

Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms

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Summary

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- Hydraulic characteristics of pteridophyte (fern and *Selaginella*) foliage were investigated to determine whether the processes of water conduction and water loss are coordinated in these early vascular plants similarly to angiosperms.
- Eight species of pteridophytes and associated woody angiosperms were examined from the sun and shade in a seasonally dry tropical forest.
- Maximum leaf hydraulic conductivity (K_{leaf}) in the four pteridophytes was within the range of the sampled shade angiosperms but much lower than that of the sun-dwelling angiosperms. Hydraulic conductivity of both angiosperm and pteridophyte leaves showed a similar response to desiccation, with K_{leaf} becoming rapidly depressed once leaf water potential fell below a threshold. Stomatal closure in angiosperms corresponded closely with the water potential responsible for 50% loss of K_{leaf} while pteridophytes were found to close stomata before K_{leaf} depression.
- The contrasting behaviour of stomata in this sample of pteridophytes suggest that this may be an intrinsic difference between pteridophytes and angiosperms, with lower safety margins in angiosperms possibly enhancing both optimization of gas exchange and xylem investment.

Key words: leaf hydraulics, cavitation, stomatal closure, ferns, stomatal optimization, vulnerability, leaf xylem.

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Introduction

Pteridophytes (the ferns and their allies) are thought to have evolved some time in the Lower Devonian, around 250 million years before the appearance of flowering plants. At this time forests of arborescent ferns and lycopods dominated the globe (Stewart & Rothwell, 1993). Disappearance of these Pteridophyte forests is generally attributed to the rise of seed plants and is indicated by a marked decline in fern and lycopod (fossil) species diversity concomitant with the radiation of gymnosperm families in the Triassic epoch (Niklas *et al.*, 1985). An important innovation that led to spermatophyte success over pteridophytes is believed to be the retention of the female gametophyte on the sporophyte parent, thereby alleviating the dependence, exhibited by pteridophytes, upon a moist environment for the growth of their nonvascular gametophyte generation (Page, 2002). Despite this, the sporophytes of many pteridophyte species

exhibit a wide range of tolerance of soil water deficit. Hence, it is not uncommon for pteridophytes (ferns, *Selaginella* and *Lycopodium*) to inhabit seasonally dry environments, albeit as understorey or ground cover (Page, 2002).

Pteridophyte water relations are potentially informative about the course of physiological evolution that led to the current vascular supremacy of angiosperms. Pteridophytes represent the first extant sporophytic generation to possess stomata unequivocally involved in the regulation of CO₂ uptake, as well as an endohydric vascular system often containing unusual vessels (Carlquist & Schneider, 2001), and an elaborate array of photosynthetic structures. The vascular system of pteridophytes is particularly interesting as it possesses primitive traits such as scalariform pitting, as well as apparently derived traits such as vessels. Clearly, pteridophytes represent a key group in the evolutionary history of vascular plants, and it is therefore surprising that so little is known about their water relations.

Studies have indicated that the hydraulic conductivity of fern stipes range from the low end of angiosperms to several orders of magnitude lower (Woodhouse & Nobel, 1982; Veres, 1990), resulting in large pressure gradients during transpiration (Nobel, 1978). These observations have been used to explain the low photosynthetic and growth rates typically exhibited by pteridophytes, suggesting inefficient delivery of water to the mesophyll as a limiting factor to photosynthesis (Woodhouse & Nobel, 1982). By contrast, a recent study demonstrated high photosynthetic rates (implying high hydraulic conductivity) and low stomatal sensitivity to changes in vapour pressure deficit (VPD) (implying the opposite) in two vessel bearing fern species (Franks & Farquhar, 1999). Hydraulic conductivity data is difficult to place in perspective without any information about xylem vulnerability to cavitation due to transpiration-induced water tension. To our knowledge, nothing is known about the vulnerability of fern vascular tissues to cavitation, a surprising omission considering the clear preference exhibited by pteridophytes for moist environments.

In this paper we examine leaf hydraulic conductivity and vulnerability in a selection of ferns to determine their hydraulic performance relative to a range of coexisting angiosperms. We focus on the relationship between stomatal closure and xylem vulnerability to test whether the responses of stomatal and xylem conductances to declining water potential are coordinated in pteridophytes as they are in angiosperms. A close correlation between these characters has been demonstrated in recent work on tropical (Brodribb & Holbrook, 2003) and temperate tree species (Nardini *et al.*, 2001; Cochard *et al.*, 2002) indicating that stomata begin to close at water potentials that initiate a decline in stem xylem conductivity (presumably due to cavitation). The similarity between stomatal and xylem responses to leaf water potential (Ψ_{leaf}) in angiosperm leaves is such that hydraulic conductivity can be significantly reduced at water potentials that induce only a 50% drop in stomatal conductance (Brodribb & Holbrook, 2003). As a result, leaves under moderate evaporational stress can be exposed daily to water potentials capable of inducing significant cavitation, requiring daily repair (Bucci *et al.*, 2003; Brodribb & Holbrook, 2004). We were interested to know if pteridophytes operate at water potentials close to their cavitation limits or whether stomatal regulation is more conservative as a result of high epidermal conductances to water vapour and low stomatal responsiveness (Heiser *et al.*, 1996).

