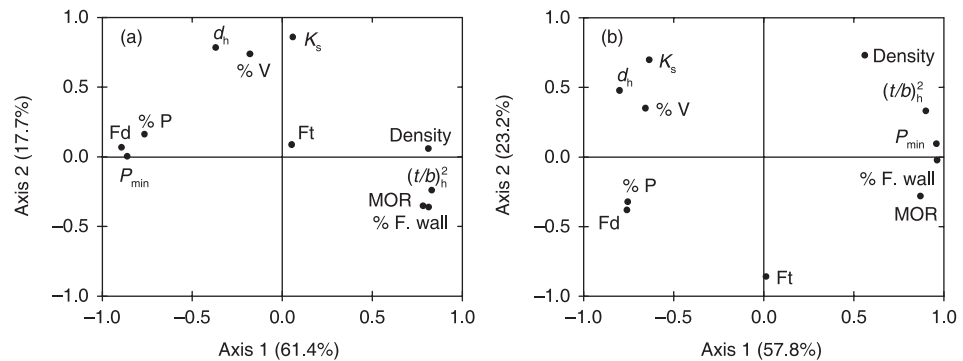


Fig. 7 Relationship among xylem traits along two axes as determined by principal component analyses on raw data (a) and phylogenetic independent contrasts (b). Xylem traits included in the analyses are as follows: minimum seasonal pressure potential (P_{\min}), xylem density, modulus of rupture (MOR), estimated vessel implosion resistance ($(t/b)_h^2$), xylem specific conductivity (K_s), hydraulic vessel diameter (d_h), fibre lumen diameter (Fd), fibre wall thickness (Ft), and percentage vessel area (% V), percentage parenchyma area (% P), and percentage fibre wall area (% F. wall) per xylem transverse area.

Lewis 1989; von Willert *et al.* 1989; Richardson & Kruger 1990; Smith & Richardson 1990). For the two *Pteronia* species, whose distributions extend into the more arid succulent karoo shrubland, similarly low values (less than -10 MPa) have been reported for individuals occurring within the succulent karoo (Midgley & van der Heyden 1999).

The greater range of P_{\min} found in this study may be due to the steep elevation and precipitation gradient of this site (Agenbag 2006). The five species experiencing the most negative P_{\min} occurred at lower elevations (lower than 660 m) at the ecotone between fynbos and succulent karoo and receive less annual precipitation than species occurring at higher elevations. For the remaining 12 species, there does not appear to be any relation between elevation and P_{\min} . For instance, the species collected in this study from the highest elevations, *Nebelia laevis* and *Brunia noduliflora*, have P_{\min} of approximately -4 MPa even though they probably receive more rainfall than many of the other species.

Species that experienced greater seasonal water stress (lower P_{\min}) had greater xylem density and greater stem mechanical strength (MOR). The xylem of species experiencing more negative P_{\min} also had xylem vessels with greater theoretical implosion resistance (i.e. $(t/b)_h^2$; Hacke *et al.* 2001). These results are consistent with what has been reported in the literature for shrubs of the Mediterranean-type climate region of California (Wagner *et al.* 1998; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Interestingly, P_{\min} is not correlated with changes in fibre properties, including fibre lumen diameter and wall thickness and transverse fibre wall area. This differs from what has been reported for the chaparral of California (Jacobsen *et al.* 2005; Jacobsen *et al.* 2006) and suggests that xylem anatomical traits associated with drought tolerance may differ between these two regions. The relationship between measurements made on bulk xylem tissues and stems, such as density and MOR, and P_{\min} appear more consistent between California and South Africa than measurements made on cellular properties (Hacke *et al.* 2001; Ackerly 2004;

Jacobsen *et al.* 2005). Thus, increased allocation of carbon resources to support xylem vessels against implosion under increasing negative pressure may be necessary at the bulk tissue level, but this may be accomplished at the cellular level by varied adjustments across cell types (e.g. vessels, tracheids, fibres or parenchyma). For example, increased cellular support against implosion can result from either increased vessel wall to lumen ratio (South Africa; this study) or increased fibre matrix support (California; Jacobsen *et al.* 2006). Both types of cellular adjustments would result in increased xylem density and MOR. Differences in cellular level adjustments and P_{\min} between South Africa and California might be due to phylogenetic differences, differences in the chemical make-up of cell walls or some yet to be determined factor.

