

1 **RUNNING TITLE : Hydraulics define death or recovery after**
2 **drought**

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5 **death in water stressed conifers.**

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1 **Hydraulic failure defines the recovery and point of death in** 2 **water stressed conifers.**

3 Tim J Brodribb and Hervé Cochard

4

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

21

22

23 Photosynthesis occurs in an aqueous environment and until evolution comes across a
24 solid-state means of fixing atmospheric CO_2 , terrestrial plant species, even those in humid
25 tropical rainforests (Engelbrecht et al., 2007), will be exposed to potentially lethal
26 desiccation. The reason for this is that in most environments competition between plants
27 forces them to engage in a dangerous balancing act between trading water for carbon at
28 the leaf while minimizing costs associated with replacing this transpired water with water
29 pulled from the soil. The job of seeking and transporting water falls upon the roots and
30 vascular system, and reduced investment in these systems comes at a cost in terms of the
31 safety and efficiency of water carriage. These conflicting demands mould the form and
32 function of vascular plants and have yielded a diverse spectrum of vascular anatomies,
33 each tuned to a specific flow capacity and drought tolerance.

1 Desiccation tolerance is at the centre of the vascular cost/benefit equation. The
2 reason for this is that a more desiccation tolerant vascular system (one that resists
3 embolism better during soil drying) is distinctly more costly to build than a sensitive
4 system (Hacke et al., 2001), yet the repercussions of vascular failure are likely to be fatal.
5 This trade-off, as with many other systems in biology, leads to functional diversity and
6 hence there is a great range in the ability of plant vascular systems to operate under the
7 variable hydraulic tensions intrinsic to pulling water from the soil to the leaf. Hydraulic
8 tension in the xylem increases as soil dries, increasing the risk of xylem dysfunction by
9 the cavitation (Tyree and Sperry, 1989) or collapse (Cochard et al., 2004) of conduits,
10 and when quantified in terms of the tension required to disable 50% of the stem xylem
11 published values range from less than 1MPa (Yangyang et al., 2007) to maxima of
12 around 15MPa (Brodribb and Hill, 1999). It is an attractive proposition to suggest that
13 xylem vulnerability to dysfunction (as expressed by Ψ_{50}) is the key trait responsible for
14 setting the drought tolerance of any species, yet the evidence for this remains
15 observational (Kolb and Davis, 1994; Brodribb and Hill, 1999; Comstock, 2000;
16 Pockman and Sperry, 2000; Tyree et al., 2003; Maherali et al., 2004; Breda et al., 2006).
17 At the same time others cite traits such as photosynthetic physiology (Hanson, 1982) and
18 senescence (Rivero et al., 2007) or combined physio-pathological processes (McDowell
19 et al., 2008) as more important limiters of plant function during drought.

20 Major progress has been made recently in our understanding of the fundamental
21 role that plant hydraulics play in governing the rate of water extraction from the soil
22 (Sperry, 2000), yet this understanding breaks down as plants approach and exceed the
23 limitations of their water transport system. Very little information is available to explain
24 the performance of plants during and after major drought events, and how these episodes
25 impact on plant survival and distribution. Theory suggests that xylem cavitation should
26 set a clear limit to the desiccation tolerance of plants such that water potentials capable of
27 reducing xylem hydraulic conductivity to approach zero should be lethal, or at least result
28 in 100% defoliation. Surprisingly there are no studies that have quantitatively linked the
29 relationship between the resistance of the xylem tissue to hydraulic tension and the
30 absolute desiccation tolerance of plants (Tyree et al., 2002). This gap in our
31 understanding of how plants respond to drought and where the limits of desiccation

1 tolerance lie for any particular species poses an enormous problem to those attempting to
2 model the impacts of changing rainfall or evaporative load on both wild and agricultural
3 plants. In this paper we examine the relationship between xylem functional limits and the
4 drought survival and recovery of plants

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

14

15 **Results**

16

17 *Drought and stomatal closure*

18

19 The diurnal course of transpiration in all plants rose from minimum values overnight to a
20 plateau which was maintained over the period 1000h to 1600h. The magnitude of this
21 transpirational plateau decreased over time as soil water content declined during drought
22 (Fig. 1). The decline in midday transpiration (E_{md}) after withholding water continued
23 until both midday and midnight transpirational fluxes were similar, signifying complete
24 stomatal closure. In all species, the response of E_{md} to decreasing midday leaf water
25 potential (Ψ_l) followed a sigmoidal trajectory, with stomata highly sensitive to a very
26 small range in Ψ_l (Fig. 2). The most sensitive stomatal response was in *Lagarostrobos*
27 *franklinii* where stomatal conductance (as inferred from E_{md}) fell from 80% of maximum
28 to 20% of maximum over the Ψ_l range -1.20MPa to -1.81MPa. *Callitris rhomboidea*
29 showed the lowest sensitivity to Ψ_l with 1.25MPa separating 20 and 80% closure. The
30 absolute sensitivity of stomata to Ψ_l was similar in all species with 50% stomatal closure
31 occurring at a mean of -1.20 ± 0.02 MPa in three of the four species, and at -1.48MPa in

1 *Callitris rhomboidea*. Following stomatal closure the mean rate of plant dehydration was
 2 similar in all plants (0.29MPa per day \pm 0.05) except in *Callitris rhomboidea* which
 3 showed a slightly higher rate of drying (0.44 MPa per day).

4
 5 *Stem and leaf vulnerability to drought*

6
 7 During desiccation a marked decline in hydraulic conductivity was observed in excised
 8 samples of both stems and leaves as hydraulic tension in the xylem increased. The degree
 9 of xylem dysfunction was related to water potential by a sigmoidal function in both stems
 10 and leaves of all species (Fig 3). Despite the relatively conservative shape of these
 11 relationships there was a huge range in xylem tolerance to water potential across the
 12 species sample. *Callitris rhomboidea* yielded the most resistant stems and leaves with
 13 50% loss of function recorded at -10.8MPa and -6.60MPa respectively; this compared
 14 with only -2.78MPa and -2.54MPa for the stems and leaves of *Dacrycarpus dacrydioides*.
 15 Leaves were always more sensitive to water stress induced dysfunction than stems, but
 16 there was a constant relationship between the two such that water potential at 50% loss of
 17 stem function ($\Psi_{\text{stem}50}$) was proportional to (and almost equal to) the water potential at
 18 95% loss of K_{leaf} ($\Psi_{\text{leaf}95}$) *i.e.* ($\Psi_{\text{stem}50} = 1.08 \Psi_{\text{leaf}95}$; $r^2 = 0.88$).

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

22
 23 *Recovery from drought*

24
 25 Plants were droughted to a variety of water potentials ranging from just past the point of
 26 80% stomatal closure, to the most severe stress approximately equal to $\Psi_{\text{leaf}95}$. Upon
 27 rewatering a universal pattern was observed whereby Ψ_{leaf} returned to a value
 28 corresponding to between 80 and 20% stomatal closure following an exponential
 29 trajectory with a half time of one to two days (Fig. 4). This pattern was repeated in all
 30 plants regardless of the degree of water stress. The final recovery of Ψ_{leaf} back to pre-
 31 stress hydration was approximately linear with a slope that was related to the level of

1 stress imposed (Fig. 4 and 5). This last phase of post drought recovery appeared to dictate
 2 the pattern of gas exchange recovery.

