Declining hydraulic efficiency as transpiring leaves desiccate: two types of response*

TIM J. BRODRIBB1,2 & N. MICHELE HOLBROOK2

1Department of Plant Science, University of Tasmania, PO Box 252-55, TAS, Australia 7001, and 2Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA, USA

ABSTRACT

The conductance of transpiring leaves to liquid water (K_leaf) was measured across a range of steady-state leaf water potentials (Ψ_leaf). Manipulating the transpiration rate in excised leaves enabled us to vary Ψ_leaf in the range -0.1 to -1.5 MPa while using a flowmeter to monitor the transpiration stream. Employing this technique to measure how desiccation affects K_leaf in 19 species, including lycophytes, ferns, gymnosperms and angiosperms, we found two characteristic responses. Three of the six angiosperm species sampled maintained a steady maximum K_leaf while Ψ_leaf remained above -1.2 MPa, although desiccation of leaves beyond this point resulted in a rapid decline in K_leaf. In all other species measured, declining Ψ_leaf led to a proportional decrease in K_leaf such that midday Ψ_leaf of unstressed plants in the field was sufficient to depress K_leaf by an average of 37%. It was found that maximum K_leaf was strongly correlated with maximum CO₂ assimilation rate, while K_leaf = 0 occurred at a Ψ_leaf slightly less negative than at leaf turgor loss. A strong linear correlation across species between Ψ_leaf at turgor loss and Ψ_leaf at K_leaf = 0 raises the possibility that declining K_leaf was related to declining cell turgor in the leaf prior to the onset of vein cavitation. The vulnerability of leaves rehydrating after desiccation was compared with vulnerability of leaves during steady-state evaporation, and differences between methods suggest that in many cases vein cavitation occurs only as K_leaf approaches zero.

INTRODUCTION

Most of the water transported in vascular plants is destined to replace leaf water sacrificed during the diffusive uptake of atmospheric CO₂ for photosynthetic carbon fixation. Irrigation of the leaf mesophyll thus represents the crux of a plant’s vascular function. Leaves make complicated demands of the vascular system, requiring close proximity between sites of water delivery and water loss while not interfering with light harvesting or the CO₂ diffusion pathway. In most plants, these demands are met by a hierarchical branching network of veins that terminate in extremely small xylem conduits, tens to hundreds of microns from the sub-stomatal cavities where the bulk of evaporation takes place (Wylie 1943; Roth-Nebelsick et al. 2001). Current research suggests that this vascular arrangement generates a large resistance to water flow through leaves, representing between 30 and 90% of the hydraulic resistance of the whole plant (Salleo, Nardini & Lo Gullo 1997; Nardini, Tyree & Salleo 2001; Sack et al. 2002). One important consequence of this is that even in plants with good access to soil water and high stem water potential, stomatal closure could be induced during the day due to water potential gradients generated in the hydraulic passage from petiole to sub-stomatal cavities. It follows therefore, that the efficiency of leaf water transport plays a governing role in processes linked to leaf water status, most importantly, stomatal behaviour and photosynthetic gas exchange.

The efficiency of water transport within leaves, or leaf hydraulic conductance (K_leaf), has been demonstrated to vary enormously between species (Tyree et al. 1999; Brodribb et al. 2005), ecological niches (Nardini & Salleo 2005; Sack, Tyree & Holbrook 2005) and seasons (Salleo et al. 2002; Brodribb & Holbrook 2003a). Among this variation, perhaps the best physiological correlate with K_leaf is stomatal conductance (Brodribb & Holbrook 2004b; Brodribb et al. 2005; Nardini, Salleo & Andri 2005). Considering that these parameters represent liquid and gas phase conductances of water moving in a serial pathway through the leaf, this correlation, and similar relationships between stem hydraulic conductance and gₛ (Nardini & Salleo 2000; Meinzer 2002) indicate that water potential gradients in non-stressed plants are relatively conservative. That is, during evolution plants appear to increase the conductance of the vascular system in order to accommodate increased transpirational demand rather than operating at increased water potential gradients. Photosynthetic performance has also been linked with K_leaf over a diverse selection of plants, although the nature of the relationship is unclear in tropical angiosperms, where values of K_leaf appear to be higher than in other species, but diurnally variable (Brodribb et al. 2005). Emerging relationships between K_leaf and anatomical characters that limit leaf gas exchange such as stomatal...
pore area index and palisade thickness (Aasamaa, Sober & Rahi 2001; Sack et al. 2003; Sack & Froel 2006) indicate that hydraulic efficiency in the leaf vascular system is highly adaptive.

While there is an ever-expanding library of data for $K_{\text{leaf}}$ variation between species, data describing the vulnerability of the whole-leaf hydraulic pathway to dysfunction under water stress remains sparse. Extensive work on stems has shown that the conductivity of the xylem is critically dependent on water potential ($\Psi$), usually declining rapidly as $\Psi$ inside the xylem apoplast falls below a threshold value (Sperry & Tyree 1988). Leaves are clearly sensitive to water stress-induced depression of hydraulic conductance (Linton & Nobel 2001; Cochard 2002; Brodribb & Holbrook 2003b; Lo Gullo et al. 2003; Brodribb & Holbrook 2004a), and due to the disproportionately large contribution leaves make to whole-plant hydraulic resistance, leaf vulnerability has the potential to dictate how plants respond to short-term water stress. This has been borne out by recent studies demonstrating a good correspondence between turgor loss, stomatal closure and leaf hydraulic dysfunction (Brodribb & Holbrook 2003b). However, the exact nature of the decline in $K_{\text{leaf}}$ with leaf water potential, remains poorly understood, hampered by a lack of techniques for probing $K_{\text{leaf}}$ while leaves are simultaneously exposed to significant negative water potentials. To date, the only methods used to examine the impact of water stress on $K_{\text{leaf}}$ have used the kinetics of $\Psi_{\text{leaf}}$ relaxation (Brodribb & Holbrook 2003b), or measured infiltration rates of droughted leaves exposed to sub-atmospheric pressures (Trifillò et al. 2003). While both these techniques have successfully shown responses of $K_{\text{leaf}}$ to drought, the conditions under which leaves are measured in both cases are rather distant from those experienced by leaves in the field. In the case of $\Psi_{\text{leaf}}$ relaxation, leaves are measured during a rapid collapse of the water potential gradient, while leaves exposed to vacuum infiltration are measured with intercellular spaces flooded and virtually no gradient in $\Psi_{\text{leaf}}$. If we are to determine whether reductions in $K_{\text{leaf}}$ in the field are likely to be rare events associated with significant plant stress, or common events that limit photosynthesis on a diurnal basis, it is desirable to measure the response of $K_{\text{leaf}}$ to desiccation in transpiring leaves exposed to a natural range of $\Psi_{\text{leaf}}$. Such conditions provide the greatest chance of capturing the full range of processes operating in leaves under natural conditions.

