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Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees

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ABSTRACT

This study examined the linkage between xylem vulnerability, stomatal response to leaf water potential (Ψ_L), and loss of leaf turgor in eight species of seasonally dry tropical forest trees. In order to maximize the potential variation in these traits species that exhibit a range of leaf habits and phenologies were selected. It was found that in all species stomatal conductance was responsive to Ψ_L over a narrow range of water potentials, and that Ψ_L inducing 50% stomatal closure was correlated with both the Ψ_L inducing a 20% loss of xylem hydraulic conductivity and leaf water potential at turgor loss in all species. In contrast, there was no correlation between the water potential causing a 50% loss of conductivity in the stem xylem, and the water potential at stomatal closure (Ψ_{SC}) amongst species. It was concluded that although both leaf and xylem characters are correlated with the response of stomata to $\Psi_{L,}$ there is considerable flexibility in this linkage. The range of responses is discussed in terms of the differing leaf-loss strategies exhibited by these species.

Key-words: cavitation; percentage loss of conductivity; pressure–volume curve; stomatal closure; tropical dry forest trees; turgor loss point; water potential.

INTRODUCTION

During the process of transpiration, water in the xylem of plants comes under a physical tension as low water potentials in leaf tissues effectively pull water from the soil through the vascular system into the leaf. This process can generate tensions of sufficient magnitude to 'seed' air bubbles through pit membranes and into the water column resulting in cavitation (Zimmermann 1983; Tyree & Sperry 1989). Once xylem cells are filled with air (embolized) they no longer function to conduct water unless they can be refilled. This has serious implications for photosynthesis and growth, which are limited by the efficiency of water supply from the soil to the leaves through the xylem (Kramer & Boyer 1995; Brodribb & Feild 2000; Hubbard *et al*. 2001). A series of recent papers have described associations between the xylem water potential at the onset of xylem cavitation and the leaf water potential (Ψ_L) triggering incipient stomatal closure (Hubbard *et al*. 2001; Nardini, Tyree & Salleo 2001; Cochard *et al*. 2002). This type of response from the stomata is believed to prevent cavitation-induced decreases in plant conductivity which, if left unchecked, could trigger 'runaway cavitation' in the xylem, depriving leaves of water supply and potentially causing leaf death (Sperry & Pockman 1993).

Our current state of knowledge is based on quantitative comparisons between declining stomatal and xylem conductivities in response to decreasing water potential in leaves and xylem. These comparisons have been made using 'vulnerability curves', which indicate the percentage decrease in xylem conductivity (or increase in acoustic emissions) due to embolism as decreasing water potentials are imposed (Tyree & Sperry 1989). Detailed examinations of xylem vulnerability in the stems, petioles and leaves of a small selection of domestic temperate trees have revealed qualitative associations between the water potential found to induce xylem cavitation in stems (Nardini & Salleo 2000; Cochard *et al*. 2002) or leaves (Salleo *et al*. 2001), and a reduction in stomatal conductance. The nature of this association remains vague, however, with uncertainty in the location and proportion of xylem cavitation required to trigger a guard cell response. The mechanism for this control of stomatal aperture also remains poorly understood, but it appears most likely that interactions between transpiration rate, bulk leaf water potential, epidermal water potential and guard cell turgor are largely responsible for maintaining Ψ_L above the threshold for xylem cavitation.

Assuming stomatal guard cells directly translate physical water potential signals in the leaf and epidermis into changes in pore aperture, and that the transduction of these signals are governed by physical attributes of the guard and epidermal cells, it seems probable that these traits might co-evolve with traits governing xylem vulnerability. The reason for this is that a decrease in xylem conductivity after cavitation will directly affect the water potential of the leaf assuming the well-supported Ohms law analogy for water flow in plants is correct. If so, we might expect conservative relations to exist between xylem vulnerability and stomatal response to Ψ_L across the majority of plant species.

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A possible mechanism linking xylem cavitation and stomatal closure may be through the effects of leaf turgor on the guard cells. This would occur if xylem cavitation led to a drop in Ψ_L in transpiring leaves such that cells began to lose turgor, triggering stomatal closure. Given that the loss of leaf turgor pressure is recognized as the initial stage of leaf wilting, and that the loss of guard cell turgor results in stomatal closure (Cowan 1977), it is generally assumed that these characters are co-ordinated (Cowan & Farquhar 1977). This assumes homogeneity and connectivity between leaf cells, including the guard cells. However recent studies focused on the epidermis illustrate that hydraulic connectivity between epidermal and guard cells may allow the stomata to act somewhat independent of whole leaf turgor (Mott, Shope & Buckley 1999; Mott & Franks 2001).