3 The recovery of gas exchange (as reflected by E_{md}) was strongly influenced by the
 4 relatively slow recovery of hydraulic conductivity following rewatering (Fig. 5). This
 5 slow recovery of E was most pronounced in plants droughted to water potentials below
 6 50% loss of K_{leaf} (Figs. 4 and 5). The inhibition of stomatal re-opening in plants
 7 recovering from these significant stresses conformed very well to a hydraulic-stomatal
 8 limitation model whereby the rate of gas exchange was a unique function of Ψ_{leaf} (Fig. 2)
 9 which was ultimately limited by whole plant hydraulic conductivity (Fig. 5). This means
 10 that the stomata responded the same to Ψ_{leaf} depression produced by hydraulic
 11 dysfunction in wet soil as they did to Ψ_{leaf} depression produced by soil drying. A
 12 synthesis of all recovery data from all plants showed very good correspondence between
 13 the observed recovery of E_{md} and the recovery of E_{md} predicted from entering measured
 14 values of Ψ_{leaf} during plant recovery into the equation $E = f(\Psi_{leaf})$ where the function $f(x)$
 15 for each species was taken from the regression equations shown in Figure 2. Regressions
 16 of % E_{md} observed vs. % E_{md} predicted yielded linear functions that were not significantly
 17 different to the same regressions fitted through data used to define $f(x)$ i.e. the data
 18 collected during the initial drought phase prior to rewatering (Fig. 6). Pooling all recovery
 19 data for all species yielded a very strong 1:1 linear regression ($r^2 = 0.70$) between % E_{md}
 20 observed and % E_{md} predicted by the hydraulic-stomatal limitation model. Only
 21 *Lagarostrobos franklinii* showed a significant deviation from the hydraulic model
 22 whereby observed E_{md} was on average 22% lower than predicted by the model (Fig 6).
 23 Importantly the relationship between observed and predicted % E_{md} was still linear in this
 24 species indicating that hydraulic limitation remained the primary limiter of gas exchange.

25 Recovery of gas exchange after rewatering was highly sensitive to minimum Ψ_{leaf}
 26 during drought. Recovery times ranged from a minimum of one day to maximum periods
 27 of over 100days (were new leaf growth was required to replace leaves damaged during
 28 drought). In order to compress the range of the recovery data we expressed the recovery of
 29 E_{md} in terms of $t_{1/2}^{-1}$, that is 1/[the time (days) required for E_{md} to return to 50% of the
 30 predrought maximum]. The advantage of this index is that $t_{1/2}^{-1}$ ranges from one,
 31 representing an overnight recovery, to zero indicating plant death. In all species $t_{1/2}^{-1}$

1 exhibited two phases, an insensitive phase followed by a linear decline to values close to
2 and occasionally reaching 0 (plant death) (Fig. 7). Fitting linear regressions to this second
3 phase of declining $t_{1/2}^{-1}$ yielded two key parameters, firstly the point at which this
4 regression = 1 was taken as the minimum Ψ_{leaf} that plants could recover gas exchange
5 overnight when rewatered. This intercept corresponded closely with the Ψ_{leaf} at 50% loss
6 of K_{leaf} ($r^2 = 0.96$). The second value derived from these regressions was the x-intercept
7 which yielded the minimum survivable water potential for each species (Ψ_{min}), and this
8 value ranged enormously from -11.4MPa in the most desiccation tolerant species
9 *Callitris rhomboidea*, to -2.40MPa in *Dacrycarpus dacrydioides*. In all species Ψ_{min} was
10 equal to the water potential at 95% loss of K_{leaf} ($r^2 = 0.88$) and 50% loss of K_{stem} ($r^2 = 0.98$;
11 Fig. 7b). The difference in Ψ_{leaf} between 100% defoliation and plant death was small in
12 each species. Only plants of *D. dacrydioides* were capable of recovering from 100%
13 defoliation, but even in this species there was a very narrow margin between Ψ_{leaf} at
14 100% leaf loss (-2.4MPa) and plant death (-2.7MPa).

15

16

1 DISCUSSION

2

3 Hydraulic function in the four conifer species examined here was found to underpin the
 4 recovery from and survival of water stress. This important result provides a functional
 5 framework for understanding how plants respond to the highly variable water stresses
 6 imposed upon the majority of plants growing in the field. Furthermore these data provide
 7 a quantitative and physiological basis for evaluating the absolute desiccation tolerance of
 8 conifer species. Xylem dysfunction and desiccation response were intimately linked by a
 9 1:1 relationship between Ψ_{\min} and both stem Ψ_{stem50} and the loss of leaf hydraulic
 10 conductivity (Ψ_{leaf95}) (Fig. 7b). Apart from the obvious physiological importance of this
 11 result, the implications for understanding drought survival and the distribution of plants
 12 are significant.

13

14 *Hydraulic limitation of drought recovery*

15

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

28

29 We found strong evidence that hydraulic limitation was the process governing
 30 gas-exchange recovery from drought in our tree sample, and specifically that this
 31 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

1 species is all the more impressive considering the enormous range of desiccation
 2 vulnerabilities represented by our species sample. Previous studies have demonstrated
 3 strong evidence for the limitation of gas exchange in non-droughted plants (Meinzer and
 4 Grantz, 1991) (Hubbard et al., 1999) (Brodribb and Feild, 2000) but here we demonstrate
 5 for the first time that the recovery of plants from water stress conforms to a hydraulic
 6 limitation model without having to invoke other factors such as plant hormones (ABA) or
 7 direct damage to leaves. The results here come from two conifer families (Podocarpaceae
 8 and Cupressaceae) although we have found recently that this type of hydraulic-mediated
 9 control of drought recovery applies equally to a group of angiosperms (Blackman et al in
 10 review). The implication of this is that hydraulic dysfunction and repair probably
 11 mediates the drought recovery of vascular plants in general.

12 Although we found an impressively strong pattern of hydraulic mediated recovery,
 13 the functions used to predict the stomatal response to Ψ_{leaf} are qualitative relationships
 14 that have been somewhat simplified to facilitate prediction. Within-species variation and
 15 osmotic adjustment are both important features which have been “smoothed” by the
 16 single sigmoid function fitted to each species. In some individuals there was evidence
 17 that during drought a degree osmotic adjustment in the leaf took place, pushing the
 18 relationship between Ψ_{leaf} and E_{md} (Fig. 2) to the right, thus enabling stomata to open at
 19 slightly lower water potentials after drought. Osmotic adjustment in response to water
 20 stress has been observed in many plants and during recovery from water stress it would
 21 have the effect of yielding higher than predicted E during the recovery phase (Fig. 8).
 22 Such osmotic adjustment could be easily accommodated in a hydraulic-stomatal
 23 limitation model, and acts in the opposite direction to the predicted effect of non-
 24 hydraulic control of plant recovery (Fig. 8).

