

# Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance

Frederick C. Meinzer<sup>\*1</sup>, Daniel M. Johnson<sup>1</sup>, Barbara Lachenbruch<sup>2</sup>, Katherine A. McCulloh<sup>2</sup> and David R. Woodruff<sup>1</sup>

<sup>1</sup>USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, Oregon 97331, USA; and <sup>2</sup>Department of Wood Science and Engineering, Oregon State University, Corvallis, Oregon 97331, USA

## Summary

1. The xylem pressure inducing 50% loss of hydraulic conductivity due to embolism ( $P_{50}$ ) is widely used for comparisons of xylem vulnerability among species and across aridity gradients. However, despite its utility as an index of resistance to catastrophic xylem failure under extreme drought,  $P_{50}$  may have no special physiological relevance in the context of stomatal regulation of daily minimum xylem pressure and avoidance of hydraulic failure under non-extreme conditions. Moreover, few studies of hydraulic architecture have accounted for the buffering influence of tissue hydraulic capacitance on daily fluctuations in xylem pressure in intact plants.

2. We used data from 104 coniferous and angiosperm species representing a range of woody growth forms and habitat types to evaluate trends in three alternative xylem hydraulic safety margins based on features of their stem xylem vulnerability curves and regulation of daily minimum stem water potential ( $\Psi_{\text{stem min}}$ ) under non-extreme conditions: (i)  $\Psi_{\text{stem min}} - P_{50}$ , (ii)  $\Psi_{\text{stem min}} - P_e$ , the difference between  $\Psi_{\text{stem min}}$  and the threshold xylem pressure at which loss of conductivity begins to increase rapidly ( $P_e$ ) and (iii)  $P_e - P_{50}$ , an estimate of the steepness of the vulnerability curve between  $P_e$  and  $P_{50}$ . Additionally, we assessed relationships between xylem capacitance, species-specific set-points for daily minimum stem water potential and hydraulic safety margins in a subset of species for which relevant data were available.

3. The three types of hydraulic safety margin defined increased with decreasing species-specific set-points for  $\Psi_{\text{stem min}}$ , suggesting a diminishing role of stem capacitance in slowing fluctuations in xylem pressure as  $\Psi_{\text{stem min}}$  became more negative. The trends in hydraulic safety were similar among coniferous and angiosperm species native to diverse habitat types.

4. Our results suggest that there is a continuum of relative reliance on different mechanisms that confer hydraulic safety under dynamic conditions. Species with low capacitance and denser wood experience greater daily maximum xylem tension and appear to rely primarily on xylem structural features to avoid embolism, whereas in species with high capacitance and low wood density avoidance of embolism appears to be achieved primarily via reliance on transient release of stored water to constrain transpiration-induced fluctuations in xylem tension.

**Key-words:** hydraulic architecture, xylem embolism, plant–water relations, transpiration

## Introduction

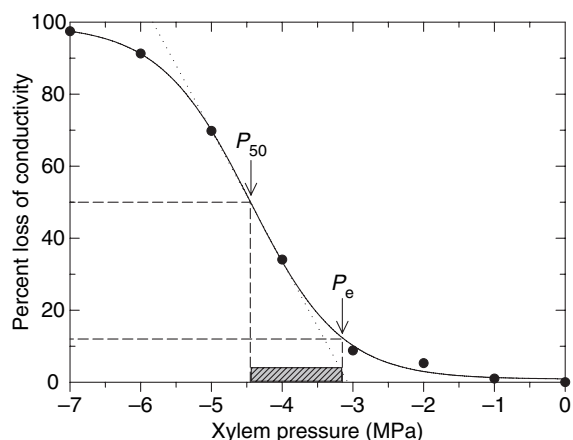
In a broad sense, stomata regulate transpiration to avoid damaging levels of leaf dehydration and to avoid hydraulic failure in the xylem of the stems to which the leaves are attached. As a consequence, stomatal control of vapour phase conductance is closely coordinated with dynamic changes in the hydraulic properties of the water transport

pathway upstream (Meinzer & Grantz 1990; Cochard *et al.* 2000; Hubbard *et al.* 2001; Mencuccini 2003). Thus, under non-extreme conditions stomatal regulation of leaf water potential ( $\Psi_L$ ) presumably constrains xylem pressure in stems to a range that does not result in excessive loss of stem conductivity from embolism. However, the hydraulic resistance of leaves is substantial (Sack & Holbrook 2006) and dynamic over a daily time scale (Bucci *et al.* 2003; Brodribb & Holbrook 2004a; Woodruff *et al.* 2007), resulting in a substantial and variable transpiration-induced disequilib-

\*Correspondence author. E-mail: fmeinzer@fs.fed.us

rium between  $\Psi_L$  and stem xylem pressure (Begg & Turner 1970; Ritchie & Hinckley 1971). Therefore, the bulk  $\Psi$  of transpiring leaves is often difficult to relate to the magnitude of xylem pressure in the adjacent stem and its impact on hydraulic function. In order to understand the potential impact of stomatal behaviour on stem hydraulics of field-grown plants, stem water potential ( $\Psi_{\text{stem}}$ ) must be estimated using techniques such as measurement of  $\Psi$  of non-transpiring leaves or shoot tips (Melcher *et al.* 2001; Bucci *et al.* 2004a) or stem psychrometry (Dixon & Tyree 1984; Scholz *et al.* 2007). Although published measurements of  $\Psi_L$  are abundant, estimates of  $\Psi_{\text{stem}}$  in transpiring plants are scarce, making it difficult to draw general conclusions about relationships between stomatal behaviour and stem hydraulic function.

The resistance of xylem to loss of function from cavitation and embolism is often assessed by generating hydraulic vulnerability curves describing the relationship between xylem pressure and loss of conductivity (e.g. Fig. 1). Vulnerability curves typically have a sigmoid shape with loss of conductivity initially increasing gradually as xylem pressure decreases followed by an abrupt transition to a much steeper, nearly linear phase, ending with a more gradual phase as loss of conductivity approaches 100%. The xylem pressure corresponding to 50% loss of conductivity ( $P_{50}$ ) is widely used for comparisons of xylem vulnerability across species, within different parts of the same individual and within species across environmental gradients (e.g. Melcher *et al.* 2001; Maherali *et al.* 2004, 2006). Another important, but less widely used point on the vulnerability curve is the threshold xylem pressure at which loss of conductivity begins to increase rapidly, often referred to as the air entry pressure ( $P_e$ ). The air entry pressure can be estimated from the  $x$ -intercept of a tangent (Fig. 1, dotted line) drawn through the midpoint of a sigmoid function fitted to the vulnerability curve data (Domec & Gartner 2001). Although specific values of  $P_{50}$  are widely



**Fig. 1.** Typical xylem vulnerability curve showing the relationship between the percent loss of hydraulic conductivity and xylem pressure. The xylem pressures corresponding to 50% loss of conductivity ( $P_{50}$ ) and the air entry threshold ( $P_e$ , see text) are shown. The horizontal shaded area corresponds to  $P_e - P_{50}$ , a measure of the steepness of the vulnerability curve with larger values of  $P_e - P_{50}$  representing a more gradual rise.

reported, values of  $P_e$  are much less frequently given and often have to be estimated from published graphs of vulnerability curves.

