Diurnal depression of leaf hydraulic conductance in a tropical tree species

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ABSTRACT

Diurnal patterns of hydraulic conductance of the leaf lamina (K_{leaf}) were monitored in a field-grown tropical tree species in an attempt to ascertain whether the dynamics of stomatal conductance (g_s) and CO_2 uptake (A_{leaf}) were associated with short-term changes in K_{leaf}. On days of high evaporative demand mid-day depression of K_{leaf} to between 40 and 50% of pre-dawn values was followed by a rapid recovery after 1500 h. Leaf water potential during the recovery stage was less than −1 MPa implying a refilling mechanism, or that loss of K_{leaf} was not linked to cavitation. Laboratory measurement of the response of K_{leaf} to Ψ_{leaf} confirmed that leaves in the field were operating at water potentials within the depressed region of the leaf 'vulnerability curve'. Diurnal courses of K_{leaf} and Ψ_{leaf} predicted from measured transpiration, xylem water potential and the K_{leaf} vulnerability function, yielded good agreement with observed trends in both leaf parameters. Close correlation between depression of K_{leaf}, g_s and A_{leaf} suggests that xylem dysfunction in the leaf may lead to mid-day depression of gas exchange in this species.

Key-words: gas exchange; leaf hydraulic conductance; mid-day depression; stomata; water use.

INTRODUCTION

The importance of the hydraulic vascular system in limiting gas exchange is well documented (Whitehead, Jarvis & Warning 1984; Brodribb & Feild 2000; Hubbard *et al.* 2001) and recent models of gas exchange dynamics from the leaf to stand level incorporate xylem functional parameters (Sperry *et al.* 1998; Buckley, Mott & Farquhar 2003; Katul, Leuning & Oren 2003). The accepted principles of xylem-limited water relations assume that under normal conditions leaf water loss is regulated such that hydrostatic water potential in the xylem approaches, but does not transgress its cavitation limit (Sperry, Alder & Eastlack 1993). Stomatal regulation of evaporation is the key process coupling water demand and supply, and recent mathematical models of stomatal behaviour illustrate how this linkage might

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operate through a combination of passive hydromechanics and active solute movement into guard cells (Buckley *et al.* 2003). It remains to be shown however, how a relationship between the mechanistically disparate processes of xylem cavitation resistance and guard cell turgor relations is manifested under natural conditions. This question is crucial for understanding how plants have adapted to a world where water availability is highly variable.

The significance of xylem cavitation in response to water stress has been borne out by studies on stems and petioles illustrating that plants appear to avoid cavitation by closing stomata at water potentials equivalent to that responsible for incipient cavitation (Salleo *et al.* 2000; Nardini, Tyree & Salleo 2001; Cochard *et al.* 2002). It makes sense that plants should avoid cavitation as it impedes water flow, and could potentially lead to runaway embolism whereby a feedback between decreasing leaf water potential and increasing cavitation would culminate in complete xylem failure (Tyree & Sperry 1988). Further supporting the concept of stomatal avoidance of cavitation is the notion that the repair of xylem embolism can only occur once water tension is relieved, at which point root pressure and capillary action are capable of collapsing emboli. Were this the case then plants could not afford to allow xylem embolism under average conditions (where plant water potential remains substantially below zero for extended periods) as accumulation of embolisms would quickly render the vascular system non-functional.

Evidence of embolism reversal under tension is sparse, although there are reports of refilling under tension in leaves (Canny 2001; Lo Gullo *et al.* 2003), roots (McCully, Huang & Ling 1998), and stems and petioles (Zwieniecki & Holbrook 1998; Bucci *et al.* 2003; Hacke & Sperry 2003). These reports sustain the possibility that embolism repair is a means by which some plants are able to operate close to the hydraulic limit of their water-conducting system without risking the compounding effects of frequent xylem cavitation. Depending on the metabolic cost associated with refilling, this may represent a legitimate strategy for optimizing the carbon economy, as cavitation resistance is likely to be a costly investment for plants (Hacke *et al.* 2001). It must be noted however, that, as yet, none of the proposed mechanisms for hydraulic isolation and refilling of embolized conduits have been shown to operate.