We selected species from the seasonally dry tropical forest of north-western Costa Rica, where large seasonal variation in soil and atmospheric water deficit were expected to foster dynamic behaviour in pteridophyte species. Experimental work was carried out on site in the Santa Rosa National Park, an area where rainfall is restricted to a 6-month period which is reliably interrupted midway by an interval of two to four rainless weeks. These fluctuating conditions result in plant species being exposed to a great range of both soil moisture and evaporative conditions. At this site, angiosperms exhibit

an impressive range of water-use 'strategies', each of which is assembled from a suite of highly variable traits, including leaf habit, leaf and stem xylem vulnerability, stomatal behaviour and root characters. Unlike angiosperms, pteridophytes are not well represented in the Santa Rosa seasonally dry forest, presumably owing to the aforementioned seasonal dynamics. There is however, a small group of hardy perennial ferns from the families Adiantaceae, Blechnaceae, Gleicheniaceae (Filicopsida) and Selaginellaceae (Lycopsida) which were chosen for examination.

Materials and Methods

Study site and plant material

All field and laboratory work was undertaken in the Santa Rosa National Park, located close to 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 (midday minimum r.h. generally between 30% and 40%) and high irradiance, all of which contribute to the high evaporative demand. Diurnal and seasonal temperature ranges are relatively small, with a mean annual temperature of 28°C. The wet season (May to December) is interrupted by a characteristic dry period of up to 1 month, typically occurring in late July to August (Janzen, 1983). This mini-summer or 'veranillo' period provides an opportunity to examine the effects of atmospheric drought while soils remain relatively hydrated. Vegetation in the park is composed of a mosaic of deciduous, evergreen and mixed forest types depending on the successional age of forest stands, with evergreen species more prominent in more mature forest patches.

Four species of pteridophytes and four associated angiosperm species were selected from open and understorey habitats in the park. Sun-adapted pteridophytes included two fern species, *Blechnum occidentale* (Blechnaceae) and *Gleichenia bifida* (Gleicheniaceae), as well as *Selaginella pallescens* (Selaginellaceae) all of which were small-stature perennial species. Only a single shade-adapted fern species, *Adiantum lunulatum* (Adiantaceae) was found in sufficient numbers to include in this study. During the dry season all species except *S. pallescens* died back to perennial rhizomes, resprouting early in the wet season. *Selaginella pallescens* behaves as a resurrection plant, retaining foliage throughout the dry season in a desiccated state until reactivated by the first rainfall of the wet season.

Four associated angiosperm species were selected from open habitats (*Simarouba glauca* (Simaroubaceae) and *Gliricidia sepium* (Fabaceae)) and understorey habitats (*Pharus latifolius* (Poaceae) and *Hirtella racemosa* (Chrysobalanaceae)). This group included one deciduous species (*Gliricidia sepium*) and one monocot grass (*Pharus latifolius*). With the exception

of *G. sepium* which occurs in deciduous forest these species are all common within patches of evergreen forest inside the park.

All sampling was carried out in the months of July and August during the mid-wet season dry period (known as the *veranillo*).

Leaf hydraulic conductivity and vulnerability to cavitation

Maximum leaf hydraulic conductivity (K_{leaf}) was measured by assessing the rehydration kinetics of leaves after detachment, as described by Brodribb and Holbrook (2003). Briefly, a detached sample leaf (bagged to prevent evaporation) is allowed to take up water through the petiole for a fixed period of time and the change in water potential during rehydration used to calculate leaf conductance. The principle underlying this approach is that the rehydrating mesophyll behaves as a capacitor, charged through a resistor ($1/K_{\text{leaf}}$).

Determination of K_{leaf} requires that the initial and final leaf water potential is measured in a sample rehydrated for a known period of time. Small branches must therefore be cut and equilibrated such that stomata are closed and all leaves are at the same water potential. After measuring the initial water potential of the excised branch, leaves were cut underwater and allowed to absorb water for a period of between 30 s and 300 s. The rehydration time was chosen to allow a relaxation of water potential to about half the initial value. Water potential of rehydrated leaves was then measured using a Scholander pressure chamber (PMS, Corvallis, OR, USA) and leaf hydraulic conductance calculated from the equation:

$$K_{\text{leaf}} = C \ln[\Psi_o/\Psi_f]/t$$

(C = leaf capacitance; Ψ_o = leaf water potential before rehydration; Ψ_f = leaf water potential after rehydration for t seconds).

