

# Hydraulic Failure Defines the Recovery and Point of Death in Water-Stressed Conifers<sup>[OA]</sup>

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This study combines existing hydraulic principles with recently developed methods for probing leaf hydraulic function to determine whether xylem physiology can explain the dynamic response of gas exchange both during drought and in the recovery phase after rewatering. Four conifer species from wet and dry forests were exposed to a range of water stresses by withholding water and then rewatering to observe the recovery process. During both phases midday transpiration and leaf water potential ( $\Psi_{\text{leaf}}$ ) were monitored. Stomatal responses to  $\Psi_{\text{leaf}}$  were established for each species and these relationships used to evaluate whether the recovery of gas exchange after drought was limited by postembolism hydraulic repair in leaves. Furthermore, the timing of gas-exchange recovery was used to determine the maximum survivable water stress for each species and this index compared with data for both leaf and stem vulnerability to water-stress-induced dysfunction measured for each species. Recovery of gas exchange after water stress took between 1 and >100 d and during this period all species showed strong 1:1 conformity to a combined hydraulic-stomatal limitation model ( $r^2 = 0.70$  across all plants). Gas-exchange recovery time showed two distinct phases, a rapid overnight recovery in plants stressed to <50% loss of leaf hydraulic conductance ( $K_{\text{leaf}}$ ) and a highly  $\Psi_{\text{leaf}}$ -dependent phase in plants stressed to >50% loss of  $K_{\text{leaf}}$ . Maximum recoverable water stress ( $\Psi_{\text{min}}$ ) corresponded to a 95% loss of  $K_{\text{leaf}}$ . Thus, we conclude that xylem hydraulics represents a direct limit to the drought tolerance of these conifer species.

Photosynthesis occurs in an aqueous environment and until evolution comes across a solid-state means of fixing atmospheric  $\text{CO}_2$ , terrestrial plant species, even those in humid tropical rainforests (Engelbrecht et al., 2007) will be exposed to potentially lethal desiccation. The reason for this is that in most environments competition between plants forces them to engage in a dangerous balancing act between trading water for carbon at the leaf while minimizing costs associated with replacing this transpired water with water pulled from the soil. The job of seeking and transporting water falls upon the roots and vascular system, and reduced investment in these systems comes at a cost in terms of the safety and efficiency of water carriage. These conflicting demands mold the form and function of vascular plants and have yielded a diverse spectrum of vascular anatomies, each tuned to a specific flow capacity and drought tolerance.

Desiccation tolerance is at the center of the vascular cost/benefit equation. The reason for this is that a more desiccation-tolerant vascular system (one that resists embolism better during soil drying) is distinctly more costly to build than a sensitive system (Hacke

et al., 2001a), yet the repercussions of vascular failure are likely to be fatal. This trade-off, as with many other systems in biology, leads to functional diversity and hence there is a great range in the ability of plant vascular systems to operate under the variable hydraulic tensions intrinsic to pulling water from the soil to the leaf. Hydraulic tension in the xylem increases as soil dries, increasing the risk of xylem dysfunction by the cavitation (Tyree and Sperry, 1989) or collapse (Cochard et al., 2004) of conduits, and when quantified in terms of the tension required to disable 50% of the stem xylem, published values range from less than 1 MPa (Yangyang et al., 2007) to maxima of around 15 MPa (Brodribb and Hill, 1999). It is an attractive proposition to suggest that xylem vulnerability to dysfunction is the key trait responsible for setting the drought tolerance of any species, yet the evidence for this remains comparative (Kolb and Davis, 1994; Brodribb and Hill, 1999; Comstock, 2000; Pockman and Sperry, 2000; Tyree et al., 2003; Maherali et al., 2004; Breda et al., 2006). At the same time others cite traits such as photosynthetic physiology (Hanson and Hitz, 1982) and senescence (Rivero et al., 2007) or combined physiopathological processes (McDowell et al., 2008) as more important limiters of plant function during drought.

Major progress has been made recently in our understanding of the fundamental role that plant hydraulics play in governing the rate of water extraction from the soil (Sperry, 2000), yet this understanding breaks down as plants approach and exceed the limitations of their water transport system. Very little

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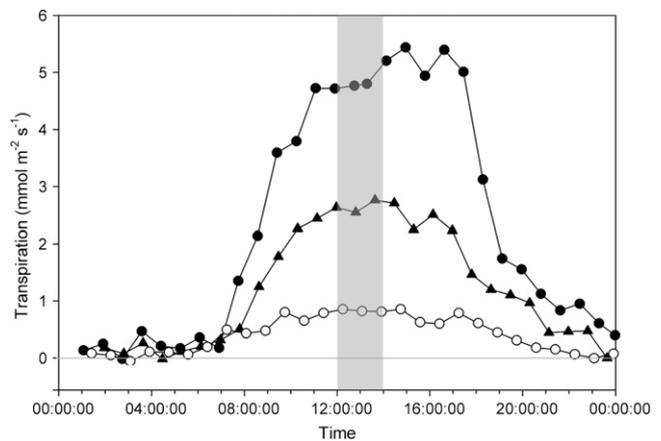
information is available to explain the performance of plants during and after major drought events, and how these episodes impact on plant survival and distribution. Theory suggests that xylem cavitation should set a clear limit to the desiccation tolerance of plants such that water potentials capable of reducing xylem hydraulic conductivity to approach zero should be lethal, or at least result in 100% defoliation. Surprisingly there are no studies that have quantitatively linked the relationship between the resistance of the xylem tissue to hydraulic tension and the absolute desiccation tolerance of plants (Tyree et al., 2002). This gap in our understanding of how plants respond to drought and where the limits of desiccation tolerance lie for any particular species poses an enormous problem to those attempting to model the impacts of changing rainfall or evaporative load on both wild and agricultural plants. In this article we examine the relationship between xylem functional limits and the drought survival and recovery of plants.

Here, we focus on the desiccation tolerance of a group of conifer trees that are apparently constrained in their distribution by the different tolerances of their stem xylem to water stress-induced cavitation (Brodribb and Hill, 1999). By first establishing the vulnerability of both stems and leaves to cavitation and then exposing whole plants to a variety of desiccation intensities we sought to determine whether xylem dysfunction plays a role in the response to desiccation and equally importantly during the postdrought recovery period. A key component of this study is to find at what point plants suffer irreversible desiccation damage, and how this cardinal point in a species' physiological compass relates to xylem function.