Despite the utility of  $P_{50}$  as an index of resistance to catastrophic xylem failure under extreme drought, it may have no special physiological relevance in the context of stomatal regulation of xylem tension under most conditions. There is increasing evidence that under non-extreme conditions stomatal control of transpiration limits maximum xylem tension to a value close to  $P_e$  for stem tissue to which the leaves are attached (Sparks & Black 1999; Brodrribb *et al.* 2003; Domec *et al.* 2008). Runaway embolism should become increasingly likely once xylem pressure falls below  $P_e$ , reaching the steep portion of the vulnerability curve because tissue hydraulic capacitance ( $C$ ) is largely depleted (Meinzer *et al.* 2008a) and no longer sufficient to adequately buffer fluctuations in tension induced by rapid changes in transpiration (see below). Thus it may be unlikely that stomatal regulation allows xylem pressure to fall substantially below species-specific values of  $P_e$ , except during periods of extreme drought that ultimately lead to branch dieback and shedding (Sperry *et al.* 1993; Sparks & Black 1999; Nardini & Salleo 2000). Both isohydric and anisohydric species would face a similar risk of runaway embolism as xylem pressure falls below  $P_e$  unless the slopes of the steeper portion of vulnerability curves systematically differ among species with these two modes of regulation of  $\Psi$ .

Although considerable attention has been paid to xylem safety and efficiency as components of hydraulic architecture,  $C$  is an additional and somewhat neglected component of hydraulic architecture that merits further study. Capacitance of plant tissues can play an important role in slowing changes in xylem pressure following transpiration-induced changes in xylem water flux (Cowan 1972; Phillips *et al.* 1997, 2004; Goldstein *et al.* 1998; Meinzer *et al.* 2004, 2008a; Scholz *et al.* 2007; Höltta *et al.* 2009). Using an Ohm's law analogy, the buffering effect of  $C$  on fluctuations in xylem pressure can be quantified in terms of the time constant ( $\tau$ ), or time required for xylem pressure to undergo 63% of its total change following a change in water flux. In practice,  $\tau$  can be calculated as the product of hydraulic resistance and capacitance ( $R \times C$ ) between a reference point within the soil and a reference point within the plant. The time constant defined in this manner is thus a dynamic rather than static property of the hydraulic pathway and is expected to fluctuate over the course of a day as changes in tissue hydration cause  $C$  and possibly  $R$  to vary (Meinzer *et al.* 2003, 2008a; Scholz *et al.* 2007). Given that time constants for stomatal responses to changing environmental variables range from a few minutes to 10 min or more (Ceulemans *et al.* 1989; Jarvis *et al.* 1999; Allen & Pearcy 2000; Powles *et al.* 2006),  $C$  through its impact on  $\tau$  for transpiration-induced fluctuations in xylem pressure may be critical for avoiding sharp decreases in xylem pressure and runaway embolism following transient increases in transpiration. Of course, sharp increases in  $R$  would increase  $\tau$ , but at the cost of catastrophic xylem failure and ensuing lethal levels of dehydration. Estimates of  $\tau$  for whole plants and plant parts are scarce, but available data for branches and whole

trees span a range from about 5 min to > 2 h (Phillips *et al.* 1997, 2004; Meinzer *et al.* 2004).

Our principal objectives in this study were to evaluate various measures of xylem hydraulic safety margins across a broad range of woody plant species and to further assess interactions between woody tissue  $C$  and hydraulic safety in a subset of nine tropical tree species for which relevant data were available (Meinzer *et al.* 2008a). Three alternative hydraulic safety margins were defined: the difference between minimum stem water potential ( $\Psi_{\text{stem min}}$ ) and  $P_{50}$ , the difference between  $\Psi_{\text{stem min}}$  and  $P_e$  and the difference between  $P_e$  and  $P_{50}$ , an estimate of the steepness of the vulnerability curve between  $P_e$  and  $P_{50}$  (Fig. 1). In order to identify key points for stomatal limitation of minimum xylem pressure when stomatal control was still fully effective, data collected only under non-extreme conditions for a given species and habitat type were included. Based on a previous study of tropical trees (Meinzer *et al.* 2008a), we hypothesized that safety margins would increase with decreasing  $\Psi_{\text{stem min}}$  and that this pattern would be associated with a declining contribution of  $C$  to buffering of transpiration-induced fluctuations in xylem pressure.

## Materials and methods

Data on xylem vulnerability to embolism, stem capacitance and  $\Psi_{\text{stem min}}$  of woody species were obtained from the literature. An extensive, but not exhaustive, search of the literature yielded partial or complete data sets for 104 woody species representing a wide range of xylem anatomy and phylogeny (see Table S1 in Supporting Information). Only data collected from field-grown plants were considered. Data obtained from experiments involving imposition of severe drought were not included. The following additional criteria for incorporation of data were applied: Three measures of xylem vulnerability to embolism were estimated from standard hydraulic vulnerability curves consisting of plots of percent loss of hydraulic conductivity (PLC) vs. stem xylem pressure (Fig. 1), or from tables of data listing key points along vulnerability curves. The three measures of vulnerability were (i) the xylem pressure corresponding to 50% loss of hydraulic conductivity ( $P_{50}$ ), (ii) the air entry threshold ( $P_e$ ) corresponding to about 12% loss of conductivity wherein species with pronounced sigmoid vulnerability curves PLC begins to rise sharply with declining xylem pressure (Domec & Gartner 2001) and (iii)  $P_e - P_{50}$ , a measure of how steeply PLC rises once  $P_e$  has been reached. Larger values of  $P_e - P_{50}$  imply a more gradual rise of PLC once xylem pressure has fallen below  $P_e$ . Stem sapwood capacitance expressed per unit sapwood volume ( $\text{kg m}^{-3} \text{MPa}^{-1}$ ) for nine co-occurring tropical tree species was obtained from Meinzer *et al.* (2008a).

Daily  $\Psi_{\text{stem min}}$  was obtained from studies in which  $\Psi$  was determined with a pressure chamber on covered, non-transpiring leaves (Begg & Turner 1970; Bucci *et al.* 2004a) and from studies in which  $\Psi$  was measured on excised previously transpiring leafy twigs (Table S1). The covered leaf method is preferable for estimating  $\Psi_{\text{stem}}$  because the hydraulic resistance of leaves often results in substantial transpiration-induced disequilibrium between stem and leaf  $\Psi$ . However, the occurrence of daily minimum plant  $\Psi$  is typically associated with marked stomatal limitation of transpiration, which would constrain the degree of disequilibrium between leaf and stem  $\Psi$ .

In three co-occurring tropical tree species for which published data were available (Meinzer *et al.* 2008a), relative values of stem  $C$  and

hydraulic resistance ( $R$ ) in relation to  $\Psi_{\text{stem}}$  were used to estimate relative time constants ( $\tau = R \times C$ ) for changes in xylem pressure following changes in xylem water flux. Relative rather than absolute values of  $\tau$  were used because the data necessary to express both  $C$  and  $R$  in units that would yield  $\tau$  in time units only were not available. The purpose of this exercise was to determine whether  $P_{50}$  and  $\Psi_{\text{stem min}}$  of different species occurred at similar points along the trajectory of  $\tau$  as  $\Psi_{\text{stem}}$  declined.