While most species had $(t/b)_h^2$ values within the range of previously reported data (approximately 0.01–0.08; Hacke *et al.* 2001; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006), there were three species that had exceptionally high $(t/b)_h^2$ values. Two of these species, *Pteronia fasciculata* and *P. paniculata*, had values that exceeded those previously reported for all woody species including conifers ($(t/b)_h^2 = 0.24$ and 0.20 , respectively; cf. Hacke *et al.* 2001). Figure 8 shows micrographs of these species in comparison with those of species with $(t/b)_h^2$ values falling within the previously reported range of values. Individuals of the two *Pteronia* species experienced pressure potentials that exceeded the range of our pressure chamber (less than -10 MPa), but their $(t/b)_h^2$ values, as well as their high MOR values, suggest that their xylem could resist implosion at pressures much more negative than -10 MPa (see Fig. 4b,c). This is consistent with prior reports of great desiccation tolerance in these species (Midgley & van der Heyden 1999). Additionally, it appears that these species have altered patterns of xylem allocation in line with the need to resist greater pressures within the xylem. This is evidenced by the relatively large $(t/b)_h^2$ values of these species for a lesser whole xylem investment compared with other species (see Figs 4a, c and 5c), which suggests they have

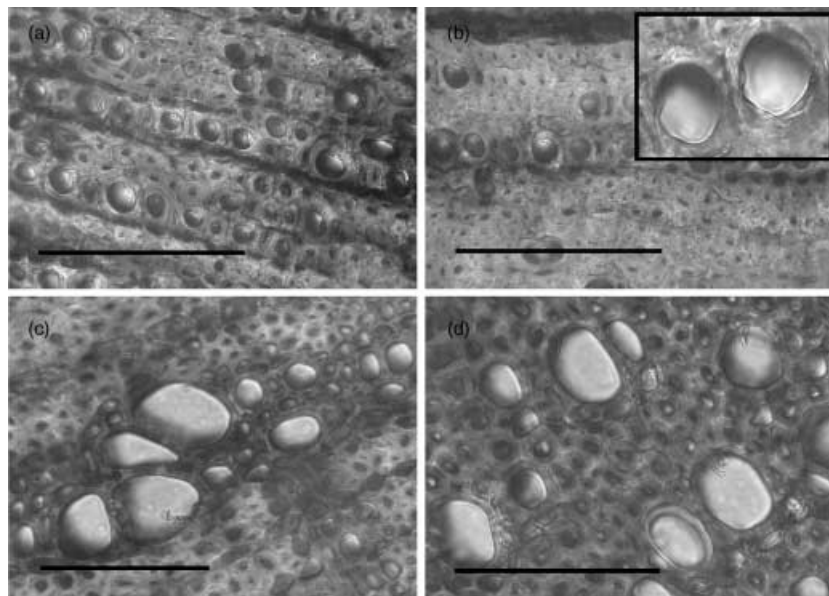


Fig. 8 Light micrographs of xylem transverse sections illustrating differences in the ratio of the double cell wall between two vessels to the vessel lumen diameter ($(tlb)_h^2$). Two of the species shown have extremely large $(tlb)_h^2$ values, *Pteronia fasciculata* (a) and *Pteronia paniculata* (b), while two other species have $(tlb)_h^2$ values that fall within the range of published values, *Aspalathus pachyloba* (c) and *Protea repens* (d). The inset in panel (b) shows two vessels from a transverse section of *P. paniculata* enlarged to a size comparable with vessels present in panels (c) and (d) to facilitate comparison of vessel wall to lumen ratios. The black bar in each panel is 100 μm in length.

preferentially developed vessels that are more implosion resistant without equivalent changes to the whole xylem tissue. This may have been necessary because these species already have the highest percentage fibre wall areas (76 and 79%) and the lowest percentage parenchyma areas (less than 3%) of all of the species examined. Thus, they may have reached the maximum xylem strength and density possible while still maintaining an adequate conductive area (i.e. vessel area). As a result, *Pteronia fasciculata* and *P. paniculata* differ from other species in the study in the effectiveness of their vessel mechanical resistance for a given xylem density (i.e. these species fall well above the trend line for density vs. $(tlb)_h^2$ in Fig. 5c).

Xylem density and MOR were correlated with xylem tissue and cellular properties, including percentage fibre wall, parenchyma, vessel area and fibre lumen diameter, and $(tlb)_h^2$, consistent with previous studies (Wagner *et al.* 1998; Hacke *et al.* 2001; Woodrum *et al.* 2003; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). However, one species, *Searsia undulata*, appeared to have relatively low MOR, fibre wall area and $(tlb)_h^2$ for its xylem density. This species also had one of the highest levels of transverse parenchyma area of the species examined. Presumably, the higher percentage of parenchyma and their associated stored starch contributed to xylem density in this species, but did not offer the mechanical strength that displaced fibre area would have, resulting in low MOR. Interestingly, while this species has a higher parenchyma and lower fibre area, which may be associated with higher capacitance (Borchert & Pockman 2005), it still experienced a relatively low P_{\min} (−6.7 MPa), suggesting high risk of cavitation. It may

be that this species differs from other species included in this study in being able to tolerate a greater degree of seasonal embolism, such as has been found in the chaparral shrub *Rhus ovata* (Pratt *et al.* 2005).

