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# The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species

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## ABSTRACT

**The hydraulic conductance of the leaf lamina ($K_{lamina}$) substantially constrains whole-plant water transport, but little is known of its association with leaf structure and function. $K_{lamina}$ was measured for sun and shade leaves of six woody temperate species growing in moist soil, and tested for correlation with the prevailing leaf irradiance, and with 22 other leaf traits. $K_{lamina}$ varied from $7.40 \times 10^{-5}$ kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for *Acer saccharum* shade leaves to $2.89 \times 10^{-4}$ kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for *Vitis labrusca* sun leaves. Tree sun leaves had 15–67% higher $K_{lamina}$ than shade leaves. $K_{lamina}$ was co-ordinated with traits associated with high water flux, including leaf irradiance, petiole hydraulic conductance, guard cell length, and stomatal pore area per lamina area. $K_{lamina}$ was also co-ordinated with lamina thickness, water storage capacitance, 1/mesophyll water transfer resistance, and, in five of the six species, with lamina perimeter/area. However, for the six species, $K_{lamina}$ was independent of inter-related leaf traits including leaf dry mass per area, density, modulus of elasticity, osmotic potential, and cuticular conductance. $K_{lamina}$ was thus co-ordinated with structural and functional traits relating to liquid-phase water transport and to maximum rates of gas exchange, but independent of other traits relating to drought tolerance and to aspects of carbon economy.**

*Key-words*: hydraulic architecture; hydraulic conductivity; hydraulic resistance; leaf water storage; specific leaf area; stomatal conductance.

## INTRODUCTION

Leaves vary tremendously in their area, thickness, shape, and in their capacities for gas exchange and for withstanding drought. This diversity is generated by phylogeny and adaptation but constrained by trait correlations. Recently, important headway has been made in elucidating leaf trait correlations relating to carbon economy (e.g. Field & Mooney 1986; Reich, Walters & Ellsworth 1997; Reich, Ellsworth & Walters 1998; Reich *et al*. 1999; Wright, Reich & Westoby 2001). The aim of this study is to test analogous links among leaf traits relating to water balance.

The hydraulic properties of the leaf lamina, the terminal component of the transpiration stream, significantly constrains whole-plant hydraulic conductance ($K_{plant}$). $K_{plant}$ defines the capacity of a plant for water use. In a given microclimate and soil water supply, it is the stomatal and boundary-layer conductances that determine the transpiration rate, while $K_{plant}$ determines the leaf water potential at that transpiration rate (Cowan 1972; Tyree & Zimmermann 2002). $K_{plant}$ thus defines how high stomatal conductance may be without desiccating the leaf, and often correlates with maximum stomatal conductance within and across species (Kuppers 1984; Meinzer & Grantz 1990; Nardini, Tyree & Salleo 2001; Bhaskar *et al*. 2002). Values in the literature suggest that the leaf lamina hydraulic conductance ($K_{lamina}$) scales linearly with $K_{plant}$, with an allometric constant of ≈4 (Fig. 1); that is $K_{lamina}$ is $\approx 4 \times K_{plant}$. Stated in another way, leaf hydraulic resistance accounts for one-quarter of the whole-plant resistance.

$K_{lamina}$ is determined by the vascular and extra-vascular pathways of transpired water (Yang & Tyree 1994; Nardini *et al*. 2001; Sack *et al*. 2002). Water enters the leaf through the petiole and flows through orders of veins in series and parallel, across the bundle sheath, and into and/or around the mesophyll cells, before evaporation into airspaces and diffusion out of the stomata. We tested whether $K_{lamina}$ was linked with 22 other leaf traits, and with the prevailing leaf irradiance. Given the hydraulic importance of $K_{lamina}$ we hypothesized it would be associated with leaf traits relating to water flux, such as petiole hydraulic conductance and stomatal size and/or density. We also tested whether $K_{lamina}$ was linked with traits associated with aspects of drought tolerance including pressure–volume curve parameters (Abrams 1988; Niinemets 2001), and cuticular conductance.

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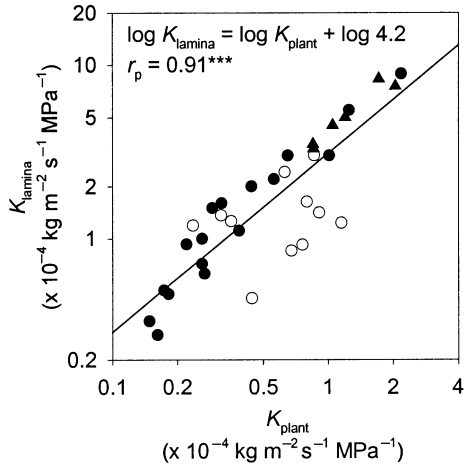


**Figure 1.** The scaling of leaf lamina and whole-plant hydraulic conductance, for 34 species; filled triangles represent herbs, filled circles woody seedlings and saplings, and open circles mature trees and shrubs ($K_{lamina}$ determined using high-pressure flow meter; $K_{plant}$ using various methods; data of Becker, Tyree & Tsuda 1999; Nardini & Tyree 1999; Nardini *et al*. 2000; Nardini & Salleo 2000; Tsuda & Tyree 2000). Standard major axis ± 95% confidence intervals of log–log data =1.21 ± 0.22.

## MATERIALS AND METHODS

### Plant material

Six species were sampled that had leaves with obvious differences in thickness, texture, and apparent desiccation tolerance. From June to August 2001 at Harvard Forest in Petersham, MA (42′54° N, 72′18° W) trees were selected of *Acer rubrum* Marsh. (Aceraceae; nomenclature follows Gleason & Cronquist 1991), *Acer saccharum* Marsh., *Betula papyrifera* L. (Betulaceae) and *Quercus rubra* L. (Fagaceae), growing at exposed sites or along roads and trails within the forest. Diameters at breast height ranged between 0.16 and 0.39 m (*A. rubrum*), 0.56–0.91 m (*A. saccharum*), 0.07–0.10 m (*Betula*) and 0.20–0.51 m (*Quercus*). Shoots were sampled 5–8 m above the ground, from the exposed part of the canopy ('sun leaves'), and the canopy interior ('shade leaves') of five trees per species. Shoots of *Hedera helix* L. (Araliaceae) and *Vitis labrusca* L. (Vitaceae) were collected from one or several individuals that covered more than 30 m of fence at the Harvard Forest (*Vitis*), or from large vines growing along a fence at the Harvard University campus (*Hedera*, sampled in October). Material collected in the field was re-cut under water and allowed to hydrate overnight by placing the cut ends of the shoots in water, and covering leaves with plastic. A 10 mM aqueous KCl solution was used to hydrate the samples to be used in hydraulic measurements, identical to the solution used for those measurements.

The prevailing light environments of the sampled leaves were measured. On overcast days the photosynthetically active radiation (PAR) incident on the leaf was measured for five experimental leaves on each plant, and simultaneously in an open area (using matched quantum sensors; LI-250 Light Meter; Li-Cor Inc., Lincoln, NE, USA), for calculation of the percentage daylight diffuse PAR (Anderson 1964).

### Leaf lamina and petiole hydraulic conductance

$K_{lamina}$ values and petiole conductances for the tree sun leaves, and for *Hedera* and *Vitis*, are reported in a previous study (Sack *et al*. 2002), with additional data for tree shade leaves collected during the same period. The three methods for determining $K_{lamina}$ compared by Sack *et al*. (2002) involve driving water flow through excised leaves, and simultaneously determining the pressure gradient driving the flow; $K_{lamina}$ is calculated as the ratio of flow rate to pressure gradient. The three methods gave statistically similar results and the data were pooled for this study. Measurements of tree shade leaves were made using two of the three methods described in Sack *et al*. (2002), the 'evaporative flux method', and the 'vacuum pump method' (after methods described in Kolb, Sperry & Lamont 1996; Nardini *et al*. 2001); $n$ = 5–7 per species per method (1–2 leaves from each study tree). The values provided by the two methods were also statistically indistinguishable in a two-way analysis of variance (log-transformed $K_{lamina}$ values, with factors species and method; $P < 0.001$ for species; $P = 0.33$ for method; $P = 0.88$ for species–method interaction), and were pooled for each species. Petiole conductance was measured for shade leaves using methods described by Sack *et al*. (2002), driving flow through petiole segments at a determined positive pressure, with $n$ = 5–8 per species. Hydraulic measurements were made in ambient temperatures of 23 ± 2 °C; for leaves that heated above ambient during measurement (by up to 2 °C), $K_{lamina}$ was reduced by 2% per °C above ambient, to normalize for the effects of temperature on viscosity (see Sack *et al*. 2002).

