Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients

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ABSTRACT

Steady-state leaf gas-exchange parameters and leaf hydraulic conductance were measured on 10 vascular plant species, grown under high light and well-watered conditions, in order to test for evidence of a departure from hydraulic homeostasis within leaves as hydraulic conductance varied across species. The plants ranged from herbaceous crop plants to mature forest trees. Across species, under standardized environmental conditions (saturating light, well watered), mean steady-state stomatal conductance to water vapour (g_w) was highly correlated with mean rate of CO_2 assimilation (A) and mean leaf hydraulic conductance normalized to leaf area (k_{leaf}). The relationship between A and g_w was well described by a power function, while that between A and k_{leaf} was highly linear. Non-linearity in the relationship between g_w and k_{leaf} contributed to an increase in the hydrodynamic (transpiration-induced) water potential drawdown across the leaf ($\Delta\Psi_{leaf}$) as k_{leaf} increased across species, although across the 10 species the total increase in $\Delta\Psi_{leaf}$ was slightly more than twofold for an almost 30-fold increase in g_w. Higher rates of leaf gas exchange were therefore associated with higher k_{leaf} and higher leaf hydrodynamic pressure gradients. A mechanistic model incorporating the stomatal hydromechanical feedback loop is used to predict the relationship between $\Delta\Psi_{leaf}$ and k_{leaf}, and to explore the coordination of stomatal and leaf hydraulic properties in supporting higher rates of leaf gas exchange.

Key-words: hydraulic conductance; hydraulic conductivity; plant evolution; stomata; stomatal control; transpiration control; water relations.

INTRODUCTION

The maximum rate of leaf gas exchange can vary severalfold across species, even amongst those growing in similar environments (Körner 1994; Larcher 2003). Leaves also exhibit considerable diversity in shape, and span several orders of magnitude in size. Despite this, leaves appear to be designed in a way that optimizes or maintains certain internal conditions (Cowan & Farquhar 1977; Parkhurst

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1986; Ball, Cowan & Farquhar 1988; Evans 1999; Farquhar, Buckley & Miller 2002). Water status is one of the most critical of these internal conditions because small deviations outside of a narrow working range can have adverse effects on key physiological processes such as photosynthesis and water transport (Boyer 1971, 1976; Hsiao *et al.* 1976; Schulze 1986; Tyree & Sperry 1989; Lawlor & Cornic 2002; Chaves, Maroco & Pereira 2003). Therefore, in order to maintain operation within narrow hydrological conditions, diversity in photosynthetic performance and leaf morphology across species must be subject to certain hydraulic constraints.

Higher rates of CO_2 assimilation are generally sustained by higher stomatal conductance to CO_2 (g_c) and, by association, stomatal conductance to water vapour (g_w). For any given evaporative demand, the transpirational water flux increases in proportion to g_w, and, with any given leaf hydraulic conductance (k_{leaf}), so too does the water potential gradient across the leaf ($\Delta\Psi_{leaf}$). If g_w simply increased to support higher rates of CO_2 assimilation in different species, and hydraulic conductance did not change, then $\Delta\Psi_{leaf}$ would increase in relative magnitude just as dramatically as g_w. Therefore, to have maintained any degree of hydraulic homeostasis over the course of vascular plant evolution, selection for increased rates of photosynthetic gas exchange had to involve increased k_{leaf}. The form of this evolutionary relationship between g_w and k_{leaf} will determine how $\Delta\Psi_{leaf}$ changes, if at all, across species as g_w changes.

There is considerable evidence in support of a general correlation between stomatal and hydraulic conductance, both within species (Meinzer & Grantz 1990; Meinzer *et al.* 1995; Saliendra, Sperry & Comstock 1995; Mencuccini & Comstock 1999; Hubbard *et al.* 2001) and to a lesser extent across species (Meinzer *et al.* 1999; Nardini & Salleo 2000; Santiago *et al.* 2004). However, less is known about the nature of this relationship within leaves, and there is no clear picture of how $\Delta\Psi_{leaf}$ changes across species with widely differing gas-exchange capacities. Furthermore, the hydraulic processes within a leaf are considerably complex, particularly with regard to the hydromechanical elements of the stomatal control mechanism. This control mechanism incorporates hydraulic conductance (Cowan 1977; Buckley, Mott & Farquhar 2003; Franks 2004) and is intimately involved in the regulation of transpiration rate, and thus $\Delta\Psi_{leaf}$. Little is known about the necessary adjustments in

the stomatal apparatus that essentially underlie the relationship between steady-state g_w, k_{leaf} and $\Delta\Psi_{leaf}$ across species.

The aim of this investigation was first to identify the form of relationship between g_w and k_{leaf} across species, and in doing so to establish the nature of any change in $\Delta\Psi_{leaf}$ as steady-state g_w changed across species. Then, with this information, the properties of the stomatal hydromechanical feedback control mechanism were investigated with the aid of a model to determine what adjustments are required to support the observed trends.

