Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species

Original Article

Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species

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ABSTRACT

Because iso- and anisohydric species differ in stomatal regulation of the rate and magnitude of fluctuations in shoot water potential, they may be expected to show differences in the plasticity of their shoot water relations components, but explicit comparisons of this nature have rarely been made. We subjected excised shoots of co-occurring anisohydric *Juniperus monosperma* and isohydric *Pinus edulis* to pressure-volume analysis with and without prior artificial rehydration. In *J. monosperma*, the shoot water potential at turgor loss (Ψ_{TLP}) ranged from −3.4 MPa in artificially rehydrated shoots to −6.6 MPa in shoots with an initial Ψ of −5.5 MPa, whereas in *P. edulis* mean Ψ_{TLP} remained at ~ −3.0 MPa over a range of initial Ψ from −0.1 to −2.3 MPa. The shoot osmotic potential at full turgor and the bulk modulus of elasticity also declined sharply with shoot Ψ in *J. monosperma*, but not in *P. edulis*. The contrasting behaviour of *J. monosperma* and *P. edulis* reflects differences in their capacity for homeostatic regulation of turgor that may be representative of aniso- and isohydric species in general, and may also be associated with the greater capacity of *J. monosperma* to withstand severe drought.

Key-words: anisohydry; drought; isohydry; osmotic potential; turgor.

INTRODUCTION

A continuum exists in the degree to which stomata regulate the minimum leaf water potential (Ψ_L). Isohydric species represent one end of the continuum and their stomata adjust to keep Ψ_L from dropping below a set value. Alternatively, anisohydric species exist at the other end of the spectrum and their stomata do not act to regulate Ψ_L at a specific value, but instead allow Ψ_L to decline as the soil dries or the vapour pressure deficit increases before reaching species-specific minimum values at which stomatal closure is complete. Although generalizations are often made about what types of plants exist at each end of the spectrum, a great deal of taxonomic diversity is actually observed along the continuum

(Tardieu & Davies 1992; Loewenstein & Pallardy 1998a,b; Tardieu & Simonneau 1998; Bonal & Guehl 2001; Pou *et al.* 2012; West *et al.* 2012; Zhang *et al.* 2013). Another generalization about the spectrum is that anisohydric plants dominate arid environments. However, there are examples of species at both ends of the spectrum co-occurring in arid regions (Linton *et al.* 1998; McDowell *et al.* 2008; Quero *et al.* 2011; Ogle *et al.* 2012; West *et al.* 2012).

There are some potential advantages to isohydry. The homeostasis associated with preventing Ψ_L from falling below a set minimum value (1) may keep the rest of the plant from experiencing embolism-inducing xylem pressures, which could minimize construction costs of the xylem if the species depends on structural avoidance of embolism; and (2) the leaves of isohydric plants would not have to undergo osmotic adjustment to maintain turgor as the soil dries, which would avoid energy costs associated with solute accumulation. The obvious drawback to isohydry is that maintaining the set Ψ_L as drought intensifies requires nearly complete stomatal closure, which severely constrains carbon assimilation.

There is considerable interest in determining if iso- versus anisohydric species are more likely to die during severe droughts. McDowell *et al.* (2008) hypothesized that isohydric species are more likely to die during long-term droughts because of their inability to sustain stomatal opening and capture new carbon. Species that tend to behave more anisohydrically often have more embolism-resistant xylem, which can be associated with maintenance of gas exchange during mild drought stress (Pou *et al.* 2012). However, Mitchell *et al.* (2012) showed that container-grown saplings of the more isohydric *Pinus radiata* were able to survive roughly twice as long as two more anisohydric *Eucalyptus* species during an imposed long-term drought because *P radiata* relied on carbohydrate reserves after early stomatal closure, whereas more prolonged stomatal opening in the *Eucalyptus* species led to more rapid dehydration and hydraulic failure. Although it is not clear how these results may apply to field-grown plants, where anisohydric *Juniperus* species co-occur with more isohydric *Pinus* species, the latter experience much greater mortality during extreme long-term drought (Breshears *et al.* 2005, 2009; McDowell *et al.* 2008; Koepke *et al.* 2010).

In addition to stomatal regulation and xylem structural characteristics, leaf biophysical characteristics can also influence a species' response to sustained drought. The principal leaf biophysical properties that have been implicated in playing key roles in maintaining leaf functioning in plants subjected to drought are the osmotic potential at full turgor ($\Psi_{\pi\,100}$), the osmotic potential and therefore water potential at the turgor loss point (Ψ_{TLP}), and the leaf bulk modulus of elasticity (ε). These leaf water relations components have traditionally been evaluated from water potential isotherms using the pressure-volume (P-V) technique (Tyree & Hammel 1972). Short- and long-term changes in ε and $\Psi_{\pi\,100}$ govern the rate at which cell turgor and volume decrease with declining Ψ_L, and can contribute to maintenance of physiological activity either through conserving turgor or protoplast volume as Ψ_L declines (Kaiser 1982; Sen Gupta and Berkowitz 1987; Berkowitz & Kroll 1988; Marshall & Dumbroff 1999; Saito & Terashima 2004). Nevertheless, in a recent meta-analysis of 317 species, it was concluded that $\Psi_{\pi\,100}$ is the major determinant of Ψ_{TLP} and that Ψ_{TLP} was a major driver of drought tolerance with no direct role for ε (Bartlett *et al.* 2012). Osmotic adjustment, the active accumulation of solutes in the symplast, as opposed to passive, dehydration-induced changes, has been proposed to play a role in turgor maintenance and therefore maintenance of stomatal opening and gas exchange (Hsiao *et al.* 1976), but not necessarily growth because of its greater sensitivity to water stress than photosynthesis (Hsiao 1973; Munns 1988; Muller *et al.* 2011).