Petiole hydraulic conductance was normalized to leaf dimensions in two ways. *Petiole conductivity* ($k_{petiole}$; units, kg $s^{-1}$ m $MPa^{-1}$) was calculated as petiole conductance × petiole length, and *petiole conductance per leaf area* ($K_{petiole}$; units, kg $s^{-1}$ $m^{-2}$ $MPa^{-1}$) as petiole conductance/lamina area. Lengths of whole petioles were estimated from known lamina areas, using petiole length versus lamina area regressions for each leaf type ($R^2$ = 0.40–0.75; $P < 0.05$; Table 1). For these regressions, sun and shade shoots were sampled from four to five study trees per species, and three to four shoots were sampled from each climber species. From each shoot, the largest and smallest leaves, and one or two average-sized leaves, were sampled. For each leaf type, this sampling protocol resulted in 10 to 16 leaves evenly spread over the typical size range.

### Leaf form and composition

Leaf form and composition were measured for each leaf type. Two sun and two shade leaves from each tree were sampled for each measurement, and two leaves from each of five different shoots of *Hedera* and *Vitis*; the two values

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**Table 1.** Mean values ± standard error for the diffuse site factors (dsf) and volumes of sun and shade leaves of each species ($n$ = 10–25 for each leaf type) and for parameters (slopes $a$ and intercepts $b$) of the linear regressions of petiole length, petiole conductivity ($k_{petiole}$), and lamina perimeter versus area

| | | | | Petiole length (m) vs lamina area ($m^2$) | | $k_{petiole}$ ($\times 10^{-5}$ kg $s^{-1}$ $m^{-1}$ $MPa^{-1}$) vs log lamina area ($cm^2$) | | Log lamina perimeter (cm) vs lamina area ($m^2$) |
|---|---|---|---|---|---|---|---|---|
| Species | Sun/shade | Mean dsf ± SE (% daylight) | Lamina volume ($cm^3$) | $a$ ± SE<br>$R^2$ | $b$ ($\times 10^{-2}$) ± SE ($n$) | $a$ ($\times 10^{-5}$) ± SE<br>$R^2$ | $b$ ($\times 10^{-7}$) ± SE ($n$) | $b$ ± SE ($n$) |
| *Acer rubrum* | Sun | 64 ± 4.8 | 0.90 ± 0.08 | 8.71 ± 1.30†<br>0.60*** | 1.92 ± 0.71† (30) | 7.38 ± 1.56†<br>0.61*** | –1.1 ± 0. 86 (12)<br>–3.2 ± 0.50 (5) | 0.575 ± 0.020 (15)<br>0.605 ± 0.029 (15) |
| | Shade | 6.3 ± 1.1 | 0.75 ± 0.09 | | | | | |
| *Acer saccharum* | Sun | 39 ± 3.3 | 1.3 ± 0.20 | 6.81 ± 1.40†<br>0.44*** | 2.82 ± 0.79† (30) | 3.60 ± 0.38†<br>0.90*** | –0.29 ± 0.18 (6)<br>–1.08 ± 0.14 (5) | 0.580 ± 0.020 (16)<br>0.606 ± 0.020 (17) |
| | Shade | 2.0 ± 0.18 | 1.2 ± 0.15 | | | | | |
| *Betula papyrifera* | Sun | 46 ± 1.6 | 0.52 ± 0.05 | 3.40 ± 0.64†<br>0.54*** | 1.16 ± 0.24† (24) | 9.58 ± 1.46†<br>0.82*** | –0.44 ± 0.34 (5)<br>–1.02 ± 0.20 (5) | 0.542† ± 0.018 (17) |
| | Shade | 12 ± 0.92 | 0.64 ± 0.04 | | | | | |
| *Hedera helix* | Shade | 14 ± 0.27 | 1.4 ± 0.25 | 10.3 ± 2.6<br>0.62** | 4.56 ± 1.49 (10) | 7.49 ± 1.69<br>0.73** | –1.7 ± 0.96 (8) | 0.660 ± 0.020 (15) |
| *Quercus rubra* | Sun | 58 ± 3.1 | 2.3 ± 0.18 | 1.73 ± 0.27†<br>0.75*** | 2.89 ± 0.25 (12)<br>1.59 ± 0.21 (15) | 34.0 ± 3.52†<br>0.79*** | –4.33 ± 2.92 (21)<br>–11.7 ± 2.24 (5) | 0.781 ± 0.020 (16)<br>0.703 ± 0.020 (16) |
| | Shade | 7.4 ± 0.82 | 2.6 ± 0.14 | | | | | |
| *Vitis labrusca* | Sun | 60 ± 3.7 | 4.2 ± 0.22 | 4.86 ± 1.51<br>0.40** | 1.44 ± 2.16 (15) | 16.2 ± 5.79<br>0.53* | –11.7 ± 11.3 (7) | 0.545 ± 0.021 (13) |

Significance levels: *$P \leq 0.05$; **$0.01 > P \geq 0.001$; ***$P \leq 0.001$. For regressions of petiole length and $k_{petiole}$ versus lamina area, species' regressions differed in slopes ($F$-ratio tests; $P < 0.001$); for log lamina perimeter versus log lamina area, all leaf types shared a common slope (0.52 ± 0.0175 SE, $R^2 = 0.92$, $P < 0.001$), and differed significantly in intercepts ($F$-ratio tests; $P < 0.001$).
†Common slope or intercept for sun and shade leaves.

for each tree or shoot were averaged, resulting in $n = 5$ for each leaf type. The leaves were hydrated overnight (as described above), weighed (for 'turgid mass'), and then placed in a vacuum flask containing distilled water, and vacuum-infiltrated >4 h. When the leaves were infiltrated and hyaline, they were towelled dry, weighed again (for 'infiltrated mass'), and then lamina volume was determined by water displacement in a graduated cylinder, to the nearest 0.05 $cm^3$, after which lamina area was determined (Li-Cor leaf area meter; Li-Cor Inc.). Leaf laminas were weighed again after drying at 70 °C for > 72 h (for 'dry mass'). From these measurements, the air–water–dry matter volumetric fractions were determined: volumes of lamina air and water were determined, respectively, as infiltrated mass – turgid mass and as turgid mass – dry mass (using the density of liquid water =1000 kg $m^{-3}$); volume of dry matter was determined as lamina volume – volumes of air and water; volumetric fractions were determined dividing by lamina volume (cf. Roderick *et al*. 1999a). Leaf density was determined as lamina dry mass/lamina volume. The density of the dry matter was determined as dry mass/volume of dry matter. Lamina thickness was determined as lamina volume/lamina area. Leaf dry mass per area (LMA) was determined as lamina area/lamina dry mass; it is also equal to lamina thickness × density (Witkowski & Lamont 1991).

Leaf outline was characterized both as perimeter/area (Talbert & Holch 1957) and perimeter$^2$/area (cf. McLellan & Endler 1998). Leaves for shape analysis were sampled according to the same protocol as described above for the regressions of petiole length versus lamina area. Each leaf was digitally scanned (using an Epson ES-1200C scanner; Epson, Long Beach, CA, USA), and the perimeter and area determined using image analysis software (ImageJ, public domain software; http://rsb.info.nih.gov/ij/). For each leaf type, log perimeter was regressed against log lamina area, because perimeter and area were found to follow a geometric power law scaling; that is, perimeter increased in proportion with the square root of area for leaves of each type. Perimeter/area was estimated from the regressions for leaves of mean lamina area (Zar 1999). Because perimeter$^2$/area is independent of leaf size, a mean value for each leaf type was calculated based on all leaves sampled for shape.

## Stomatal density, guard cell length and stomatal pore area index

Stomatal densities and guard cell lengths were determined by microscopic measurement of impressions from abaxial nail varnish peels taken centrally, midway between midrib and margin. This method could not be used for the papillate *Vitis* leaf; for this species, leaves were gently macerated, and sections of abaxial epidermis were removed and inverted (following Grubb, Grubb & Miyata 1975), and microscopic measurements were made on these sections. Counts were averaged for four locations per peel; peels were made for one sun and one shade leaf for each study tree, from two leaves from each of five shoots of *Hedera* and one leaf from each of three shoots of *Vitis*. Total stomatal pore area index (SPI; a dimensionless index of stomatal pore area per lamina area) was calculated as stomatal density × guard cell length$^2$.

