

Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees

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Received: 12 January 2005

Accepted: 17 February 2005

Summary

• The leaf hydraulic conductance (K_{leaf}) is a major determinant of plant water transport capacity. Here, we measured K_{leaf} and its basis in the resistances of leaf components, for fully illuminated leaves of five tree species that regenerate in deep shade, and five that regenerate in gaps or clearings, in Panamanian lowland tropical rainforest. We also determined coordination with stomatal characters and leaf mass per area.

• K_{leaf} varied 10-fold across species, and was 3-fold higher in sun- than in shade-establishing species. On average, 12% of leaf hydraulic resistance ($= 1/K_{\text{leaf}}$) was located in the petiole, 25% in the major veins, 25% in the minor veins, and 39% outside the xylem. Sun-establishing species had a higher proportion of leaf resistance in the xylem. Across species, component resistances correlated linearly with total leaf resistance.

• K_{leaf} correlated tightly with indices of stomatal pore area, indicating a coordination of liquid- and vapor-phase conductances shifted relative to that of temperate woody species.

• Leaf hydraulic properties are integrally linked in the complex of traits that define differences in water use and carbon economy across habitats and vegetation zones.

Key words: Barro Colorado Island (BCI), biological networks, high pressure flowmeter (HPFM), leaf hydraulics, shade tolerance, vasculature.

New Phytologist (2005) **167**: 403–413

© *New Phytologist* (2005) doi: 10.1111/j.1469-8137.2005.01432.x

Introduction

The leaf is a major bottleneck in the whole-plant liquid flow pathway, and thus the hydraulic conductance of the leaf ($K_{\text{leaf}} = 1/\text{hydraulic resistance}$) is an important physiological parameter (Tyree *et al.*, 1999; Nardini & Salleo, 2000; Sack *et al.*, 2002, 2003; Brodribb *et al.*, 2005; Sack & Tyree, 2005). Previous studies have shown that K_{leaf} is coordinated with stomatal pore area, and consequently with maximum rates of gas exchange (Sack *et al.*, 2003; Brodribb & Holbrook, 2004; Brodribb *et al.*, 2005). Differences in K_{leaf} may be an important factor in the differential function of species across habitats. However, little work has been carried out to characterize the differences in K_{leaf} across ecological types, although large differences might exist. For instance, within given plant canopies, shade

leaves have lower K_{leaf} than sun leaves (Sack *et al.*, 2003; Lo Gullo *et al.*, 2004). Also, for a given species, plants grown in low irradiance can have lower K_{leaf} (Engelbrecht *et al.*, 2000). One study indicated a higher K_{leaf} for five species in open sites than for three species in shaded sites; the species varied widely in life forms (Brodribb & Holbrook, 2004). The first goal of this study was to determine the variation in K_{leaf} for species of tropical rainforest trees that establish in contrasting irradiances. By hypothesis, species of high irradiance, selected for high rates of gas exchange and evaporative demand, should have higher K_{leaf} than species that establish in deep shade.

Recent work has pioneered understanding of the basis of K_{leaf} in the pathways of water flow through the leaf (Tyree *et al.*, 2001; Cochard *et al.*, 2004; Gascó *et al.*, 2004; Sack *et al.*, 2004). The leaf hydraulic resistance (R_{leaf}) can be partitioned

into additive components: the hydraulic resistances of the petiole, major veins, minor veins, and the extra-xylem flow pathways across the living tissues of bundle sheath and between and/or around mesophyll cells to evaporation sites in the leaf. The second goal of this study was to partition R_{leaf} for diverse species, allowing us to determine whether variation across species in R_{leaf} arises from differences in all components, or can be attributed to differences in a single component. Which components dominate R_{leaf} will have important implications for leaf function. If the resistance of the xylem is an important determinant of overall R_{leaf} , xylem cavitation will have a marked influence on R_{leaf} . Additionally, a substantial proportion of overall resistance in the xylem suggests the possibility of functional consequences for the diversity of venation architectures observed among extant plant species (Sack & Tyree, 2005). We hypothesized that species that establish in shade rather than in sun might differ in the partitioning of R_{leaf} . By hypothesis, species that establish in higher irradiance would have a lower R_{leaf} , potentially by having a lower resistance both inside and outside the xylem. If low-resistance xylem transport is associated with a high vascular construction cost (McCulloh *et al.*, 2003), then sun-establishing species may achieve a lower R_{leaf} primarily via a low resistance outside of the xylem, and thus show a higher proportion of R_{leaf} in the xylem than shade-establishing species.

Finally, we determined the coordination of K_{leaf} with stomatal characters and leaf mass per area (LMA). Recent work has indicated close coordination of hydraulic and gas exchange characters. For sun and shade leaves of woody temperate species, K_{leaf} was closely coordinated with stomatal pore area per leaf area (Sack *et al.*, 2003). Across species, K_{leaf} is correlated with maximum stomatal conductance and gas exchange rates per leaf area (Asanmaa *et al.*, 2001; Brodribb *et al.*, 2005). However, K_{leaf} has been previously reported to be independent of LMA (Tyree *et al.*, 1999; Nardini, 2001; Sack *et al.*, 2003), a trait that is coordinated with a complex of traits bearing on leaf and plant carbon economy (Wright *et al.*, 2004). The coordination of K_{leaf} and other traits has not previously been compared for species of contrasting ecological function.

In lowland tropical rainforest on Barro Colorado Island, Panama, tree leaves vary greatly in size, shape, texture, lifespan and venation architecture. Tree species contrast in their ranges of regeneration irradiance, from those that persist as seedlings and saplings for decades in shaded understorey (< 1–2% daylight) to those that establish in gaps and clearings (Condit *et al.*, 1996; Leigh, 1999; Wright *et al.*, 2003). In this study, we investigated leaf hydraulic properties for species varying in their regeneration irradiance.

Materials and Methods

Study site, species and sampling method

The study was conducted in lowland tropical rainforest on Barro Colorado Island, Republic of Panama (9°9' N, 79°51' W;

for extensive background see Leigh, 1999), on tree leaves sampled from January to April 2003. Study species were selected to span a wide range of leaf sizes, shapes, textures and lifespans, as well as two categories of typical irradiance during juvenile establishment ('regeneration irradiance'), as reported in observations and plot data (Condit *et al.*, 1996; R. Condit, Smithsonian Tropical Research Institute, Panama, pers. comm.). Study species that persist as juveniles in shaded understorey (henceforth 'shade-establishing') were *Calophyllum longifolium* Willd. (Clusiaceae; nomenclature follows Croat, 1978, Tree Atlas of the Panama Canal Watershed; <http://ctfs.si.edu/webatlas/maintreecatlas.html>; Stevens, 2005), *Dendropanax arboreus* (L.) Dec. & Planch. (Araliaceae), *Posoqueria latifolia* (Rudge) R. & S. (Rubiaceae), *Protium tenuifolium* Engler ssp. *sessiliflorum* (Rose) Porter (Burseraceae), and *Swartzia simplex* (Sw.) Spreng. var. *ochracea* (A. DC.) Cowan (Fabaceae); species that typically establish in high irradiance (henceforth 'sun-establishing') were *Annona glabra* L. (Annonaceae), which establishes in open water, and four species common in gaps or clearings: *Cordia alliodora* (R. & P.) Cham. (Boraginaceae), *Lindackeria laurina* Presl. (Achariaceae), *Miconia argentea* (Sw.) DC. (Melastomataceae), and *Terminalia amazonia* (J. E. Gmel.) Exell (Combretaceae).