Studies on xylem dysfunction and refilling have been confined almost exclusively to woody parts of plants or to petioles, whereas the leaf lamina has attracted relatively little attention. This is surprising as the leaf lamina represents one of the least conductive regions of the plant, contributing between 30 and 80% of the whole plant hydraulic resistance (Salleo, Nardini & Lo Gullo 1997; Nardini & Salleo 2000; Sack *et al*. 2003) over a distance of rarely more than 1% of the total hydraulic path length. Only recently has the vulnerability of leaf venation been investigated, and the results of these studies suggest that leaf xylem operates very close to its cavitation threshold (Nardini & Salleo 2000; Brodribb & Holbrook 2003a; Lo Gullo *et al*. 2003). For example, in a survey of four tropical forest trees it was shown that leaf hydraulic conductivity was significantly reduced at water potentials responsible for inducing only 50% stomatal closure (Brodribb & Holbrook 2003a). In the field, a reduction in stomatal conductance to 50% of maximum during conditions of high evaporative demand is not uncommon for tropical or temperate species and these data raise the possibility that depression in leaf xylem conductivity may be a regular part of leaf function.

Here we investigate the diurnal behaviour of leaves of a seasonally dry tropical forest tree *Simarouba glauca*, previously identified as a species in which minimum diel leaf water potential approaches the cavitation threshold for petioles (Brodribb *et al*. 2003). Focusing at the leaf level, our goals were to determine if leaf hydraulic conductivity behaved in a dynamic fashion during diurnal fluctuations in evaporative demand, and how these dynamics related to stomatal control of water loss and assimilation (A_{leaf}). *Simarouba glauca* is an ideal subject for this type of study being a deep-rooted, evergreen tree, and as such the variation in pre-dawn leaf water potential is small relative to the large fluctuations in leaf water potential in response to seasonal and diurnal changes in atmospheric vapour pressure. Our investigations were undertaken in Pacific northern Costa Rica, where the climate is characterized by a strong dry season of approximately 6 months, during which time rainfall is typically absent and evaporative demand high. Previous studies in this region have indicated significant losses in stem and leaf hydraulic conductivity and associated declines in photosynthetic capacity during the dry season in a range of associated species (Brodribb, Holbrook & Gutiérrez 2002, Brodribb *et al*. 2003). Strong depression of stomatal conductance and photosynthesis after a morning peak has been noted in several of the common evergreen species during the dry season and this behaviour in *S. glauca* was the focus of investigations here.

MATERIALS AND METHODS

Study site and plant material

This investigation was undertaken in the Santa Rosa National Park, located close to the Northern Pacific coast of Costa Rica (10°52′ N, 85°34′ W, 285 m above sea level).

Mean annual rainfall in the park is 1528 mm; however, more than 90% of this falls between the months of May and December, resulting in a pronounced dry season. The dry season is accompanied by strong trade winds, low relative humidity (mid-day minimum relative humidity generally between 30 and 40%) and high irradiance, all of which contribute to the high evaporative demand. Diurnal and seasonal temperature ranges are relatively small, with a mean annual temperature of 28 °C. The wet season (May to December) is interrupted by a characteristic dry period of up to a month, typically occurring in late July to August (Janzen 1983). This mini-summer or 'veranillo' period provides an opportunity to examine the effects of atmospheric drought while soils remain relatively hydrated. Vegetation in the park is comprised of a mosaic of deciduous, evergreen and mixed forest types depending on the successional age of forest stands, with evergreen species more prominent in more mature forest patches.

The common evergreen tree species *Simarouba glauca* (Simaroubiaceae) was chosen for this study for its compound leaves (allowing within-leaf sampling) and deep root system which enables efficient access to water year round. *Simarouba glauca* trees grow to about 25 m in height and are successful over a range of soil types from deep clay vertisols to sand and serpentine soils. This species produces large compound leaves approximately 20 cm in length comprised of 12–16 pinnae each approximately 5 cm in length. *Simarouba glauca* has been included in several recent studies of tree species in Santa Rosa National Park, and hence information on leaf water relations, stem xylem vulnerability and seasonal gas exchange and water relations are already available.

All sampling was made from two adjacent trees which produced synchronous leaf flushes during 2003. Leaf age has been shown to produce a strong effect on both K_{leaf} and photosystem II (PSII) electron transport capacity (Brodribb & Holbrook 2003a), and as such it was imperative to use replicate trees with identical leaf age structure. Our sampling strategy was designed to minimize the period between sampling so as to glean the maximum information about short-term changes in gas exchange and hydraulic conductance of leaves.

