Leaf hydraulics and drought stress: response, recovery and survivorship in four woody temperate plant species

CHRISTOPHER J. BLACKMAN, TIMOTHY J. BRODRIBB & GREGORY J. JORDAN

School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia

ABSTRACT

Efficient conduction of water inside leaves is essential for leaf function, yet the hydraulic-mediated impact of drought on gas exchange remains poorly understood. Here we examine the decline and subsequent recovery of leaf water potential (Ψleaf), leaf hydraulic conductance (Kleaf), and midday transpiration (E) in four temperate woody species exposed to controlled drought conditions ranging from mild to lethal. During drought the vulnerability of Kleaf to declining Ψleaf varied greatly among the species sampled. Following drought, plants were rewatered and the rate of E and Kleaf recovery was found to be strongly dependent on the severity of the drought imposed. Gas exchange recovery was strongly correlated with the relatively slow recovery of Kleaf for three of the four species, indicating conformity to a hydraulic-stomatal limitation model of plant recovery. However, there was also a shift in the sensitivity of stomata to Ψleaf suggesting that the plant hormone abscisic acid may be involved in limiting the rate of stomatal reopening. The level of drought tolerance varied among the four species and was correlated with leaf hydraulic vulnerability. These results suggest that species-specific variation in hydraulic properties plays a fundamental role in steering the dynamic response of plants during recovery.

Key-words: drought stress; gas exchange; leaf hydraulics; leaf hydraulic conductance; leaf water potential; recovery; survivorship; transpiration; vulnerability.

INTRODUCTION

Drought is a major environmental stress that adversely affects plant growth (Kramer & Boyer 1995) and strongly influences plant distribution patterns (Engelbrecht et al. 2007; Kursar et al. 2009). Given that current climate change models predict drought to increase in frequency and severity in several regions around the world (IPCC, 2007), there is an increasingly urgent need to better understand the impact of drought on plant function and particularly the physiological mechanisms underlying plant responses during and in recovery from water stress. To date, much of the literature in this area has focused on plant biochemical and molecular processes related to drought-stress (Reddy, Chaitanya & Vivekanandan 2004; Flexas et al. 2008). A number of recent studies, however, have explored the physiological responses of plants to drought, identifying plant hydraulics as one of the principal governors of gas exchange under water stress as well as during the recovery period following rewatering (Sperry 2000; Meinzer 2002; Brodribb & Cochard 2009; Resco et al. 2009).

Water flowing through the vascular system of plants moves down a water potential gradient from the roots to the sites of evaporation in the leaves. Under drought conditions, water potential becomes more negative throughout the plant. This creates increased hydraulic tension within the xylem, which can result in xylem cavitation and an associated reduction in hydraulic conductance once species-specific thresholds are transgressed (Sperry & Tyree 1988; Tyree & Sperry 1989). Plants have been shown to pre-empt this cavitation-induced decrease in hydraulic conductance by closing their stomata, thus avoiding increases in xylem tension sufficient to cause runaway embolism (Nardini & Salleo 2000; Cochard et al. 2002; Brodribb & Holbrook 2003). However, it is now accepted that many plants in the natural environment regularly operate at water potentials very close to their cavitation threshold (Lo Gullo et al. 2003; Brodribb & Holbrook 2004a), and furthermore, are capable of recovering lost hydraulic conductance (Holbrook & Zwieniecki 1999; Tyree et al. 1999; Melcher et al. 2001; Bucci et al. 2003; Brodribb & Holbrook 2004a), although the exact mechanisms underlying such repair remain unresolved (Tyree et al. 1999; Bucci et al. 2003).

While the vulnerability of plant hydraulic conductance to drought-induced cavitation and its ecological implications have received significant attention, most studies have been conducted on stems, which is surprising considering that the leaf constitutes a substantial part (~30%) of whole plant hydraulic resistance (Sack & Holbrook 2006), and thus can constrain maximum stomatal conductance and photosynthetic capacity (Brodribb et al. 2005). The onset of leaf hydraulic dysfunction also corresponds closely to turgor loss and stomatal closure (Brodribb & Holbrook 2003), suggesting that leaf hydraulic vulnerability has the potential to dictate how plants respond to short-term water stress. A small number of studies have explored the vulnerability of leaf hydraulic conductance (Kleaf) to drought (Nardini, Tyree & Salleo 2001; Brodribb & Holbrook 2003), and shown a progressive decline of Kleaf as leaf water potential (Ψleaf) falls. Importantly, further studies have also described...
the recovery of $K_{\text{leaf}}$ following rewatering after drought (Lo Gullo et al. 2003; Trifilo et al. 2003), and determined that, in conifers, the recovery of $K_{\text{leaf}}$ steers the recovery of fundamental physiological processes such as gas exchange (Brodribb & Cochard 2009).