MATERIALS AND METHODS

Plant material

Ten C_3 plant species known to exhibit widely different photosynthetic capacities were randomly chosen for the study. The leaves of the plants had developed under full sunlight and well-watered conditions, in either their natural environment, or in a glasshouse. For the leaf hydraulic conductance measurements on the rain forest canopy trees [*Acmena graveolens* (F. M. Bailey) L. S. Smith, *Argyrodendron peralatum* (Bailey) Edlin, *Dysoxylum pettigrewianum* Bailey and *Syzygium sayeri* (F. muell.) B. Hyland], leaves were collected pre-dawn from the top of the forest canopy at Cape Tribulation, Australia, using the Australian Canopy Crane. The leaves were immediately placed into plastic bags and brought to the laboratory for the measurement of the leaf hydraulic conductance within a few hours at the time of collection. Leaf gas-exchange measurements for these species were carried out *in situ* on leaves that had developed under high-light intensity at the top of the rain forest canopy (see below). The remaining plant species (*Eucalyptus ptychocarpa* F. Muell., *Idiospermum australiense* (Diels) S. T. Blake, *Nephrolepis exaltata* (L.) Schott, *Psilotum nudum* (L.) Beuv., *Triticum aestivum* L. and *Vicia faba* L.) were grown in a glasshouse (30/25 °C day/night temperature, high humidity, well watered) and fertilized with a slow-release fertilizer (Osmocote; Grace-Sierra Pty Ltd, Castle Hill, Australia). These plants were transferred to the laboratory in the evening prior to leaf gas-exchange and hydraulic measurements, and were well watered. *E. ptychocarpa* is a small, dry-land tree; *I. australiense* is a tropical rain forest sub-canopy tree; *N. exaltata* is a fern; *T. aestivum* and *V. faba* are herbaceous crop plants and *P. nudum* is a vascular cryptogam. For *P. nudum*, whole, branching, photosynthetic stems were treated as single 'leaves', with projected area used in calculations.

Leaf gas exchange

Variability in both the hydraulic and gas-exchange environments was minimized by ensuring that the plants were maximally hydrated, and by conducting gas-exchange measurements under standardized environmental conditions. All measurements were carried out with an open-flow

leaf gas-exchange analyser (model LI-6400, Li-Cor, Lincoln, NB, USA). The leaf chamber conditions were controlled at the following levels: ambient CO_2 concentration, 350 μmol mol^{-1}; leaf temperature, 30 °C, photosynthetically active radiation (PAR), 1000 μmol m^{-2} s^{-1}; leaf-to-air water vapour mole fraction difference (Δw), 0.01 mol mol^{-1}. In the laboratory, the plants were allowed to equilibrate and attain maximal hydration overnight, and the following morning the leaves were clamped into the gas-exchange chamber under the standardized conditions until g_w reached a steady state, at which point the g_w was recorded. This usually took 2–3 h for each leaf. The same procedure was followed for measurements on the rain forest canopy leaves from the crane gondola, although a steady state was usually reached within 30–60 min because of the pre-exposure of the leaves to sunlight. The measurements were made on four leaves from four individuals, and were conducted between approximately 0900 and 1500 h to coincide with the natural period of maximal photosynthetic activity.

Measurement of k_{leaf}

Following the electrical analogue approach (Tyree & Cheung 1977; Roberts 1979; Nobel & Jordan 1983; Schulte 1993), the leaf hydraulic system is represented by a single bulk capacitance (C_{leaf}) in series with a single bulk hydraulic conductance k_{leaf}. C_{leaf} [in moles per meter squared (leaf) per megapascal] represents the water stored in the leaf tissue, and k_{leaf} [in moles per metre squared (leaf) per second per megapascal] is the hydraulic conductance to water flow between the leaf water-storage tissue (mostly mesophyll and epidermal cells) and the petiole xylem, which includes the entire laminar vasculature, down to the petiole (Fig. 1). In the intact leaf, water flows from the petiole, through the xylem to the storage tissue to replace the water lost through transpiration. In this method, the lamina of a detached, fully hydrated leaf is pressurized to force a small amount of water in the reverse direction (i.e. from the storage tissue to the xylem). The rate of water efflux from the tip of the petiole (J_w) [in moles per metre squared (leaf)
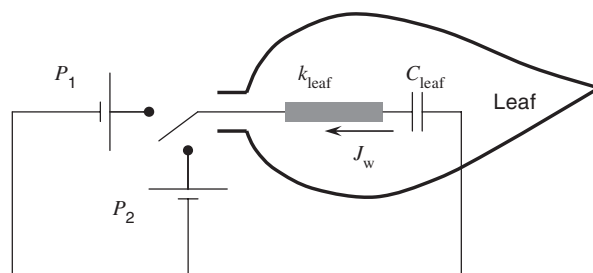

Figure 1. Electrical analogue model of the pathway of water flow (J_w, representing electrical current) from a fully hydrated mesophyll (C_{leaf}, representing electrical capacitance) through bulk leaf hydraulic conductance (k_{leaf}), when a step change in pressure (P_1 to P_2, representing a voltage change) is applied to a detached leaf. The technique for the measurement of k_{leaf} is based on this model. See Materials and methods for further explanation.

per second] as it discharges from the storage tissue, at any instant t following a step increase in pressure, is given by

$$J_w(t) = k_{leaf}\Delta P e^{\frac{1}{C_{leaf}/k_{leaf}}}, \tag{1}$$

where ΔP (in megapascals) is the difference between the initial pressure (P_1) and final pressure (P_2) of the step change. At $t = 0$, J_w is maximal, i.e.

