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

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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 (Ψ_{leaf}), leaf hydraulic conductance (K_{leaf}), and midday transpiration (E) in four temperate woody species exposed to controlled drought conditions ranging from mild to lethal. During drought the vulnerability of K_{leaf} to declining Ψ_{leaf} varied greatly among the species sampled. Following drought, plants were rewatered and the rate of E and K_{leaf} 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 K_{leaf} 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 Ψ_{leaf} 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,

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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 ($\geq 30\%$) 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 (K_{leaf}) to drought (Nardini, Tyree & Salleo 2001; Brodribb & Holbrook 2003), and shown a progressive decline of K_{leaf} as leaf water potential (Ψ_{leaf}) falls. Importantly, further studies have also described

the recovery of K_{leaf} following rewatering after drought (Lo Gullo *et al.* 2003; Trifilo *et al.* 2003), and determined that, in conifers, the recovery of K_{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_{leaf} dysfunction and its influence on gas exchange with declining Ψ_{leaf} and find the level of Ψ_{leaf} that induces irreversible drought damage. Importantly, the study also aimed to identifying the speed of K_{leaf} recovery following rewatering and testing the possible link between the recovery of K_{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). *Tasmannia 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 (Ψ_{leaf}), leaf hydraulic conductance (K_{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

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

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

associated with direct solar radiation. During drought, Ψ_{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 Ψ_{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 Ψ_{leaf}, plants were debagged and rewatered to full capacity soil moisture each morning during the recovery period. After each plant was rewatered, Ψ_{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 Ψ_{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 Ψ_{leaf} measured every 5 d.

Leaf water potential (Ψ_{leaf}) and transpiration (E)

Stomatal responses to Ψ_{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 Ψ_{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 Ψ_{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 their 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 Ψ_{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 Ψ_{leaf} relaxation upon leaf rehydration. Initial Ψ_{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 Ψ_{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 Ψ_{leaf} was measured with the pressure chamber and K_{leaf} calculated from the ratio of the initial to final Ψ_{leaf} and the capacitance of the leaf.

$$K_{leaf} = C_{leaf} \, \mathrm{In}[\Psi_o / \Psi_f]/t$$

where Ψ_o = initial water potential (MPa); Ψ_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 Ψ_{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 Ψ_{leaf} and two sample leaves were recut under water, leaving at least three quarters of the leaf to rehydrate, and final Ψ_{leaf} measured following equilibration. Importantly, this method of determining the response of K_{leaf} to declining Ψ_{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 Ψ_{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 Ψ_{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 ($\pm$1.74) and VPD of 2.23 kPa ($\pm$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 Ψ_{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 Ψ_{leaf} was determined by closely monitoring the decline in Ψ_{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 Ψ_{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 Ψ_{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 $y = 100/(1 + e^{a(\Psi leaf-b)})$, where y = % loss of K_{max}, was fitted to K_{leaf} verses Ψ_{leaf} data from each species. K_{max} was defined as the mean K_{leaf} at $\Psi_{leaf} > -1.25$ MPa for *H. lissosperma* and at $\Psi_{leaf} > -1$ MPa for the remaining three species. An exponential 4-parameter sigmoid function of the form $y = y_0 + a/(1 + e^{(-(\Psi leaf-x0)/b)})$ was fitted to midday *E* versus Ψ_{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 Ψ_{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 Ψ_{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 Ψ_{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 (Ψ_{leaf}) all species displayed a similar pattern whereby midday transpiration followed a sigmoidal decline with declining Ψ_{leaf}, reflecting a highly sensitive stomatal response to small changes in Ψ_{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 Ψ_{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 Ψ_{leaf} was similar in all species with 50% of stomatal closure occurring at a mean of −1.18 $\pm$ 0.08 MPa. Throughout the drought treatment midday *E* and Ψ_{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_{leaf}) in excised samples showed a marked decline as hydraulic tension in the xylem increased. When K_{leaf} was plotted as a function of Ψ_{leaf}, a sigmoid response was observed in all species, with an initial plateau followed by a decline in K_{leaf} to a minimum value as Ψ_{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_{leaf} (Ψ_{50}) recorded at −1.41 MPa in *A. moschatum*, to the least vulnerable species *H. lissosperma*, with Ψ_{50} recorded at −2.87 MPa (Fig. 2).