Despite the wealth of information about hydraulic vulnerability, particularly on stems, there remains very little information about how this variable xylem trait is related to plant injury or death during drought (McDowell et al. 2008). Following strong evidence linking drought-induced hydraulic failure and shoot die-back in wild plant populations (Davis et al. 2002; Breda et al. 2006), Brodribb & Cochard (2009) found that xylem hydraulic failure corresponded directly with point of death in a number of conifer species. However, for angiosperm plants, there are no studies that show how the resistance to xylem dysfunction translates to absolute drought tolerance.

Here, we focus on the response of leaf hydraulic conductance and gas exchange of four temperate woody angiosperm species to drought stress. These species are distributed from rainforest to dry sclerophyll forest and are thereby expected to span a range of tolerances to desiccation. Specifically, we sought to identify the nature of $K_{\text{leaf}}$ dysfunction and its influence on gas exchange with declining $\Psi_{\text{leaf}}$ and find the level of $\Psi_{\text{leaf}}$ that induces irreversible drought damage. Importantly, the study also aimed to identifying the speed of $K_{\text{leaf}}$ recovery following rewatering and testing the possible link between the recovery of $K_{\text{leaf}}$ with the recovery of leaf gas exchange.

**MATERIALS AND METHODS**

**Plant material**

Four woody angiosperm species native to Tasmania, Australia, were used in the study (Table 1). Based on both climatic distribution patterns from rainforest to dry sclerophyll forest and phylogenetic histories, the choice of these species was designed to maximize the range of possible plant responses to drought stress. *Atherosperma moschatum* (Atherospermataceae) is a cool-temperate rainforest tree that is widespread and abundant in wetter parts of Tasmania (638–2766 mm annual rainfall) as well as occurring in wet microsites in areas of south-eastern Australia (Sommerville & Read 2008). *Tasmania lanceolata* (Winteraceae) is a vessel-less angiosperm that ranges from wet forest to wet alpine vegetation (733–2978 mm annual rainfall). *Lomatia tinctoria* (Proteaceae) is widespread and frequent in moderately dry forests from sea level to 1200 m (503–2124 mm annual rainfall). *Hakea lissosperma* (Proteaceae) is widespread and frequent in regions of moderate rainfall at sea level, and on mountains reaching 1300 m (>800 mm annual rainfall). It is a terete-leaved member of a large genus of predominantly dry climate species with deeply encrypted stomata that have been reconstructed as having evolved in response to dry climates (Jordan et al. 2008).

Plants were grown from seed or cuttings collected in areas typical of each species’ habitat. Up to 10 plants 3–4 years of age and between 30 and 50 cm in height were potted in eight parts composted pine bark and 2 parts coarse river sand with added slow release fertiliser (Osmocote) suitable for Australian native plants and added lime to adjust pH, in 1.8 L pots and acclimated in a controlled glasshouse environment (18 h days at 25/10 °C day/night) 2 weeks prior to the drought treatment. Five plants of each species were used to monitor leaf hydraulics and gas exchange during drought and following recovery after rewatering, while the remaining four to five plants of each species were used to determine the point at which drought damage becomes irreversible (see further discussion).

Humidity in the glasshouse was controlled at 50% using a De Longhi (Italy) DHE-PC de-humidifier regulated by a Dixell (Italy) XH260V-500CO humidity sensor and controller. Throughout the experiment, temperature and humidity were monitored with a Vaisala humidity probe and logged on a Campbell CR10X data logger. Lighting in the growth chamber was unfiltered natural light, with sodium vapour lamps (providing 300–500 mmol quanta m$^{-2}$ s$^{-1}$ at the leaf surface) used to extend the photoperiod to 18 h.

**Drought treatment and recovery**

Initially, leaf water potential ($\Psi_{\text{leaf}}$), leaf hydraulic conductance ($K_{\text{leaf}}$), assimilation (A) and transpiration (E) were measured over 3 d in five or six well-watered replicate individuals. These values were used to represent pre-drought stress levels of each parameter for each individual drought treatment. The roots and soil of plants were then double-bagged and tied closely around the base of the stem to prevent any loss of water from the soil and allowed to dehydrate by withholding water. Each pot was also wrapped in aluminium foil to prevent soil heating

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**Table 1.** Morphological and physiological characteristics of each species measured under normal pre-drought stress conditions ($n = 5$). Nomenclature follows Buchanan (2008)

