Stomatal Closure during Leaf Dehydration, Correlation with Other Leaf Physiological Traits[1]

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The question as to what triggers stomatal closure during leaf desiccation remains controversial. This paper examines characteristics of the vascular and photosynthetic functions of the leaf to determine which responds most similarly to stomata during desiccation. Leaf hydraulic conductance (K_{leaf}) was measured from the relaxation kinetics of leaf water potential (Ψ_l), and a novel application of this technique allowed the response of K_{leaf} to Ψ_l to be determined. These "vulnerability curves" show that K_{leaf} is highly sensitive to Ψ_l and that the response of stomatal conductance to Ψ_l is closely correlated with the response of K_{leaf} to Ψ_l. The turgor loss point of leaves was also correlated with K_{leaf} and stomatal closure, whereas the decline in PSII quantum yield during leaf drying occurred at a lower Ψ_l than stomatal closure. These results indicate that stomatal closure is primarily coordinated with K_{leaf}. However, the close proximity of Ψ_l at initial stomatal closure and initial loss of K_{leaf} suggest that partial loss of K_{leaf} might occur regularly, presumably necessitating repair of embolisms.

Stomata appear in the fossil record approximately 400 million years ago (Edwards et al., 1998) at approximately the same time as the evolution of an internal water conducting system in plants. Stomatal evolution is believed to be a response to selective pressure to optimize the ratio of CO_2 uptake to water lost during photosynthesis (Raven, 2002). The evolution of internal conduits for water transport added a level of complexity to optimizing gas exchange during photosynthesis, because of the dependence of water supply capacity upon the water potential in the plant (Sperry et al., 2002). This complexity is evidenced by the variable effects of leaf water potential (Ψ_l) and vapor pressure deficit on stomatal movements among species. Although stomatal aperture responds predictably to guard cell turgor (Franks et al., 1995), the relationships between guard cell turgor and either transpiration (E) or mesophyll turgor are still hypothetical (Buckley and Mott, 2002). Amid mechanistic debate as to the process of stomatal closure, the fundamental question of why stomata close remains unanswered. Given that stomata may predate the evolution of xylem (Edwards et al., 1998; Raven, 2002), it is appropriate to question whether it is vascular or other tissues that provide the trigger for stomatal closure.

We focus here on the question of what sets the point of stomatal closure in leaves. That is to say which aspect of a plant's physiology is sufficiently sensitive to decreasing Ψ_l that it requires stomata to be closed and photosynthesis sacrificed to protect from loss of function and damage. A key assumption here is that traits responsible for determining the stomatal response to leaf desiccation are coordinated with physiological characters dictating the sensitivity of the metabolic or transport machinery of the plant to water stress. Candidates for these coordinated traits are likely be located in or near the leaf, because transduction of signals from far upstream of the leaves is generally slow relative to the half-time for stomatal responses to perturbations in leaf water balance (Tardieu and Davies, 1993). Additionally, it would be expected that among these traits, adaptation to sustain lower Ψ_l would come at a significant cost. Features such as the vulnerability of leaf xylem to cavitation and the resistance of leaf cells to collapse fulfill these criteria in that they are prone to failure (either structural or functional) under conditions of low water content and are both costly to augment. However, it is clear that photosynthesis in most species becomes irreversibly depressed when leaf relative water content (RWC) falls to around 70% (Lawlor and Cornic, 2002), and thus the resistance of the photosynthetic apparatus to desiccation is also a potential trigger for stomatal closure.

In this paper, we examine the vascular and photosynthetic apparatus of the leaf to test whether stomatal closure is correlated with the water-stress tolerance of different leaf tissues or functions. This work follows a number of studies that have demonstrated similarity between the response of both stomatal conductance (g_s) and stem xylem cavitation to decreasing Ψ_l (Salleo et al., 2000; Hubbard et al., 2001; Cochard et al., 2002). It is likely that this correlation between stomatal closure and xylem cavitation will be most prominent in

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the leaf, given that leaf minor veins appear more prone to cavitation than stems (Nardini et al., 2001), and that leaves represent a large proportion of the whole plant hydraulic resistance (Nardini, 2001; Brodribb et al., 2002). Surprisingly there have been few studies that have quantified the effect of Ψ_l on leaf hydraulic conductance (K_{leaf}) in woody plants (Nardini et al., 2001), probably due to technical difficulties in measuring the hydraulic conductance of the leaf.

Here, we quantify the relationship between Ψ_l on K_{leaf} by examining the kinetics of Ψ_l relaxation in rehydrating leaves. A number of studies have examined the dynamics of pressure equilibration in leaves to estimate components of their hydraulic resistance. For example, Cruiziat et al. (1980) and Tyree et al. (1981) estimated K_{leaf} from the kinetics of water flow into dehydrated sunflower leaves, whereas Nobel and Jordan (1983) used the time constant for water potential equilibration following overpressurization to estimate leaf mesophyll transfer resistance. In this study, we measured the rate of relaxation of Ψ_l during the rehydration of leaves desiccated to different water potentials, enabling the quantitative determination of leaf vulnerability to cavitation.

