Stomatal Closure during Leaf Dehydration, Correlation with Other Leaf Physiological Traits

Tim J. Brodribb* and N. Michele Holbrook

Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts

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 ($\psi_l$), and a novel application of this technique allowed the response of $K_{leaf}$ to $\psi_l$ to be determined. These "vulnerability curves" show that $K_{leaf}$ is highly sensitive to $\psi_l$ and that the response of stomatal conductance to $\psi_l$ is closely correlated with the response of $K_{leaf}$ to $\psi_l$. 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 $\psi_l$ than stomatal closure. These results indicate that stomatal closure is primarily coordinated with $K_{leaf}$. However, the close proximity of $\psi_l$ 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 ($\psi_l$) 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 $\psi_l$ 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 to 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 $\psi_l$ 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 $\psi_l$ (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

---

1 This work was supported by the National Science Foundation (grant no. IBN 0212792) and by the Andrew Mellon Foundation.

* Corresponding author; e-mail brodribb@fas.harvard.edu; fax 617–496–5854.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.103.023879.
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 $\Psi_l$ 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 $\Psi_l$ on $K_{leaf}$ by examining the kinetics of $\Psi_l$ 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 $\Psi_l$ 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:

$$V_f = V_o e^{-t/RC}$$

where $V_o$ is the initial potential, $V_f$ is the potential after charging for $t$ seconds, $R$ is the resistance ($=1/K$), and $C$ 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 $\Psi_l$ is determined. An important requirement for the accurate determination of $K_{leaf}$ is that the initial (pre-rehydration) $\Psi_l$ 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 $\Psi_l$ as well as the time of rehydration enabled $K_{leaf}$ to be calculated:

$$K_{leaf} = \frac{C \ln(\Psi_o/\Psi_f)}{t}$$

where $C$ is leaf capacitance, $\Psi_o$ is $\Psi_l$ before rehydration, and $\Psi_f$ is $\Psi_l$ 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 $\Psi$. 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 stomatal and photosynthetic behavior among these species (Brodribb et al., 2003) making them ideal for comparative study.

RESULTS

Stomatal Closure

A general pattern in the stomatal response to $\Psi$, was seen in all species, whereby $g_s$ was responsive to $\Psi_l$ only over a narrow range of $\Psi_l$ (Fig. 2). As a result, the transition from 90% to 20% of maximum $g_s$ in each species occurred over a band of $\Psi_l$ less than 1 MPa. Despite this rapid transition, most species exhibited a continuous response of $g_s$ to $\Psi_l$, and only Quercus oleoides developed a plateau where $g_s$ was not sensitive $\Psi_l$. Variation between species was expressed in the initial $\Psi_l$ that produced strong decreases in $g_s$, and the range of $\Psi_l$ to which stomatal aperture appeared to respond. The point of stomatal closure (defined here as the $\Psi_l$ 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, $\Psi_l$ relaxed (became less negative) exponentially over time as pre-

![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 $\Psi_l$ (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.](http://www.plantphysiol.org)
Figure 2. The relationship between $\Psi_f$ 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 $\Psi_f$ 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 $\Psi_f$ continued until $\Psi_f$ reached around $-0.1$ to $-0.3$ MPa, after which it became slower and nonexponential as $\Psi_f$ 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 $\Delta \Psi_f$ without $\Psi_f$ rising above $-0.3$MPa.

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

Leaf Vulnerability

In all species, $K_{leaf}$ decreased precipitously once $\Psi_f$ 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 $\Psi_f$, $K_{leaf}$ 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 $\Psi_f$ 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 $\Psi_f$ to which $g_s$ responded was evident (Fig. 4). The $\Psi_f$ at turgor loss was also closely correlated with the transition from minimum to maximum $K_{leaf}$ ($r^2 = 0.86$ for $\Psi_f$ at turgor loss versus $\Psi_f$ 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 $\Psi_f$ relaxation remained equivalent to preturgor loss values. In fact, the relaxation of $\Psi_f$ in leaves desiccated below the turgor loss point was extremely slow relative to leaves at higher $\Psi_f$ (Fig. 3), and hence, the calculated $K_{leaf}$ also declined at around this point.

Photosynthetic Response to $\Psi_f$

PSII quantum yield at 1,800 $\mu$mol 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 $\Psi_f$ 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 $\phi_{PSII}$ did not appear to be reversible. Minimum values of $\phi_{PSII}$ were around 0.1, and unlike leaves rehydrated before reaching this low level of fluorescence,
Stomatal Closure and Correlated Physiological Traits

Stomatal Closure and Correlated Physiological Traits

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

DISCUSSION

$K_{\text{leaf}}$

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

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

Figure 3. Typical rehydration kinetics for S. glauca leaves. Single points represent $\Psi_i$ of leaflets during rehydration of a single compound leaf. All curves are exponential, and the slope is used to calculate $K_{\text{leaf}}$. 
The rehydration technique employed here produced values of $K_{\text{leaf}}$ 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).