<table>
<thead>
<tr>
<th>Species</th>
<th>LMA (g m$^{-2}$)</th>
<th>max $K_{\text{leaf}}$ (mmol m$^{-2}$ s$^{-1}$ MPa)</th>
<th>max $E$ (mmol m$^{-2}$ s$^{-1}$) $\times 10^{-4}$</th>
<th>max A (μmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tasmania lanceolata</em> (Poir) A.C.Sm</td>
<td>161.53 ± 10.97</td>
<td>10.55 ± 0.58</td>
<td>2.3 ± 0.2</td>
<td>5.85 ± 0.59</td>
</tr>
<tr>
<td><em>Hakea lissosperma</em> R. Br.</td>
<td>769.23 ± 35.53</td>
<td>25.46 ± 1.99</td>
<td>15 ± 0.11</td>
<td>29.93 ± 0.99</td>
</tr>
<tr>
<td><em>Lomatia tinctoria</em> R. Br.</td>
<td>243.9 ± 14.86</td>
<td>12.07 ± 0.78</td>
<td>7.2 ± 0.63</td>
<td>10.10 ± 0.77</td>
</tr>
<tr>
<td><em>Atherosperma moschatum</em> Labill</td>
<td>136.98 ± 0.33</td>
<td>11.48 ± 1.1</td>
<td>3.01 ± 0.12</td>
<td>6.15 ± 0.27</td>
</tr>
</tbody>
</table>

associated with direct solar radiation. During drought, $\Psi_{leaf}$, $K_{leaf}$ and $E$ were initially monitored daily and then every third or fourth day. The plants of each species were exposed to a range of drought intensities. Minimum water stress corresponded to leaf water potentials associated with ~50% loss of leaf hydraulic conductance based on the relationship between $K_{leaf}$ and $\Psi_{leaf}$ (see further discussion). Maximum water stress corresponded to leaf water potentials associated with the onset of visual signs of drought damage (leaf shrinkage and/or necrosis). Upon reaching the target $\Psi_{leaf}$, plants were debagged and rewatered to full capacity soil moisture each morning during the recovery period. After each plant was rewatered, $\Psi_{leaf}$, $K_{leaf}$ and $E$ were measured daily for the first 2–3 d and then every third or fourth day over a period of 4 to 5 weeks or until the plant reached and remained at 100% recovery. The leaves chosen for measuring $\Psi_{leaf}$ and $K_{leaf}$ were always those that showed the least sign of drought. For the purposes of the study, plant ‘recovery’ from drought was defined as plants that were able to recover at least 50% of maximum pre-stress values of each plant parameter measured. In order to determine if soil dehydration was uniquely responsible for the observed changes in plant function, well watered control plants of each species were maintained throughout the period of the experiment and midday $E$ and $\Psi_{leaf}$ measured every 5 d.

**Leaf water potential ($\Psi_{leaf}$) and transpiration ($E$)**

Stomatal responses to $\Psi_{leaf}$ during both drought and recovery were established for each species to ascertain whether the recovery of gas exchange following rewatering was limited by leaf hydraulic repair. For the determination of $\Psi_{leaf}$, two leaves of each plant were sampled at about 1300 h and immediately double-bagged to prevent any water loss, and transported to the lab. Each leaf was wrapped in moist paper towel inside aluminium foil and inserted into a Scholander pressure chamber and $\Psi_{leaf}$ measured (PMS, Albany, OR, USA).

Plant transpiration ($g \ m^{-2} \ s^{-1}$, $E$) was determined gravimetrically over a 1–2 h period between 1100 h and 1300 h, during which time transpiration rates for all species was at its maximum (data not shown). Throughout the experiment, vapour pressure deficit (VPD) remained constant between 0800 h and 1700 h and, therefore, $E$ was approximately proportional to stomatal conductance apart from small variations in leaf temperature. Each time leaves were taken to measure $\Psi_{leaf}$ and $K_{leaf}$ they were scanned using a flatbed scanner. Final leaf area was calculated at the end of the experiment by scanning a sub-sample of the remaining leaves from each plant. Leaf areas were measured using image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA) and transpiration adjusted for the loss of leaf area over the experimental period.

**Response of $K_{leaf}$ to desiccation**

Leaf ‘vulnerability curves’ were constructed for each species by measuring $K_{leaf}$ in excised leaves rehydrated from a range of water potentials during the course of the drought treatment. We employed the technique of Brodribb & Holbrook (2003) to calculate $K_{leaf}$ from the kinetics of $\Psi_{leaf}$ relaxation upon leaf rehydration. Initial $\Psi_{leaf}$ was determined by measuring two leaves neighbouring the sample leaf with the pressure chamber. The sample leaf was then cut under water and allowed to rehydrate for 30, 60, 90 or 120 s depending on the initial $\Psi_{leaf}$ and immediately wrapped in moist paper towel and double-bagged for 5 min to allow for equilibration of spatial gradients in water potential. Final $\Psi_{leaf}$ was measured with the pressure chamber and $K_{leaf}$ calculated from the ratio of the initial to final $\Psi_{leaf}$ and the capacitance of the leaf.

$$K_{leaf} = C_{leaf} \ln[\Psi_{w}/\Psi_{f}] / t$$

where $\Psi_{w}$ = initial water potential (MPa); $\Psi_{f}$ = final water potential (MPa); $t$ = duration of rehydration (s); $C_{leaf}$ = leaf capacitance (mmol m$^{-2}$ MPa$^{-1}$).