$$J_{w0} = k_{leaf}\Delta P. \tag{2}$$

Equation 2 may be rearranged to give an expression for k_{leaf}. Thus,

$$k_{leaf} = \frac{J_{w0}}{\Delta P}. \tag{3}$$

Following the step change in pressure, J_w diminishes exponentially from J_{w0} to 0 as t approaches infinity. J_{w0} may be estimated as the volume of sap expressed (Δv) (in moles) per unit leaf area (A_{leaf}) (in metres squared) over time interval t, provided that t is much less than the time constant for the discharge of C_{leaf}. This time constant, which is the time taken for 63% of the total sap discharge, was found to be in the order of 60–120 s for the leaves in this study, so J_{w0} was estimated using

$$J_{w0} \approx \frac{\Delta v}{10A_{leaf}}, \tag{4}$$

where Δv was the volume of sap expressed in the first 10 s following the step increase in pressure.

The above model and methodology, although based on classical plant tissue water relations theory, rely on the accuracy of the conductance–capacitance analogue for leaves. Errors could arise if the pathway of water efflux from the leaf, back through the petiole, is different from that of transpiration-driven water influx, but there is little evidence as yet to suggest this is the case.

The protocol for obtaining J_{w0} and k_{leaf} was as follows: A leaf was severed from a fully hydrated plant and placed in a Scholander-type pressure chamber (Soilmoisture Equipment, model 3005, Santa Barbara, CA, USA). The chamber pressure was increased to the balance pressure (P_1) and allowed to equilibrate for about 2 min. The chamber pressure was then rapidly increased to P_2, and the sap expressed in the first 10 s was collected and weighed on an analytical balance to obtain Δv, hence J_{w0} from Eqn 4, and then k_{leaf} from Eqn 3. ΔP was about 0.5 MPa (accurately measured to ± 0.01 MPa). Leaf area was measured with a leaf area meter (model AM100, ADC Plant Science Instrumentation, Hoddesdon, England). The measurements were carried out on 4–10 leaves per species.

Calculating $\Delta \Psi_{leaf}$

The $\Delta \Psi_{leaf}$ was obtained using the equations for transpiration rate (E) (in moles per metre squared per second) and J_w (in moles per metre squared per second). Thus,

$$E = g_w \times \Delta w, \tag{5}$$

$$J_w = k_{leaf} \times \Delta \Psi_{leaf}. \tag{6}$$

In the steady state, $E = J_w$, and so under the same conditions

$$\Delta \Psi_{leaf} = \frac{g_w \Delta w}{k_{leaf}}. \tag{7}$$

Equation 5 applies when the leaf boundary layer conductance is much larger than g_w, as was the case here. Otherwise, g_w in Eqn 5 should be replaced by the sum of the stomatal and the boundary layer conductance in series.

RESULTS

Across the 10 species, mean $\Delta \Psi_{leaf}$ was found to increase in a non-linear fashion with increasing k_{leaf} (Fig. 2). The smallest estimated $\Delta \Psi_{leaf}$ for these conditions was found in *P. nudum* at 0.22 ± 0.01 MPa, which had also the lowest k_{leaf}, 1.30 ± 0.21 mmol m^{-2} s^{-1} MPa^{-1}. Mean $\Delta \Psi_{leaf}$ approached a maximum of about 0.55 MPa as mean k_{leaf} increased towards a maximum of 15.4 mmol m^{-2} s^{-1} MPa^{-1} in *T. aestivum*. A similar pattern was observed in the relationship between $\Delta \Psi_{leaf}$ and mean steady-state g_w (Fig. 3), with *P. nudum* and *T. aestivum* again at either extremes. There was a strong positive, although non-linear, relationship between mean g_w and k_{leaf} (Fig. 4). The solid lines passing through the data in Figs 2–4 (main graph) are the result of fitting the stomatal hydraulic feedback model that is
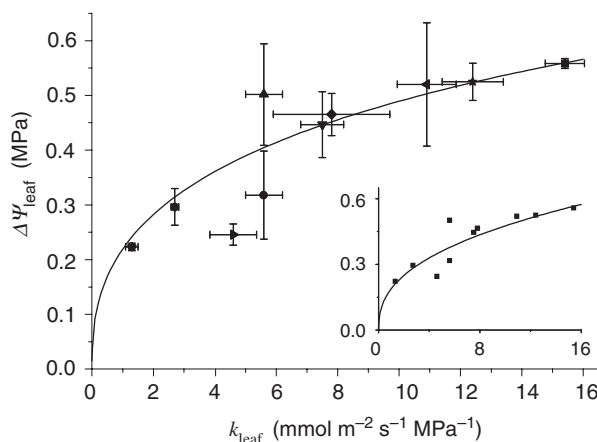