K_{leaf} was calculated by assuming that the rehydration of desiccated leaves is equivalent to the charging of a capacitor through a resistor:

$$Vf = Voe^{-t/RC}$$

where V_o is the initial potential, V_f is the potential after charging for t seconds, R is the resistance (=1/K), C is capacitance (Fig. 1), and t is a period of recharge. Desiccated leaves are detached underwater from their subtending branch or stem and allowed to rehydrate for known periods of time, after which the final Ψ_l is determined. An important requirement for the accurate determination of K_{leaf} is that the initial (pre-rehydration) Ψ_l be measured on adjacent leaves rather than leaves to be rehydrated. For reasons unknown to us, pressurization in a pressure chamber substantially alters the ability of the leaf to rehydrate. Leaves previously measured in a pressure chamber show little or no tendency to rehydrate through their petiole. Measurement of pre- and post-rehydration Ψ_l as well as the time of rehydration enabled K_{leaf} to be calculated:

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

where C is leaf capacitance, Ψ_o is Ψ_l before rehydration, and Ψ_f is Ψ_l after rehydration for t seconds.

By examining leaf vulnerability, turgor loss point, and loss of quantum yield of photosynthesis during leaf desiccation, we were able to determine which of these characters conformed most closely to the stomatal response to Ψ_l. Variation in these relationships was examined among a group of phenologically diverse species to ascertain whether correlations between stomatal and leaf physiological parameters were conserved between species. To maximize the diversity of phenology and physiology of our sample, two deciduous and two evergreen species were selected from the seasonally dry forest of northwest Costa Rica. Previous work has illustrated a diversity of hydraulic and photosynthetic behavior among these species (Brodribb et al., 2003) making them ideal for comparative study.

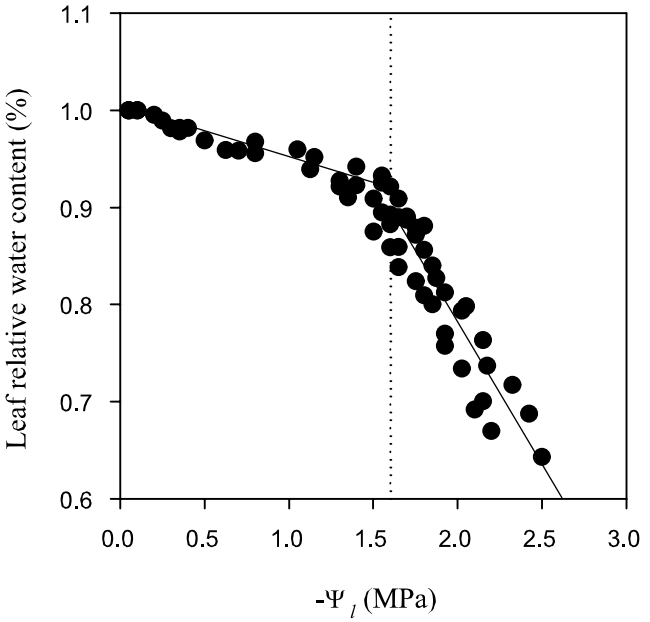

Figure 1. The two-phase function fitted to pressure volume data for five *Gliricidia sepium* leaves. Leaf capacitance (C_{leaf}) was calculated from the slope of the relationship between leaf RWC and Ψ_l (see "Materials and Methods"). Low C_{leaf} was found in all species before the turgor loss point (dotted line). Post turgor loss, C_{leaf} increased substantially.

RESULTS

Stomatal Closure

A general pattern in the stomatal response to Ψ_l was seen in all species, whereby g_s was responsive to Ψ_l only over a narrow range of Ψ_l (Fig. 2). As a result, the transition from 90% to 20% of maximum g_s in each species occurred over a band of Ψ_l less than 1 MPa. Despite this rapid transition, most species exhibited a continuous response of g_s to Ψ_l, and only *Quercus oleoides* developed a plateau where g_s was not sensitive Ψ_l. Variation between species was expressed in the initial Ψ_l that produced strong decreases in g_s and the range of Ψ_l to which stomatal aperture appeared to respond. The point of stomatal closure (defined here as the Ψ_l at which g_s fell below 20% of maximum g_s) ranged from −1.65MPa in *Simarouba glauca* to −2.95MPa in *Q. oleoides*. High minimum leaf g_s in *Rhedera trinervis* appeared to result from an inability to completely close stomata (Fig. 2).