Although many studies have applied the P-V technique to characterize leaf water relations components of species representing a range of hypothesized drought tolerance, few, if any, have explicitly compared species representing different points along the isohydry/anisohydry continuum. Two coniferous species, the anisohydric *Juniperus monosperma* and isohydric *P. edulis*, co-occur over large areas of the semiarid southwestern United States. In anisohydric species such as *J. monosperma*, leaf tissue produced under relatively mesic conditions must cope with large seasonal variations in Ψ after it is fully formed and mature. In contrast, more stringent stomatal regulation in species such as *P. edulis* strongly constrains seasonal variation in plant Ψ (West *et al.* 2007; Plaut *et al.* 2012; Limousin *et al.* 2013). Based on their contrasting modes of regulation of plant Ψ and previous indications that some anisohydric species can show rapid adjustments in leaf water relations components, we hypothesized that leaf water relations components would exhibit greater seasonal and shorter term plasticity in the anisohydric *J. monosperma* than in the co-occurring isohydric *P. edulis*. To address this hypothesis, we conducted P-V analyses on non-rehydrated and rehydrated shoots collected over a 16 month period during which shoot Ψ of *J. monosperma* varied from ~ −1 to −6 MPa and that of *P. edulis* from ~ −1 to −3 MPa.

MATERIALS AND METHODS

Site and plant material

The plant material was collected at the Los Alamos National Laboratory Plant Survival and Mortality (SUMO) site in Los Alamos, New Mexico (35°48′48″N 106°17′58″W, elevation 2150 masl). The site is located on the eastern side of Jemez Mountains on Pajarito Plateau at the transition zone from ponderosa pine (*P. ponderosa* Doug. ex Laws.) dominated forest to piñon-juniper woodland, and is dominated by piñon pine (*P. edulis* Engelm.) and juniper [*J. monosperma* (Engelm.) Sarg.], with the occasional ponderosa pine tree at the vicinity of the site and Gambel oak (*Quercus gambelii* Nutt.) shrubs among the trees.

Mean annual temperature (1981–2012) is 11 °C with the coldest month, January, averaging −2 °C and the warmest month, July, averaging 20 °C. Mean annual precipitation is 470 mm of which 48% falls during the North American Monsoon between July and September. Soils are Hackroy clay loam derived from volcanic tuff (Soil Survey Staff, Natural Resource Conservation Service, United States Department of Agriculture; http://websoilsurvey.sc.egov .usda.gov/App/HomePage.htm) with depth ranging from 40 to 80 cm.

The SUMO experiment consists of five treatments each containing five mature piñon pine and juniper trees: drought, elevated temperature, combined drought and elevated temperature, control and chamber control. The drought treatment is imposed using plastic troughs made of polycarbonate sheet attached to rails approximately 1.5 m above the ground. These troughs direct approximately 50% of precipitation away from the 40×40 m drought treatment area. The elevated temperature treatment is achieved using open top chambers and industrial scale air conditioning units that maintain the temperature 5 °C above ambient continuously. The chamber control treatment uses similar chambers where the temperature is kept at ambient. The treatments were initiated in early June 2012. Samples for the present study were collected only from plants growing in the control and drought treatment plots.

PV analyses

Small branches (30–50 cm) containing healthy, non-necrotic shoot tips were excised shortly after sunrise and immediately sealed in three to four layers of plastic bags to minimize post-excision changes in hydration prior to P-V analyses. Sample collection dates were March, June, July 2012 and June 2013 for *P. edulis* and April, June, July 2012 and March 2013 for *J. monosperma*. Upon arrival at the laboratory, healthy, non-necrotic shoot tips were rapidly excised and prepared for P-V analyses and the remaining material was re-sealed in the plastic bags and stored in a refrigerator at 4–5 °C for additional P-V analyses over a subsequent 2–3 day period. No time-related trends in initial shoot Ψ or other characteristics determined from P-V curves were detected over this time interval. Paired shoots from three to six individuals were either used in their original, non-rehydrated condition or subjected to a 1.5 to 3 h hydration treatment after re-cutting their bases under water. The portion of the shoot that had been under water was removed prior to determination of its P-V curve to minimize impacts of oversaturation on the shape of the P-V curve, which results in a gradual rather than

steep initial decline in $1/\Psi$ as relative water deficit (RWD) increases; the so-called 'plateau effect' (e.g. Parker & Pallardy 1987; Kubiske & Abrams 1990; Dichio *et al*. 2003). After a rapid determination of initial shoot Ψ with a pressure chamber and weight to 0.0001 g, Ψ and weight were recorded periodically while shoots were allowed to dry slowly on the laboratory bench. As with other conifers, the dehydration process sometimes required up to 48 h to obtain a sufficient number of points on the P-V curve. Initial Ψ of non-rehydrated shoots was always within the range of predawn and midday shoot Ψ measured in the field (see below).

Because sample saturated weights are necessary for determination of relative water content (RWC) or RWD for P-V analyses, saturated weights of non-rehydrated and rehydrated samples were estimated by extrapolating linear regressions fitted to the pre-turgor loss portions of plots of sample fresh weight versus Ψ to $\Psi =$ zero. When P-V curve data are presented as sample weight against Ψ rather than $1/\Psi$, the pre-turgor loss portion is linear unless substantial oversaturation has occurred. For rehydrated samples, the fresh weight corresponding to the initial balance pressure was not included in the regression analysis to eliminate the potential influence of the plateau effect on estimates of saturated weights. In plots of sample weight against Ψ, the plateau effect is manifested as a steep initial decline in fresh weight with decreasing Ψ, which leads to overestimation of the saturated weight if these points are included in the regression. However, inspections of plots of fresh weight versus water potential for rehydrated samples indicated no evidence of a rehydration-induced plateau in many cases and a negligible influence on saturated weight estimates of excluding or including the initial balance pressure data in the regression (Supporting Information Fig. S1). Plots of $1/\Psi$ versus sample weight loss were updated during P-V curve determination to ensure that at least three to five data points on the linear portion of the curve had been obtained. Later, regressions were fitted to the linear portions of plots of $1/\Psi$ versus RWD beginning with the three lowest values of $1/\Psi$ and adding points until the coefficient of determination reached a maximum. The P-V curves and the regressions fitted to their linear portions were then used to estimate several bulk tissue water relations characteristics. The osmotic potential at saturation ($\Psi_{\pi\,100}$) was estimated from the extrapolation of the linear regression to the $1/\Psi$ axis at RWD = 0. The RWD and water (and osmotic) potential at the turgor loss point (Ψ_{TLP}) were estimated from the intersection of the linear portion of the curve with a negative exponential function fitted to the non-linear portion. Turgor was estimated from the difference between shoot Ψ at a given RWD and the corresponding value of osmotic potential derived from the extrapolation of the linear regression. The mean modulus of elasticity (ε, MPa) was estimated as the slope of a regression fitted to the nearly linear portion of the relationship between turgor and RWC, which comprised the points on the initial portion of the P-V curve where turgor > 0 MPa (Supporting Information Fig. S2). Successive points were included until the coefficient of determination for a linear fit was maximized. In a subset of P-V curves

