

# Interrelations among pressure–volume curve traits across species and water availability gradients

Tanja I. Lenz\*, Ian J. Wright and Mark Westoby

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

## Correspondence

\*Corresponding author,  
e-mail: tlenz@bio.mq.edu.au

Received 10 October 2005; revised 14 December 2005

doi: 10.1111/j.1399-3054.2006.00680.x

Pressure–volume (P–V) curves for leaves or terminal shoots summarize leaf-level responses to increasing water deficit. P–V curve traits and field-measured shoot xylem pressures were characterized across 62 species from four sites differing in rainfall and soil phosphorus. Within-species variation in the measured traits was small relative to differences among species and between environments. P–V curve traits tended to differ with site rainfall but not with soil phosphorus. Turgor loss points (TLPs) varied widely and averaged more negative in species from lower-rainfall sites. Differences between species in TLP were driven mainly by differences in solute potential, rather than by differences in cell wall elasticity. Among species at individual sites, species seemed to vary in leaf-response strategy reflected in TLP independently from water-uptake strategy reflected in predawn xylem pressures and in xylem pressure drop from predawn to midday.

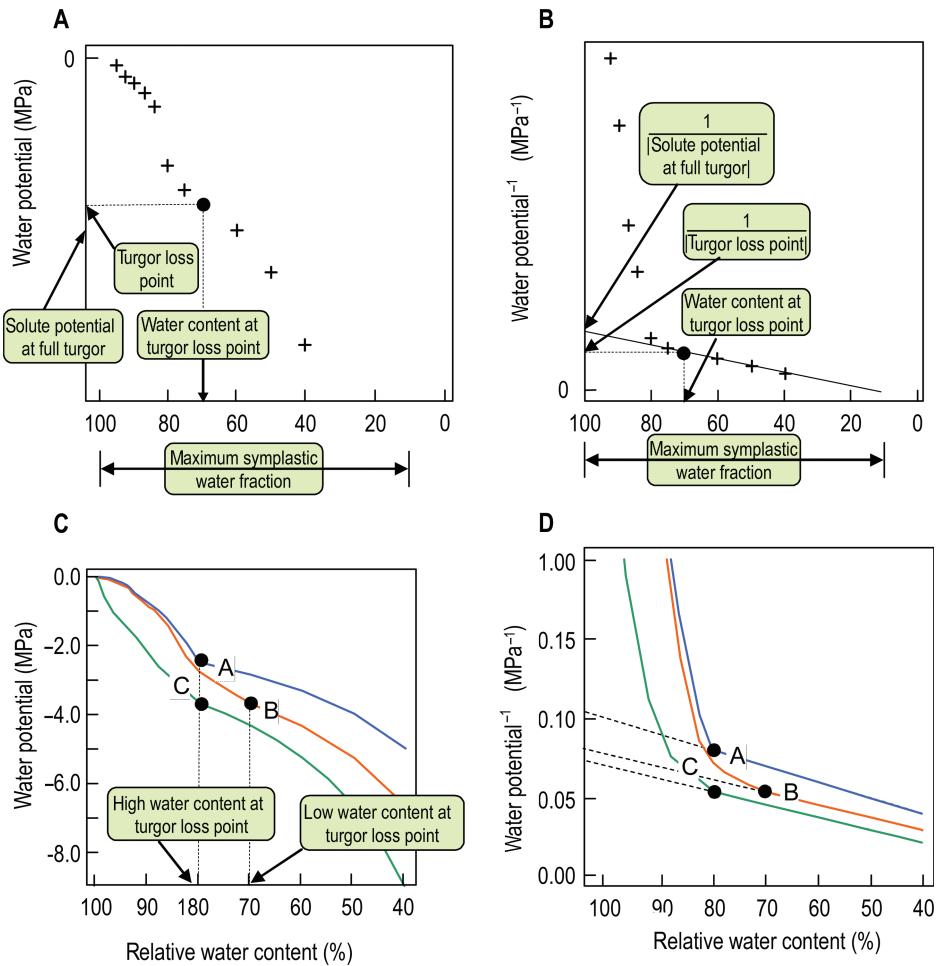
## Introduction

One aspect of a plant species strategy in the face of fluctuating water supply is the maintenance of cell turgor (Tyree and Jarvis 1982). As leaves dry out, their cell volumes shrink, and turgor pressure and water potential decrease (become more negative). At the ‘turgor loss point’ (TLP; Fig. 1A), the turgor pressure is zero, the cell wall is relaxed and the cell water potential is equal to the cell osmotic potential (Schulze et al. 2005). Moisture–release curves describe the decrease in water potential in the leaf as a function of decreasing relative water content (RWC) (Fig. 1A). They are produced slowly by drying an excised shoot on the bench. Several parameters can be estimated from the moisture–release curve. Actual estimation is performed from the pressure–volume (P–V) curve, a plot of inverse water

potential (Fig. 1B), which by definition declines linearly with RWC below the TLP (Tyree and Hammel 1972).

Thus, working from lower right in Fig. 1B, maximum symplastic water fraction  $W_s$  is estimated by extrapolating the straight-line section to very large negative water potential (1 water potential<sup>-1</sup> approaches zero); TLP is estimated as the point where the line becomes non-linear; solute potential at full turgor ( $SP_0$ ) is estimated by extrapolating the straight-line section to 100% RWC. The bulk modulus of elasticity ( $\epsilon$ ) estimates cell wall elasticity and is determined from the non-linear slope from zero water potential towards TLP of the pressure potential component of the P–V curve (i.e. via a Höfler diagram, not shown). These traits differ genetically between species (Kozlowski and Pallardy 2002) and also individual plants can adjust them over time. Different species lose

**Abbreviations** –  $\epsilon_{\text{max-TLP}}$ , bulk modulus of elasticity from full turgor to turgor loss;  $g_s$ , stomatal conductance;  $K_{\text{whole plant}}$ , whole plant conductivity;  $\psi_{\text{MD}}$ , midday shoot water potential;  $\psi_{\text{PD}}$ , predawn shoot water potential; P–V, pressure–volume; RWC, relative water content; SMA, standardized major axes; soil P, soil phosphorus;  $SP_0$ , solute potential at full turgor; SWC, shoot water content at full hydration; TLP, turgor loss point; VPD, vapour pressure deficit;  $W_s$ , symplastic water fraction;  $W_{\text{TLP}}$ , water content at turgor loss.