'Safety margins', defined as the difference between Ψ_{leaf} at 80% stomatal closure and the Ψ_{leaf} at 50% loss of K_{leaf} (Brodribb & Holbrook 2004b) were also variable between species, ranging from a margin of 1.27 MPa in
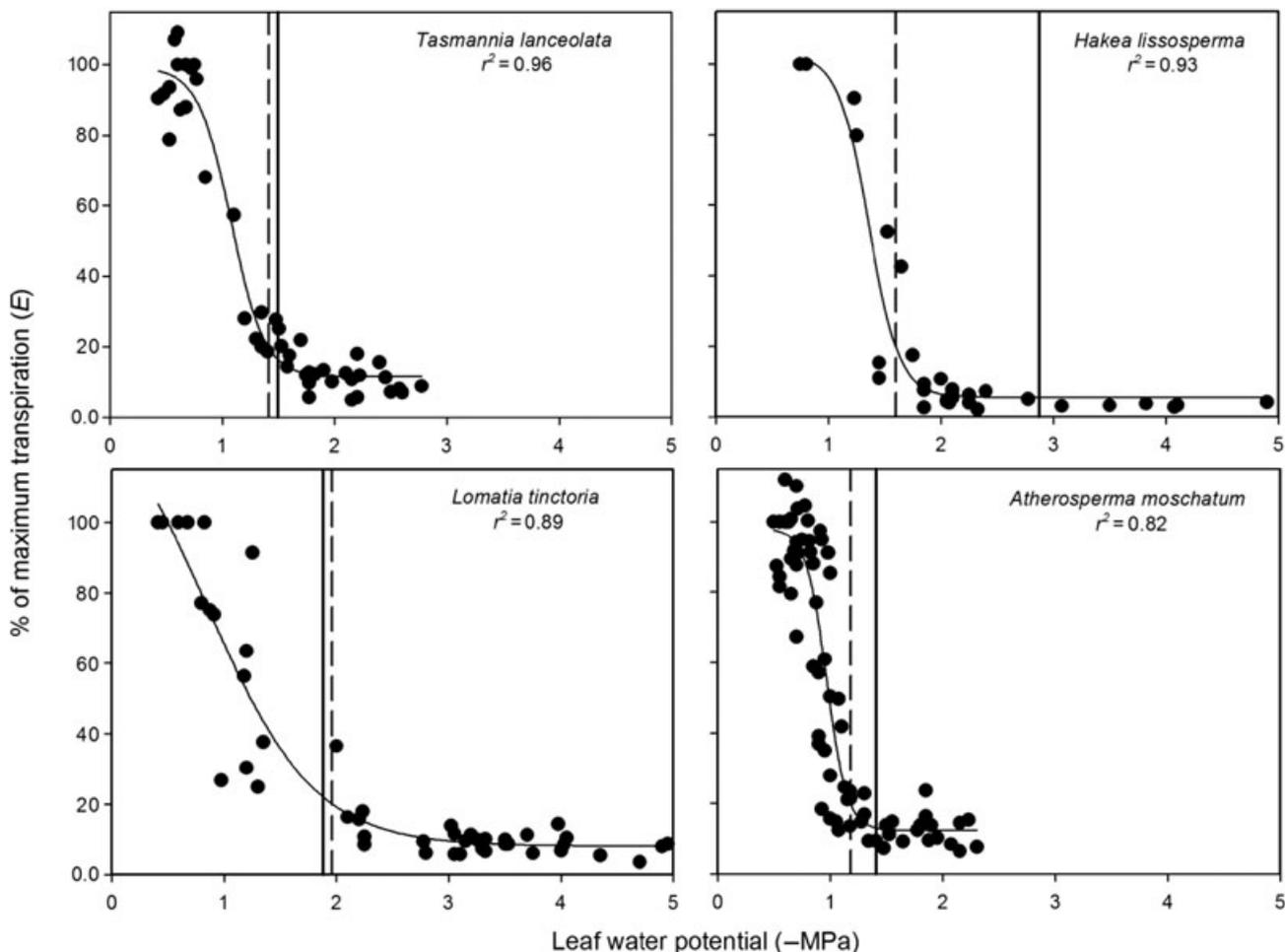
Figure 1. Compiled data showing the response of midday transpiration (E) to increasingly negative Ψ_{leaf} as soil became drier during the drought treatment (in view of constant VPD throughout the experiment, E was taken as being proportional to stomatal conductance). Curves fitted are sigmoidal functions in each case. Solid vertical lines indicate the Ψ_{leaf} at 50% loss of K_{leaf} (Ψ_{50}). Dashed vertical lines indicate the Ψ_{leaf} at 80% stomatal closure.