determined on rehydrated and non-rehydrated shoots from six individuals of *J. monosperma*, the tissue symplastic water fraction (SWF) was estimated from the *x*-intercept at $1/\Psi = 0$ of the regression fitted to the linear portion of the relationship between $1/\Psi$ and RWD. These values of SWF were then used to calculate symplast solute content on a dry weight basis (N_s, Tyree *et al*. 1978) to determine whether differences in $\Psi_{\pi\,100}$ between rehydrated and non-rehydrated shoots were largely attributable to true osmotic adjustment (changes in symplast solute content) or hydration-induced changes in symplast volume.

Thermocouple psychrometry

Initial P-V analyses of non-rehydrated and rehydrated *J. monosperma* shoots suggested that rehydration induced large changes in both $\Psi_{\pi\,100}$ and Ψ_{TLP}. To test for artefacts associated with application of the P-V method to rehydrated shoots of this species, bulk tissue osmotic potential of non-rehydrated and rehydrated shoots was measured independently via thermocouple psychrometry. Subsamples of tissue from the same non-rehydrated and rehydrated shoots from the same six individuals used for the P-V-based estimates of SWF and N_s described above were sealed in vials, frozen in liquid N_2, thawed to room temperature and subsamples placed in cups of screen cage thermocouple psychrometer chambers (JRD Merrill, Logan, UT, USA) connected to a digital psychrometer meter (Psypro, Wescor Inc., Logan, UT, USA). Prior to sealing the chambers, the tissue was crushed with a metal rod to release cell sap. The chambers were then sealed inside a plastic bag and submerged in an insulated water bath where they were allowed to equilibrate for a minimum of 4 h. A minimum of three measurements were then made at 30 min intervals to ensure that equilibration had occurred. The psychrometers had been previously calibrated using solutions of known osmolality. Bulk tissue Ψ_π determined in this manner was compared with the estimates derived from regressions fitted to the linear portions of P-V curves determined on the same material.

Field measurements of water potential

Pre-dawn and midday shoot water potentials of each tree in the SUMO experiment were measured monthly using Scholander-type pressure chambers (PMS Instruments, Albany, OR, USA). Two healthy twigs were collected from south-facing branches before sunrise and between 1130 and 1300 h, placed in a humidified plastic bag and stored in a cooler until water potential was measured within 2 h of collection.

Data analysis

Least squares regressions were fitted to linear portions of P-V curves as described above. The significance of treatment-related (rehydration versus no rehydration) differences in leaf water relations components was evaluated with one-way analysis of variance (ANOVA). The significance of relationships

between shoot water relations components was evaluated first with one-way ANOVA and linear regressions were fitted to the data when these relationships were significant ($P < 0.05$).

RESULTS

Seasonal courses of midday shoot Ψ were consistent with anisohydric behaviour in *J. monosperma* and isohydric behaviour in *P. edulis* (Fig. 1). Midday shoot Ψ varied by about 3.6 MPa in *J. monosperma* and only 0.9 MPa in *P. edulis* during the study period, which was characterized by pronounced soil drying and rewetting cycles as manifested in seasonal courses of predawn shoot Ψ in *J. monosperma*. The level of tissue hydration as reflected in water potential at the initiation of P-V curves had pronounced effects on multiple shoot water relations components in *J. monosperma* but not in *P. edulis* (Fig. 2). For shoots collected from the same *J. monosperma* plant when shoot water potential was −2.4 MPa at the time of excision, $\Psi_{\pi\,100}$ was −3.1 MPa in a non-rehydrated shoot and −2.0 MPa in a rehydrated ($\Psi = -0.03$ MPa) shoot (Fig. 2a,b). The shoot water potential at turgor loss was −5.0 MPa in the non-rehydrated shoot and −3.8 MPa in the rehydrated shoot. The initial shoot Ψ of −2.4 MPa fell approximately in the upper third of the range

of predawn and midday shoot Ψ observed in *J. monosperma* at the study site during the study (Fig. 1). For *P. edulis* shoots collected from the same plant when shoot Ψ was −1.8 MPa at the time of excision, $\Psi_{\pi\,100}$ was −1.5 MPa in a non-rehydrated shoot and −1.7 MPa in a rehydrated ($\Psi = -0.1$ MPa) shoot (Fig. 2c,d). Values of Ψ_{TLP} were identical for both shoots (−2.5 MPa). Shoot Ψ of *P. edulis* ranged from about −1 to −3 MPa during the study period. There was no significant difference in Ψ_{TLP} of paired rehydrated and non-rehydrated shoots of *P. edulis* on any of the sampling dates (Fig. 1). In contrast, differences in Ψ_{TLP} of rehydrated and non-rehydrated *J. monosperma* shoots were highly significant ($P \le 0.01$) on all three dates when this comparison was made (Fig. 1). In *J. monosperma*, the Ψ_{TLP} of non-rehydrated samples appeared to track seasonal changes in midday Ψ, whereas Ψ_{TLP} of rehydrated shoots was nearly constant seasonally.

When P-V curve data for *J. monosperma* were combined across sampling dates and rehydration treatments, highly significant ($P < 0.0001$) linear relationships were observed between initial shoot Ψ and Ψ_{TLP}, the relative water deficit at turgor loss and bulk modulus of elasticity (Fig. 3). Over a range of initial shoot Ψ from ~0 to −5.5 MPa, Ψ_{TLP} decreased from −3.2 to −6.4 MPa, the relative water deficit at turgor loss increased from 0.21 to 0.40 and the bulk shoot ε decreased from 11 to 2.8 MPa. In contrast to *J. monosperma*, there was no significant relationship between initial shoot Ψ and Ψ_{TLP} in *P. edulis* (Fig. 4). The mean Ψ_{TLP} of *P. edulis* was −3.05 MPa over a range of initial shoot Ψ from −0.1 to −2.26 MPa. Relationships between initial shoot Ψ and the relative water deficit at turgor loss and the bulk tissue ε were also nonsignificant in *P. edulis* (Supporting Information Fig. S3).