The process responsible for reduced $K_{\text{leaf}}$ at low water potential is assumed to be the same as in stems, that is, embolism derived from air-seeding of the pit membrane (Zimmermann 1983), and this is supported by observation of embolism in petioles (Bucci et al. 2003) and in veins (Canny 2001; Sallee et al. 2001). However, other dynamic processes are also thought to affect the efficiency of water flow through leaves, including aquaporin activity (Nardini et al. 2005) and conduit deformation under water stress (Cochard et al. 2004; Brodribb & Holbrook 2005). To comprehend the likely contribution of these (and other) factors in the decline of $K_{\text{leaf}}$ with $\Psi_{\text{leaf}}$, it is essential to comprehensively understand the response of $K_{\text{leaf}}$ to $\Psi_{\text{leaf}}$. Here we examine the relationship between $K_{\text{leaf}}$ and $\Psi_{\text{leaf}}$ in plants spanning a large range of morphological and anatomical complexity, from lycopod to angiosperm. We investigate $K_{\text{leaf}}$ under conditions that closely replicate those experienced by leaves in situ by measuring $\Psi_{\text{leaf}}$ under known conditions of leaf transpiration (Tyree et al. 1999). In a novel application of this technique, we were able to create a large range of transpiration (E) in each species by using a variable fan to force water loss, thus giving a measure of $K_{\text{leaf}}$ at a range of $\Psi_{\text{leaf}}$. Data from this steady-state technique are compared with $K_{\text{leaf}}$ vulnerability to dehydration determined by the non-steady-state pressure relaxation technique (Brodribb & Holbrook 2003b), to indicate the processes responsible for impeding water flow at low water potential.

**MATERIALS AND METHODS**

**Plant material**

A list of 19 species, designed to span a large climatic as well as morphological and phylogenetic range, was sampled in temperate forest in Hobart, Australia, and Harvard Forest, USA and tropical forest at Santa Rosa National Park, Costa Rica; Lake Eacham, Australia; and Mt. Dzumac, New Caledonia. Among this selection were six lycophytes, two ferns, five gymnosperms (including a cycad) and six angiosperms (see Table 1). All gymnosperm and angiosperm leaves were collected from small trees (< 4 m) in full sun, while only two of the fern species were collected in the sun and the other six collected in forest understory. Only healthy mature leaves of a similar age were used in each species sample so as to minimize within species variation to a minimum.

**$K_{\text{leaf}}$ determined by evapotranspiration**

This method calculates $K_{\text{leaf}}$ of a leaf transpiring at a known steady-state as the ratio of transpiration flux over the pressure differential between water entering the leaf and steady-state $\Psi_{\text{leaf}}$ (Boyer 1974). Excised, transpiring leaves were connected to a flowmeter that measured the transpiration stream as it was sucked into the leaf. A modified flowmeter similar to those used to measure the hydraulic conductivity of excised twigs (Brodribb & Feild 2000) was used where a filtered, degassed 0.01 M KCl solution passed from a reservoir, through a capillary tube and into the petiole of the sample leaf. A pressure transducer (PX-136; Omega Engineering Inc. Stamford, CT, USA) measured the water pressure between a calibrated capillary tube and the leaf, and this pressure (sub-atmospheric due to the suction created by the leaf) was logged and converted into a flow rate. The length of the capillary tube was tailored to the type of leaf being measured such that the pressure in the flowmeter remained in the range −0.05 to −0.15 bars, thus avoiding tube cavitation while generating sufficient pressure to allow accurate calculation of flow.