**Fig. 1.** (A) A hypothetical moisture release curve and the corresponding pressure–volume curve (B) illustrating the relationship among the underlying traits; (C) moisture release curves and (D) corresponding inverse pressure–volume curves of a P–V curve with high turgor loss point A, a P–V curve with low turgor loss point B due to low solute potential at full turgor and a P–V curve with low turgor loss point C due to a high cell wall elasticity (low modulus of elasticity). Note that x-axes are reversed.

turgor at a water potential between  $-1.00$  and  $-4.08$  MPa (Bannister 1986, Sobrado 1986, Sack et al. 2003). This point corresponds more or less to the water potential at which stomates are closing or closed (Brodribb et al. 2003, Burghardt and Riederer 2003). With further desiccation, the leaf or part thereof is permanently damaged, e.g. permanent damage to 50% of the leaf area occurred between  $-3.3$  and  $-7.7$  MPa in 23 New Zealand plant species (Bannister 1986).

Hypothetical species with different P–V curves are illustrated in Fig. 1C, D. Species with high TLP show a shallow decline in leaf water potential as RWC decreases (TLP at point A in Fig. 1C, D, blue lines). Species can have lower TLP via a higher concentration of solutes (Fig. 1C, D, orange lines). This lowers TLP (point B in Fig. 1C, D) by lowering  $SP_0$ . Alternatively, TLP can be lower due to less elastic cell walls (high  $\epsilon$ ) (TLP at point C in Fig. 1C, D, green lines). When cell walls are less elastic, water potential drops faster for a given decline in RWC. In this case,  $W_{TLP}$  can remain unchanged (Fig. 1C).

Numerous articles since the 1970s have described these changes in P–V curve traits within and between small numbers of species. Given that P–V curve traits are at least partially genetically determined and are important as part of a species' drought–response strategy, then species in general experiencing different soil moisture availability and soil phosphorus (soil P) availability might be expected to vary in these traits, in predictable ways and independent of their evolutionary history. In this study, we quantified P–V curve traits and water potentials of a total of 62 species from four vegetation types. Specifically, we tested the following hypotheses:

- (1) Species with lower TLPs have lower  $SP_0$ s and/or higher  $\epsilon$ .
- (2) Species with less access to soil moisture or lower soil moisture status at midday have P–V curve traits associated with drought tolerance, e.g. lower TLPs and  $SP_0$ s.
- (3) Species at the low soil P sites have higher  $\epsilon$ , irrespective of access to soil moisture. Low soil P levels are

associated with species with high leaf mass per area (Beadle 1954, Fonseca et al. 2000), which in turn are associated with decreased cell wall elasticity (Niinemets 2001).

We used both cross-species and phylogenetic analyses for testing these hypotheses. Phylogenetic analyses test whether evolutionary divergences are correlated consistently in different phylogenetic lineages.

## Methods

### Field sites and species selection

Four field sites were chosen in New South Wales, Australia (Wright et al. 2001, Wright et al. 2002), so as to contrast in rainfall (1220 at Sydney vs 387 mm year<sup>-1</sup> inland) and soil type (coarser textured, low P soil and finer textured, high P soil) (Appendix S1). All sites fall within a common latitudinal band and experience mean annual temperature of 17.5°C and relatively aseasonal rainfall. Each site supports a different vegetation type (Appendix S1). The species in the low soil P sites are on average more sclerophyllous (higher leaf mass per area) (Wright et al. 2002). At each vegetation type, 16–18 woody, evergreen, non-climbing taxa ('species', hereafter) were sampled, giving a total of 62 species from 17 families (Appendix S2). Three subspecies of *Dodonaea viscosa* were treated as separate entities, as were two variants of *Senna artemisioides* that have distinct leaf types but that were not distinguished in the current taxonomic treatment of this species (Randell and Barlow 1998). Four species were sampled at both vegetation types in the low-rainfall zone. At the high-rainfall vegetation types, species were chosen randomly from previously compiled species lists; at the low-rainfall (and low diversity) vegetation types, only species with sufficient individuals (minimum of five) were sampled. Further details of study sites and species-selection criteria were given by Wright et al. (2001, 2002).

### Environmental conditions

P–V curves were determined for each species between July and October 2004, with some additional sampling in November 2004 (Appendix S1). Rainfall, temperature, relative humidity and soil moisture during sampling periods varied somewhat among vegetation types (Appendix S1). Variation among species in the measured traits may have reflected plastic responses to differing environmental conditions among vegetation types as well as inherent trait differences among species (our primary interest). All P–V curve traits reported here were inspected to see whether the species-mean trait varied

with sampling date in each vegetation type. Only species-mean  $W_{TLP}$  varied with sampling date in two of the vegetation types (Appendix S1).

### Trait measurements

For the P–V curves, one shoot was cut from each of at least three individuals of a randomly selected subgroup of species per vegetation type and collection. Whole leaves were used rather than shoots for *Eucalyptus haemostoma*, whereas part leaves were used for *Macrozamia communis*, a large-leaved cycad. Shoots or petioles were cut underwater, wherever possible. In tall plants (some individuals of *Acacia doratoxylon*, *Brachychiton populneus*, *Eucalyptus* spp. and *Santalum acuminatum*), branches were first cut to at least 0.5 m and the shoots were then recut underwater. The shoots or leaves were hydrated in potable tap water (room temperature and kept in the dark for 24 h); then they were either processed or refrigerated at 7°C in the dark until processed (<48 h). After 48 h, any remaining shoots were discarded.