Our principal goal was to determine how stomatal closure, xylem cavitation and the loss of cell turgor in the leaf were related, and whether a common relationship existed amongst coexisting tree species that expressed contrasting hydraulic and leaf characteristics. To test these relationships across the widest range of leaf types we chose tree species from a dry tropical forest, selecting a range of species encompassing the range from deciduous to brevi-deciduous and evergreen. Deciduousness in the species investigated here coincides with the period of low water availability during the dry season, and hence there is a good chance that leaf shedding may be linked to xylem hydraulic characteristics.

MATERIALS AND METHODS

Field site

This investigation was conducted in the Santa Rosa National Park, located on the Northern Pacific coast of Costa Rica (10°52′ N, 85°34′ W, 285 m above sea level). Mean annual rainfall in the park is 1528 mm. However, more than 90% of this falls between the months of May and December, resulting in a pronounced dry season. The dry season is accompanied by strong trade winds, low relative humidity and high irradiance, all of which produce a high evaporative demand. Diurnal and seasonal temperature ranges are relatively small.

Vegetation in the park comprises a heterogeneous mosaic consisting of various stages of regeneration from former pastures as well as some small areas of primary forest. Evergreen and deciduous species can be found at all successional stages, however, the percentage cover by evergreen species is greatest in the mature forest, and deciduous species tend to be more dominant in earlier successional stages.

Plant material

We chose eight species, four of which are deciduous, two evergreen, and two are classified as brevi-deciduous. In brevi-deciduous species an annual exchange of leaves occurs, at which time all leaves are shed and a flush of new leaves immediately follows. The deciduous species were: *Bursera simaruba* (Burseraceae), *Calycophyllum candidissimum* (Rubiaceae), *Enterolobium cyclocarpum* (Fabaceae), and *Rhedera trinervis* (Verbenaceae). Evergreen species were: *Simarouba glauca* (Simarubaceae), *Quercus oleoides* (Fagaceae) and brevi-deciduous species: *Hymenaea courbaril* (Fabaceae), and *Sweitenia macrophylla* (Meliaceae). All experimental trees were located on level, open ground, ensuring good access to fully illuminated branches.

Xylem vulnerability

Stem xylem vulnerability was determined by bench drying in all species, and verified by pressure injection in three species. Bench drying involved cutting and bagging several branches from each of three trees per species early in the morning. Approximately 10 leaves per branch were sealed into plastic bags and wrapped with aluminium foil to prevent water loss. These non-transpiring leaves served to measure the water potential in the xylem of thetest branches. The branches were allowed to desiccate during the course of the day and periodic measurements of percentage embolism and Ψ_L were made during drying. In species with compound leaves the petiole was used for vulnerability measurements, whereas in species with simple leaves, new, green branches similar in dimension to the petioles of compound leaf species were used. An earlier study showed no evidence to suggest strong vulnerability segmentation in these species (Brodribb, Holbrook & Gutiérrez 2002).

Embolism was quantified using branch or petiole segments of approximately 2 cm (shorter than the shortest vessels). These segments were excised under water and their conductivity measured by flowing filtered (to 0.1 μm) water from a reservoir 30 cm above the segment, through the segment and onto a computer-interfaced balance. Reservoir pressure (3 kPa) was low enough to ensure that embolisms could not be flushed from the open vessels during initial measurement (Cochard *et al.* 2000). After determining the initial flow rate, embolisms were flushed out of the segment by injecting water at approximately 100 kPa for 20 s. Flow rate was then re-measured under identical conditions and the difference between initial and flushed flow rates expressed as a percentage of the flushed (non-embolized) state. This figure was described as the percentage loss in conductivity (PLC). Segments were cut as far distal to the initial cut as possible on branches to avoid including embolisms induced by cutting in the field, and in species with large compound leaves, petioles were used. The xylem water potential was determined by measuring the water potential of bagged leaves attached to the sample branch. Water potential readings were made with a pressure chamber (PMS, Corvallis, OR, USA). Generally six to 10 measurements were made from the branches of each of three replicate trees per species, with the branches being discarded once the leaves became too desiccated to yield reliable Ψ_L measurements. Plots of PLC versus Ψ_L were made for each replicate tree, with cumulative normal distribution curves fitted (Brodribb & Hill 1999). The Ψ_L corresponding to 20% (Ψ_{20}) and 50% PLC (Ψ_{50}) was read from these curves and a mean value calculated for each species.