Both *P. edulis* and *J. monosperma* exhibited highly significant linear relationships between $\Psi_{\pi\,100}$ and Ψ_{TLP} (Fig. 5). However, values of $\Psi_{\pi\,100}$ and Ψ_{TLP} converged as $\Psi_{\pi\,100}$ declined in *P. edulis* and diverged in *J. monosperma*. Thus, increasing osmotic solute content in *P. edulis* had a diminishing effect on depression of Ψ_{TLP} and a steadily increasing or amplified effect in *J. monosperma*. The relationship between $\Psi_{\pi\,100}$ and Ψ_{TLP} in *J. monosperma* was associated with a significant positive correlation between Ψ_{TLP} and ε ($P < 0.0001$) indicating that Ψ_{TLP} became more negative with increasing tissue elasticity (Fig. 6).

Additional P-V analyses carried out on shoots collected from six *J. monosperma* plants on the same date indicated in addition to $\Psi_{\pi\,100}$ being substantially more negative in non-rehydrated shoots, their estimated tissue symplastic water fraction and symplastic solute content were significantly higher than in rehydrated shoots, consistent with hydration-induced changes in $\Psi_{\pi\,100}$ reflecting true osmotic adjustment (increase in symplastic solute content) rather than passive concentration of solutes due to shrinkage of the symplast (Table 1). Thus, key shoot water relations characteristics of *J. monosperma* were highly dynamic and tightly coordinated with variations in Ψ of intact and excised shoots. For the same six plants, bulk tissue osmotic potentials of non-rehydrated and rehydrated shoots estimated from regression equations fitted to the linear portion of P-V curves agreed reasonably
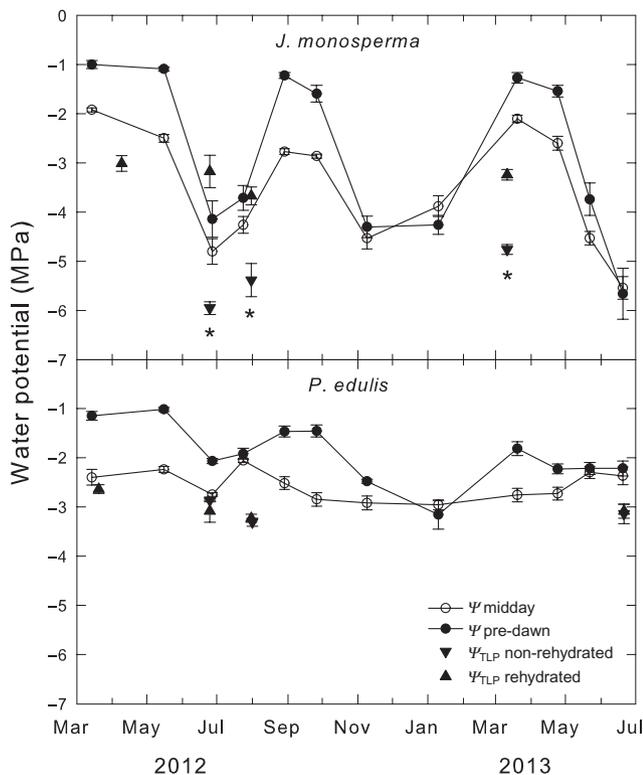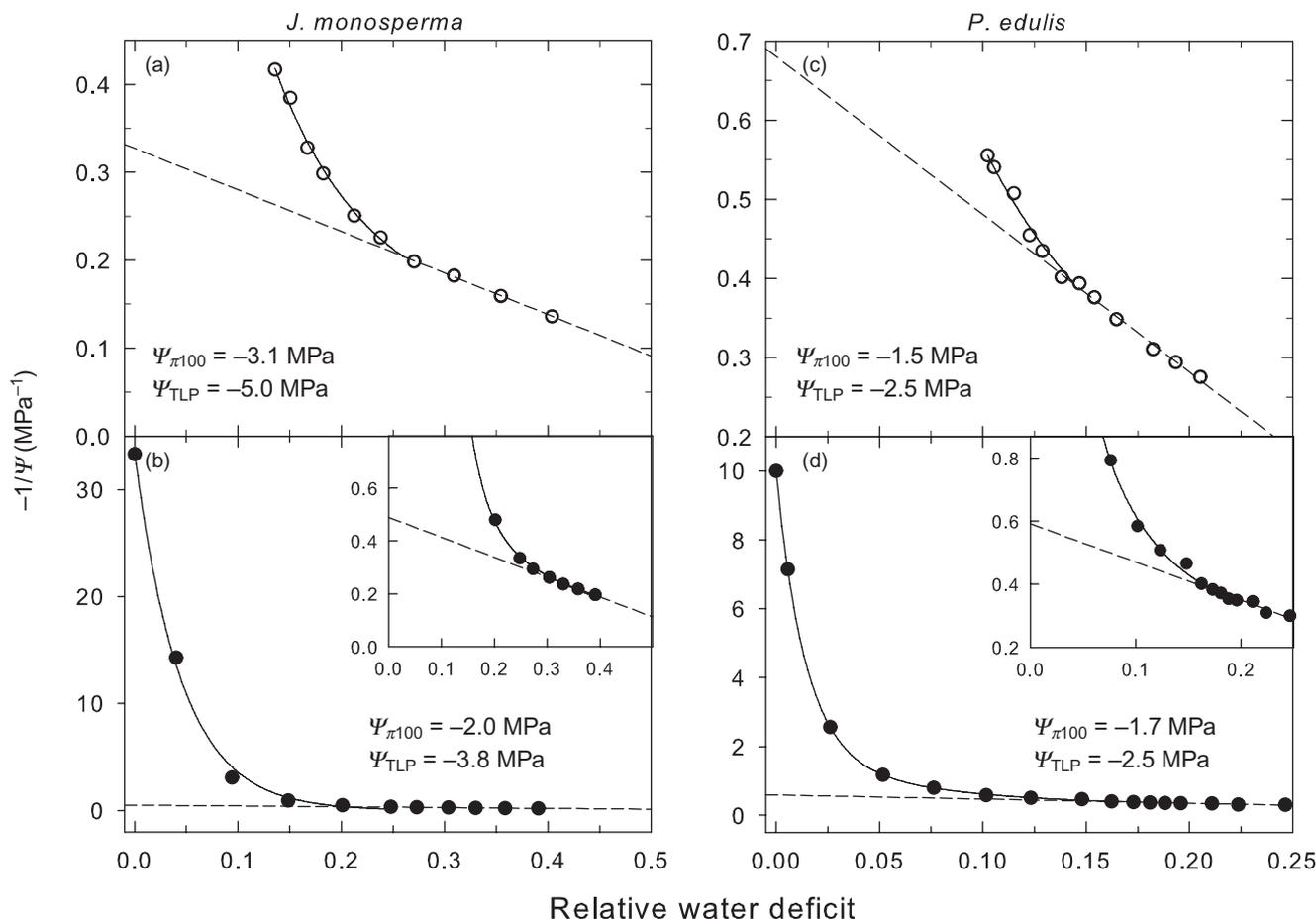