## RESULTS

### Drought and Stomatal Closure

The diurnal course of transpiration in all plants rose from minimum values overnight to a plateau that was maintained over the period 10 AM to 4 PM. The magnitude of this transpirational plateau decreased over time as soil water content declined during drought (Fig. 1). The decline in midday transpiration ( $E_{md}$ ) after withholding water continued until both midday and midnight transpirational fluxes were similar, signifying complete stomatal closure. In all species, the response of  $E_{md}$  to decreasing midday leaf water potential ( $\Psi_l$ ) followed a sigmoidal trajectory, with stomata highly sensitive to a very small range in  $\Psi_l$  (Fig. 2). The most sensitive stomatal response was in *Lagarostrobos franklinii* where stomatal conductance (as inferred from  $E_{md}$ ) fell from 80% of maximum to 20% of maximum over the  $\Psi_l$  range  $-1.20$  MPa to  $-1.81$  MPa. *Callitris rhomboidea* showed the lowest sensitivity to  $\Psi_l$  with 1.25 MPa separating 20% and 80% closure. The absolute sensitivity of stomata to  $\Psi_l$  was similar in all species with 50% stomatal closure occurring at a mean of  $-1.20 \pm 0.02$  MPa in three of the four species,



**Figure 1.** Examples of diurnal patterns of whole-plant transpiration in a single individual of *A. arenarius* during several weeks of withholding water. The three plots show data while unstressed ( $\Psi_{\text{leaf}} = -1.15$  MPa; black circles), moderately stressed ( $\Psi_{\text{leaf}} = -1.65$  MPa; triangles), and stressed to >80% stomatal closure ( $\Psi_{\text{leaf}} = -2.85$  MPa; white circles).  $E_{md}$  was measured during the shaded time interval.

and at  $-1.48$  MPa in *C. rhomboidea*. Following stomatal closure the mean rate of plant dehydration was similar in all plants ( $0.29$  MPa per day  $\pm 0.05$ ) except in *C. rhomboidea* that showed a slightly higher rate of drying ( $0.44$  MPa per day).

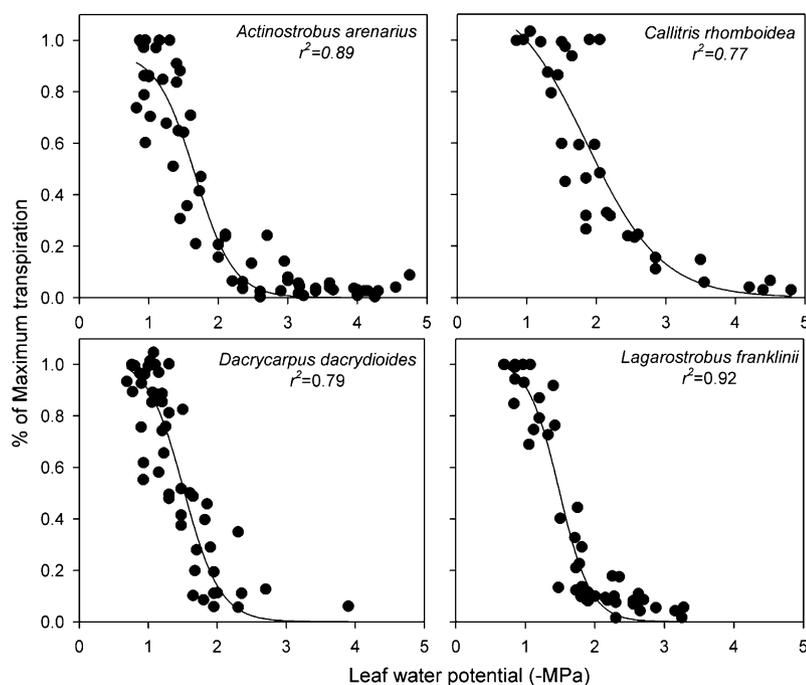
### Stem and Leaf Vulnerability to Drought

During desiccation a marked decline in hydraulic conductivity was observed in excised samples of both stems and leaves as hydraulic tension in the xylem increased. The degree of xylem dysfunction was related to water potential by a sigmoidal function in both stems and leaves of all species (Fig. 3). Despite the relatively conservative shape of these relationships there was a huge range in xylem tolerance to water potential across the species sample. *C. rhomboidea* yielded the most resistant stems and leaves with 50% loss of function recorded at  $-10.8$  MPa and  $-6.60$  MPa, respectively; this compared with only  $-2.78$  MPa and  $-2.54$  MPa for the stems and leaves of *Dacrycarpus dacrydioides*. Leaves were always more sensitive to water-stress-induced dysfunction than stems, but there was a constant relationship between the two such that water potential at 50% loss of stem function ( $\Psi_{\text{stem}50}$ ) was proportional to (and almost equal to) the water potential at 95% loss of  $K_{\text{leaf}}$  ( $\Psi_{\text{leaf}95}$ ), i.e.  $\Psi_{\text{stem}50} = 1.08 \Psi_{\text{leaf}95}$  ( $r^2 = 0.88$ ).

Stomatal closure (50%) preceded 50% stem xylem dysfunction by between 1.7 MPa (*D. dacrydioides*) and 9.1 MPa (*C. rhomboidea*) and there was no relationship between stomatal closure and xylem failure in either stems or leaves.

### Recovery from Drought

Plants were droughted to a variety of water potentials ranging from just past the point of 80% stomatal



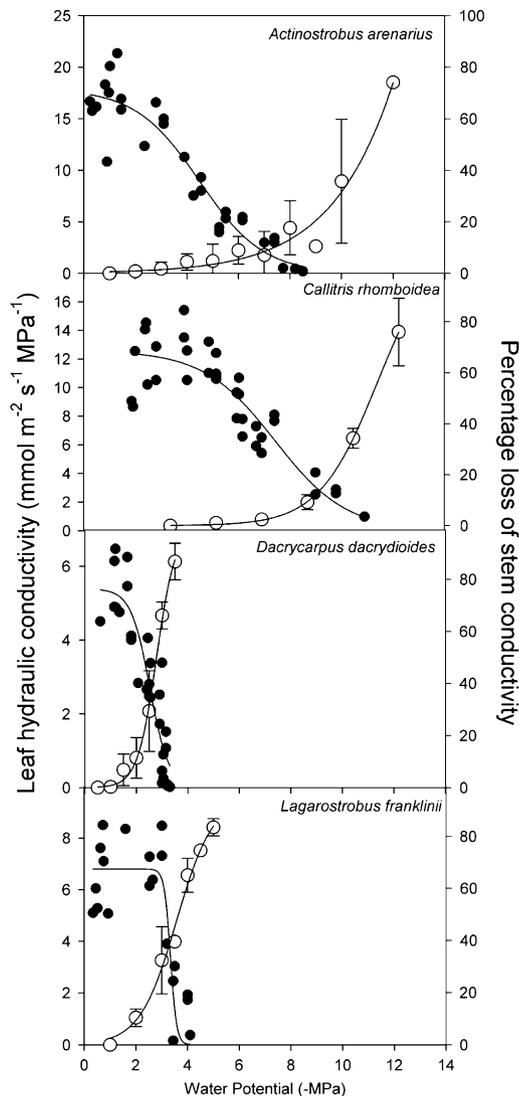
**Figure 2.** Pooled data ( $n = 5$ ) showing the response of transpiration (proportional to stomatal conductance under the controlled vapor pressure growth regime) to increasingly negative  $\Psi_{\text{leaf}}$  as soil dried during the drought treatment. Regressions are sigmoidal functions in each case, and these regression functions were used to define the stomatal dependence upon  $\Psi_{\text{leaf}}$  to evaluate the degree of hydraulic limitation during drought recovery (see Fig. 5A).