Figure 2. The relationship between leaf hydrodynamic (transpiration-induced) water potential drawdown ($\Delta \Psi_{leaf}$) and k_{leaf} as mean k_{leaf} increases across 10 C$_3$ vascular plant species that were grown and compared under similar environmental conditions (high-light intensity, well-watered condition). The symbols denote the mean species $\Delta \Psi_{leaf}$ calculated from the measured g_w and k_{leaf} using Eqn 7 (350 μmol mol^{-1} ambient CO$_2$ concentration, 30 °C leaf temperature, 1000 μmol m^{-2} s^{-1} PAR, 0.01 mol mol^{-1} leaf-to-air water vapour mole fraction difference (Δw); well-watered plants). The solid line is $\Delta \Psi_{leaf}$ predicted for given k_{leaf} using the feedback model developed in the Discussion. The inset shows $\Delta \Psi_{leaf}$ predicted from k_{leaf} using Eqn 7 and g_w obtained from the fitting of a simple power function to g_w versus k_{leaf} data (see details in Discussion). ▼, *Acmena graveolens*; ▲, *Argyrodendron peralatum*; ◆, *Dysoxylum pettigrewianum*; ★, *Eucalyptus ptychocarpa*; ▶, *Idiospermum australiense*; ●, *Nephrolepis exaltata*; ⬟, *Psilotum nudum*; ●, *Syzygium sayeri*; ■, *Triticum aestivum*; ◀, *Vicia faba*. PAR, photosynthetically active radiation.
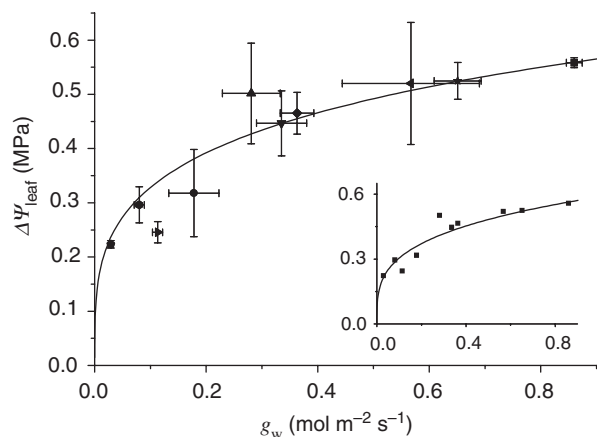
Figure 3. The relationship between the leaf hydrodynamic water potential drawdown ($\Delta\Psi_{\text{leaf}}$) and the steady-state stomatal conductance to water vapour (g_{w}) across 10 C$_3$ vascular plant species. Steady-state g_{w} was measured under the same controlled environmental conditions for all species. The solid line is $\Delta\Psi_{\text{leaf}}$ predicted using the feedback model described in the Discussion. The inset shows $\Delta\Psi_{\text{leaf}}$ predicted using Eqn 7 and g_{w} obtained from the fitting of a simple power function to g_{w} versus k_{leaf} data (see details in Discussion). ▼, *Acmena graveolens*; ▲, *Argyrodendron peralatum*; ◆, *Dysoxylum pettigrewianum*; ★, *Eucalyptus ptychocarpa*; ▶, *Idiospermum australiense*; ●, *Nephrolepis exalata*; ⬟, *Psilotum nudum*; ●, *Syzygium sayeri*; ■, *Triticum aestivum*; ◀, *Vicia faba*. Conditions as for Fig. 2.

Figure 4. Steady-state stomatal conductance to water vapour (g_{w}) versus leaf hydraulic conductance (k_{leaf}) for the 10 species. The solid line is g_{w} predicted for given k_{leaf} using the feedback model. The inset shows g_{w} obtained from the fitting of a simple power function to g_{w} versus k_{leaf} data (see details in Discussion). ▼, *Acmena graveolens*; ▲, *Argyrodendron peralatum*; ◆, *Dysoxylum pettigrewianum*; ★, *Eucalyptus ptychocarpa*; ▶, *Idiospermum australiense*; ●, *Nephrolepis exalata*; ⬟, *Psilotum nudum*; ●, *Syzygium sayeri*; ■, *Triticum aestivum*; ◀, *Vicia faba*. Conditions as for Fig. 2.

developed in the Discussion. Overall, the values of k_{leaf} spanned the typical range measured with a variety of techniques (Table 1).

The steady-state CO$_2$ assimilation rate (A) (in micromoles per metre squared per second) under the standardized conditions varied across species by about the same factor as g_{w} and k_{leaf}, ranging from a mean of 2.47 ± 0.29 in *P. nudum* to a mean of 23.5 ± 0.24 in *T. aestivum*. There was also a strong positive, non-linear correlation between g_{w} and A (Fig. 5). The basic non-linear form of the steady-state-g_{w}-versus-A relationship is noteworthy because it suggests that the leaf intercellular CO$_2$ concentration tends to be higher in plants with higher A, as was observed in a previous comparison of the gas-exchange characteristics of 13 species (Franks & Farquhar 1999). This has important
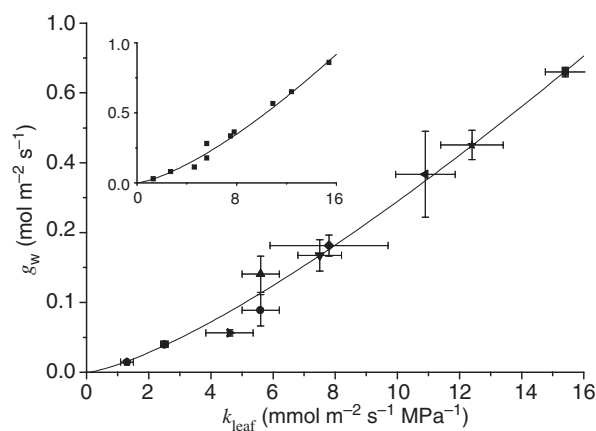
implications for water economy and nitrogen-use efficiency. Although driven by a complex mechanism, the relationship between g_{w} and A in Fig. 5 was modelled empirically as a simple power function by regressing log g_{w} on log A ($g_{\text{w}} = 0.0056A^{1.6}$, $r^2 = 0.95$, $P < 0.0001$, with g_{w} in moles per metre squared per second and A in micromoles per metre squared per second). There was a strong positive correlation between A and k_{leaf}, with linear regression of A on k_{leaf} yielding $A = 1.6k_{\text{leaf}}$, $r^2 = 0.95$, $P < 0.0001$ (Fig. 6).