Mean leaf capacitance (C_{leaf}) was measured at each of the sampling periods from the slope of the pressure–volume relationship. The relationship between Ψ_l and water volume in the leaf was quantified using the bench drying technique (Tyree & Hammel, 1972). Branches were cut underwater in the morning and rehydrated until Ψ_l was > -0.05 MPa, after which six leaves per species were detached for pressure–volume (PV) determination. Leaf weight and Ψ_l were measured periodically during slow desiccation of sample leaves in the laboratory. Desiccation of leaves continued until leaf water potentials stopped falling, or began to rise due to cell damage. Owing to the elasticity of the cell walls, leaf capacitance pre- and post-turgor loss are quite different. The capacitance function was defined by measuring the turgor loss point from the inflection point of the graph of $1/\Psi_l$ vs relative water content (RWC), and then using this value as the intersection of linear regressions fitted through data either side of the turgor loss point. Slopes of these curves yielded the leaf capacitance function in terms of RWC.

Calculation of K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) requires that leaf capacitance, as determined by the pressure volume curve ($\delta\text{RWC}/\delta\Psi_l$, MPa^{-1}), be expressed in absolute terms and normalized by leaf area. To do this, the capacitance calculated from the PV curve must be multiplied by the saturated mass of water in the leaf and then divided by leaf area (Koide *et al.*, 1991). In practice, the ratios of leaf dry weight : leaf area and saturated mass of water : leaf dry weight were determined for each species, and used to calculate the leaf area normalized absolute capacitance:

$$C_{\text{leaf}} = \delta\text{RWC}/\delta\Psi_l \times (\text{DW}/\text{LA}) \times (\text{WW}/\text{DW})/\text{M}$$

(C_{leaf} = area normalized leaf capacitance; DW = leaf dry weight (g); LA = leaf area (m^2); WW = mass of leaf water at 100% RWC (g); M = molar mass of H_2O (g mol^{-1})).

The response of K_{leaf} to declining Ψ_{leaf} , commonly referred to as the xylem vulnerability, was determined for each species. This involved cutting approximately 20 branches (from five individuals per species) in the early morning when Ψ_{leaf} was high and carefully dehydrating and equilibrating branches to a range of water potentials from close to -0.5 MPa to around 2 MPa below the turgor loss point of the leaf. K_{leaf} was then measured on leaves from each of the equilibrated branches as described above. The resultant data for K_{leaf} at a range of Ψ_{leaf} was plotted up and a cumulative normal probability curve fitted (Brodribb & Hill, 1999).

Maximum stomatal conductance

Diurnal courses of stomatal conductance indicated that maximum stomatal conductance ($g_{s \text{ max}}$) in Santa Rosa trees occurred between 08:00 hours and 10:00 hours when humidity was still high and irradiance was generally close to saturating (Brodribb & Holbrook, 2004). Thus, we measured $g_{s \text{ max}}$ at 09:00–10:00 hours using a null balance porometer (Li-1600; Licor, Lincoln, NE, USA). The porometer was nulled at ambient humidity and two healthy leaves from 10 individuals of each species sampled for g_s . Mean g_s from these measurements was defined as $g_{s \text{ max}}$ for each species.

Stomatal closure and safety margin

The leaf water potential responsible for complete stomatal closure in each species was determined by measuring rates of water loss was measured on detached leaves allowed to desiccate very slowly at high humidity. Leaf water potential during desiccation was calculated from changes in RWC.

Four leaves were detached after sunset and allowed to rehydrate overnight with petioles in water. Next morning, four or five leaves were weighed and placed under a weak light of approximately $30 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in a humidified box at 75–80% r.h. Leaves were allowed to desiccate over several hours while leaf mass was monitored by regularly removing leaves from the box and weighing on an analytical balance.

Leaf transpiration (E) typically increased initially as stomata opened, then dropped as stomata closed in response to falling Ψ_{leaf} . Once E had stabilized at a minimum rate ($g_s < 10 \text{ mmol m}^{-2} \text{ s}^{-1}$, as measured with a porometer) for at least 2 h, leaves were placed in a drying oven at 70°C for 1 wk and dry weight measured. Plots of E vs time generally showed clear transitions from declining E to stable minimum E (assumed to be 100% closed stomata) and the water potential at this transition was calculated from the previously established relationship between RWC and Ψ_{leaf} .

To assess the degree of xylem protection against cavitation afforded by stomatal closure, a 'safety margin' between stomatal closure and the depression of K_{leaf} was defined. This term was quantified as the difference between the Ψ_{leaf} at complete stomatal closure and Ψ_{leaf} at 50% loss of K_{leaf} .