closure, to the most severe stress approximately equal to  $\Psi_{\text{leaf}95}$ . Upon rewatering, a universal pattern was observed whereby  $\Psi_{\text{leaf}}$  returned to a value corresponding to between 80% and 20% stomatal closure following an exponential trajectory with a half time of 1 to 2 d (Fig. 4). This pattern was repeated in all plants regardless of the degree of water stress. The final recovery of  $\Psi_{\text{leaf}}$  back to prestress hydration was approximately linear with a slope that was related to the level of stress imposed (Figs. 4 and 5). This last phase of postdrought recovery appeared to dictate the pattern of gas-exchange recovery.

The recovery of gas exchange (as reflected by  $E_{\text{md}}$ ) was strongly influenced by the relatively slow recovery of hydraulic conductivity following rewatering (Fig. 5). This slow recovery of  $E$  was most pronounced in plants droughted to water potentials below 50% loss of  $K_{\text{leaf}}$  (Figs. 4 and 5). The inhibition of stomatal reopening in plants recovering from these significant stresses conformed very well to a hydraulic-stomatal limitation model whereby the rate of gas exchange was a unique function of  $\Psi_{\text{leaf}}$  (Fig. 2) that was ultimately limited by whole-plant hydraulic conductivity (Fig. 5). This means that the stomata responded the same to  $\Psi_{\text{leaf}}$  depression produced by hydraulic dysfunction in wet soil as they did to  $\Psi_{\text{leaf}}$  depression produced by soil drying. A synthesis of all recovery data from all plants showed very good correspondence between the observed recovery of  $E_{\text{md}}$  and the recovery of  $E_{\text{md}}$  predicted from entering measured values of  $\Psi_{\text{leaf}}$  during plant recovery into the equation  $E = f(\Psi_{\text{leaf}})$  where the function  $f(x)$  for each species was taken from the regression equations shown in Figure 2. Regressions of %  $E_{\text{md}}$  observed versus %  $E_{\text{md}}$  predicted

yielded linear functions that were not significantly different to the same regressions fitted through data used to define  $f(x)$ , i.e. the data collected during the initial drought phase prior to rewatering (Fig. 6). Pooling all recovery data for all species yielded a very strong 1:1 linear regression ( $r^2 = 0.70$ ) between %  $E_{\text{md}}$  observed and %  $E_{\text{md}}$  predicted by the hydraulic-stomatal limitation model. Only *L. franklinii* showed a significant deviation from the hydraulic model whereby observed  $E_{\text{md}}$  was on average 22% lower than predicted by the model (Fig. 6). Importantly the relationship between observed and predicted %  $E_{\text{md}}$  was still linear in this species, indicating that hydraulic limitation remained the primary limiter of gas exchange.

Recovery of gas exchange after rewatering was highly sensitive to minimum  $\Psi_{\text{leaf}}$  during drought. Recovery times ranged from a minimum of 1 d to maximum periods of over 100 d (where new leaf growth was required to replace leaves damaged during drought). To compress the range of the recovery data we expressed the recovery of  $E_{\text{md}}$  in terms of  $t_{1/2}^{-1}$ , that is  $1/[\text{the time (days) required for } E_{\text{md}} \text{ to return to 50\% of the predrought maximum}]$ . The advantage of this index is that  $t_{1/2}^{-1}$  ranges from one, representing an overnight recovery, to zero indicating plant death. In all species  $t_{1/2}^{-1}$  exhibited two phases, an insensitive phase followed by a linear decline to values close to and occasionally reaching zero (plant death; Fig. 7). Fitting linear regressions to this second phase of declining  $t_{1/2}^{-1}$  yielded two key parameters, first the point at which this regression = 1 was taken as the minimum  $\Psi_{\text{leaf}}$  that plants could recover gas exchange overnight when rewatered. This intercept corresponded



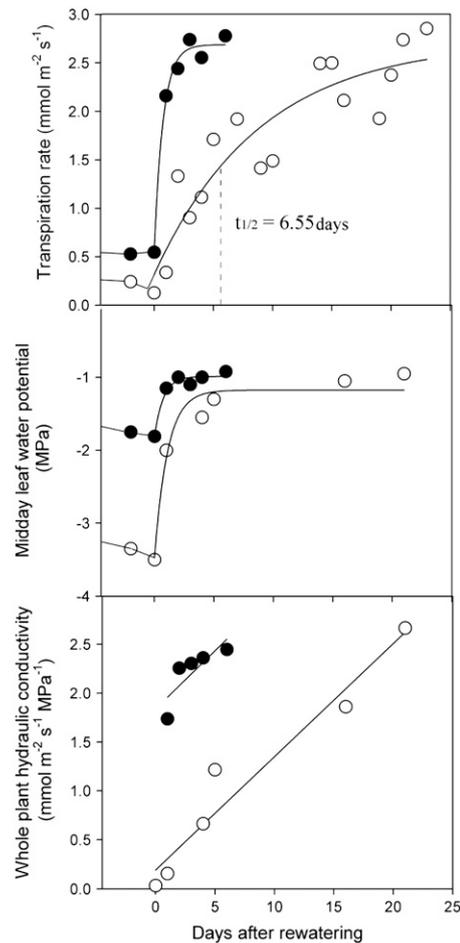
**Figure 3.** Simultaneous plots of declining  $K_{leaf}$  and increasing percentage loss of  $K_{stem}$  in response to increasingly negative water potential. Leaf data are pooled from three plants exposed to gradually increasing water stress while stem data are means ( $n = 4$ ) from excised branches exposed to a range of hydraulic tensions induced by centrifuge. Sigmoid functions are fitted to both stem and leaf data and were used to predict 50% and 95% loss of function in stems and leaves.