Shoots were left to dry on the bench between measurements and were weighed to 0.001 g immediately before and after water potential was measured with a pressure chamber (Model 1000, PMS Instruments, Corvallis, OR). Replicate shoots were sampled in a random interspersed order. We discarded shoots that did not hydrate to >–0.2 MPa, except in *Dodonaea triquetra* and *Hakea teretifolia*, which consistently did not hydrate to >0.3 MPa. The mean initial water potential of all shoots used was –0.11 MPa.

Water potential was measured using standard procedures (Turner 1981). Three replicate P–V curves were measured for all species, with the following exceptions: *Synoum glandulosum*, *Banksia marginata*, *Grevillea buxifolia*, *Grevillea speciosa* and *Persoonia levis* (four replicates); *Corymbia gummifera* and *Gompholobium glabratum* (two replicates). TLP was determined from the start of the straight line from plots of inverse balance pressure vs shoot fresh mass (Fig. 1B). Calculation of the other traits (Table 1) followed Schulte and Hinckley (1985) and Kubiske and Abrams (1990, correction for overhydration) with the exception of  $\epsilon$ . Due to the non-linear relationship between pressure potential at full turgor and TLP, the slope of this section of the curve and thus  $\epsilon$  is not constant. Customarily,  $\epsilon$  is calculated from the slope of the maximum hydration part of the pressure potential component of the P–V curve (i.e. via a Höfler diagram). We used instead the average slope of the moisture release curve from full turgor down to TLP, defined as  $\epsilon_{max-TLP}$ . One reason was that this measure best reflects any influence of elasticity

**Table 1A.** Partitioning of variation (% sums of squares) of the traits into vegetation type, species within vegetation type and replicate within species (nested ANOVA). Species that were sampled in both low-rainfall vegetation types were treated as individual species. \*Tests on log-transformed data.

Trait	Solute potential at full turgor (MPa)	Turgor loss point (MPa)	Water content at turgor loss (%)	Symplastic water fraction (%)	Bulk modulus of elasticity (MPa)	Shoot water content after hydration (%)	Predawn water potential (MPa) <sup>a</sup>	Midday water potential (MPa) <sup>a</sup>
Vegetation	23.35	41.30	21.16	70.04	6.13	2.90	81.05	75.98
Species	57.73	44.47	50.06	17.24	70.16	83.98	12.61	18.19
[vegetation]								
Replicate	18.93	14.23	28.46	12.71	23.71	13.11	6.34	5.82
[species]								

**Table 1B.** Mean pressure-volume curve traits  $\pm$  SE and water potentials of 16–18 woody species in each of two high- and low-rainfall vegetation types (P, soil phosphorus). Different letters indicate significant differences between vegetation types at  $\alpha = 0.05$  (MANOVAs, ANOVAs, Tukey-Kramer HSD tests). \*Tests on log-transformed data.

Trait	Solute potential at full turgor (MPa)	Turgor loss point (MPa)	Water content at turgor loss (%)	Symplastic water fraction (%)	Bulk modulus of elasticity (MPa)	Shoot water content after hydration (%)	Predawn water potential (MPa)*	Midday water potential (MPa)*
High rain, high P	-1.94 $\pm$ 0.10 <sup>ab</sup>	-2.48 $\pm$ 0.11 <sup>a</sup>	83.17 $\pm$ 1.44 <sup>a</sup>	76.69 $\pm$ 2.37 <sup>a</sup>	14.55 $\pm$ 2.05 <sup>a</sup>	58.47 $\pm$ 1.59 <sup>a</sup>	-0.52 $\pm$ 0.05 <sup>a</sup>	-0.99 $\pm$ 0.10 <sup>a</sup>
High rain, low P	-1.74 $\pm$ 0.11 <sup>a</sup>	-2.31 $\pm$ 0.13 <sup>a</sup>	86.15 $\pm$ 1.24 <sup>a</sup>	60.51 $\pm$ 3.39 <sup>b</sup>	14.34 $\pm$ 1.20 <sup>a</sup>	56.22 $\pm$ 1.95 <sup>a</sup>	-0.67 $\pm$ 0.04 <sup>b</sup>	-1.24 $\pm$ 0.06 <sup>a</sup>
Low rain, high P	-2.31 $\pm$ 0.12 <sup>b</sup>	-3.37 $\pm$ 0.15 <sup>b</sup>	76.46 $\pm$ 1.61 <sup>b</sup>	75.53 $\pm$ 2.52 <sup>a</sup>	10.70 $\pm$ 0.90 <sup>a</sup>	56.10 $\pm$ 1.60 <sup>a</sup>	-2.96 $\pm$ 0.21 <sup>c</sup>	-3.68 $\pm$ 0.24 <sup>b</sup>
Low rain, low P	-2.45 $\pm$ 0.12 <sup>bc</sup>	-3.45 $\pm$ 0.15 <sup>b</sup>	77.28 $\pm$ 1.58 <sup>b</sup>	77.59 $\pm$ 2.88 <sup>a</sup>	12.52 $\pm$ 1.27 <sup>a</sup>	55.01 $\pm$ 2.03 <sup>a</sup>	-3.14 $\pm$ 0.19 <sup>c</sup>	-4.12 $\pm$ 0.22 <sup>b</sup>

differences on TLP. The second reason was that existing guidelines for selecting this portion of the curve (Koide et al. 1989) were insufficient to provide a consistent determination of  $\epsilon$  at full hydration for a large number of species. Note that throughout this article, the term 'elasticity' is used in its traditional sense, while  $\epsilon$  refers to the slope of the change in turgor pressure with changing RWC, which increases with decreasing elasticity.