Results

Hydraulic conductivity and vulnerability

A large range of leaf hydraulic and stomatal conductances was found amongst the fern and angiosperm species investigated. The lowest values of K_{leaf} were observed in the leaves of shade ferns and angiosperms ($0.74 \pm 0.11 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ in *A. lunulatum* and $2.65 \pm 0.6 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ in *P. latifolius*). Leaf conductances to vapour and liquid phase water were closely correlated amongst species and across light environments in angiosperms ($r^2 = 0.99$; Fig. 1). Liquid and vapour phase conductances in ferns were also positively correlated but the slope of the regression was not significant ($r^2 = 0.68$). Although the range of K_{leaf} observed among

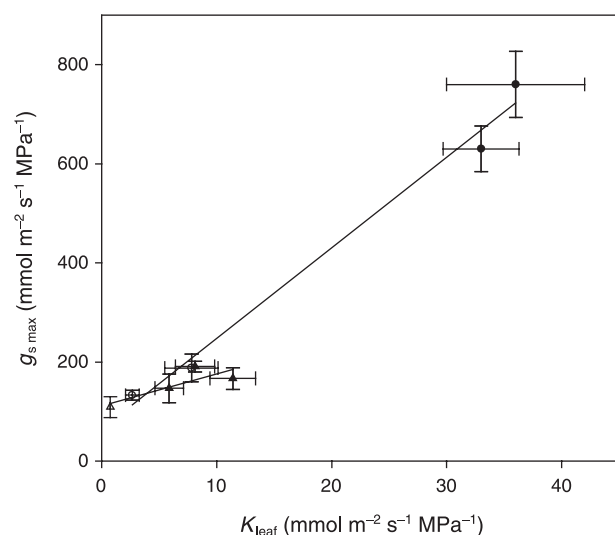


Fig. 1 Mean maximum hydraulic (K_{leaf} ; $n = 5$) and vapour phase conductances ($g_{s \text{ max}}$; $n = 10 \pm \text{SD}$) for the leaves of four angiosperms (circles) and four pteridophytes (triangles) from sunny (closed) and shaded (open) habitats. Linear regressions are fitted to both angiosperm ($r^2 = 0.99$; $P < 0.001$) and fern ($r^2 = 0.68$; $P = 0.17$) data.

pteridophyte species fell within the range for angiosperms, the difference between sun and shade angiosperms was much larger than that of the pteridophyte species. Hence, maximum K_{leaf} in the two sun-grown angiosperms (33 and $36 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$) was considerably greater than that of the sun-grown pteridophytes (5.9 – $11.4 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$).

Unlike hydraulic conductance, vulnerability to cavitation showed no relationship with either K_{leaf} or plant type (pteridophyte or angiosperm), except for the fact that the most cavitation resistant leaves, those of the understorey fern *A. lunulatum*, also had the lowest hydraulic conductance. The response of K_{leaf} to Ψ_{leaf} exhibited a characteristic shape all species, with a plateau followed by a rapid decline in K_{leaf} to a minimum value as Ψ_{leaf} progressively decreased (Fig. 2). The range of Ψ_{leaf} to which K_{leaf} was responsive was relatively conservative among the eight species, always falling in the range -1 to -3 MPa . No systematic differences were apparent between the responses of pteridophyte and angiosperm xylem to imposed desiccation.

Stomatal closure and safety margin

The transpiration rate in all species responded similarly to slow desiccation. Initial rates were typically low, followed by an increase in E as stomata opened (Fig. 3), then a decline as leaf desiccation and falling Ψ_{leaf} caused stomatal closure. As a result of the low light and high humidity, maximum transpiration rates during desiccation were less than 10% of field maxima. Because rates of E were maintained as low as possible, the closure phase was prolonged over a minimum of 60 min, thus allowing precise determination of Ψ_{leaf} at complete stomatal closure. The response of stomata to Ψ_{leaf} was closely correlated with the response of K_{leaf} to Ψ_{leaf} in both pteridophytes and angiosperms (Fig. 4), however, the regressions between these parameters were significantly different in the two groups (ANOVA). As a result, margins between stomatal closure and loss of K_{leaf} were different in the two groups (Fig. 5). The dynamics of pteridophyte stomatal closure appeared much more conservative than that of the associated angiosperms (Fig. 5), with complete stomatal closure 0.3 – 0.8 MPa before the 50% loss of K_{leaf} . By contrast, the angiosperms investigated produced safety margins close to or less than zero, suggesting that a significant loss of K_{leaf} preceded complete stomatal closure. The angiosperm *Simarouba glauca* exhibited the lowest safety in terms of stomatal closure, with stomata closing at a water potential found to reduce K_{leaf} by approximately 80%. In pteridophytes there was evidence of a linear dependence of safety margin upon Ψ_{leaf} a 50% loss of K_{leaf} with more vulnerable species producing smaller safety margins, while angiosperms showed no such relationship.

Discussion

Pteridophyte leaves were found to conduct water less efficiently than the angiosperm sun leaves investigated here, but fell within