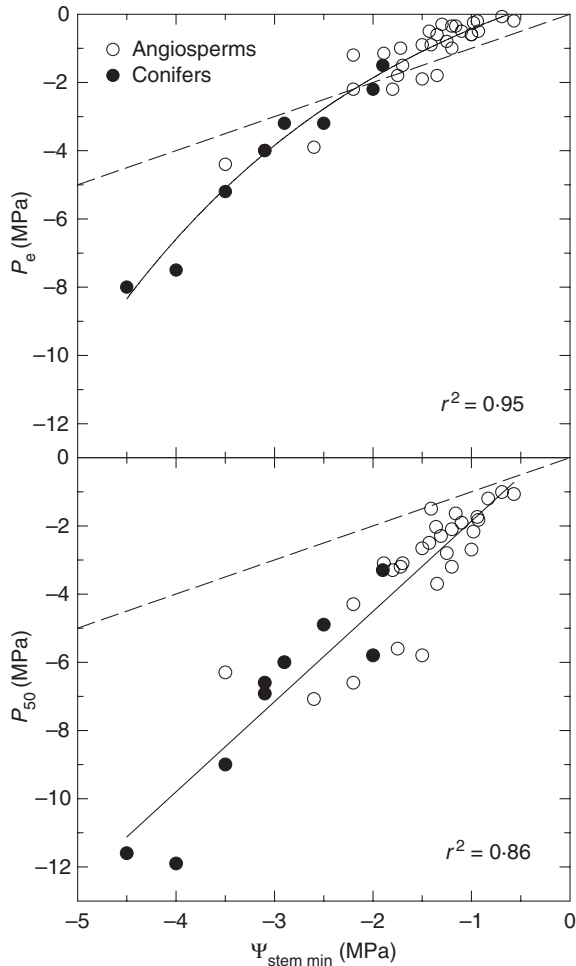
The significance of relationships between  $\Psi_{\text{stem}}$  and  $P_e$ ,  $P_{50}$  and  $P_e - P_{50}$  and between  $P_{50}$  and  $P_e$  was evaluated using one-way ANOVA. Differences in the dependence of  $P_{50}$  on  $P_e$  among three groups of species provisionally identified *post hoc* were assessed by pair-wise ANCOVA after it was determined that the regressions were heterogeneous ( $F = 12.5, P < 0.0001$ ).

## Results

Observations compiled for nine coniferous and 28 angiosperm species showed that daily  $\Psi_{\text{stem min}}$  measured when soil water deficits were non-extreme was tightly coordinated with both  $P_e$  and  $P_{50}$  in a similar manner across species and across both conifers and angiosperms ( $P < 0.0001$ , Fig. 2). The relationship between  $P_e$  and  $\Psi_{\text{stem min}}$  was close to the 1:1 line over a relatively wide range of species values of  $\Psi_{\text{stem min}}$ . For species having  $\Psi_{\text{stem min}}$  more negative than about  $-3$  MPa,  $P_e$  began to decline noticeably more quickly than  $\Psi_{\text{stem min}}$ . In the species with the least negative values of  $\Psi_{\text{stem min}}$ ,  $P_e$  was less negative than  $\Psi_{\text{stem min}}$ . In contrast,  $P_{50}$  was always more negative than  $\Psi_{\text{stem min}}$ , and decreased more than 2.5 times faster than  $\Psi_{\text{stem min}}$  with declining species-specific values of  $\Psi_{\text{stem min}}$ .

The hydraulic safety margin expressed as  $\Psi_{\text{stem min}} - P_{50}$  was always positive and increased about 10-fold over the range of  $\Psi_{\text{stem min}}$  observed (Fig. 3a). The safety margin expressed as  $\Psi_{\text{stem min}} - P_e$  was negative or near zero for species with  $\Psi_{\text{stem min}}$  less negative than about  $-2.5$  MPa and increased to about 4 MPa in species with the lowest values of  $\Psi_{\text{stem min}}$  observed (Fig. 3b). A third safety margin,  $P_e - P_{50}$ , based on species-specific xylem vulnerability curve characteristics alone, increased from about 1 to 4 MPa as  $\Psi_{\text{stem min}}$  declined from about  $-0.5$  to  $-4.5$  MPa ( $P < 0.0001$ , Fig. 3c). Greater values of  $P_e - P_{50}$  indicate a more gradual loss of hydraulic conductivity as xylem pressure falls below  $P_e$ .

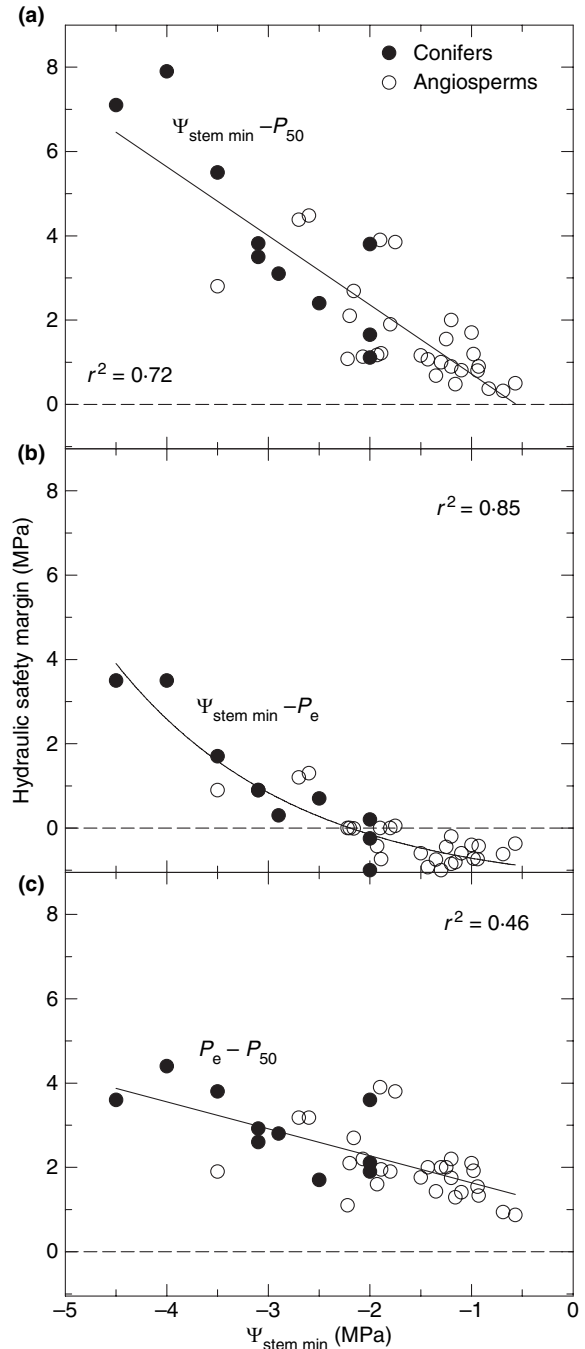
Xylem vulnerability curve data were found for more species than were data for  $\Psi_{\text{stem}}$ . Variation in  $P_{50}$  was linearly related to that of  $P_e$ , but three distinct groups of species appeared to emerge based on trajectories of regressions fitted to relationships between  $P_e$  and  $P_{50}$  ( $P < 0.0001$  for all regressions, Fig. 4). Among 16 species of conifers and 47 species of angiosperms native to wet, mesic and seasonally dry habitats, the slope of the relationship between  $P_{50}$  and  $P_e$  was 1.42, indicating that the slope of the steeper portion of the vulnerability curve became more gradual as  $P_e$  declined (Fig. 4a). In two other groups of species, the slopes of the relationships between  $P_{50}$  and  $P_e$  were not significantly different from 1.0, indicating that the difference between  $P_e$  and  $P_{50}$  was constant over the entire range of  $P_e$  observed (Fig. 4b,c). In 12 members of Cupressaceae (e.g. *Juniperus* spp.) native to



**Fig. 2.** Relationship between stem xylem vulnerability properties and daily minimum stem water potential ( $\Psi_{\text{stem min}}$ ) for several coniferous and angiosperm species.  $P_e$  is the air entry pressure and  $P_{50}$  is the pressure causing 50% loss of hydraulic conductivity (see Fig. 1). Dashed lines represent the 1:1 relationships. Species and references are listed in Table S1.

semi-arid and arid zones and 14 angiosperm species native to Mediterranean climate zones,  $P_{50}$  was about 4.5 MPa more negative than  $P_e$  over a broad range of  $P_e$  (Fig. 4b). Among 15 vesselleless angiosperm species,  $P_{50}$  was only about 1 MPa more negative than  $P_e$  (Fig. 4c). The slopes of the regressions in Fig. 4b,c were both significantly different from that in Fig. 4a ( $P \leq 0.01$ ) and they differed significantly in their offset from the 1:1 line ( $P < 0.0001$ ).