A problem with the evapotranspiration method is that...
Gymnosperms

Species Family K

Angiosperms

Journal compilation © 2006 Blackwell Publishing Ltd, © 2006 The Authors

Parameters include the type of regression found to best describe the response of \( K_{leaf} \) to decreasing \( \Psi_{leaf} \); habitat from which leaves were sampled; \( X \)-intercept of the linear function \( K_{leaf} = f(\Psi_{leaf}) + c \) showing the water potential at which \( K_{leaf} = 0 \) (only in species with linear response functions); mean maximum \( K_{leaf} \) (mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\)); mean maximum stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\)) and mean maximum instantaneous rate of CO\(_2\) uptake (\( \mu \)mol m\(^{-2}\) s\(^{-1}\)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>( K_{leaf} ) response</th>
<th>Habitat</th>
<th>( \Psi_{leaf} ) at ( K_{leaf} = 0 )</th>
<th>( K_{leaf} ) max.</th>
<th>g. max.</th>
<th>A max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selaginella longipinnae</td>
<td>Selaginellaceae</td>
<td>Linear</td>
<td>Shade, tropical</td>
<td>1.80</td>
<td>2.03</td>
<td>0.0728</td>
<td>1.70</td>
</tr>
<tr>
<td>Selaginella pallescens</td>
<td>Selaginellaceae</td>
<td>Linear</td>
<td>Sun, tropical</td>
<td>2.50</td>
<td>4.83</td>
<td>0.2445</td>
<td>6.15</td>
</tr>
<tr>
<td>Tectaria confluis</td>
<td>Tectariaceae</td>
<td>Linear</td>
<td>Shade, tropical</td>
<td>2.45</td>
<td>3.19</td>
<td>0.0719</td>
<td>1.85</td>
</tr>
<tr>
<td>Gymnosperms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callitris rhomboidea</td>
<td>Cupressaceae</td>
<td>Linear</td>
<td>Sun, temperate</td>
<td>4.01</td>
<td>11.30</td>
<td>0.1684</td>
<td>12.31</td>
</tr>
<tr>
<td>Cycas media</td>
<td>Cycadaceae</td>
<td>Linear</td>
<td>Sun, tropical</td>
<td>1.85</td>
<td>7.65</td>
<td>0.093</td>
<td>9.65</td>
</tr>
<tr>
<td>Pinus strobus</td>
<td>Pinaceae</td>
<td>Linear</td>
<td>Sun, temperate</td>
<td>2.10</td>
<td>10.20</td>
<td>0.1719</td>
<td>9.80</td>
</tr>
<tr>
<td>Retropphyllum comptonii</td>
<td>Podocarpaceae</td>
<td>Linear</td>
<td>Sun, tropical</td>
<td>1.43</td>
<td>4.58</td>
<td>0.0711</td>
<td>5.72</td>
</tr>
<tr>
<td>Tsuga canadensis</td>
<td>Pinaceae</td>
<td>Linear</td>
<td>Sun, temperate</td>
<td>1.61</td>
<td>6.60</td>
<td>0.1083</td>
<td>8.89</td>
</tr>
<tr>
<td>Angiosperms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eucalyptus globulus</td>
<td>Myrtaceae</td>
<td>Linear</td>
<td>Sun, temperate</td>
<td>2.97</td>
<td>13.20</td>
<td>0.3592</td>
<td>16.85</td>
</tr>
<tr>
<td>Byrsonima crassifolia</td>
<td>Malpigiaceae</td>
<td>Sigmoid</td>
<td>Sun, tropical</td>
<td>–</td>
<td>17.19</td>
<td>0.4488</td>
<td>15.59</td>
</tr>
<tr>
<td>Curatella americana</td>
<td>Dillenaceae</td>
<td>Linear</td>
<td>Sun, tropical</td>
<td>2.30</td>
<td>21.10</td>
<td>0.4043</td>
<td>15.21</td>
</tr>
<tr>
<td>Dalbergia reflexa</td>
<td>Fabaceae</td>
<td>Sigmoid</td>
<td>Sun, tropical</td>
<td>–</td>
<td>19.50</td>
<td>0.6619</td>
<td>19.02</td>
</tr>
<tr>
<td>Genipa americana</td>
<td>Rubiaceae</td>
<td>Linear</td>
<td>Sun, tropical I</td>
<td>2.55</td>
<td>17.00</td>
<td>0.4161</td>
<td>14.29</td>
</tr>
<tr>
<td>Rehdera trinervis</td>
<td>Verbenaceae</td>
<td>Sigmoid</td>
<td>Sun, tropical</td>
<td>–</td>
<td>20.6</td>
<td>0.4118</td>
<td>17.03</td>
</tr>
</tbody>
</table>

Rapid hydration of leaves often results in the hydropassive closure of stomata, thus arresting transpiration. This was overcome here by minimizing the time between leaf excision and connection to the flowmeter (to typically 60–180 s), avoiding excessive leaf wetting, and by driving transpiration with heated air as soon as the leaf was connected to the flowmeter. These measures avoided a rapid rise in \( \Psi_{leaf} \) as leaves were connected to the flowmeter, thus eliminating hydropassive stomatal closure.

Leaves were measured between 1000 and 1530 h and were maintained in full sun (1700–2000 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\)) after sampling and during measurement. Leaves were always acclimated to sunlight for a minimum of 2 h prior to measurement. The sampling process involved cutting a small branch underwater, trying to avoid leaf wetting. The sample leaf was carried to the flowmeter where it was re-cut under the perfusing solution at the petiole and rapidly connected to the flowmeter. A heat gun (HG 1100; Makita, Aichi, Japan) was then used to create the desired transpiration flow by modifying the airflow and/or temperature of the leaf. Leaves were maintained in full sun at temperatures ranging from 25 °C to a maximum of between 38 and 40 °C; the upper temperature limit for each species was determined by the maximum temperature found to produce a reversible 20% decrease in CO\(_2\) uptake in the leaves of each species. This was considered a reasonable test to ensure that membrane fluidity was minimally impacted by temperature. Leaf temperature was measured by two fine wire thermocouples held in contact with the adaxial surface of the sample leaf by a coarse nylon mesh. Temperatures of the water entering the leaf as well as the flowmeter were also measured by thermocouples, and the whole system (including the leaf) maintained as isothermal as possible (by directing a regulated proportion of the heat gun flow over the flowmeter) to avoid problems associated with temperature gradients. Because of the complicating effect of temperature on \( K_{leaf} \) calculation (due to viscosity effects and membrane fluidity), the leaf temperature range for each species was confined to 5 °C. In order to minimize the correlation between leaf temperature and E, airflow over the leaf was modulated to ensure that the warmest leaves of each species were measured at both low and high steady-state evaporation rates. To avoid any \( K_{leaf} \) signal generated by circadian rhythmicity (Nardini et al. 2005), leaves were measured between 1000 and 1530 h, and the imposed evaporative gradient was varied such that equal numbers of leaves in the morning and afternoon were exposed to low and high \( \Psi_{leaf} \). Sample size per species ranged between 30 and 80 replicate leaves from a minimum of five plants.

