

# Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit

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## Summary

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- Motivated by the urgent need to understand how water stress-induced embolism limits the survival and recovery of plants during drought, the linkage between water-stress tolerance and xylem cavitation resistance was examined in one of the world's most drought resistant conifer genera, *Callitris*.
- Four species were subjected to drought treatments of –5, –8 and –10 MPa for a period of 3–4 wk, after which plants were rewatered. Transpiration, basal growth and leaf water potential were monitored during and after drought.
- Lethal water potential was correlated with the tension producing a 50% loss of stem hydraulic conductivity. The most resilient species suffered minimal embolism and recovered gas exchange within days of rewatering from –10 MPa, while the most sensitive species suffered major embolism and recovered very slowly. The rate of repair of water transport in the latter case was equal to the rate of basal area growth, indicating xylem reiteration as the primary means of hydraulic repair.
- The survival of, and recovery from, water stress in *Callitris* are accurately predicted by the physiology of the stem water-transport system. As the only apparent means of xylem repair after embolism, basal area growth is a critical part of this equation.

## Introduction

Vascular plants employ the cohesive forces between water molecules as a means of connecting transpiring leaves, via the xylem vascular system, to a reliable source of water in the soil. Unfortunately, this connection also means that plants are vulnerable to the generation of enormous tensions when soil water becomes depleted. Water tensions sufficient to crush cells, cavitate the water transport system and kill tissues are commonly experienced by plants in natural systems exposed to drought (Tyree & Sperry, 1989). Accordingly, the water transport system is considered as a primary site of adaptation involved in determining the ability of woody plants to survive under different rainfall regimes (Brodribb & Hill, 1999; Hacke *et al.*, 2001; Maherali *et al.*, 2004; Kursar *et al.*, 2009). Most research into the drought-adaptive potential of the xylem water transport system is directed towards the modification of xylem tissue to resist cavitation failure under water stress.

Drought-induced failure of the hydraulic system has often been associated with the sudden death of plants

exposed to critical water stress (Davis *et al.*, 2002; Tyree *et al.*, 2002), and in this respect the hydraulic system should dictate limits of species tolerance of water stress. Indeed, the ecological potential of using xylem vulnerability to cavitation as an index of drought tolerance is widely recognized and has stimulated the characterization of hundreds of plant species in terms of the water tension required to induce a 50% loss of xylem conduction. Yet, despite this interest in xylem function, the relationship between xylem vulnerability and plant drought tolerance has only rarely been quantified (Brodribb & Cochard, 2009). As such, there is a significant gap between our knowledge of xylem cavitation and its application as an ecological tool.

Determining precisely what magnitude of water tension constitutes a lethal water stress is complicated by the existence of a cavitation feedback as water tensions approach the specific functional limits of the xylem (Tyree & Sperry, 1988). In trees exposed to drought, leaf water potential ( $\Psi_1$ ) initially tends to decline gradually because stomatal closure reduces the daily rate of leaf water loss. However, if drought is severe then  $\Psi_1$  can fall sufficiently to cause major

embolism of the xylem, potentially impeding water transport such that water supply is insufficient to sustain water loss from leaves with closed stomata. At this point a feedback between declining  $\Psi_1$  and xylem conductivity will cause rapid foliage death as leaves become hydraulically disconnected from the plant. Thus, understanding the significance of catastrophic xylem dysfunction as a cause of plant death during drought is critically important because xylem vulnerability underpins proposed ecological trade-offs between wood density, conductivity and growth rate (Hacke *et al.*, 2001; Ackerly, 2004; Chave *et al.*, 2009; Poorter *et al.*, 2010).

Fatal water stress ( $\Psi_d$ ) in any species can be operationally defined as the transition point during drought where  $\Psi_1$  passes from a recoverable to nonrecoverable water stress upon soil rewetting. For example, a recent study demonstrated that  $\Psi_d$  in a sample of conifer saplings corresponded to water tensions sufficient to cause a 50% loss of conductivity in the stem or a 95% loss of leaf hydraulic conductivity (Brodribb & Cochard, 2009). While the use of post-drought gas-exchange recovery data as an index of the severity of water stress holds significant potential for defining survival limits, the actual mechanism of water transport and gas exchange recovery remains unclear. Slow, hydraulically limited recovery of conifer saplings from sub-lethal water stress suggests that xylem cavitation in conifers is either very slow to repair or that the damaged water transport system must be replaced by new xylem tissue (Brodribb & Cochard, 2009). Uncertainty remains as to the speed and effectiveness of xylem repair after drought-induced cavitation or indeed whether conifer tracheids can be repaired at all. Some studies have shown that rapid repair of xylem cavitation can occur in roots (Kaufmann *et al.*, 2009) and stems (Holbrook *et al.*, 2001) upon rewatering while others maintain that repair is possible even under moderate water tension (Holbrook & Zwieniecki, 1999; McCully, 1999). Identifying how xylem recovers after drought damage is fundamental to understanding the impact of water transport function of plant drought survival, providing key insights into evolution of drought tolerance.

Our aim in this study was to investigate the relationships between xylem vulnerability and plant recovery from extreme water stress in *Callitris* (Cupressaceae) an Australian conifer genus known to be highly resistant to drought (Claytongreene, 1983; Attiwill & Claytongreene, 1984). *Callitris* provides an ideal model system because the species span a wide range of seasonally dry climates characterized by episodic and occasionally severe droughts driven by ENSO (El Niño Southern Oscillation) climate mode. We were particularly interested to investigate the role of basal area growth as a means of recovering hydraulic function in plants where embolism had been induced *in planta* by exposure to water stress. By comparing the dynamics of basal growth and gas

exchange during the recovery period we were able to determine how vascular cambial activity responds to drought, and whether xylem reiteration explains the rate of plant functional repair.