Vessel lengths were measured in branchlets to determine the minimum sample size possible to excise while still preventing emboli from entering the sample leaf tissue. Small branches spanning two internode lengths were sampled in the case of *A. moschatum*, and detached individual leaves were used for *T. lanceolata*, *L. tinctoria* and *H. lissosperma*. When individual leaves were used to calculate the kinetics of $\Psi_{leaf}$ relaxation, up to four leaves adjacent to each other on the plant were excised at the base of the petiole and immediately double bagged. Two leaves were then used to measure initial $\Psi_{leaf}$ and two sample leaves were recut under water, leaving at least three quarters of the leaf to rehydrate, and final $\Psi_{leaf}$ measured following equilibration. Importantly, this method of determining the response of $K_{leaf}$ to declining $\Psi_{leaf}$ generated very similar results to that using larger branch material from field-based plants for each species (data not shown).

**Pressure–volume (PV) relations**

For each species, one leaf from each of five replicates used in the experiment was sampled for determination of leaf turgor dynamics and leaf capacitance ($C_{leaf}$) from PV analysis (Tyree & Hammel 1972). While under pre-drought conditions, fully expanded leaves were sampled early in the morning when $\Psi_{leaf}$ was high (>0.1 MPa) and allowed to slowly desiccate in the laboratory. During this process, leaf weight and water potential were measured periodically. Leaf capacitance was calculated in terms of relative water content (RWC) from the initial (linear) slopes of the relationship between RWC and $\Psi_{leaf}$. Calculation of $K_{leaf}$ (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) requires the leaf capacitance normalised by leaf area. To do this, the capacitance calculated from the PV curve was multiplied by the saturated mass of water in the leaf and then divided by leaf area (Brodribb & Holbrook 2003).

**Assimilation (A)**

Assimilation ($A$) was measured using a portable gas analyser (Li-6400; Li-Cor, Lincoln, NE, USA), with a light
intensity of 1500 μmol quanta m⁻¹ s⁻¹ at a mean air temperature of 28.9 °C (± 1.74) and VPD of 2.23 kPa (± 0.44). A was measured between 1100 and 1300 h throughout the experiment. For _T. lanceolata_, _L. tinctoria_ and _A. moschatum_, A was measured on two healthy 1-year-old leaves. Each leaf was tagged and used for additional measurements during the experiment. On each day, assimilation was measured for _H. lissosperma_, three of the least drought affected 1-year-old terete leaves were removed from the plant and immediately placed in the gas analyser. In all cases, A was adjusted for leaf area.

**Point of death**

The capacity of each of the four species to survive water deficit was determined by estimating the critical \( \Psi_{\text{leaf}} \) beyond which a rewatered plant will not recover. Four to five plants were rewatered from a narrow range of leaf water potentials associated with a range of different degrees of extensive drought damage. Subsequently, some plants died and others, while suffering extensive leaf damage, were recoverable. This narrow range of \( \Psi_{\text{leaf}} \) was determined by closely monitoring the decline in \( \Psi_{\text{leaf}} \) as soil dehydration increased coupled with close visual observation of impending plant death estimated by signs of extensive leaf damage and necrosis. The minimum \( \Psi_{\text{leaf}} \) of each plant was measured from two of the least drought-damaged leaves immediately prior to rewatering. The proportion of dead or damaged leaves was measured on plants that were recoverable once it was clear which leaves had died from the drought stress imposed. As a result, we calculated the point of plant death by fitting a quadratic curve through the percent leaf death data for recoverable plants, which included a data point for 100% leaf death associated with the closest \( \Psi_{\text{leaf}} \) recorded for an individual that subsequently died (suffered 100% leaf death). The x intercept of the quadratic curve through 100% leaf death was determined as the point of plant death.