For each species, three reproductively mature trees 5–10 m high were located, and leaves of the most recent mature cohort were sampled from fully exposed branches at a height of > 3 m. Leaves of *C. alliodora* and *M. argentea* were sampled in the main laboratory clearing, early in the dry season, when soil was still moist. For other species, trees were sampled that were growing in moist soil with branches overhanging the water at the edge of the island, and were accessed by boat using pole pruners. For compound-leaved *P. tenuifolium*, the terminal leaflet was used. For each measured variable, measurements for each tree were averaged before calculation of a species mean (i.e. $n = 3$ per species).

Leaf hydraulic conductance and resistance

Material collected in the field was recut under water and allowed to hydrate overnight by placing the cut ends of the branches in 10 mM KCl solution (flow solution for the hydraulic measurements), and covering leaves with plastic. Measurements of leaf hydraulic properties were made using the high-pressure flowmeter (HPFM; Tyree *et al.*, 1993; Nardini *et al.*, 2001; Sack *et al.*, 2002). Hydraulic measurements were made on one to three leaves per individual; five to eight per species. Hydraulic resistance (R_{leaf}) is used in discussing the partitioning of resistances in the leaf, because resistances add in series; more generally, hydraulic conductance ($K_{leaf} = 1/R_{leaf}$) is used for examining the coordination with aspects of leaf structure that are potentially linked with conductance to gas exchange. The HPFM measures K_{leaf} as the flow rate driven through a leaf for a given pressure driving force. Pressurized (0.5–0.6 MPa) flow solution (degassed 10 mM KCl in distilled water, refiltered to 0.2-µm pore diameter on introduction to the system) was forced through a system of tubing,

which included a high-resistance segment, into the petiole (which had been previously cut under water and attached by compression fitting), through the leaf, and eventually out of the stomata. The high-resistance tubing was a 145-cm segment of red polyetheretherketone (PEEK) tubing (0.125 mm internal diameter; Upchurch Scientific, Oak Harbor, WA, USA); its hydraulic resistance (R_p) was determined from the slope of delivery pressure vs flow rate measured with an analytic balance (0.1 mg; Mettler AG104; Mettler-Toledo GmbH, Greifensee, Switzerland). Pressure transducers (Omega PX-180; Omega Engineering, Stamford, CT, USA) before (P_1) and after (P_2) the high-resistance tubing allowed calculation of the flow rate as equal to $(P_1 - P_2)/R_p$. K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was calculated by dividing the flow rate by the pressure drop across the leaf (P_2) and normalizing by the area of the leaf lamina determined using a leaf area meter (Li-Cor 3100C; Li-Cor, Lincoln, NE, USA). To prevent growth of microorganisms, the entire tubing system was bleached and rinsed at regular intervals (approx. 4–5 d of measurement). K_{leaf} was recorded once measurements were stable to a coefficient of variation < 5% for 5 min, which took typically 30–45 min for an intact leaf and 10–15 min after veins were cut. All measured leaves were submerged in a temperature-controlled water bath ($25 \pm 2^\circ\text{C}$). To determine K_{leaf} values representative of high irradiance (Sack *et al.*, 2002, 2004; Tyree *et al.*, 2005), leaves were illuminated during measurement ($> 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation inside the water bath; LI-250 light meter; Li-Cor Biosciences, Lincoln, NE, USA). Hydraulic conductance values were standardized for the effects of the slight temperature variation on the viscosity of water by correcting to a value for 25°C (Weast, 1974; Yang & Tyree, 1993).

Partitioning the leaf hydraulic resistance

Leaves were measured before and after applying each of two vein cutting treatments (five to eight leaves per species). Following the method of Sack *et al.* (2004), the resistance downstream of the minor veins, the extra-xylem resistance ($R_{\text{outside xylem}}$), was removed by making 1–2-mm incisions at random locations across the lamina with a scalpel. Only minor veins (i.e. the smallest visible reticulate orders of veins embedded in the lamina; fourth order or higher) were severed and at least 150 cuts were made per leaf, on average $1\text{--}8 \text{ cuts cm}^{-2}$, depending on species, which tests demonstrated was in excess of the number needed to remove the extra-xylem resistance for these species (Sack *et al.*, 2004; see following paragraph). The resistance of the leaf xylem (R_{xylem}) was calculated as $R_{\text{leaf}} - R_{\text{outside xylem}}$. Next, to determine the resistance of the minor veins ($R_{\text{minor veins}}$), all the visible tertiary veins were cut throughout the lamina, allowing solution to leak out, and the decline in resistance was measured. The resistance of the petiole was measured after removing the lamina (R_{petiole}). The resistance of the major veins ($R_{\text{major veins}}$) was calculated as $R_{\text{xylem}} - R_{\text{minor veins}} - R_{\text{petiole}}$. The cuts to tertiary veins were

made centrally. Assuming that water leaves the tertiary veins into the minor veins evenly along their length, $R_{\text{major veins}}$ thus includes the 'average' axial conductance of the tertiary veins, and $R_{\text{minor veins}}$ includes the resistance to water movement between major and minor veins. All hydraulic resistances were normalized by leaf area and have the same units as R_{leaf} .