## Materials and Methods

### Plant material and growth conditions

Species from the successful Australian conifer genus *Callitris* (Cupressaceae) were chosen for study because of their reported drought tolerance (Attiwill & Claytongreene, 1984). The four species; *Callitris collumellaris* F. Muell., *Callitris gracilis* R. T. Baker, *Callitris preisii* Miq. and *Callitris rhomboidea* R. Br. ex A. Rich. & Rich. all extend into semi-arid woodland across much of the Australian continent where they typically form small gregarious communities (generally 5–15 m) embedded in larger tracts of *Eucalyptus*-dominated vegetation. Seedling plants were collected from nursery stock in Tasmania, Western Australia and Queensland and 30 individuals of each species repotted into 5-l pots with a soil mixture of eight parts composted pine bark and two parts coarse river sand with added slow release fertilizer and dolomite lime to adjust pH. Plants were initially established in randomized blocks for 12 months in a glasshouse in Hobart (at the University of Tasmania, Australia), then, 6 months before commencing drought treatment, at a height of *c.* 1 m, plants were transferred to a controlled environment cell with 18-h daylength at 25°C : 10°C day : night temperatures. Daytime relative humidity was maintained at 40% in the cells using a dehumidifier unit coupled to a humidity sensor. Temperature, light, and humidity were continuously monitored and logged.

### Xylem vulnerability to cavitation

In addition to control plants, three plants from each species were maintained well watered throughout the experiment for determination of xylem vulnerability. The main axis of each of these individuals was harvested 50 mm above the soil early in the morning and trimmed to 400 mm in length. Stems were wrapped in moist paper towel and plastic and immediately posted to France where vulnerability was determined using the Cavitron method (Cochard, 2002). Samples arrived 2 d after postage and were immediately refrigerated at 2°C. Within 2 wk after receipt of the samples, xylem cavitation was assessed with the Cavitron, a centrifuge technique following the procedure described by Cochard (2002). Before measurement, all branches were cut under water to a standard length of 0.27 m. Centrifugal force was used to generate negative pressure in the xylem and to provoke water stress-induced cavitation using a custom rotor mounted on a 'late' centrifuge (HS18; MSE

Scientific, London, UK). Xylem conductance was measured under negative pressure using a reference ionic solution of 10 mmol l<sup>-1</sup> KCl and 1 mmol l<sup>-1</sup> CaCl<sub>2</sub> in deionized water. Xylem pressure ( $P$  in MPa) was first set to a reference pressure (-2 MPa) and the sample maximal conductance ( $K_{\max}$  in m<sup>2</sup> MPa<sup>-1</sup> s<sup>-1</sup>) was determined. The xylem pressure was then set to a more negative pressure by steps of 1 or 2 MPa depending on the species and subsequently returned to a reference pressure to determine the new sample conductance  $K$ . Data acquisition and processing were performed using the Cavisoft software (Cavisoft v1.5, University of Bordeaux, Bordeaux, France). The percentage loss of conductance (PLC) of the stem was determined at each pressure step following the equation:

$$\text{PLC} = 100 \times \left(1 - \frac{K}{K_{\max}}\right) \quad \text{Eqn 1}$$

The relation between  $P$  and PLC represents the vulnerability curve of the sample. Vulnerability curves were determined on two to three different samples for each species. A sigmoid function (Pammenter & Van der Willigen, 1998) was fitted to the data from each species using the following equation:

$$\text{PLC} = \frac{100}{\left(1 + \exp\left(\frac{S}{25 \times (P - P_{50})}\right)\right)} \quad \text{Eqn 2}$$

where  $P_{50}$  (MPa) is the xylem pressure inducing 50% loss of conductance and  $S$  (% MPa<sup>-1</sup>) is the slope of the vulnerability curve at the inflexion point. These two parameters were averaged for each species.

### Drought treatment

Three individuals of each species were exposed to three different water-stress treatments and a single control plant was used for each species in each treatment. Leaf water potential ( $\Psi_l$ ) was used to monitor the level of plant water deficit during treatments. Three target levels of water stress (-5 MPa, -8 MPa and -10 MPa) were imposed with the intention of exposing plants to sublethal and lethal water deficits. Water stress was created by withholding water, but rapid soil drying was prevented by exposing plants to a mild (-4 MPa) predrought after which plants were rewatered for 2 d before being allowed to dry down to the target  $\Psi_l$ . Regular sampling of  $\Psi_l$  was carried out on two small branchlets per plant which were removed and balance pressure carefully determined in a Scholander pressure chamber (Soilmoisture Corp. Santa Barbara, CA, USA) with a dissecting microscope fitted. To ensure that plants from the most extreme treatment did not experience a greater period of time with closed stomata than other treatments, all plants

were controlled such that plants experienced 4 wk with stomata > 90% closed. This required plants to be maintained at target  $\Psi_l$  for longer periods in the less severe treatments. Hence, water loss and  $\Psi_l$  were monitored during plant drying and pot-mass targets for individual plants were determined at each treatment. Once the target mass and  $\Psi_l$  were reached, daily addition of small quantities of water ensured maintenance of target  $\Psi_l$ . Following the completion of 4 wk of drought at the different stress levels, pots were soaked in water for an hour and daily watering was resumed.