Leaf Rehydration

Following detachment underwater, Ψ_l relaxed (became less negative) exponentially over time as pre-

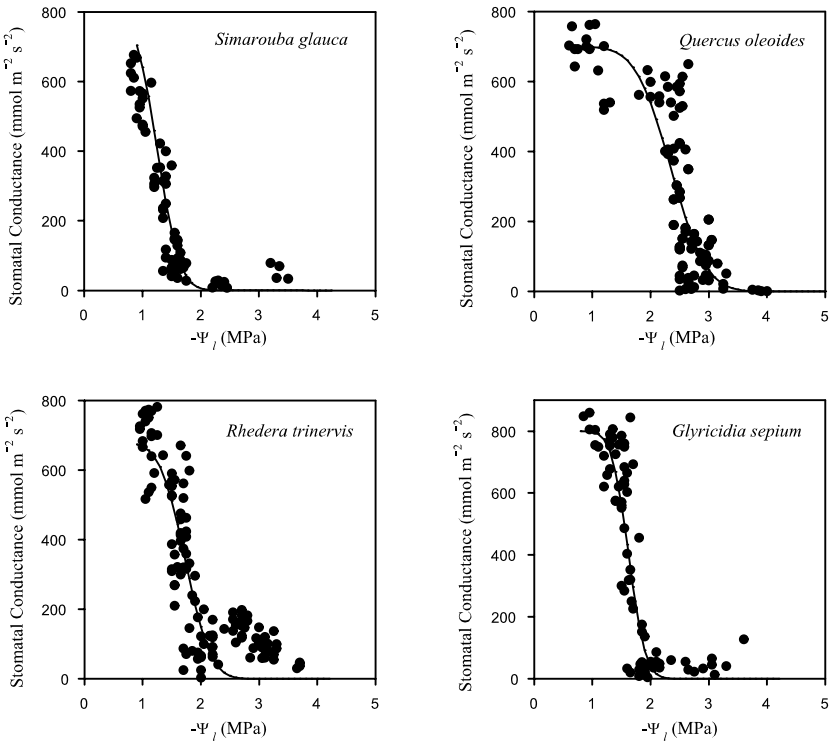

Figure 2. The relationship between Ψ_l and g_s in evergreen (*S. glauca* and *Q. oleoides*) and deciduous (*R. trinervis* and *Gliricidia sepium*) species. Data were collected from six trees of each species on sunny days. A range of Ψ_l was measured by surveying g_s under different evaporative conditions. Minimum g_s was measured on detached branches. Curves are cumulative normal distributions.

dicted from the behavior of a simple resistor/capacitor circuit (Fig. 3). In all species, this exponential increase of Ψ_l continued until Ψ_l reached around -0.1 to -0.3 MPa, after which it became slower and nonexponential as Ψ_l approached zero. The optimal period over which to measure relaxation in the four species studies was 15 to 30 s, because this resulted in a large $\Delta\Psi_l$ without Ψ_l rising above -0.3MPa.

As Ψ_o became more negative, the slope of the Ψ_l relaxation curve became shallower in all species, indicating a decrease in K_{leaf} (Fig. 3). At very low water potentials (less than -4MPa), leaves rehydrated extremely slowly as K_{leaf} approached zero.

Leaf Vulnerability

In all species, K_{leaf} decreased precipitously once Ψ_l fell below a threshold value. Mean maximum values of K_{leaf} varied between species from a high of 24.1 mmol m^{-2} s^{-1} MPa^{-1} in *S. glauca* to a low of 16.7 mmol m^{-2} s^{-1} MPa^{-1} in *R. trinervis*. Variation in maximum K_{leaf} within a species was relatively large, with SDS between 15% and 19%, and as a result only *R. trinervis* and *S. glauca* were significantly different in mean K_{leaf}. At low Ψ_l, K_{leaf} fell to minimum values of between 2% and 20% of the mean maximum K_{leaf} for each species (Fig. 4).

Differences in the shape of the response of K_{leaf} to Ψ_l were seen in the slope of the transition between maximum and minimum K_{leaf}, with the two deciduous species, *Gliricidia sepium* and *R. trinervis*, exhibiting much more rapid transitions than the two evergreen species. A clear correspondence between this transition zone and the region of Ψ_l to which g_s responded was evident (Fig. 4). The Ψ_l at turgor loss was also closely correlated with the transition from minimum to maximum K_{leaf} ($r^2 = 0.86$ for Ψ_l at turgor loss versus Ψ_l at 50% loss of K_{leaf}). This result occurred despite the fact that leaf capacitance (C_{leaf}) was up to nine times greater in leaves after turgor loss than the same leaf preturgor loss (Fig. 1). The effect of this high capacitance post turgor loss would be to yield much higher calculated values for K_{leaf} if the slope of Ψ_l relaxation remained equivalent to preturgor loss values. In fact, the relaxation of Ψ_l in leaves desiccated below the turgor loss point was extremely slow relative to leaves at higher Ψ_l (Fig. 3), and hence, the calculated K_{leaf} also declined at around this point.