Several methods for partitioning leaf resistance have been described in the literature, and there has been some recent debate about them. The method of Sack *et al.* (2004) used here has been questioned because < 5% of the total minor vein segments are severed in removing $R_{\text{outside xylem}}$ (Cochard *et al.*, 2004). However, repetitive cutting demonstrated that this is sufficient to remove downstream resistance (Sack *et al.*, 2004). The method has been recently confirmed for leaves of *Coffea arabica* by Gascó *et al.* (2004). In that study, up to 500 cuts were made, for leaves of unspecified size, but still representing the severing of < 5% of the total minor vein segments. The higher number of cuts required might reflect the use of smaller incisions or a difference between the leaves of *C. arabica* and those used in Sack *et al.* (2004) and this study. To confirm that sufficient cuts were used, the percentage xylem resistance ($R_{\text{xylem}}/R_{\text{leaf}}$), as determined by the above method, was plotted against the number of cuts per leaf area for each species. For each species, measured leaves varied in area by 2-fold on average, and venation density was invariant across leaf size (L. Sack & K. Frole, unpublished). The percentage xylem resistance was invariant with increasing number of cuts per leaf area [average Spearman rank correlation coefficient (r_s) = 0.06; P = 0.59, n = 5–8; see e.g. plot for *L. laurina*, Fig. 1], indicating that the number of cuts was sufficient to remove the extra-xylem resistance (Sack *et al.*, 2004). It is possible that the $R_{\text{minor veins}}$ might have been to some degree underestimated (and $R_{\text{outside xylem}}$ overestimated, because the minor veins were severed such that water could now exit the severed ends of the veins proximal to the minor vein endings within areoles, as

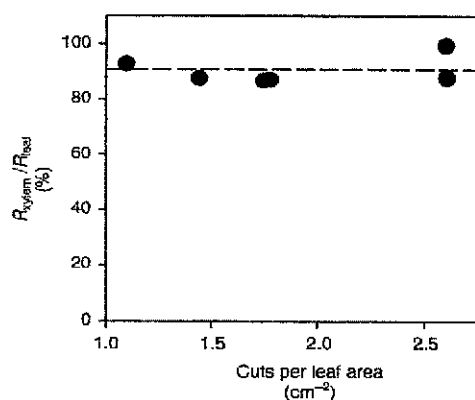


Fig. 1 Plot of the percentage of leaf hydraulic resistance in the xylem [resistance of the leaf xylem (R_{xylem})/resistance of the leaf (R_{leaf})] against the number of small incisions severing minor veins, for leaves of *Lindackeria laurina*. The mean value is given as the dashed line.

all species had free ending veins within areoles but *C. longifolium*. However, the error in estimation would be expected to be low, if, as previously argued (Canny, 1990), water exits the minor veins to surrounding mesophyll along most of their length in the reticulate network, and not simply from the smallest free ending veins.

The partitioning of leaf resistances in this study is based on the approximation that leaf flow pathway components act in series, that is, that the bulk of water moves through each order of venation consecutively, and eventually out of the minor veins into parallel extra-xylem pathways. We note that this partitioning method, like other cutting or freezing and thawing methods, yields only an approximation of the true component resistances. A complete understanding of component resistances will only be obtained when experiments parameterize fully explicit leaf models that account for the complex pattern of series and parallel pathways, and the fact that flow through the leaf xylem is analogous to flow through 'leaky' pipes. There is some degree of water movement from major veins of any order to minor veins, as well as some degree of water movement out of the xylem along its length into the mesophyll (Wei *et al.*, 1999; Nardini *et al.*, 2001; Cochard *et al.*, 2004; Sack *et al.*, 2004).

Leaf structure and stomatal measurements

Leaf mass per area (*LMA*) was determined as lamina mass after drying for more than 72 h at $> 70^{\circ}\text{C}$, divided by lamina area (Li-Cor leaf area meter; Li-Cor Biosciences; five leaves per individual and 15 leaves per species). Stomatal densities and lengths of guard cells and stomatal pores were determined by microscopic measurement of impressions from abaxial nail varnish peels, taken centrally in the leaf, midway between the midrib and the margin, for leaves preserved in formalin acetic acid. This method could not be used for the papillate leaves of *C. alliodora* and *M. argentea*. Two leaves were measured per individual (six leaves per species); for each measured leaf, measurements were averaged from four measurements from each of three different peels. Total stomatal pore area index (*SPi*; a dimensionless index of stomatal pore area per lamina area; Sack *et al.*, 2003) was calculated in two ways, either as stomatal density $\times$ guard cell length² (*SPi_{gcl}*) or as stomatal density $\times$ stomatal pore length² (*SPi_{sp}*).

Statistics

Differences among species, and between the two regeneration irradiances, were determined using a general linear model, with species nested within regeneration irradiance category (Minitab Release 14; Minitab Inc., State College, PA, USA). Parametric correlation and Spearman rank correlation coefficients (r_p and r_s) and regression coefficients between tightly correlated variables were calculated (using Minitab Release 14; Sokal & Rohlf, 1995). For comparison of relationships among leaf traits of tropical and temperate species, regression lines were compared. First slopes were compared; when slopes

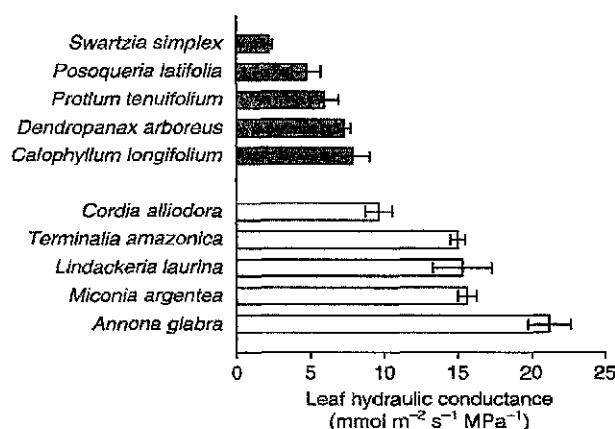


Fig. 2 Leaf hydraulic conductance (K_{leaf}) for tropical rainforest tree species that regenerate principally in understorey (closed bars) or in gaps or clearings (open bars). Means $\pm$ standard errors are shown; three individuals per species.

were the same, tests were made for differences in intercepts (using GENSTAT 7th edn, VSN International Ltd., Herts, UK; Zar, 1999).

Results

Leaf hydraulic architecture and its correlation with seedling regeneration irradiance

Values for K_{leaf} varied approx. 10-fold across the study species, ranging from 2.2 ± 0.27 mmol m⁻² s⁻¹ MPa⁻¹ [mean $\pm$ standard error (SE)] for *S. simplex* to 21.2 ± 1.49 mmol m⁻² s⁻¹ MPa⁻¹ for *A. glabra*. K_{leaf} values were on average approx. 3-fold higher for sun-establishing than for shade-establishing species (Table 1; Fig. 2).