DISCUSSION

The data show an increase in $\Delta\Psi_{\text{leaf}}$ as k_{leaf} increases across species (Fig. 2). Because k_{leaf} was found to be highly correlated with g_{w} and A (Figs 4 & 6), it can be concluded that higher rates of leaf gas exchange were associated with higher leaf hydrodynamic pressure gradients. The most plausible mechanism for this trend is a predominantly

Table 1. A sample of published leaf hydraulic conductance (k_{leaf}) measurements (mmol m^{-2} s^{-1} MPa^{-1}) using a variety of techniques, all involving measures of water influx or efflux via the petiole of detached leaves

k_{leaf}	Plant material	Technique	Author/s
1.4–16	10 species (fern, angiosperm tree, herbaceous)	Capacitive discharge	This study
4.4	*Fagus grandifolia*	Capacitive discharge	Tyree & Cheung (1977)
1.8; 4.1	*Cornus florida*; *Ilex opaca*	Capacitive discharge	Roberts (1979)
5.5–50	12 species (9 angiosperms, 3 conifers)	HPFM	Becker, Tyree & Tsuda (1999)
2.2–21	16 species (evergreen & deciduous trees)	HPFM	Nardini (2001)
6.9	*Prunus laurocerasus*	Vac.	Nardini, Tyree & Salleo (2001)
6.9–16	Six species (woody temperate angiosperms)	HPFM, Vac., Leaf evap.	Sack *et al.* (2002)
1.9; 8.3	*Laurus nobilis*; *Juglans regia*	HPFM	Cochard, Nardini & Coll (2004)
1.0–36	Eight species (fern, angiosperm)	Capacitive recharge	Brodribb & Holbrook (2004b)

HPFM, water influx using high-pressure flow meter; Vac., water influx with leaf under vacuum; Leaf evap., evaporation-driven water influx.
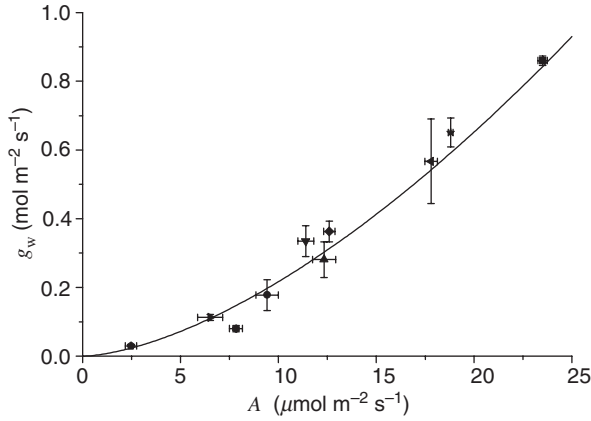
Figure 5. Steady-state stomatal conductance to water vapour (g_w) versus CO_2 assimilation rate (A) across the 10 species. The relationship is well described by a power function (solid line, $g_w = 0.0056A^{1.6}$). ▼, *Acmena graveolens*; ▲, *Argyrodendron peralatum*; ◆, *Dysoxylum pettigrewianum*; ★, *Eucalyptus ptychocarpa*; ▶, *Idiospermum australiense*; ●, *Nephrolepis exalata*; ⬟, *Psilotum nudum*; ●, *Syzygium sayeri*; ■, *Triticum aestivum*; ◄, *Vicia faba*. Conditions as for Fig. 2.

non-linear relationship between maximum g_w and k_{leaf} across species, whereby g_w increases at a greater rate than k_{leaf}.

Modelling g_w and $\Delta\Psi_{leaf}$ versus k_{leaf}

If the correlation between g_w and k_{leaf} was linear, then for any given Δw, $\Delta\Psi_{leaf}$ would remain constant at a value determined by the ratio $g_w : k_{leaf}$. The plot of g_w versus k_{leaf} in Fig. 4, although well characterized by a linear function on account of relatively subtle curvature, is best described by a non-linear function. Thus, $\Delta\Psi_{leaf}$ appears to increase