Figure 1. Seasonal courses of predawn and midday shoot water potential (Ψ) in co-occurring *J. monosperma* and *P. edulis* trees. Estimates of the shoot water potential at the turgor loss point (Ψ_{TLP}) obtained from pressure-volume curves for rehydrated and non-rehydrated shoots excised on different dates are shown. Asterisks indicate significant differences ($P \le 0.01$) in Ψ_{TLP} between rehydrated and non-rehydrated shoots.

Figure 2. Representative pressure-volume curves for a non-rehydrated (a) and rehydrated (b) shoot collected from the same *J. monosperma* plant on 12 March 2013 and a non-rehydrated (c) and rehydrated (d) shoot collected from the same *P. edulis* plant on 24 June 2013. The shoot water potentials at the time of excision in a and c were −2.4 MPa and −1.8 MPa, and −0.03 and −0.1 MPa after rehydration in b and d. Note difference in *x*- and *y*-axis scales among the panels. Osmotic potentials at saturation ($\Psi_{\pi\,100}$) and the water potential at the turgor loss point (Ψ_{TLP}) are shown. Insets in b and d show expanded axis scales for the region of the curve near turgor loss.

well with independent measurements of osmotic potential of frozen and thawed tissue using thermocouple psychrometry (Fig. 7). The 95% confidence interval of the linear regression fitted to the data in Fig. 7 included the 1:1 line.

DISCUSSION

Consistent with our hypothesis, our results showed that in the anisohydric *J. monosperma* key shoot water relations characteristics such as $\Psi_{\pi\,100}$, Ψ_{TLP} and ε exhibited a high degree of apparent plasticity in response to changes in tissue hydration over short timescales. In contrast, these shoot properties appeared to be nearly static in the isohydric *P. edulis*. We suggest that this contrasting behaviour of *J. monosperma* and *P. edulis* may be associated with their modes of regulation of plant water status and may be representative of anisohydric and isohydric species in general. However, testing this hypothesis would require systematic comparisons of species predetermined to occupy different positions along the anisohydry/isohydry continuum. Based on the P-V relationships obtained here, estimated minimum values of shoot

turgor were always above zero MPa for *J. monosperma* and periodically near zero for *P. edulis*, respectively, over the range of shoot Ψ observed in the field during the study period (Fig. 1). Extrapolation of the P-V relationships (Figs 3 & 4) suggests that *J. monosperma* and *P. edulis* would ultimately lose turgor at shoot water potentials of approximately −8.2 and −3.3 MPa, respectively. Xylem pressures reported to cause 50% loss of hydraulic conductivity in *J. monosperma* (−11.6 to −12 MPa) and *P. edulis* (−4.5 MPa) branches are considerably more negative than these values (Linton *et al.* 1998; Pockman & Sperry 2000; Willson *et al.* 2008). Thus, turgor loss and potentially irreversible dehydration damage to photosynthetic shoots may occur well in advance of levels of stem hydraulic dysfunction reported to be essentially irreversible in conifers (Brodribb & Cochard 2009; Brodribb *et al.* 2010). Massive dehydration-induced leaf death would likely compromise plant carbon balance unless a new cohort of leaves were produced, which would require alleviation of drought conditions.

The behaviour exhibited by *J. monosperma* was similar to that observed in the extremely anisohydric desert shrub
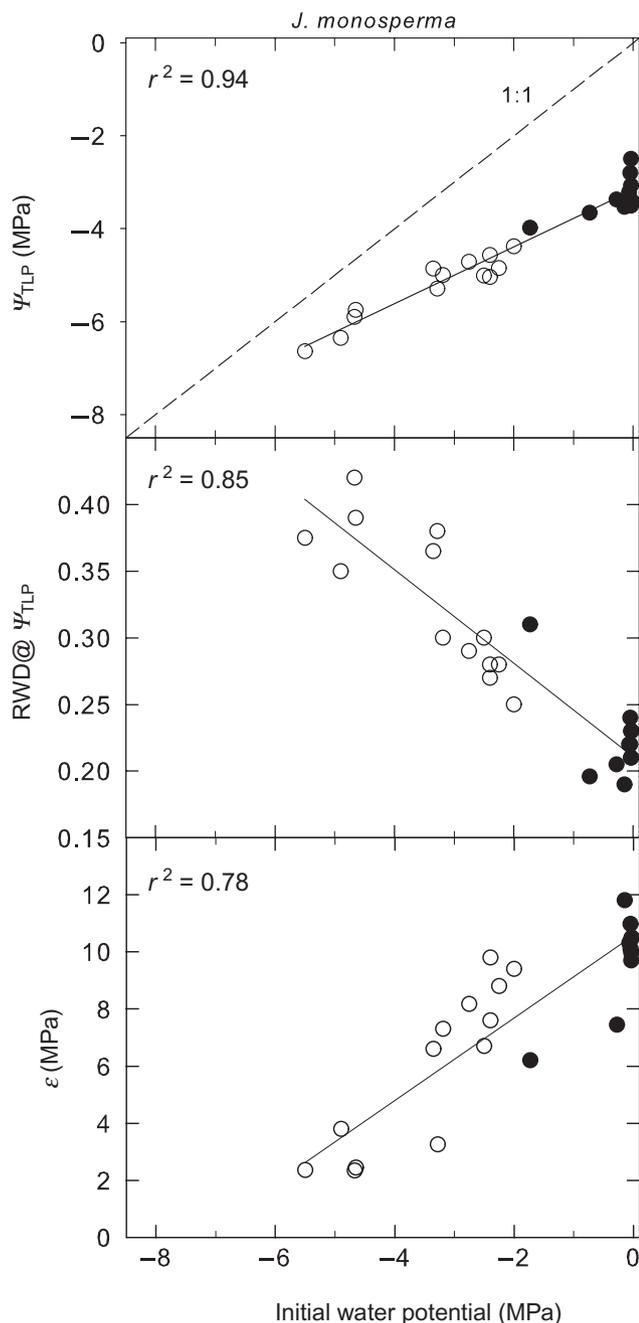
Figure 3. Relationships between initial shoot water potential and (a) the water potential at the turgor loss point (Ψ_{TLP}), (b) the relative water deficit at the turgor loss point (RWD@Ψ_{TLP}), and (c) the bulk tissue modulus of elasticity (ε) for ($\bigcirc$) non-rehydrated and ($\bullet$) rehydrated *J. monosperma* shoots excised on different dates during 2012–2013.