**Statistics**

A three-parameter sigmoidal regression function of the form \( y = 100/(1 + e^{(-y_{\Psi_{\text{leaf}}-b})}) \), where \( y \) = % loss of \( K_{\text{max}} \), was fitted to \( K_{\text{leaf}} \) versus \( \Psi_{\text{leaf}} \) data from each species. \( K_{\text{max}} \) was defined as the mean \( K_{\text{leaf}} \) at \( \Psi_{\text{leaf}} > -1.25 \) MPa for _H. lissosperma_ and at \( \Psi_{\text{leaf}} > -1 \) MPa for the remaining three species. An exponential 4-parameter sigmoid function of the form \( y = y_0 + a(1 + e^{(-y_{\Psi_{\text{leaf}}-b})}) \) was fitted to midday \( E \) versus \( \Psi_{\text{leaf}} \) data for all species. A multiple parameter sigmoid was preferred because it made no assumption of isohydric or anisohydric stomatal response (Tardieu & Simonneau 1998). This function defined the hydraulic model for stomatal response to \( \Psi_{\text{leaf}} \) and was used to test whether species conformed to hydraulic limitation of gas exchange during recovery from drought. Correspondence to a hydraulic limitation model was determined by plotting \( E \) against \( \Psi_{\text{leaf}} \) during the recovery from drought then calculating correlation coefficient (\( r^2 \)) based on the empirically determined hydraulic model for each species. Any systematic deviation from the predicted response of stomata to \( \Psi_{\text{leaf}} \) during recovery was determined by fitting a quadratic curve through the residuals. Significance in the slope or intercept of the quadratic regression was interpreted as indicating systematic deviation from the model. To calculate the time for 50% recovery of \( E \) after rewatering, an exponential rise to maximum function of the form \( y = y_0 + a(1 - e^{-bx}) \) where \( y = \% \) of pre-stress maximum was fitted to the recovery data of each individual. The inverse of resultant x intercept (in days) was calculated. Regression and curve analysis was undertaken using SigmaPlot (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Mean maximum leaf hydraulic conductance and mean maximum leaf gas exchange (\( E \) and \( A \)) were strongly correlated (\( r^2 \geq 0.91 \)) across the species samples in healthy plants prior to the drought treatment (Table 1).

**Dynamics of plant response to drought**

When exposed to decreasing midday leaf water potential (\( \Psi_{\text{leaf}} \)) all species displayed a similar pattern whereby midday transpiration followed a sigmoidal decline with declining \( \Psi_{\text{leaf}} \), reflecting a highly sensitive stomatal response to small changes in \( \Psi_{\text{leaf}} \) (Fig. 1). _A. moschatum_ displayed the most sensitive stomatal response where stomatal conductance (as inferred from midday \( E \)) fell from 80% of maximum to 20% of maximum over the \( \Psi_{\text{leaf}} \) range -0.84 MPa to -1.18 MPa. The lowest sensitivity was in _L. tinctoria_ with 1.17 MPa separating 20% and 80% closure. _L. tinctoria_ was the most sensitive in terms of the onset of stomatal closure with 20% closure occurring at -0.79 MPa, whereas 20% closure occurred at -1.2 MPa in _H. lissosperma_. The absolute sensitivity of stomata to \( \Psi_{\text{leaf}} \) was similar in all species with 50% of stomatal closure occurring at a mean of -1.18 ± 0.08 MPa. Throughout the drought treatment midday \( E \) and \( \Psi_{\text{leaf}} \) did not change significantly in control plants of each species indicating that soil dehydration in the droughted plants was uniquely responsible for the observed changes in plant function (data not shown).

During the drought treatment, leaf hydraulic conductance (\( K_{\text{leaf}} \)) in excised samples showed a marked decline as hydraulic tension in the xylem increased. When \( K_{\text{leaf}} \) was plotted as a function of \( \Psi_{\text{leaf}} \), a sigmoid response was observed in all species, with an initial plateau followed by a decline in \( K_{\text{leaf}} \) to a minimum value as \( \Psi_{\text{leaf}} \) declined (Fig. 2). Despite the relatively similar sigmoidal character of these relationships the species varied markedly in vulnerability to decreasing water potential, ranging from 50% loss of \( K_{\text{leaf}} \) (\( \Psi_50 \)) recorded at -1.41 MPa in _A. moschatum_, to the least vulnerable species _H. lissosperma_, with \( \Psi_50 \) recorded at -2.87 MPa (Fig. 2).

‘Safety margins’, defined as the difference between \( \Psi_{\text{leaf}} \) at 80% stomatal closure and the \( \Psi_{\text{leaf}} \) at 50% loss of \( K_{\text{leaf}} \) (Brodribb & Holbrook 2004b) were also variable between species, ranging from a margin of 1.27 MPa in _H. lissosperma_ to 1.17 MPa in _L. tinctoria_.
H. lissosperma to −0.08 MPa in Lomatia tinctoria. This least conservative ‘safety margin’ suggests that some loss of $K_{\text{leaf}}$ precedes complete stomatal closure in *L. tinctoria*. No significant relationship was found between stomatal closure and the onset of $K_{\text{leaf}}$ dysfunction across the species sample.