A second method of xylem vulnerability testing was used in three species. This involved an adaptation of the pressure injection method (Sperry & Pockman 1993) in which branches were cut underwater, bagged and stored with the cut end underwater until Ψ_L was > –0.02 MPa. Leaf laminas distal to the test segment were cut away leaving only the midrib and first-order veins and branches sealed into a pressure chamber so that the dissected leaves were inside and the rest of the branch outside. Test pressures were applied for 3 min after which branch segments were removed from the chamber, excised underwater, and PLC measured as described above.

Leaf water relations

Stomatal response to Ψ_L was measured in all species under natural conditions as well as using excised branched to determine the behaviour of stomata under extreme drought. Evergreen species were surveyed during the months of May (end of the dry season) until July (wet season), and deciduous species measured in June and July once the leaves were fully expanded. All measurements were made on four trees of each species and under conditions of full sun. Stomatal conductance (g_s) was measured using a Li-Cor 1600 porometer (Li-Cor Inc., Lincoln, NE, USA) at different times of the day between 0900 and 1400 hours in order to include a maximum range of leaf water potentials. Stomatal conductance was recorded from a series of marked leaves that were subsequently removed and bagged for later determination of Ψ_L. The relationship between Ψ_L and g_s was plotted and curves fitted assuming a cumulative normal probability distribution. We defined the water potential at 50% g_s as the Ψ_L at stomatal closure (Ψ_{sc}) because the incipient stages of stomatal closure were difficult to distinguish due to variation between leaves, whereas by contrast Ψ_L at 50% g_s was very distinct.

In all species, Ψ_L was surveyed (when leaves were present) approximately every 20 d over the course of 1 year. Measurements of four tagged trees of each species were made at midday while Ψ_L was at its minimum diurnal value.

The water potential at the leaf turgor loss point was measured by pressure–volume analysis using the bench drying technique described by (Koide *et al.* 1991). Measurements were made on two occasions in evergreen species and once for deciduous species. The evergreen and bevi-deciduous species were sampled once at the end of the dry season (May) and once during the wet season (June – July). The deciduous species were sampled only once in June – July after the beginning of the wet season when the leaves were fully mature. Branches were cut underwater in the morning and rehydrated until Ψ_L was > –0.05 MPa, after which four leaves per species were detached, and pressure–volume relations determined. For each species the process of weighing and measuring Ψ_L in leaves was continued until leaf water potentials began to rise due to cell damage. All leaves selected were of approximately uniform age across all species, to avoid the possible effects of leaf age. All measurements of pressure–volume relations overlapped with measurements of g_s and xylem vulnerability.

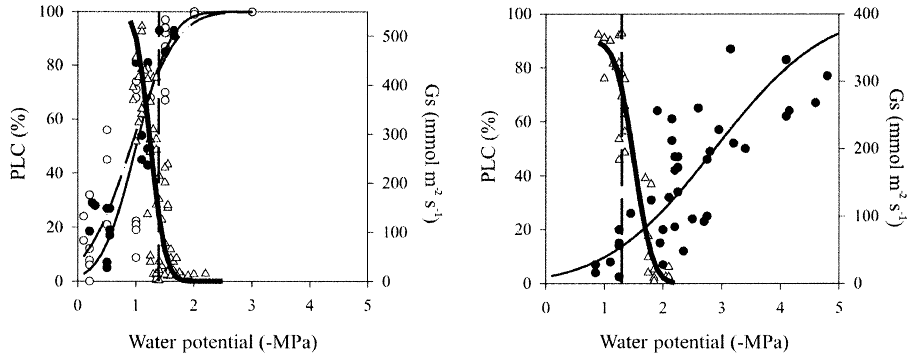
RESULTS

Little variation in the water potential at turgor loss point (Ψ_{TLP}) was observed within species, although between species more than a two-fold range in Ψ_{TLP} was measured. The four deciduous species exhibited the highest Ψ_{TLP} (from –1.30 to –1.82 MPa in *C. candidissimum* and *E. cyclocarpum*, respectively), whereas brevi-deciduous and evergreen species exhibited lower Ψ_{TLP} (Table 1). No significant change in Ψ_{TLP} was found between the end of the wet season and early dry season in the non-deciduous species, and hence data for each species were pooled.