Figure 4. Relationship between the turgor loss point estimated from pressure-volume curves and the initial water potential for ($\bullet$) rehydrated and ($\bigcirc$) non-rehydrated shoots of *P. edulis* collected on different dates during 2012–2013.

Larrea tridentata in which Ψ_{TLP} of intact field-grown plants was reported to change by as much as 2 MPa diurnally (Meinzer *et al.* 1988) and by 3 MPa in excised shoots subjected to rehydration treatments in the laboratory (Meinzer *et al.* 1986). Under natural conditions, *J. monosperma*, *L. tridentata* and other anisohydric species frequently experience levels of plant Ψ predicted to be below (more negative
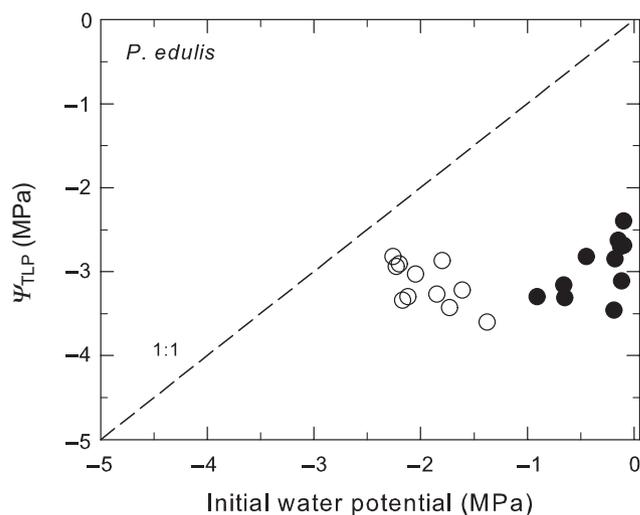
than) their Ψ_{TLP} based on P-V analyses of rehydrated samples. It is highly unlikely that the measurable rates of photosynthetic gas exchange and growth observed under these conditions can occur if plants are indeed operating at zero or even negative turgor (see below) as has been inferred in some studies.

Predictions of zero or negative turgor in woody species growing under natural field conditions are often based on comparisons of plant Ψ with Ψ_{TLP} estimated from P-V analyses of excised, rehydrated leaf or shoot samples (Odening *et al.* 1979; Monson & Smith 1982; Nilsen *et al.* 1984; Davis & Mooney 1986; Abrams *et al.* 1990; Grammatikopoulos 1999; Mitchell *et al.* 2008). Paradoxically, these predictions seem to be more frequent for species considered to be highly drought tolerant. In *L. tridentata*, for example, stomatal opening and positive net photosynthesis are maintained at plant Ψ as low as −8 MPa (Odening *et al.* 1974), considerably lower than estimates of Ψ_{TLP} from P-V curves determined on rehydrated shoots (Monson & Smith 1982; Nilsen *et al.* 1984). It had even been suggested that *L. tridentata* was able to grow at 'non-positive' values of turgor (Odening *et al.* 1979). Current understanding of biophysical control of cell expansion in plants cannot provide an explanation for how growth could occur at negative values of turgor. Subsequent P-V analyses of non-rehydrated *L. tridentata* shoots suggested that turgor was maintained to at least −8.1 MPa, consistent with observations of physiological activity in the field (Meinzer *et al.* 1986). The ability of living plant cells to develop negative turgor has been controversial (Tyree 1976; Rhizopoulou 1997). The puzzling frequency of its apparent occurrence in highly drought-tolerant species has been attributed to the ability of small thick-walled cells in scleromorphic leaves to resist collapse (Oertli *et al.* 1990). However, Tyree (1976) pointed to pitfalls in the analysis and interpretation of some types of data from which negative values of turgor are
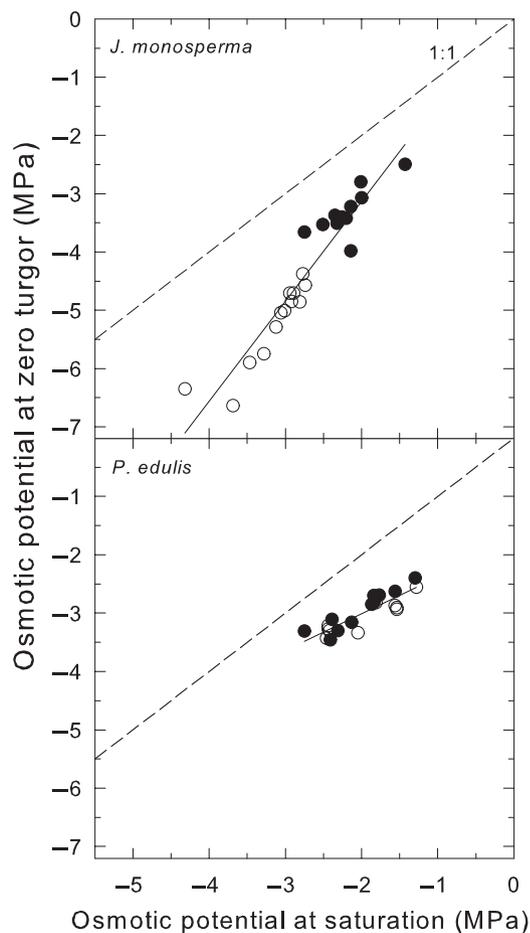
Figure 5. Relationships between the osmotic potential at zero turgor (turgor loss point) and the osmotic potential at saturation determined from pressure-volume curves for (●) rehydrated and (○) non-rehydrated shoots of *J. monosperma* and *P. edulis*. Regressions are significant at *P* < 0.0001.