## Pressure–volume curve parameters and water storage capacitance

Pressure–volume curve parameters were determined for one sun and one shade leaf from each tree, and one leaf from each of five shoots of *Hedera* and *Vitis*. The leaves were dried on a laboratory bench, and alternately weighed and measured for water potential with a pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Subsequently dry mass was determined after more than 72 h at 70 °C. Pressure–volume curve parameters were calculated (Koide *et al*. 2000): osmotic potential at full turgor and at the turgor loss point ($\pi_{ft}$ and $\pi_{tlp}$), modulus of elasticity at full turgor ($\epsilon_{ft}$), and relative capacitance at full turgor ($C_{ft}$; ΔRWC/Δ leaf water potential, between full turgor and turgor loss point). 'Plateau' effects' associated with leaf airspace infiltration were found for some of the leaves, and corrected (Kubiske & Abrams 1991). For parameters calculated from slopes of two 'dependent variables', i.e. for $\epsilon_{ft}$ and $C_{ft}$, standard major axes were used (Sokal & Rohlf 1995). Leaf-area specific capacitance was calculated as $C_{ft}$ × (lamina turgid mass – lamina dry mass)/lamina area, using mean values ($C_{ft}$*, in units of kg $MPa^{-1}$ $m^{-2}$). The transfer resistance ($R_t$) linking water stored in mesophyll cells with the vasculature was measured following the method described by Nobel & Jordan (1983). In this method, the kinetics of the decline of water potential were followed for leaves repeatedly pressurized for 2 s to 0.2 MPa above turgid water potential, in a pressure bomb (Nobel & Jordan 1983). Analysing the kinetics yields the time constant for water exchange ($t_{1/2}$). $R_t$ was calculated assuming that $t_{1/2}$ is equal to $R_t \times C_{ft}$*. Standard errors for $C_{ft}$* and $R_t$ were determined by propagation of error (Beers 1957).

## Cuticular conductance

Cuticular conductance (= 'minimum conductance', $g_{min}$ *sensu* Kerstiens 1996) was determined for one sun and one shade leaf from each tree, and one leaf from each of five shoots of *Hedera* and *Vitis*. Hydrated leaves were dried on a laboratory bench, at PAR < 10 $\mu$mol photons $m^{-2}$ $s^{-1}$, for 6–8 h. The leaves were weighed at intervals of 10–30 min. Cuticular transpiration was measured as the slope of water loss versus time; the slope often became shallower within the first hour of drying, suggesting progressive stomatal closure, followed by a highly linear decline for hours ($R^2 > 0.995$), suggesting closed stomata; the slope of the decline from 2 to 4 h was used to estimate cuticular transpiration. The value of $g_{min}$ was calculated as cuticular transpiration/mole fraction gradient in water vapour from the leaf to air, assuming the leaf internal air to be fully saturated (Pearcy, Schulze & Zimmermann 2000). Ambient temperature and relative humidity (RH), measured at

30 min intervals (LI-1600; LiCor Inc.), fluctuated minimally during the measurements (i.e. respectively, ± 1 °C and ±0.5–2%). Mean temperature and RH during measurement were 22 °C and 35% for *Acer* spp. and *Quercus*, 22 °C and 45% for *Betula* and *Vitis* and 25 °C and 12.5% for *Hedera*. The differences in RH for measurement of different species were not in the range that would significantly affect $g_{min}$ (Schreiber *et al*. 2001).

### Statistics

Species and sun–shade differences were determined using two-way ANOVAs after log-transformation of data to increase heteroscadicity and to model for multiplicative effects (Minitab Release 13.32; Minitab Inc., State College, PA, USA). Relationships between petiole conductivity and petiole length versus lamina area, and between log lamina perimeter and log lamina area were determined using regression analyses. The regressions for each species' sun and shade leaves were compared; when slopes were the same, tests were made for differences in intercepts (using Genstat 5th edition; Zar 1999). Similarly, different species' regressions were compared.

For leaf traits, parametric correlation and Spearman rank-correlation coefficients ($r_p$ and $r_s$; Sokal & Rohlf 1995) were calculated using Minitab Release 13.32. We confirmed that correlations between lamina area-normalized variables were not due to auto-correlation (Niklas 1994), by testing again after removing the area dependence of one or both variables by multiplying or dividing by lamina area.

Allometric relationships were determined using standard major axes and $r_p$ for log-transformed data (Sokal & Rohlf 1995). Standard errors were calculated as for ordinary linear regression (Niklas 1994; Sokal & Rohlf 1995).

## RESULTS

### Comparisons of tree sun and shade leaves

Tree sun leaves had significantly higher $K_{lamina}$ than shade leaves (Fig. 2; Table 2), strikingly in *A. saccharum* (sun leaves 67% higher), and in *Q. rubra* (48% higher). Petiole hydraulic conductance was also higher for sun leaves. For each species petiole conductivity ($k_{petiole}$, conductance × petiole length, in kg m s$^{-1}$ MPa$^{-1}$) was linearly related to lamina area ($R^2$ = 0.53–0.90; $P < 0.05$; Table 1). The regressions of $k_{petiole}$ versus lamina area coincided in slope for sun and shade leaves of each species, with the sun leaves having higher intercepts (*F*-ratio tests; $P < 0.05$; Table 1). As the leaves of larger lamina area have longer petioles (Table 1), for each leaf type *petiole conductance per leaf area* ($K_{petiole}$; conductance/lamina area, in kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$) was invariant with leaf size (i.e. no significant trend with increasing leaf area). $K_{petiole}$ was significantly higher for sun than shade leaves (up to 2.3 × higher, for *A. rubrum*; Table 2; Fig. 2).

The differences in $K_{lamina}$, $k_{petiole}$ and $K_{petiole}$ coincided with previously characterized sun–shade differences in other traits (e.g. Givnish 1988; Abrams & Kubiske 1990; Niinemets & Kull 1994) as well as with several sun–shade differences that are novel. Sun leaves were smaller and thicker than shade leaves (Fig. 3a & b; Table 2); the two effects compensated, leading to a similar lamina volume (Tables 1 & 2). Sun leaves generally had higher perimeter/area than shade leaves (Table 2), up to 36% greater, for *Quercus* (Fig. 3d), and higher stomatal pore area index, up to 54% higher, again in *Quercus,* due to higher stomatal densities, because guard cell length was statistically similar (Fig. 4a–c). Sun leaves had lower (i.e. more negative) $\pi_{tlp}$ than shade leaves and, in three of four tree species, higher $\pi_{ft}$ and lower $R_t$ (Fig. 5a & d).

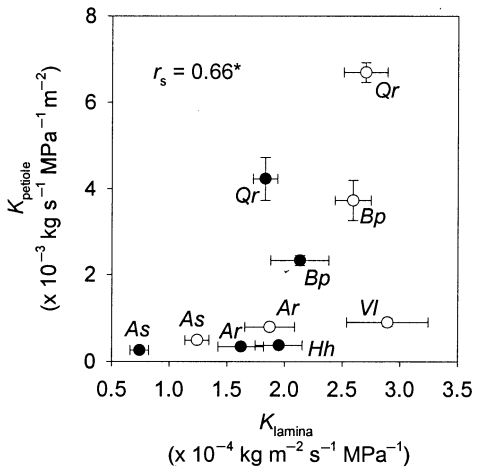


**Figure 2.** Co-ordination of petiole and lamina hydraulic conductance. Open symbols, sun leaves; filled symbols, shade leaves. Species symbols and *n* for $K_{lamina}$ (sun/shade): *Ar*, *Acer rubrum*, 16/11; *As*, *Acer saccharum*, 18/12; *Bp*, *Betula papyrifera*, 16/10; *Hh*, *Hedera helix* 16; *Qr*, *Quercus rubra* 24/10; *Vl*, *Vitis labrusca*, 18. *n*-values for $K_{petiole}$ as for $k_{petiole}$ (Table 1). Error bars = 1 SE. Significance levels as in Table 1.

Sun leaves were denser than shade leaves, with a significantly smaller volumetric fraction of air (Fig. 6a & b), a difference balanced by their slightly (and non-significantly) higher volumetric fraction of water and/or dry matter (Fig. 6a, Table 2). Sun leaves also had denser dry matter in each species but *A. rubrum* (Fig. 6c; Table 2). With their greater thickness and density, sun leaves had ~10% to 110% higher LMA than shade leaves (Fig. 6b; Table 2). In *A. saccharum* and *Quercus*, sun leaves had lower $g_{min}$ than shade leaves (Fig. 6d).

### Comparisons across all leaf types

#### *$K_{lamina}$: variation and linkage with prevailing leaf irradiance*

$K_{lamina}$ values varied approximately four-fold, ranging from $7.40 \times 10^{-5}$ kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for *A. saccharum* shade leaves to $2.89 \times 10^{-4}$ kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for *Vitis* sun leaves (Fig. 2). $K_{lamina}$ rank-correlated with leaf irradiance ($P < 0.05$), which ranged from 2% daylight for *A. saccharum* shade leaves to 64% daylight for *A. rubrum* sun leaves (Tables 1 & 3).