### Water potentials

To obtain an estimate of access to soil moisture and midday water status that could be compared across species within vegetation types, predawn (04:00–07:20 hours, depending on sunrise) and midday (12:00–14:00 hours) shoot water potentials were measured around the dates when P–V curve samples were taken. Measurements were made over 2 days; half of the species (randomly selected) were measured each day. One replicate shoot was collected from each of five individuals per species, if possible from individuals not growing close to each other (only four individuals could be found of *D. triquetra*). Replicates of each species were interspersed throughout the measurement period. Healthy terminal shoots were cut at 0.5–1.5 m height or up to 3 m height (some individuals of *Eucalyptus* spp., *S. acuminatum*, *B. populneus* and *A. doratoxylon*) with secateurs, placed into a plastic bag containing a moist paper towel, sealed and stored in the dark. Within 10 min, shoots were trimmed by <1 mm with a razor blade and the balance pressure was measured in the pressure chamber as described above. Data exploration showed no consistent linear or non-linear change in 64 of the 66 species from the first to the fifth replicate. Thus  $\psi_{MD}$  is likely to be a reasonable representative of minimum  $\psi$ .

### Statistical analysis

Variation in each measured trait was decomposed into vegetation type, species and individual plant components (nested ANOVA variance components, type I sums of squares). The species term consistently accounted for a larger share of total variation than the individual-within-species term (Table 1A), hence species-mean trait values were calculated for each species at each vegetation type. Most P–V curve traits and  $\log_{10}$ -transformed [water potentials] were normally distributed (Shapiro-Wilk test) and had homogeneous variances between vegetation types (Brown-Forsythe test). MANOVA was used to test for differences in traits between vegetation types, followed by ANOVA and Tukey-Kramer HSD tests. All tests used  $\alpha = 0.05$  for significance.

### Cross-species analyses

Relationships among species-mean trait values were summarized by fitting standardized major axis (SMA) slopes (Sokal and Rohlf 1995), and their significance was tested for using Pearson's correlations test. Differences between SMA slopes fitted to individual vegetation types were tested with the SMA analogue of ANCOVA (i.e. slopes tested for heterogeneity; where deemed homogeneous, a common slope was fitted and intercept differences were tested for). Group shifts (i.e. changes in the position of the slopes between groups) along these common slopes were also identified using the 'SMATR' software package (Falster et al. 2003). For illustrative purposes, 68% data distribution ellipses (i.e. mean  $\pm 1\text{SD}$ ) are also given in Fig. 2. These serve the dual purposes of illustrating the strength of trait correlations (the more elongate the ellipse, the stronger the correlation) and of emphasizing any mean shifts between the groups of data (Wright et al. 2002).

### Phylogenetic analyses

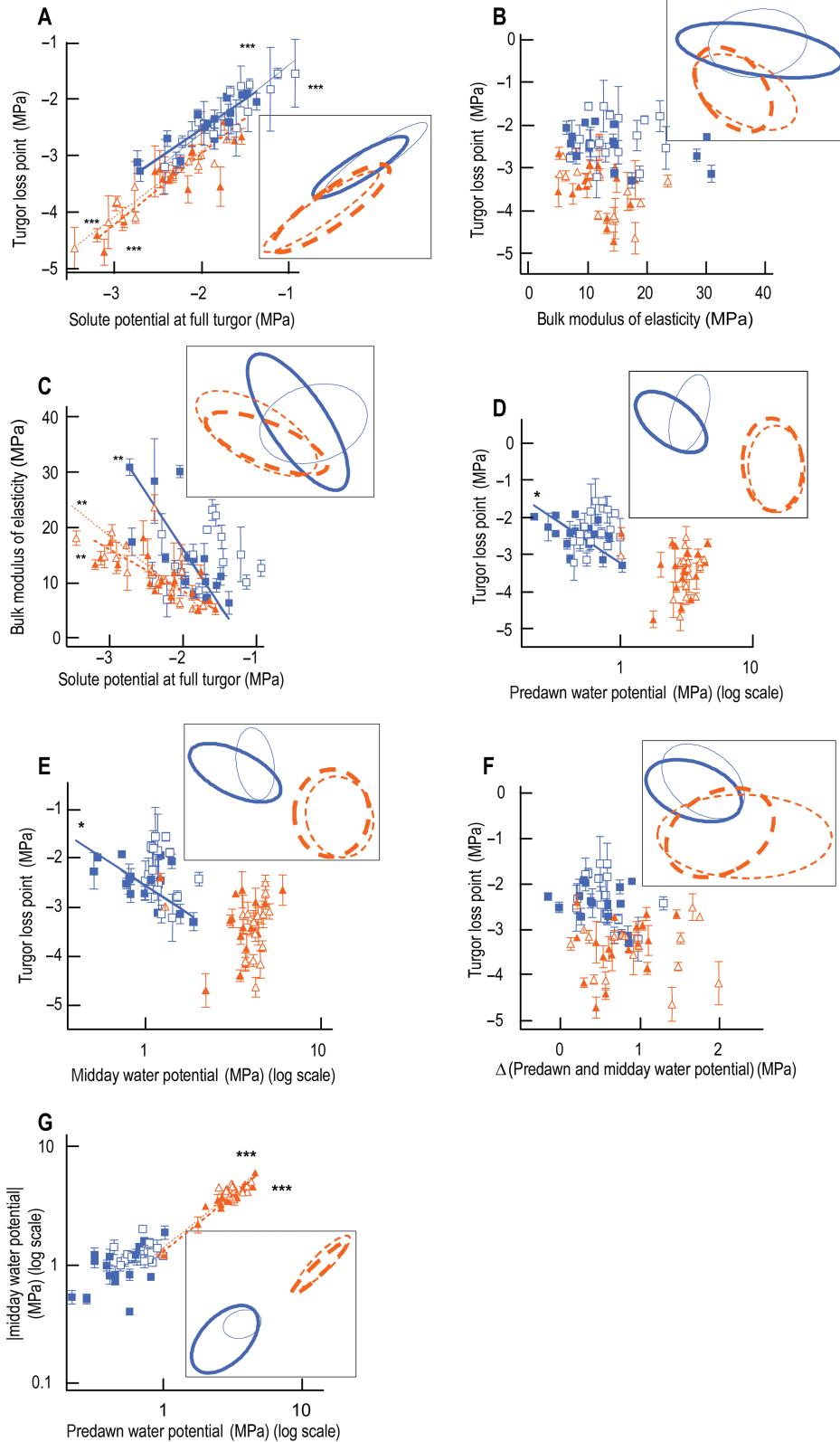
A complementary question to asking whether the traits of present-day species are correlated with one another is to ask whether trait divergences have been correlated through evolutionary history (Harvey and Pagel 1991). These 'correlated divergence' analyses were run for each trait pair that had shown significant relationships in the cross-species analysis. Details of these analyses are described in Appendix S2.