### Gas exchange, hydraulic conductance and growth

Water loss was measured gravimetrically from whole plants, with the pots bagged and covered in foil to prevent water loss from the soil. Pot weights were determined at 11:00 h and again at 12:00 h and the difference attributed to transpiration. Transpiration rate should have been closely proportional to stomatal conductance because humidity and temperature were controlled in the glasshouse cells. Whole-plant hydraulic conductance was calculated in plants recovering from drought using the following equation:

$$K_{\text{plant}} = E / \Delta\Psi \quad \text{Eqn 3}$$

( $E$ , transpiration rate (mmol s<sup>-1</sup>);  $\Delta\Psi$ , water potential gradient from soil to leaf). Soils were watered to capacity during recovery from drought, and hence  $\Delta\Psi$  was equivalent to  $-\Psi_l$ . Both  $K_{\text{plant}}$  and  $E$  were expressed on a whole-plant basis, thereby averaging the hydraulic impact of drought over the whole crown (not normalized to leaf area). Replicate plants for each species were selected with very similar size and mass thereby ensuring minimal variation in the initial rates of  $E$  and  $K_{\text{plant}}$  before drought.

Basal diameter growth was measured for 12 months before the drought treatment, during drought and for several months after rewatering using a laser micrometer (Metralight, San Mateo, CA, USA) mounted onto a jig designed to hold plants in the same position for scanning. Pots were marked with two points 90° apart which were used to align the main stem for two repeatable perpendicular diameter measurements. The basal area of each stem was determined from these the two diameter measurements. All plants were measured every 2 wk during the hour predawn to avoid diurnal fluctuations in diameter. More frequent measurements were made immediately after rewatering droughted plants in order to differentiate swelling caused by rehydration from growth. Diameter measurements included the bark, but the bark thickness was < 5% of the basal diameter and hence it was assumed that the overwhelming majority of diameter growth resulted from the xylem. Growth and gas exchange parameters were also monitored

in a control individual for each species throughout the length of the experiment. Occasional measurements of leaf level  $\text{CO}_2$  and water vapour exchange were made before, during and after drought using a portable open gas exchange system (Li-6400; Li-Cor Inc., Lincoln, NE, USA).

Between 2 and 3 months after drought, all ( $n = 3$ ) *C. rhomboidea* and *C. columellaris* plants from the  $-10$  MPa treatment as well as controls for these species were harvested to determine the degree of embolism recovery that had occurred in the months following rewatering. Plants that were transpiring maximally at 11 : 00 h were cut at soil level, then immediately recut under water, shaved with a razor and transferred (underwater) to a filtered solution of the fluorescent tracer sulphorhodamine G. Plants were left to transpire in the sun for 15–30 min before they were recut *c.* 50 mm above the original cut, at the same height diameter measurements were made. Stem sections were then photographed under visible and UV light to quantify the amount of xylem tissue that was actively transporting water.

#### Lethal water potential ( $\Psi_d$ )

Calculation of the lethal level of water stress for each species was undertaken using the methodology of Brodribb & Cochard (2009) whereby the dynamics of gas exchange recovery after rewatering was used to determine the severity of the water stress. Plotting the inverse of the time taken for transpiration rate to return to 50% of predrought levels ( $t_{1/2}$ ) against minimum  $\Psi_1$  during the drought period yields a linear relationship, the  $x$ -intercept of which defines the lethal water stress (Brodribb & Cochard, 2009). Transpiration recovery data from all individuals in all treatments were pooled together and linear regressions fitted through data from each species to find  $\Psi_d$ .

#### Hydraulic recovery by stem growth

*C. rhomboidea* was the only species predicted to suffer a significant loss of xylem function in the stems after the  $-10$  MPa drought treatment, hence we examined the dynamics of hydraulic recovery in this species to determine whether xylem regrowth explained the observed rate of hydraulic recovery. The predicted rate of xylem repair by basal growth was calculated from the mean basal growth rate recorded in each individual after plants had rehydrated to above  $-2$  MPa. Stem basal area was assumed to be proportional to plant hydraulic conductance and the initial percentage loss of stem hydraulic conductance was taken as the stable initial value after plants had rehydrated above  $-2$  MPa and stomata had reopened. Hydraulic recovery was linear and the mean recovery rate in % per day was compared with the basal area growth rate in % per day for each of the three replicate plants.