H. lissosperma to –0.08 MPa in *Lomatia tinctoria*. This least conservative 'safety margin' suggests that some loss of K_{leaf} precedes complete stomatal closure in *L. tinctoria*. No significant relationship was found between stomatal closure and the onset of K_{leaf} dysfunction across the species sample.

Recovery from drought

The most negative Ψ_{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_{leaf} for each species but occurred before incipient leaf death. With the exception of *H. lissosperma*, which recovered Ψ_{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

(Ψ_{leaf} between stomatal closure and about 80% loss K_{leaf}) rehydrated rapidly such that Ψ_{leaf} returned to pre-stress hydration overnight, while plants subjected to greater drought-stress required a longer time period for Ψ_{leaf} to recover (Fig. 3). This slower Ψ_{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_{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_{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
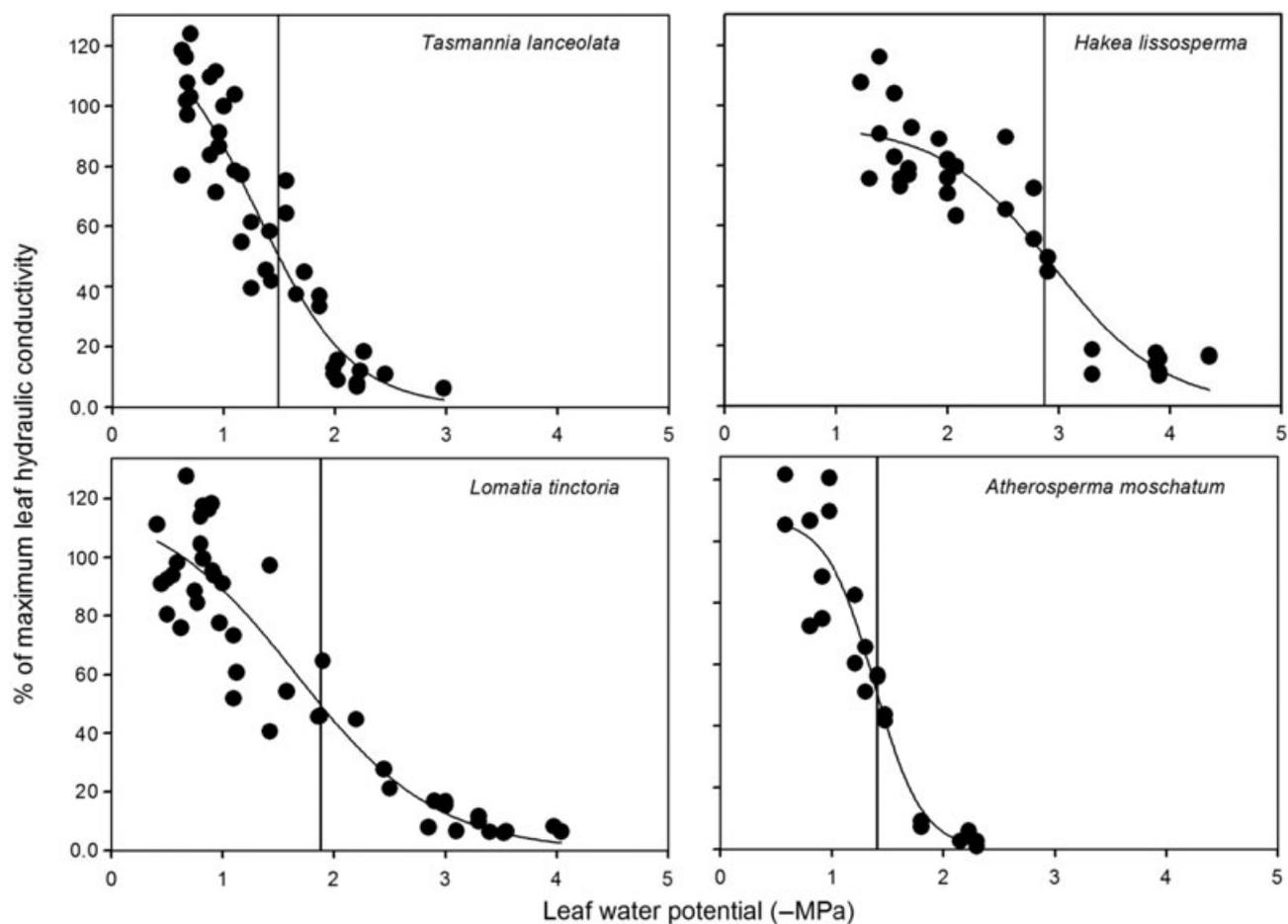
Figure 2. Responses of leaf hydraulic conductance (K_{leaf}) to declining leaf water potential (Ψ_{leaf}) during dehydration in the four species investigated. Curves fitted are sigmoidal functions. Solid vertical lines indicate the Ψ_{leaf} at 50% loss of K_{leaf} (Ψ_{50}).