## Results

### P–V curve traits

We first consider TLP and what traits were mainly responsible for its variation across species. Species with lower TLP should be able to continue growth at lower leaf water potentials. Species-mean TLPs varied from ca  $-1.5$  to nearly  $-5$  MPa. The strongest predictor of a more negative TLP was a more negative osmotic potential at full turgor ( $\text{SP}_0$ ) (Fig. 2A, Table 2A). TLP was not associated with  $\epsilon_{\text{max-TLP}}$  or elasticity (Fig. 2B, Table 2A). Rather, increased elasticity was associated with less negative  $\text{SP}_0$  within vegetation types (Fig. 2C, Table 2A). Species in low-rainfall vegetation types were distinct from the high-rainfall vegetation types in a combination of higher elasticity and more negative  $\text{SP}_0$ , more so than in either of these dimensions taken individually (Fig. 2C, inset).

TLP was shifted lower for species occurring at low-rainfall vegetation types but was not different between soil types in either rainfall zone (Table 1B). The down-shift in TLP at low rainfall was strongly related to more



**Fig. 2.** Relationships between pairs of water-use traits (Table 1) of 16–18 species each in two high-rainfall vegetation types (■ + bold solid line, high P soil; □ + regular solid line, low P soil) and two low-rainfall vegetation types (▲ + bold dashed line, high P soil; △ + regular dashed line, low P soil). Error bars represent  $\pm 1\text{SE}$ ; fitted lines are SMA slopes where correlations are significant at  $\alpha = 0.05$  (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.0001$ , Table 2). Ellipses show 68% confidence intervals. For shifts between slopes, see Table 2B.

**Table 2.** Relationships across species between pairs of pressure-volume curve traits ( $SP_0$ , solute potential at full turgor; TLP, turgor loss point;  $\epsilon_{\max-TLP}$ , bulk modulus of elasticity;  $\psi_{MD}$ , midday shoot water potential;  $\psi_{PD}$ , predawn shoot water potential) between high and low rainfall and between high and low soil phosphorus (P). (a) SMA slopes fitted through four vegetation types, with intercepts (IC) and Pearson's  $r^2$ . The SMA slopes of the correlations between  $\epsilon_{\max-TLP}$  and TLP, between  $SP_0$  and  $\epsilon_{\max-TLP}$ , and between  $\psi_{MD}$  and TLP are significantly different between vegetation types at  $\alpha = 0.05$ . (b) P-values of the shifts in SMA slope estimations in elevation and along slope between trait pairs. <sup>#</sup>No test for shift in elevation or along slope since slopes differ (Warton & Weber likelihood ratio). <sup>ns</sup> $P > 0.05$ ; <sup>\*</sup> $P < 0.05$ ; <sup>\*\*</sup> $P < 0.01$ ; <sup>\*\*\*</sup> $P < 0.0001$ .

(a)		High rain, high soil P			High rain, low soil P			Low rain, high soil P			Low rain, low soil P		
		x-axis	y-axis	slope	IC	$r^2$	slope	IC	$r^2$	slope	IC	$r^2$	slope
$SP_0$	TLP	1.1	-0.5	0.79***	1.2	-0.2	0.83***	1.3	-0.5	0.76**	1.2	-0.6	0.86***
$\epsilon_{\max-TLP}$	TLP			0.17 <sup>ns</sup>			0.01 <sup>ns</sup>			0.17 <sup>ns</sup>			0.22 <sup>ns</sup>
$SP_0$	$\epsilon_{\max-TLP}$	-20.2	-24.5	0.54**			0.03 <sup>ns</sup>	-7.6	-6.9	0.55**	-10.3	-12.7	0.42**
log $\psi_{PD}$	TLP	-2.4	-3.3	0.28*			0.17 <sup>ns</sup>			0.01 <sup>ns</sup>			0.01 <sup>ns</sup>
log $\psi_{MD}$	TLP	-2.3	-2.6	0.28*			0.05 <sup>ns</sup>			0.01 <sup>ns</sup>			0.01 <sup>ns</sup>
$\psi_{PD} - \psi_{MD}$	TLP			0.12 <sup>ns</sup>			0.16 <sup>ns</sup>			0.10 <sup>ns</sup>			0.01 <sup>ns</sup>
log $\psi_{PD}$	log $\psi_{MD}$			0.24 <sup>ns</sup>			0.03 <sup>ns</sup>	1.0	0.1	0.93***	1.0	0.1	0.78***
(b)		Shift between rain (high P)			Shift between rain (low P)			Shift between P (high rain)			Shift between P (low rain)		
		x-axis	y-axis	Elevation	Slope	Elevation	Slope	Elevation	Slope	Elevation	Slope	Elevation	Slope
$SP_0$	TLP	***	**	***	***	ns	ns	ns	ns	ns	ns	ns	ns
$\epsilon_{\max-TLP}$	TLP	#(**)		***	**	#(*)		ns		ns		ns	
$SP_0$	$\epsilon_{\max-TLP}$	#(**)		***	ns	ns	ns	ns	ns	ns	ns	ns	ns
log $\psi_{PD}$	TLP	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns
log $\psi_{MD}$	TLP	***	***	***	***	#(**)		ns		ns		ns	
$\psi_{PD} - \psi_{MD}$	TLP	***	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	ns
log $\psi_{PD}$	log $\psi_{MD}$	**	***	ns	***	ns	*	ns		ns		ns	