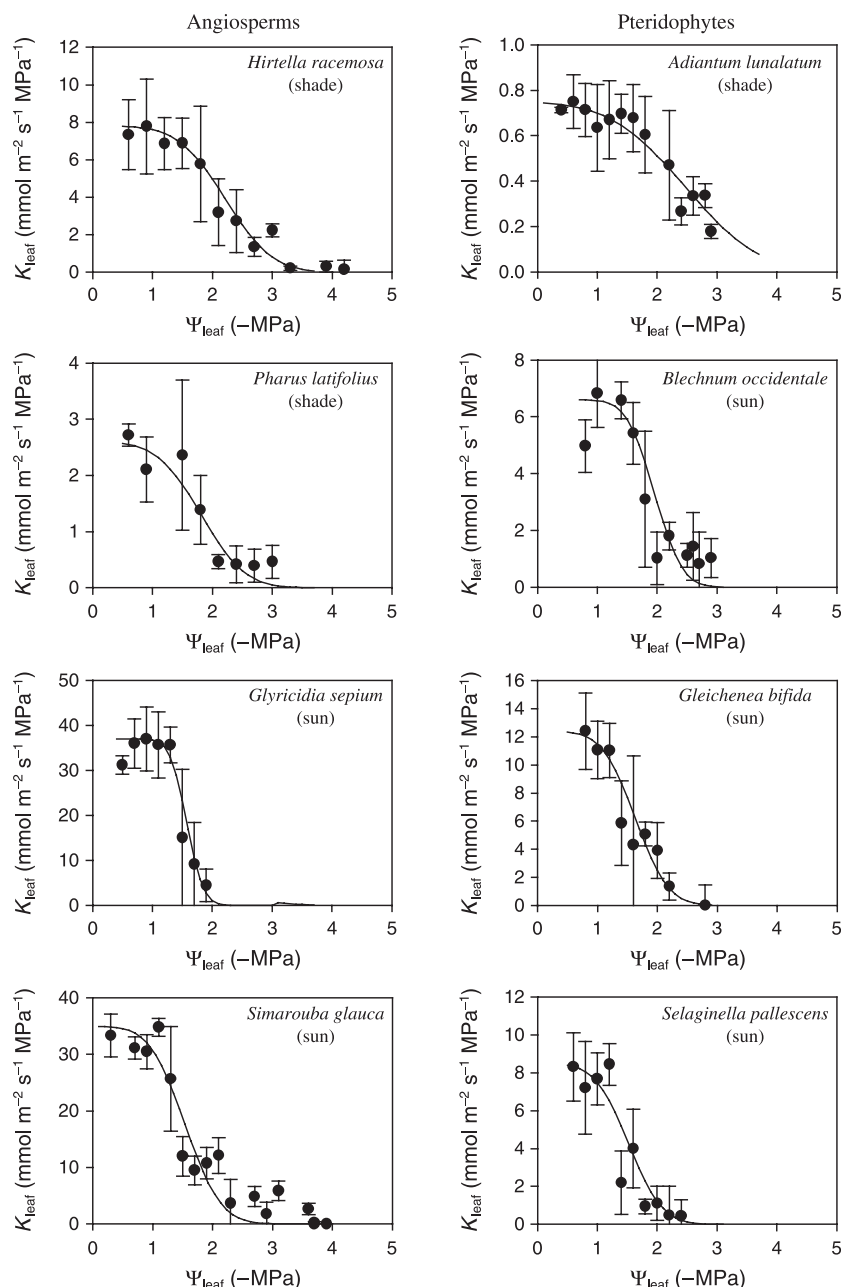


Fig. 2 Responses of leaf xylem hydraulic conductivity to declining leaf water potential during desiccation in the eight species investigated. Data are pooled from three individuals of each species and each point represents a mean \pm SD ($n = 5$). Curves fitted are cumulative normal distributions and are highly significant in each case ($P < 0.01$).

the range of shade angiosperm species sampled. Although the hydraulic conductance of both angiosperm and pteridophyte leaves responded similarly to imposed water stress, the relationship between stomatal closure and xylem dysfunction was distinctly different in the two groups of plants. Pteridophytes proved more conservative than angiosperms, with stomatal closure substantially pre-empting xylem dysfunction.

Maximum hydraulic conductivities determined here for the pteridophytes *G. bifida* and *S. pallescens* of 7.8 and 11.4 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, are small relative to values of K_{leaf} from tropical canopy angiosperms which fall in the range of 15–35 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ (Brodribb & Holbrook, 2003). Compared

with other studies, however, the values for K_{leaf} in these sun-exposed pteridophyte species are at the high end of the range for temperate angiosperms which extends from around 4–16 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ (Sack *et al.*, 2003). Given that vessels have not been reported from the lamina of fern leaves (Carlquist & Schneider, 2001) it seems initially surprising that a tracheid-based vein network could supply water with a similar efficiency to angiosperms, where leaf venation is composed of conduits highly specialized to maximize axial flow. It must be remembered however, that the value of K_{leaf} expressed here is a conductance normalized to leaf area, and as such, low intrinsic conductivity of veins can be compensated by a large