H. lissosperma, the recovery of E conformed to a hydraulic limitation model in all species (Fig. 5) whereby E is a unique function of Ψ_{leaf} (Fig. 1). Although the correlation between E and Ψ_{leaf} 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 Ψ_{leaf} 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/[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 Ψ_{leaf} 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 Ψ_{leaf} 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/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 Ψ_{leaf} fell below a threshold for all species (Fig. 6). The minimum Ψ_{leaf} from which point the effects of drought stress became irreversible varied among the four species ranging from −10 MPa in the most desiccation-tolerant species *H. lissosperma*, to −3.5 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 (Ψ_{50}) and the absolute drought tolerance for the four species (Fig. 7).
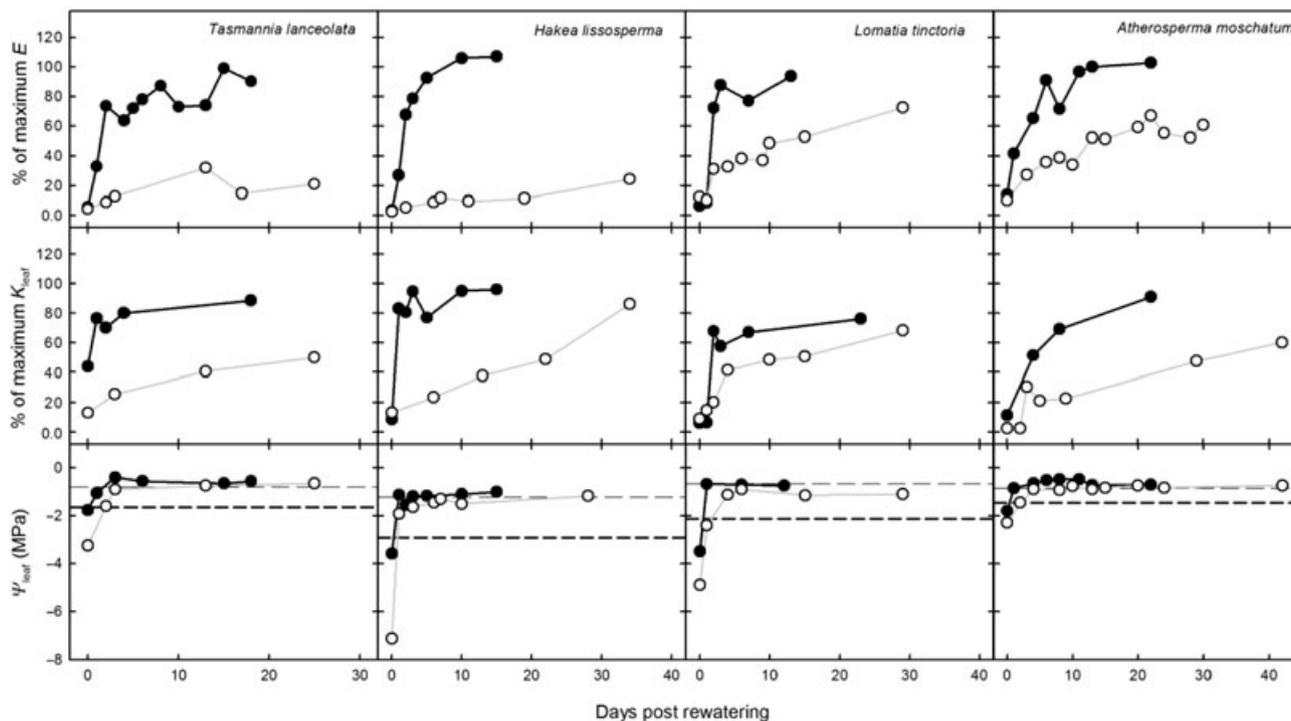
Figure 3. Patterns of recovery over time in moderately (just beyond Ψ_{50}) (solid circles) and severely (but with minimal drought-induced leaf death) (open circles) drought-stressed individuals of each of the four species examined. Detailed for each species is the recovery of leaf water potential (Ψ_{leaf}), K_{leaf} and E. Both K_{leaf} and E are expressed as a percentage of maximum pre-drought values. The mildly stressed plants of each species show a rapid recovery of each function following drought relief in contrast to the more severely stressed plants, which experience much slower recovery. The heavy and light horizontal dashed lines in the Ψ_{leaf} plots represent Ψ_{50} and the Ψ_{leaf} associated with 20% stomatal closure respectively.