Trees flushed during the early dry season (December 2002) and again during the early wet season (early June 2003). Diurnal courses of K_{leaf}, stomatal conductance, leaf and stem xylem water potential and CO_2 uptake were sampled at the end of the dry season (21–23 April 2003, 5-month-old leaves), early wet season (13–16 June 2003, 7-month-old leaves) and finally during the dry interruption of wet season (14–15 August 2003, 2-month-old leaves). The first two sampling periods used the same cohort of leaves whereas the final sampling period (August 2003) used leaves from the June cohort.

Leaf hydraulic conductance

Sampling days for hydraulic and gas exchange measurements were selected for minimum cloud cover. In April and

August this was not a problem, however, during the wet season (June) afternoon cloud, usually accompanied by rain, was a regular daily feature and hence K_{leaf} measurements could only be accompanied by gas exchange data until mid-day when cloud or rain truncated the record. On each of the sampling days, 21 leaflets were covered with plastic wrap and aluminium foil and these leaflets used to assay xylem water potential (Ψ_x) every 100–120 min ($n = 3$ per sample).

The value of K_{leaf} was measured by assessing the rehydration kinetics of leaves after detachment, as described by Brodribb & Holbrook (2003a). Briefly, a detached sample leaf (non-evaporating) is allowed to take up water through the petiole for a fixed period of time and the change in water potential during rehydration used to calculate leaf conductance. The principle being that the rehydrating mesophyll behaves as a capacitor, charged through a resistor ($1/K_{leaf}$). Using this technique, intensive sampling of trees over 2 d enabled K_{leaf} to be determined every hour between 0600 and 1800 h during the three sample periods. At each time interval three fully exposed leaves were selected from the most recent cohort of leaves and cut and carefully bagged in the dark for 30 min while leaflet water potential was allowed to equilibrate and stomata close. From each leaf, two leaflets were harvested as an estimate of the initial leaf water potential. Assuming agreement in the samples for initial water potential, two remaining leaves were placed underwater and cut from the rachis. Leaflets were allowed to absorb water for 10 and 20 s after which their petioles were dabbed dry on paper towel, and the leaflets placed in plastic bags to prevent water loss. The value of Ψ_l was immediately measured using a Scholander pressure chamber (PMS, Corvallis, OR, USA). Leaf conductance was then calculated from the equation:

$$K_{leaf} = C \ln(\Psi_o/\Psi_f)/t \qquad (1)$$

where C is the leaf capacitance; Ψ_o is the leaf potential prior to rehydration; Ψ_f is the leaf water potential after rehydration for t seconds.

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

Calculation of K_{leaf} (mmol m^{-2} s^{-1} MPa^{-1}) requires that leaf capacitance 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 pressure–volume 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:

$$C_{leaf} = \delta RWC/\delta\Psi_l \times (DW/LA) \times (WW/DW)/M \qquad (2)$$

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

Vulnerability curves

Vulnerability of leaf hydraulic conductance to leaf water potential was measured for the April and August sampling periods. Ten to 20 sun leaves were removed from trees pre-dawn and allowed to desiccate on a bench (at 25 °C) for periods of 5–400 min. After desiccation, leaves were bagged in the dark for 30 min after which K_{leaf} was measured as above and plotted against the initial leaf water potential. Vulnerability curves were produced by fitting cumulative probability plots to the data as described in (Brodribb & Hill 1999).

Leaf gas exchange

During the three sampling periods stomatal conductance was measured as frequently as possible during daylight hours in order to obtain the maximum information about diurnal stomatal dynamics. An Li-1600 porometer (LiCor Inc., Lincoln, NE, USA) was used as it provided the most rapid instantaneous estimate of g_s without modifying ambient conditions of humidity, temperature or light around the leaf. Five leaflets were labelled and g_s measured every 30 min between 0600 and 1800 h. Half an hour was considered the highest sampling frequency possible without significantly perturbing the stomata and depressing g_s. Air temperature, ambient humidity, leaf temperature and irradiance were also recorded at each time period.

Instantaneous determination of CO_2 uptake was made only in the August sampling period using the same labelled leaves as above. A portable infrared gas analyser (Li-6400; LiCor Inc.) was used under ambient light and temperature conditions and measurements of CO_2 and water vapour exchange made every 30 min.