**Recovery from drought**

The most negative $\Psi_{\text{leaf}}$ imposed on plants of each species from which the recovery of gas exchange and leaf hydraulic conductance was closely monitored was −3.25 MPa for *T. lanceolata*, −7.1 MPa for *H. lissosperma*, −4.9 MPa for *L. tinctoria* and −2.3 MPa for *A. moschatum* (Fig. 3), all of which significantly exceeded 80% loss of $K_{\text{leaf}}$ for each species but occurred before incipient leaf death. With the exception of *H. lissosperma*, which recovered $\Psi_{\text{leaf}}$ relatively quickly regardless of the severity of the drought treatment, the rate of recovery of leaf water potential following rewatering was dependent on the level of drought severity imposed. Thus plants exposed to moderate drought-stress ($\Psi_{\text{leaf}}$ between stomatal closure and about 80% loss $K_{\text{leaf}}$) rehydrated rapidly such that $\Psi_{\text{leaf}}$ returned to pre-stress hydration overnight, while plants subjected to greater drought-stress required a longer time period for $\Psi_{\text{leaf}}$ to recover (Fig. 3). This slower $\Psi_{\text{leaf}}$ recovery in severely stressed plants combined with reduced leaf hydraulic conductivity appeared to influence the recovery of gas exchange in these species. The speed of recovery of both $K_{\text{leaf}}$ and $E$ was dependent on the extent of the drought imposed for all species, with the most drought stressed individuals of each species taking significantly longer to recover $K_{\text{leaf}}$ and $E$ (Fig. 3).

Following rewatering, the recovery of gas exchange (as inferred by midday $E$) was strongly correlated ($P < 0.0001$) with the relatively slow recovery of leaf hydraulic conductance in all species (Fig. 4). For all species, the recovery of midday transpiration was significantly correlated ($P < 0.0001$) with plant assimilation ($A$) during recovery ($r^2 = 0.85$, *T. lanceolata*; $r^2 = 0.82$, *H. lissosperma*; $r^2 = 0.78$, *L. tinctoria*; $r^2 = 0.73$, *A. moschatum*). With the exception of

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*Figure 1.* Compiled data showing the response of midday transpiration ($E$) to increasingly negative $\Psi_{\text{leaf}}$ as soil became drier during the drought treatment (in view of constant VPD throughout the experiment. $E$ was taken as being proportional to stomatal conductance). Curves fitted are sigmoidal functions in each case. Solid vertical lines indicate the $\Psi_{\text{leaf}}$ at 50% loss of $K_{\text{leaf}}$ ($\Psi_{50}$). Dashed vertical lines indicate the $\Psi_{\text{leaf}}$ at 80% stomatal closure.
H. lissosperma, the recovery of $E$ conformed to a hydraulic limitation model in all species (Fig. 5) whereby $E$ is a unique function of $\Psi_{st}$ (Fig. 1). Although the correlation between $E$ and $\Psi_{st}$ was significantly based on a hydraulic model in three of the four species, there was evidence of hysteresis in all species. An apparent increase in the sensitivity of $E$ to $\Psi_{st}$ during recovery from drought was confirmed by analysis of the regression residuals which exhibited a significant slope in all species (Fig. 5).

The rate of gas exchange recovery following rewatering was highly dependent on the severity of drought-stress. The time to recovery ranged from 1 d for plants moderately stressed, to more than 20 d for plants that suffered visual leaf damage or death during severe drought. In order to compress the range of the recovery data, we expressed the recovery of midday $E$ in terms of $1/t_{1/2}$; that is, $1/1$[the time (days) required for midday $E$ to return to $50\%$ of the pre-stress maximum]. Using this scale, a $1/t_{1/2}$ of one would indicate rapid overnight recovery while zero would indicate plant death. In all species the relationship between $\Psi_{st}$ under stress and $1/t_{1/2}$ displayed three distinct phases: an insensitive phase where plants would recover gas exchange overnight when rewatered (this phase must be inferred because exposures to moderate $\Psi_{st}$ were insufficient to close stomata by $50\%$); a linear decline in $1/t_{1/2}$ to approximately $0.1$ (10 d); and a third shallower gradient between $0.1$ and zero (Fig. 6). Plants rewatered from minimum leaf water potentials that required 5 d for $50\%$ recovery ($1/\text{days} = 0.2$), recovered with minimal leaf death. Beyond this level of drought stress, (associated with the third phase in the plots) the percent leaf death accelerated rapidly to the point that gas exchange recovery to pre-stress maximums would have required sufficient time for new plant growth to replace damaged leaves during drought.

The extent of drought-induced plant injury, as determined by the proportion of drought-induced leaf death per plant leaf area, increased as $\Psi_{st}$ fell below a threshold for all species (Fig. 6). The minimum $\Psi_{st}$ from which point the effects of drought stress became irreversible varied among the four species ranging from $-10 \text{ MPa}$ in the most desiccation-tolerant species H. lissosperma, to $-3.5 \text{ MPa}$ in T. lanceolata (Fig. 6). A significant positive relationship ($r^2 = 0.97$) was shown to occur between the water potential associated with $50\%$ loss of leaf hydraulic conductance ($\Psi_{50}$) and the absolute drought tolerance for the four species (Fig. 7).
DISCUSSION

Although the four species examined here showed large differences in drought resistance, a number of general patterns emerged among the species in terms of the response of plants during and in recovery from water stress. In all the experimental species, hydraulic dysfunction and stomatal closure were symptomatic of plant shutdown during the onset of drought. Post-drought recovery of gas-exchange, meanwhile, appeared to be strongly limited by the rate of leaf hydraulic repair in three of the four species; although a slight hysteresis in the recovery of leaf gas exchange was caused by a shift in the sensitivity of stomata to \( \Psi_{\text{leaf}} \) during the recovery phase in all species, possibly due to the action of abscisic acid (ABA). In contrast to conifers which showed a strong association between leaf hydraulic failure and plant death (Brodribb & Cochard 2009), the loss of hydraulic function in the leaves of angiosperms studied here did not correspond with leaf or plant death. However, leaf vulnerability and plant tolerance of desiccation were strongly correlated in our sample indicating the probability of an adaptive linkage between these traits.