**Table 2.** Mean squares and significance of effects in analyses of variance for individual leaf traits

| Trait | One-way ANOVA testing for species differences, using sun leaves for trees | | Two-way ANOVA testing for species differences and sun–shade differences, for the four tree species | | | |
|---|---|---|---|---|---|---|
| | Species (d.f. = 5) | Error mean squares (d.f.) | Species (d.f. = 3) | Sun–shade (d.f. = 1) | Species × Sun–shade (d.f. = 3) | Error mean squares (d.f.) |
| $K_{lamina}$ | 0.361*** | 0.0372 (102) | 0.946*** | 0.493*** | 0.0364 | 0.0268 (109) |
| $K_{petiole}$ | 3.04*** | 0.0115 (53) | 4.25*** | 0.816*** | 0.0169 | 0.0090 (56) |
| Lamina volume | 0.506*** | 0.0127 (24) | 0.721*** | 0.00015 | 0.0163 | 0.0101 (32) |
| Lamina area | 0.354*** | 0.0109 (24) | 0.695*** | 0.0756** | 0.00315 | 0.00812 (32) |
| Lamina thickness | 0.262*** | 0.00326 (24) | 0.0324*** | 0.0788*** | 0.0120** | 0.00226 (32) |
| Perimeter$^2$/area | 0.582*** | 0.00755 (87) | 1.02*** | 0.00010 | 0.0882*** | 0.0138 (120) |
| Stomatal density | 0.375*** | 0.00458 (27) | 1.10*** | 0.0214* | 0.00459 | 0.00451 (32) |
| Guard cell length | 0.200*** | 0.0100 (27) | 0.656*** | 0.00397 | 0.00170 | 0.00152 (32) |
| Stomatal pore area index | 0.233** | 0.0510 (27) | 0.638*** | 0.0741** | 0.0164 | 0.00871 (32) |
| Relative $C_{ft}$ | 0.111** | 0.0273 (24) | 0.142*** | 0.0276 | 0.0688 | 0.0174 (32) |
| $t^1/_2$ | 0.100*** | 0.00526 (24) | 0.204*** | 0.001 | 0.0261* | 0.00676 (32) |
| Lamina density | 0.0968*** | 0.00247 (24) | 0.0633*** | 0.101*** | 0.00736 | 0.00360 (32) |
| LMA | 0.0381*** | 0.00558 (24) | 0.0781*** | 0.359*** | 0.0374** | 0.00641 (32) |
| Vol fraction air | 0.278*** | 0.000657 (24) | 0.340*** | 0.143*** | 0.0136 | 0.00653 (32) |
| Vol fraction water | 0.00734** | 0.00175 (24) | 0.0201*** | 0.00216 | 0.00470 | 0.00213 (32) |
| Vol fraction dry matter | 0.109*** | 0.0103 (24) | 0.0470** | 0.00591 | 0.0302* | 0.00696 (32) |
| Density of dry matter | 0.0250 | 0.0116 (24) | 0.0380** | 0.0612* | 0.0167 | 0.00840 (32) |
| $\epsilon_{ft}$ | 0.160*** | 0.0161 (24) | 0.246*** | 0.131* | 0.0484 | 0.0270 (32) |
| $\pi_{ft}$ | 0.0662*** | 0.00642 (24) | 0.128*** | 0.0544** | 0.0452** | 0.00702 (32) |
| $\pi_{tlp}$ | 0.0388*** | 0.00295 (24) | 0.0929*** | 0.0575*** | 0.00145 | 0.00343 (32) |
| $g_{min}$ | 0.941*** | 0.00748 (24) | 0.132*** | 0.0023 | 0.0248 | 0.0108 (32) |

LMA, leaf dry mass per area. *$P$ <0.05; ** 0.01 ≥ $P$ > 0.001; ***$P$ ≤ 0.001.

### *Petiole hydraulics and $K_{lamina}$*

$K_{petiole}$ differed approximately 26-fold, from *A. saccharum* shade leaves to *Quercus* sun leaves (Fig. 2). $K_{petiole}$ was rank-correlated with $K_{lamina}$ (Fig. 2; Table 3). The absence of a parametric correlation between $K_{petiole}$ and $K_{lamina}$ (Fig. 2; Table 3) means that the percentage of the area-normalized leaf resistance accounted for by the petiole varies significantly, from 4% in *Quercus* sun and shade leaves to 34% in *Hedera (*median for all leaf types, 19%).

### *Lamina dimensions and $K_{lamina}$*

The study leaves differed significantly in size and shape: by approximately six-fold in area, two-fold in thickness, eight-fold in volume, three-fold in perimeter$^2$/area, and two-fold in perimeter/area (Fig. 3a–d; Tables 1 & 2). $K_{lamina}$ was independent of lamina area (Fig. 3b), volume, perimeter$^2$/area (Fig. 3c) and of perimeter/area (Fig. 3d), but significantly correlated with lamina thickness (Fig. 3a; Table 3); analysed allometrically, $K_{lamina} \propto$ lamina thickness$^{1.75 \pm 0.46\ SE}$ ($r_p$ = 0.67; $P$ = 0.034). In addition, excluding *Vitis*, $K_{lamina}$ correlated with perimeter/area for the remaining five species (Fig. 3d).

### *Leaf stomatal traits and $K_{lamina}$*

Stomatal density varied approximately six-fold, from 82 per mm$^2$ in *Betula* shade leaves to 494 per mm$^2$ in *Quercus* sun leaves, and guard cell length varied approximately four-fold, from 10 $\mu$m in *A. rubrum* to 42 $\mu$m in *Betula* (Fig. 4a & b; Table 2). Stomatal density correlated negatively with guard cell length (Table 3). Analysed allometrically, stomatal density $\propto$ guard cell length$^{-1.3 \pm 0.25\ SE}$ ($r_p$ = –0.85; $P$ = 0.002). This relation is not sufficiently compensatory as to equalize stomatal pore area index (SPI, the product of stomatal density and guard cell length$^2$); the study leaves differed by approximately six-fold (Fig. 4c; Table 2). Differences in SPI were driven by differences in guard cell length ($r_p$ = 0.68; $P$ = 0.03), rather than by differences in stomatal density ($r_s$ = –0.15; $P$ = 0.68; $r_p$ = –0.28; $P$ = 0.43). $K_{lamina}$ was uncorrelated with stomatal density (Fig. 4a), rank-correlated with guard cell length (Fig. 4b; Table 3), and tightly correlated with SPI ($r_p$ = 0.93; $P$ < 0.001; Fig. 4c; Table 3). Analysed allometrically, $K_{lamina} \propto$ guard cell length$^{0.83 \pm 0.22\ SE}$ ($r_p$ = 0.64; $P$ = 0.044), and $K_{lamina} \propto$ SPI$^{0.74 \pm 0.08\ SE}$ ($r_p$ = 0.95; $P$ < 0.001).

### *Leaf water storage traits and $K_{lamina}$*

Both relative capacitance ($C_{ft}$) and leaf-area specific capacitance ($C_{ft}$*) varied approximately four-fold, time constants approximately 2.5-fold, and transfer resistance ($R_t$) approximately seven-fold (Table 2; Fig. 5a–d). $C_{ft}$* was negatively related to $R_t$ ($r_s$ = 0.84; $P$ = 0.002; $r_p$ = 0.67; $P$ = 0.033). Notably, $K_{lamina}$ was unrelated to $C_{ft}$, but positively correlated with $C_{ft}$* (Fig. 5b); $K_{lamina}$ was correlated with water mass