closely with the  $\Psi_{leaf}$  at 50% loss of  $K_{leaf}$  ( $r^2 = 0.96$ ). The second value derived from these regressions was the  $x$  intercept that yielded the minimum survivable water potential for each species ( $\Psi_{min}$ ), and this value ranged enormously from  $-11.4$  MPa in the most desiccation-tolerant species *C. rhomboidea*, to  $-2.40$  MPa in *D. dacrydioides*. In all species  $\Psi_{min}$  was equal to the water potential at 95% loss of  $K_{leaf}$  ( $r^2 = 0.88$ ) and 50% loss of  $K_{stem}$  ( $r^2 = 0.98$ ; Fig. 7B). The difference in  $\Psi_{leaf}$  between 100% defoliation and plant death was small in each species. Only plants of *D. dacrydioides* were capable of recovering from 100% defoliation, but even in this species there was a very narrow margin be-

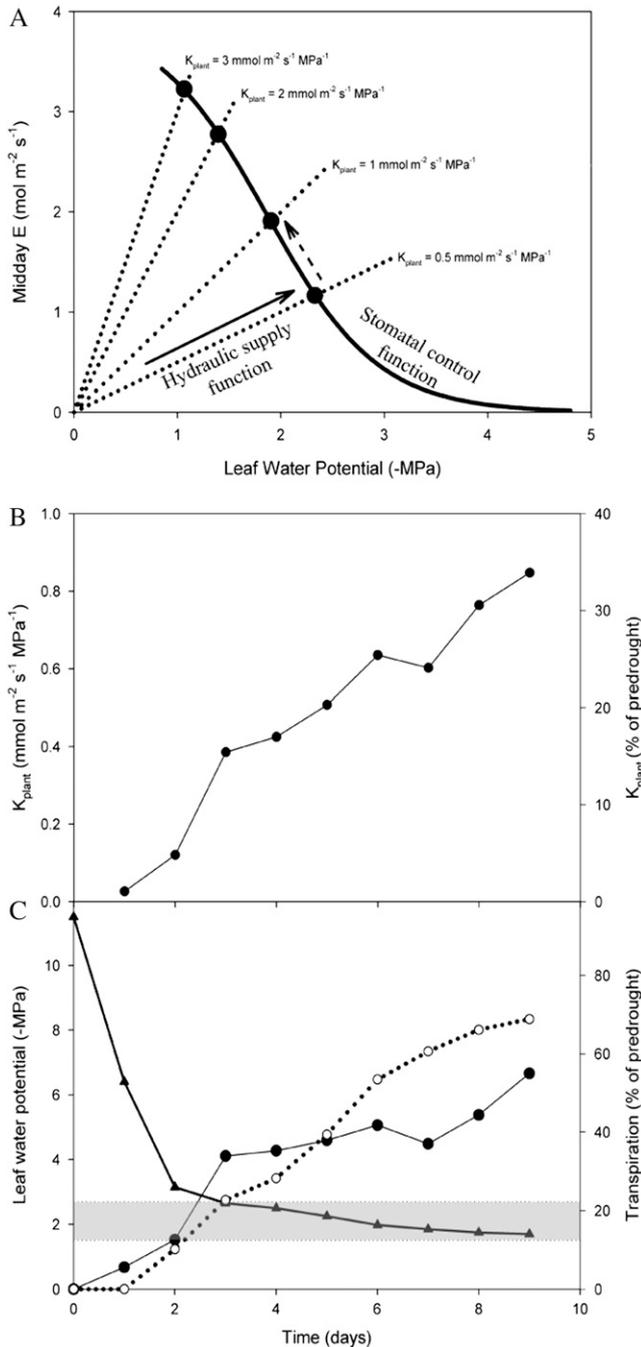
tween  $\Psi_{leaf}$  at 100% leaf loss ( $-2.4$  MPa) and plant death ( $-2.7$  MPa).

## DISCUSSION

Hydraulic function in the four conifer species examined here was found to underpin the recovery from and survival of water stress. This important result provides a functional framework for understanding how plants respond to the highly variable water stresses imposed upon the majority of plants growing in the field. Furthermore these data provide a quantitative and physiological basis for evaluating the absolute desiccation tolerance of conifer species. Xylem dysfunction and desiccation response were intimately linked by a 1:1 relationship between  $\Psi_{min}$  and



**Figure 4.** An example of recovery from mild (black circles) and severe (white circles) water stress in rewatered plants of *L. franklinii*. The mildly stressed plant shows a minimal reduction of  $K_{plant}$  and is able to rapidly recover leaf hydration and gas exchange. By contrast the severely stressed plant experiences profound depression of  $K_{plant}$  that recovers slowly, thus limiting gas-exchange recovery, which has a  $t_{1/2}$  of 6.5 d. Although  $\Psi_{leaf}$  recovers relatively quickly in both plants, it remains limiting during recovery of the severely stressed plant, thus preventing stomatal reopening.



**Figure 5.** Modeled and measured recovery data for a *C. rhomboidea* plant subject to a stress sufficient to reduce  $K_{leaf}$  by approximately 90%. A, According to the hydraulic-stomatal limitation model, in fully hydrated soils  $E$  will be equal to the intersection of a hydraulic supply function (defined by  $K_{plant}$ ) and the stomatal control function (determined empirically from the regression equations in Fig. 2). B, The observed recovery of whole-plant hydraulic conductivity after rewetting. C, The predicted (white circles, dotted line) recovery of midday  $E$  closely matches the observed (black circles, unbroken line) dynamic as the rewatered plant initially rehydrates rapidly to the edge of the stomatal control window (shown as the gray region, representing the  $\Psi_{leaf}$  range responsible for a 20% to 80% reduction in stomatal aperture) then slowly thereafter, thus limiting stomatal conductance and gas exchange. Predicted % $E$  is calculated from entering the

both stem  $\Psi_{stem50}$  and the loss of leaf hydraulic conductivity ( $\Psi_{leaf95}$ ; Fig. 7B). Apart from the obvious physiological importance of this result, the implications for understanding drought survival and the distribution of plants are significant.