25 By demonstrating conservation of the $E(\Psi_{\text{leaf}})$ function both during and post-
 26 drought, the data tend to negate the possibility of an ABA modification of the stomatal
 27 sensitivity to Ψ_{leaf} in these species (*cf.* Wilkinson and Davies, 2002). Under conditions of
 28 ABA induced stomatal closure, Ψ_{leaf} would quickly rise to close to zero after rewatering
 29 due to the low E and hydrated soil, then gradually decline as ABA concentration declined
 30 over time, and stomata reopened (Fig. 8). This type of response was not found to occur in

1 any individual, thus emphasizing the fundamental nature of the hydraulic-mediated
2 stomatal recovery from drought.

3

4

5 *Recovery of K_{plant}*

6

7 All species showed a similar pattern whereby recovery from mild water stress (Ψ_{leaf}
8 between stomatal closure and 50% loss of leaf conductivity) was very different from the
9 behaviour of plants subject to stresses beyond 50% loss of K_{leaf} . Plants rewatered after
10 mild water stress recovered gas exchange very quickly (overnight) despite that fact that in
11 some cases significant depression of K_{leaf} had occurred (Fig. 4, 7a). Two explanations
12 could account for this observation, the first of which is that plants were able to rapidly
13 and fully rehydrate overnight thus refilling embolised conduits in the leaf (Milburn and
14 McLaughlin, 1974). This concept of rapid embolism reversal in conifers is an important
15 and controversial issue given that there is evidence that cavitation leading to aspiration of
16 the torus/margo pit complex is non-reversible (Sperry and Tyree, 1990). The other, most
17 parsimonious explanation for this rapid recovery phase is that the initial loss in leaf
18 hydraulic conductivity may not be associated with xylem cavitation. Good evidence
19 exists to suggest that xylem tissue collapse (Cochard et al., 2004; Brodribb and Holbrook,
20 2005) and loss of leaf turgor (Brodribb and Holbrook, 2006; Kim and Steudle, 2007) may
21 both play a part in the loss of K_{leaf} in a variety of plants. Furthermore, we have observed
22 xylem cell collapse in the leaves of two of the four species in this study (both
23 Cupressaceae species) making cell collapse a strong candidate for the incipient (rapidly
24 reversible) stage of K_{leaf} depression.

25 The timing of gas exchange recovery in plants exposed to water potentials
26 sufficient to induce >50% loss of K_{leaf} was strongly influenced by the magnitude of water
27 stress (Fig. 7a). The shape of this relationship suggests that the rate of repair of K_{plant} in
28 these individuals was nonlinear, decreasing exponentially as Ψ_{leaf} approached lethal
29 values. This slow repair of K_{plant} is likely to represent the refilling of embolised conduits,
30 which could occur under capillary force overnight when Ψ_{leaf} was found to increase to

1 close to zero in rewatered plants (unpublished data). Direct evidence of xylem refilling
 2 came from examining dyed and frozen stems of both *Callitris rhomboidea* and
 3 *Actinostrobus arenarius* which had recovered from water stresses sufficient to kill
 4 approximately 50% of the foliage. After 3 weeks recovery we found that most (>80%) of
 5 the xylem in these stems was functional as opposed to <50% during drought. The
 6 observation that the efficiency of conduit refilling decreased as Ψ_{leaf} approached Ψ_{min} is
 7 significant as this leads to a rapid increase in $t_{1/2}$ and hence a rapid transition from
 8 recoverable to non-recoverable water stress (Fig. 7). Given the heterogeneous pattern of
 9 leaf damage observed after severe drought in these species (T. Brodribb *pers. obs.*) it is
 10 highly probable that severe stress causes an increasing heterogeneity in Ψ_{leaf} of the plant
 11 canopy as some branches approach zero conductivity before others. Clearly as K_{plant}
 12 approaches zero the ability of branches to rehydrate decreases rapidly, and it is probable
 13 that an increasing proportion of branches with catastrophic loss of conductivity contribute
 14 to the observed pattern of rapidly increasing recovery time. Recovery times greater than
 15 50 days appeared to be attributable to new sapwood growth in branches where hydraulic
 16 conductivity had approached zero. The likelihood of canopy Ψ_{leaf} heterogeneity during
 17 severe drought is greatly accentuated in older plants as the variation in age (Brodribb and
 18 Holbrook, 2003), position and history (Hacke et al., 2001) of different leaf cohorts
 19 increases. Hence the ability of large plants in the field to recover from drought might be
 20 expected to decrease more gradually plants approach Ψ_{min} than for the young potted
 21 plants observed here.

22 Although both stem and leaf vulnerability were very significantly correlated with
 23 Ψ_{min} , K_{leaf} vulnerability was most strongly implicated as the causal parameter driving
 24 drought response and recovery kinetics. There are several reasons for this conclusion, the
 25 first of which is that $t_{1/2} = 0$ (Ψ_{min} , or plant death) corresponded closely with the Ψ_{leaf} at
 26 95% loss of K_{leaf} . Such a marked loss of K_{leaf} would impact very significantly upon K_{plant}
 27 because leaves represent a disproportionately large resistance compared with stems (Sack
 28 and Holbrook, 2006). By contrast Ψ_{min} correlated with only a 50% loss of K_{stem} , the
 29 impact of which upon K_{plant} would be relatively small. Other tissues, in particular roots,
 30 have always been considered as candidates for the vulnerability-limiting-tissue due to
 31 what is often found to be their high vulnerability to cavitation (Kolb et al., 1996; Sperry

1 and Ikeda, 1997). The evidence here suggests that tissues upstream of the leaf were either
2 similar to, or more resistant to cavitation than the leaves, emphasizing the ecological
3 importance of leaf vulnerability.

4
5
6 *Ecological implications*

7
8 These data have a number of important ecological implications, the most fundamental of
9 which is that xylem vulnerability, particularly that of the leaf, can be used to place a
10 definitive limit on the physical tolerance of conifer species to desiccation. This idea has
11 been mooted in the past and there have been several attempts to define how vulnerability
12 to cavitation might limit the dry end of plant distributions (Sperry et al., 1998; Brodribb
13 and Hill, 1999), however here for the first time we present a means of identifying
14 precisely when a plant can be expected to die during exposure to extreme drought.
15 Furthermore it should be possible, using the principles of hydraulic-mediated recovery
16 from drought, to model the long term dynamics of gas exchange during drought cycles in
17 the field. Such a model would need to incorporate information about water release
18 characteristics of different soils (Sperry et al., 1998) though rather than using stem
19 vulnerability as the foundational component of the model it would use a combination of
20 leaf vulnerability and stomatal response functions to predict transpiration and
21 assimilation recovery.

22 Important questions remain not the least of which is the fundamental question of
23 why stomata close when they do. Much discussion revolves around the issue of whether
24 xylem vulnerability to cavitation defines how stomata respond to Ψ_{leaf} (Bunce, 2006). In
25 this respect it is interesting to note the rather large “safety margin” between the Ψ_{leaf} at
26 stomatal closure and hydraulic dysfunction observed here for these conifer species. This
27 large safety margin places conifers in a similar category to ferns (Brodribb and Holbrook,
28 2004) and distinguishes them from angiosperms which close stomata very close to the
29 onset of K_{leaf} dysfunction. The evolutionary implications of such a functional schism high
30 in the phylogeny of vascular plants would be of great significance.