Photosynthetic Response to Ψ_l

PSII quantum yield at 1,800 μmol quanta m^{-2} s^{-1} decreased from maximum values of 0.35 to 0.45 to minimum values less than 0.1 as RWC and water potential decreased. Quantum yield responded to Ψ_l in a similar fashion to g_s and K_{leaf}, with an initial nonsensitive phase followed by a decline to a minimum. The initial part of this decline was reversible, presumably due to increasing non-photochemical quenching resulting from factors such as falling CO_2 concentration in the leaf. However, the final loss of ϕ_{PSII} did not appear to be reversible. Minimum values of ϕ_{PSII} were around 0.1, and unlike leaves rehydrated before reaching this low level of fluorescence,

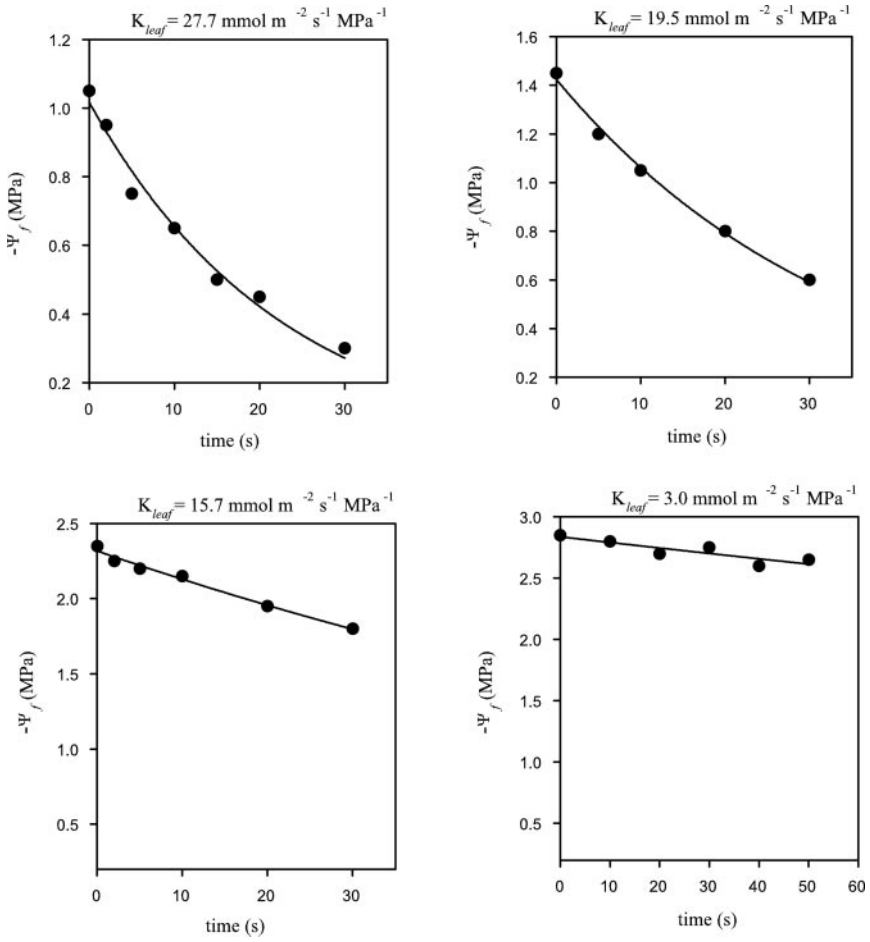

Figure 3. Typical rehydration kinetics for *S. glauca* leaves. Single points represent Ψ_l of leaflets during rehydration of a single compound leaf. All curves are exponential, and the slope is used to calculate K_{leaf}.

ϕ_{PSII} in leaves desiccated to this point could not be revived by rehydration. In all species except *Gliricidia sepium* the decline in ϕ_{PSII} occurred at lower Ψ_l than either stomatal closure or loss of K_{leaf}, such that complete stomatal closure occurred at water potentials above that which caused depression of ϕ_{PSII} (Fig. 5).

Relationships between Leaf Traits and Stomatal Closure

Stomatal closure was closely correlated with the decline in K_{leaf} during desiccation. Examination of the slopes of regressions between stomatal, hydraulic, turgor, and photosynthetic responses to Ψ_l indicated that stomatal closure corresponded most closely with the initial loss of K_{leaf} (Table I). A relationship with turgor loss was also evident, but the slope of Ψ_l at turgor loss versus Ψ_l at stomatal closure was less than 1, indicating that stomata tended to close before the turgor loss point. The depression of ϕ_{PSII} below 0.10 occurred at water potentials significantly lower than stomatal closure, and the slope of the relationship between Ψ_l at stomatal closure, and Ψ_l at $\phi_{PSII}<0.10$ was significantly different to 1 ($P < 0.01$).

DISCUSSION

K_{leaf}

Analysis of Ψ_l relaxation kinetics provides an efficient means of assessing the hydraulic conductance of leaves as well as the response of leaf conductance to decreasing Ψ_l. Calculated values of K_{leaf} from rehydration were very similar to conductances measured on some of the same species by different techniques. Maximum values of K_{leaf} measured by vacuum infiltration (Nardini et al., 2001) and pressure drop during E in *R. trinervis*, for example, were 15 and 25mmol m^{-2} s^{-1} MPa^{-1}, respectively (Brodribb and Holbrook, 2003), which compares favorably with the mean K_{leaf} of 16.7 mmol m^{-2} s^{-1} MPa^{-1} for *R. trinervis* measured here. Becker et al. 1999 found a mean value of 17.2 mmol m^{-2} s^{-1} MPa^{-1} for the K_{leaf} of 10 tropical trees measured by a high-pressure flowmeter (Tyree et al., 1995), this value also compares well with the mean value of K_{leaf} of 20.4 mmol m^{-2} s^{-1} MPa^{-1} from the four species measured here. The K_{leaf} of the tropical species studied here was higher than values of K_{leaf} for temperate species, which have been shown to fall in the range of