The half-time for water potential equilibration after alteration in E was either measured from the kinetics of \( \Psi_{leaf} \) relaxation in excised leaves rehydrating underwater, or taken from previous work (Brodribb & Holbrook 2003b). Among the species sampled here, the range of \( \Psi_{leaf} \) relaxation half-times was 8 to 40 s, thus requiring that leaves were maintained at steady state (<3% change in

© 2006 The Authors
Journal compilation © 2006 Blackwell Publishing Ltd, Plant, Cell and Environment, 29, 2205–2215
flow over 20 s for at least 3 min before being removed from the flowmeter and immediately sealed into humidified plastic bags that were then placed into a pressure chamber (SoilMoisture, Santa Barbara, CA, USA) for determination of $\Psi_{\text{leaf}}$. The balance pressure was determined with a pressure gauge ($\pm$ 0.1 psi), and a dissecting microscope used to scrutinize the cut end of the petiole for the exact moment of sap exudation. Precise determination of $K_{\text{leaf}}$ requires that $\Psi_{\text{leaf}}$ measured by the pressure chamber accurately reflects the driving force behind hydraulic flow in the leaf. We were therefore careful to test for changes in the balance pressure of leaves after the initial determination of $\Psi_{\text{leaf}}$ (approximately 20 s after removal from the flowmeter) as this might reflect redistribution of water within the leaf due to possible tissue compartmentalization. Typically leaves were measured immediately after removal from the flowmeter, and then re-measured 5 min (and occasionally 10 min) later to determine the extent of possible drift in $\Psi_{\text{leaf}}$ due to heterogeneous pressure distribution in the leaf. In about 3% of leaves, drift in the balance pressure was observed to exceed 5%, in which case it was assumed that insufficient time had elapsed for steady-state $\Psi_{\text{leaf}}$ to establish, and the reading was discarded. Following determination of $\Psi_{\text{leaf}}$, leaf area was measured with a digital camera (Nikon, Tokyo, Japan) and image analysis software (Image J, National Institute of Health, USA). In the case of the lycopods and Selaginella, whole shoots were used, and leaf area was measured with all leaves/microphylls removed.

Clogging of the cut end of the petiole proved to be problematic in some species, particularly those with resins in the petiole. To minimize the impact of clogging, the cortex was removed where possible, and the petiole cut several times in clean perfusing solution to ensure that the initial influx of water into the leaf did not carry with it resins or chemicals likely to block the xylem. In the case of species where resin canals were distributed throughout the petiole/stem, a branch was cut and all leaves removed except the target leaf. This relatively large segment of stem was then attached to the flowmeter applying the principle that providing an excess of xylem pathways to the leaf should overcome localized flow interruption. In general, leaves were removed and re-cut if the flow had not stabilized after 10 min.

During the flowmeter measurements, leaf temperature, air temperature, capillary tube temperature, relative humidity and pressure transducer voltage were all logged at 5 s intervals and the data stored on a datalogger (CR-10X; Campbell Scientific Inc., Logan, UT, USA). The flow into the leaf was determined by multiplying the pressure differential across the capillary tube by its hydraulic conductance (Eqn 1), and $K_{\text{leaf}}$ by dividing the steady-state flow by $\Psi_{\text{leaf}}$ (Eqn 2).

$$F = \Delta P K_{\text{tube}} 1/V_{\text{tube}},$$

where $F$ = flow into petiole (mmol s$^{-1}$); $\Delta P$ = the pressure differential across the capillary tube (MPa); $K_{\text{tube}}$ = capillary tube hydraulic conductance (mmol s$^{-1}$ MPa$^{-1}$); $V_{\text{tube}}$ = viscosity of water in capillary tube relative to 20 °C.

$$K_{\text{leaf}} = F V_{\text{leaf}} / (\Delta \Psi_{\text{leaf}} A_{\text{leaf}}),$$

where $K_{\text{leaf}}$ = leaf hydraulic conductance (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$); $V_{\text{leaf}}$ = viscosity of water in the sample leaf relative to 20 °C; $\Delta \Psi_{\text{leaf}}$ = tube to leaf water potential difference (MPa); $A_{\text{leaf}}$ = leaf area (m$^2$).

**Vulnerability determined by $\Psi_{\text{leaf}}$ relaxation**

In a sub-sample of five species (*Byronima, Callitris, Curatella, Genipa and Rehdera*), the response of $K_{\text{leaf}}$ to $\Psi_{\text{leaf}}$ was measured by water potential relaxation to provide a comparison with data collected under steady-state evaporation. Six branches from three trees of each species were collected in the morning and each branch subdivided into three to four samples, all of which were allowed to desiccate to a range of water potentials from approximately −0.4 to −3.0 MPa before being carefully bagged to arrest water loss. We employed the technique of Brodribb & Holbrook (2003b) to calculate $K_{\text{leaf}}$ from the kinetics of $\Psi_{\text{leaf}}$ relaxation in leaves rehydrated through the petiole. Initial $\Psi_{\text{leaf}}$ was determined either by measuring leaves neighbouring the sample leaf, or in large leaves by sampling a small part of the target leaf prior to rehydration. Sample leaves were then cut at the petiole, while underwater, and allowed to rehydrate in full sun for 30, 60 or 90 s depending on the initial $\Psi_{\text{leaf}}$. Final $\Psi_{\text{leaf}}$ was measured with the pressure chamber and $K_{\text{leaf}}$ calculated from the ratio of initial to final $\Psi_{\text{leaf}}$ and the leaf capacitance (Eqn 3).

$$K_{\text{leaf}} = C_{\text{leaf}} \ln[\Psi_{\text{f}}/\Psi_{\text{i}}]/(t V_{\text{leaf}}),$$

where $\Psi_{\text{i}}$ = initial water potential (MPa); $\Psi_{\text{f}}$ = final water potential (MPa); $t$ = duration of rehydration (s); $C_{\text{leaf}}$ = leaf capacitance (mmol m$^{-2}$ MPa$^{-1}$).