31
32

1 MATERIALS AND METHODS

2

3 **Plant Material**

4

5 Four species of conifer trees were selected to cover a broad range of drought sensitivity.
6 *Actinostrobus arenarius* (Cupressaceae) grows in semi-arid woodland in Western
7 Australia, *Callitris rhomboidea* (Cupressaceae) extends from dry open woodland to moist
8 coastal habitats in eastern Australia, *Dacrycarpus dacrydioides* (Podocarpaceae) grows in
9 ever-wet rainforest in New Zealand and *Lagarostrobos franklinii* (Podocarpaceae) is
10 restricted to wet forest in Tasmania. All individuals were grown from seed collected in
11 native forests and grown for several years under non-heated glasshouse conditions in
12 Hobart, Tasmania. Twelve healthy individuals of each species were chosen such that all
13 plants were similar in size (between 50cm and 1m tall) and age (between 3 years and 5
14 years old). Eight of these plants were used in drought experiments while 3 were sampled
15 for stem vulnerability. Plants were potted in a high conductivity soil such that soil
16 hydraulic conductivity was unlikely to be limiting during the droughting or recovery
17 phases of measurement (Sperry et al., 1998). All individuals were potted into a mix of 8
18 parts composted pine bark, 2 parts coarse river sand and 1 part peat moss with added slow
19 release fertiliser (Osmocote) in 1.8 litres pots. Two months prior to the commencement of
20 measurements plants were moved to a controlled environment glasshouse cell and grown
21 under 18h days at 25°C/10°C day /night in a controlled glasshouse environment.
22 Humidity in the glasshouse was controlled at 50% using a De Longhi (Italy) DHE-PC de-
23 humidifier regulated by a Dixell (Italy) XH260V-500CO humidity sensor and controller.
24 Throughout the experiment, temperature and humidity were monitored with a Visalia
25 humidity probe and logged on a Campbell CR10X datalogger. Lighting in the growth
26 chamber was unfiltered natural light, with sodium vapour lamps (providing 300-500
27 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at the leaf surface) used to extend the photoperiod to 18h.

28

29 **Leaf vulnerability**

30

1 Leaf vulnerability was determined in three plants of each species during the gradual
 2 imposition of water stress by withholding water. During this drought phase branches were
 3 removed periodically to measure K_{leaf} as Ψ_{leaf} declined from midday values around -
 4 1MPa to minimum water potentials associated with leaf death over a period of 4 to 8
 5 weeks. Branches were sampled around midday, and prior to removing the sample branch
 6 the mean Ψ_{leaf} was determined from two adjacent shoots. A small sunlit branch
 7 (approximately 15cm²) was then removed and bagged before being quickly transferred
 8 back to the laboratory (approximately 2 minutes transfer time). Using a modified
 9 rehydration technique we recut the branch underwater and immediately connected it to a
 10 micro-flowmeter (Brodribb and Holbrook, 2006). Branches remained connected to the
 11 flowmeter under 1000 μ mol quanta m⁻² illumination at 22°C with flow rate logged every
 12 1s for 60s, after which the branch was immediately disconnected and wrapped in moist
 13 paper and foil and transferred to a pressure chamber for determination of final Ψ_{leaf} . K_{leaf}
 14 was determined at the two instantaneous points corresponding to the initial and final Ψ_{leaf}
 15 using equation 1 (based on Ohm's law analogy where the pressure gradient across the
 16 excised branchlet is equal to $-\Psi_{leaf}$).

17

$$18 \quad 1. \quad K_{leaf} = -\frac{I}{A_l \Psi_{leaf}}$$

19 Where I = instantaneous flow rate into the leaf (mmol s⁻¹); A_l = projected leaf area

20

21 Initial and final K_{leaf} did not tend to vary by more than 10% and hence were combined to
 22 produce a mean K_{leaf} measurement at the initial Ψ_{leaf} value. In cases where initial and
 23 final K_{leaf} differed by more than 20% (occasionally in hydrated leaves), a third technique
 24 was employed whereby branches were allowed to come to an evaporational steady-state
 25 for 120 to 180s while connected to the flowmeter, then disconnected and Ψ_{leaf}
 26 immediately measured. In very dehydrated leaves it was often necessary to recut the stem
 27 several times before maximum flow was initiated. This may have been due to localized
 28 embolism around the initial cut, or may alternatively be due to displacement of the torus
 29 under large pressure gradients (Hacke et al., 2004). In order to overcome this artefact

1 dehydrated stems that showed low flow were recut five times or until flow remained
2 steady after recutting.

3

4 **Stem vulnerability**

5

6 Xylem cavitation was assessed with the Cavitron technique (Cochard, 2002), a technique
7 derived from the centrifuge method of (Alder et al., 1997). The principle of the technique
8 is to use centrifugal force to increase the water tension in a xylem segment and, at the
9 same time, measure the decrease in its hydraulic conductance. The curve of percentage
10 loss of xylem conductance (PLC) versus xylem water tension represents the sample
11 vulnerability to cavitation. Vulnerability curves were determined on three to five different
12 samples for each species. The samples were collected on well watered plants in Tasmania,
13 defoliated, wrapped in wet paper and shipped to France by express air mail (arriving
14 within 3days). In France, the samples were re-cut under water to 0.28-m long segments
15 and installed in the Cavitron. Xylem pressure was first set to a reference pressure (-0.5
16 MPa or -1 MPa) and the sample maximal conductance (K_{\max}) was determined. The xylem
17 pressure was then set to a more negative pressure and the new sample conductance K was
18 determined. The sample percent loss of conductance was then computed as $PLC = 100(1 -$
19 $K/K_{\max})$. The procedure was repeated for more negative pressures (with -0.5 to -2 MPa
20 step increments) until PLC reached at least 90% or down to -12MPa (pressures less -
21 12MPa could not be generated without serious risk of fracturing the rotor). Rotor velocity
22 was monitored with an electronic tachymeter (10 rpm resolution) and xylem pressure was
23 adjusted at $ca \pm 0.1$ MPa.

24

25 **Gas exchange during drought**

26

27 Plant gas exchange was monitored daily using a computer-interfaced balance to measure
28 whole-plant water loss. Pots were double bagged and plants weighed to an accuracy of \pm
29 0.01g (Mettler-Toledo PG5002-S, Greifensee, Switzerland) between 1100h and 1300h.
30 Transpiration was calculated by the loss of weight of each plant between measurements
31 divided by the total leaf area of the plant. Leaf area was measured at the conclusion of the

1 experiment by compressing the entire plant between glass plates on a light box and
2 photographing the projected leaf area. Normalization of E_{md} to leaf area could be
3 problematic in species where leaf drop occurred as a normal response to drought,
4 however in these species leaf senescence was only observed under very severe stress, in
5 which case leaf drop was close to 100%. Hence in plants exposed to these water potential
6 extremes, leaf area was measured prior to stress exposure and E_{md} normalized to this pre-
7 drought leaf area.