The response of leaf hydraulic conductance to dehydration in each of the four species was similar to that observed for other plant species (Brodribb & Holbrook 2003; Brodribb & Holbrook 2006), in following a sigmoidal trajectory whereby \( K_{\text{leaf}} \) declined as \( \Psi_{\text{leaf}} \) approached turgor loss point. Whilst the most conventional explanation for water-stress induced decline in \( K_{\text{leaf}} \) is xylem cavitation, it should be noted that either turgor loss, which could lead to reduced extra-xylary conductance (Brodribb & Holbrook 2004b; Knipfer & Steudle 2008), or xylem collapse (Cochard et al. 2004; Brodribb & Holbrook 2005) are also plausible explanations for xylem dysfunction. Indeed, the rapid reversibility of \( K_{\text{leaf}} \) dysfunction in moderately drought-stressed plants in the present study suggests these other processes may be operating.

The recovery from drought stress in three of our four woody angiosperm species was found to conform to a hydraulic-stomatal limitation model whereby the recovery of gas exchange was strongly mediated by the recovery of leaf hydraulic conductance (Figs 4 & 5). This mode of recovery was particularly evident in the more severely stressed plants (>80% loss of \( K_{\text{leaf}} \)) where any influence of the plant drought-response hormone, ABA, would have had little effect beyond three days after drought relief (Davies & Zhang 1991). That this hydraulic flow restriction was localized in the leaf is evidenced by the fact that \( K_{\text{leaf}} \) measurements were made on excised leaves yet they measured a sufficient depression in conductance during recovery to account for gas exchange depression. A number of recent studies have highlighted the control that hydraulic limitations impose on gas exchange recovery in plants rehydrated from a range of drought severities (Lo Gullo et al. 2003; Lo Gullo et al. 2003).
Brodribb & Cochard 2009; Resco et al. 2009). Data here adds to this body of evidence by showing a distinctive pattern of hydraulic limitation in angiosperm leaves recovering from severe drought. Importantly, the dynamics of gas exchange recovery observed here differ from those observed in conifers where recovery from drought conformed to a hydraulically limited pattern without any indication of hysteresis.

Although we found a good correlation between $K_{\text{leaf}}$ and $E$ in three of the four species following drought relief, a shift in the relationship between $E$ and $\Psi_{\text{leaf}}$ following rewatering in all species (Fig. 5) suggests that additional factors may also contribute to the dynamics of gas exchange recovery. A possible candidate involves direct damage to the leaf photosynthetic apparatus, although in the current study a reduction in leaf internal CO$_2$ concentration was observed in recovering individuals (data not published) suggesting that damage to photosynthetic apparatus was not driving stomatal closure (Farquhar & Sharkey 1982). The other possible candidate is the accumulation of the plant hormone ABA in leaves, which is commonly associated with stomatal closure (Davies & Zhang 1991; Wilkinson & Davies 2002; Lovisolo et al. 2008). In the current study, we observed that stomatal conductance was reduced following rewatering despite $\Psi_{\text{leaf}}$ returning relatively quickly to pre-drought hydration levels (Fig. 5). This change in correlation between $E$ and $\Psi_{\text{leaf}}$ suggests that an accumulation of ABA may increase the sensitivity of stomata to leaf water potential in these species during the early stages of plant recovery. ABA induced stomatal closure has been suggested to help promote gradual hydraulic repair by limiting the rate of stomatal reopening (Lovisolo et al. 2008). The impact of ABA on stomatal closure during drought is well known (Wilkinson & Davies 2002) but the interaction between ABA and hydraulic signalling during drought recovery appears to be variable between species (Brodribb & Cochard 2009; Resco et al. 2009) and dependant on the severity of the drought imposed.