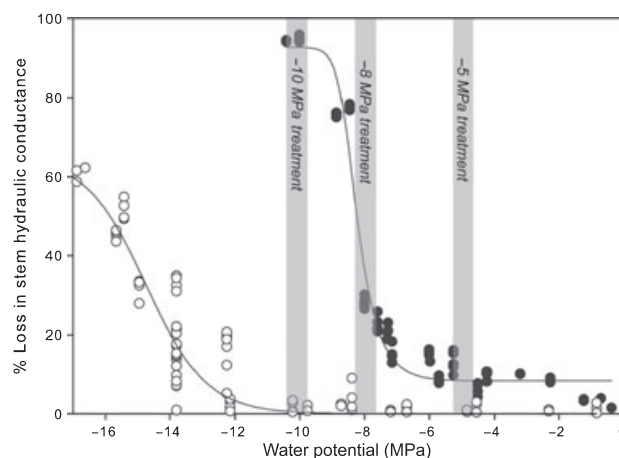
## Results

### Xylem vulnerability

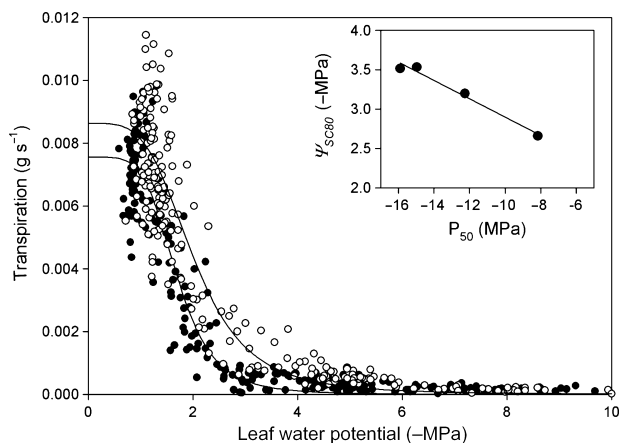
The four *Callitris* species all possessed extremely cavitation resistant stem xylem. Water potentials required to induce a 50% loss of hydraulic conductivity ( $P_{50}$ ) in the main axis ranged from  $-8.2$  MPa in *C. rhomboidea* to  $-15.9$  MPa in *C. columellaris*. As a result, the most severe drought treatment at  $-10$  MPa was predicted to induce markedly different degrees of embolism in the different species, with *C. rhomboidea* predicted to suffer  $> 80\%$  loss of conductivity while *C. preisii* and *C. columellaris* were predicted to lose  $< 10\%$  of hydraulic function (Fig. 1; see the Supporting Information, Fig. S1).

### Drought and gas exchange dynamics

Transpiration rates in all species were highly sensitive to drought, with a general pattern of incipient stomatal closure after  $\Psi_1$  fell below  $-1.5$  MPa (Fig. 2). Despite similar initial stomatal sensitivity to  $\Psi_1$ , more cavitation-resistant species closed stomata more slowly as  $\Psi_1$  continued to fall, leading to substantial differences in  $\Psi_1$  at 80% stomatal closure. A highly significant linear correlation was observed between  $\Psi_1$  at 80% stomatal closure and  $P_{50}$  (Fig. 2) but there remained a very large margin between stomatal closure and  $P_{50}$  in all species. Once stomata were closed  $\Psi_1$  tended to decline very slowly, such that the minimum target  $\Psi_1$  of  $-10$  MPa was achieved 3–4 wk after water was withheld. The reapplication of water after drought led to gas exchange recovery in all plants, but the rates of recovery were variable



**Fig. 1** Stem xylem vulnerability for the most vulnerable (*Callitris rhomboidea*, closed circles) and least vulnerable (*Callitris columellaris*, open circles) study species. Fitted sigmoid curves were used to determine the water potential required to produce a 50% loss of stem hydraulic conductivity ( $P_{50}$ ). The range of leaf water potentials observed at the three drought treatments are shown as grey bars.