negative osmotic potentials at full turgor  $SP_0$  (Fig. 2A). However, the relationships between TLP and  $SP_0$  also differed in elevation (i.e. the  $y$ -intercept); low-rainfall species having TLP ca 0.5 MPa lower at a given  $SP_0$  (Fig. 2A, Table 2B). It is possible to create a lower TLP for the same  $SP_0$  in two ways. Firstly, decreasing  $W_s$  shifts the TLP down (see Fig. 1B). In this case,  $W_{TLP}$  stays similar. Secondly, increasing the elasticity (decreasing  $\epsilon$ , i.e. decreasing the slope between full turgor and TLP) shifts the TLP down, but also across. This lowers  $W_{TLP}$  and potentially increases  $W_s$ . In this dataset, low-rainfall species had higher  $W_s$  ( $W_s$ , low rain =  $76.5\% \pm 1.9\text{SE}$ ) than high-rainfall species ( $W_s$ , high rain =  $68.6\% \pm 2.5\text{SE}$ ; ANOVA:  $P = 0.01$ ) and  $W_{TLP}$  also (Table 1). Consequently, low-rainfall species also had a higher measure of elasticity between full turgor and TLP or a lower  $\epsilon_{\max-TLP}$  ( $\epsilon_{\max-TLP}$ , low rain =  $12.1 \text{ MPa} \pm 0.8\text{SE}$ ) than did high-rainfall species ( $\epsilon_{\max-TLP}$ , high rain =  $15.3 \text{ MPa} \pm 1.2\text{SE}$ ; ANOVA:  $P = 0.03$ ).

Overall, the main differences in mean P–V curve traits occurred between the species of the high- and low-rainfall vegetation types rather than between the different soil types. However, there was considerable variation in most traits within each set of co-occurring species. The

ranges of all the P–V curve traits overlapped considerably between vegetation types, such that 30–93% of the variation was not explainable by vegetation type (Table 1A).

#### Variation in P–V curve traits with regard to water availability

Predawn water potentials ( $\psi_{PD}$ ) are often interpreted as indicating relative access to soil moisture. Thus, mean differences in  $\psi_{PD}$  between vegetation types largely reflect differences in soil moisture at the time of sampling. Indeed, species at the low-rainfall vegetation types had lower  $\psi_{PD}$ , on average (Table 1). Still, differences between species within vegetation types might be expected to reflect relative access to water by deeper roots ('effective' rooting depth), and thus generally reflect differences in water-use strategy.

Contrary to expectations, there was no clear tendency within vegetation types for species with lower TLPs to operate at lower  $\psi_{PD}$  (Fig. 2D), nor did they consistently run down to lower midday water potentials ( $\psi_{MD}$ ) or drop more in water potential during the day than species with higher TLPs (Fig. 2E, F). A negative correlation between water potentials and TLPs existed at only one of four vegetation types (high rainfall, high soil P; Fig. 2D, E, Table 2A).

Species that had less negative  $\psi_{MD}$  tended also to recover to higher water potentials overnight (Fig. 2G).  $\psi_{MD}$  and  $\psi_{PD}$  were closely correlated at the low-rainfall vegetation types and across vegetation types. In other words, low-rainfall species varied mainly in whether they operated at higher or lower water potential overall, rather than in the amplitude of the difference between  $\psi_{PD}$  and  $\psi_{MD}$ . In high-rainfall species, on the other hand,  $\psi_{MD}$  was independent of  $\psi_{PD}$ .

### Phylogenetic analyses

Phylogenetic analyses confirmed that within vegetation types, most evolutionary divergences in the traits were correlated similar to present-day trait values (Appendix S5). The majority of phylogenetic divergences between species pairs from different vegetation types were also in the same direction as the general cross-species patterns (Appendix S6).

### Discussion

With few exceptions (e.g. Bannister 1986, Kubiske and Abrams 1990, Krasser and Kalapos 2000, Corcuera et al. 2002), most studies of P–V curve traits have focused on plasticity and adaptation within species or across <10 species at a time. Our aim here was to compare widely across species, and this came at the expense of repetition over seasons and years. We found that P–V curve traits varied predominantly between vegetation types and between species within vegetation types, more than within species and independent of phylogenetic relationships between species. This discussion proceeds on the premise that the traits express ecological differences between species, at least with regard to their relative rankings, if not the absolute values.

### Between-species and within-species comparison in response to water stress

Most existing literature about P–V curve traits deals with how individual plants respond to water stress. Constitutive differences between species that normally operate under different degrees of water stress need not necessarily show the same patterns. Still, responses of individual plants over time provide a reasonable working hypothesis for what might be expected across species. In response to water stress, species generally lower their TLP and their  $SP_0$  (independent of their  $W_s$ ), although sometimes there is no change (Kubiske and Abrams 1994, Ashraf and Yasmin 1995, Kozlowski and Pallardy 1997). For other P–V traits, there has been little consistency. Species have been observed to

increase (Joly and Zaerr 1987, Major and Johnsen 1999), maintain (Sobrado 1986, Anderson and Helms 1994, Ashraf and Yasmin 1995) or decrease cell wall elasticity (Chimenti and Hall 1994, Dicho et al. 2003, Saito and Terashima 2004) in response to drought. The same is the case for  $W_s$  (Joly and Zaerr 1987, Girma and Krieg 1992, Chimenti and Hall 1994, Anísko and Lindstrom 1996, Dicho et al. 2003).

Across species in our study, TLPs averaged significantly lower in low-rainfall than in high-rainfall species. Between and within vegetation types, downward shifts in TLPs were associated with downward shifts in  $SP_0$ . The cost of a low  $SP_0$  is that the high solute concentration can interfere with cell metabolism. Organic compatible solutes protect membranes and proteins from low  $SP_0$  (Schulze et al. 2005) but can be energetically expensive (Patakas et al. 2002). The lower  $W_{TLP}$  associated with low  $SP_0$  also results in more water being lost before the cells lose turgor. Nevertheless, low TLP was more closely associated with low  $SP_0$  rather than with decreased cell wall elasticity, despite large variability in  $\epsilon$ . Also, a downward shift in elasticity also combined with a downward shift in  $SP_0$  to lower the TLP, but this occurred mainly between species in a vegetation type. These results differ from those reported by Niinemets (2001), who concluded that elasticity was globally the most important source of differences between species in leaf adaptations to water limitation. Several other studies have found (with various degrees of strength) that  $SP_0$  is more closely associated with TLP than is elasticity (Bannister 1986, Burghardt and Riederer 2003, Sack et al. 2003).