**Pressure–volume (PV) relations**

Two leaves from each of three replicates of each species were sampled for determination of leaf turgor dynamics and leaf capacitance from PV analysis (Tyree & Hammel 1972). Mean leaf capacitance ($C_{\text{leaf}}$) for each species was measured from six fully expanded leaves using the slope of the leaf pressure–volume relationship (Tyree & Hammel 1972). Branches were cut underwater in the morning and rehydrated until $\Psi_{\text{leaf}}$ was > −0.05 MPa, after which leaves were detached for PV determination. Leaf weight and water potential were measured periodically during slow desiccation of sample leaves in the laboratory. The initial (linear) slopes of the relative water content (RWC) versus $\Psi_{\text{leaf}}$ curves yielded the leaf capacitance function in terms of RWC. Calculation of $K_{\text{leaf}}$ (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) requires that leaf capacitance be calculated in absolute terms and normalized by leaf area. To do this, the capacitance calculated from the PV curve was multiplied by the saturated mass of water in the leaf and then divided by leaf area (Koide et al. 1991; Brodribb & Holbrook 2003b). Leaf areas were measured as...
projected areas with a digital camera and image analysis software.

Maximum assimilation and field \( \Psi_{\text{leaf}} \)

Mean maximum assimilation rate was measured in each species from a sample of 10 leaves. Measurements were made at 0700–0800 h while plants were maximally hydrated. A portable gas exchange system (Li-6400; Li-Cor, Lincoln, NE, USA) was used to measure \( \text{CO}_2 \) uptake, transpiration and stomatal conductance in a ventilated cuvette which provided a saturating quantum flux of 1800 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \) during measurements. Leaf temperature was not controlled, but remained in the range 25–36 °C in all measurements. Small shoots from lycopods, Selaginella and some conifer species were dissected and photographed following removal from the cuvette to ensure that the total projected leaf area within the cuvette was measured.

In the same sub-sample of species used for determination of vulnerability by \( \Psi_{\text{leaf}} \) relaxation, diurnal trends in field \( \Psi_{\text{leaf}} \) were measured during the wet season under conditions of high soil moisture availability (pre-dawn \( \Psi_{\text{leaf}} \approx 0 \)). Three leaves from three trees of each Costa Rican species were sampled hourly from 0800 to 1500 h on two cloudless days (2 and 18 August 2005), while 10 leaves of the Australian fern species (Tectaria confluens) were sampled between 1200 and 1500 h on two dry sunny days (20 and 22 November 2005).

Additionally, a transpiration rate of 34 mmol m\(^{-2}\) s\(^{-1}\) at a transpiration rate of close to 30 mmol m\(^{-2}\) s\(^{-1}\), even at the very high transpiration rate of close to 30 mmol m\(^{-2}\) s\(^{-1}\). By contrast, the second leaf (closed circles) initially exceeds \( \Psi_{\text{leaf}} \) of this leaf. An exponential sigmoid function of the form \( y = 100/(1 + e^{(\Psi_{\text{leaf}}+b)}) \), where \( y = \% \) loss of \( \Psi_{\text{leaf}} \) fitted (Pammenter & Van der Willigen 1998), and \( K_{\text{max}} \) was defined as the mean \( \Psi_{\text{leaf}} \) at \( \Psi_{\text{leaf}} > -1 \) MPa. Curves were fitted using the statistical package JMP (SAS Inst., Cary, NC, USA).

**RESULTS**

Application of the heat gun to modify the temperature and boundary layer of sample leaves was highly effective in producing a large range of \( E \) in all species (Fig. 1). Maximum flow rates were achieved by the combination of high airflow over the leaf and high leaf temperature (\( T_{\text{max}} = 40 ^{\circ} \text{C} \) in tropical tree species). Immediately upon directing heated air over the leaf, the water flow into the petiole was observed to rise rapidly to a maximum value, stabilizing typically after 60–90 s (Fig. 2). Leaves exposed to higher airflow and air temperature reached progressively higher steady-state \( E \). Maximum transpirational fluxes induced were up to three times larger than maximum \( E \) measured in the field (unpublished data). Once a threshold rate of evaporation was exceeded however, stomatal closure was triggered and \( E \) reached a steady-state below its initial maximum (Fig. 2). Increasing \( E \) was associated with a decline in \( \Psi_{\text{leaf}} \), however, the relationship between \( E \) and

![Figure 2](image2.png)
ψrst was non-linear in most species due to a disproportionate decrease in ψrst as E increased. All species exhibited a maximum limit for E, above which stomatal closure was apparently triggered by low leaf water potential (Fig. 2). Maintaining forced evaporation from the leaf once stomatal closure was initiated resulted in low leaf water potentials accompanied by significantly reduced E (Fig. 2). Drift in balance pressure of leaves 10 min after removal from the flowmeter was less than 5% indicating a high degree of pressure homogeneity in the leaf while transpiring at steady state.

The relationship between E and ψrst was non-linear in all species measured, resulting in two characteristic patterns in the response of Kleaf to ψrst. Most species, including all sampled conifers, cycads, lycophytes ferns and three of the six angiosperms, exhibited a linear decline in Kleaf with ψrst as leaves were exposed to increasingly large evaporative fluxes (Fig. 3). A second relationship was characterized in the remaining three angiosperms sampled, where the response of Kleaf to ψrst presented a more ‘typical’ sigmoidal shape as seen in stem xylem vulnerability curves (Fig. 3). Linear or sigmoid regressions fitted to each respective Kleaf versus ψrst plot yielded highly significant correlation coefficients in all cases (r² > 0.72; P < 0.01).

Measurements of ψrst in all angiosperms and a single fern in the field indicated that for the species with a linear response of Kleaf to ψrst, field ψrst at midday would result in a depression of Kleaf by an average of 37 ± 6% (SD; n = 4 species) of maximum Kleaf expected at ψrst = 0 MPa (Fig. 3). In the angiosperm species exhibiting a sigmoidal response of Kleaf to ψrst, midday values of ψrst (with fully hydrated soil) remained above the threshold inducing significant depression of Kleaf (Fig. 3).