As predicted, K_s was correlated with increased hydraulic vessel diameter (d_h) and the exponent of the power fit relating these two variables was not different from 4, consistent with the Hagen-Poiseuille equation (Tyree & Zimmermann 2002). Thus, among these species, vessel diameter is a strong predictor of K_s regardless of interspecific differences in vessel area (8.6–18.7%) or possible differences in pitted area and vessel sculpturing. Increased K_s , which is associated with greater potential growth rate (Vander Willigen & Pammenter 1998), varied independent of xylem traits associated with strength (fibre and tissue parameters) or drought tolerance (P_{\min} and $(tlb)_h^2$), consistent with previous findings (Woodrum *et al.* 2003; Jacobsen *et al.* 2005; Kern *et al.* 2005).

Species of the genus *Leucadendron* are dioecious, with some species displaying vegetative dimorphism between sexes, such as differences in leaf morphology and branching, while in other species the sexes display little vegetative divergence (Bond & Midgley 1988; Bond & Maze 1999; Rebelo 2001). The two *Leucadendron* species included in this study, *L. laureolum* and *L. salignum*, are vegetatively dimorphic. Consistent with this, the sexes in *L. salignum* also display xylem trait dimorphism. Conversely, the sexes in *L. laureolum* do not appear to vary in the xylem traits we examined.

Males of *L. salignum* have greater xylem-specific conductivity (K_s), hydraulic vessel diameters (d_h), and percentage vessel area, suggesting that for a given xylem area they have more efficient hydraulic conductivity

compared with females. Males also have a lower percentage of fibre wall area for a given xylem area due to a combination of fibre displacement in favour of increased vessel area and greater fibre lumen diameter. These changes in cell type abundance apparently do not affect whole stem strength or drastically alter carbon allocation, as males and females do not differ in stem mechanical strength (MOR) or xylem density. Consistent with what has been reported for other *Leucadendron* species (Bond & Midgley 1988; Bond & Maze 1999), males of *L. salignum* at our study site appear to have smaller leaves, thinner branches and greater branch ramification than females. Selection for greater ramification in males may be due to sexual selection for greater inflorescence number (Bond & Midgley 1988; Geber 1995; Bond & Maze 1999), as inflorescences are terminal in *L. salignum*. It may be that selection for greater inflorescence number has affected xylem hydraulic traits. Males, with more ramified and narrow branches, may have increased hydraulic conductivity per xylem area (higher K_s) as a means to maintain flow to leaves through a smaller xylem area. Additionally, although it was not measured in this study, smaller leaves in males may also lead to greater leaf specific hydraulic conductivity (K_l) in males vs. females. These results suggest a link between floral and hydraulic traits.

Correlations among phylogenetic independent contrasts (PICs) of xylem characteristics generally agree with correlations obtained from the raw trait values that did not incorporate phylogenetic relatedness. Thus, many of the traits that are correlated using raw data have also experienced correlated evolutionary change across the phylogeny, suggesting that they may be functionally related. Evolutionary changes in xylem density are correlated with evolutionary changes in P_{\min} , MOR, fibre lumen diameter, $(t/b)_h^2$ and fibre wall thickness, supporting utility of xylem density in prediction of other xylem traits. Similarly, K_s and d_h contrasts were correlated, supporting a functional relationship between these hydraulic traits.

Unlike the raw trait regression, percentage vessel area contrasts were correlated with K_s contrasts. This suggests that these traits may be functionally related as predicted (i.e. greater vessel area could mean either larger vessels or a greater number of vessels, both of which would be predicted to increase flow; Ewers 1985). However, it should be noted that PIC analyses should be viewed with caution, especially where they vary from raw analyses, because of the uncertainty in phylogeny topography and branch length and non-random species sampling (Donoghue & Ackerly 1996; Ackerly 2000).

Principal components analyses of both raw trait values and PICs confirm the above relationships between a suite of mechanical and drought tolerance xylem traits and a suite of hydraulic traits. The mechanical and drought tolerance traits include xylem density, MOR, P_{\min} , $(t/b)_h^2$, fibre lumen diameter, percentage fibre wall area, and percentage parenchyma area. The hydraulic traits include K_s , d_h , and percentage vessel area. We found

no evidence of a trade-off between these hydraulic and the mechanical drought traits.

The great variability in P_{\min} found among species suggests that water use and availability may be important factors in the structuring of fynbos and succulent karoo evergreen woody shrub communities of the Mediterranean-type climate region of South Africa. The range of P_{\min} among the species included in this study is similar to the evergreen sclerophyllous shrubs of the Mediterranean-type climate region of California and suggests that co-occurring shrubs in these regions may similarly utilize water resources in spite of differences in rainfall reliability among the regions (Cowling *et al.* 2005). The ease of measuring xylem density and its use in predicting xylem characteristics as well as P_{\min} , may make it a useful tool in estimating the xylem characteristics and drought tolerance of large numbers of species. This may be particularly useful in South Africa, where the region is vulnerable to changes in climate and where plant distributions and diversity are likely to be impacted (McCarthy *et al.* 2001; Lovett *et al.* 2005; McClean *et al.* 2005).

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