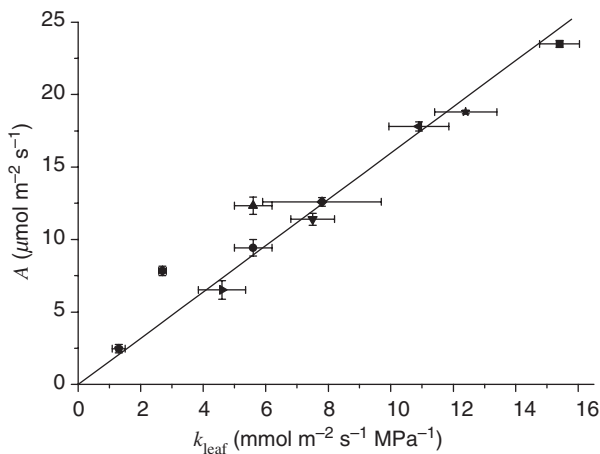**Figure 6.** The steady-state CO_2 assimilation rate (A) versus leaf hydraulic conductance (k_{leaf}) for the 10 species, showing a highly linear relationship. ▼, *Acmena graveolens*; ▲, *Argyrodendron peralatum*; ◆, *Dysoxylum pettigrewianum*; ★, *Eucalyptus ptychocarpa*; ▶, *Idiospermum australiense*; ●, *Nephrolepis exalata*; ⬟, *Psilotum nudum*; ●, *Syzygium sayeri*; ■, *Triticum aestivum*; ◄, *Vicia faba*. Conditions as for Fig. 2.

with g_w and k_{leaf} across species (Figs 2 & 3). A similar non-linearity was observed between g_w and whole plant leaf area-specific hydraulic conductance across a wide range of species in a survey and analysis of published data (Mencuccini 2003). The various environmental conditions under which the studies in that survey were conducted led to considerable noise in the final data compilation, but the similarity between the trend observed for whole plants (Mencuccini 2003) and for leaves (this study) is evidence that despite the specialized tissue structure of leaves, they may be constructed in accordance with general, whole-plant hydraulic scaling laws.

There are several ways that an increased g_w can be achieved (e.g. increasing aperture via larger guard cells and/or turgor pressures, increased stomatal density or reduced epidermal mechanical advantage over guard cells). Of these, stomatal size and density are the most widely documented variant stomatal properties contributing to differing leaf diffusive conductance (see surveys in Jones 1992; Willmer & Fricker 1996; Larcher 2003), although there are no specific patterns: high stomatal conductance is exhibited in species with both high densities of small stomata and low densities of large stomata. Regardless of the means, however, the resulting relationship between maximum g_w and k_{leaf} determines the pattern of $\Delta\Psi_{leaf}$ versus k_{leaf}.

The observed relationship between g_w, k_{leaf} and $\Delta\Psi_{leaf}$ can be modelled empirically by applying a simple power function relationship between g_w and k_{leaf} of the form

$$g_w = c \times k_{leaf}^{m}, \tag{8}$$

where c and m are constants. Fitting Eqn 8 to the data in Fig. 4 (regressing log g_w on log k_{leaf}) resulted in $c = 0.02$ and $m = 1.4$ ($r^2 = 0.97$; $P < 0.0001$). The result of this fit is illustrated in Fig. 4 (inset, solid line). Using the obtained g_w to calculate $\Delta\Psi_{leaf}$ from Eqn 7, a similarly accurate description is obtained for measured $\Delta\Psi_{leaf}$ versus k_{leaf} and g_w (Figs 2 & 3 inset, solid lines). Despite its simplicity and accuracy in describing the results, however, this empirical model casts little light on the processes underlying the observed trends. These are better explored with the application of a mechanistic model.

Although its characterization remains difficult, the stomatal hydromechanical feedback loop has long been recognized as the foundation of leaf transpiration control (Raschke 1970; Farquhar & Cowan 1974; Meidner 1975, 1976; Cowan 1977; Farquhar 1978; Mott & Parkhurst 1991; Jones 1998; Whitehead 1998). The hydraulic conductance to water flow through the leaf, k_{leaf}, which includes the xylem and extra-xylary components, is an integral and dominant component of this control system (Haefner, Buckley & Mott 1997; Mott & Franks 2001; Buckley & Mott 2002; Buckley *et al.* 2003; Franks 2004). The general form of the stomatal hydromechanical feedback system is summarized in Fig. 7, which is adapted from Franks & Farquhar (1999) and Franks (2004), and follows the theory set out in the seminal stomatal control papers by Farquhar & Cowan (1974) and Cowan (1977). The system is structured around the basic hydromechanical interaction of the epidermis and
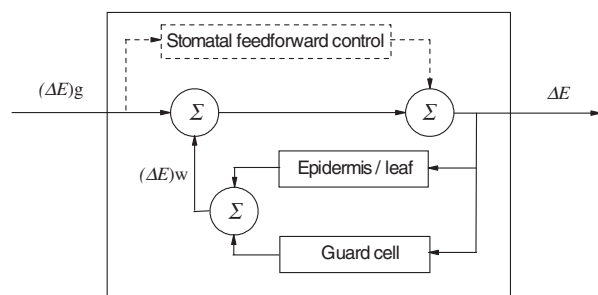
Figure 7. The overall structure of the feedback model used to predict the steady-state stomatal conductance to water vapour (g_w) and the leaf hydrodynamic water potential drawdown ($\Delta\Psi_{leaf}$) as a function of the leaf hydraulic conductance (k_{leaf}). See Discussion for further details.

the stomatal guard cells, with the feedback element comprising essentially the interdependence of guard cell turgor and transpiration rate. Although the presence of a feedforward control component is often evident in leaf gas-exchange data (Cowan 1977; Farquhar 1978), its effects are not usually manifested at low Δw (Monteith 1995), so here, for simplicity, the feedforward element was omitted. Its action in relation to the feedback loop, however, is indicated in Fig. 7 (dotted lines).