Figure 6. Relationship between the water potential at the turgor loss point (Ψ_{TLP}) and the bulk tissue modulus of elasticity (ε) for (○) non-rehydrated and (●) rehydrated *J. monosperma* shoots excised on different dates during 2012–2013. Data taken from Fig. 3.

inferred and noted that in P-V analyses, substantially negative values of turgor should be manifested as a distinct non-linearity in the normally linear post-turgor loss portion of P-V curves.

We suggest that the dynamic nature of shoot water relations components, particularly Ψ_{TLP}, evident from P-V analyses in *J. monosperma* is a manifestation of normal homeostatic physiological regulation rather than a hydration-induced artefact. Anisohydric species such as *J. monosperma* show large seasonal variation in minimum plant Ψ and rapid short-term variations in Ψ after drought-breaking precipitation events. The impact of variation in tissue hydration on P-V curve characteristics in drought tolerant, anisohydric species such as *J. monosperma* may only become an artefact when results obtained from excised shoots at a given level of hydration are applied to intact plants operating at a different level of hydration.

Concerns about effects of sample rehydration on the outcome of P-V analyses have focused largely on artefacts associated with oversaturation of tissues, which affects the initial shape of the P-V curve at values of Ψ close to zero. This
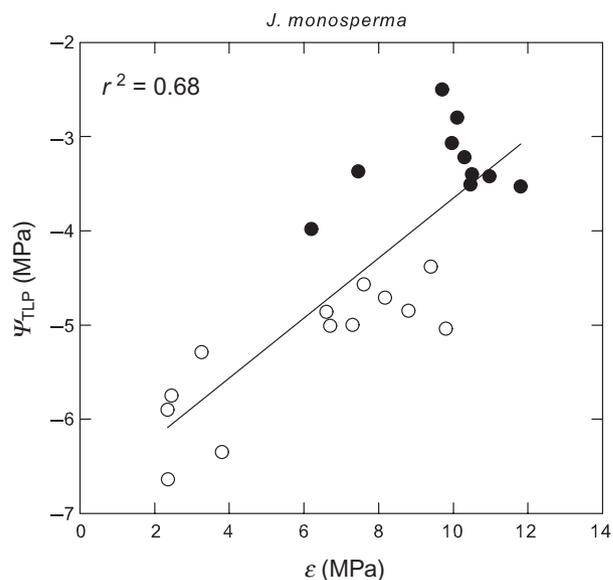
so-called plateau effect can result in significant overestimates of tissue saturated weights and therefore of RWD and ultimately of $\Psi_{\pi\ 100}$ because of the extrapolation involved (Parker & Pallardy 1987; Kubiske & Abrams 1990; Dichio *et al.* 2003). Oversaturation of tissue can result from the long rehydration times of up to 24 h or more employed in some studies. However, we limited rehydration times of *J. monosperma* and *P. edulis* to 1.5 to 3 h and removed the portion of the sample that had been in direct contact with water prior to determination of P-V curves. This procedure resulted in small to undetectable plateau effects. Because estimates of Ψ_{TLP} depend on identifying the transition from the non-linear to linear portion of the P-V curve rather than extrapolation of the linear portion to RWD = 0, variation in the level of tissue hydration corresponding to the initial point on the P-V curve should not introduce artefacts into estimates of Ψ_{TLP}. Thus, in *J. monosperma* and other drought-tolerant anisohydric species, rapid changes in Ψ_{TLP} with natural changes in Ψ in intact plants and with artificial rehydration treatments of excised shoots likely represent a physiological adaptation for coping with a large range of plant Ψ that can fluctuate rapidly.

In addition to the well-studied phenomenon of osmotic adjustment, bulk tissue elastic properties (ε) can undergo reversible changes over various timescales in mature leaves. Seasonal changes in ε have been documented in a number of species with ε increasing under drier conditions in some species (Bowman & Roberts 1985; Meinzer *et al.* 1990; Nardini *et al.* 1999; Mitchell *et al.* 2008) and decreasing in others (Moore 1988; Meinzer *et al.* 1988; Mitchell *et al.* 2008; Scholz *et al.* 2012). It is conceivable that apparently contrasting responses of ε to drought based on P-V analyses may be

Condition	Initial Ψ (MPa)	$\Psi_{\pi\,100}$ (MPa)	SWF	N_s (mOsmol g^{-1})
Non-rehydrated	−2.38 ± 0.11	−2.90 ± 0.05	0.71 ± 0.01	0.60 ± 0.02
Rehydrated	−0.05 ± 0.01	−2.12 ± 0.08	0.64 ± 0.01	0.52 ± 0.01

Table 1. Initial water potential, osmotic potential at full turgor ($\Psi_{\pi\,100}$), symplastic water fraction (SWF) and symplastic solute content per tissue dry mass (N_s) for non-rehydrated and rehydrated shoots from six *J. monosperma* plants sampled on 11 March 2013

All values (±SE) within columns are significantly different at $P \leq 0.03$.

partly attributable to rapid, rehydration-induced adjustments in ε in some species, but not in others. Adjustments in ε can also occur over much shorter than seasonal timescales (e.g. Kubiske & Abrams 1991; Saliendra & Meinzer 1991; Marshall & Dumbroff 1999; Saito & Terashima 2004), including during rehydration of samples for P-V analysis (Meinzer *et al*. 1986; Evans *et al*. 1990; Kubiske & Abrams 1991) as in *J. monosperma*. The mechanisms responsible for reversible changes in ε of fully expanded cells have not been elucidated, but changes in cell wall chemistry, particularly proteins, have been implicated (Bozarth *et al*. 1987; Marshall & Dumbroff 1999).