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 Ψ_{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_{leaf} declined as Ψ_{leaf} approached turgor loss point.

Whilst the most conventional explanation for water-stress induced decline in K_{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_{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_{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_{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;
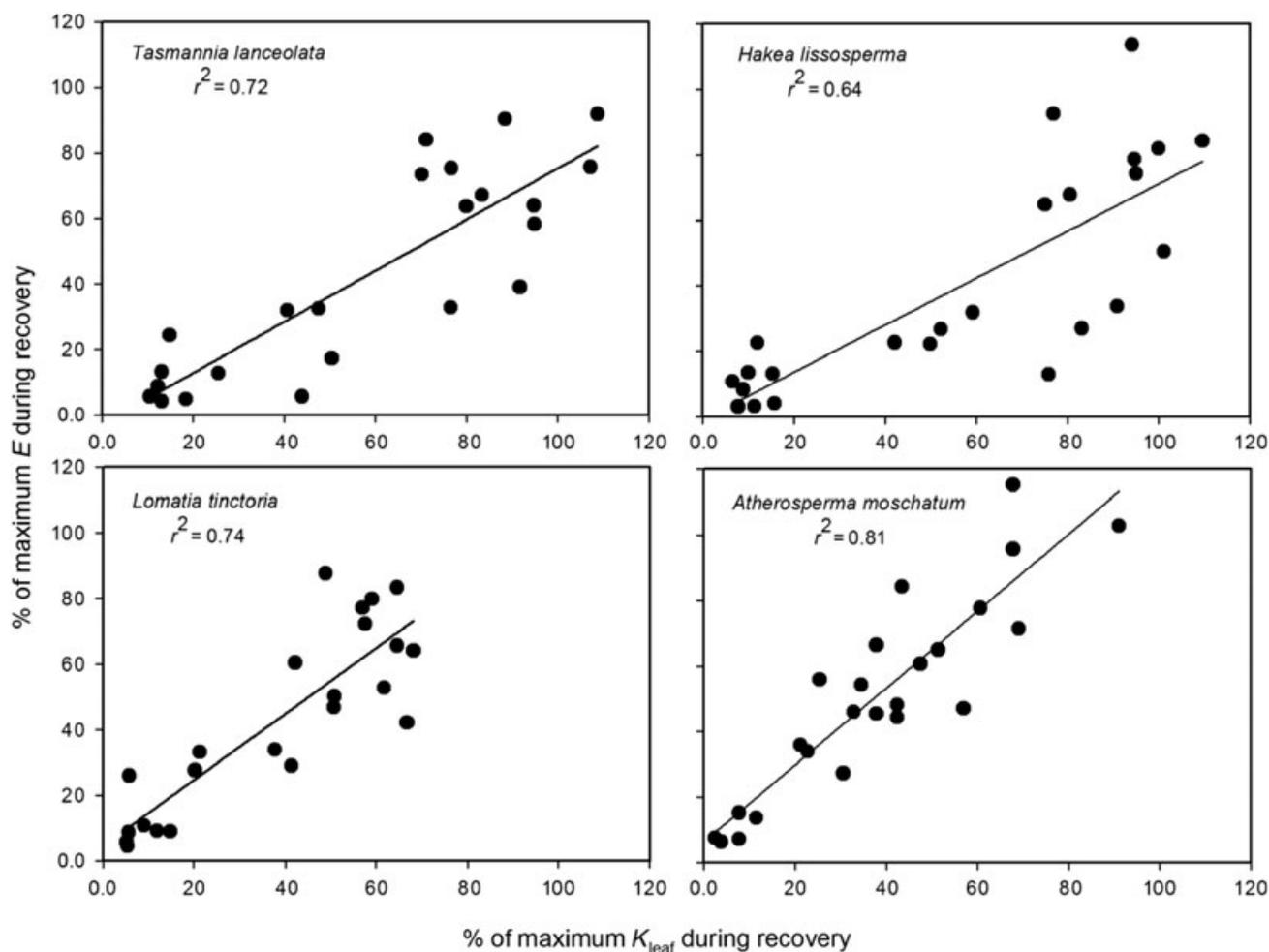
Figure 4. Plots of the relationship between percentage of maximum (pre-stress) values of midday transpiration (E) and percentage of maximum values of leaf hydraulic conductance (K_{leaf}) following rewatering. With the exception of *H. lissosperma*, all plants showed a good correlation ($P < 0.0001$) between percent max E and percent max K_{leaf} during recovery.