Xylem vulnerability was highly variable between species and the mean water potential at 50% loss of xylem conductivity (Ψ_{50}) ranged from a maximum of –1.00 MPa in *B. simaruba* to a minimum of –3.03 MPa in *Q. oleoides* (Table 1). In the three species in which air injection was used to verify Ψ_{50} determined by bench drying, the mean value of Ψ_{50} by air injection was consistently slightly higher than that for bench drying (Fig. 1). However the difference in means was less than 0.4 MPa in each case and the shape

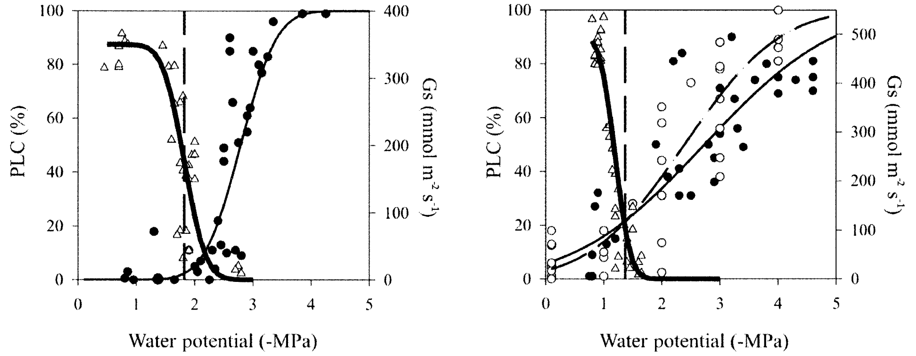
Table 1. Leaf habit, mean water potential at 50% stomatal closure (Ψ_{SC}) leaf turgor loss (Ψ_{TLP}), mean water potential at 20% xylem cavitation (Ψ_{20}) and 50% xylem cavitation (Ψ_{50}) in the eight species investigated

	Leaf habit	Ψ_{SC} (MPa)	Ψ_{TLP} ± SE (MPa)	Mean Ψ_{20} (MPa)	Ψ_{50} ± SE (MPa)
Bursera simaruba	Deciduous	–1.25	–1.39 ± 0.01	–0.61	–1.00 ± 0.03
Calycophyllum candidissimum	Deciduous	–1.54	–1.30 ± 0.05	–1.67	–2.87 ± 0.11
Enterolobium cyclocarpum	Deciduous	–1.82	–1.82 ± 0.01	–1.39	–2.73 ± 0.03
Rhedera trinervis	Deciduous	–1.20	–1.37 ± 0.09	–1.29	–2.80 ± 0.22
Hymenaea courbaril	Brevi-deciduous	–2.41	–2.17 ± 0.06	–2.30	–3.00 ± 0.17
Sweitenia macrophylla	Brevi-deciduous	–1.74	–2.15 ± 0.05	–1.65	–2.20 ± 0.18
Simarouba glauca	Evergreen	–1.33	–2.21 ± 0.01	–1.85	–2.00 ± 0.06
Quercus oleoides	Evergreen	–2.76	–3.12 ± 0.01	–2.65	–3.03 ± 0.23