8 Throughout the experiment vapour pressure deficit (VPD) remained constant during the
9 day and therefore E_{md} was closely proportional to stomatal conductance apart from small
10 variations in leaf temperature.

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

22 23 **Recovery measurements**

24
25 Once the above targets for droughted Ψ_{leaf} had been reached, plants were
26 rewatered overnight until soils became saturated. During the subsequent recovery period
27 plants were watered daily to full soil capacity in the morning then bagged at midday to
28 avoid water-logging of pots. Ψ_{leaf} and E_{md} were monitored every 1-3 days depending on
29 the recovery rate. Whole-plant hydraulic conductivity at midday (K_{plant}) was calculated
30 on the assumption that soils were fully saturated and hence water potential at the root was
31 close to zero. Under these circumstances:

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

2

3 **Statistics**

4

5 Stomatal response to Ψ_{leaf} was a key component of the hydraulic model and was
6 determined by using regression fitting software (Sigmaplot, SPSS Inc.) to fit a sigmoid

7 function of the form $y = \frac{a}{1 + e^{\frac{x-x_0}{b}}}$

8 to the pooled (n=5) E_{md} vs Ψ_{leaf} data collected for each species during the initial drought
9 treatment. Transpiration data were normalized as percentage data for each species in
10 order to reduce the effects of within species variation. Vulnerability curves for leaves and
11 stems used the same function as above and again data were pooled from three replicate
12 plants. In the case of stomatal and K_{leaf} vulnerability the parameter b was negative whilst
13 in the case of stem % loss of conductivity data b was positive. Estimates of Ψ_{leaf} at 20, 50
14 and 95% stomatal limitation and losses of hydraulic conductivity were made from the
15 respective regression equations with their attendant standard errors.

16

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18

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21

22

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- 5 porous angiosperms. *Plant Cell and Environment* **177**: 558-568
- 6

1

2 FIGURE LEGENDS

3

4 **Figure 1.** Examples of diurnal patterns of whole plant transpiration in a single individual
5 of *Actinostrobos arenarius* during several weeks of withholding water. The three plots
6 show data whilst unstressed ($\Psi_{\text{leaf}} = -1.15\text{MPa}$; closed circles) moderately stressed (Ψ_{leaf}
7 = -1.65MPa ; triangles), and stressed to $>80\%$ stomatal closure ($\Psi_{\text{leaf}} = -2.85\text{MPa}$; open
8 circles). Midday transpiration (E_{md}) was measured during the shaded time interval.

9

10

11

12 **Figure 2.** Pooled data ($n=5$) showing the response of transpiration (proportional to
13 stomatal conductance under the controlled vapour pressure growth regime) to
14 increasingly negative Ψ_{leaf} as soil dried during the drought treatment. Regressions are
15 sigmoidal functions in each case, and these regression functions were used to define the
16 stomatal dependence upon Ψ_{leaf} in order to evaluate the degree of hydraulic limitation
17 during drought recovery (see fig. 5a).

18

19

20

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

27

28

29

30 **Figure 4.** An example of recovery from mild (closed circles) and severe (open circles)
31 water stress in rewatered plants of *Lagarostrobos franklinii*. The mildly stressed plant

1 shows a minimal reduction of K_{plant} and is able to rapidly recover leaf hydration and gas
 2 exchange. By contrast the severely stressed plant experiences profound depression of
 3 K_{plant} which recovers slowly, thus limiting gas exchange recovery, which has a $t_{1/2}$ of 6.5
 4 days. Although Ψ_{leaf} recovers relatively quickly in both plants, it remains at limiting
 5 during recovery of the severely stressed plant, thus preventing stomatal reopening.

6

7

8

9 **Figure 5.** Modeled and measured recovery data for a *Callitris rhomboidea* plant subject
 10 to a stress sufficient to reduce K_{leaf} by approximately 90%. (a) According to the hydraulic-
 11 stomatal limitation model, in fully hydrated soils E will be equal to the intersection of a
 12 hydraulic supply function (defined by K_{plant}) and the stomatal control function
 13 (determined empirically from the regression equations in Fig. 2). (b) The observed
 14 recovery of whole-plant hydraulic conductivity after rewatering. (c) The predicted (open
 15 circles, dotted line) recovery of midday E closely matches the observed (closed circles,
 16 unbroken line) dynamic as the rewatered plant initially rehydrates rapidly to the edge of
 17 the stomatal control window (shown as the grey region, representing the Ψ_{leaf} range
 18 responsible for a 20% to 80% reduction in stomatal aperture) then slowly thereafter, thus
 19 limiting stomatal conductance and gas exchange. Predicted % E is calculated from
 20 entering the measured Ψ_{leaf} (triangles) into the stomatal control function equation % $E = f$
 21 (Ψ_{leaf}) shown in (a).

22

23

24

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

1

2

3

4 **Figure 7.** (a) The relationship between recovery time (plotted as $t_{1/2}^{-1}$) and final Ψ_{leaf}
 5 prior to rewatering in all individuals of *A. arenarius* (open circles), *C. rhomboidea*
 6 (closed circles), *D. dacrydioides* (closed triangles) and *Lagarostrobos franklinii* (open
 7 triangles). Recovery time showed two phases, the first phase was insensitive to Ψ_{leaf}
 8 ($1/t_{1/2}=1$) and the second highly dependent. Linear regressions are fitted through this
 9 second phase as $t_{1/2}$ fell from one (overnight recovery of $t_{1/2}$) to 0 (plant death). The x-
 10 intercept of these regressions was defined as the minimum recoverable water potential
 11 (Ψ_{min}). (b) Shows the very highly significant 1:1 relationships between Ψ_{min} derived from
 12 (a) and 50% loss of K_{stem} ($r^2=0.98$) and 95% loss of K_{leaf} ($r^2=0.94$), symbols as in (a).
 13 Correlation coefficients are for regression lines forced through the origin.

14

15

16

17 **Figure 8.** Examples of measured (open circles) and modeled (lines) recovery trajectory of
 18 transpiration in a *Lagarostrobos franklinii* plant over 20 days following rewatering from
 19 drought (-3.5MPa). Three curves depict three models of stomatal-hydraulic behavior; the
 20 hydraulic-stomatal limitation model with a fixed $E=f(\Psi_{\text{leaf}})$ (bold line); a hydraulic-
 21 stomatal limitation model with osmotic adjustment to promote stomatal opening at lower
 22 Ψ_{leaf} (dotted line); a non-hydraulic limited recovery where stomatal sensitivity to Ψ_{leaf} is
 23 enhanced or non-existent post drought *e.g.* as might occur if ABA was limiting stomatal
 24 aperture (dashed line). The measured recovery response for this individual and all
 25 individuals (Fig. 6) was best described by the constant $E=f(\Psi_{\text{leaf}})$ function.

26

27

Figure 1.