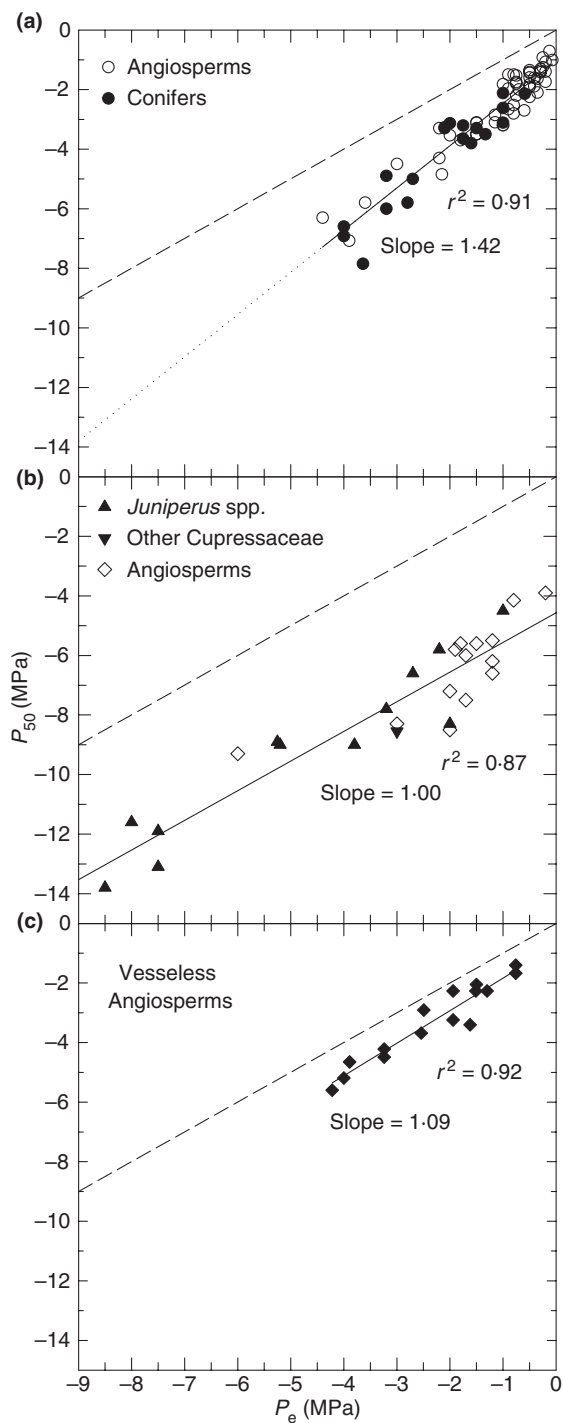
Minimum  $\Psi_{\text{stem}}$  showed a strong positive correlation ( $P = 0.0002$ ) with sapwood capacitance among nine tropical tree species (Fig. 5a). Minimum daily  $\Psi_{\text{stem}}$  was about 1 MPa less negative in the species with the highest  $C$  ( $410 \text{ kg m}^{-3} \text{ MPa}^{-1}$ ) than in the species with the lowest  $C$  ( $83 \text{ kg m}^{-3} \text{ MPa}^{-1}$ ), consistent with the buffering effect on xylem pressure of transient withdrawal of water from internal storage. Among the same nine species, the difference between  $P_e$  and  $P_{50}$  decreased in a linear fashion ( $P < 0.0001$ ) with increasing sapwood  $C$  (Fig. 5b), indicating that the slope of the steeper portion of the xylem vulnerability curve was more abrupt in species with high  $C$ .



**Fig. 3.** Relationships between three types of hydraulic safety margins and daily minimum stem water potential ( $\Psi_{\text{stem min}}$ ). (a) The difference between  $\Psi_{\text{stem min}}$  and the xylem pressure causing 50% loss of conductivity ( $P_{50}$ ). (b) the difference between  $\Psi_{\text{stem min}}$  and the air entry threshold ( $P_e$ ) and (c)  $P_e - P_{50}$ . Horizontal dashed lines represent safety margins of zero. Species and references are listed in Table S1.

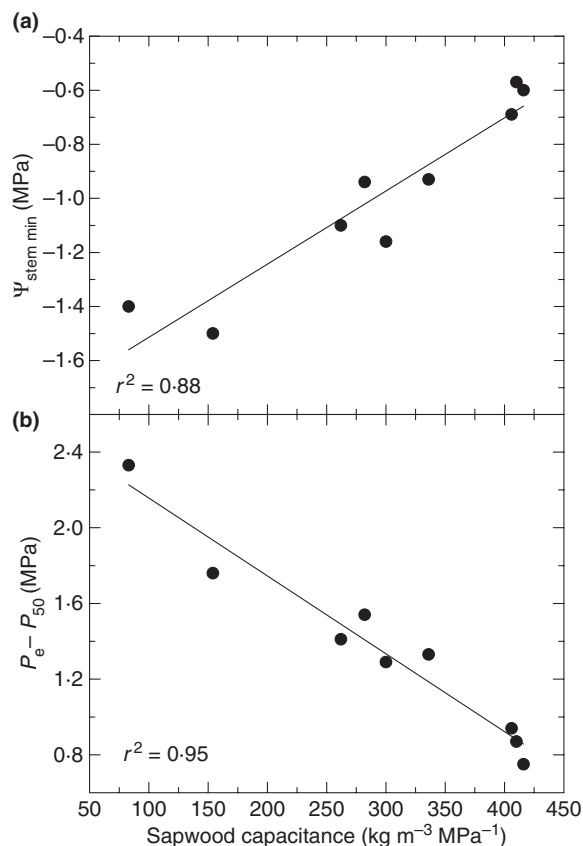
The dependence of relative  $C$  and relative hydraulic resistance on  $\Psi_{\text{stem}}$  was characterized for three tropical tree species with high, intermediate and low values of sapwood  $C$  and daily  $\Psi_{\text{stem min}}$  (Fig. 6). As expected, relative  $C$  initially declined sharply as  $\Psi_{\text{stem}}$  decreased followed by an asymptotic approach to a minimum value of  $C$ , whereas the initial increase in relative  $R$  associated with embolism was gradual





**Fig. 4.** Relationships between the xylem pressure causing 50% loss of stem hydraulic conductivity ( $P_{50}$ ) and the air entry pressure ( $P_e$ ) for several coniferous and angiosperm species. The dashed lines represent 1:1 relationships. Species and references are listed in Table S1.

followed by a steep rise. Regardless of species-specific values of  $\Psi_{\text{stem min}}$  and  $C$ ,  $\Psi_{\text{stem min}}$  occurred near or just after the transition from a steep to gradual decline in  $C$  as  $\Psi_{\text{stem}}$  declined, and was associated with a relatively narrow range of increase in relative resistance (30–39%). Overall, the patterns in Fig. 6 implied that the relative time constant ( $R \times C$ ) for a response in xylem tension following a change in xylem water