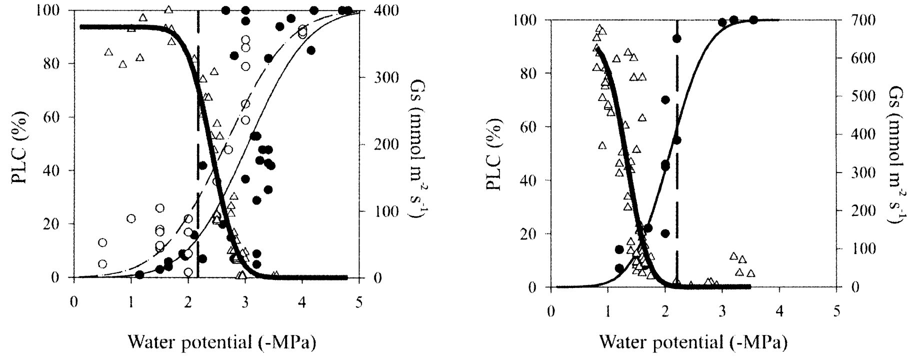
(a) *Bursera simaruba*

(b) *Calycophyllum candidissimum*



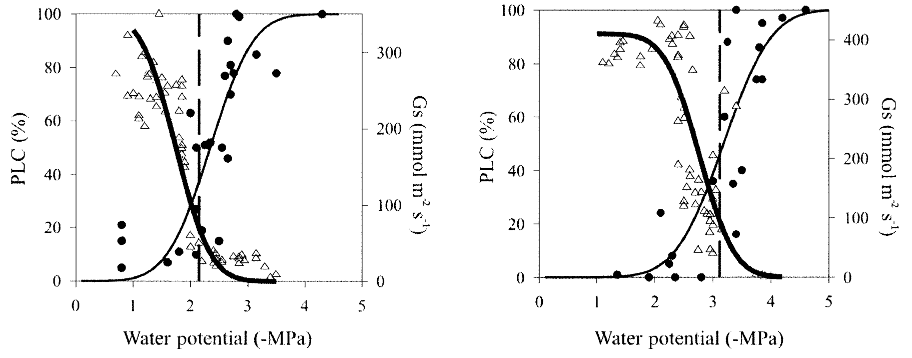
(c) *Enterolobium cyclocarpum*

(d) *Rhedera trinervis*



(e) *Hymenaea courbaril*

(f) *Simarouba glauca*



(g) *Sweitenia macrophylla*

(h) *Quercus oleoides*

of vulnerability curves derived from bench drying and air seeding also appeared similar (Fig. 1). Unfortunately the xylem of most species was found to contain embolisms at the time of collection despite the fact that measurements were made during the wet season. However, even in species in which native embolism was significant such as *B. simaruba* (Fig. 1), the native embolism never exceeded 30% and was usually much lower. The difference between Ψ_{50} and Ψ_{20} in most species was less than 0.5 MPa, however, in the three deciduous species *C. candidissimum*, *E. cyclocarpum* and *R. trinervis*, a much more gradual transition between 20 and 50% PLC was observed, leading to a difference of more than 1 MPa between Ψ_{50} and Ψ_{20} (Table 1). Although the most vulnerable species was deciduous, and the least vulnerable species evergreen, there was no clear link between leaf habit and xylem vulnerability (Table 1).

A general pattern in the stomatal response to leaf water potential was seen in all species, in which stomatal conductance was responsive to Ψ_L only over a narrow range of Ψ_L (Fig. 1). As a result, the transition from 90 to 20% of maximum g_s in each species occurred over a band of Ψ_L that was less than 1 MPa. Despite this rapid transition, most species exhibited a continuous response of g_s to Ψ_L as evidenced by the fact that many species did not achieve a plateau in g_s at high Ψ_L, and that midday g_s during the rainy season was commonly maintained at about 40–50% of maximum. Variation between species was expressed in the water potential that produced strong decreases in g_s and the range of Ψ_L to which stomatal aperture appeared to respond. All deciduous species were found to be isohydric (Tardieu & Simonneau 1998) during the wet season, and the non-deciduous species anisohydric with the exception of *S. glauca*. To distinguish natural variation in maximum g_s within species from stomatal closure resultant from low Ψ_L we defined this threshold as the Ψ_L causing 50% stomatal closure (Ψ_{sc}). A large amount of variation in Ψ_{sc} was evident between species, with the range extending from –1.20 MPa in *R. trinervis* to –2.76 MPa in *Q. oleoides*. The highest sensitivity to Ψ_L was observed in deciduous species in which g_s responded to a range of less than 0.5 MPa in Ψ_L, whereas g_s in some evergreens, such as *Q. oleoides*, responded to a range of more than 2.5 MPa (Fig. 1).