These species differences arose from variation in each component of R_{leaf} : petiole, major veins, minor veins, and pathways outside the xylem (Table 1). The percentage of R_{leaf} in the petiole varied 10-fold across species, from $2.5 \pm 0.9\%$ (mean $\pm$ SE) in *S. simplex* to $26 \pm 5.4\%$ in *P. latifolia* (Fig. 3). The percentage of R_{leaf} in other components showed less variation. The percentage of R_{leaf} in major veins ranged from $10 \pm 1.4\%$ in *C. longifolium* to $44 \pm 6.4\%$ in *M. argentea*. The percentage of R_{leaf} in minor veins ranged from $12 \pm 2.5\%$ in *D. arboreus* to $47 \pm 4.7\%$ in *P. tenuifolium*; *C. longifolium* had no visible minor veins, but a midrib and close-set parallel second-order veins (Fig. 3). The percentage of R_{leaf} in the xylem ranged from $26 \pm 4.8\%$ for *C. longifolium* to $89 \pm 1.1\%$ for *L. laurina*, and thus the percentage of R_{leaf} outside the xylem ranged from $11 \pm 1.1\%$ for *L. laurina* to $74 \pm 4.8\%$ for *C. longifolium*. Averaging across species, the petiole accounted for $12 \pm 2.6\%$ of R_{leaf} (Fig. 3), and the major and minor veins each constituted twice the resistance of the petiole: $25 \pm 3.7\%$ and $25 \pm 4.0\%$ of R_{leaf} respectively. Thus, the resistances inside and outside of the xylem were on the same order, with on average $64 \pm 6.6\%$ of R_{leaf} in the xylem, and $34 \pm 6.6\%$ outside the xylem.

Table 1 Mean squares and significance of effects in analyses of variance (species, nested within regeneration irradiance) for individual leaf traits

Trait	Regeneration irradiance (df = 1)	Species (df)	Error (df)
K_{leaf}	714.1***	32.7*** (8)	3.33 (20)
$R_{petiole}$	0.00155***	0.000534*** (8)	0.0000369 (20)
$R_{major\ veins}$	0.00298***	0.00225*** (8)	0.0000518 (20)
$R_{minor\ veins}$	0.00647***	0.00290*** (8)	0.000164 (20)
R_{xylem}	0.0312***	0.0100*** (8)	0.000238 (20)
$R_{outside\ xylem}$	0.0468***	0.00960*** (8)	0.00158 (20)
% R_{leaf} in petiole	0.0028	0.023*** (8)	0.00268 (20)
% R_{leaf} in major veins	0.148***	0.0263* (8)	0.00795 (20)
% R_{leaf} in minor veins	0.0418*	0.0482*** (8)	0.00622 (20)
% R_{leaf} in and outside xylem	0.243**	0.115*** (8)	0.0188 (20)
Stomatal density	338	195710*** (6)	3140 (16)
Guard cell length	11.378	86.215*** (6)	3.667 (16)
Stomatal pore length	40.9***	60.2*** (6)	1.786 (16)
SPI_{gcl}	0.01704**	0.0209*** (6)	0.00117 (16)
SPI_{spl}	0.01174***	0.000736* (6)	0.000236 (16)
LMA	8617***	4006*** (8)	133 (20)

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

df, degrees of freedom; K_{leaf} , leaf hydraulic conductance; $R_{petiole}$, $R_{major\ veins}$, $R_{minor\ veins}$, R_{xylem} , $R_{outside\ xylem}$, hydraulic resistances of the petiole, major veins, minor veins, xylem and pathways of water outside the xylem, respectively; SPI_{gcl} , stomatal pore area index calculated as stomatal density $\times$ guard cell length²; SPI_{spl} , stomatal pore area index calculated as stomatal density $\times$ stomatal pore length²; LMA, leaf mass per area.

Leaf hydraulic architecture differed significantly between the two categories of regeneration irradiance. The percentage of R_{leaf} in the major veins was on average twice as high in sun-establishing species than in shade-establishing species (on average $32 \pm 5.2\%$ vs $18 \pm 2.8\%$ of R_{leaf} , respectively; Fig. 3; Table 1), and the percentage of R_{leaf} in the minor veins was higher for sun-establishing species (on average $29\% \pm 2.3$ SE vs. $22\% \pm 7.7$ SE of R_{leaf} , respectively; Fig. 3, Table 1). These differences drove a substantially higher percentage of R_{leaf} in the xylem for sun-establishing species than for shade-establishing species (on average $70 \pm 7.4\%$ vs $52 \pm 9.9\%$ of R_{leaf} in the xylem; Fig. 3; Table 1). There was no systematic difference between sun- and shade-establishing species in the proportion of R_{leaf} in the petiole (Table 1).

Across species, a tight correlation was observed between R_{leaf} and its components. Only $R_{petiole}$ was not parametrically correlated with R_{leaf} , although the two were rank-correlated (Fig. 4a). $R_{major\ veins}$, $R_{minor\ veins}$, R_{xylem} and $R_{outside\ xylem}$ scaled linearly with R_{leaf} (Fig. 4b–e). As in the case of R_{leaf} , higher values were found for shade- than for sun-establishing species in all components. The total resistances of the petiole, major veins, minor veins, overall xylem, and pathways outside the xylem were 2–5 times higher for shade-establishing species than for sun-establishing species (Table 1; Fig. 4a–e).

Coordination of leaf hydraulic conductance, stomatal traits and leaf structure

Across species, stomatal characters were highly variable; species varied 6-fold in stomatal density, 2-fold in the lengths of guard cells and of stomatal pores, and 3-fold in stomatal pore

area indices based on guard cell and stomatal pore lengths (Fig. 5a–e). Stomatal characters were interrelated across species. There was a tight relationship between guard cell length and stomatal pore length [data in Fig. 5b and c; guard cell length (μm) = $1.08 \times$ stomatal pore length (μm) + 10.6; $R^2 = 0.88$; $P = 0.001$]; on average, guard cell length was $1.8 \times$ stomatal pore length, although in *P. tenuifolium* guard cell length was $2.4 \times$ stomatal pore length. Stomatal density was negatively related to guard cell length and stomatal pore length [stomatal density (mm^{-2}) = $-35.9 \times$ guard cell length (μm) + 1372; $R^2 = 0.58$; $P = 0.029$; stomatal density = $-43.1 \times$ stomatal pore length (μm) + 1058; $R^2 = 0.63$; $P = 0.019$]. Systematic differences were found among sun- and shade-establishing species, with sun leaves having on average 1.2-, 1.2- and 1.7-fold larger values for stomatal pore length, SPI_{gcl} and SPI_{spl} , respectively, but no systematic differences were found for stomatal density or guard cell lengths (Table 1).