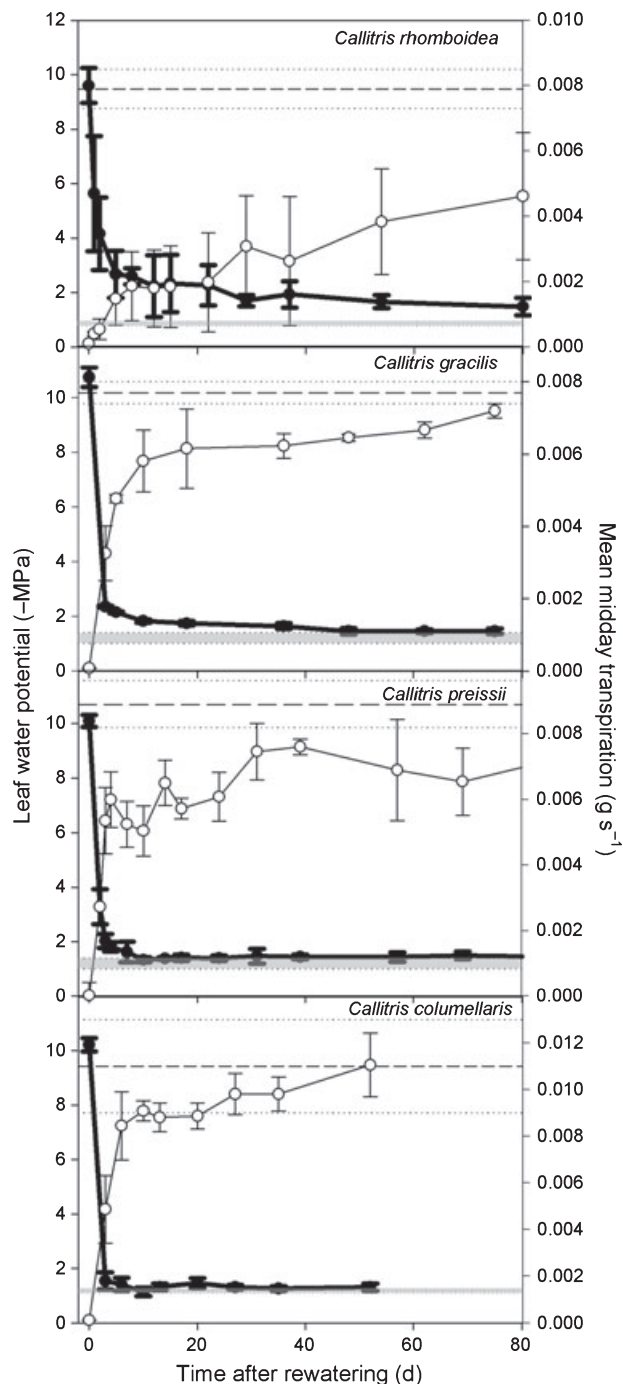


**Fig. 2** Pooled data showing the decrease in whole-plant transpiration as mean leaf water potential ( $\Psi_l$ ) declined from  $-1$  MPa to  $-10$  MPa over a period of 3–4 wk in *Callitris rhomboidea* (closed circles) and *Callitris columellaris* (open circles). Sigmoid curves provided highly significant ( $P < 0.001$ ) fits to the data, and were the most appropriate form because transpiration rates did not continue to increase when plants were hydrated above  $-1$  MPa. Differences in the dynamics of stomatal closure between species were noticeable at  $\Psi_l$  below  $-2$  MPa, and species were characterized by the  $\Psi_l$  at which transpiration was reduced by 80% of maximum ( $\Psi_{SC80}$ ). A significant linear regression ( $r^2 = 0.98$ ;  $P < 0.01$ ) described the relationship between of cavitation resistance ( $P_{50}$ ) and  $\Psi_{SC80}$  in the four *Callitris* species (inset).

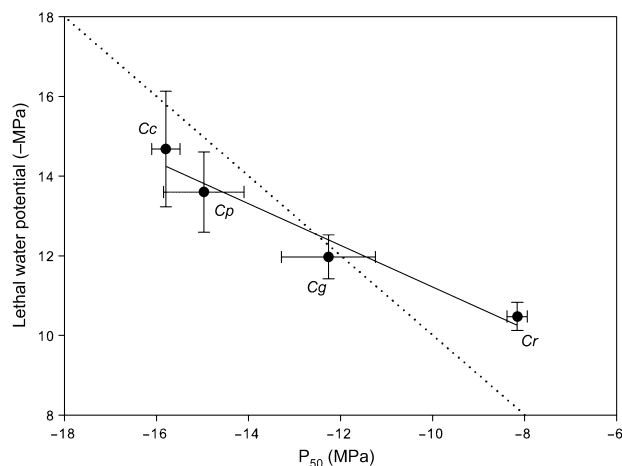
depending upon the species and the magnitude of the stress. In all individuals recovering from drought, transpiration rates remained below predrought levels until such time as  $\Psi_l$  returned to control levels (Fig. 3). Analysis of transpiration recovery data to find the lethal  $\Psi_d$  revealed a range from  $-10.5$  MPa in *C. rhomboidea* to  $-14.7$  MPa in *C. columellaris*. Across species there was close to a 1 : 1 correlation between  $\Psi_d$  and stem  $P_{50}$  (Fig. 4). No individual of any species died after recovery from a drought stress of  $-10$  MPa, but recovery of gas exchange and  $\Psi_l$  in plants of *C. rhomboidea* was extremely slow after this extreme treatment (Fig. 3). *Callitris rhomboidea* was also the only species where  $P_{50}$  and  $\Psi_d$  were close to the highest stress treatment ( $-10$  MPa).

### Growth rates

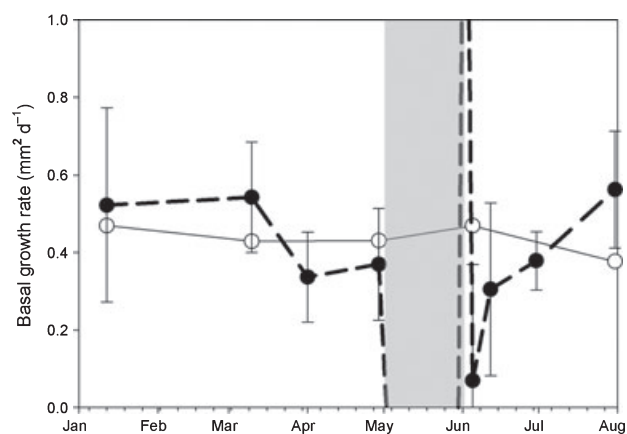
Mean basal area growth rates of control plants from each treatment ranged from a maximum of  $0.60 \pm 0.03$  mm<sup>2</sup> d<sup>-1</sup> in *C. rhomboidea* to a minimum of  $0.22 \pm 0.06$  mm<sup>2</sup> d<sup>-1</sup> in *C. preissii*. Overall, a slight decrease in growth rates of control plants was recorded in all species over the 8 months in the glasshouse cells. Withholding water caused stems to shrink by up to 16% as  $\Psi_l$  declined (*C. rhomboidea* at  $-10$  MPa), and this shrinkage was reversed within 3–5 d after rewatering as  $\Psi_l$  relaxed (Fig. 5). After rehydration, basal area growth recovered to control levels in the period 2–4 wk after rewatering in *C. columellaris* and *C. preissii* regardless of the intensity



**Fig. 3** Mean leaf water potential ( $\Psi_l$ ; closed circles and bold lines) and transpiration rates (open circles) in plants droughted to  $-10$  MPa for 3 wk and then rewatered on day zero. Plants are ranked in order of recovery speed. Slowest to recover was *Callitris rhomboidea*, where  $\Psi_l$  had not returned to control levels (grey line) after 80 d. As a result this species exhibited a protracted hydraulically limited recovery of gas exchange that had not reached predrought levels (dashed lines  $\pm$  SD) even 80 d post-rewatering. The most resilient species, *Callitris columellaris*, responded very quickly to rewatering, with  $\Psi_l$  returning to control levels within 3–5 d and a similarly rapid recovery of transpiration to within 80% of predrought levels over the same period.