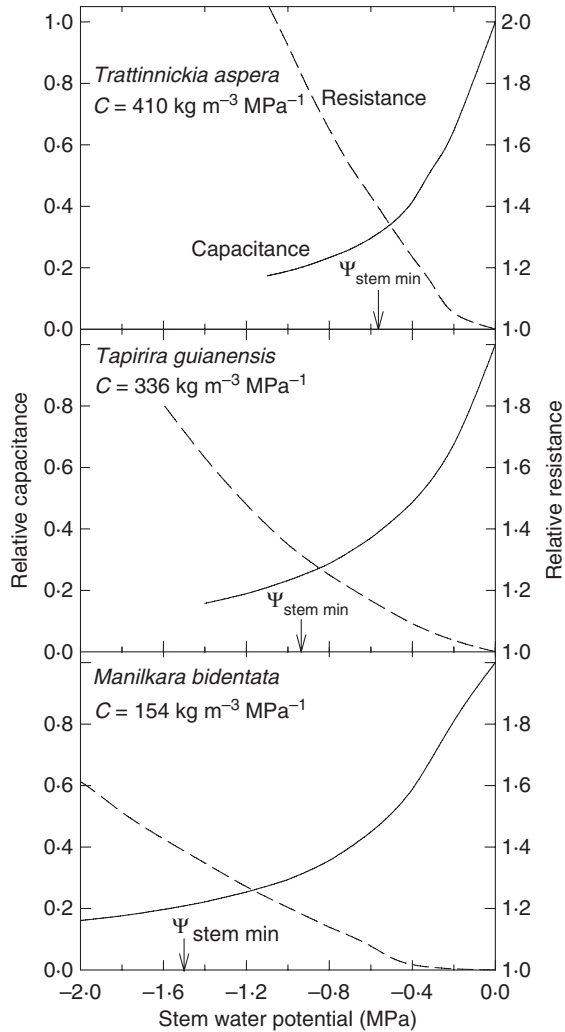


**Fig. 5.** Relationship between stem sapwood capacitance and (a) daily minimum stem water potential ( $\Psi_{\text{stem min}}$ ) and (b) the stem hydraulic safety margin defined as the difference between the air entry pressure and the pressure causing 50% loss of conductivity ( $P_e - P_{50}$ ) for upper canopy branches of nine tropical tree species (Table S1).

flux initially declined sharply as  $\Psi_{\text{stem}}$  decreased followed by a gradual approach to a minimum value of  $\tau$  (Fig. 7). Across three species representing a broad range of sapwood  $C$ ,  $\Psi_{\text{stem min}}$  was consistently associated with the transition from a steep to a gradual, nearly linear decline in  $\tau$  as  $\Psi_{\text{stem}}$  decreased. Consistent with the data shown in Fig. 3a, the difference between the  $\Psi_{\text{stem min}}$  and  $P_{50}$  increased with decreasing species-specific values of sapwood  $C$  and minimum  $\Psi_{\text{stem}}$  (Fig. 7).

## Discussion

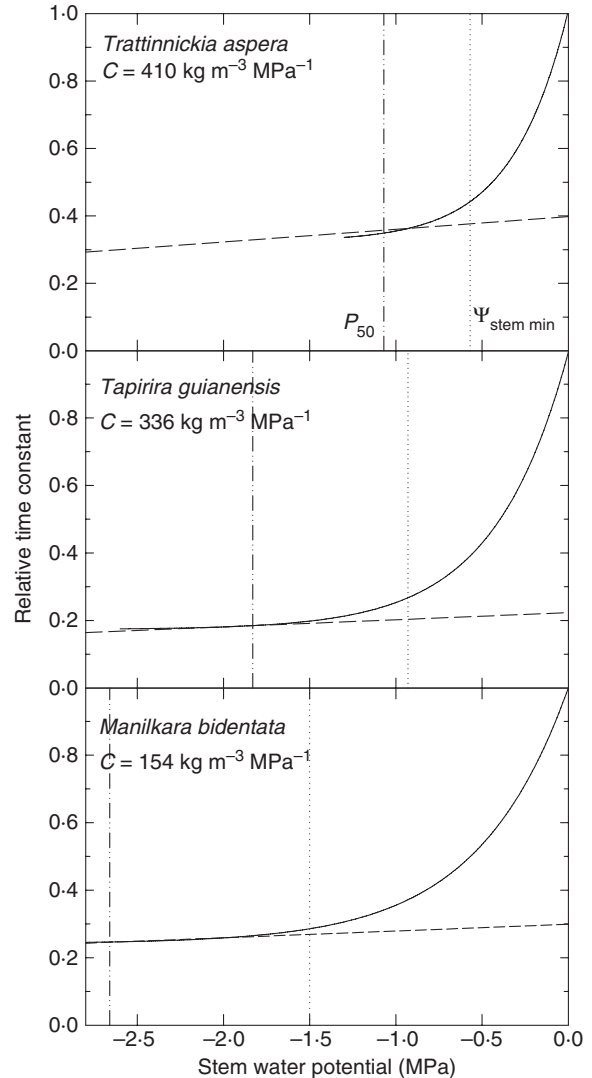
Even under non-extreme conditions of adequate soil moisture and moderate vapour pressure deficit, the potential risk of catastrophic xylem failure is high, unless stomatal behaviour is tightly coordinated and integrated with the properties of the hydraulic pathway upstream from the leaves as they change over the course of the day. We found a notable degree of convergence in trends of xylem hydraulic safety over a broad range of  $\Psi_{\text{stem min}}$  among 37 coniferous and angiosperm species native to diverse habitat types. The three types of hydraulic safety margin defined increased with decreasing species-specific values of  $\Psi_{\text{stem min}}$ . This pattern probably reflects a diminishing role of  $C$  in slowing fluctuations in  $\Psi_{\text{stem}}$  as  $\Psi_{\text{stem}}$  becomes more negative. Nevertheless, stomatal control interacts with  $C$  to determine species-specific set-



**Fig. 6.** The dependence of relative capacitance and relative hydraulic resistance on stem water potential for three co-occurring tropical tree species. The arrows indicate mean daily minimum stem water potentials ( $\Psi_{\text{stem min}}$ ). Absolute values of capacitance ( $C$ ) are shown.

points for  $\Psi_{\text{stem min}}$ , and therefore hydraulic safety margins. Overall, the patterns observed were consistent with earlier suggestions that although coordination between stomatal behaviour and stem hydraulic capacity generally avoids catastrophic hydraulic failure, tolerance of a certain degree of embolism may enhance productivity under some conditions, especially if loss of stem hydraulic function is reversible over a relatively short time scale (Jones & Sutherland 1991; Vogt 2001; Brodrribb & Holbrook 2004b; Sperry 2004).

Taken together, our results suggest that here is a continuum of relative reliance on different mechanisms that confer hydraulic safety under dynamic conditions. Species with low capacitance and denser wood experience greater daily maximum xylem tension and appear to rely primarily on xylem structural features to avoid embolism, whereas in species with high capacitance and low wood density, avoidance of embolism appears to be achieved primarily via reliance on transient release of stored water to constrain fluctuations in xylem tension.