Given that $\Delta\Psi_{leaf}$ is driven by transpiration rate, the model determines the actual change in transpiration rate (ΔE) for a given Δw, and hence $\Delta\Psi_{leaf}$ via Eqn 7. ΔE is determined by the sum of $(\Delta E)_g$ (the potential change in transpiration rate if g_w was fixed) and $(\Delta E)_w$ (the change in transpiration rate due to the feedback effect of E on g_w). Full details of these basic structural features of the model are given in (Franks 2004). Here, the model is extended by incorporating a relationship between g_w and k_{leaf}, whereby the maximum g_w increases as a power function of k_{leaf}.

During the model-fitting process, several variables were held constant at values typical of well-watered plants under high-light intensity: epidermal osmotic pressure $\Pi_e = 0.8$ MPa; guard cell osmotic pressure $\Pi_g = 3.5$ MPa (based on an average estimated maximum guard-cell turgor of 3–4 MPa) (Franks, Cowan & Farquhar 1998; Franks & Farquhar 2001; Franks 2003). Assuming there is evaporation from the leaf-interior surfaces of guard cells (see Franks 2004 for an outline of this rationale), the ratio of the drawdown in water potential between petiole and the sites of evaporation on the guard cell walls and that from the petiole to the epidermis was set at 2:1. Because of the mechanical advantage of the epidermis over the guard cells, the reduction in g_w at maximum epidermal turgor relative to g_w at zero epidermal turgor was fixed at 20%, which is a typical mid-range value (Franks 2003). The evaporation potential, Δw, was fixed at 0.01, as per experimental conditions (see Materials and methods). The stomatal conductance was calculated from modelled stomatal aperture (a) using

$$g_w = d \times k_{leaf}{}^m \times a, \tag{9}$$

where d and m are constants. Thus, g_w across species was predicted on the basis of two processes: (1) the within-

species hydromechanical feedback mechanism (Fig. 7) that determines actual aperture, and hence g_w, as an environmentally determined reduction from maximum g_w for that species; and (2) an across-species component defined by a power relationship between maximum aperture (hence, maximum g_w) and k_{leaf}. Strictly speaking, it is the whole-plant hydraulic conductance to which g_w is mechanistically coupled, but k_{leaf} is used as a proxy here to compare the hydraulic systems of leaves across species.

The equations in the feedback model were solved simultaneously using an iterative procedure. With m fixed at 1.4 (as per the empirical curve fit), the model was fitted for $k_{leaf} = 0.2$–16 mmol m^{-2} s^{-1} MPa^{-1} by adjusting d so as to minimize the mean square residual for $\Delta\Psi_{leaf}$. The final value of d, a scaling factor, is somewhat arbitrary because it depends on the sensitivity of a to guard cell turgor ($\partial a/\partial P_g$) which, as previously noted, varies widely across species on account of different sizes of guard cells and, potentially, different guard-cell-wall properties. Fixing $\partial a/\partial P_g$ at 3 μm/MPa resulted in $d = 0.002$. The model was found to fit the data well (Fig. 8). The modelled $\Delta\Psi_{leaf}$ is plotted against k_{leaf} in Fig. 2 (solid line). The plots of the modelled $\Delta\Psi_{leaf}$ against g_w and the modelled g_w against k_{leaf} are shown, respectively, as solid lines in Figs 3 and 4.

The model in the form described above predicts that the relative stomatal sensitivity to Δw (or E) increases as k_{leaf} and g_w increase across species. Controlled gas-exchange measurements (Franks & Farquhar 1999; Comstock 2000) have shown the opposite trend (i.e. species with higher g_w were, overall, less sensitive to Δw). There is at least one simple mechanism for this: decreasing sensitivity with increasing g_w and k_{leaf} will result if $\partial a/\partial P_g$ declines in association with increasing g_w and k_{leaf}, which require also that Π_g increases with g_w and k_{leaf}. One practical advantage of decreasing rather than increasing sensitivity is improved rather than diminished stability of the feedback mechanism in conditions of higher evaporative demand. Configuring the model with these characteristics results in the same quality of fit to the data, although in the absence of any data on changes in either $\partial a/\partial P_g$ or Π_g with increasing g_w or k_{leaf} it is better not to speculate here on the exact form of this interaction. It is noted, however, that if species with higher g_w are generally less sensitive to Δw, then the trend of increasing $\Delta\Psi_{leaf}$ across species (Figs 2 & 3) will be even more pronounced at higher Δw.