**Comparison of methods**

In five species from each of the vulnerability classes described earlier, the response of Kleaf to ψrst was tested using the pressure relaxation method to help pinpoint the tissue responsible for inhibiting Kleaf as ψrst declines. All species including those with linear responses under steady-state flow (above) exhibited sigmoid-shaped responses of Kleaf to ψrst when vulnerability was measured by relaxation (Fig. 4). Despite the contrasting shape of vulnerability curves in two of the four species, the extrapolated maximum Kleaf (at ψrst = 0) derived by each method was similar, except in *Byrsonima crassifolia* where maximum Kleaf determined by relaxation was significantly higher than that measured by evaporative flux (24.4 mmol m⁻² s⁻¹ MPa⁻¹ versus 17.3 mmol m⁻² s⁻¹ MPa⁻¹, respectively).

Among the 16 out of 19 species that demonstrated a linear response of Kleaf to ψrst, the X-intercept of the Kleaf versus ψrst function was calculated to determine the water potential at which Kleaf = 0. This analysis produced a range of ψrst from −1.4 MPa in the rain forest conifer *Retrophyllum comptonii* to −4.0 MPa in the dry forest conifer *Callitris rhomboidea*. A strong correlation between the water potential at turgor loss and ψrst at Kleaf = 0 was observed (r² = 0.77; P < 0.01), however, the slope of this relationship (0.6) indicates that in most species, bulk leaf turgor loss occurred before Kleaf reached zero (Fig. 5).

© 2006 The Authors

*Figure 3. Changes in Kleaf in response to decreasing ψrst under forced evaporation.* Two species (angiosperms *Rehdera trinervis* and *Byrsonima crassifolia*) show sigmoidal responses to ψrst while the other two (the angiosperm *Genipa americana* and fern *Tectaria confluens*) show linear responses to decreasing ψrst. Mean minimum diurnal ψrst for each of these species measured in the field during the wet season is shown as a vertical line (with dotted SD). According to these data, both species with linear Kleaf response to ψrst should exhibit significant diurnal depression of Kleaf on sunny days.
K_leaf versus instantaneous CO₂ uptake

Maximum values of K_leaf for each species were strongly correlated with mean maximum CO₂ assimilation rate (Fig. 6). The best fit for this correlation was a curve describing an exponential rise to a maximum assimilation rate of 19.7 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), that is, \( A = -3.5 + 23.2(1 - e^{-0.1K_{leaf}}) \). This indicates a decreasing sensitivity of maximum CO₂ uptake to increasing K_leaf at values of K_leaf above 10 mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\). The angiosperms represented here are all sun plants with K_leaf > 10 mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\), and hence in this sample of angiosperms, CO₂ fixation was relatively insensitive to variation in K_leaf. By contrast, the non-angiosperm sample, which included many understory species, demonstrated a high sensitivity of CO₂ uptake to K_leaf.

DISCUSSION

In a potent demonstration of the limitations of the leaf vascular system, we found that excised leaves given unimpeded access to water were easily desiccated, by forced evaporation, to water potentials low enough to induce stomatal closure. Manipulating leaf evaporation enabled the creation of a large range of steady-state \( \Psi_{\text{leaf}} \) from which the response of K_leaf to \( \Psi_{\text{leaf}} \) could be calculated for the first time in transpiring leaves. Declining leaf water potential compromised the conductance of whole leaves to liquid water in the range of water potentials likely to be experienced by leaves in the field. While the impact of low leaf water potential was detrimental to all 19 species sampled here, the manner in which K_leaf responded to \( \Psi_{\text{leaf}} \) fell into two distinct patterns. From a sample size of 19 species, 16 species demonstrated a linear decline in K_leaf with \( \Psi_{\text{leaf}} \). The remaining three species, all angiosperms, presented a sigmoidal response to \( \Psi_{\text{leaf}} \) when K_leaf was measured under conditions of steady-state evaporation.
classic sigmoid response of $K_{leaf}$ to desiccation where $K_{leaf}$ declined rapidly beyond a threshold $\Psi_{leaf}$.

Among the few techniques for measuring $K_{leaf}$ vulnerability to desiccation, this novel technique of using transpiring leaves provides the most realistic measure of leaf performance under natural conditions. Other techniques such as vacuum infiltration and pressure relaxation can provide information about loss of $K_{leaf}$ by embolism, if it is assumed that emboli repair slowly, but cannot be used to determine the influence of flow or pressure-dependent processes upon $K_{leaf}$. It could be argued that the high maximum rates of evaporation induced here are also likely to create an unrealistic environment for the leaf; however, in all species at least half of $K_{leaf}$ determinations were made with leaves transpiring within their natural range. Hence, the water potential gradients and vapour fluxes in this half of the leaves measured were equivalent to those known to occur at midday on plants with good access to water. The only shortcoming of this technique is that it is not possible to lower the delivery pressure of water at the petiole to less than approximately −30 kPa, and hence the effect of drying soil cannot be directly simulated.

Setting aside this limitation for a moment and focusing on undroughted plants, we can say with confidence that the data presented here indicate that $K_{leaf}$ in many species is likely to change continually with variations in transpiration during the course of a day. Such excursions have been observed in direct measurements of $K_{leaf}$ (Bucci et al. 2003; Brodribb & Holbrook 2004a), although most techniques currently used for measuring $K_{leaf}$ might mask $K_{leaf}$ depression due to the fact that leaves are measured only while fully hydrated. Among the angiosperm species found here to exhibit linear vulnerability to $K_{leaf}$, diurnal variation in $\Psi_{leaf}$ under conditions of abundant soil moisture would produce an expected diurnal depression of $K_{leaf}$ in the order of 37% (e.g. *Genipa americana*; Fig. 3). Assuming some degree of isohydry in leaves of these species, a 37% reduction in $K_{leaf}$ would lead to a similar reduction in sustainable water loss from the leaf, and hence in CO$_2$ assimilation compared with the expected rate if $K_{leaf}$ was to remain maximal (Franks 2006). While the impact of a labile $K_{leaf}$ on gas exchange is relatively easy to predict, it is critical to understand the physiology of the process leading to $K_{leaf}$ depression so as to determine how different species manifest differences in the vulnerability of leaves to impaired hydraulic function, and whether the depression of $K_{leaf}$ is reversible.