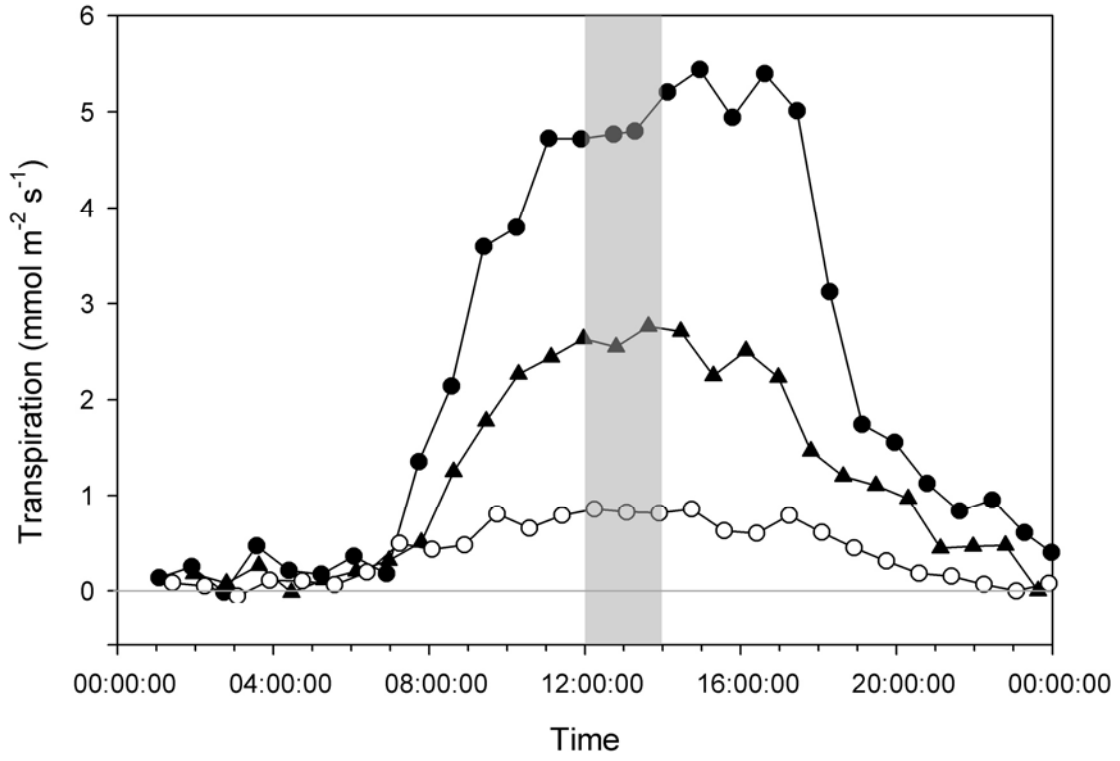


Figure 2.

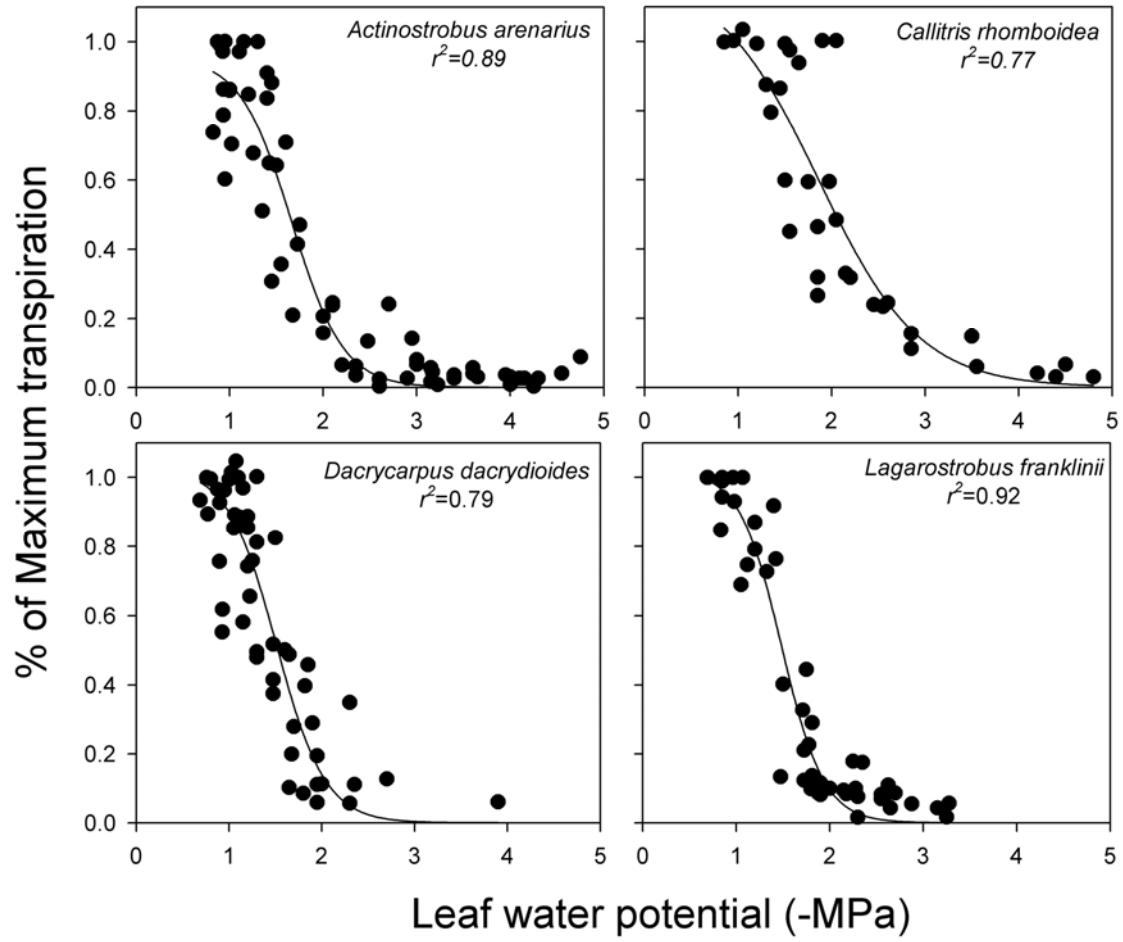


Figure 3.