Modelling leaf water potential and K_{leaf}

In order to test the predicted versus observed diurnal patterns of K_{leaf} and Ψ_{leaf} in response to measured diurnal

trends in leaf transpiration a standard hydraulic application of Ohm's law was employed:

$$\Psi_{leaf} = \Psi_x - E/K_{leaf}. \tag{3}$$

Using Eqn 3, the diurnal variation in K_{leaf} and its effect on leaf water potential were modelled using leaf transpiration (E) data, stem xylem water potential (Ψ_x), with $K_{leaf} = f(\Psi_{leaf})$ where f was the previously established vulnerability function. Leaf water potential was modelled assuming steady-state conditions. We assumed that the vulnerability function was reversible without hysteresis. Due to the fact that K_{leaf} and Ψ_{leaf} were not independent it was necessary to find solutions to Eqn 3 by iteration. This was done using Visual Basic for Applications (Microsoft, Washington, USA) where 1000 iterations was sufficient to yield stable solutions for K_{leaf} and Ψ_{leaf} at each time period.

RESULTS

Dynamics in liquid and gas phase conductance

Very large differences in stomatal and xylem dynamics were apparent between leaves of *S. glauca* in the wet and dry seasons. Dry season dynamics were characterized by a concomitant decline in stomatal and leaf hydraulic conductance after a morning peak, followed by afternoon recovery of both K_{leaf} and g_s (Fig. 1). Data from 22 April (late dry season) indicated a rapid early morning rise in g_s followed

by an equally rapid stomatal closure after 0900 h as vapour pressure deficit (VPD) increased to peak daily values. In all leaves g_s was reduced to between 40 and 5% of the morning maximum by 1100 h. During this same period K_{leaf} was observed to fall from early morning highs of between 27 and 42 mmol m^{-2} s^{-1}MPa^{-1} (mean 34 mmol m^{-2} s^{-1}MPa^{-1}) to minimum values of between 11 and 20 mmol m^{-2} s^{-1}MPa^{-1} (Fig. 1). Afternoon saw a recovery of g_s in all leaves to approximately 50% of maximum by 1500 h. The afternoon recovery of g_s was not accompanied by large changes in VPD, which generally fell after 1500 h. A rapid recovery in K_{leaf} was also observed after 1400 h when values returned to around the early morning maximum.

Leaves in the wet season (June) exhibited very different stomatal and K_{leaf} dynamics. A much smaller diurnal range in VPD resulted in higher g_s in all of the marked leaflets (same leaflets as above). Stomatal conductance peaked at the high value of approximately 600 mmol m^{-2} s^{-1} at between 0900 and 1000 h, and in contrast to the dry season, this high conductance was more or less maintained until the early afternoon when rain truncated measurement. No dynamic changes in K_{leaf} were observed, although mean values were only approximately half the dry season (April) maximum, presumably due to the effects of leaf age on K_{leaf} (these leaves were already 7 months old at the time of measurement and fell at 9–10 months of age).

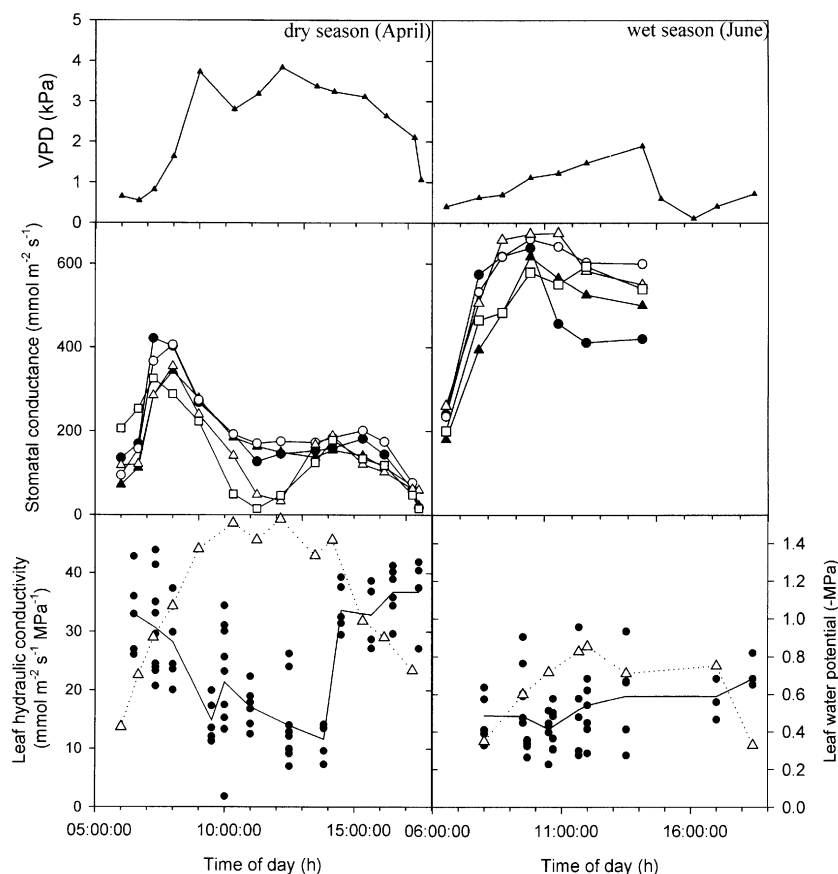The second cohort of leaves measured during the mini dry season in August showed similar dynamics to leaves