Despite a three-fold difference in annual rainfall, average cell wall elasticity did not differ between vegetation types. Neither was there any evidence of a decrease in elasticity with lower soil P and the parallel increase in leaf mass per area at these vegetation types (Wright et al. 2002). Elasticity is ambiguous as a drought adaptation, as both increases and decreases in  $\epsilon$  can be interpreted as drought adaptation (Tyree and Karamanos 1981, Niinemets 2001). Low elasticity (i.e. high  $\epsilon$ ) allows a cell to drop faster in water potential for the same loss of water. Thus, a species with high average  $\epsilon$  is able to draw more water from the soil for a smaller loss of water than a species with low  $\epsilon$ . Further, the osmotic potential is kept relatively steady during changes in water content (Tyree and Karamanos 1981). Alternatively, high cell wall elasticity enables a cell to maintain turgor pressure and hence continue growth, despite losing water and thus volume.

For  $W_s$  as for elasticity, alternative arguments can be invoked about drought response. If during a drought, plants were to lose apoplastic water in preference to

symplastic water, then  $W_s$  would increase. On the other hand, plants could decrease the solute potential of the symplast and thus the TLP by pumping water from the symplast into the apoplast, lowering  $W_s$ . Zwiazek (1991) hypothesized that this could be achieved through depositing hydrophilic compounds into the cell walls (apoplast). The subsequent movement of water into the cell wall would not only lower  $W_s$  but also decrease the cell wall elasticity. Empirically, observed changes in  $W_s$  when individual plants are droughted have not been consistent. In our study results, species at low rainfall averaged marginally higher  $W_s$  than those at high rainfall, but there was strong overlap across the rainfall zones.

### Variation in P–V curve traits with regard to water availability

Within each of the four vegetation types, species-mean predawn and midday water potentials were closely correlated with each other but varied independently from the species-mean TLPs at three of the four vegetation types. There was considerable interspecific variation in both types of traits which, on the face of it, should have given us sufficient power to detect any general trends. This result contrasts somewhat with more controlled studies, where e.g. the same individuals were sampled simultaneously for all traits. Considering six tree species in Brazil, Bucci et al. (2004) found that species and individuals that were operating at lower water potentials also had lower TLPs, while there was a trend ( $P = 0.076$ ) to the same in Myers et al. (1997, re-analysis using Datathief (Tummers 1999). Kubiske and Abrams (1994) showed significant relationships between  $\psi_{MD}$  and TLP in three communities during a dry year.

The close correlation between  $\psi_{PD}$  and  $\psi_{MD}$  meant that the difference between  $\psi_{MD}$  and  $\psi_{PD}$  varied little across species within low-rainfall vegetation types. By analogy with Ohm's law,  $\psi_{MD} - \psi_{soil} = (g_s \times VPD / K_{whole\ plant})$ , where  $g_s$  represents stomatal conductance, VPD the vapour pressure deficit (in mol fraction),  $K_{whole\ plant}$  the whole plant conductance and  $\psi_{soil}$  the soil water potential. The product of  $g_s$  and VPD is the transpiration rate. Assuming that  $\psi_{soil}$  can be approximated by  $\psi_{PD}$ , the equation becomes:  $\psi_{MD} - \psi_{PD} = (g_s \times VPD / K_{whole\ plant})$ . Given that the average VPD is similar for the different species within a vegetation type, the fact that the difference between midday and predawn water potential differed little between species implies that ratios of transpiration to whole plant conductance were similar across species within a vegetation type, irrespective of differences in access to soil water. In other words, stomatal conductance rates scale with whole plant conductances for species within

a site (Nardini and Salleo 2000, Sack et al. 2005) and the slope of this relationship varies with the terms that affect the remainder of the equation: overall site humidity and overall site soil moisture availability (Nardini and Salleo 2000; Sack et al. 2005).

### Conclusions

Species did vary widely in P–V curve traits, within as well as between vegetation types. Two principal conclusions emerged from this study. First, between-species variation in TLP arose predominantly from differences in solute potential rather than from differences in elasticity or symplastic water content. Second, species varied in TLP and solute potential more or less independently of variation in access to water or rooting depth, as indicated by predawn water potential differences within vegetation types. It appears that different leaf-level adaptations for responding as leaf water potential declines can be found in any combination with different whole-plant adaptations for investing in root depth to gain access to water.

**Acknowledgements** – Many thanks go to Elizabeth Lindsay, Muhammad Masood, Barbara Rice, Robyn Sinclair and Michael Wastell, who assisted with sample collection and processing. The MS benefited from suggestions by Lawren Sack. This study was supported by Australian Research Council.

### Supplementary material

The following material is available to download from <http://www.blackwell-synergy.com/loi/ppl>

**Appendix S1.** Details of (a) the study site conditions and (b) sampling dates.

**Appendix S2.** Details of phylogenetic analyses.

**Appendix S3.** Phylogenetic tree of the 62 study species.

**Appendix S4.** Sets of phylogenetically independent contrasts for four vegetation types, two each with low and high rainfall and two each with low and high phosphorus soils, in New South Wales, Australia.

**Appendix S5.** *P*-values of tests that slopes are different from zero (Model I regression, through the origin) of evolutionary divergences between pairs of water use traits in four vegetation types.

**Appendix S6.** Rainfall contrasts between species at high and low soil phosphorus (P) levels and soil (P) contrasts between species at high- and low-rainfall levels for correlations between various traits of 16–18 species each in two high-rainfall and two low-rainfall vegetation types.