**Fig. 4** Lethal water potential, as determined by gas exchange recovery kinetics and stem resistance to cavitation were strongly positively correlated ( $r^2 = 0.95$ ;  $P < 0.05$ ) across the four *Callitris* species (*Cc*, *Callitris columellaris*; *Cg*, *Callitris gracilis*; *Cp*, *Callitris preissii*; *Cr*, *Callitris rhomboidea*).

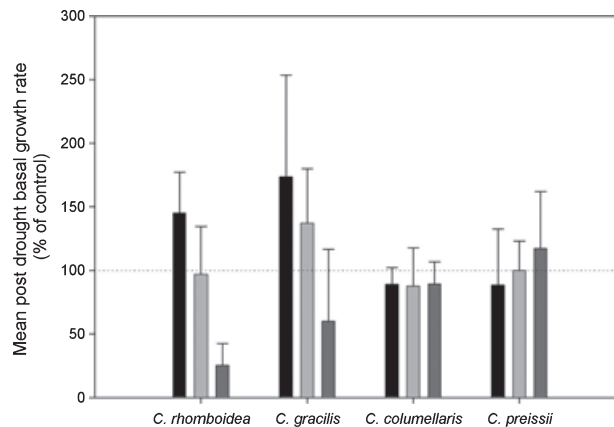


**Fig. 5** Mean basal area growth rate ( $n = 3$ ) of droughted (closed circles) and control (open circles) *Callitris columellaris* plants over 8 months. Water was withheld from droughted plants in May and plants maintained at  $-10$  MPa until early June (tinted box) causing stem shrinkage (negative growth). In this species, rewatering after the  $-10$  MPa treatment caused stems to swell for several days, after which normal growth resumed within 2–3 wk.

of drought (Fig. 6). Reductions in growth rate occurred in individuals of *C. gracilis* and *C. rhomboidea* exposed to the most severe treatment, with *C. rhomboidea* exhibiting the most significant reduction to 25% of control growth rate 1 month after rewatering.

### Hydraulic repair

In agreement with predictions of the vulnerability curves for *Callitris*, only *C. rhomboidea* plants were found suffer a sustained loss of whole-plant hydraulic conductance ( $K_{\text{plant}}$ ) after rewatering. These losses ranged from 82% to 95% of



**Fig. 6** Mean basal area growth rates ( $\pm$  SD;  $n = 3$ ) relative to undroughted controls 1 month after rewatering from the three drought treatments ( $-5$  MPa (closed bars),  $-8$  MPa (light tinted bars) and  $-10$  MPa (dark tinted bars)). Only *Callitris rhomboidea* and *Callitris gracilis* suffered declines in mean post-drought growth rate below control levels. These species also exhibited a boost in growth after mildest drought treatment.

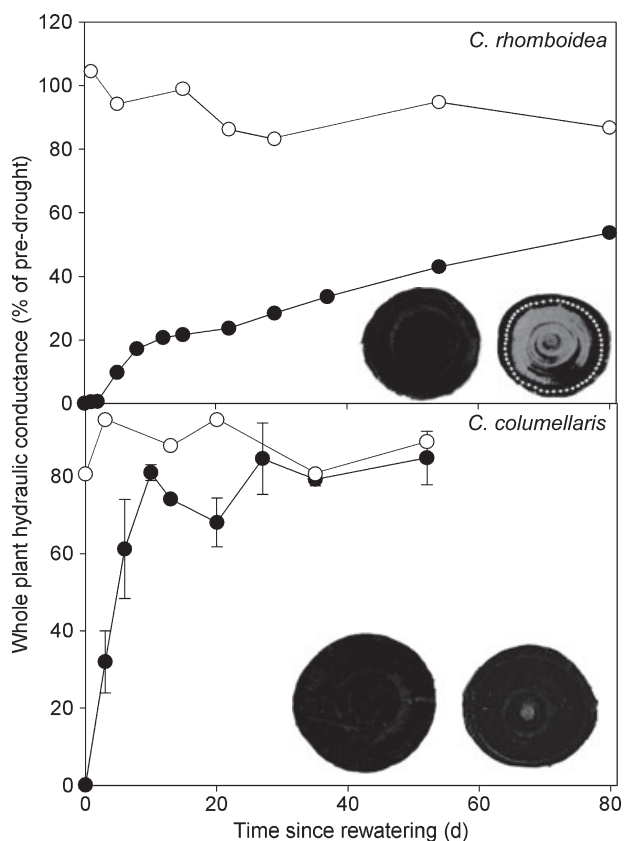
predrought  $K_{\text{plant}}$  and the repair of this damage was a slow linear process that took  $> 2$  months to complete (Fig. 7). Comparing the measured rate of hydraulic recovery with the expected rate based on post-drought basal area growth rate in *C. rhomboidea* showed that the rate of xylem replacement by new basal area growth was correlated with, but slightly slower than the rate of measured hydraulic recovery in each of the three individuals from the  $-10$  MPa treatment (Fig. 8).

### Discussion

Stems of *Callitris* species were found to be extraordinarily resistant to hydraulic dysfunction under water stress, ranking this alongside the most cavitation-resistant genera ever measured (Willson *et al.*, 2008). Extreme cavitation resistance in *Callitris* was found to be associated with equally outstanding resistance of gas exchange and growth to severe water deficit. Three of the four species measured recovered gas exchange and growth within days of rewatering even after the extreme drought treatment of exposing plants to  $\Psi_1$  close to  $-10$  MPa for several weeks. However, when significant embolism was induced *in planta* we found that the dynamics of gas exchange recovery matched the rate of hydraulic repair owing to xylem regrowth.