### Hydraulic Limitation of Drought Recovery

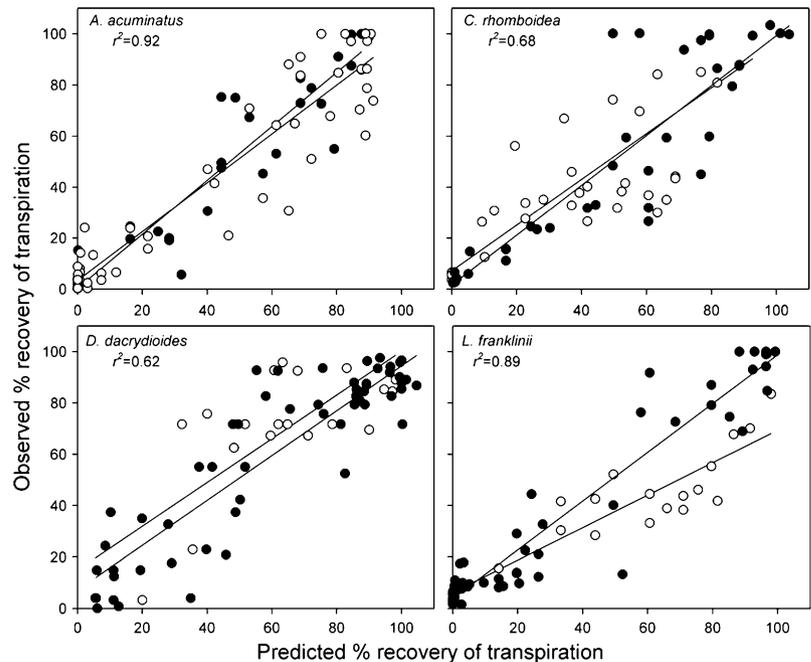
The recovery from water stress in our four conifer species conformed to a hydraulic-stomatal limitation model whereby the response of stomata to  $\Psi_{leaf}$  was the same function during poststress reopening of stomata in wet soil as it was during soil drying (Fig. 6). This scenario means that slow recovery of plant hydraulic conductivity after drought limits the recovery of leaf gas exchange because in saturated soils  $E$  and  $K_{plant}$  determine  $\Psi_{leaf}$  according to the expression:  $-\Psi_{leaf} = E/K_{plant}$ . Hence if a plant suffers a reduction of  $K_{plant}$  during drought, then following rewetting the model would predict that  $\Psi_{leaf}$  will be much more sensitive to  $E$ , and hence stomatal opening will quickly be limited by  $E = f(\Psi_{leaf})$ . Effectively, the realized  $E_{md}$  will be the intersection of the hydraulic supply function (straight line, Fig. 5A) and the stomatal control function (sigmoid curve, Fig. 5A). Recovery of  $K_{plant}$  allows gradually higher  $E_{md}$  to be achieved until  $\Psi_{leaf}$  is nonlimiting at maximum stomatal opening.

We found strong evidence that hydraulic limitation was the process governing gas-exchange recovery from drought in our tree sample, and specifically that this hydraulic-stomatal limitation model could account for over 70% of the variation in gas exchange during the recovery from all levels of drought. This conformity across all species is all the more impressive considering the enormous range of desiccation vulnerabilities represented by our species sample. Previous studies have demonstrated strong evidence for the limitation of gas exchange in nondroughted plants (Meinzer and Grantz, 1991; Hubbard et al., 1999; Brodrribb and Feild, 2000), but here we demonstrate that the recovery of plants from water stress conforms to a hydraulic limitation model without having to invoke other factors such as plant hormones (abscisic acid [ABA]) or direct damage to leaves. The results here come from two conifer families (Podocarpaceae and Cupressaceae) although we have found recently that this type of hydraulic-mediated control of drought recovery applies equally to a group of angiosperms (T.J. Brodrribb, unpublished data). The implication of this is that hydraulic dysfunction and repair probably mediates the drought recovery of vascular plants in general.

Although we found an impressively strong pattern of hydraulic-mediated recovery, the functions used to predict the stomatal response to  $\Psi_{leaf}$  are qualitative relationships that have been somewhat simplified to

measured  $\Psi_{leaf}$  (triangles) into the stomatal control function equation % $E = f(\Psi_{leaf})$  shown in A.

**Figure 6.** Predicted and observed recovery of  $E_{\text{md}}$  (white circles) in all plants after rewatering from all levels of drought. Predicted and observed % $E_{\text{md}}$  are shown simultaneously (black circles) for plants during the droughting phase as well to provide a comparative data set showing stomatal control of gas exchange under limiting soil water content. All plants showed good correlation between observed and predicted % $E_{\text{md}}$  during drought recovery. Only in *L. franklinii* was there any significant difference in the slopes between recovery and droughting datasets.



facilitate prediction. Within-species variation and osmotic adjustment are both important features that have been smoothed by the single sigmoid function fitted to each species. In some individuals there was evidence that during drought a degree of osmotic adjustment in the leaf took place, pushing the relationship between  $\Psi_{\text{leaf}}$  and  $E_{\text{md}}$  (Fig. 2) to the right, thus enabling stomata to open at slightly lower water potentials after drought. Osmotic adjustment in response to water stress has been observed in many plants and during recovery from water stress it would have the effect of yielding higher than predicted  $E$  during the recovery phase (Fig. 8). Such osmotic adjustment could be easily accommodated in a hydraulic-stomatal limitation model, and acts in the opposite direction to the predicted effect of nonhydraulic control of plant recovery (Fig. 8).

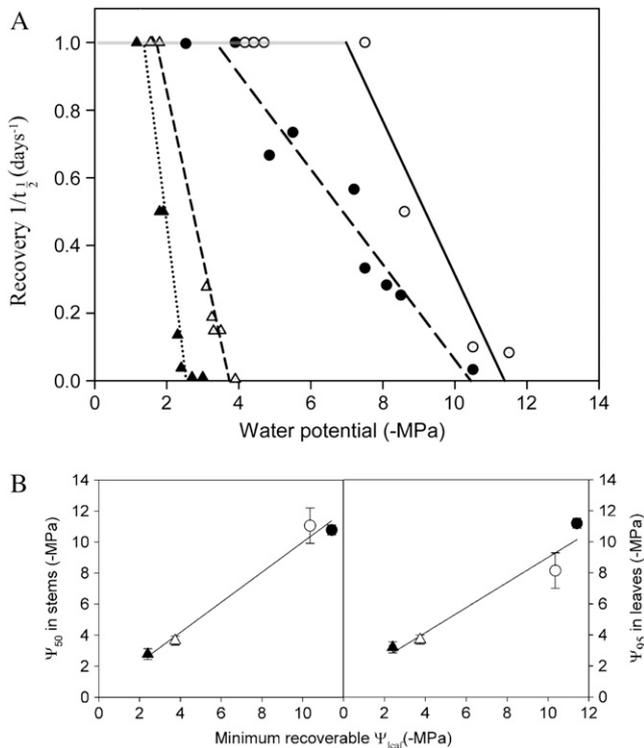
By demonstrating conservation of the  $E(\Psi_{\text{leaf}})$  function both during and postdrought, the data tend to negate the possibility of an ABA modification of the stomatal sensitivity to  $\Psi_{\text{leaf}}$  in these species (compare with Wilkinson and Davies, 2002). Under conditions of ABA-induced stomatal closure,  $\Psi_{\text{leaf}}$  would quickly rise to close to zero after rewatering due to the low  $E$  and hydrated soil, then gradually decline as ABA concentration declined over time, and stomata reopened (Fig. 8). This type of response was not found to occur in any individual, thus emphasizing the fundamental nature of the hydraulic-mediated stomatal recovery from drought.