Total stomatal pore area indices were strongly correlated with K_{leaf} , indicating a coordination of liquid-phase and vapor-phase conductances within the leaf, as previously found for temperate woody species (Fig. 5d and e). Across all species, stomatal pore index based on guard cell length (SPI_{gcl}) was correlated with K_{leaf} , excepting the outlier *P. tenuifolium* (Fig. 5d; $R^2 = 0.66$; $P = 0.025$). The relationship was stronger when calculating stomatal pore area index based on stomatal pore length (SPI_{spl} ; Fig. 5e). These relationships arose despite the absence of a relationship between K_{leaf} and either stomatal density ($r_p = -0.08$; $P = 0.86$; Fig. 5a) or the length of guard cells or of stomatal pores ($r_p = 0.36$ and 0.49 , respectively, and $P = 0.38$ and 0.22 ; Fig. 5b and c). Because of the intercorrelation of the hydraulic resistances of components of the leaf,

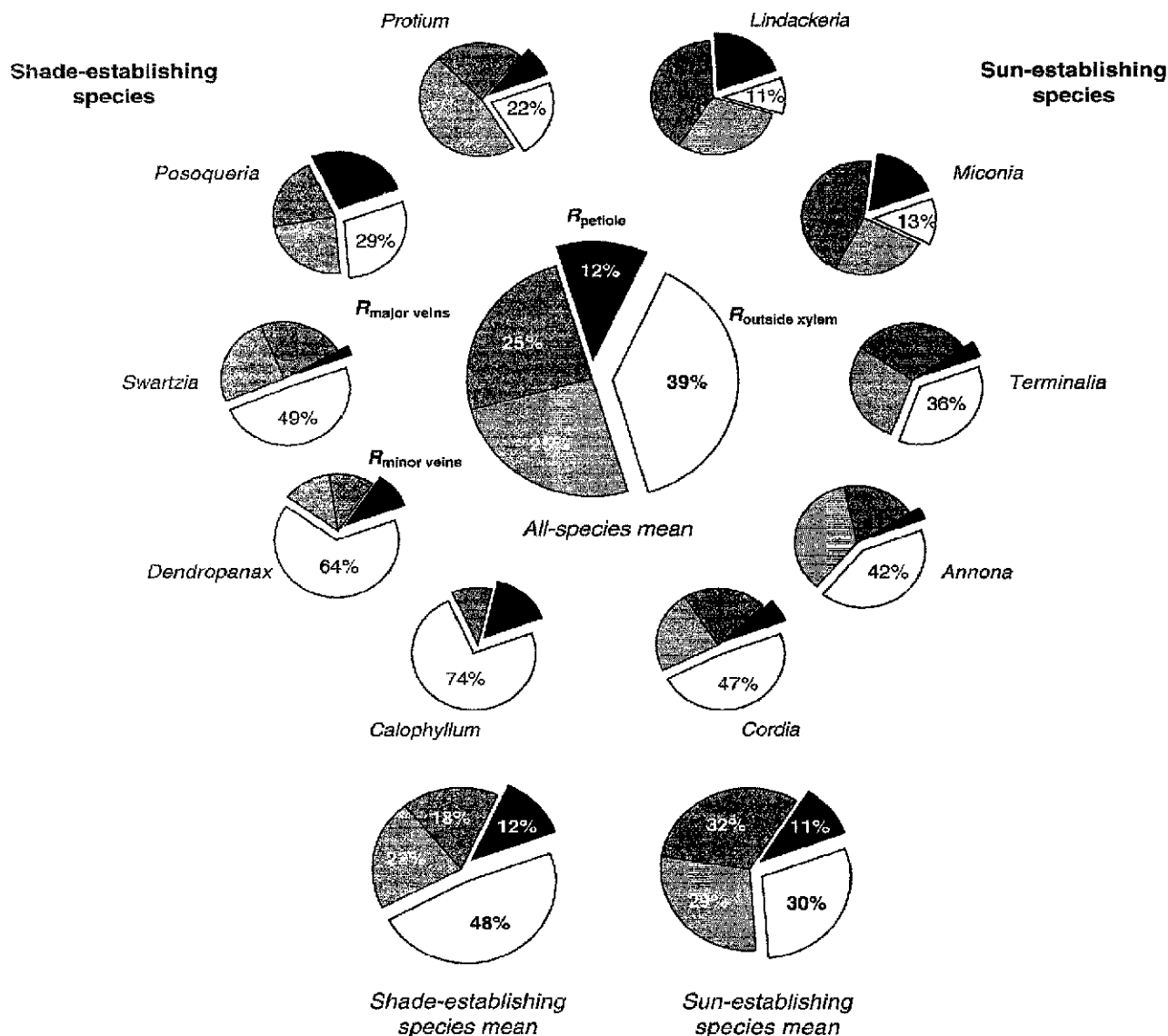


Fig. 3 Partitioning of leaf hydraulic resistance ($R_{\text{leaf}} = 1/K_{\text{leaf}}$, where K_{leaf} is the leaf hydraulic conductance) into resistances of petiole (R_{petiole}), major veins ($R_{\text{major veins}}$), minor veins ($R_{\text{minor veins}}$) and pathways outside the xylem ($R_{\text{outside xylem}}$) for 10 tropical rainforest tree species. Species are grouped by typical regeneration irradiance, with five species that typically persist in shaded understorey on the left, and five that establish in gaps or clearings on the right.

these too were significantly correlated with stomatal pore area indices. $R_{\text{major veins}}$, $R_{\text{minor veins}}$, R_{xylem} and $R_{\text{outside xylem}}$ were strongly negatively correlated with SPI_{sp} (respectively, $r_p = -0.76$, $P = 0.028$; $r_p = -0.76$, $P = 0.048$; $r_p = -0.79$, $P = 0.019$; $r_p = -0.84$, $P = 0.009$; data log-transformed for linearity).

The relationship of stomatal pore area with K_{leaf} for the tropical rainforest species was compared with that previously determined for temperate woody species (Sack *et al.*, 2003). Excepting *P. tenuifolium*, the coordination for tropical rainforest species showed the same trajectory as for temperate

woody species, with similar slopes ($P = 0.45$), but a higher intercept ($P < 0.001$; Fig. 5d). Thus, tropical rainforest species had higher SPI_{sp} for a given K_{leaf} than temperate woody species.

Across species, LMA varied 3-fold, from $66 \pm 7.7 \text{ g m}^{-2}$ (mean $\pm$ SE) for *L. laurina* to $200 \pm 1.2 \text{ g m}^{-2}$ for *C. longifolium* (Fig. 6). As previously reported for tropical rainforest trees (Bongers & Popma, 1988), sun-establishing species had lower LMA than shade-establishing species ($80 \pm 5 \text{ g m}^{-2}$ vs $114 \pm 22 \text{ g m}^{-2}$, respectively; Fig. 6; Table 1). Comparing