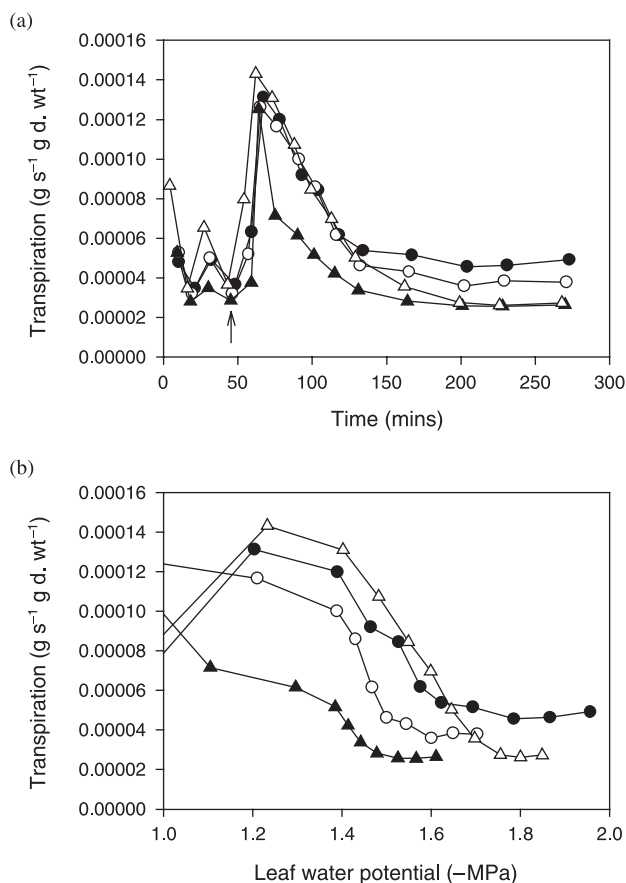


Fig. 3 Data showing the response of transpiration rate in four leaves of the sun angiosperm *Gliricidia sepium* to desiccation. (a) illustrates the dynamics of E over time as leaves were allowed to desiccate slowly at a constant high humidity. Transpiration rate increased initially after leaves were exposed to a weak light (arrowed), then fell as stomata presumably responded to dropping leaf water potential (b) shows the relationship between leaf transpiration (E) and leaf water potential in the same four leaves. In both cases a clear transition from declining E to a stable minimum is evident, and the transition between these phases was defined as the water potential at complete stomatal closure.

ratio of xylem tissue per unit leaf area. Conifers overcome low specific conductivity of wood in a similar fashion, by supporting small (relative to angiosperm) leaf areas, thus enabling leaf specific hydraulic conductivities (and photosynthetic rates) in the range of associated angiosperms (Brodribb & Feild, 2000). Pinnules of both *G. bifida* and *S. pallescens* were extremely narrow (1–2 mm) leading us to suggest that high K_{leaf} in these species derives from a large ratio of vein tissue to photosynthetic tissue.

The hydraulic conductivity of fern leaves exhibited a similar dependence upon Ψ_{leaf} as angiosperm leaves, with a rapid decline in K_{leaf} observed in the range of -1 to -3 MPa of xylem tension. There was no evidence that the xylem of pteridophytes was more susceptible to dysfunction under water stress than that of angiosperms. In fact the most impervious

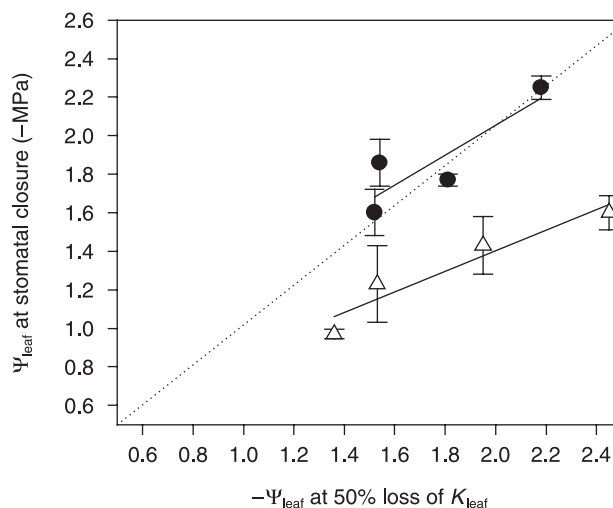


Fig. 4 Relationship between mean leaf water potential at stomatal closure, and at 50% loss of leaf hydraulic conductivity in angiosperms (circles) and pteridophytes (triangles). The linear regression for angiosperms ($r^2 = 0.76$) showed a 1 : 1 relationship between these parameters while the slope of the regression for pteridophytes ($r^2 = 0.91$) was significantly less than that for angiosperms ($P < 0.05$; ANOVA). The lower slope of this regression in pteridophytes indicates the tendency for pteridophyte leaves to close stomata before K_{leaf} becomes depressed rather than after, as in angiosperms.

xylem in this group of dry tropical forest species was the understorey maidenhair fern *Adiantum lunulatum*. These data indicate that pteridophyte sporophytes probably have a similar potential to extract water from dry soil as angiosperms, suggesting that the pteridophyte preference for the moist understorey probably does not derive from intrinsically drought sensitive xylem. Interestingly, there are also reports that many fern gametophytes are highly desiccation tolerant (Pickett, 1914; Page, 2002), raising the possibility that low representation of ferns in seasonally dry environments stems from some other aspect of fern physiology.