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_{leaf} and E in three of the four species following drought relief, a shift in the relationship between E and Ψ_{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 Ψ_{leaf} returning relatively quickly to pre-drought hydration levels (Fig. 5). This change in correlation between E and Ψ_{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
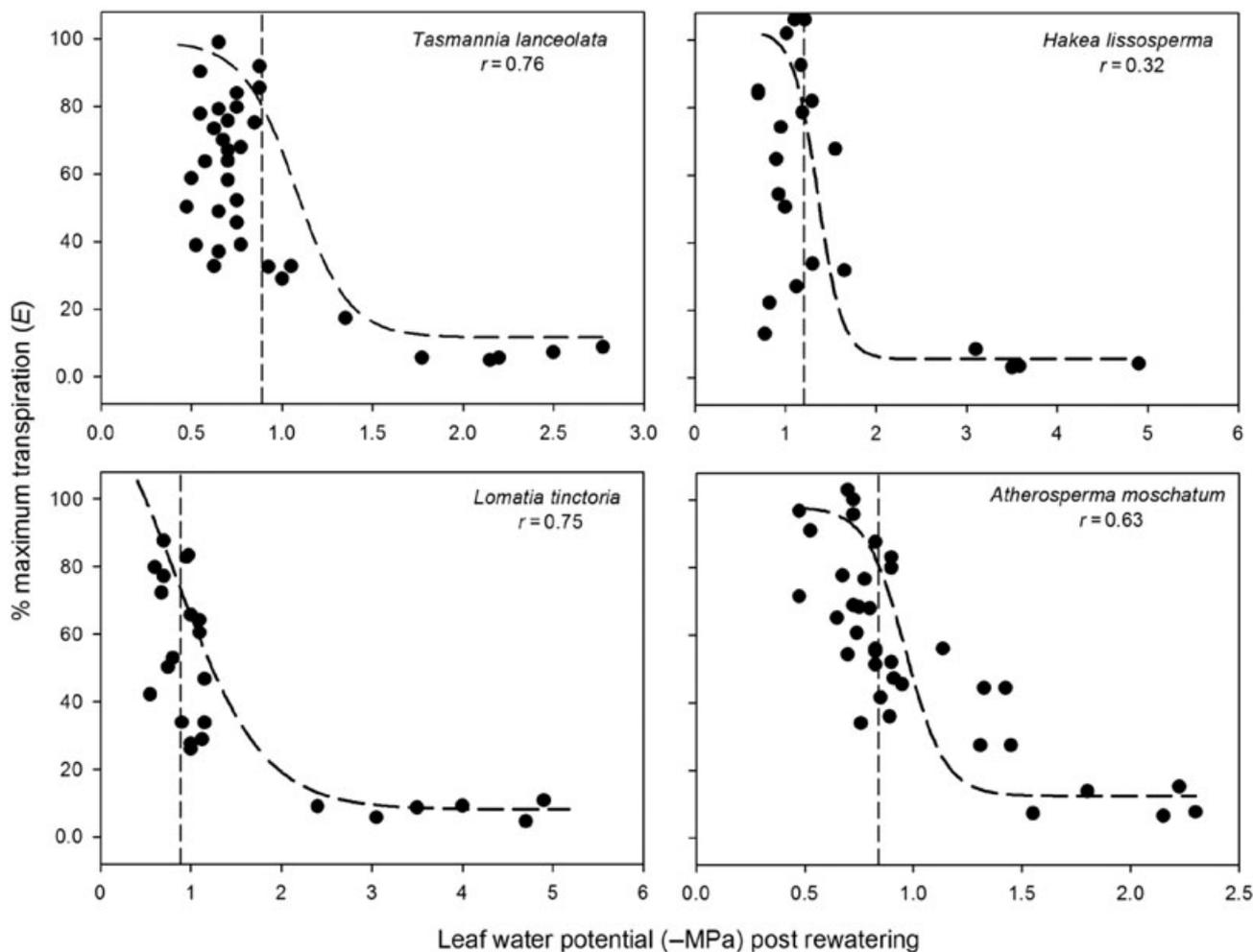
Figure 5. Leaf water potential (Ψ_{leaf}) recovery versus midday transpiration (*E*) recovery in plants following soil rehydration. The heavy dashed sigmoidal function in each plot represents the relationship between *E* and Ψ_{leaf} during soil drying (see Fig. 1). For each species, the proportion of 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 Ψ_{leaf} during recovery corresponded significantly (*P* < 0.05) to this model. The thin dashed vertical line represents the Ψ_{leaf} associated with 20% stomatal closure during the drought phase. A significant increase in the sensitivity of stomata to Ψ_{leaf} during drought recovery suggests that the plant hormone ABA may interact with the hydraulic signal to inhibit gas exchange recovery.