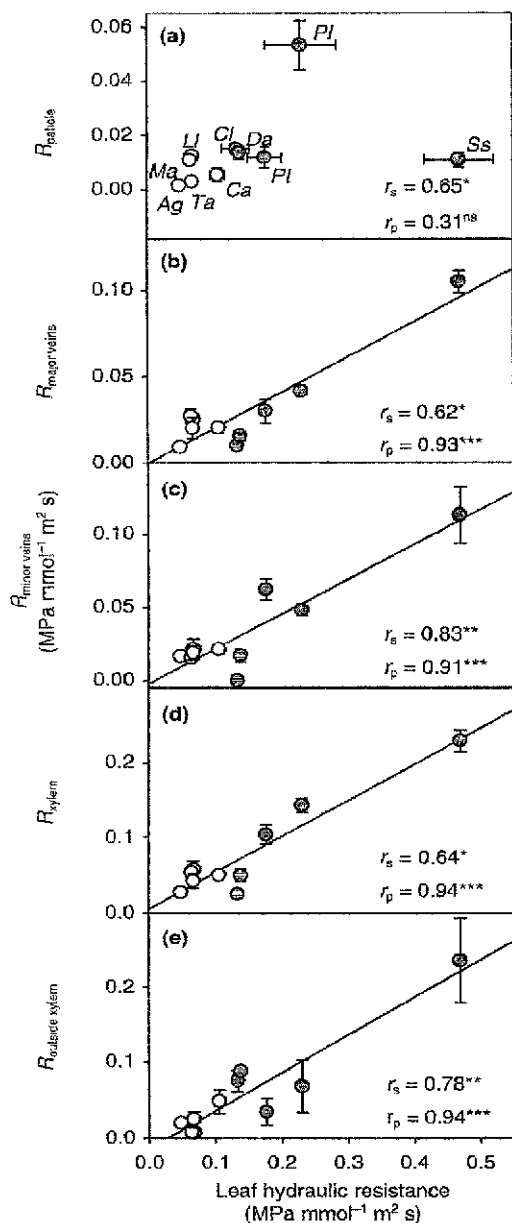


Fig. 4 Coordination of the hydraulic resistance of the leaf (R_{leaf}) and of (a) the petiole (R_{petiole}), (b) the major veins ($R_{\text{major veins}}$), (c) the minor veins ($R_{\text{minor veins}}$), (d) the leaf xylem (R_{xylem}), and (e) pathways of water outside the xylem ($R_{\text{outside xylem}}$) in tropical rainforest tree species. Symbols represent means $\pm$ standard errors (x-error bars in panel a only), for three individuals per species. Symbols: Ag, *Annona glabra*; Cl, *Calophyllum longifolium*; Ca, *Cordia alliodora*; Da, *Dendropanax arboreus*; Li, *Lindackeria laurina*; Ma, *Miconia argentea*; Pl, *Posoqueria latifolia*; Pt, *Protium tenuifolium*; Ss, *Swarzia simplex*, and Ta, *Terminalia amazonia*. Open and closed symbols indicate sun- and shade-establishing species, respectively. ^{ns}, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

the two regeneration irradiance categories, a higher K_{leaf} was coordinated with a lower LMA (Fig. 6). However, across all the study species, K_{leaf} was independent of LMA ($r_p = -0.27$; $P = 0.45$; Fig. 6). This independence was maintained when values were included for previously studied temperate woody species (Sack *et al.*, 2003; Fig. 6). Each of the components of R_{leaf} (R_{petiole} , $R_{\text{major veins}}$, $R_{\text{minor veins}}$, R_{xylem} and $R_{\text{outside xylem}}$) was also independent of LMA ($r_p = -0.20$ to 0.28 ; $P = 0.43$ to 0.79).

Discussion

Leaf hydraulic conductance correlates with regeneration irradiance

Tree leaves of shade-establishing species had lower K_{leaf} than those of sun-establishing species. Because the leaf is a major hydraulic bottleneck in the plant (Nardini & Salleo, 2000; Brodribb *et al.*, 2003; Sack *et al.*, 2003; Sack & Tyree, 2005), the lower K_{leaf} of shade-establishing species will be associated with lower maximum rates of gas exchange (see Discussion of stomatal pore characters below). Thus, for a given level of leaf area allocation, the lower K_{leaf} will be linked with lower whole-plant relative growth rates typical for shade-adapted forest species relative to light-demanding species (Givnish, 1988; Walters & Reich, 1999). Presumably, a high K_{leaf} requires a substantial investment in vascular tissue and membrane aquaporins. Fast-growing, sun-establishing species would receive a high return on this investment, whereas shade-establishing species would not, as these tend toward an ecology based on slow relative growth rates, low rates of carbon assimilation and respiration, and tissue costs amortized over long tissue lifespans (Coley, 1988; Kitajima, 1994; Walters & Reich, 1999).

The range of K_{leaf} values for the tropical trees in this study ($2.2\text{--}21\text{ mmol m}^{-2}\text{ s}^{-1}\text{ MPa}^{-1}$) extends that reported for temperate species (cf. $5\text{--}14\text{ mmol m}^{-2}\text{ s}^{-1}\text{ MPa}^{-1}$ for sun leaves of temperate woody species; Sack *et al.*, 2003). The 10-fold range of K_{leaf} , from the shade-tolerant legume *S. simplex* to the water-establishing *A. glabra*, is the largest reported for a single growth form in a single vegetation type. This range in K_{leaf} is equal to that previously shown in comparing eight species of shade and sun pteridophytes and angiosperms from Costa Rica (Brodribb & Holbrook, 2004), and it is double the range reported for five angiosperms in a forest in Costa Rica, or in a forest in Chile (Brodribb *et al.*, 2005). In fact, the range of K_{leaf} values found here for Barro Colorado Island (BCI) trees was equal to that of the combined data for 20 species of angiosperms, pteridophytes and conifers from Chile and Costa Rica (Brodribb *et al.*, 2005). The exceptional range of K_{leaf} values in this study is probably associated with the inclusion of species with strongly contrasting regeneration irradiances.

Our findings indicate that juvenile ecology is associated with leaf functional differences even in mature plants. Indeed, an increasing number of leaf traits important to seedling ecology are recognized as persistent in mature plants and