It appears likely that there are similar mechanisms of recovery after severe drought events among the four plant species examined. Desiccation of plants to water potentials

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**Figure 4.** Plots of the relationship between percentage of maximum (pre-stress) values of midday transpiration ($E$) and percentage of maximum values of leaf hydraulic conductance ($K_{\text{leaf}}$) following rewatering. With the exception of $H. lissosperma$, all plants showed a good correlation ($P < 0.0001$) between percent max $E$ and percent max $K_{\text{leaf}}$ during recovery.
between stomatal closure and approximately 80% loss of \( K_{\text{leaf}} \) caused little long term effect once water was reapplied. These plants typically recovered \( K_{\text{leaf}} \) and gas exchange over a 24 h period. Similar rapid recovery times for the restoration of \( K_{\text{leaf}} \) have been reported for plant species dehydrated just passed leaf turgor loss point (Lo Gullo et al. 2003; Trifilo et al. 2003; Brodribb & Cochard 2009). However, we found here that droughting plants further beyond this 80% loss of \( K_{\text{leaf}} \) produced a lasting depression of hydraulic and gas exchange function that took much longer to repair. These distinctly different recovery kinetics suggest that different processes are responsible for short and long term xylem dysfunction. The very slow recovery of \( K_{\text{leaf}} \) recorded for the severely stressed plants of each species probably reflects cavitation in stem xylem, which is likely to require zero or positive root pressure for repair (Ewers, Cochard & Tyree 1997). Rehydration of the whole plant to this point may take some time to establish even in wet soil if cavitation is widespread throughout the plant. The mechanism responsible for the relatively quick recovery of \( K_{\text{leaf}} \) in the moderately dehydrated plants is more difficult to explain. It has been suggested that a related decline in \( K_{\text{leaf}} \) with leaf turgor prior to the onset of cavitation might lead to rapidly recoverable loss of \( K_{\text{leaf}} \) (Brodribb & Holbrook 2006) or alternatively xylem collapse cannot be ruled out as a short-term limiter of \( K_{\text{leaf}} \) (Cochard et al. 2004; Brodribb & Holbrook 2005).

A number of recent studies have focused on the drought resistance of different plant species individually and within an ecological context by comparing vulnerability curves between species (Brodribb & Hill 1999; Hacke, Sperry & Pittermann 2000; Pockman & Sperry 2000; Maherali, Pockman & Jackson 2004). The general pattern is that plants with low vulnerability to loss of hydraulic conductance

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**Figure 5.** Leaf water potential (\( \Psi_{\text{leaf}} \)) recovery versus midday transpiration (\( E \)) recovery in plants following soil rehydration. The heavy dashed sigmoidal function in each plot represents the relationship between \( E \) and \( \Psi_{\text{leaf}} \) during soil drying (see Fig. 1). For each species, the proportion of the variance (based on the hydraulic model) of observed \( E \) explained by this regression is given \( (r) \). With the exception of *H. lissosperma*, the response of \( E \) to \( \Psi_{\text{leaf}} \) during recovery corresponded significantly \( (P < 0.05) \) to this model. The thin dashed vertical line represents the \( \Psi_{\text{leaf}} \) associated with 20% stomatal closure during the drought phase. A significant increase in the sensitivity of stomata to \( \Psi_{\text{leaf}} \) during drought recovery suggests that the plant hormone ABA may interact with the hydraulic signal to inhibit gas exchange recovery.
are drought tolerant (Pockman & Sperry 2000; Choat, Sack & Holbrook 2007). However, although there is a clear relationship between complete loss of leaf hydraulic conductance and plant death in a number of conifer species (Brodribb & Cochard 2009) the current study is the first to show how the capacity to resist leaf xylem dysfunction ($\Psi_{\text{leaf}}$) under water stress correlates with absolute drought tolerance in angiosperms (Fig. 7). An important distinction between the conifer data set and the angiosperm species used here is that absolute drought tolerance in conifers was related to $\Psi_{\text{leaf}}$ (open circles) for the four species studied. Recovery time showed three distinct phases, the first phase was insensitive to $\Psi_{\text{leaf}}$ (1/days = 1), the second was dependent whereby recovery time (1/days) declined linearly from 1 (overnight) to values approaching 0.1 (10 days). The third phase was represented by a shallow decline from 0.1 to 0 (plant death). This third phase was characterised by a concomitant increase in percent leaf death. Quadratic curves are fitted to the percentage leaf death data. The heavy dashed line shows the $\Psi_{\text{leaf}}$ at 80% loss of leaf hydraulic conductance.

In these angiosperms is probably associated with massive xylem dysfunction in the stem or roots.

While drought damage in adult plants may involve an interaction between hydraulic limitation and carbon balance (McDowell et al. 2008), the survival of seedling and sapling plants with relatively shallow root systems is likely to be dominated by hydraulic failure. Given that mortality in these early stages of plant recruitment is very high, it is likely that hydraulic constraints delimit the recruitment niche for any species, and hence should define the rainfall limits of plant distributions. The four species in the current experiment showed a wide range of tolerance in terms of the absolute minimum $\Psi_{\text{leaf}}$ from which plants could recover some plant function. Considering that some of these species occur in similar habitats, projected future climates with more frequent and intense drought events can be expected to have a disproportionate affect on plant species that occupy the same environment.


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