There are several candidates for producing impaired flow under conditions of decreasing $\Psi_{leaf}$: the most standard is cavitation of the vein xylem. A large body of evidence indicates that xylem cavitation in the stem is common under water stress, and the role of desiccation-induced cavitation in leaves is supported by observation of embolism in major veins of plants (Canny 2001; Cochard 2002; Bucci et al. 2003). Certainly, xylem cavitation is inevitable at some point as leaves desiccate, and studies of water potential relaxation data (including those shown here), indicate a dramatic loss of $K_{leaf}$ at water potentials less than −2 MPa. However, it seems less likely that cavitation is the cause of rapid linear decline in water potential observed here in most species at $\Psi_{leaf} > −1$ MPa. The only suggestion of leaf vein embolism in this range of $\Psi_{leaf}$ comes from a reported decrease in dye infiltration of fine veins; however, this was not typically linked to a decrease in $K_{leaf}$ (Salleo et al. 2001; Trifilo et al. 2003). Indeed it seems improbable that leaves should invest carbon into a fine vein network that becomes embolized by water potentials equivalent to the midday $\Psi_{leaf}$ of well-watered plants. Such highly vulnerable fine veins would only function under conditions of low evaporation such as low light or high humidity; conditions under which the enhanced $K_{leaf}$ they confer will be of little service to the leaf.

We contend that the decline of $K_{leaf}$ at water potentials above the leaf turgor loss point may arise from either turgor-related changes in the conductivity of tissue downstream from the xylem conduits, or a change in the proportion of evaporation from different hydraulic compartments of the leaf. While both turgor and osmotic signals are thought to induce changes in membrane properties (Lew 1996; Heidecker et al. 2003), turgor is a better candidate for signalling changes in tissue conductivity as it changes in proportion with $\Psi_{leaf}$, while osmotic potential changes in a non-linear fashion. Several features of the parenchymatous bundle sheath that surrounds the minor veins make it the probable location for such a turgor-sensitive governor in the leaf hydraulic pathway (Sack & Holbrook 2006). It is thought that much of the transpiration stream is shunted through the bundle sheath symplast by a suberized layer in perpendicular walls of bundle sheath cells (Van Fleet 1950; Canny 1990; Sack, Streeter &
bulk leaf turgor and $K_{\text{leaf}}$ could be defined (Fig. 7). Most species showed sigmoidal vulnerability functions, a linear relationship between $\Psi_{\text{leaf}}$ and $K_{\text{leaf}}$. In all species (except the three angiosperms displaying sigmoidal vulnerability functions), a linear relationship between average leaf turgor pressure and $K_{\text{leaf}}$ is observed here. The concept of a turgor-limited passage through the bundle sheath is supported here by a good correlation between the leaf turgor loss point and the $\Psi_{\text{leaf}}$ at which $K_{\text{leaf}}$ fell to zero (Fig. 5). In all species (except the three angiosperms displaying sigmoidal vulnerability functions), a linear relationship between bulk leaf turgor and $K_{\text{leaf}}$ could be defined (Fig. 7). Most species however, conserved a small proportion of leaf conductance at zero leaf turgor. This is expected considering that xylem collapse is xylem collapse (Cochard et al. 2004). As such, bundle sheath cells are probably the first living cells traversed by water passing from the stem to leaf. If this is so, then the hydraulic conductivity of this gateway into the mesophyll is susceptible to changes in membrane properties and possibly cell turgor. The concept of a turgor-limited passage through the bundle sheath is supported here by a good correlation between the leaf turgor loss point and the $\Psi_{\text{leaf}}$ at which $K_{\text{leaf}}$ fell to zero (Fig. 5). In all species (except the three angiosperms displaying sigmoidal vulnerability functions), a linear relationship between bulk leaf turgor and $K_{\text{leaf}}$ could be defined (Fig. 7). Most species however, conserved a small proportion of leaf conductance at zero leaf turgor. This is expected considering that roughly 30% of leaf hydraulic resistance is due to resistances between the fine veins and the sites of evaporation (Sack et al. 2003). As such, while the leaf is transpiring, the bundle sheath cells will be at a higher water potential than those cells downstream at the evaporating end of the hydraulic pathway (the cells assumed to contribute the bulk of $\Psi_{\text{leaf}}$). Hence, bundle sheath cells may retain a small proportion of turgor while cells at the sites of evaporation are at turgor loss.

The discrepancy between pressure relaxation kinetics and steady-state evaporation techniques can also be reconciled if $K_{\text{leaf}}$ is sensitive to bundle sheath turgor. All measurements of leaves, both here and in previous studies (Brodribb & Holbrook 2003b, 2004a) have shown that leaf vulnerability to $K_{\text{leaf}}$ depression is best described by a sigmoid function whenever it is measured by water potential relaxation techniques. Interestingly, this includes those species found here to express a linear response when measured under steady-state evaporation conditions (Fig. 4). If it is assumed that a significant proportion of the hydraulic resistance of leaves lies downstream of the bundle sheath (Boyer 1974; Salleo et al. 2003; Sack et al. 2004), then during pressure relaxation measurements the water potential of the bundle sheath will rise much more rapidly than the average leaf water potential (as measured by the pressure bomb). This would have the effect of relieving the flow inhibition created by reduced bundle sheath turgor early in the rehydration time course, thus erroneously inflating the measured $K_{\text{leaf}}$ at low initial leaf water potentials. Vulnerability responses generated by pressure relaxation are therefore likely to indicate the onset of vein cavitation while being relatively insensitive to perturbations in $K_{\text{leaf}}$ caused by changes in bundle sheath turgor.