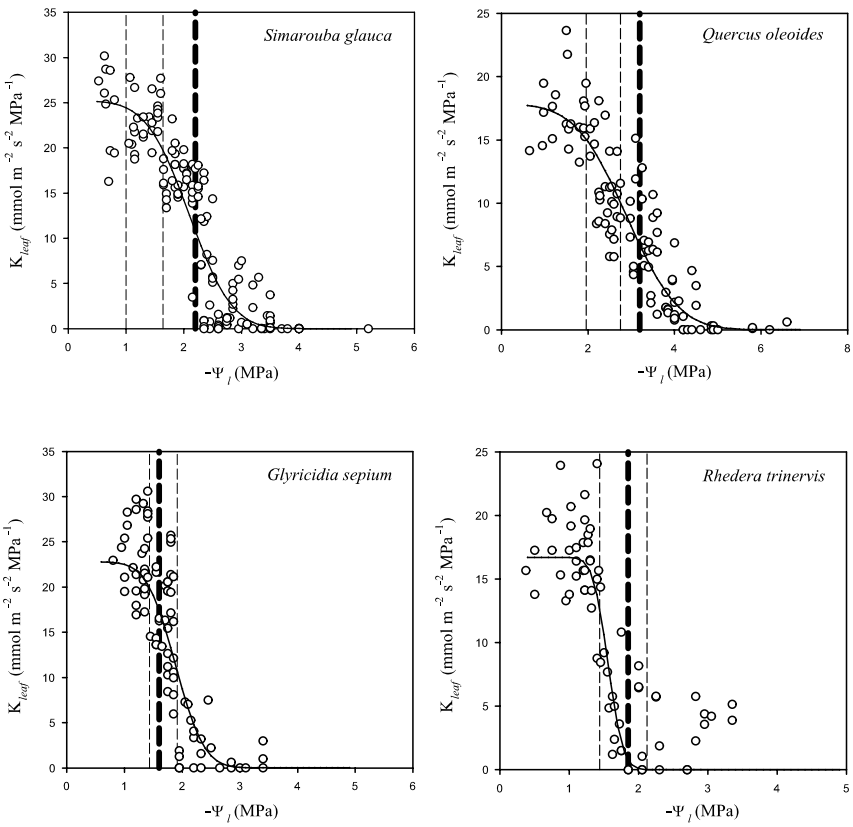

Figure 4. Response of K_{leaf} to Ψ_l in each of the four species studied. Each point represents the average K_{leaf} from two leaves per branch, and a cumulative normal distribution curve is fitted to the data. Dotted lines indicate the Ψ_l at 80% and 20% of maximum g_s, and the heavy dotted line shows the Ψ_l at turgor loss.

5 to 20 mmol m^{-2} s^{-1} MPa^{-1} (Nardini, 2001; Sack et al., 2002).

The rehydration technique employed here produced values of K_{leaf} similar to those measured by other techniques such as the high pressure flowmeter and vacuum infiltration, both of which potentially allow water to bypass the mesophyll symplast. Given that the pathway measured during leaf rehydration includes the transfer resistance from the apoplast into the mesophyll symplast, this agreement suggests that the mesophyll transfer component of leaf resistance is low. Several recent studies support this conclusion, suggesting that the majority of the water potential drop across the leaf occurs in the venation (Sack et al., 2002; Zwieniecki et al., 2002; but see Tyree et al., 1981).

K_{leaf} was highly sensitive to desiccation, declining rapidly as Ψ_l approached the turgor loss point. Although it cannot be determined which part of the pathway from petiole to mesophyll is responsible for this decline in K_{leaf}, recent evidence from leaf acoustic emissions and dye infiltration have suggested that leaf minor veins are susceptible to cavitation (Salleo et al., 2001). We assume that losses in K_{leaf} observed here represent cavitation for two reasons, firstly because the response of K_{leaf} in *S. glauca* to Ψ_l here is very similar to the response of petioles of the same species to water-stress induced cavitation measured by flushing embolisms from the xylem (Brodribb et al., 2003). Second, the precipitous decline in K_{leaf} observed as Ψ_l fell below a critical value is indicative of a process of rapid conduit blockage, and the most parsimonious explanation of this is cavitation. The close proximity of the Ψ_l at incipient loss of K_{leaf} and Ψ_l at 50% stomatal closure was surprising and appears to indicate that leaves closely approach and even cross the leaf cavitation threshold on an average day of sunny conditions. This would also suggest that cavitation in leaf veins might be a regular occurrence, requiring the ability to refill cavitated conduits to maintain photosynthetic capacity of the leaf. Leaves provide probably the best environment for refilling of embolized conduits (Salleo et al., 2000; 2001) due to the relative abundance of inorganic ions and other osmolytes that could be used to generate positive pressures (Holbrook and Zwieniecki, 1999), as well as possessing large amounts of metabolic energy to drive ion movement. Hence, it is plausible that to minimize leaf resistance, the leaf xylem is constructed with large pores in inter-conduit pit membranes enhancing conductivity, but increasing the risk of air-seeding through pit membranes (Sperry and Tyree, 1988).