$K_{\text{leaf}}$ was highly sensitive to desiccation, declining rapidly as $\Psi_t$ 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 $K_{\text{leaf}}$, 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 $K_{\text{leaf}}$ observed here represent cavitation for two reasons, firstly because the response of $K_{\text{leaf}}$ in *S. glauca* to $\Psi_t$ 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 $K_{\text{leaf}}$ observed as $\Psi_t$ 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 $\Psi_t$ at incipient loss of $K_{\text{leaf}}$ and $\Psi_t$ 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**

$K_{\text{leaf}}$ and $g_s$ both showed very similar responses to $\Psi_t$ (Fig. 4; Table I), whereas leaf turgor loss occurred midway through stomatal closure (Fig. 4), and damage to PSII (as indicated by of $\Phi_{\text{PSII}}$) occurred at a
substantially lower $\Psi_l$. 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 $\Psi_l$ is extremely rapid and feed-forward (enabling relaxation of $\Psi_l$ 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 $\Psi_l$ 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 $\Psi_l$ could might be that rapid decreases in $\Psi_l$ 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 $\Psi_l$ and whether guard cell movements are con-

Table 1. Slopes ± se of regressions between cardinal points on the relationships between $\Psi_l$ and stomatal closure, leaf vulnerability, loss of $\phi_{psat}$ and the turgor loss point

<table>
<thead>
<tr>
<th></th>
<th>$\Psi_l$ at 50% of $g_{max}$</th>
<th>$\Psi_l$ at 20% of $g_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi_l$ at turgor loss</td>
<td>1.04 ± 0.085</td>
<td>1.21 ± 0.096</td>
</tr>
<tr>
<td>$\Psi_l$ at 20% loss of $K_{leaf}$</td>
<td>0.76 ± 0.084</td>
<td>0.89 ± 0.089</td>
</tr>
<tr>
<td>$\Psi_l$ at $\phi_{psat}$ &lt; 0.1</td>
<td>0.456 ± 0.063**</td>
<td>0.534 ± 0.068**</td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.031**</td>
<td>0.13 ± 0.040**</td>
</tr>
</tbody>
</table>

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

Stomatal Closure and Correlated Physiological Traits
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 $\Psi_f$. 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, *Glicidia sepium* (Fabaceae) and *Rhederia trinervis* (Verbenaceae), and two evergreen, *Simarouba glauca* (Simaroubaceae) and *Quercus oleoides* (Fagaceae). All are tree-forming species, with *Glicidia* sp. and *Simarouba* sp. both producing compound leaves approximately 20 to 30 cm in length and *Rhederia* 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 $\Psi_f$ 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 $\Psi_f$**

To determine the time course of $\Psi_f$, 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 $\Psi_f$. If these leaves differed in $\Psi_f$ 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 under water using a razor blade. Leaf laminae 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. $\Psi_f$ 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 $\beta - 1$. If these leaves differed from the turgor loss point, the relationship between $\Psi_f$, 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 $\Psi_f$ was ≥0.05 MPa, after which six leaves per species were detached for PV determination. Leaf weight and $\Psi_f$ were measured periodically during slow desiccation of sample leaves in the laboratory. Desiccation of leaves continued until $\Psi_f$ stopped falling or began to rise due to cell damage. Due to the elastic cell walls, $C_{leaf}$ pre- and post-turgor loss are quite different. It was found that the relationship between $\Psi_f$ 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 $1/\Psi_f$ 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 $C_{leaf}$ be determined by the pressure volume curve ($\psi_{RWC}/\Psi_f$, 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 (Koida 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:

$$C_{leaf} = \frac{RWC}{\Psi_f} \times \frac{1}{(DW/LA)} \times \frac{(WW/DW)/M}{M}$$

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 $\Psi_f$ 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 $\Psi_f$. Periodically, branches were bagged and placed in the dark for 30 min to ensure stomata were closed and $\Psi_f$ was homogenous among leaves. Two leaves were then removed to gauge the $\Psi_f$ 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 $\Psi_f$. Branches were progressively desiccated during the course of a single day, and $K_{leaf}$ was monitored as $\Psi_f$ dropped. In a few cases ($<5$) rehydration spanned the $\Psi_f$ 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 $\Psi_f$ and $\Psi_l$ 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 $\Psi_f$. 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 $\Psi_s$ during desiccation. Branches were collected early in the morning and allowed to desiccate under uniformly shaded conditions (photosynthetic photon flux density of 1,000 mol 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 mol quanta m$^{-2}$ s$^{-1}$ for 90 s, and the quantum yield of PSII ($\phi_{PSII}$) was measured with a single saturating flash to the middle of the adaxial surface of the leaf (avoiding veins). Leaf temperature remained between 24°C and 28°C during measurement. $\Psi_s$ of the sample leaf was then immediately measured giving a single $\phi_{PSII}$ and $\Psi_s$ 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 $\phi_{PSII}$ 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 $\Psi_s$ at which $\phi_{PSII}$ 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 Corin, 2002). Hence $\phi_{PSII} = 0.1$ was considered to be the initial damage point for PSII.

Stomatal Closure

Stomatal response to $\Psi_s$ was measured in all species under natural conditions as well as using excised branches 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 $\Psi_s$. $g_s$ was recorded from a series of marked leaves that were subsequently removed and bagged for later determination of $\Psi_s$. The relationship between $\Psi_s$ 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 $\Psi_s$ 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 ($K_{s,leaf}$ vulnerability, turgor loss point, and $\phi_{PSII}$ sensitivity) exhibited a relationship to $\Psi_s$, 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 $\Psi_s$ and $\phi_{PSII}$, $\Psi_s$ and $g_s$, and $\phi_{PSII}$ and $g_s$ were calculated in all species under natural conditions as well as using excised branches 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 $\Psi_s$. $g_s$ was recorded from a series of marked leaves that were subsequently removed and bagged for later determination of $\Psi_s$. The relationship between $\Psi_s$ 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 $\Psi_s$ where the fitted curve for $g_s$ fell from 90% to 20% of maximum.

ACKNOWLEDGMENTS

We acknowledge the help and support of Maria Marta Chavarria and Rojel Blanco of Parque Nacional Santa Rosa.

Received April 24, 2003; returned for revision May 18, 2003; accepted May 18, 2003.

LITERATURE CITED


Holbrook NM, Zwie Niecki MA (1999) Xylem refilling under tension: Do we need a miracle? Plant Physiol 120: 7–10


Stomatal Closure and Correlated Physiological Traits