## References

Anderson PD, Helms JA (1994) Tissue water relations of *Pinus ponderosa* and *Arctostaphylos patula* exposed to various levels of soil moisture depletion. *Can J For Res* 24: 1495–1502

Anísko T, Lindstrom OM (1996) Cold hardiness and water relations parameters in *Rhododendron* cv Catawbiense Boursault subjected to drought episodes. *Physiol Plant* 98: 147–155

Ashraf M, Yasmin N (1995) Responses of four arid zone grass species from varying habitats to drought stress. *Biol Plant* 37: 567–575

Bannister P (1986) Drought resistance, water potential and water content in some New Zealand plants. *Flora* 178: 23–40

Beadle NCW (1954) Soil phosphate and the delimitation of plant communities in Eastern Australia. *Ecology* 35: 370–375

Brodrribb TJ, Holbrook NM, Edwards EJ, Gutierrez MV (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant Cell Environ* 26: 443–450

Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M (2004) Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. *Tree Physiol* 24: 891–899

Burghardt M, Riederer M (2003) Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. *J Exp Bot* 54: 1941–1949

Chimenti CA, Hall AJ (1994) Responses to water stress of apoplastic water fraction and bulk modulus of elasticity in sunflower (*Helianthus annuus* L.) genotypes of contrasting capacity for osmotic adjustment. *Plant Soil* 166: 101–107

Corcuera L, Camarero JJ, Gil-Pelegrin E (2002) Functional groups in *Quercus* species derived from the analysis of pressure-volume curves. *Trees* 16: 465–472

Dichio B, Xiloyannis C, Angelopoulos K, Nuzzo V, Bufo SA, Celano G (2003) Drought-induced variations of water relations parameters in *Olea europaea*. *Plant Soil* 257: 381–389

Falster DS, Warton DI, Wright IJ (2003) (S)MATR: standardised major axis tests and routines. Version 1.0, available at <http://www.bio.mq.edu.au/ecology/SMATR>

Fonseca CR, Overton JM, Collins B, Westoby M (2000) Shifts in trait-combinations along rainfall and phosphorus gradients. *J Ecol* 88: 964–977

Girma FS, Krieg DR (1992) Osmotic adjustment in sorghum I. Mechanisms diurnal osmotic potential changes. *Plant Physiol* 99: 577–582

Harvey PH, Pagel MD (1991) The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford

Joly RJ, Zaerr JB (1987) Alteration of cell-wall water content and elasticity in douglas-fir during periods of water deficit. *Plant Physiol* 83: 418–422

Koide RT, Robichaux RH, Morse SR, Smith CM (1989) Plant water status, hydraulic resistance and capacitance. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) *Plant Physiological Ecology: Field Methods and Instrumentation*. Chapman & Hall, London, pp 161–183

Kozlowski TT, Pallardy SG (1997) *Physiology of Woody Plants*. Academic Press, San Diego

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *Bot Rev* 68: 270–334

Krasser D, Kalapos T (2000) Leaf water relations for 23 angiosperm species from steppe grasslands and associated habitats in Hungary. *Community Ecol* 1: 123–131

Kubiske ME, Abrams MD (1990) Pressure-volume relationships in non-rehydrated tissue at various water deficits. *Plant Cell Environ* 13: 995–1000

Kubiske ME, Abrams MD (1994) Ecophysiological analysis of woody species in contrasting temperate communities during wet and dry years. *Oecologia* 98: 303–312

Major JE, Johnsen KH (1999) Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. II. Temporal trends and response to varying soil water conditions. *Tree Physiol* 19: 375–382

Myers BA, Duff GA, Eamus D, Fordyce IR, O'Grady A, Williams RJ (1997) Seasonal variation in water relations of trees of differing leaf phenology in a wet-dry tropical savanna near Darwin, northern Australia. *Aust J Bot* 45: 225–240

Nardini A, Salleo S (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees-Struct Funct* 15: 14–24

Niinemets Ü (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82: 453–469

Patakas A, Nikolaou N, Zioziou E, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. *Plant Sci* 163: 361–367

Randell BR, Barlow BA (1998) *Senna*, Flora of Australia. 12: 89–138. CSIRO Australia, Melbourne

Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant Cell Environ* 26: 1343–1356

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

Saito T, Terashima I (2004) Reversible decreases in the bulk elastic modulus of mature leaves of deciduous *Quercus* species subjected to two drought treatments. *Plant Cell Environ* 27: 863–875

Schulte PJ, Hinckley TM (1985) A comparison of pressure-volume curve data analysis techniques. *J Exp Bot* 36: 1590–1602

Schulze ED, Beck E, Müller-Hohenstein K (2005) *Plant Ecology*. Springer, Berlin

Sobrado MA (1986) Aspects of tissue water relations and seasonal changes of leaf water potential components of evergreen and deciduous species coexisting in tropical dry forests. *Oecologia* 68: 413–416

Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York

Tummers B (1999) Datathief II. Available at <http://www.nikhef.nl/~keeshu/datathief/>

Turner NC (1981) Techniques and experimental approaches for the measurements of plant water status. *Plant Soil* 58: 339–366

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J Exp Bot* 23: 267–282

Tyree MT, Jarvis PG (1982) Water in tissues and cells. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) *Physiological Plant Ecology II: Water Relations and Carbon Assimilation II*. Springer-Verlag, Berlin, pp 35–77

Tyree MT, Karamanos AJ (1981) Water stress as an ecological factor. In: Grace JB, Ford ED, Jarvis PG (eds) *Plants and Their Atmospheric Environment*. Blackwell Scientific Publications, Oxford, pp 237–261

Wright IJ, Reich PB, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct Ecol* 15: 423–434

Wright IJ, Westoby M, Reich PB (2002) Convergence towards higher leaf mass per area has different consequences for leaf lifespan in dry and nutrient-poor habitats. *J Ecol* 90: 534–543

Zwiazek JJ (1991) Cell wall changes in white spruce (*Picea glauca*) needles subjected to repeated drought stress. *Physiol Plant* 82: 513–518

Edited by V. Hurry