A striking difference was revealed between the pteridophytes and angiosperms examined here when the water potential at stomatal closure relative to xylem dysfunction during desiccation was compared. We found in all pteridophytes that during slow desiccation, the leaf water potential that corresponded to complete stomatal closure was above the threshold responsible for significant depression of xylem conductivity. In other words, stomata appeared to close in order to prevent any loss of hydraulic conductance in the leaf. By contrast, the angiosperms investigated here delayed stomatal closure until leaf water potential had fallen below that responsible for 50% loss of K_{leaf} . This surprising result appears to leave the foliage of angiosperms vulnerable to depression of K_{leaf} during any rapid excursion in the transpiration rate that leads to a drop in Ψ_{leaf} . The only means of avoiding K_{leaf} depression in these angiosperms would be if their stomata responded continuously to Ψ_{leaf} such that an early reduction of stomatal aperture as Ψ_{leaf} began to drop might prevent

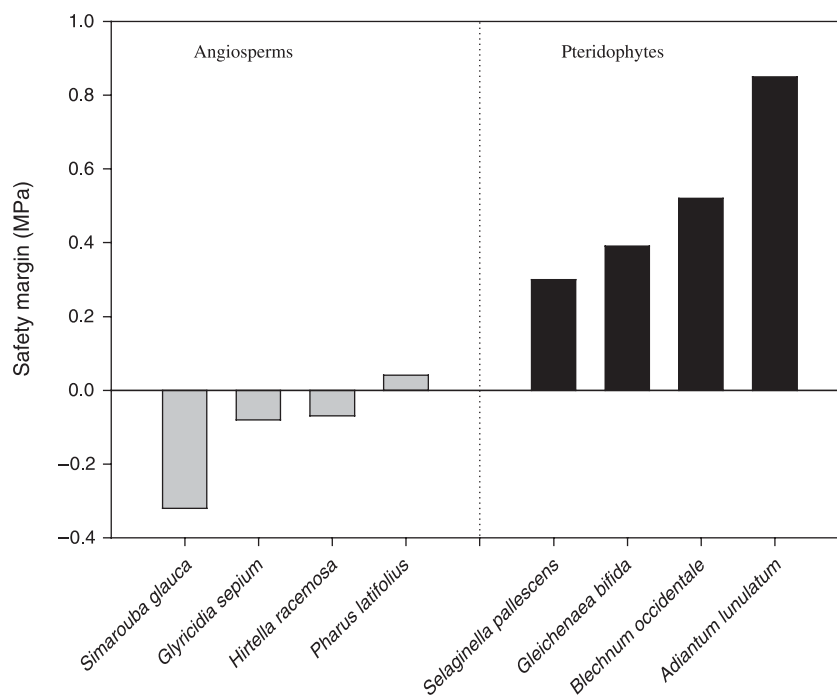


Fig. 5 Safety margin in each of the four pteridophytes and angiosperms, measured as the difference between Ψ_{leaf} at stomatal closure minus the Ψ_{leaf} at 50% loss of leaf hydraulic conductivity. Pteridophytes all exhibited large safety margins, while angiosperms typically exhibited negative safety margins, indicating that stomatal closure occurred after more than 50% depression of leaf hydraulic conductivity.

leaves from ever reaching water potentials capable of inducing K_{leaf} depression. This is not the case, however, as angiosperms studied in this area have been shown to produce a threshold response of stomata to Ψ_{leaf} (Brodribb *et al.*, 2003), meaning that leaves of these angiosperms are prone to frequent depression of K_{leaf} whenever soil or atmospheric water deficit increases. Recent studies showing reversible midday depression of K_{leaf} in several angiosperms including *S. glauca* (Brodribb & Holbrook, 2003; Bucci *et al.*, 2003) provide testimony to the minimal safety margin with which many angiosperms seem to operate.

We believe that the relationship between stomatal closure and K_{leaf} depression is adaptive in angiosperms, allowing greater efficiency in water use and xylem investment than their pteridophyte predecessors. These efficiencies would only be realized if depressions in K_{leaf} were rapidly reversible and did not incur large metabolic costs. The former condition appears to be met in the leaves examined to date (Bucci *et al.*, 2003; Brodribb & Holbrook, 2004), while the metabolic costs of reversing K_{leaf} depression are yet to be ascertained. The small safety margin in angiosperm leaves would allow maximum efficiency in xylem investment due to the fact that construction of cavitation resistant xylem is costly (Hacke *et al.*, 2001) and maximum stomatal opening is obviously beneficial (in terms of carbon gain). Hence, it is clear that the closer operating leaf water potential comes to the failure tension of the xylem the more optimal is the utilization of xylem investment. The second possible advantage for leaves operating within the region of K_{leaf} depression is that gas exchange may be better optimized in terms of Assimilation (δA)/ δE if K_{leaf} falls in response to increased VPD (Brodribb & Holbrook, 2004; T. Buckley pers. comm.).