Figure 1. Diurnal courses of leaf-atmospheric VPD, stomatal conductance, K_{leaf} (closed circles) and Ψ_{leaf} (open triangles) in two adjacent trees in the dry season (22 and 23 April) and wet season (13 and 15 June). Both stomatal conductance (middle panel) in each of five leaves (each with unique symbol) and K_{leaf} (lower panel) became profoundly depressed in the April leaves, whereas no such dynamic can be observed in the wet season. Full recovery of K_{leaf} was observed in leaflets during the afternoon whereas Ψ_{leaf} remained less than −1 MPa. The same five leaves were used for g_s measurements in April and June, and symbols for each leaf are consistent. June measurements of g_s were truncated soon after mid-day by consistent rainfall. Each data point for K_{leaf} represents a single leaflet whereas Ψ_{leaf} data represent means of 5–10 leaflets.

measured in April (Fig. 2). Depression of both g_s and K_{leaf} was evident between 1000 and 1400 h, although less profound than that recorded in April. Photosynthetic rates as measured by CO_2 uptake followed stomatal conductance dynamics with a strong mid-day depression followed by partial recovery after 1400 h.

A comparison of leaf xylem vulnerability between April and August illustrated that although the shape of the relationship remained constant, leaves at the end of the dry season (April) lost more conductivity than those during the veranillo in August (Fig. 3).
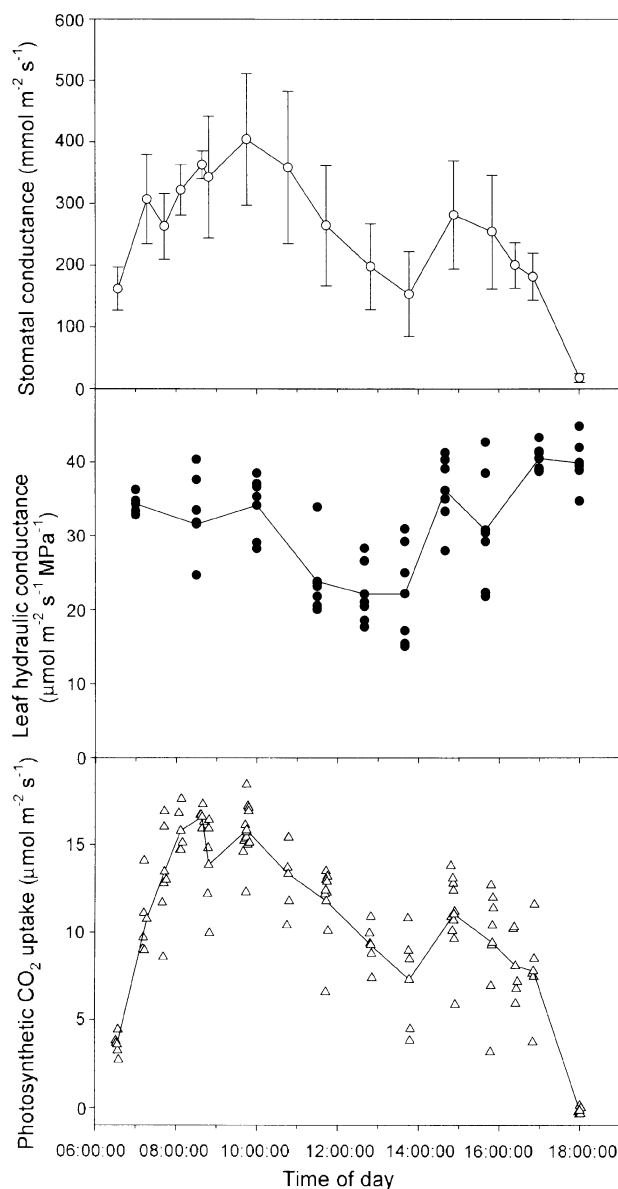