**Fig. 7.** Calculated relative time constants for changes in stem water potential in response to changes in xylem water flux for three co-occurring tropical tree species. Time constants were estimated as the product of resistance and capacitance ( $R \times C$ ) from the data shown in Fig. 6. Vertical lines represent daily minimum stem water potential ( $\Psi_{\text{stem min}}$ ) and the stem xylem pressure corresponding to 50% loss of hydraulic conductivity ( $P_{50}$ ). Horizontal dashed lines represent tangents drawn through the nearly linear portions of the curves at low values of stem water potential.

#### STOMATAL CONTROL OF STEM XYLEM PRESSURE

Minimum  $\Psi_{\text{stem}}$  was close to  $P_e$  over a wide range of  $\Psi_{\text{stem min}}$  from about  $-0.5$  to  $-3$  MPa (Fig. 2). This finding is consistent with earlier reports that when soil water availability is adequate, stomata constrain  $\Psi_{\text{stem}}$  to minimum values at or less negative than  $P_e$  (Sparks & Black 1999; Brodrribb *et al.* 2003; Domec *et al.* 2008). In species with  $P_e$  below about  $-4$  MPa, stomatal regulation of  $\Psi_{\text{stem}}$  was more conservative resulting in a safety margin ( $\Psi_{\text{stem min}} - P_e$ ) of nearly 4 MPa in the species with the lowest values of  $P_e$  (cf. Figs 2a and 3b). Stomatal regulation of stem xylem pressure at values substantially more negative than  $P_e$  implies an increased risk of runaway embolism as xylem pressure begins to traverse the steep

portion of the sigmoid hydraulic vulnerability curve where tissue  $C$  has effectively been exhausted, causing a sharp reduction in the time constant for changes in xylem pressure in response to changes in transpiration. The effectiveness of stomata in constraining stem xylem pressure diminishes as the time constant for flow-induced changes in xylem pressure decreases relative to that for reductions in stomatal conductance. The trend towards higher values of  $\Psi_{\text{stem min}} - P_e$  in species with lower  $\Psi_{\text{stem min}}$  is consistent with this interpretation because the relative role of  $C$  in buffering changes in  $\Psi_{\text{stem}}$  diminishes as  $\Psi_{\text{stem}}$  declines (Figs 5 and 7). The specific mechanisms by which stomata respond to and control  $\Psi_L$  to result in species-specific values of  $\Psi_{\text{stem min}}$  are not known. Regardless of the mechanisms, the coordination of leaf and stem  $\Psi$  set-points optimizes reliance on hydraulic capacitance of stem tissue (Meinzer *et al.* 2008a).

The data presented for daily  $\Psi_{\text{stem min}}$  represent pressure chamber measurements on non-transpiring leaves attached to transpiring shoots as well as conventional measurements on transpiring leaves or leafy shoots. However, the hydraulic resistance of leaves is substantial, leading to pronounced uncoupling or disequilibrium between leaf and stem  $\Psi$  in transpiring shoots (Begg & Turner 1970; Meinzer *et al.* 2001; Melcher *et al.* 2001; Bucci *et al.* 2004a). Thus, a violation of our assumption that  $\Psi_L$  is approximately equal to  $\Psi_{\text{stem}}$  when  $\Psi_L$  is at its daily minimum value and stomata have partly closed (see Materials and methods) would yield estimates of  $\Psi_{\text{stem min}}$  that are too negative. If actual species values of  $\Psi_{\text{stem min}}$  were less negative than those shown in some cases, the overall trend of increasing hydraulic safety margins with declining  $\Psi_{\text{stem min}}$  would remain the same, but stomatal regulation of  $\Psi_{\text{stem}}$  in relation to  $P_e$  and other key points for stem hydraulic function would appear to be even more conservative. Nevertheless, most of the data for  $\Psi_{\text{stem min}} \geq -2$  MPa were obtained from non-transpiring leaves (Table S1) reinforcing the inference that the safety margin defined as  $\Psi_{\text{stem min}} - P_e$  was actually negative for values of  $\Psi_{\text{stem min}} \geq -2$  MPa. The impact of stomatal behaviour on the hydraulic function of stems to which leaves are attached cannot be assessed fully without knowledge of  $\Psi_{\text{stem}}$ , but explicit attempts to estimate  $\Psi_{\text{stem}}$  using techniques such as pressure chamber measurements on non-transpiring leaves or stem psychrometry are rather scarce in the literature. Studies aimed at describing the integration of stomatal and stem hydraulic function in intact plants should incorporate methods designed to estimate the  $\Psi$  of the stems supporting the leaves.

#### HYDRAULIC SAFETY MARGINS

The air-seeding pressure,  $P_{50}$ , is widely used as a comparative index of xylem hydraulic safety. Physiologically, however, attainment of  $P_{50}$  indicates that nearly catastrophic hydraulic failure has already occurred and that the risk of further run-away embolism is acute because xylem pressure is operating along the steepest portion of the vulnerability curve (Tyree & Sperry 1988). Thus, the relevance of  $P_{50}$  as an indicator of resistance to hydraulic failure may be restricted to episodic

extreme drought conditions under which it becomes physically impossible for stomata to constrain xylem pressure above or around  $P_e$ . These conditions are expected to lead to branch dieback and shedding, and in some cases death of the entire plant, which would ultimately limit species distributions along aridity gradients. In the present study we have identified three alternative hydraulic safety margins referenced to species-specific values of daily  $\Psi_{\text{stem min}}$  set by stomatal control of transpiration (Fig. 3). All three types of safety margins increased with declining species-specific values of  $\Psi_{\text{stem min}}$ . Interestingly, the safety margin defined as  $\Psi_{\text{stem min}} - P_e$  was negative in species having values of  $\Psi_{\text{stem min}}$  down to about  $-2$  MPa, implying that branches of these species may regularly experience a substantial loss of their conductivity even when soil water availability is not severely restricted. Consistent with this, native loss of conductivity was about 36% among 20 co-occurring tropical tree species having mean daily values of  $\Psi_{\text{stem min}}$  between  $-0.7$  and  $-1.5$  MPa (Santiago *et al.* 2004; Meinzer *et al.* 2008a). Even higher levels of native embolism were found in two co-occurring Mediterranean *Quercus* species (Tognetti *et al.* 1998). The extent to which daily losses of conductivity may be reversed overnight is unclear because branch samples in the studies cited were collected after the onset of transpiration in the morning. However, results of other studies suggest that a certain fraction of stem embolism may be readily reversible over diel and seasonal cycles (e.g. Zwieniecki & Holbrook 1998; Melcher *et al.* 2001; Vogt 2001) and that in leaves embolism reversal may be even more rapid with recovery of hydraulic conductance occurring during the afternoon while xylem pressure is still well below zero (Bucci *et al.* 2003; Brodribb & Holbrook 2004a; Woodruff *et al.* 2007).

In contrast to  $\Psi_{\text{stem min}} - P_e$ ,  $\Psi_{\text{stem min}} - P_{50}$  was always positive and increased by a factor of about 10 over the observed range of  $\Psi_{\text{stem min}}$ . The absence of negative values of  $\Psi_{\text{stem min}} - P_{50}$  reinforces the notion that stomatal regulation normally prevents stem xylem pressure from traversing the steep portion of the vulnerability curve. Increasing values of the safety margin defined as  $P_e - P_{50}$  indicate a progressively more gradual slope of the vulnerability curve once the air entry threshold has been crossed, which can be regarded as a second line of defence against catastrophic xylem failure once stomatal regulation can no longer prevent xylem pressure from entering the steeper portion of the vulnerability curve. The specific xylem structural features responsible for the observed behaviour of  $P_e - P_{50}$  are not known, but could certainly be examined. Most likely they relate to the incidence of safety features in the population of cells contributing to embolism avoidance, which could include not only tracheids and vessels, but also parenchyma and fibre cells.