#### Recovery of $K_{\text{plant}}$

All species showed a similar pattern whereby recovery from mild water stress ( $\Psi_{\text{leaf}}$  between stomatal

closure and 50% loss of leaf conductivity) was very different from the behavior of plants subject to stresses beyond 50% loss of  $K_{\text{leaf}}$ . Plants rewatered after mild water stress recovered gas exchange very quickly (overnight) despite the fact that in some cases significant depression of  $K_{\text{leaf}}$  had occurred (Figs. 4 and 7A). Two explanations could account for this observation, the first of which is that plants were able to rapidly and fully rehydrate overnight, thus refilling embolized conduits in the leaf (Milburn and McLaughlin, 1974). This concept of rapid embolism reversal in conifers is an important and controversial issue given that there is evidence that cavitation leading to aspiration of the torus/margo pit complex is nonreversible (Sperry and Tyree, 1990). The other, most parsimonious explanation for this rapid recovery phase is that the initial loss in leaf hydraulic conductivity may not be associated with xylem cavitation. Good evidence exists to suggest that xylem tissue collapse (Cochard et al., 2004; Brodribb and Holbrook, 2005) and loss of leaf turgor (Brodribb and Holbrook, 2006; Kim and Steudle, 2007) may both play a part in the loss of  $K_{\text{leaf}}$  in a variety of plants. Furthermore, we have observed xylem cell collapse in the leaves of two of the four species in this study (both Cupressaceae species), making cell collapse a strong candidate for the incipient (rapidly reversible) stage of  $K_{\text{leaf}}$  depression.

The timing of gas-exchange recovery in plants exposed to water potentials sufficient to induce >50% loss of  $K_{\text{leaf}}$  was strongly influenced by the magnitude of water stress (Fig. 7A). The shape of this relationship suggests that the rate of repair of  $K_{\text{plant}}$  in these individuals was nonlinear, decreasing exponentially as  $\Psi_{\text{leaf}}$  approached lethal values. This slow repair of  $K_{\text{plant}}$  is likely to represent the refilling of embolized

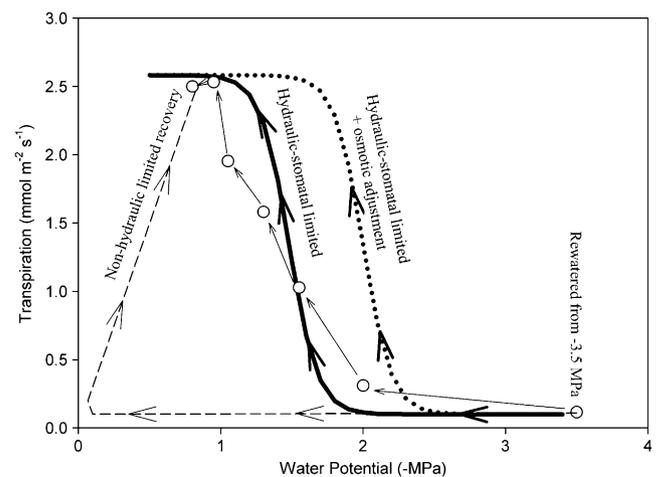


**Figure 7.** A, The relationship between recovery time (plotted as  $t_{1/2}^{-1}$ ) and final  $\Psi_{\text{leaf}}$  prior to rewatering in all individuals of *A. arenarius* (white circles), *C. rhomboidea* (black circles), *D. dactyloides* (black triangles), and *L. franklinii* (white triangles). Recovery time showed two phases, the first phase was insensitive to  $\Psi_{\text{leaf}}$  ( $1/t_{1/2} = 1$ ) and the second highly dependent. Linear regressions fitted through this second phase as  $t_{1/2}$  fell from 1 (overnight recovery of  $t_{1/2}$ ) to 0 (plant death). The x intercept of these regressions was defined as the minimum recoverable water potential ( $\Psi_{\text{min}}$ ). B, Shows the very highly significant 1:1 relationships between  $\Psi_{\text{min}}$  derived from A and 50% loss of  $K_{\text{stem}}$  ( $r^2 = 0.98$ ) and 95% loss of  $K_{\text{leaf}}$  ( $r^2 = 0.94$ ), symbols as in A. Correlation coefficients are for regression lines forced through the origin.

conduits, which could occur under capillary force overnight when  $\Psi_{\text{leaf}}$  was found to increase to close to zero in rewatered plants (T.J. Brodrribb, unpublished data). Direct evidence of xylem refilling came from examining dyed and frozen stems of both *C. rhomboidea* and *Actinostrobus arenarius* that had recovered from water stresses sufficient to kill approximately 50% of the foliage. After 3 weeks recovery we found that most (>80%) of the xylem in these stems was functional as opposed to <50% during drought. The observation that the efficiency of conduit refilling decreased as  $\Psi_{\text{leaf}}$  approached  $\Psi_{\text{min}}$  is significant as this leads to a rapid increase in  $t_{1/2}$  and hence a rapid transition from recoverable to nonrecoverable water stress (Fig. 7). Given the heterogeneous pattern of leaf damage observed after severe drought in these species (T. Brodrribb, personal observation), it is highly probable that severe stress causes an increasing heterogeneity in  $\Psi_{\text{leaf}}$  of the plant canopy as some branches approach zero conductivity before others. Clearly as  $K_{\text{plant}}$  ap-

proaches zero the ability of branches to rehydrate decreases rapidly, and it is probable that an increasing proportion of branches with catastrophic loss of conductivity contribute to the observed pattern of rapidly increasing recovery time. Recovery times greater than 50 d appeared to be attributable to new sapwood growth in branches where hydraulic conductivity had approached zero. The likelihood of canopy  $\Psi_{\text{leaf}}$  heterogeneity during severe drought is greatly accentuated in older plants as the variation in age (Brodrribb and Holbrook, 2003), position, and history (Hacke et al., 2001b) of different leaf cohorts increases. Hence the ability of large plants in the field to recover from drought might be expected to decrease more gradually as plants approach  $\Psi_{\text{min}}$  than for the young potted plants observed here.