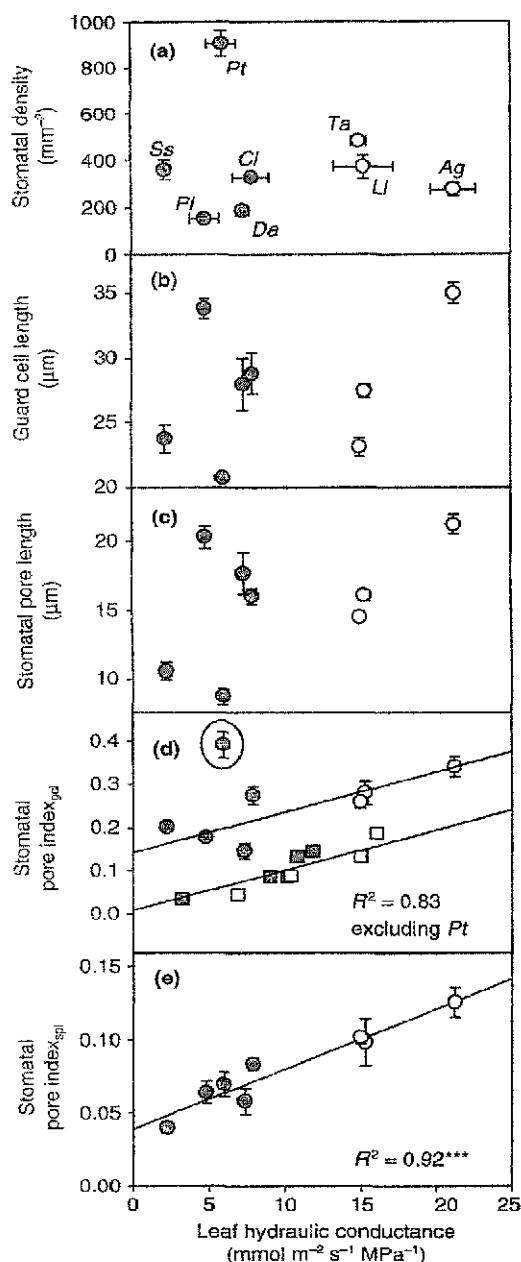


Fig. 5 Coordination of leaf hydraulic conductance (K_{leaf}) and (a) stomatal density, (b) guard cell length, (c) stomatal pore length, (d) stomatal pore index based on guard cell length (SPI_{gcl} = stomatal density $\times$ guard cell length²) and (e) stomatal pore index based on stomatal pore length (SPI_{spl} = stomatal density $\times$ stomatal pore length²). Symbols: Ag, *Annona glabra*; Cl, *Calophyllum longifolium*; Ca, *Cordia alliodora*; Da, *Dendropanax arboreus*; Ll, *Lindackeria laurina*; Ma, *Miconia argentea*; Pt, *Protium tenuifolium*; Ss, *Swartzia simplex*, and Ta, *Terminalia amazonia*. Open and closed circles indicate sun- and shade-establishing species, respectively. In (d), the linear regression model includes tropical species (excluding *Protium tenuifolium*; circled) and temperate

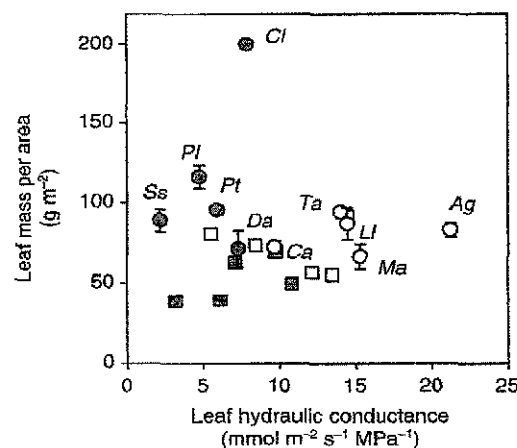


Fig. 6 The independence of leaf hydraulic conductance (K_{leaf}) and leaf mass per area. Symbols: Ag, *Annona glabra*; Cl, *Calophyllum longifolium*; Ca, *Cordia alliodora*; Da, *Dendropanax arboreus*; Ll, *Lindackeria laurina*; Ma, *Miconia argentea*; Pt, *Protium tenuifolium*; Ss, *Swartzia simplex*, and Ta, *Terminalia amazonia*. Open and closed circles indicate sun- and shade-establishing species, respectively. Squares represent comparative values for temperate woody species; open and closed squares, respectively, represent sun and shade leaves (from Sack *et al.*, 2003).

potentially constrain mature plant function. In a meta-analysis of woody species of temperate and Mediterranean zones, seedling and adult leaf values were correlated across species for leaf size, *LMA*, and stomatal densities (Cornelissen *et al.*, 2003). Thus, barring cases of strong heteroblasty, the function of mature plants will be partially determined by characters important in juvenile ecology. This work indicates that K_{leaf} is involved in the complex of traits underlying species differences in growth and responses to irradiance, which potentially contribute to the coexistence of large numbers of species in tropical forest (Walters & Reich, 1999; Sack & Grubb, 2001; Wright, 2002; Poorter & Aerts, 2003).

Partitioning of leaf hydraulic resistance: variation across contrasting regeneration irradiances

This study confirms previous work on partitioning resistances of flow pathways in the leaf, in showing that resistances within the xylem are on the same order as those outside of the xylem (Gascó *et al.*, 2004; Sack *et al.*, 2004). Indeed, the average partitioning found in this study was similar to that previously shown for two temperate deciduous tree species (Sack *et al.*, 2004). Notably, a substantial resistance was found in the petiole (on average 12% of R_{leaf}), and even higher values were found in both the major and minor veins (both 25%) and

species (open and closed squares represent, respectively, values for sun and shade leaves; from Sack *et al.*, 2003); $SPI_{gcl} = 0.00902K_{leaf} + 0.0283$ for temperate species, 0.145 for tropical trees). In (e), $SPI_{spl} = 0.00410K_{leaf} + 0.0393$.

outside the xylem (39%). The substantial resistance in the xylem may have arisen from the hydraulic resistance to flow through the conduits in the xylem, as well as from the resistance to flow between conduits: conduit endings were found in the midrib or in the secondary or tertiary veins, depending on species (data not shown). The resistance outside the xylem may arise from resistance to flow out of the xylem, across the living tissues of the bundle sheath, and then through the apoplast and/or symplast and finally out of the stomata. We note that a recent study has proposed that there is negligible resistance in the leaf xylem, because a computer model indicated negligible resistance when scaling up from dimensions of xylem conduits in cross-sections in different vein orders (Cochard *et al.*, 2004). Notably, that model, while pioneering, did not include estimates of the resistance to water movement between xylem conduits, or in junctions between vein orders; the attempt to fit the model findings to hydraulic measurements indicated that such resistance was considerable. We note that several studies have reported lower xylem resistances than the average found in this study when treatments were applied to remove extra-xylem resistance by cutting major veins, or freezing the leaf (e.g. Tyree *et al.*, 2001; Trifilo *et al.*, 2003; Cochard *et al.*, 2004). As previously argued (Sack *et al.*, 2004), these methods may have opened up pathways so that water would have leaked out of the vein system proximally to the minor veins, and thus underestimated xylem resistance. We note that the outstanding debate is focused on how much resistance is in the minor veins, and how best to measure it. However, even leaving aside the question of resistance in the minor veins, our study indicates substantial resistance in the petiole and major veins, and so our findings do not support any general concept of leaf hydraulics in which there is negligible resistance in the xylem.

Across species, there was a strong scaling among the hydraulic resistances of the components of the leaf pathways. This finding indicates the strong overall importance of the leaf xylem, and of the pathways outside the xylem, in determining species differences in K_{leaf} . This finding suggests a substantial need for further research on how resistances arise inside and outside the xylem. For the xylem resistance, research is needed to determine the potential linkage with the immensely variable leaf venation architecture.