Stomatal Closure

K_{leaf} and g_s both showed very similar responses to Ψ_l (Fig. 4; Table I), whereas leaf turgor loss occurred midway through stomatal closure (Fig. 4), and damage to PSII (as indicated by of ϕ_{PSII}) occurred at a

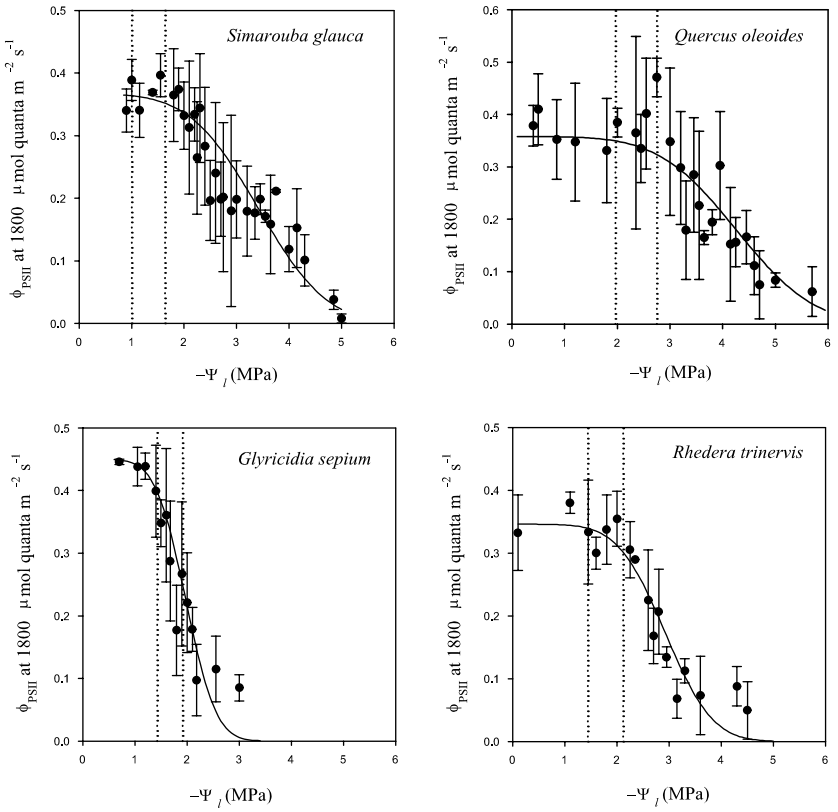

Figure 5. Decreasing quantum yield of PSII during leaf desiccation of detached branches. Each point represents the means ± SD of three to five leaves. Curves are cumulative normal distributions, and dotted lines indicate the Ψ_l at 80% and 20% of maximum g_s.

substantially lower Ψ_l. This supports the idea that stomatal closure occurs as a protective mechanism against xylem cavitation (Tyree and Sperry, 1988), although the safety margin, especially in the two deciduous species was extremely small. A similar relationship between stomatal closure and stem cavitation was described in a group of tropical deciduous species (Brodribb et al., 2003), although a larger safety margin for the stem xylem meant that stomata were completely closed before a 50% loss of stem conductivity had occurred.

Given that leaves represent a large resistor in the hydraulic pathway through the plant, it is surprising that this resistor should also be susceptible to desiccation-induced decline in conductance. The lack of a safety margin in these species suggests that either the stomatal response to Ψ_l is extremely rapid and feed-forward (enabling relaxation of Ψ_l to stem xylem water potential after sudden increases in evapotranspiration) or, as mentioned above, that cavitation and refilling occur daily. Considering that these requirements, not to mention the loss of photosynthesis during stomatal closure, would be costly to the plant, the other alternative of increasing the cavitation resistance of the xylem must represent an even greater cost. A close link between leaf turgor loss and loss of K_{leaf} shown here indicates that a higher modulus of elasticity and greater osmotic potential of leaf cells would be required to support lower Ψ_l as well as greater lignification of upstream xylem (Hacke et al., 2001).

Another possibility is that the leaf vascular system rather than being a weak link in the hydraulic pathway requiring protection, has evolved to cavitate early as a means of sensitizing the stomata to changes in evaporation. In this role, the leaf vascular system could amplify the effect of increasing E on the water potential of guard cells. The only danger in such an augmentation of the rate of response of Ψ_l could might be that rapid decreases in Ψ_l are known to induce a transient opening of stomata due to loss of subsidiary cell turgor (Tardieu and Davies, 1993). What is required to verify such speculation is a clearer understanding of the response of guard cells to Ψ_l, and whether guard cell movements are con-

Table I. *Slopes ± SE of regressions between cardinal points on the relationships between Ψ_l and stomatal closure, leaf vulnerability, loss of ϕ_{PSII}, and the turgor loss point*

Leaf vulnerability was closest to a 1:1 relationship with stomatal closure, whereas Ψ_l at turgor loss, although exhibiting a smaller slope, was also not significantly different to a 1:1 relationship with stomatal closure. Loss of ϕ_{PSII} was furthest from a 1:1 relationship with stomatal closure. **, A regression significantly different to 1:1 ($P < 0.01$; t test).