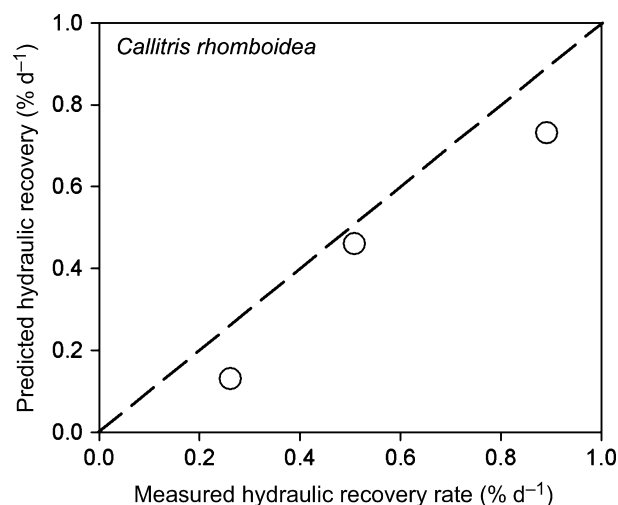
### Recovery from extreme water deficit without embolism

After exposure to extreme water deficits in the range  $-10$  to  $-11$  MPa no evidence of damage to gas exchange or basal area growth rate was observed in *C. columellaris* or *C. preissii*. According to centrifuge measurements, neither of



**Fig. 7** Recovery of plant hydraulic conductance after plants were rewatered from the most extreme  $-10$  MPa drought treatment (closed circles) compared with undroughted controls (open circles). Data are shown for a single plant of the most vulnerable species (*Callitris rhomboidea*) and mean data for the least vulnerable (*Callitris columellaris*) species. Slow recovery in *C. rhomboidea* was associated with substantial embolism in the stem, as shown by a large proportion of inactive wood (white areas) in stem cross-sections where tracheids failed to take up dye (control, left; treatment, right). New wood can be seen in the cross-section of the droughted *C. rhomboidea* stem as active xylem that had grown during the recovery phase (dotted ring shows the position of the growth compression ring produced by the drought treatment). Both control (left) and treatment (right) stems of *C. columellaris* showed almost 100% dye uptake indicating full functionality of the xylem before and after drought.

these two species was susceptible to embolism at the  $-10$  MPa drought treatment, and the prompt restoration of predrought transpiration over a period of 1–5 d upon rewatering confirmed that hydraulic damage was minimal in these species (Fig. 3). Furthermore, close to 100% of xylem was stained when stem segments were infused with dye after droughting (T.J. Brodrribb, unpublished), further supporting the maintenance of hydraulic integrity during drought. The short period of depressed gas exchange immediately following rewatering was associated with the slow rehydration of the plant back to control levels of water deficit. According to the relationship between  $\Psi_1$  and stomatal conductance (Fig. 2), *Callitris* stomata were unable to open fully until  $\Psi_1$



**Fig. 8** Measured recovery rates of hydraulic conductance in three *Callitris rhomboidea* individuals rehydrated from  $-10$  MPa, compared with the predicted rates of hydraulic recovery calculated from the mean growth rate in stem basal xylem area measured in the same plants over the same period. Measured recovery was correlated with predicted rate but was consistently faster.

climbed above  $-1.5$  MPa, and hence even in plants unaffected by embolism, a phase of rehydration following rewatering prevents the immediate recovery of gas exchange. That rehydration should be delayed for a period of several days after rewatering suggests that a reversible loss of hydraulic conductance occurs in a part of the plant that is more vulnerable to dysfunction than the stem. Leaves are an obvious candidate and have already been shown to be considerably more vulnerable than the stem in *C. rhomboidea* (Brodrribb & Cochard, 2009). Furthermore there is good evidence to suggest that losses of hydraulic conductivity in leaves of conifers are caused by reversible processes such as cell collapse (Cochard *et al.*, 2004) or turgor loss (Brodrribb & Holbrook, 2006; Kim & Steudle, 2007).

Rapid recovery of water potential, gas exchange and growth after exposure to  $-10$  MPa in two of our four *Callitris* species not only indicates an extreme level of xylem resistance to cavitation, but also an extraordinary desiccation tolerance of other plant systems including photosynthetic and cambial tissues. By comparison, the highly drought-tolerant Californian chaparral boasts the most negative water potentials yet measured in native vegetation, but even the most resilient of the chaparral species were found to suffer dieback at water potentials between  $-10$  and  $-11$  MPa (Davis *et al.*, 2002). At the dry limits of *C. columellaris* and *C. preissii* distributions, rainfall is similar to that of the dry Californian chaparral scrub, but it appears that the relatively the long-lived evergreen tree habit of *Callitris* demands even greater drought resistance than the shorter-lived more opportunistic flora of the chaparral. The correlation between  $P_{50}$  and lethal water potential demonstrated here for *Callitris* species, illustrates that stem

cavitation resistance remains a significant selective factor even at the extreme end of the vulnerability spectrum. Given that soil water is almost completely exhausted at water potentials below  $-8$  MPa, survival time after this point will depend on plant capacitance, cuticular conductance and cavitation resistance.