Implications

For a given allocation of carbon to the leaf vascular system, the photosynthetic potential of leaves will be constrained by the hydraulic conductivity of the xylem. Accordingly, higher rates of photosynthetic gas exchange, and correspondingly higher stomatal conductance to CO_2 and water vapour, must be supported by higher hydraulic conductance. This is more readily achieved in vessel-bearing angiosperms because of the higher hydraulic conductivity of xylem that is built with vessels (Givnish 1986; Patino, Tyree & Herre 1995; Sperry 2003; Franks & Brodribb
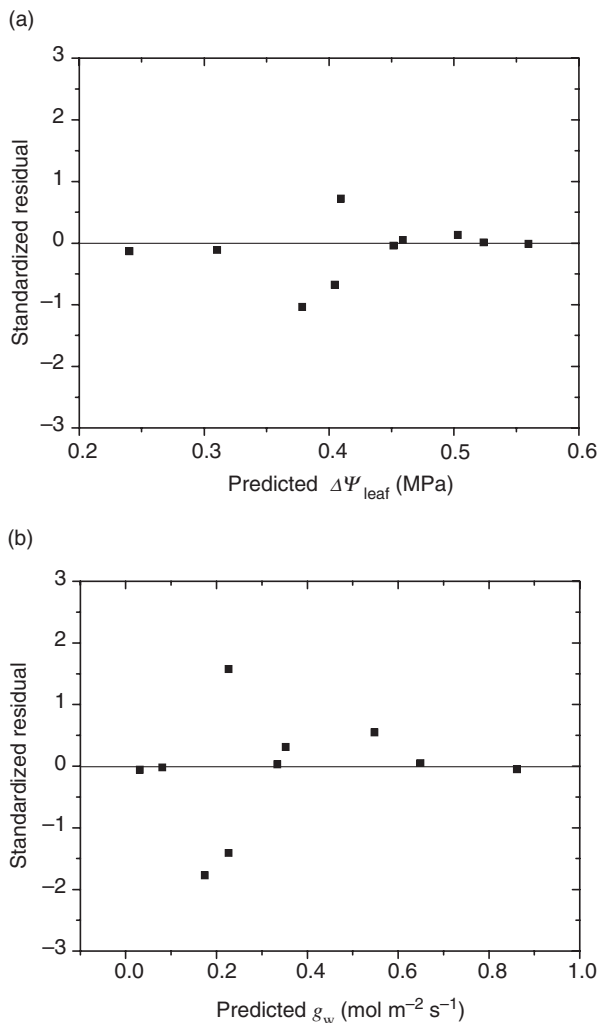
(a)

(b)

Figure 8. Plot of standardized residual for the feedback model fit versus the predicted leaf hydrodynamic water potential drawdown ($\Delta\Psi_{leaf}$) (a) and the steady-state stomatal conductance to water vapour (g_w) (b).

2005). However, although there is a continuum of evolutionary improvements in the hydraulic capacity of tracheary elements, xylem often comprises a mixture of functionally different elements (Bailey & Tupper 1918; Zimmermann 1983; Baas 1986; Carlquist 1988), so the trends in Figs 4 and 6 cannot be easily divided into hydraulically primitive or advanced subgroups. Consistent with the theory of evolution towards increased hydraulic capacity, however, is the appearance of *P. nudum* (an ancient and hydraulically inferior vascular species) and *T. aestivum* (a more recent and hydraulically superior species) at opposite extremes of the plots in Figs 4 and 6.

An open question at this point is why, if indeed the pattern of terrestrial plant adaptation has been one of generally increasing photosynthetic gas-exchange capacity, the steady-state $\Delta\Psi_{leaf}$ has tended to creep up, as is suggested by Figs 2 and 3. There are no direct advantages to larger $\Delta\Psi_{leaf}$. In fact, larger water potential gradients in the leaf

are likely to impact adversely on several physiological properties. The range of $\Delta\Psi_{leaf}$ measured here across the 10 species is, however, comparatively small, increasing a little more than twofold for a 30-fold increase in g_w. This poses the question as to what might be the constraints on $\Delta\Psi_{leaf}$. Cavitation and air embolisms in the leaf xylem begin to occur at about −0.5 MPa xylem water potential (Milburn 1974; Kikuta *et al.* 1997; Nardini *et al.* 2001), and diurnal reduction and recovery of leaf hydraulic conductance, possibly as a result of hydrodynamically induced xylem cavitation, has been observed in well-watered plants (Brodribb & Holbrook 2004a). Keeping $\Delta\Psi_{leaf}$ to a minimum would help to minimize these adverse effects. Another possible constraint on $\Delta\Psi_{leaf}$ is its potential influence on plasmodesmal function. It has been shown that passage through the plasmodesmata can be blocked if the pressure differential between two cells exceeds a certain threshold, which has been measured at about 0.2 MPa (Cote, Thain & Fensom 1987; Oparka & Prior 1992; Reid & Overall 1992). Minimizing $\Delta\Psi_{leaf}$ will help keep the pressure differential between any two cells below this threshold.

The overall trend of increasing $\Delta\Psi_{leaf}$ across species might be tied to the selection process that led to more recent, high-productivity plant taxa. With much of vascular plant evolution, particularly those with broad leaves, occurring over a period of generally declining atmospheric CO_2 concentration (Beerling, Osborne & Chaloner 2001; Crowley & Berner 2001), tolerance of larger leaf water-potential gradients would have allowed g_w to increase more rapidly than k_{leaf}, thus facilitating higher rates of leaf gas exchange despite declining atmospheric CO_2 concentration. The saturating nature of $\Delta\Psi_{leaf}$ with increasing k_{leaf} across species implies that $\Delta\Psi_{leaf}$ does constitute some form of constraint. Further study of the hydraulic components of the stomatal control mechanism and of physiological sensitivities to $\Delta\Psi_{leaf}$ will help clarify this.

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