Figure 2. Dynamics of g_s ($n = 5$–10), K_{leaf} and Assimilation on two consecutive days (14–15 August) of high evaporative demand during the short rainless break in the wet season (soils fully hydrated). Depression of gas exchange and hydraulic conductance are clearly evident, although not as profound as in the late dry season (cf. Fig. 1). Afternoon recovery of K_{leaf} coincides with a moderate increase in g_s and A_{leaf}.
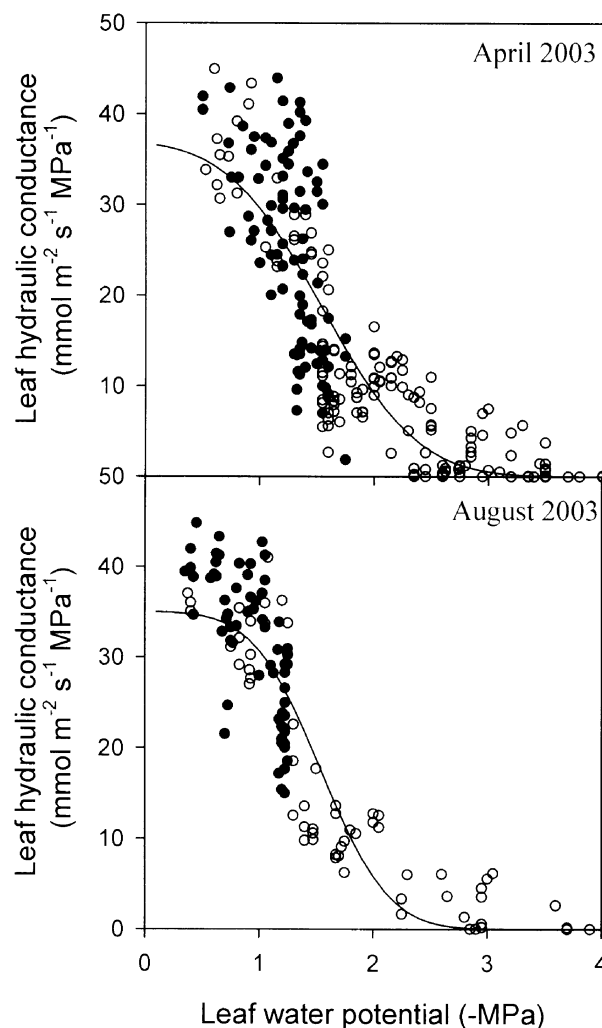

Figure 3. Vulnerability of K_{leaf} to Ψ_{leaf} induced depression in *Simarouba glauca* leaves slowly bench dried in the dark (open circles). Vulnerability curves in April and August were virtually identical, with 50% loss incurred at −1.55 and −1.54 MPa, respectively. Field data (filled circles) are overlaid for K_{leaf} during the April and August observation periods. Field data conformed to the same vulnerability relationship although the April field data appeared somewhat scattered, possibly indicating slight hysteresis in the recovery of K_{leaf} during the afternoon.