One final possible explanation for a linear decline in $K_{\text{leaf}}$ during desiccation is xylem collapse (Cochard et al. 2004). This phenomenon has only been observed in a few species of conifers (Cochard et al. 2004; Brodribb & Holbrook 2005) while its importance in other plant families is unknown. Two pieces of evidence tend to rule against this as a likely factor however. Firstly, the water potentials shown to initiate cell collapse in the xylem are substantially more negative than $-1\ MPa$, yet we see strong depression of $K_{\text{leaf}}$ in all species in the $\Psi_{\text{leaf}}$ range $0$ to $-1\ MPa$. Secondly, xylem collapse appears to respond sigmoidally to declining $\Psi_{\text{leaf}}$, making it an unlikely candidate for producing the linear sensitivity of $K_{\text{leaf}}$ to $\Psi_{\text{leaf}}$ observed here.

Interestingly, we found that three species among our sample of 19 demonstrated sigmoidal responses of $K_{\text{leaf}}$ to declining $\Psi_{\text{leaf}}$ when measured using both steady-state evaporation and water potential relaxation techniques (Fig. 4). These three species, all angiosperms, showed good agreement between techniques in maximum $K_{\text{leaf}}$ as well as the threshold $\Psi_{\text{leaf}}$ leading to $K_{\text{leaf}}$ depression (Fig. 4). This may indicate that the conductivity of leaf tissue in these angiosperms is insensitive to turgor, or that the turgor of the bundle sheath is maintained by osmotic adjustment, thereby maintaining maximum $K_{\text{leaf}}$ until the cavitation threshold is reached. Considering the obvious benefit for the plant in avoiding $K_{\text{leaf}}$ depression under average field $\Psi_{\text{leaf}}$, it is probable that this behaviour is adaptive, and possibly restricted to angiosperms. The disadvantage of such a threshold in $K_{\text{leaf}}$ sensitivity to $\Psi_{\text{leaf}}$ is that once a minimum value of $\Psi_{\text{leaf}}$ is transgressed, $K_{\text{leaf}}$ falls precipitously, presumably by xylem cavitation. Avoiding cavitation thus requires closely regulated isohydry (Tardieu & Simonneau 1998), a condition that is satisfied by each of the angiosperms found here to express sigmoidal vulnerability (T.J. Brodribb, unpublished data).

By contrast, a linear decrease in $K_{\text{leaf}}$ during drought may attenuate the impact of drying soil upon leaf conductance by expanding the range of leaf water potentials to which stomatal must respond to maintain leaf evaporation within the limits defined by $K_{\text{leaf}}$. In doing so, the decline in $\Psi_{\text{leaf}}$ in response to drought should be slowed, and hence $K_{\text{leaf}}$ and $\Psi_{\text{leaf}}$ are less likely to plummet catastrophically as might be the case with a sigmoidal vulnerability. This concept is
supported by observations of stomatal closure in response to leaf water potentials significantly higher than those found to initiate leaf vein cavitation in a number of ferns species (Brodribb & Holbrook 2004b).

These two types of $K_{\text{leaf}}$ dynamics in angiosperms are strongly reminiscent of the two types of photosynthetic responses to water deficit described by Lawlor & Cornic (2002). Photosynthetic data mostly from crop plants suggest that in one group of species, CO$_2$-saturated assimilation declines in proportion with declining RWC, while in the other group maximum assimilation is insensitive to changes in RWC (while RWC > 75%). These similarities in $K_{\text{leaf}}$ and photosynthetic responses to RWC may not be coincidental but rather indicate coordinated limitation of these two processes.

Much interest has been generated recently by correlations between $K_{\text{leaf}}$ and leaf anatomical traits such as vein density, and palisade thickness (Aasamaa et al. 2001; Sack & Froë 2006). It is hoped that these correlations will enable the prediction of leaf gas exchange properties and hence ecological character from leaf anatomy. As such it is important to consider that in most of the species measured here, $K_{\text{leaf}}$ declines linearly with $\Psi_{\text{leaf}}$, and hence the $K_{\text{leaf}}$ of that species measured at $\Psi_{\text{leaf}} = 0$ MPa will not provide an accurate indication of the $K_{\text{leaf}}$ expressed in transpiring leaves even with fully hydrated soil. Even in terms of understanding the relationship between maximum CO$_2$ fixation and leaf hydraulics, it must be borne in mind that maximum CO$_2$ fixation in the field can only be realized under conditions of maximum irradiance and moderate air movement, conditions that must result in $\Psi_{\text{leaf}}$ falling significantly below zero. For this reason, $K_{\text{leaf}}$ will always be depressed below maximum whenever leaves are photosynthesizing maximally (except in species with sigmoid vulnerability). This may be part of the explanation for the weak correlation between CO$_2$ uptake and $K_{\text{leaf}}$ in angiosperms (Fig. 6), given that half the measured angiosperms displayed linear- and half-sigmoidal vulnerability.

In summary, we note that the hydraulic efficiency of leaves of each species examined in this study decreased in a predictable manner as transpirational flux increased. Between species however, the response of $K_{\text{leaf}}$ to transpiration was highly variable. All leaves measured under well-watered conditions in the field operated at water potentials capable of inducing some degree of $K_{\text{leaf}}$ depression, indicating that the phenomenon is probably widespread among plants. Combining this knowledge with the fact that the leaves contribute a large proportion of the whole-plant hydraulic resistance, it is clear that the vulnerability of leaves to $K_{\text{leaf}}$ depression represents a defining character of a plant’s physiology.

ACKNOWLEDGMENTS

We thank The Arnold Arboretum of Harvard University, the National Science Foundation (IBN 0212792), The Australian Research Council, The Harvard Forest, The National Geographic Society and the Andrew W. Mellon Foundation. We also thank Jill Britton for field support and the staff of Parque Nacional Santa Rosa.

REFERENCES


Received 22 May 2006; received in revised form 28 July 2006; accepted for publication 18 August 2006