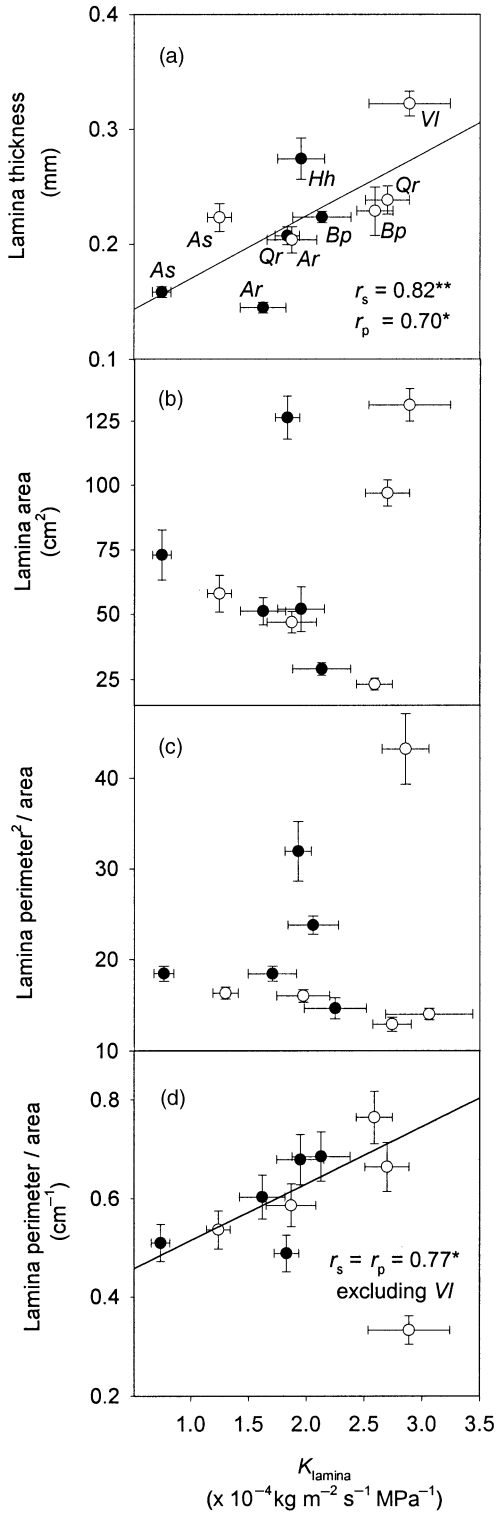


**Figure 3.** Co-ordination of $K_{lamina}$ and leaf dimensions: (a) lamina thickness; (b) area; (c) perimeter$^2$/area; and (d) perimeter/area. Symbols as in Fig. 2; in (d), vertical error bars are 95% confidence intervals.

per unit area, which contributed strongly to species differences in $C_{ft}$*, as reported for three desert species (Nobel & Jordan 1983). $K_{lamina}$ correlated negatively with $R_t$ (Fig. 5d; Table 3). As $R_t$ might represent a component of the overall lamina hydraulic resistance (the inverse of $K_{lamina}$; see Discussion), $R_t$ was considered as a percentage of $1/K_{lamina}$. $R_t$ accounted for 14% of the lamina hydraulic resistance in *Vitis* and for 60% in *Hedera*, but for most leaves was close to the median value, 28%.

### *Traits associated with leaf drought tolerance*

Leaves varied significantly in composition (Fig. 6a, Table 2), and in parameters associated with leaf drought

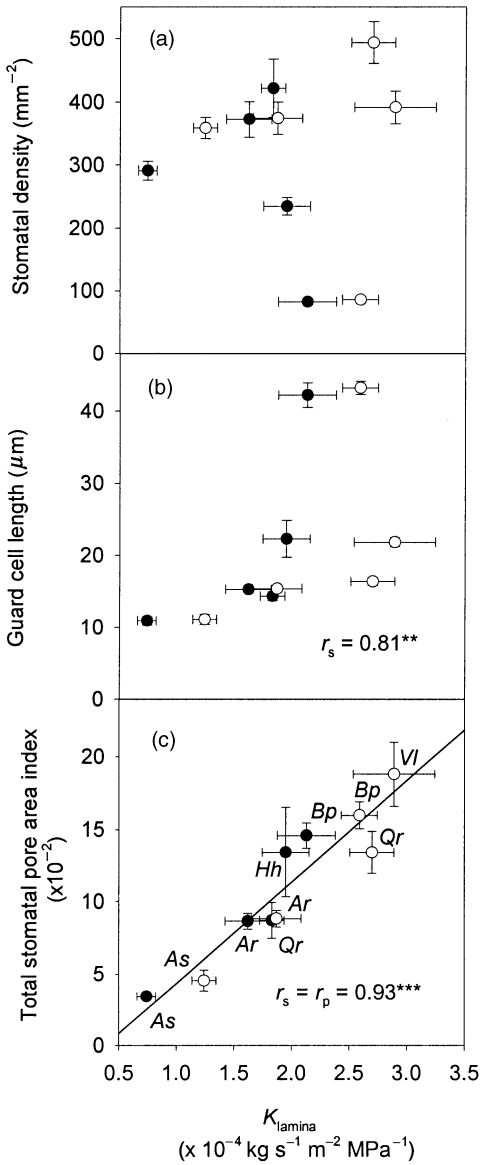


**Figure 4.** Co-ordination of $K_{lamina}$ and stomatal traits: (a) stomatal density; (b) guard cell length; and (c) total stomatal pore area index. Symbols as in Fig. 2.

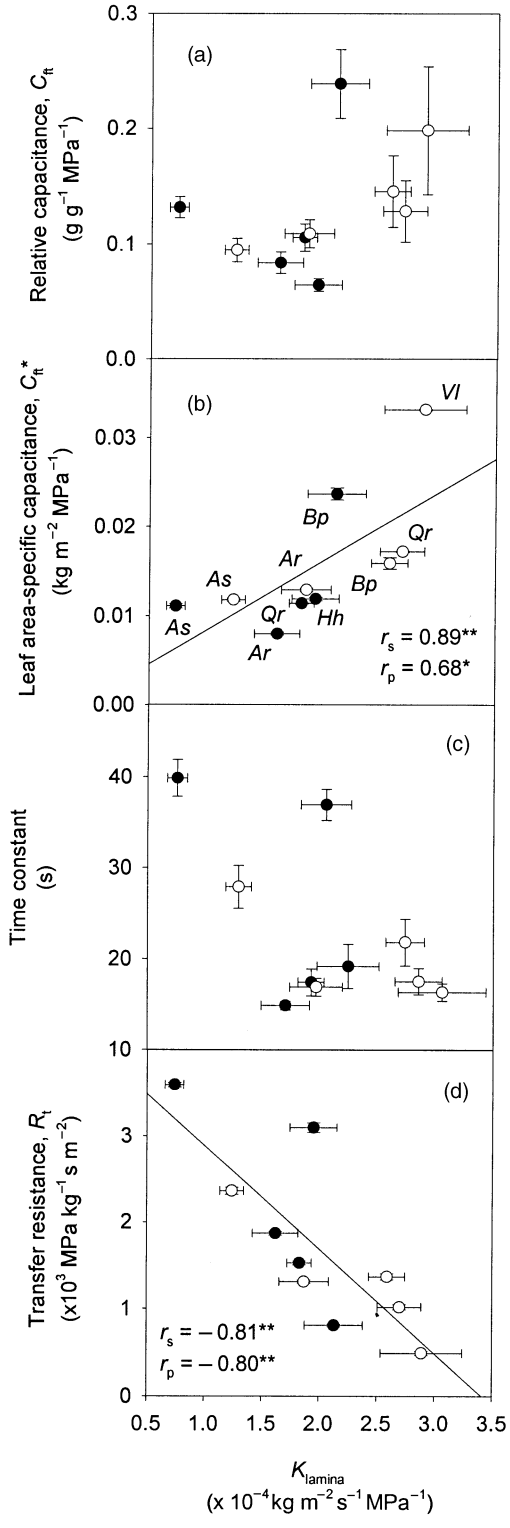


**Figure 5.** Co-ordination of $K_{lamina}$ and leaf water storage: (a) relative capacitance; (b) leaf area-specific capacitance; (c) time constant; and (d) transfer resistance. Symbols as in Fig. 2.

tolerance (Fig. 6c–e). The value of $g_{min}$ varied approximately 16-fold from *Hedera* to *Vitis* (Fig. 6d). Values for $g_{min}$ were in the middle range of values for temperate herbs and trees, with *Vitis* having an exceptionally high, and *Hedera* an exceptionally low value (cf. median value of approximately 3 mmol m$^{-2}$ s$^{-1}$ for more than 100 species; Kerstiens 1996). $K_{lamina}$ was independent of leaf volumetric composition, density, and LMA, and of traits associated with leaf drought tolerance, $\epsilon_{ft}$, $\pi_{ft}$, $\pi_{tlp}$, and $g_{min}$. However, there were notable correlations among these traits, independent of $K_{lamina}$. Leaf density was strongly positively linked with volumetric fraction of dry matter, and negatively with volumetric fraction of air; it was independent of the volumetric fraction of water. Leaf density was the chief driver of LMA (Fig. 6b); LMA was uncorrelated with leaf thickness in the studied leaves. $\epsilon_{ft}$ was strongly correlated with the density of the leaf dry matter (Fig. 6c), and negatively correlated with $g_{min}$ (Fig. 6d), $\pi_{ft}$ and $\pi_{tlp}$ (Fig. 6e). $\epsilon_{ft}$ was also negatively correlated with $C_{ft}$ ($r_s = 0.78$; $P = 0.008$; $r_p = -0.70$; $P = 0.024$), but independent of $C_{ft}$*.