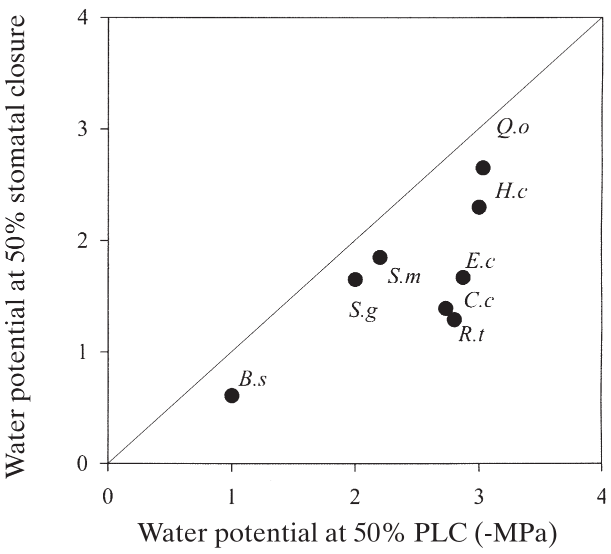


Figure 3. Mean water potential at 50% loss of xylem conductivity (Ψ_{50}) versus water potential producing a 50% reduction in stomatal conductance (Ψ_{SC}). No significant correlation was observed, although this was largely due to the poor correlation within the deciduous species. Species labelled as in Fig. 2.

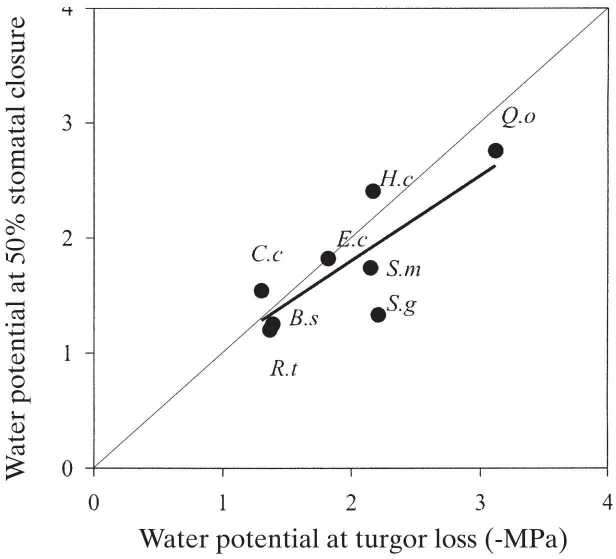


Figure 2. Mean water potential at turgor loss versus (Ψ_{TLP}) versus water potential at 50% stomatal closure (Ψ_{SC}). A highly significant linear regression is shown ($r^2 = 0.65$) that had a slope not significantly different to a 1 : 1 relationship (dotted line). Species are labelled on the graph; *Bursera simaruba* (*B.s*), *Calycophyllum candidissimum* (*C.c*) *Enterolobium cyclocarpum* (*E.c*) *Hymenaea courbaril* (*H.c*), *Rhedera trinervis* (*R.t*), *Simarouba glauca* (*S.g*), *Sweitenia macrophylla* (*S.m*) and *Quercus oleoides* (*Q.o*).

A strong correlation ($r^2 = 0.65$; $P < 0.01$) was found between mean Ψ_{sc} and Ψ_{TLP} for each species (Fig. 2). The regression between these parameters was not significantly different to a 1 : 1 relationship (analysis of covariance). There was no correlation between the water potential at 50% cavitation (Ψ_{50}) and Ψ_{sc} (Fig. 3), however, water potential at incipient cavitation (Ψ_{20}) strongly correlated with mean Ψ_{sc} ($r^2 = 0.74$; $P < 0.01$; Fig. 4). The lack of cor-

Figure 1. Simultaneous plots of the response of stomatal conductance (△) and xylem cavitation to water potential, in deciduous (a–d) and non-deciduous species (e–h). Response of xylem cavitation (PLC) to water potential was measured by bench drying in all species (●) and verified by embolism induction through air injection (○) in three species, *Bursera simaruba*, *Calycophyllum candidissimum* and *Hymenaea courbaril*. Mean water potential at turgor loss point is shown for each species as a dotted vertical line. Curves are fitted to vulnerability data from bench drying (thin line), air seeding (dotted line), and stomatal response data (thick line) assuming a cumulative normal distribution.