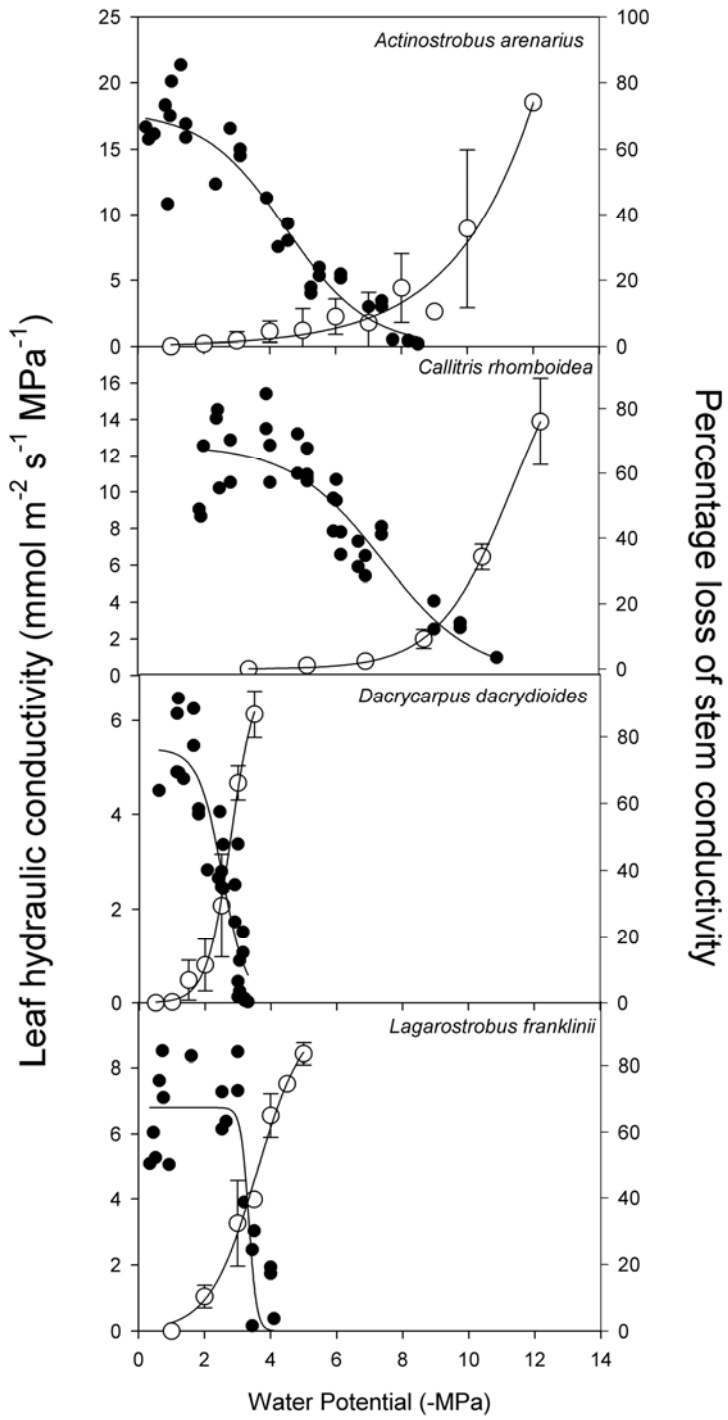


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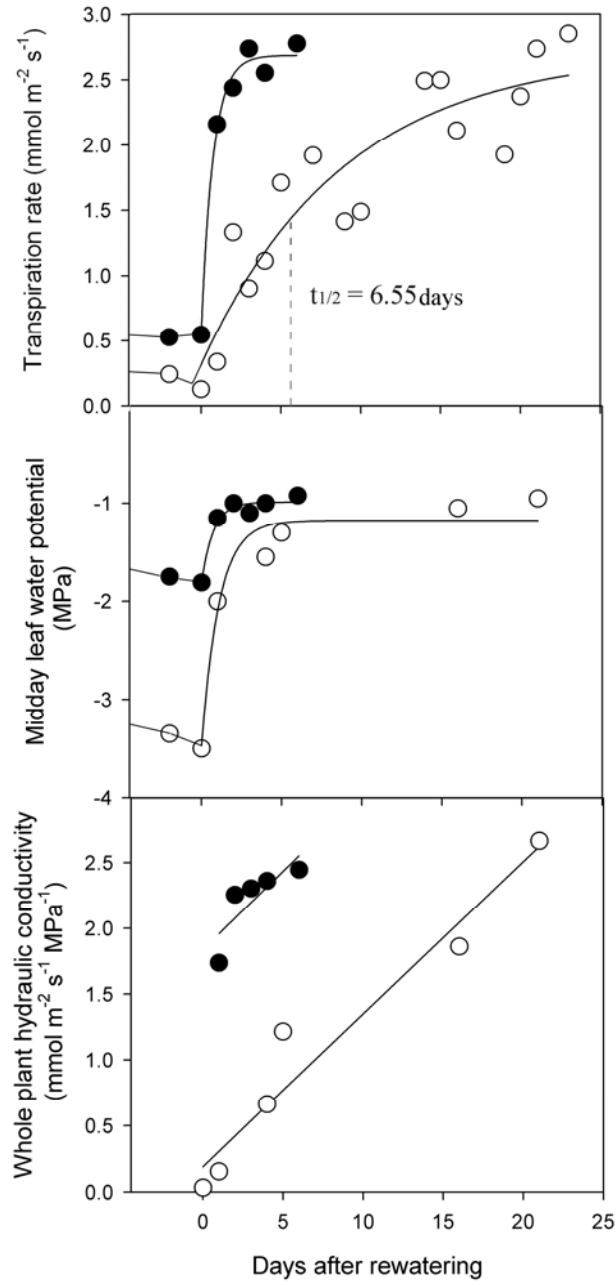


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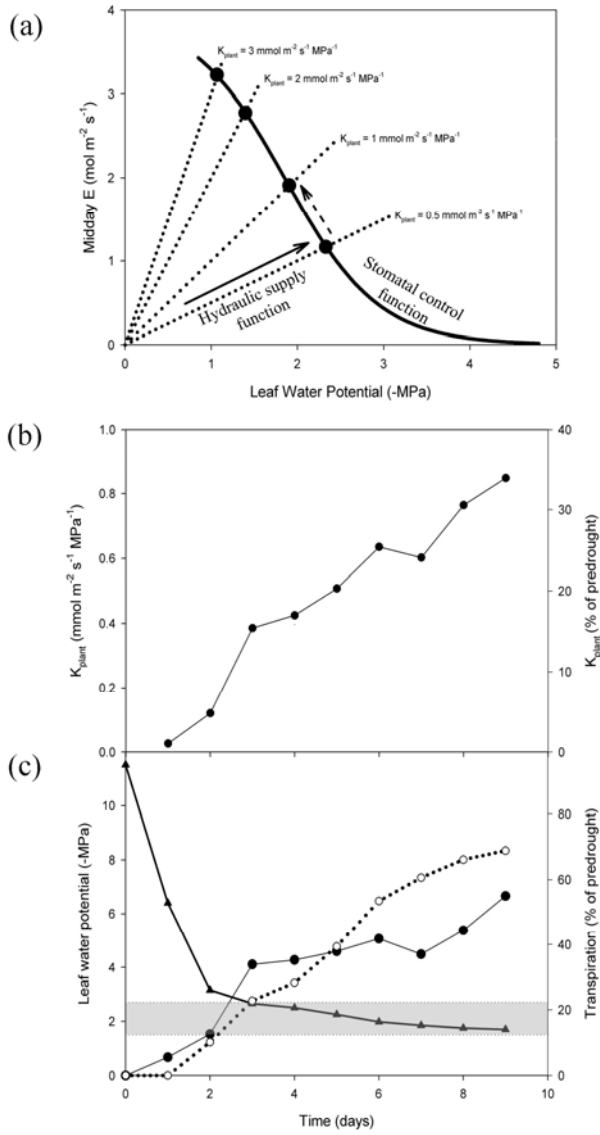