### Recovery after drought-induced cavitation

The only *Callitris* species predicted to experience major stem xylem cavitation during the  $-10$  MPa drought treatment was *C. rhomboidea*, and the recovery of gas exchange in this species was markedly slower than that observed in the other *Callitris* species (or in *C. rhomboidea* plants droughted to less severe water stresses). Although the initial rapid rehydration of *C. rhomboidea* plants rewatered from  $-10$  MPa was similar to other species, a second phase of very slow rehydration from  $-2$  MPa back to control  $\Psi_1$  over a period of months was consistently observed (Figs 3, 7). Similar dynamics have been observed in a number of other conifer and angiosperm species recovering from severe drought (Blackman *et al.*, 2009; Brodribb & Cochard, 2009; Resco *et al.*, 2009) with slow hydraulic damage and repair commonly cited as the cause. Data here concur with this hydraulic explanation, indicating an initial rapid rehydration phase occurs with closed stomata followed by a second phase where plants with significantly reduced hydraulic conductances gradually reopen stomata. In agreement with previous studies of conifer species there was no evidence of nonhydraulic reduction of gas exchange as might be expected as a result of ABA accumulation (Brodribb & Cochard, 2009). It should be noted that these hydraulic and gas exchange dynamics are not attributable to significant losses in foliar area, which remained below 10% for the plants in question (T.J. Brodribb, pers. obs.).

The dynamics of hydraulic repair during the slow phase of gas exchange recovery revealed that a linear process of repair was capable of restoring between 15% and 20% of lost hydraulic function per month in *C. rhomboidea*. Such a slow process was considered unlikely to represent xylem refilling, which in angiosperms typically refills embolized conduits within hours or days (Bucci *et al.*, 2003; Salleo *et al.*, 2004). Instead of xylem repair in these conifers we found that xylem replacement was the most likely mode of hydraulic recovery. Measured rates of basal area growth in *C. rhomboidea* after drought were capable of accounting for most of the observed recovery of hydraulic conductance and gas exchange, as demonstrated by a model of hydraulic recovery predicted by area growth (Fig. 7). This is an important result because controversy remains about whether tracheids are able to be refilled after cavitation (Sobrado *et al.*, 1992; Edwards *et al.*, 1994). Our results suggest that tracheid embolism in *Callitris* is nonreversible after drought, probably because of the irreversible aspiration

of the torus–margo pit structure (Utsumi *et al.*, 2003). This conclusion was supported by dye infusions of stems of *C. rhomboidea* rewatered after drought (Fig. 7). Two months after recovery from  $-10$  MPa, all three individuals of *C. rhomboidea* showed clear evidence of major embolism of the stems as well as some peripheral xylem regrowth (Fig. 7). Although the stem represents only a small part of the whole-plant resistance, the growth in basal area is probably representative of cambial growth throughout the plant from root to shoot. Therefore, the basal area increment would represent the change in resistance of the entire woody portion of the whole plant hydraulic system.

Our data indicate that stem embolism and stem growth are fundamental determinants of drought damage and recovery in *Callitris*. This conclusion probably also holds for a diversity of conifers and angiosperms that have demonstrated similar gas exchange recovery dynamics (Blackman *et al.*, 2009; Brodribb & Cochard, 2009). Stem vulnerability may be more informative about survival limits of conifers than root and leaf hydraulics because although both these organs tend to be more vulnerable than the stems, the hydraulic conductance of roots and leaves responds dynamically to multiple factors in addition to  $\Psi_1$ . By contrast, the nonreversibility of stem embolism appears to constitute a predictable physical limit to the water transport capability of conifers under stress. Furthermore, if individuals are reliant on xylem reiteration to recover from major water stress, then the rate of stem diameter growth and the impact of drought on vascular cambial activity are of major significance for plant (and ecosystem) productivity. This is apparent in our data where neither *C. columellaris* nor *C. preissii* showed any decline in growth rate after being droughted to  $-10$  MPa, but *C. rhomboidea* basal area growth rate declined to 25% of maximum after recovery from  $-10$  MPa. The interdependence of hydraulic conductivity, gas exchange and growth probably mean that above a threshold, apparently between 50% and 90% embolism, the concomitant reduction in hydraulic conductance, gas exchange and growth will result in either very slow recovery from drought or death. Whether this death results directly from embolism in the stem or correlated losses of hydraulic conductance in the more vulnerable leaves (Brodribb & Cochard, 2009) requires further study. Either way, the use of recovery dynamics of gas exchange ( $t_{1/2}$ ) as an indicator of proximity to death (Brodribb & Cochard, 2009) is likely to provide an accurate index of lethal  $\Psi_1$ .

### Plant scale

It is possible that the mode of damage during drought may be different at different plant stages because seedlings and small saplings with shallow root systems are much more likely to experience acute water stress. At the early sapling



stage examined here, there is a strong likelihood that plants in the field will encounter close to lethal water stress in the field during the first years of growth, and hence the significance of cavitation and hydraulic recovery as a limiter of growth and survival is paramount. As plants increase in size, however, there is increasingly the risk of chronic drought stress, resulting from extended periods of moderate water stress, causing unfavourable carbon balance (McDowell *et al.*, 2008). Understanding the prevalence of acute and chronic drought stress in natural environments will be of major importance for predicting productivity and population dynamics of plant communities exposed to drought stress. In this respect *Callitris* in arid Australia presents an ideal model system.

## Conclusion

*Callitris* trees constitute some of the most resilient trees in Australia, the driest vegetated continent. This resilience is clearly reflected in the extraordinary ability of *Callitris* stems to resist cavitation under water stress. Our data demonstrate the critical influence of hydraulics in dictating not only the point of lethal water stress, but also the dynamics of recovery from extreme drought. Great potential lies in the use of water transport physiology to predict growth and mortality in a continent such as Australia where the climate is characterized by low and unpredictable rainfall.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Vulnerability curves for three replicate stems of *Callitris gracilis* and *Callitris preissii*.

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