It is interesting that when all vulnerability curve data were pooled, two types of relationships between  $P_e$  and  $P_{50}$  emerged, one in which there was an increasing departure from the 1:1 relationship with declining  $P_e$  (Fig. 4a), and one in which there was a constant offset from the 1:1 line (Fig. 4b,c). The patterns observed suggest that phylogeny and evolutionary adaptations to certain habitat types were important deter-

minants of relationships between  $P_e$  and  $P_{50}$ . Among 15 vesselless angiosperms,  $P_e - P_{50}$  remained nearly constant at about 1 MPa over a 3.5 MPa range of  $P_e$ , whereas  $P_e - P_{50}$  was about 4.5 MPa over an 8 MPa range of  $P_e$  in 28 species of Cupressaceae and angiosperms native largely to arid and Mediterranean climate zones. In other conifers and angiosperms native to more mesic climate zones  $P_e - P_{50}$  increased with declining  $P_e$ . The species represented in Fig. 4 do not entirely overlap with those in Fig. 3 because published data on  $\Psi_{\text{stem}}$  were not available for many of the species in Fig. 4. The overall trends in hydraulic safety margins were consistent with a diminishing role of  $C$  as  $\Psi_{\text{stem}}$  declined (Fig. 5), increasing the likelihood that stomatal responses would not be rapid enough to avoid catastrophic hydraulic failure unless safety margins increased or were consistently large.

#### CAPACITANCE AND HYDRAULIC SAFETY

Stomata regulate xylem pressure under dynamic conditions in which water released into the transpiration stream via discharge of capacitance comes into play. Therefore,  $C$  must be considered in attempts to understand how intact plants avoid hydraulic failure under the dynamic conditions that prevail in the field. The buffering influence of  $C$  can be viewed as a dynamic component of overall hydraulic safety because it varies with  $\Psi_{\text{stem}}$  over daily and seasonal cycles. In contrast, properties such as  $P_e$  and  $P_{50}$  are static over the daily time scale of stomatal regulation. The patterns reported here are consistent with a partial reliance on  $C$  to confer a margin of hydraulic safety rather than sole reliance on xylem structural features that govern properties such as  $P_e$  and  $P_{50}$ . Species-specific values of  $\Psi_{\text{stem min}}$  and  $C$  were positively correlated (Fig. 5a). Additionally, previous studies have noted positive correlations between  $C$  and  $P_{50}$  and between  $C$  and  $\Psi_{\text{stem min}} - P_{50}$  (Domec & Gartner 2001; Pratt *et al.* 2007; Meinzer *et al.* 2008a). Even the slope of the steeper portion of the vulnerability curve ( $P_e - P_{50}$ ) was steepest in species with the highest  $C$  (Fig. 5b). Thus, declining species-specific values of  $C$  imply increasing reliance on xylem structural features that confer adequate hydraulic safety margins via changes in features such as  $P_e$ ,  $P_{50}$  and  $P_e - P_{50}$ .

Capacitive release of water via xylem cavitation can contribute to buffering of fluctuations in xylem pressure despite the negative impact of cavitation on hydraulic conductivity (Höltta *et al.* 2009). However, capacitive release of water from wood does not necessarily imply a trade-off involving reduced conducting efficiency. In conifers, cavitation of latewood tracheids can supply water to the transpiration stream without a significant loss of sapwood hydraulic conductivity because the conductivity of the more cavitation-resistant earlywood tracheids is about an order of magnitude greater than that of latewood tracheids (Domec & Gartner 2001, 2002). In diffuse-porous angiosperms, living axial and ray parenchyma can constitute up to 40–75% of sapwood volume (Panshin & de Zeeuw 1980; Chapotin *et al.* 2006) making these tissues a potentially important source of  $C$ . In several tropical tree species water released via cavitation of vessels accounted for only about 15% of total daily reliance on  $C$  (Meinzer *et al.* 2008a).

When stem tissue is fully hydrated, species with higher values of  $C$  are expected to show greater time constants for changes in xylem pressure in response to changes in transpiration. These attributes would partly compensate for relatively long time constants of several minutes for stomatal closure (Ceulemans *et al.* 1989; Jarvis *et al.* 1999; Allen & Pearcy 2000; Powles *et al.* 2006), permitting safety margins based on features such as  $P_e$  and  $P_{50}$  to appear small. However, as  $\Psi_{\text{stem min}}$  and  $P_e$  are approached, the hydraulic system becomes nearly inelastic because  $C$  is essentially exhausted (Figs 6 and 7) causing time constants for transpiration-induced changes in xylem pressure to approach minimum values, thereby making it difficult for stomata to respond quickly enough to dampen abrupt decreases in xylem pressure that could trigger rampant embolism. The inverse relationship between dynamic and static components of hydraulic safety represents a trade-off whose common denominator is likely to be wood density. Several studies have noted inverse relationships between wood density and  $C$  (Meinzer *et al.* 2003, 2008b; Pratt *et al.* 2007; Scholz *et al.* 2007), minimum shoot  $\Psi$  as well as variation in shoot  $\Psi$  (Stratton *et al.* 2000; Meinzer 2003; Bucci *et al.* 2004b; Jacobsen *et al.* 2007, 2008), xylem specific conductivity (Stratton *et al.* 2000; Bucci *et al.* 2004b; Meinzer *et al.* 2008b) and  $P_{50}$  (Hacke *et al.* 2001; Pratt *et al.* 2007). However, some studies have not detected a dependence of some of these traits on wood density (e.g. Jacobsen *et al.* 2007; Meinzer *et al.* 2008b).

#### Acknowledgements

This research was supported by National Science Foundation grants IBN 9905012 and IOB 0544470.

#### References

- Allen, M.T. & Pearcy, R.W. (2000) Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. *Oecologia*, **122**, 470–478.
- Begg, J.E. & Turner, N.C. (1970) Water potential gradients in field tobacco. *Plant Physiology*, **46**, 343–346.
- Brodribb, T.J. & Holbrook, N.M. (2004a) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant Cell and Environment*, **27**, 820–827.
- Brodribb, T.J. & Holbrook, N.M. (2004b) Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. *New Phytologist*, **162**, 663–670.
- Brodribb, T.J., Holbrook, N.M., Edwards, E.J. & Gutierrez, M.V. (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell and Environment*, **26**, 443–450.
- Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C. & Sternberg, L.D.L. (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell and Environment*, **26**, 1633–1645.
- Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C., Hinojosa, J.A., Hoffmann, W.A. & Franco, A.C. (2004a) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology*, **24**, 1119–1127.
- Bucci, S.J., Goldstein, G., Meinzer, F.C., Scholz, F.G., Franco, A.C. & Bustamante, M. (2004b) Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. *Tree Physiology*, **24**, 891–899.
- Ceulemans, R., Hinckley, T.M. & Impens, I. (1989) Stomatal response of hybrid poplar to incident light, sudden darkening and leaf excision. *Physiologia Plantarum*, **75**, 174–182.