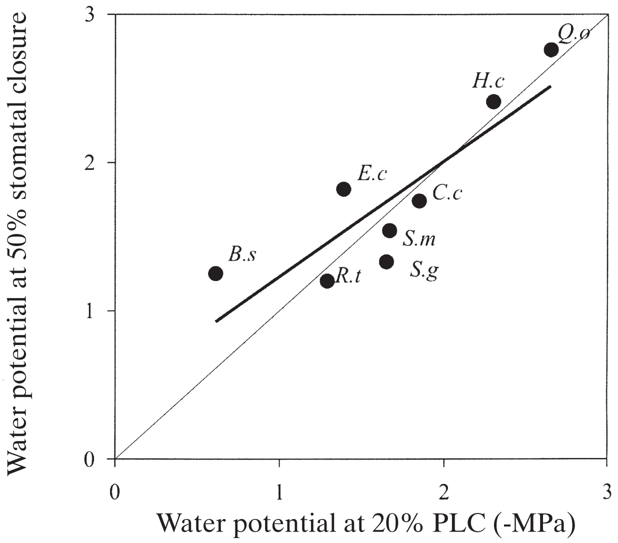


Figure 4. Mean water potential at 20% loss of xylem conductivity (Ψ_{20}) versus Ψ_{SC}. A strong linear correlation ($r^2 = 0.76$) is illustrated, and a 1 : 1 line drawn for comparison.

relation between Ψ_{50} and Ψ_{sc} was due to the cavitation-resistant xylem expressed in three of the deciduous species, all of which produced relatively soft leaves with high Ψ_{TLP} and Ψ_{SC}. Examination of two of these species (*C. candidissimum* and *R. trinervis*) towards the end of the wet season (November) immediately prior to the start of leaf shedding, illustrated a significant hardening of leaves. The shape of the stomatal response to Ψ_L of these species in November was much closer to the shape of their vulnerability curves (Fig. 5).

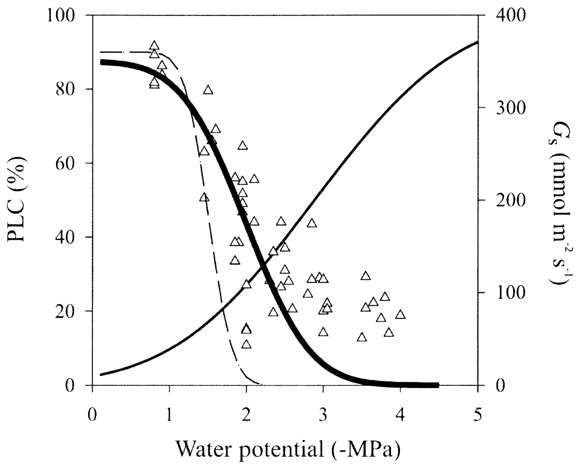
DISCUSSION

The data presented here illustrate that among this phenologically diverse group of species, stomatal closure and xylem cavitation are linked in terms of a correlation between the water potential at incipient cavitation (Ψ_{20}) and stomatal closure (Ψ_{sc}; Fig. 4). This agrees with the results of several studies in which qualitative relationships were described between xylem cavitation and stomatal closure in temperate northern-hemisphere species (Sperry & Saliendra 1994; Nardini & Salleo 2000; Nardini *et al.* 2001; Cochard *et al.* 2002) and in conifers (Brodribb & Hill 1999).

Although Ψ_{TLP} was strongly correlated with the threshold water potential inducing 50% stomatal closure (Fig. 2), it was surprising to note that this leaf trait was not uniquely associated with stomatal closure. In general Ψ_{TLP} fell between the incipient and final stages of stomatal closure (Fig. 1), but strong variation was noted between species. In *H. courbaril* for example, Ψ_{TLP} occurred at the earliest stage of stomatal closure, but in *S. glauca* leaf cells were found to lose turgor at a water potential which induced 99% stomatal closure (Fig. 1). Thus in species such as *S. glauca*, most of the stomatal response to Ψ_L occurs as mesophyll cell turgor declines, whereas in Hymenaea, the stomatal closure occurs after the bulk of leaf cells reach zero turgor pressure. Such uncoupling between stomatal closure and bulk leaf water status suggest that the pressure–volume relation for guard cells is different to that of the bulk leaf, or that guard cells are somewhat hydraulically isolated from the rest of the leaf. These conclusions are supported by evidence for the co-ordination between stomata whereas bulk leaf conditions remain unchanged (Mott & Franks 2001).