	Ψ_l at 50% of g_s max.	Ψ_l at 20% of g_s max.
Ψ_l at 20% loss of K_{leaf}	1.04 ± 0.085	1.21 ± 0.096
Ψ_l at turgor loss	0.76 ± 0.084	0.89 ± 0.089
Ψ_l at $\phi_{PSII} < 0.1$	0.456 ± 0.063**	0.534 ± 0.068**

trolled by a passive loss of turgor in concert with the surrounding cells, or by an activated ion pump from the subsidiary cells.

This paper provides the first coordinated examination of how the stomatal, photosynthetic, and hydraulic systems in the leaf respond to changes in Ψ_l. The data presented here showed a remarkably consistent proximity between the point of initial leaf cavitation and stomatal closure. By contrast, stomatal closure did not appear to be closely linked to the water potential at which irreversible damage to photosynthetic apparatus ($\phi_{PSII} < 0.1$) occurred. Although turgor loss was also closely associated with stomatal closure, the physiological impact of turgor loss is unclear given that photosynthesis was not irreversibly damaged until water potential fell substantially below the turgor loss point. These data point to vulnerability of the xylem in leaf veins as a primary trigger for stomatal closure, although the mechanism for this trigger remains unknown.

MATERIALS AND METHODS

Study Site

This investigation was undertaken 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 1,528 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 contribute to generate a high evaporative demand. Diurnal and seasonal temperature ranges are relatively small, with a mean annual temperature of 28°C.

We chose four species: two deciduous, *Gliricidia sepium* (Fabaceae) and *Rhedera trinervis* (Verbenaceae), and two evergreen, *Simarouba glauca* (Simaroubaceae) and *Quercus oleoides* (Fagaceae). All are tree-forming species, with *Gliricidia* sp. and *Simarouba* sp. both producing compound leaves approximately 20 to 30 cm in length and *Rhedera* sp. and *Quercus* sp. both with simple leaves 10 to 20 cm in length. Leaf age was monitored on tagged branches, and only leaves 4 to 6 months old were selected for experiments. All data were collected during the mid-late wet season from July to September.

K_{leaf}

Measurement of K_{leaf} was made under non-steady-state conditions using the rate of relaxation of Ψ_l in leaves detached from the stem under water to calculate the leaf conductance from Equation 2 (see above). This calculation requires knowledge of C_{leaf}, mass of water per unit leaf area, and leaf dry mass per unit area for each species.

Relaxation of Ψ_{leaf}

To determine the time course of Ψ_{leaf} relaxation, a number of small branches bearing eight to 10 leaves in a tight cluster were cut from single trees and allowed to slowly desiccate in the laboratory. Using data for the vessel length of each of the four species (T. J. Brodribb, unpublished data), branches were cut of sufficient length that emboli did not extend in to the petioles of sample leaves. Once a branch had reached approximately −1 MPa, the branch was placed in a plastic bag in the dark for approximately 1 h to minimize variation in water potential between leaves. Two leaves were then harvested as an estimate of the initial Ψ_l. If these leaves differed in Ψ_l by more 0.10 MPa, the branch was discarded. Leaves were rehydrated by submerging their subtending branch in filtered tap water such that the petioles of the target leaves could be cut simultaneously underwater using a razor blade. Leaf laminas were maintained dry to avoid possible uptake of water through the epidermis or stomata. Leaves were allowed to absorb water for a predetermined period of time after which their petioles were dabbed dry on paper towel, and the leaves placed in plastic bags to prevent water loss. Ψ_l was immediately measured using a Scholander pressure chamber (PMS, Corvallis, OR).

To test the applicability of the one-compartment rehydration model (charging of a single capacitor through a resistor), we rehydrated leaves (all with the same initial water potential) for varying lengths of time. A least squares exponential regression was then fitted to the plot of final water potential versus rehydration time. According to Equation 1, the exponent from this regression is equal to $-K_{leaf}\, t/C_{leaf}$.

C_{leaf}

C_{leaf} was measured from the slope of the pressure-volume relationship for each species. The relationship between Ψ_l and water volume in the leaf was quantified using the bench drying technique (Tyree and 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 PV determination. Leaf weight and Ψ_l were measured periodically during slow desiccation of sample leaves in the laboratory. Desiccation of leaves continued until Ψ_ls stopped falling or began to rise due to cell damage. Due to the elasticity of the cell walls, C_{leaf} pre- and post-turgor loss are quite different. It was found that the relationship between Ψ_l and leaf RWC could be closely approximated by a two-phase linear equation intersecting at the turgor loss point (e.g. Fig. 1). The capacitance function was defined by measuring the turgor loss point from the inflection point of the graph of $1/\Psi_l$ versus 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 C_{leaf} function in terms of RWC.