### *Inter-relations of traits related to $K_{lamina}$*

Traits correlated with $K_{lamina}$ were themselves significantly intercorrelated. $K_{petiole}$ was unrelated to most traits apart from $K_{lamina}$, although it was inversely rank-correlated with $R_t$ (Table 3). Leaf dimensions, water storage, and stomatal traits were co-ordinated: leaf thickness and perimeter/area (excluding *Vitis*) were rank-correlated and/or parametrically correlated with $C_{ft}$*, $R_t$, guard cell length and SPI. Leaf thickness was positively related to SPI; analysed allometrically, SPI $\propto$ lamina thickness$^{2.37 \pm 0.63\ SE}$ ($r_p = 0.66$; $P = 0.038$). $C_{ft}$*, like $K_{lamina}$, rank-correlated with prevailing leaf irradiance.

## DISCUSSION

Trait co-ordination may occur in two ways. Traits are *structurally co-ordinated* if they share an anatomical basis. Traits are *functionally co-ordinated* if they are co-selected in a given environment; they may be structurally independent (Givnish 1987; Niklas 1994). For the six species studied, all measured while growing in moist soil, $K_{lamina}$, $K_{petiole}$, $1/R_t$, lamina thickness and SPI were apparently functionally co-ordinated, reflecting a co-selection of traits that bear on the ability of the leaf to support high maximum stomatal conductance ($g_{max}$) and high rates of transpiration and photosynthesis per unit area. $K_{lamina}$ was apparently also structurally and/or functionally co-ordinated with lamina perimeter/area and traits influencing leaf water storage capacity. In contrast, $K_{lamina}$ was independent of traits associated with the ability of leaves to minimize water loss in desiccating conditions ($g_{min}$) or to cope with low leaf water potentials ($\pi_{tlp}$, $\varepsilon_{ft}$).

### Co-ordination of leaf traits associated with liquid phase transport

In the six species, there was co-ordination among leaf traits associated with liquid-phase water flux, $K_{lamina}$, $K_{petiole}$, and $1/R_t$. The values of $K_{petiole}$ and $K_{lamina}$ and were higher in sun than shade leaves, as previously reported for grapevine

**Table 3.** Correlation coefficients of leaf traits linked with $K_{lamina}$

| | $K_{lamina}$ | $K_{petiole}$ | dsf | Th | P/A | gcl | SPI | $C_{ft}$* | $R_t$ |
|---|---|---|---|---|---|---|---|---|---|
| $K_{lamina}$ | | ***0.66*** | ***0.66*** | ***0.82*** | ***0.77*** | ***0.81*** | ***0.93*** | ***0.89*** | ***–0.81*** |
| $K_{petiole}$ | 0.55 | | *0.44* | *0.46* | *0.22* | *0.43* | *0.52* | *0.59* | ***–0.67*** |
| dsf | 0.62 | 0.45 | | *0.55* | *0.37* | *0.42* | *0.56* | ***0.71*** | *–0.62* |
| Th | **0.70** | 0.12 | 0.52 | | *0.62* | ***0.66*** | ***0.79*** | ***0.78*** | *–0.46* |
| P/A | **0.77** | 0.28 | 0.35 | 0.51 | | ***0.93*** | ***0.87*** | ***0.68*** | *–0.45* |
| gcl | 0.53 | 0.23 | 0.08 | 0.28 | **0.82** | | ***0.92*** | ***0.72*** | *–0.55* |
| SPI | **0.93** | 0.33 | 0.42 | **0.74** | **0.90** | **0.68** | | ***0.86*** | ***–0.72*** |
| $C_{ft}$* | **0.68** | 0.12 | 0.45 | **0.75** | 0.56 | 0.44 | **0.75** | | ***–0.84*** |
| $R_t$ | **–0.80** | –0.48 | –0.57 | –0.40 | –0.39 | –0.43 | **–0.67** | **–0.67** | |

Italicized values are $r_s$; values in normal font are $r_p$. Bold-faced values significant at $P < 0.05$. dsf: diffuse site factor; Th: lamina thickness; P/A: lamina perimeter/area for leaves of mean area; gcl: guard cell length; SPI: stomatal pore area index; $C_{ft}$*: leaf area-specific capacitance; $R_t$: transfer resistance. For P/A, outlier *Vitis* was excluded.

(Schultz & Matthews 1993), and $R_t$ was lower. Across all leaves, $K_{petiole}$ rank-correlated with $K_{lamina}$, reflecting the serial arrangement of the petiole and leaf lamina. Additionally, $K_{lamina}$ correlated negatively with $R_t$, a possible case of structural co-ordination, as the resistance between xylem and mesophyll cells may be a component of the same transpiration pathways through the leaf that are described by $K_{lamina}$. $R_t$ was in the median case 28% of lamina hydraulic resistance, and indeed, recent experiments have shown extra-vascular resistance to be approximately –30% of leaf hydraulic resistance (unpubl. data for *A. saccharum* and *Q. rubra*), although other studies estimated a higher percent-

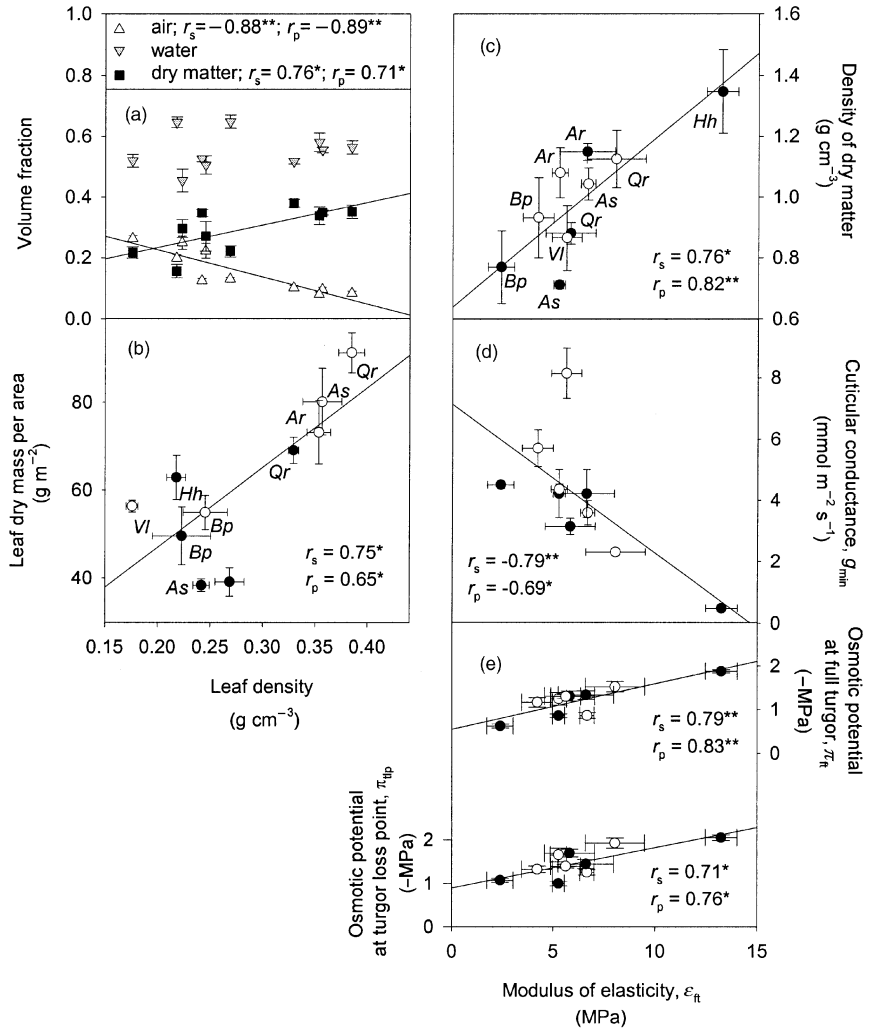


**Figure 6.** Co-ordination of traits relevant to leaf drought tolerance, independent of $K_{lamina}$: (a) leaf volumetric fractions of air, water and dry matter and (b) leaf dry mass per area (LMA) versus leaf density; (c) density of dry matter; (d) cuticular conductance; and (e) osmotic potential at full and zero turgor versus modulus of elasticity. Symbols as in Fig. 2.

age (e.g. Martre, Cochard & Durand 2001). The high value for $R_t$ relative to lamina hydraulic resistance for *Hedera* (60%) could correspond to its distinctive hydraulic design; the *Hedera* leaf has no bundle sheath extensions, and has an exceptionally low minor vein density, possibly indicating a larger extra-vascular component in the transpirational path (Wylie 1943, 1951; Sheriff & Meidner 1974).