- Chapotin, S.M., Razanameharizaka, J.H. & Holbrook, N.M. (2006) A biomechanical perspective on the role of large stem volume and high water content in baobab trees (*Adansonia* spp., Bombacaceae). *American Journal of Botany*, **93**, 1251–1264.
- Cochard, H., Martin, R., Gross, P. & Bogeat-Triboulot, M.B. (2000) Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. *Journal of Experimental Botany*, **51**, 1255–1259.
- Cowan, I.R. (1972) An electrical analogue of evaporation from, and flow of water in plants. *Planta*, **106**, 221–226.
- Dixon, M.A. & Tyree, M.T. (1984) A new stem hygrometer, corrected for temperature-gradients and calibrated against the pressure bomb. *Plant, Cell and Environment*, **7**, 693–697.
- Domec, J.-C. & Gartner, B.L. (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees*, **15**, 204–214.
- Domec, J.-C. & Gartner, B.L. (2002) How do water transport and storage differ in coniferous earlywood and latewood? *Journal of Experimental Botany*, **53**, 2369–2379.
- Domec, J.-C., Lachenbruch, B., Meinzer, F.C., Woodruff, D.R., Warren, J.M. & McCulloh, K.A. (2008) Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 12069–12074.
- Goldstein, G., Andrade, J.L., Meinzer, F.C., Holbrook, N.M., Cavelier, J., Jackson, P. & Celis, A. (1998) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell and Environment*, **21**, 397–406.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D. & McCulloh, K.A. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia*, **126**, 457–461.
- Höltta, T., Cochard, H., Nikinmaa, E. & Mencuccini, M. (2009) Capacitive effect of cavitation in xylem conduits: results from a dynamic model. *Plant, Cell and Environment*, **32**, 10–21.
- Hubbard, R.M., Ryan, M.G., Stiller, V. & Sperry, J.S. (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell and Environment*, **24**, 113–121.
- Jacobsen, A.L., Agenbag, L., Esler, K.J., Pratt, R.B., Ewers, F.W. & Davis, S.D. (2007) Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology*, **95**, 171–183.
- Jacobsen, A.L., Pratt, R.B., Davis, S.D. & Ewers, F.W. (2008) Comparative community physiology: nonconvergence in water relations among three semi-arid shrub communities. *New Phytologist*, **180**, 100–113.
- Jarvis, A.J., Young, P.C., Taylor, C.J. & Davies, W.J. (1999) An analysis of the dynamic response of stomatal conductance to a reduction in humidity over leaves of *Cedrella cordata*. *Plant, Cell and Environment*, **22**, 913–924.
- Jones, H.G. & Sutherland, R.A. (1991) Stomatal control of xylem embolism. *Plant, Cell and Environment*, **14**, 607–612.
- Maherali, H., Pockman, W.T. & Jackson, R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology*, **85**, 2184–2199.
- Maherali, H., Moura, C.F., Caldeira, M.C., Willson, C.J. & Jackson, R.B. (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant, Cell and Environment*, **29**, 571–583.
- Meinzer, F.C. (2003) Functional convergence in plant responses to the environment. *Oecologia*, **134**, 1–11.
- Meinzer, F.C. & Grantz, D.A. (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant, Cell and Environment*, **13**, 383–388.
- Meinzer, F.C., Clearwater, M.J. & Goldstein, G. (2001) Water transport in trees: current perspectives, new insights and some controversies. *Environmental and Experimental Botany*, **45**, 239–262.
- Meinzer, F.C., James, S.A., Goldstein, G. & Woodruff, D.R. (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant, Cell and Environment*, **26**, 1147–1155.
- Meinzer, F.C., James, S.A. & Goldstein, G. (2004) Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. *Tree Physiology*, **24**, 901–909.
- Meinzer, F.C., Woodruff, D.R., Domec, J.-C., Goldstein, G., Campanello, P.I., Gatti, M.G. & Villalobos-Vega, R. (2008a) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia*, **156**, 31–41.
- Meinzer, F.C., Campanello, P.I., Domec, J.C., Gatti, M.G., Goldstein, G., Villalobos-Vega, R. & Woodruff, D.R. (2008b) Constraints on physiological function associated with branch architecture and wood density in tropical forest trees. *Tree Physiology*, **28**, 1609–1617.
- Melcher, P.J., Goldstein, G., Meinzer, F.C., Yount, D.E., Jones, T., Holbrook, N.M. & Huang, C.X. (2001) Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics of embolism formation and repair. *Oecologia*, **126**, 182–192.
- Mencuccini, M. (2003) The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant Cell and Environment*, **26**, 163–182.
- Nardini, A. & Salleo, S. (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees*, **15**, 14–24.
- Panshin, A.J. & de Zeeuw, C. (1980) *Textbook of Wood Technology*. McGraw-Hill, New York.
- Phillips, N., Nagchaudhuri, A., Oren, R. & Katul, G. (1997) Time constant for water transport in loblolly pine trees estimated from time series of evaporative demand and stem sapflow. *Trees*, **11**, 412–419.
- Phillips, N.G., Oren, R., Licata, J. & Linder, S. (2004) Time series diagnosis of tree hydraulic characteristics. *Tree Physiology*, **24**, 879–890.
- Powles, J.E., Buckley, T.N., Nicotra, A.B. & Farquhar, G.D. (2006) Dynamics of stomatal water relations following leaf excision. *Plant, Cell and Environment*, **29**, 981–992.
- Pratt, R.B., Jacobsen, A.L., Ewers, F.W. & Davis, S.D. (2007) Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist*, **174**, 787–798.
- Ritchie, G.A. & Hinckley, T.M. (1971) Evidence for error in pressure-bomb estimates of stem xylem potentials. *Ecology*, **30**, 534–536.
- Sack, L. & Holbrook, N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology*, **57**, 361–381.
- Santiago, L.S., Goldstein, G., Meinzer, F.C., Fisher, J.B., Machado, K., Woodruff, D. & Jones, T. (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia*, **140**, 543–550.
- Scholz, F.G., Bucci, S.J., Goldstein, G., Meinzer, F.C., Franco, A.C. & Miralles-Wilhelm, F. (2007) Biophysical properties and functional significance of stem water storage tissues in neotropical savanna trees. *Plant Cell and Environment*, **30**, 236–248.
- Sparks, J.P. & Black, R.A. (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiology*, **19**, 453–459.
- Sperry, J.S. (2004) Coordinating stomatal and xylem functioning – an evolutionary perspective. *New Phytologist*, **162**, 568–570.
- Sperry, J.S., Alder, N.N. & Eastlack, S.E. (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *Journal of Experimental Botany*, **44**, 1075–1082.
- Stratton, L., Goldstein, G. & Meinzer, F.C. (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. *Plant, Cell and Environment*, **23**, 99–106.
- Tognetti, R., Longobucco, A. & Raschi, A. (1998) Vulnerability of xylem to embolism in relation to plant hydraulic resistance in *Quercus pubescens* and *Quercus ilex* co-occurring in a Mediterranean coppice stand in central Italy. *New Phytologist*, **139**, 437–447.
- Tyree, M.T. & Sperry, J.S. (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology*, **88**, 574–580.
- Vogt, U.K. (2001) Hydraulic vulnerability, vessel refilling, and seasonal courses of stem water potential of *Sorbus aucuparia* L. and *Sambucus nigra* L. *Journal of Experimental Botany*, **52**, 1527–1536.
- Woodruff, D.R., McCulloh, K.A., Warren, J.M., Meinzer, F.C. & Lachenbruch, B. (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell and Environment*, **30**, 559–569.
- Zwieniecki, M.A. & Holbrook, N.M. (1998) Diurnal variation in xylem hydraulic conductivity in white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). *Plant, Cell and Environment*, **21**, 1173–1180.

Received 29 December 2008; accepted 13 March 2009

Handling Editor: David Whitehead

## Supporting information

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

**Table S1.** Stem xylem vulnerability characteristics and daily minimum stem water potential.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.