Figure 6

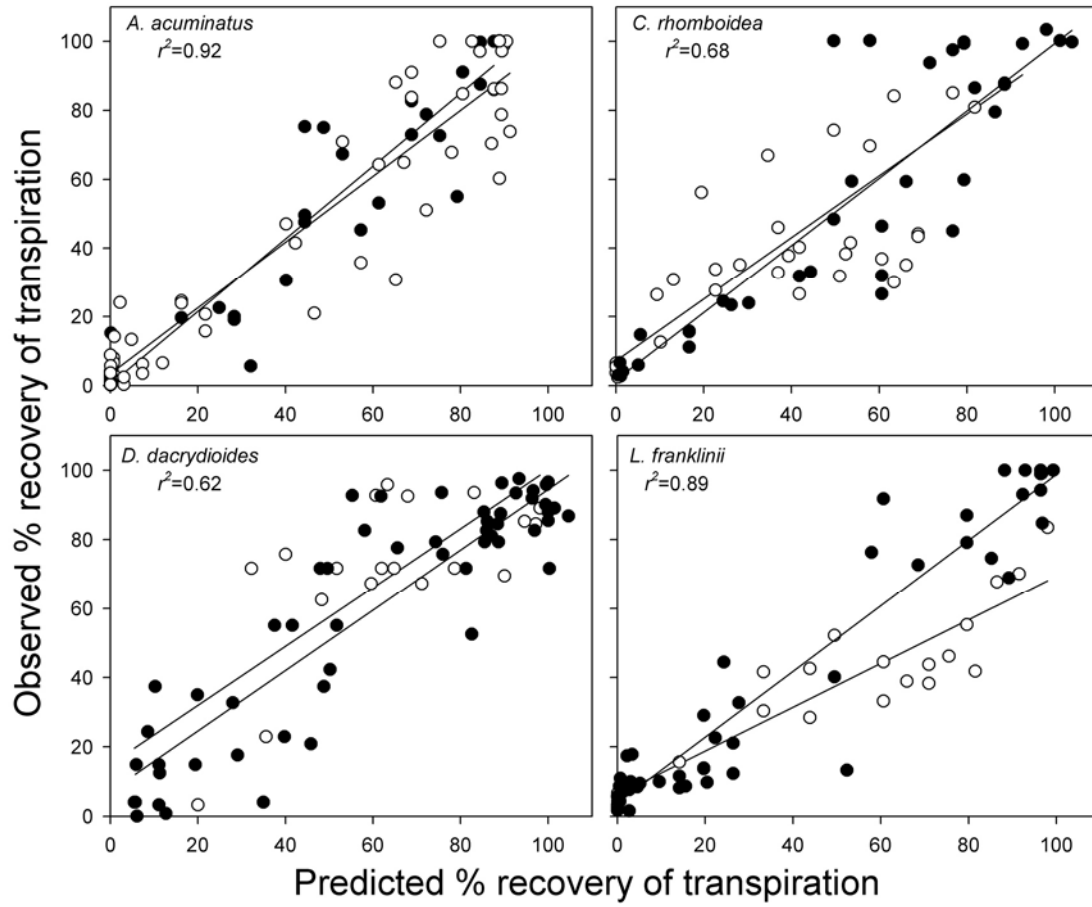


Figure 7

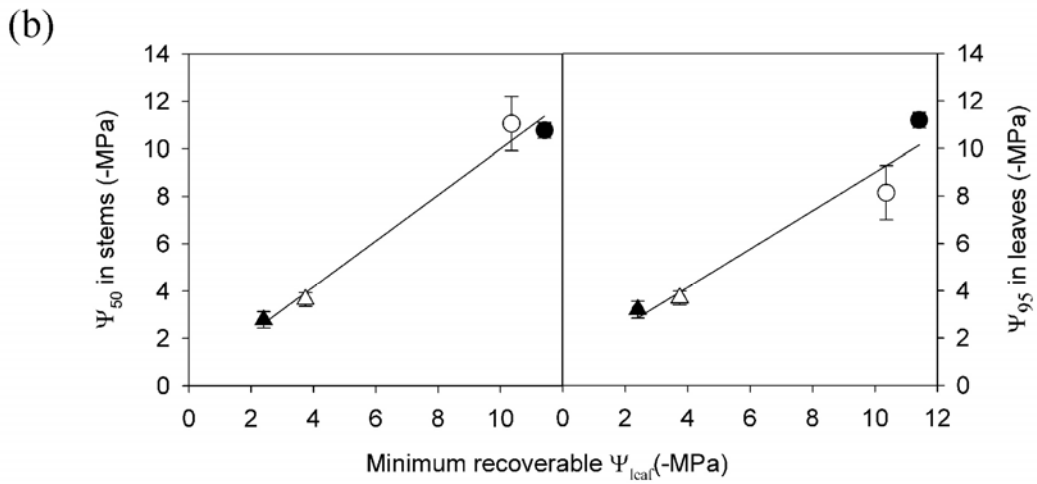
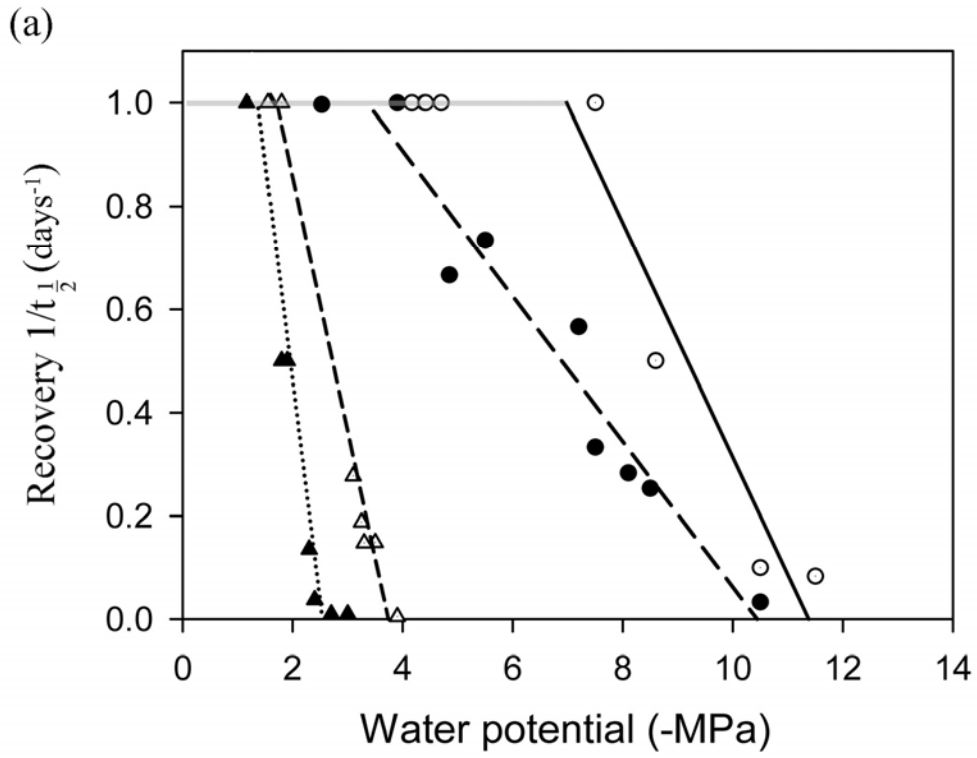


Figure 8