Modelled interaction of K_{leaf} with Ψ_{leaf} and mid-day depression of A

The diurnal progress of K_{leaf} and Ψ_{leaf} was modelled from observed rates of E and by defining $K_{leaf} = f(\Psi_{leaf})$ where f was the regression between K_{leaf} and Ψ_{leaf} in leaf desiccated in the laboratory (regression Fig. 3). Results matched quite closely the observed diurnal behaviour of both K_{leaf} and Ψ_{leaf} (Fig. 4). Some disparity was evident in the April data where the observed decline in K_{leaf} was greater than predicted, and as a result leaf water potentials were more negative than predicted. This discrepancy was greatest in the latter half of the day after recovery of K_{leaf}.
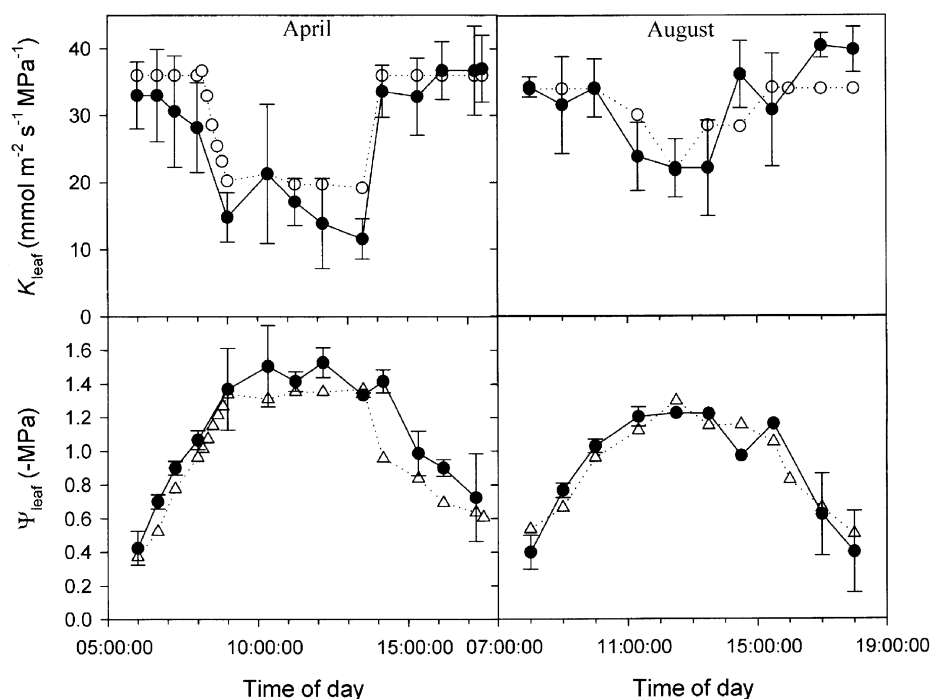
Figure 4. Modelled versus observed ($\pm$ SD; n = 5–10) diurnal behaviour of K_{leaf} and Ψ_{leaf} during the April and August observation periods. Predictions from the model (open symbols) were in good agreement with the observed values (filled symbols) for both K_{leaf} and Ψ_{leaf} during the day, most predicted values falling within the ± 1 SD bars shown.

DISCUSSION

The results here illustrate that the tropical tree *S. glauca* experienced diurnal depression and recovery of leaf hydraulic conductivity under conditions of moderate evaporative stress even when soils were hydrated. These results compliment earlier data (Brodribb & Holbrook 2003a) showing that a sample of dry tropical tree species all operated close to, or within the region of K_{leaf} depression due to leaf water deficit. Although several studies have observed diurnal cycles of cavitation and recovery in the petiole (Zwieniecki *et al.* 2000; Bucci *et al.* 2003), this is the first time that the leaf lamina has been shown to undergo diurnal cycles of loss and recovery of hydraulic conductance.

It is somewhat surprising that leaves should experience losses in hydraulic conductivity during periods of high evapotranspiration as this must lead to positive feedback between K_{leaf} and Ψ_{leaf} risking catastrophic xylem failure. Leaves of *S. glauca* appeared to avoid this scenario by a combination of rapid stomatal response to evaporation (Fig. 1) as well as high maximum K_{leaf} which damps the initial response of leaf water potential to changes in VPD. Maximum values of K_{leaf} in *S. glauca* of around 37 mmol m^{-2} s^{-1} MPa^{-1} are among the highest values measured for tree species (cf. maximum for temperate species of 17 mmol m^{-2} s^{-1} MPa^{-1} in *Vitis* (Sack *et al.* 2003)), and even after a 50% loss of K_{leaf}, the residual conductivity is sufficient to sustain rapid recovery of Ψ_L if stomata close and upstream xylem is not compromised.

The diurnal loss and recovery of K_{leaf} in *S. glauca* appears to proceed without the significant hysteresis expected for a relatively slow refilling process (Hacke & Sperry 2003).