Calculation of K_{leaf} (mmol m^{-2} s^{-1} MPa^{-1}) requires that C_{leaf} as determined by the pressure volume curve ($\delta RWC/\delta\Psi_l$, 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:

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

where DW is leaf dry weight (g), LA is leaf area (m^2), WW is mass of leaf water at 100% RWC (g), and M is molar mass of water (g mol^{-1}).

Response of K_{leaf} to Desiccation

"Vulnerability curves" of each species were constructed by measuring K_{leaf} in leaves rehydrated from a range of initial water potentials. Branches were cut early in the morning while Ψ_l was high, and most leaves were removed except for terminal clusters of four to eight leaves. These branches were then allowed to desiccate very slowly ensuring all leaves remained at similar Ψ_l. Periodically, branches were bagged and placed in the dark for 30 min to ensure stomata were closed and Ψ_l was homogenous among leaves. Two leaves were then removed to gauge the Ψ_l of the leaves remaining on the branch, after which two further leaves were detached with their petioles underwater and allowed to rehydrate as described above. The standard rehydration period was between 15 and 30 s. For each sample K_{leaf} was calculated using Equation 2, and the mean of the two samples was used as the K_{leaf} for the branch at the specified Ψ_l. Branches were progressively desiccated during the course of a single day, and K_{leaf} was monitored as Ψ_l dropped. In a few cases (<5) rehydration spanned the Ψ_l at turgor loss. Because C_{leaf} differs pre- and post turgor loss, in these circumstances, the value of C_{leaf} was apportioned depending on the relative distances of Ψ_o and Ψ_f from the turgor loss point. This approximation averages the capacitance during the relaxation period rather than more correctly applying two separate decay curves to either side of the turgor loss point. However, because of the short rehydration period, the loss of accuracy was very small relative to maximum values of K_{leaf}.

Vulnerability curves were generated by plotting K_{leaf} against Ψ_l. The distribution of vulnerabilities of conductive elements in the leaf was assumed to be normal, and hence, a cumulative normal probability curve was fitted to the data.

Response of Photosynthetic Capacity to Desiccation

Chlorophyll fluorescence of PSII was used to measure the sensitivity of photosynthesis to Ψ_l during desiccation. Branches were collected early in the morning and allowed to desiccate under uniform partially shaded conditions (photosynthetic photon flux density of 1,000–1,500 μmol quanta m^{-2} s^{-1}). Leaves were measured in the light to quantify depression of photosynthesis under conditions experienced in the field. Periodically, leaves were removed and placed in the leaf clip of a MiniPam (Walz, Effeltrich, Germany) where they were exposed to an actinic light intensity of 1,800 μmol quanta m^{-2} s^{-1} for 90 s, and the quantum yield of PSII (ϕ_{PSII}) was measured with a single saturating flash to the middle of the adaxial surface of the leaf (avoiding veins). Leaf temperature remained between 25°C and 28°C during measurement. Ψ_l of the sample leaf was then immediately measured giving a single ϕ_{PSII} and Ψ_l per leaf. A minimum of five branches per species were measured, resulting in at least three measurements per 0.1 MPa from −0.5 MPa until ϕ_{PSII} fell below 0.1. As with the vulnerability data, cumulative normal probability plots were fitted to the data, and the point of nonreversible photosynthetic damage was defined as the Ψ_l at which ϕ_{PSII} fell below 0.1. Leaves with yields below 0.1 did not recover maximum dark-adapted quantum yield after rehydration (T. J. Brodribb, unpublished data), in approximate agreement with the general rule indicating 70% RWC as the mean threshold for photosynthetic damage (Lawlor and Cornic, 2002). Hence $\phi_{PSII} = 0.1$ was considered to be the initial damage point for PSII.

Stomatal Closure

Stomatal response to Ψ_l was measured in all species under natural conditions as well as using excised branched to determine the behavior of stomata under extreme drought. All species were surveyed during the months of August and September 2002. Measurements were made on six trees of each species and under conditions of full sun. g_s was measured using a porometer (1600, LI-COR, Lincoln, NE) at different times of the day between 9 AM and 2 PM to include a maximum range of Ψ_ls. g_s 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 were fitted assuming a cumulative normal probability distribution. We defined the response zone of g_s as the region of Ψ_l where the fitted curve for g_s fell from 90% to 20% of maximum.

Statistical Analysis

To test which of the three measured leaf parameters (K_{leaf} vulnerability, turgor loss point, and ϕ_{PSII} sensitivity) exhibited a relationship to Ψ_l most similar to that of g_s, cardinal points in the response functions of each of these relationships were compared. Slopes of the regressions between Ψ_l at early (20%) and mid (50%) stomatal closure, and Ψ_l responsible for early (20%) loss of K_{leaf}, turgor loss, and decline of ϕ_{PSII} below 0.10 were compared by analysis of variance with regressions forced through the origin. Using the SE for the slopes of these regressions, a t test was used to determine whether slopes were significantly different from 1.

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