The data presented here indicate that although both xylem cavitation and turgor loss are associated with the water potential at stomatal closure, they are not necessarily correlated and neither exerts unique control. Instead, it

(a) *Calycophyllum candidissimum*



(b) *Rhedera trinervis*

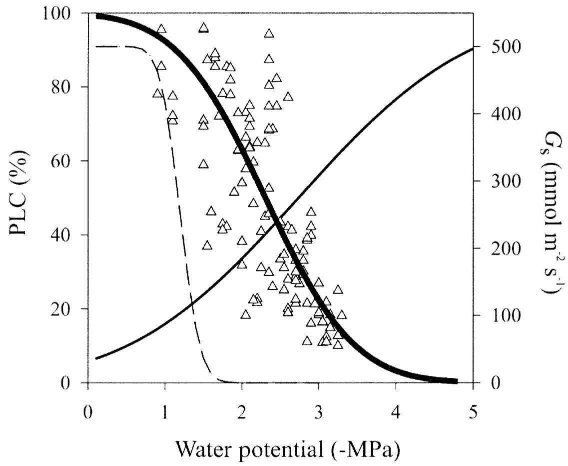


Figure 5. Stomatal response to Ψ_L in *Calycophyllum candidissimum* (a) and *Rhedera trinervis* (b) at the end of the wet season (May). Curves are fitted to the stomatal response data for May (thick line) stomatal responses of young leaves in July (dotted lines). Also shown are vulnerability curves (single thin line) for each species as measured at the start of the wet season (July).

appears that a complex association exists between xylem cavitation, Ψ_{TLP} and stomatal aperture. This follows given that the proposed mechanism linking stomatal closure and xylem cavitation is not direct, and is thought to operate via the effects of cavitation-induced decreases in xylem conductivity on Ψ_L. Therefore the basic principal revolves around a 'set point' in Ψ_L that once reached, initiates stomatal closure. Recent research has shown that this set point is breached in transpiring leaves at a similar water potential to that which initiates xylem cavitation in leaves or branch tips (Nardini *et al*. 2001; Cochard *et al*. 2002). However, the role of xylem vulnerability in the process of stomatal closure is likely to be complex, and responsive to anatomical features of both the leaf and xylem. Correlations between leaf and xylem anatomy have been shown to exist (Aasamaa, Sober & Rahi 2001), although the characteristics of this linkage are almost certainly subject to evolutionary selection. Under exposure to contrasting environments, evolutionary forces acting on the linkage between xylem hydraulics and guard cell properties would be expected to yield variability. This is especially so when considered in the light of variation in hydraulic strategies that are known to exist even amongst co-occurring species (Kolb & Davis 1994; Jarbeau, Ewers & Davis 1995; Nardini, Lo Gullo & Salleo 1999; Brodribb *et al*. 2002). The large discrepancy between Ψ_{50} and Ψ_{SC} in three of the four deciduous species examined here (Fig. 3) provides evidence of this variation.

It was observed here that in some species, 50% stomatal closure occurred at water potentials up to 1.4 MPa above Ψ_{50} (*C. candissisimum*; Fig. 1), whereas in others, Ψ_{SC} occurred at water potentials less than Ψ_{50} (*B. simaruba*; Fig. 1). The weak linkage between Ψ_{50} and Ψ_{SC} in early wet season leaves of the deciduous species may result from a differential ability of xylem and leaf tissue to adapt to changing water potentials during the wet season. It appears that for *C. candidissimum* and *R. trinervis*, the xylem produced during the early wet season possesses a vulnerability relationship tuned to resisting cavitation during the drier end of the wet season rather than conditions faced during the early wet season. Leaves of these species appear able to adapt to changes in water availability by altering Ψ_{SC} (Fig. 5) and almost certainly osmotic adjustment. As a result Ψ_{SC} and Ψ_{50} at the end of the dry season are much better matched (Fig. 5).

In a related study, Brodribb *et al*. (2002) illustrated contrasting hydraulic strategies among seasonally dry forest trees including the drought deciduous species examined here. They showed that within the large range of seasonal patterns in hydraulic conductivity, *C. candidissimum* exhibited the highest relative xylem conductivity of all deciduous species during the dry season. This was interpreted as evidence of significant water retention in the xylem during the dry months (Brodribb *et al*. 2002). Considered in the light of data presented here it also seems likely that the large margin by which stomatal closure in *C. candidissimum* precedes Ψ_{50}, enables leaf shedding to be decoupled to some degree from xylem dysfunction. In contrast, the highly vulnerable xylem in *B. simaruba* is consistent with the large amount of native embolism measured in this species (Fig. 1a) as well as the observation that wood conductivity declined during a period of unusually low rainfall during the wet season (Brodribb *et al*. 2002). Such remarkable variation in deciduous strategies has not previously been described. However, further studies are needed to understand what determines the water potential at stomatal closure, and how this is linked to the water status of both the photosynthetic cells and the xylem.

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