Dual physiological roles for adjustments in ε have been proposed. Decreased elasticity (higher ε) may enhance the impact of osmotic adjustment on turgor via greater resistance of cell walls to deformation as water is taken up in response to an osmotic gradient. On the other hand, increased elasticity can also contribute to the maintenance of turgor as RWC and Ψ decline, which has been suggested to help sustain photosynthesis in drought-stressed plants (Kaiser 1982; Berkowitz & Kroll 1988; Santakumari & Berkowitz 1990; Meinzer *et al*. 1993). In *J. monosperma*, the positive correlation between Ψ_{TLP} and ε (Fig. 6) and the increasing impact of $\Psi_{\pi\,100}$ on Ψ_{TLP} as $\Psi_{\pi\,100}$ declined (Fig. 5) were
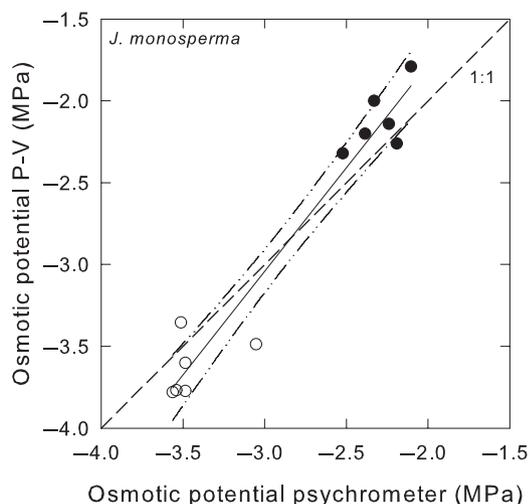

Figure 7. Relationship between osmotic potential determined from pressure-volume curves and osmotic potential measured in psychrometer chambers on frozen, thawed and crushed tissue samples from (●) rehydrated and (○) non-rehydrated shoots from six individuals of *J. monosperma*. The diagonal dashed line represents a 1:1 relationship and the dashed-dotted lines represent the 95% confidence interval for the solid regression line ($r^2 = 0.94$, $P < 0.0001$).

consistent with adjustments in both $\Psi_{\pi\,100}$ and ε contributing to turgor maintenance as shoot Ψ decreased. In contrast, no significant relationship between ε and Ψ_{TLP} was found in two multi-species surveys of P-V curve data (Lenz *et al*. 2006; Bartlett *et al*. 2012). However, these surveys relied largely on P-V data generated from artificially rehydrated samples, potentially obscuring relationships between ε and Ψ_{TLP} in species showing rapid changes in these traits with changing hydration.

In the present study, estimates of bulk tissue osmotic potential based on psychrometric measurements of Ψ in crushed tissue previously frozen in liquid N$_2$ were reasonably close to those derived from P-V curves (Fig. 7). At first sight, this may seem somewhat surprising given that dilution of cell contents with apoplasmic water in killed tissue typically yields consistently less negative psychrometric and osmometric estimates of tissue $\Psi\pi$ than those obtained from P-V curves (Tyree 1976; Markhart *et al*. 1981). However, the unavoidable presence of woody tissue in the samples used for P-V analyses likely biased our estimates of the SWF of living tissue towards lower than actual values (Neufeld & Teskey 1986). Additionally, the apoplastic dilution effect would be diminished by the presence of substantial apoplastic solute concentrations as has been inferred in some species (Meinzer *et al*. 1986; Evans *et al*. 1990). It has been suggested that rapid, rehydration-induced changes in $\Psi_{\pi\,100}$ and Ψ_{TLP} inferred from P-V analyses in some species may be associated with redistribution of solutes between the symplast and apoplast (Meinzer *et al*. 1986; Evans *et al*. 1990; Saliendra & Meinzer 1991).

Conducting P-V analyses exclusively on fully rehydrated samples continues to be the standard protocol for characterizing shoot water relations components despite mounting evidence that the results can be misleading when applied to intact, field-grown plants experiencing a broad range of tissue Ψ. Thus, our results and those of others have implications concerning the types of inferences that can be drawn from broad ecological and taxonomic surveys of species in which P-V curve parameters have been derived solely from rehydrated samples. The dynamic responses of leaf biophysical properties to changes in hydration observed in *J. monosperma* and other species may not be restricted to drought-adapted anisohydric species (e.g. Saliendra & Meinzer 1991). Therefore, to detect dynamic behaviour that contributes to homeostasis of leaf physiological functions in the intact plant, we suggest that P-V analyses be routinely conducted on samples spanning a range of hydration.

In addition to highlighting the potential misinterpretation of data from P-V curve analyses that can arise from artificial rehydration of shoots of drought-adapted species, our results point to a potential mechanism for enhancing tree survival in

habitats that experience large and rapid fluctuations in soil moisture availability. The ability of drought-adapted species like *J. monosperma* to adjust tissue elasticity may represent a key adaptation that allows them to avoid damaging cell deformation during times of relatively high moisture availability, while being able to maintain cell turgor and photosynthesis under conditions of substantially lower soil moisture availability.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Typical relationships between sample fresh weight and water potential for non-rehydrated (○) and rehydrated (●) shoots of *J. monosperma* (upper) and *P. edulis* (lower) collected from the same individuals. Extrapolation of the regressions to the *y*-axis at zero water potential yields an estimate of tissue saturated weight. Note that the initial data point for the rehydrated *J. monosperma* shoot shows evidence of a small oversaturation effect.

Figure S2. Typical relationships between turgor and relative water content in non-rehydrated (upper panels) and rehydrated (lower panels) shoots of *P. edulis* and *J. monosperma*. The slopes of the regression lines are estimates of the bulk tissue modulus of elasticity (ε). Values of initial shoot water potential (Ψ) are shown.

Figure S3. Plots of the relative water deficit at the turgor loss point (RWD@TLP) and the tissue bulk modulus of elasticity (ε) versus the initial shoot water potential for rehydrated (●) and non-rehydrated (○) shoots of *P. edulis*. Neither relationship was statistically significant.