This was evidenced both by the agreement between equilibrated vulnerability curves in the laboratory and field K_{leaf} measurements (Fig. 3), and by the accurate prediction of K_{leaf} and Ψ_L made from a model that assumed no hysteresis in the conductance/water potential relationship (Fig. 4). These data do not support the concept of a threshold water potential necessary for recovery, and suggest that lag time for recovery is either very short or absent. Two possibilities arise from these observations, first that refilling after cavitation is an extremely rapid, ongoing process in the leaf, and second that the observed losses in K_{leaf} are due to processes other than cavitation and refilling. The existing evidence for afternoon recovery of petiole hydraulic conductivity comes from studies that have included either conduit staining or percentage embolism data in support of the cavitation/refilling mechanism (Zwieniecki & Holbrook 1998; Bucci *et al.* 2003). These techniques are difficult to apply to the venation within the leaf lamina; however, acoustic emissions (Nardini *et al.* 2001; Lo Gullo *et al.* 2003) and cryoscanning electon microscopy (Canny 2001) of leaves both suggest that the same process of bubble formation occurs in the leaf venation as in other parts of the plant vascular system.

An alternative explanation for diurnal cycles in K_{leaf} other than classical cavitation is the idea that conduits may deform under tension, reversibly constricting the hydraulic flow through the leaf (Cochard *et al.* 2004). The observations by Cochard *et al.* of tracheid collapse in *Pinus* at moderate leaf water potentials, followed by a rapid recovery upon rehydration, may explain the K_{leaf} recovery observed here. One observation that does not appear consistent with this hypothesis is the fact that the kinetics of water potential relaxation in dehydrated leaves of *S. glauca* follow a single

exponential function, suggesting constant K_{leaf} during rehydration to water potentials close to zero (Brodribb & Holbrook 2003a). However, it is possible that the reversal of a hydromechanical restriction to flow in the leaf might occur with a time constant greater than the 60 s generally allowed for water potential relaxation measurements, but less than the 30 min between measurements here.

Mechanism aside, it remains to explain the advantage to leaves in producing a vascular system that is vulnerable to cavitation during normal daily function. The answer to this question in the case of *S. glauca* probably lies in the relationship between the guard cell environment and the evaporative environment. The high hydraulic conductivity of *S. glauca* leaves means that the guard cell water potential is likely to be dominated by the water potential of the upstream xylem and soil. As a result, even large changes in the evaporative environment of the leaf will impact minimally on the water potential of the guard cells, and this may impede the responsiveness of stomata to changes in evaporational flux. It is possible then that a reversible loss of hydraulic conductivity in the leaf may be an adaptive means of amplifying the evaporative demand signal to the stomata in order to expedite a stomatal response. The major benefit of this would be protection of upstream xylem; a realistic motivation considering that the petiole xylem in *S. glauca* is only slightly more resistant to cavitation than the leaf (Brodribb *et al.* 2003) and may be more difficult to repair.

Viewing the close correspondence between K_{leaf} depression and mid-day depression of leaf gas exchange it is tempting to propose a causal relationship. Verification of such an hypothesis requires that the influence of VPD on stomatal aperture be accounted for independent of the effects of K_{leaf}. Given that stomatal conductance generally responds to VPD in such a way as to maintain homeostasis in Ψ_{leaf} (Oren *et al.* 1999), mid-day depression of g_s and A would be expected to accompany a mid-day peak of VPD even in the absence of K_{leaf} depression. In the case of *S. glauca* we believe that the very high maximum values of K_{leaf} would be sufficient to dampen the direct stomatal response to VPD (Franks & Farquhar 1999) to a much greater degree than was observed here (Fig. 1). The reduction of g_s from values around 400 mmol m^{-2} s^{-1} in the morning to values close to zero at mid-day was common in *S. glauca* (Fig. 1) as was an afternoon increase in g_s whereas VPD remained constant. These observations defy the predictions of a hydraulic response to VPD unless a mid-day depression and recovery of K_{leaf} is invoked. We suggest therefore that K_{leaf} depression does play a part in the strong mid-day depression of gas exchange observed in *S. glauca*. Furthermore, it has been shown that the leaves of many species operate close to the margin of cavitation (Kikuta *et al.* 1997; Brodribb & Holbrook 2003a,b; Lo Gullo *et al.* 2003) and as such may also suffer diurnal decline of K_{leaf} and associated depression of diurnal assimilation. The potential for a widespread impact of K_{leaf} depression on the dynamics of diurnal gas exchange warrants a detailed understanding of this phenomenon.

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