Although both stem and leaf vulnerability were very significantly correlated with  $\Psi_{\text{min}}$ ,  $K_{\text{leaf}}$  vulnerability was most strongly implicated as the causal parameter driving drought response and recovery kinetics. There are several reasons for this conclusion, the first of which is that  $t_{1/2} = 0$  ( $\Psi_{\text{min}}$ , or plant death) corresponded closely with the  $\Psi_{\text{leaf}}$  at 95% loss of  $K_{\text{leaf}}$ . Such a marked loss of  $K_{\text{leaf}}$  would impact very significantly upon  $K_{\text{plant}}$  because leaves represent a disproportionately large resistance compared with stems (Sack and Holbrook, 2006). By contrast  $\Psi_{\text{min}}$  correlated with only a 50% loss of  $K_{\text{stem}}$ , the impact of which upon  $K_{\text{plant}}$  would be relatively small. Other tissues, in



**Figure 8.** Examples of measured (white circles) and modeled (lines) recovery trajectory of transpiration in a *L. franklinii* plants over 20 d following rewatering from drought (-3.5 MPa). Three curves depict three models of stomatal-hydraulic behavior: the hydraulic-stomatal limitation model with a fixed  $E = f(\Psi_{\text{leaf}})$  (bold line); a hydraulic-stomatal limitation model with osmotic adjustment to promote stomatal opening at lower  $\Psi_{\text{leaf}}$  (dotted line); and a nonhydraulic limited recovery where stomatal sensitivity to  $\Psi_{\text{leaf}}$  is enhanced or nonexistent postdrought, e.g. as might occur if ABA was limiting stomatal aperture (dashed line). The measured recovery response for this individual and all individuals (Fig. 6) was best described by the constant  $E = f(\Psi_{\text{leaf}})$  function.

particular roots, have always been considered as candidates for the vulnerability-limiting tissue due to what is often found to be their high vulnerability to cavitation (Kolb et al., 1996; Sperry and Ikeda, 1997). The evidence here suggests that tissues upstream of the leaf were either similar to, or more resistant to cavitation than the leaves, emphasizing the ecological importance of leaf vulnerability.

## Ecological Implications

These data have a number of important ecological implications, the most fundamental of which is that xylem vulnerability, particularly that of the leaf, can be used to place a definitive limit on the physical tolerance of conifer species to desiccation. This idea has been mooted in the past and there have been several attempts to define how vulnerability to cavitation might limit the dry end of plant distributions (Sperry et al., 1998; Brodribb and Hill, 1999), however we present a means of identifying precisely when a plant can be expected to die during exposure to extreme drought. Furthermore it should be possible, using the principles of hydraulic-mediated recovery from drought, to model the long-term dynamics of gas exchange during drought cycles in the field. Such a model would need to incorporate information about water release characteristics of different soils (Sperry et al., 1998), though rather than using stem vulnerability as the foundational component of the model it would use a combination of leaf vulnerability and stomatal response functions to predict transpiration and assimilation recovery.

Important questions remain, not the least of which is the fundamental question of why stomata close when they do. Much discussion revolves around the issue of whether xylem vulnerability to cavitation defines how stomata respond to  $\Psi_{\text{leaf}}$  (Bunce, 2006). In this respect it is interesting to note the rather large safety margin between the  $\Psi_{\text{leaf}}$  at stomatal closure and hydraulic dysfunction observed here for these conifer species. This large safety margin places conifers in a similar category to ferns (Brodribb and Holbrook, 2004) and distinguishes them from angiosperms that close stomata very close to the onset of  $K_{\text{leaf}}$  dysfunction. The evolutionary implications of such a functional schism high in the phylogeny of vascular plants would be of great significance.

## MATERIALS AND METHODS

### Plant Material

Four species of conifer trees were selected to cover a broad range of drought sensitivity. *Actinostrobus arenarius* (Cupressaceae) grows in semiarid woodland in western Australia, *Callitris rhomboidea* (Cupressaceae) extends from dry open woodland to moist coastal habitats in eastern Australia, *Dacrycarpus dacrydioides* (Podocarpaceae) grows in ever-wet rainforest in New Zealand, and *Lagarostrobos franklinii* (Podocarpaceae) is restricted to wet forest in Tasmania. All individuals were grown from seed collected in native forests and grown for several years under nonheated glasshouse conditions in

Hobart, Tasmania. Twelve healthy individuals of each species were chosen such that all plants were similar in size (between 50 cm and 1 m tall) and age (between 3 and 5 years old). Eight of these plants were used in drought experiments while three were sampled for stem vulnerability. Plants were potted in a high conductivity soil such that soil hydraulic conductivity was unlikely to be limiting during the droughting or recovery phases of measurement (Sperry et al., 1998). All individuals were potted into a mix of eight parts composted pine bark, two parts coarse river sand, and one part peat moss with added slow release fertilizer (Osmocote) in 1.8-L pots. Two months prior to the commencement of measurements plants were moved to a controlled-environment glasshouse cell and grown under 18-h days at 25°C/10°C day/night in a controlled glasshouse environment. Humidity in the glasshouse was controlled at 50% using a De Longhi DHE-PC dehumidifier regulated by a Dixell 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 datalogger. Lighting in the growth chamber was unfiltered natural light, with sodium vapor lamps (providing 300–500  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at the leaf surface) used to extend the photoperiod to 18 h.

### Leaf Vulnerability

Leaf vulnerability was determined in three plants of each species during the gradual imposition of water stress by withholding water. During this drought phase branches were removed periodically to measure  $K_{\text{leaf}}$  as  $\Psi_{\text{leaf}}$  declined from midday values around  $-1$  MPa to minimum water potentials associated with leaf death over a period of 4 to 8 weeks. Branches were sampled around midday, and prior to removing the sample branch the mean  $\Psi_{\text{leaf}}$  was determined from two adjacent shoots. A small sunlit branch (approximately 15  $\text{cm}^2$ ) was then removed and bagged before being quickly transferred back to the laboratory (approximately 2 min transfer time). Using a modified rehydration technique we recut the branch underwater and immediately connected it to a microflowmeter (Brodribb and Holbrook, 2006). Branches remained connected to the flowmeter under 1,000  $\mu\text{mol quanta m}^{-2}$  illumination at 22°C with flow rate logged every 1 s for 60 s, after which the branch was immediately disconnected and wrapped in moist paper and foil and transferred to a pressure chamber for determination of final  $\Psi_{\text{leaf}}$ .  $K_{\text{leaf}}$  was determined at the two instantaneous points corresponding to the initial and final  $\Psi_{\text{leaf}}$  using equation 1 (based on Ohm's law analogy where the pressure gradient across the excised branchlet is equal to  $-\Psi_{\text{leaf}}$ ):

$$K_{\text{leaf}} = - \frac{I}{A_1 \psi_{\text{leaf}}}$$

where  $I$  = instantaneous flow rate into the leaf ( $\text{mmol s}^{-1}$ );  $A_1$  = projected leaf area.