Despite the general scaling, species differed significantly in the partitioning of whole-leaf resistance. Shade-establishing species had higher resistances in each component of the leaf, excepting the petiole. Further, shade-establishing species in general had a significantly higher proportion of total resistance outside the xylem than sun-establishing species. This difference is consistent with previous work showing a high resistance outside the xylem (62–75% of R_{leaf}) in *C. arabica*, a species domesticated from shaded habitat (Gascó *et al.*, 2004). This difference is also expected from the hypothesis that highly conductive xylem requires a substantial vascular construction cost (McCulloh *et al.*, 2003). Conversely, highly conductive

flow paths out of the xylem might require a lower construction cost, potentially being based on a greater permeability for exchange between xylem and surrounding mesophyll (Salleo *et al.*, 2003). The conductivity of these paths and those through the mesophyll might require a greater activation of aquaporins (Tyree *et al.*, 2005). Species selected for a lower R_{leaf} would thus, by hypothesis, possess reduced resistance in each component of R_{leaf} , and the reduction would be more pronounced outside the xylem, as that reduction would not entail the construction of highly conductive xylem. The balance of costs and benefits associated with investing in xylem and extra-xylem tissue, in terms of hydraulic conductance and overall carbon balance, is an important topic for further research; notably, investment in xylem in many species also contributes to mechanical support of leaf display for light capture (Niinemets & Sack, 2005).

Coordination of leaf hydraulic conductance with stomatal characters and LMA

As reported previously for temperate species (Sack *et al.*, 2003), K_{leaf} correlated with SPI . The previously used index SPI_{gcl} (Sack *et al.*, 2003) was correlated with K_{leaf} only when *P. tenuifolium*, which had an exceptionally large guard cell relative to its stomatal pore length, was excluded. The correlation of K_{leaf} and SPI_{gpl} was strong and predictive. As reported previously for temperate woody species (Sack *et al.*, 2003), this coordination arose despite a lack of coordination between K_{leaf} and stomatal density, the dimensions of the stomatal pore or the guard cell length: none of these characters alone was functionally linked with K_{leaf} across species. The coordination of K_{leaf} and SPI will result in a strong coordination of K_{leaf} with stomatal conductance, and maximum rates of gas exchange (Aasamaa *et al.*, 2001; Brodribb & Holbrook, 2004; Brodribb *et al.*, 2005).

While many recent studies have indicated coordination across species of leaf or stem hydraulic conductances and leaf gas exchange (e.g. Brodribb & Feild, 2000; Nardini & Salleo, 2000; Santiago *et al.*, 2004; Brodribb *et al.*, 2005), there has been no analysis of differences in this coordination across vegetation zones or functional types. In this study, the relationship of K_{leaf} with SPI_{gcl} for tropical tree species differed from that found previously for temperate woody species, providing the first indication that this coordination, and by extension that of K_{leaf} and maximum rates of gas exchange, differs across vegetation types. A theoretical expectation for this coordination can be developed based on the idea that species of a given life form and vegetation type converge in aspects of their water balance. Rearranging the Ohm's law analogy for the soil–plant–atmosphere continuum, $\Psi_{\text{leaf}} - \Psi_{\text{soil}} = E/K_{\text{plant}} = VPD \times g/K_{\text{plant}}$, where Ψ_{leaf} , Ψ_{soil} , E , K_{plant} , VPD and g represent, respectively, the leaf and soil water potential, the transpiration rate, the whole-plant (soil-to-leaf) hydraulic conductance, the leaf-to-air vapor pressure difference, and the leaf conductance to water vapor:

$$g = K_{\text{plant}} \times (\Psi_{\text{leaf}} - \Psi_{\text{soil}}) / VPD \quad \text{Eqn 1}$$

In the simplest case, if species of a given life form and vegetation type converge in a relatively narrow range of Ψ_{leaf} during their peak rates of gas exchange, and in Ψ_{soil} and VPD during peak function, there must then follow coordination between g and K_{plant} . Additionally, if K_{leaf} scales with K_{plant} across species as previously indicated (Sack *et al.*, 2003), there will follow coordination of g and K_{leaf} . Assuming that boundary layer conductance is relatively constant across species, maximum stomatal conductance would be coordinated with K_{leaf} . Thus, for plants of a given functional type, convergence in aspects of water balance during periods of peak function will drive coordination of K_{leaf} with stomatal pore area per leaf area and thus with maximum stomatal conductance. However, tropical rainforest tree species might be expected to experience a higher Ψ_{soil} and/or Ψ_{leaf} and/or a lower VPD than temperate woody species during peak activity. Thus, tropical rainforest species would be expected to have a higher right-hand term in Eqn 1, and thus to be enabled to possess a higher g (and SPI) for a given K_{plant} (and K_{leaf}) than temperate woody species. Of course, we note that, in nature, the coordination described above will not be perfect, even within a vegetation type, as differences in factors such as rooting depth and vulnerability to cavitation will result in species differences in Ψ_{soil} , Ψ_{leaf} , K_{plant} and/or K_{leaf} . Additionally, species may diverge in the season during which they manifest peak activity, which would further destabilize the coordination. Our findings indicate the need for further work on the coordination of hydraulics and gas exchange within and across vegetation types.

Across species, K_{leaf} was independent of LMA , as previously found for temperate deciduous species and Mediterranean species (Tyree *et al.*, 1999; Nardini, 2001; Sack *et al.*, 2003). In turn, LMA is linked with many important aspects of leaf functional ecology; notably, it is positively correlated across species with leaf lifespan, and negatively correlated with leaf nitrogen concentration per mass. Along with the maximum rate of photosynthesis per leaf area (A_{area}), LMA determines the maximum rate of photosynthesis per leaf mass ($A_{\text{mass}} = A_{\text{area}} / LMA$; Sack *et al.*, 2003; Wright *et al.*, 2004; Niinemets & Sack, 2005). A previous study has shown that K_{leaf} is correlated across species with A_{area} (Brodribb *et al.*, 2005), and thus it is linked with carbon economy, although orthogonal to LMA . Our data indicate that leaf hydraulic properties are linked in the complex of traits that define differences in water use and carbon economy across habitats and vegetation zones.

Acknowledgements

We thank Rebecca Barron and Eric Manzano for technical assistance; Oris Acevedo and Daniel Millan and researchers and staff at Barro Colorado Island for logistical support; and Hervé Cochard, Rick Condit, Egbert Leigh, Joe Wright and

Gerhard Zotz for helpful discussion. LS was supported by a Short-term Fellowship at the Smithsonian Tropical Research Institute and a Putnam Fellowship at the Arnold Arboretum of Harvard University. NMH acknowledges the support of The Andrew W. Mellon Foundation.

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