It is possible that the 'premature' stomatal closure observed in the fern species here is associated with protection of vulnerable xylem in the rhizome. The xylem in roots is commonly more vulnerable to cavitation than stems (Sperry & Ikeda, 1997; Linton *et al.*, 1998) and has been suggested as a trigger stomatal closure in some tree species. Given that fern vessels have been identified most commonly in the xylem of the rhizome, it is possible that rhizomes of ferns are more cavitation sensitive than the veins of fern fronds. However, the persistence of rhizomes throughout the dry season in these species suggests this is not the case, and that xylem in the rhizome is the most resilient vascular tissue in these plants.

Hence, it appears that despite similar magnitudes of K_{leaf} and vulnerabilities of xylem in pteridophytes and angiosperms, there may be fundamental differences in the coordination between stomata and xylem in these plant groups. These differences may explain why liquid- and vapour-phase conductance of water in the leaves of these angiosperms is highly correlated while in ferns the correlation is much weaker (Fig. 1). It is probable that the small safety margin exercised by the angiosperms here may yield enhanced efficiency of xylem investment and water use. This may in turn provide a part of the explanation for low success of pteridophytes relative to angiosperms in seasonally and perennially dry habitats.

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References

- Brodrribb TJ, Feild TS. 2000. Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment* 23: 1381–1388.
- Brodrribb TJ, Hill RS. 1999. The importance of xylem constraints in the distribution of conifer species. *New Phytologist* 143: 365–372.
- Brodrribb TJ, Holbrook NM. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* 132: 2166–2173.
- Brodrribb TJ, Holbrook NM. 2004. Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant, Cell & Environment* (In press.)
- Brodrribb TJ, Holbrook NM, Edwards EJ, Gutiérrez MV. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant Cell & Environment* 26: 443–450.
- Bucci SJ, Scholtz FG, Goldstein G, Meinzer FC, Sternberg 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 & Environment* 26: 1633–1645.
- Carlquist S, Schneider EL. 2001. Vessels in ferns: structural, ecological, and evolutionary significance. *American Journal of Botany* 88: 1–13.
- Cochard H, Coll L, Le Roux X, Ameglio T. 2002. Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. *Plant Physiology* 128: 282–290.
- Franks PJ, Farquhar GD. 1999. A relationship between humidity response, growth form and photosynthetic operating point in C_3 plants. *Plant, Cell & Environment* 22: 1337–1349.
- Hacke U, Sperry JS, Pockman WT, Davis SD, McCulloch A. 2001. Trends in wood density and structure are linked to the prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- Heiser T, Giers A, Bennert HW. 1996. *In situ* gas exchange measurements and the adaptation to light regime of three species of *Lycopodium*. In: Camus JM, Gibby M, Johns RJ, eds. *Pteridology in perspective*. Kew, UK: Royal Botanical Gardens, 599–610.
- Janzen DH. 1983. *Costa Rican natural history*. Chicago, MI, USA: University of Chicago Press.
- Koide RT, Robichaux RH, Morse SR, Smith CM. 1991. Plant water status, hydraulic resistance and capacitance. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW, eds. *Plant physiological ecology*. New York, NY, USA: Chapman & Hall, 161–183.
- Linton MJ, Sperry JS, Williams DG. 1998. Limits to water transport in *Juniperus osteosperma* and *Pinus edulis*; implications for drought tolerance and regulation of transpiration. *Functional Ecology* 12: 906–911.
- Nardini A, Tyree MT, Salleo S. 2001. Xylem cavitation in the leaf of *Prunus laurocerasus* L. and its impact on leaf hydraulics. *Plant Physiology* 125: 1700–1709.
- Niklas KJ, Tiffney BH, Knoll AH. 1985. Patterns in vascular plant diversification: an analysis at the species level. In: Valentine JW, ed. *Phanerozoic diversity patterns: profiles in macroevolution*. Princeton, NJ, USA: Princeton University Press.
- Nobel PS. 1978. Microhabitat, water relations and photosynthesis of a desert fern, *Notholaena parryi*. *Oecologia* 31: 293–309.
- Page CN. 2002. Ecological strategies in fern evolution: a neopteridological overview. *Review of Palaeobotany and Palynology* 119: 1–33.
- Pickett FL. 1914. Some ecological considerations of certain fern prothallia. *American Journal of Botany* 1: 477–498.
- Sack L, Cowan PD, Jaikumar N, Holbrook NM. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* 26: 1343–1356.
- Sperry JS, Ikeda T. 1997. Xylem cavitation in roots and stems of Douglas fir and White fir. *Tree Physiology* 17: 275–280.
- Stewart WN, Rothwell GW. 1993. *Paleobotany and the evolution of plants*. Cambridge, UK: Cambridge University Press.
- Tyree MT, Hammel HT. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* 23: 267–282.
- Veres JS. 1990. Xylem anatomy and hydraulic conductance of Costa Rican Blechnum ferns. *American Journal of Botany* 77: 1610–1625.
- Woodhouse R, Nobel P. 1982. Stipe anatomy, water potentials, and xylem conductances in seven species of ferns (Filicopsida). *American Journal of Botany* 69: 135–140.



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