between stomatal closure and approximately 80% loss of K_{leaf} caused little long term effect once water was reapplied. These plants typically recovered K_{leaf} and gas exchange over a 24 h period. Similar rapid recovery times for the restoration of K_{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_{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_{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_{leaf} in the moderately dehydrated plants is more difficult to explain. It has been suggested that a related decline in K_{leaf} with leaf turgor prior to the onset of cavitation might lead to rapidly recoverable loss of K_{leaf} (Brodribb & Holbrook 2006) or alternatively xylem collapse cannot be ruled out as a short-term limiter of K_{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
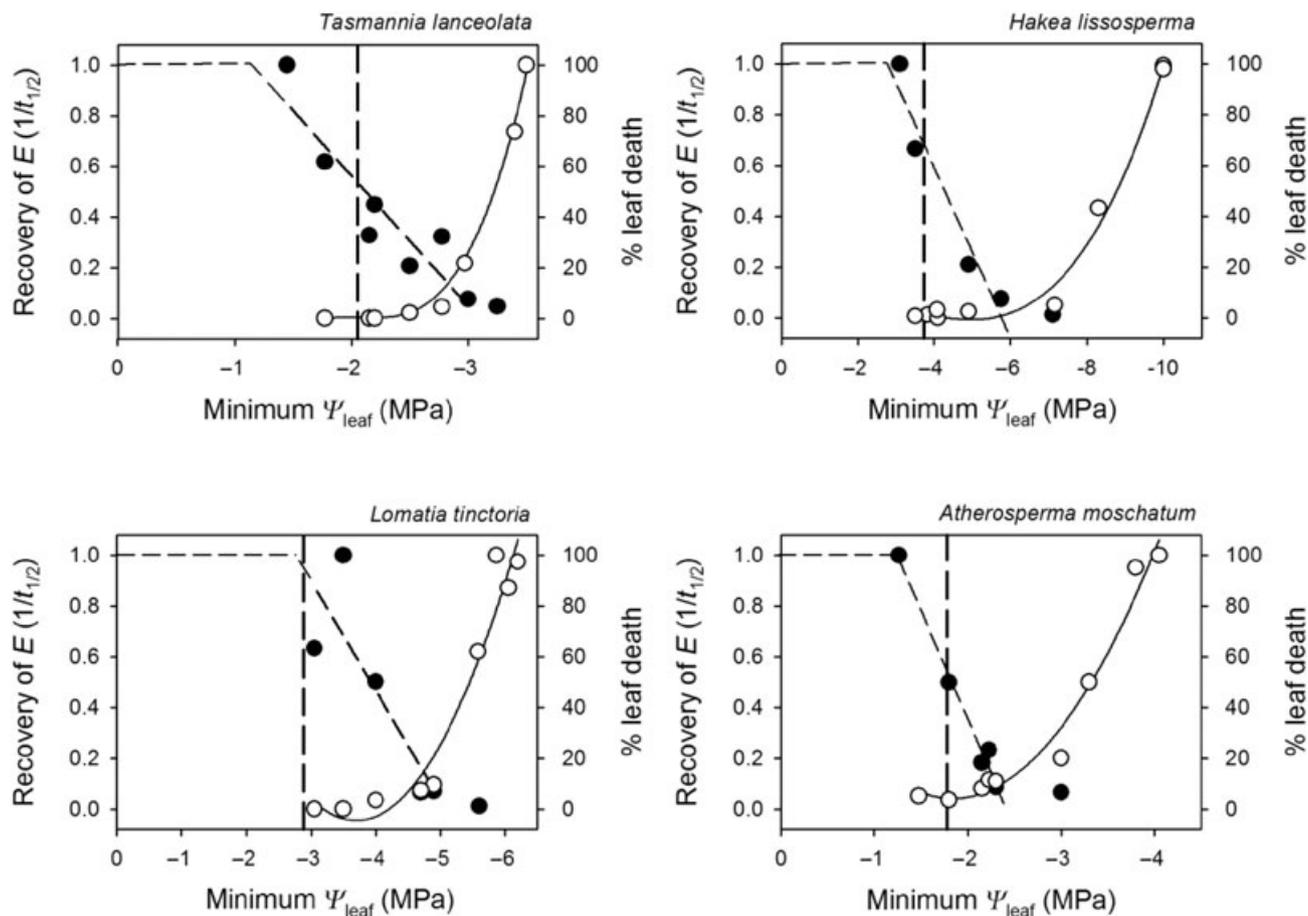
Figure 6. The time (1/days) required for 50% recovery of maximum (pre-stress) E after rewatering in individual plants dehydrated to a range of negative leaf water potentials Ψ_{leaf} (solid circles) and the percentage of leaf death recorded for individual plants dehydrated to a range of Ψ_{leaf} (open circles) for the four species studied. Recovery time showed three distinct phases, the first phase was insensitive to Ψ_{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 Ψ_{leaf} at 80% loss of leaf hydraulic conductance.