Initial and final  $K_{\text{leaf}}$  did not tend to vary by more than 10% and hence were combined to produce a mean  $K_{\text{leaf}}$  measurement at the initial  $\Psi_{\text{leaf}}$  value. In cases where initial and final  $K_{\text{leaf}}$  differed by more than 20% (occasionally in hydrated leaves), a third technique was employed whereby branches were allowed to come to an evaporational steady state for 120 to 180 s while connected to the flowmeter, then disconnected and  $\Psi_{\text{leaf}}$  immediately measured. In very dehydrated leaves it was often necessary to recut the stem several times before maximum flow was initiated. This may have been due to localized embolism around the initial cut, or may alternatively be due to displacement of the torus under large pressure gradients (Hacke et al., 2004). To overcome this artifact dehydrated stems that showed low flow were recut five times or until flow remained steady after recutting.

### Stem Vulnerability

Xylem cavitation was assessed with the Cavitrone technique (Cochard, 2002), a technique derived from the centrifuge method of Alder et al. (1997). The principle of the technique is to use centrifugal force to increase the water tension in a xylem segment and, at the same time, measure the decrease in its hydraulic conductance. The curve of percentage loss of xylem conductance (PLC) versus xylem water tension represents the sample vulnerability to cavitation. Vulnerability curves were determined on three to five different samples for each species. The samples were collected on well-watered plants in Tasmania, defoliated, wrapped in wet paper, and shipped to France by express air mail (arriving within 3 d). In France, the samples were recut under water to 0.28-m long segments and installed in the Cavitrone. Xylem pressure was first set to a reference pressure ( $-0.5$  MPa or  $-1$  MPa) and the sample

maximal conductance ( $K_{\max}$ ) was determined. The xylem pressure was then set to a more negative pressure and the new sample conductance  $K$  was determined. The sample percent loss of conductance was then computed as  $PLC = 100(1 - K/K_{\max})$ . The procedure was repeated for more negative pressures (with  $-0.5$  to  $-2$  MPa step increments) until PLC reached at least 90% or down to  $-12$  MPa (pressures less  $-12$  MPa could not be generated without serious risk of fracturing the rotor). Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution) and xylem pressure was adjusted at approximately  $\pm 0.1$  MPa.

## Gas Exchange during Drought

Plant gas exchange was monitored daily using a computer-interfaced balance to measure whole-plant water loss. Pots were double bagged and plants weighed to an accuracy of  $\pm 0.01$  g (Mettler-Toledo PG5002-S) between 1100 and 1300 h. Transpiration was calculated by the loss of weight of each plant between measurements divided by the total leaf area of the plant. Leaf area was measured at the conclusion of the experiment by compressing the entire plant between glass plates on a light box and photographing the projected leaf area. Normalization of  $E_{\text{md}}$  to leaf area could be problematic in species where leaf drop occurred as a normal response to drought, however in these species leaf senescence was only observed under very severe stress, in which case leaf drop was close to 100%. Hence in plants exposed to these water potential extremes, leaf area was measured prior to stress exposure and  $E_{\text{md}}$  normalized to this predrought leaf area.

Throughout the experiment vapor pressure deficit remained constant during the day and therefore  $E_{\text{md}}$  was closely proportional to stomatal conductance apart from small variations in leaf temperature.

Plants were droughted by withholding water while the pots and soil were bagged and covered in foil to prevent excess heating of the roots. During droughting  $\Psi_{\text{leaf}}$  was measured initially on a daily basis, then every 2 to 4 d immediately prior to transpiration measurements by removing two small shoots (equivalent to approximately 0.2% of the total leaf area) and immediately bagging them for measurement with a Scholander pressure chamber. Minimum water potentials were targeted at 80% stomatal closure, 50% loss of  $K_{\text{leaf}}$ , 95% loss of  $K_{\text{leaf}}$ , and 50% loss of  $K_{\text{stem}}$ . Individuals that were exposed to the most severe drought were all previously exposed to one cycle of moderate drought and rewatered. This was done to ensure that plants were hardened prior to severe desiccation, and thus yielded an accurate measure of the maximum drought tolerance of each species.

## Recovery Measurements

Once the above targets for droughted  $\Psi_{\text{leaf}}$  had been reached, plants were rewatered overnight until soils became saturated. During the subsequent recovery period plants were watered daily to full soil capacity in the morning then bagged at midday to avoid water logging of pots.  $\Psi_{\text{leaf}}$  and  $E_{\text{md}}$  were monitored every 1 to 3 d depending on the recovery rate. Whole-plant hydraulic conductivity at midday ( $K_{\text{plant}}$ ) was calculated on the assumption that soils were fully saturated and hence water potential at the root was close to zero. Under these circumstances:

$$K_{\text{plant}} = E_{\text{md}}/\Psi_{\text{leaf}}$$

## Statistics

Stomatal response to  $\Psi_{\text{leaf}}$  was a key component of the hydraulic model and was determined by using regression fitting software (Sigmaplot, SPSS Inc.) to fit a sigmoid function of the form

$$y = \frac{a}{1 + e^{-\frac{x-x_0}{b}}}$$

to the pooled ( $n = 5$ )  $E_{\text{md}}$  versus  $\Psi_{\text{leaf}}$  data collected for each species during the initial drought treatment. Transpiration data were normalized as percentage data for each species to reduce the effects of within species variation. Vulnerability curves for leaves and stems used the same function as above and again data were pooled from three replicate plants. In the case of stomatal and  $K_{\text{leaf}}$  vulnerability the parameter  $b$  was negative while in the case of stem % loss of conductivity data  $b$  was positive. Estimates of  $\Psi_{\text{leaf}}$  at 20%, 50%, and 95% stomatal limitation and losses of hydraulic conductivity were made from the respective regression equations with their attendant ses.

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