## Co-ordination of liquid phase transport traits and gas exchange traits

For the six species, traits related to liquid phase transport through the leaf were co-ordinated with traits related to gas exchange per leaf area. In sun versus shade leaves and across leaf types, $K_{lamina}$ was co-ordinated with both external conditions (irradiance), and internal traits (lamina thickness and SPI) that are associated with high maximum rates of gas exchange per leaf area. Thicker leaves are likely to have more internal mesophyll surface area per lamina area (Turrell 1936, 1944; Jackson 1967; Nobel, Zaragoza & Smith 1975; Chabot & Chabot 1977; James, Smith & Vogelmann 1999; but see Slaton, Hunt & Smith 2001) as well as higher nitrogen content and more photosynthetic machinery per area (Grubb 1984; Field & Mooney 1986; Koike 1988; Niinemets 1999; Shipley & Lechowicz 2000), whereas SPI is a determinant of maximum stomatal conductance [$g_{max} \propto$ SPI/(stomatal depth + stomatal pore radius); Nobel 1999]. SPI scales with lamina thickness as well as with $K_{lamina}$ (SPI $\propto$ lamina thickness$^{2.37 \pm 0.63\ SE}$ in this study; SPI $\propto$ lamina thickness$^{2.0 \pm 0.17}$ in our analysis of published data for 84 species; $r_p = 0.43$; $P < 0.001$; data from Abrams & Kubiske 1990; Bongers & Popma 1990). As both the thickness of the lower epidermis (an approximate index of stomatal depth), and guard cell length (linearly related to stomatal pore radius) tend to increase linearly with lamina thickness (thickness of lower epidermis $\propto$ lamina thickness$^{0.92 \pm 0.06\ SE}$ for 188 species; $r_p = 0.44$; $P < 0.001$; guard cell length $\propto$ lamina thickness$^{1.09 \pm 0.096\ SE}$ for 84 species; $r_p = 0.34$; $P < 0.001$; our analysis of the data of Wylie 1951; Philpott 1953; Powers 1967; Abrams & Kubiske 1990; Bongers & Popma 1990; Roderick *et al*. 1999b), higher SPI is likely to drive a higher $g_{max}$. These correlations between $K_{lamina}$ and gas exchange-related traits extend the framework of previously reported correlations between branch (and whole-plant) hydraulic conductivities, stomatal dimensions, $g_{max}$, and mid-day rates of transpiration and photosynthesis per leaf area (Nardini & Salleo 2000; Aasamaa & Sober 2001; Aasamaa, Sober & Rahi 2001; Bhaskar *et al*. 2002; Meinzer 2002). Such relationships indicate an optimization of hydraulic and gas exchange capacities (see Rosen 1967; Cody 1974).

One surprising finding of this study was that stomatal density *per se* is not a reliable index of $K_{lamina}$ or SPI, across the study species. Whereas the higher SPI observed in sun than shade leaves was due largely to variation in stomatal density rather than guard cell length, across leaf types, higher SPI was driven primarily by longer guard cells and not by stomatal density (also true in our analysis of the data of Bongers & Popma 1988; Abrams & Kubiske 1990). Thus, across leaf types, stomatal density *per se* was unrelated to other water flux traits, including lamina thickness (see Beerling & Kelly 1996; also supported by our analysis of the data of Abrams & Kubiske 1990; Bongers & Popma 1990). We note the general inverse relation of stomatal density and guard cell length (see also Salisbury 1927; Grubb *et al*. 1975; Wood 1934; Sack, Marañón & Grubb 2003). One suggested explanation is that the ratio of guard cells to epidermal cells is roughly constant across leaves, and that epidermal cells increase in size at the same rate as guard cells (Salisbury 1927); thus, larger stomata would be spaced further apart, and SPI would be constant. This geometric scaling holds as a central trend [for 84 species, stomatal density $\propto$ guard cell length$^{-2.1 \pm 0.13\ SE}$; $r_p = 0.53$; $P < 0.001$; data of Abrams & Kubiske (1990) and Bongers & Popma (1990)], but with extensive scatter. A weaker scaling was found in this study, leading to substantial variation in SPI (see also Poole *et al*. 1996).

Water storage capacitance ($C_{ft}$*) was co-ordinated with $K_{lamina}$, lamina thickness, and SPI. Apparently across the study species there is a structural co-ordination of lamina thickness and $C_{ft}$* via the thickness of cells that contribute to water mass per unit area (Shipley 1995; Lamont & Lamont 2000; Vendramini *et al*. 2002). Additionally, $K_{lamina}$, $C_{ft}$* and $R_t$ may be *functionally* co-ordinated. A high $C_{ft}$* and low $R_t$ may contribute to the ability of the leaf to endure fluctuating root supply and transpirational demand, minimizing transient fluctuations in mesophyll water potential. A linkage between high $K_{lamina}$ and high $C_{ft}$* might account for the finding that at a given transpiration rate excised leaves of high $K_{lamina}$ close their stomata relatively slowly (Aasamaa & Sober 2001). This function of water storage (analogous to that of a capacitor in an electronic circuit) is alternative to that in semi-desert succulents; that is, for drought survival. Notably, $C_{ft}$* was uncorrelated across the study species with drought tolerance traits such as $\epsilon_{ft}$ and $g_{min}$.

## Co-ordination of $K_{lamina}$ and lamina perimeter/area

The co-ordination of $K_{lamina}$ and lamina perimeter/area in five of the six species might be *both* structural and functional. Both $K_{lamina}$ and leaf shape may be structurally associated with the venation properties (Thoday 1931; Yang & Tyree 1994; Jones 1995; Dengler & Kang 2001; Nardini *et al*. 2001; Sack *et al*. 2002; Zwieniecki *et al*. 2002). The venation acts as an 'irrigation system', supplying water relatively equitably across the lamina; the lower orders of major veins (e.g. midrib and secondary veins) are 'supply veins' of low axial resistance, whereas the higher-order veins leak relatively more to the mesophyll, and have a higher axial resistance (Canny 1990; Zwieniecki *et al*. 2002; Sack, Cowan & Holbrook 2003). Thus, in more entire leaves, the larger areas of mesophyll that are far from the supply veins may

be supplied with relatively low conductance, thus bringing down the overall $K_{lamina}$ Further, these less well-supplied mesophyll regions in entire leaves are prone to desiccation under high evaporative demand or limited water supply (Thoday 1931; Zwieniecki *et al*. 2002). Leaves with higher perimeter/area, by contrast, would have all mesophyll regions closer to supply veins. Furthermore, leaves with higher perimeter/area tend to have a thinner boundary layer over the bulk of the lamina, which enhances convective cooling and gas exchange at low windspeeds (Vogel 1968, 1970; Givnish 1987; Canny 1990). *Vitis* broke the trend between $K_{lamina}$ and perimeter/area; the co-ordination is not in all cases inherent. It is noteworthy that the *Vitis* leaf is compound as a primordium, and expands into a simple leaf, possibly indicating an ancestrally compound leaf with a high perimeter/area (Bharathan *et al*. 2002).

Perimeter/area is related to perimeter$^2$/area $\times$ 1/area, where perimeter$^2$/area is an index of intrinsic (size-independent) shape. Thus, a high perimeter/area, and its benefits described above, arise not only from a more complex shape *per se*, but also from a smaller leaf. One previous study (Sisó, Camarero & Gil-Pelegrín 2001) found $K_{lamina}$ to be linked with 'fractal dimension', a correlate of perimeter$^2$/area (McLellan & Endler 1998), in eight *Quercus* species. Our result indicates no relationship independent of leaf size.