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 (Ψ_{50}) 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 Ψ_{leaf} associated with 95% loss of leaf hydraulic conductance (Brodribb & Cochard 2009). In contrast, plant death observed in the current species sample did not corresponded to Ψ_{leaf} associated with near complete loss of leaf hydraulic conductance (Fig. 6). In fact, plant death occurred well beyond major leaf hydraulic dysfunction (by several MPa in *H. lissosperma*) suggesting that the final minimum water potential connected to irrecoverable drought damage 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 Ψ_{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.
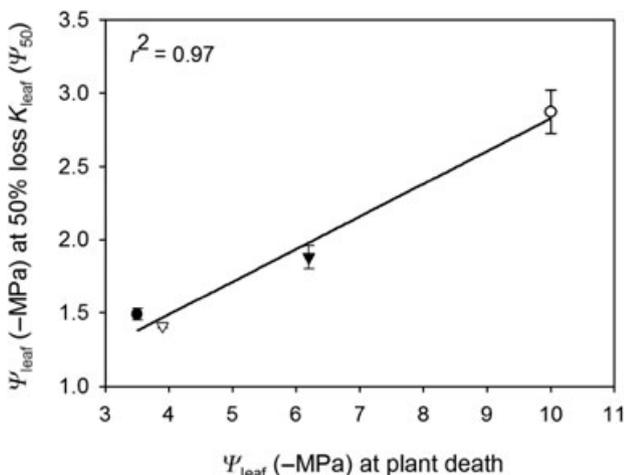
Figure 7. The relationship between the Ψ_{leaf} at 50% loss of K_{leaf} (Ψ_{50}) and the absolute drought tolerance (Ψ_{leaf}) of the four species investigated (linear regression: $r^2 = 0.97$). Solid circle, *Tasmannia lanceolata*, open circle, *Hakea lissosperma*, solid triangle, *Lomatia tinctoria*, open triangle, *Atherosperma moschatum*.

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