## $K_{lamina}$ and drought tolerance: associated or independent?

A high $K_{lamina}$ may confer drought tolerance by allowing a higher leaf water potential ($\psi_{leaf}$) at a given transpiration rate and soil water supply (Tsuda & Tyree 2000). However, for the study leaves, $K_{lamina}$ and traits associated with water flux were independent of traits associated with turgor maintenance at low leaf water potential. $K_{lamina}$ in this study was measured for plants in moist soil, and it is possible that during drought $K_{lamina}$ may decline due to xylem cavitation (Kikuta *et al*. 1997; Nardini *et al*. 2001; Salleo *et al*. 2001), and may be more closely related to drought tolerance traits. For the six species studied $K_{lamina}$ was uncorrelated with $\pi_{ft}$, $\pi_{tlp}$, $\varepsilon_{ft}$, lamina density, leaf dry mass per area (LMA), and $g_{min}$. These traits are partially inter-related, suggesting co-selection by desiccating conditions. $\varepsilon_{ft}$ was strongly linked with the density of the leaf dry matter, which may reflect denser cell walls. Leaf density, which derives from a high volumetric fraction of dry matter, and a low volumetric fraction of air (also see Niinemets 1999), drove LMA, which, representing a low surface area: mass ratio, augments the effects of low $g_{min}$ (Hadley & Smith 1990; Sack, Marañón & Grubb 2003). A low LMA may also contribute to a longer leaf lifespan (see below). Finally, $\varepsilon_{ft}$ was negatively correlated with $\pi_{ft}$ and $\pi_{tlp}$ (see also Niinemets 2001), and with $g_{min}$. Notably, sun leaves tended to show greater modification for high water flux than shade leaves, and simultaneously, features contributing to greater leaf drought tolerance. Across leaf types, an independence of leaf traits associated with water flux and those associated with drought tolerance would explain why, in well-watered conditions, drought-tolerant species can have $g_{max}$ (and, indeed, maximum relative growth rates) similar to or higher than those of species confined to moist areas (Maximov 1931; Fernandez & Reynolds 2000; Sack 2000; Wright *et al*. 2001).

## The role of $K_{lamina}$ in carbon economy

Leaf hydrology has potential implications for plant carbon economy. As shown above, $K_{lamina}$ may be co-ordinated with photosynthetic rate per unit leaf area. However, $K_{lamina}$ may be orthogonal to traits that are also important in carbon economy, such as LMA, which can strongly influence photosynthetic rate per unit leaf mass (Evans 1972; Lichtenthaler 1985; Field & Mooney 1986; Koike 1988; Poorter & Van der Werf 1998; Reich *et al*. 1999; Wright *et al*. 2001; Shipley 2002) and leaf lifespan (Nardini 2001; but see Sobrado 1998). We note that leaf lifespan and LMA are often correlated (Reich *et al*. 1999; Wright & Westoby 2002); together they may thus represent an axis of variation orthogonal to $K_{lamina}$ and water flux traits. *Hedera*, which has a long-lived leaf, had a $K_{lamina}$ in the same range as the five deciduous species. The independence of LMA and $K_{lamina}$ found for the species in this study was previously reported in two studies of other species (Tyree *et al*. 1999; Salleo & Nardini 2000). However, we note that across large species sets, lamina thickness and LMA are often positively correlated (Shipley 1995; Niinemets 1999; Vendramini *et al*. 2002). Thus, if the co-ordination of $K_{lamina}$ and lamina thickness is general, $K_{lamina}$ might be loosely correlated with LMA for large sets of species. However, because lamina density is also a strong determinant of LMA (Witkowski & Lamont 1991; Niinemets 1999), and it is orthogonal to $K_{lamina}$, an LMA–$K_{lamina}$ correlation would likely be weak at best. For published data sets in which SPI correlated with lamina thickness, it was unrelated to LMA (our analysis of the data of Abrams & Kubiske 1990; Bongers & Popma 1990). Further, the relationship of $K_{lamina}$ and lamina thickness might shift with increasing proportions of leaf sclerenchyma (cf. Wright & Westoby 2002). Thick, long-lived scleromorphic leaves are not expected to have proportionally higher $K_{lamina}$ than the deciduous species in this study.

Our understanding of leaf traits and their evolution will be enhanced by further studies of leaf hydrology, both in terms of mechanism, and to determine generality across larger sets of species, in different growth conditions. There is potential to integrate $K_{lamina}$ in the framework of traits linking physiology to performance for different species (Grubb 2002). For example, differences in the $K_{lamina}$ of *Quercus rubra* relative to coexisting *Acer* species are associated with its higher $g_{max}$ and photosynthetic rate per unit leaf area (Jurik 1986; L. Sack, unpublished data). An exciting field for study is the possible co-ordination of leaf hydrology with characteristics of other systems and organs, including resistance to xylem embolism, efficiency of nutrient transport, and root uptake capacities; all of

which contribute to overall plant performance in given microclimates.

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## REFERENCES

Aasamaa K. & Sober A. (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* **44,** 65–73.

Aasamaa K., Sober A. & Rahi M. (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28,** 765–774.

Abrams M.D. (1988) Sources of variation in osmotic potentials with special reference to North American tree species. *Forest Science* **34,** 1030–1046.

Abrams M.D. & Kubiske M.E. (1990) Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade-tolerance rank. *Forest Ecology and Management* **31,** 245–253.

Anderson M.C. (1964) Studies of the woodland light climate. 1. The photographic computation of light conditions. *Journal of Ecology* **52,** 27–41.

Becker P., Tyree M.T. & Tsuda M. (1999) Hydraulic conductances of angiosperms versus conifers: similar transport sufficiency at the whole-plant level. *Tree Physiology* **19,** 445–452.

Beerling D.J. & Kelly C.K. (1996) Evolutionary comparative analyses of the relationship between leaf structure and function. *New Phytologist* **134,** 35–51.

Beers Y. (1957) *Introduction to the Theory of Error.* Addison-Wesley, Reading, MA, USA.

Bharathan G., Goliber T.E., Moore C., Kessler S., Pham T. & Sinha N.R. (2002) Homologies in leaf form inferred from KNOXI gene expression during development. *Science* **296,** 1858–1860.

Bhaskar R., Tirado-Fernandez R., Cavender-Bares J., Zlatnick D., Preston K. & Ackerly D. (2002) Influences of whole plant water transport and drought on gas exchange within a chaparral community. *Ecological Society of America, Annual Meeting, Tucson, AZ.*

Bongers F. & Popma J. (1988) Is exposure-related variation in leaf characteristics of tropical rain forest species adaptive? In *Plant Form and Vegetation Structure* (eds M.J.A. Werger, P.J.M. van der Aart, H.J. During & J.T.A. Verhoeven), pp. 191–200. SPB Academic, The Hague, The Netherlands.

Bongers F. & Popma J. (1990) Leaf characteristics of the tropical rainforest flora of Los Tuxtlas, Mexico. *Botanical Gazette* **151,** 354–365.

Canny M.J. (1990) What becomes of the transpiration stream? *New Phytologist* **114,** 341–368.

Chabot B.F. & Chabot J.F. (1977) Effects of light and temperature on leaf anatomy and photosynthesis in *Fragaria vesca*. *Oecologia* **26,** 363–377.

Cody M.L. (1974) Optimization in ecology. *Science* **183,** 1156–1164.

Cowan I.R. (1972) Electrical analog of evaporation from, and flow of water in plants. *Planta* **106,** 221–226.

Dengler N. & Kang J. (2001) Vascular patterning and leaf shape. *Current Opinion in Plant Biology* **4,** 50–56.

Evans G.C. (1972) *The Quantitative Analysis of Plant Growth.* Blackwell Publishing, Oxford, UK.

Fernandez R.J. & Reynolds J.F. (2000) Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? *Oecologia* **123,** 90–98.

Field C.B. & Mooney H.A. (1986) The photosynthesis – nitrogen relationship in wild plants. In *On the Economy of Plant Form and Function* (ed. T.J. Givnish), pp. 25–55. Cambridge University Press, Cambridge, UK.

Givnish T.J. (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* **106,** 131–160.

Givnish T.J. (1988) Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology* **15,** 63–92.

Gleason H.A. & Cronquist A. (1991) *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2nd edition. New York Botanical Garden, Bronx, New York, USA.

Grubb P.J. (1984) Some growth points in investigative plant ecology. In *Trends in Ecological Research for the 1980s* (eds J.H. Cooley & F.B. Golley), pp. 51–74. Plenum, New York, USA.

Grubb P.J. (2002) Leaf form and function – towards a radical new approach. *New Phytologist* **155,** 317–320.

Grubb P.J., Grubb E.A.A. & Miyata I. (1975) Leaf structure and function in evergreen trees and shrubs of Japanese warm temperate rain forest. 1. Structure of lamina. *Botanical Magazine-Tokyo* **88,** 197–211.

Hadley J.L. & Smith W.K. (1990) Influence of leaf surface wax and leaf-area to water-content ratio on cuticular transpiration in western Conifers, USA. *Canadian Journal of Forest Research* **20,** 1306–1311.

Jackson L.W.R. (1967) Effect of shade on leaf structure of deciduous tree species. *Ecology* **48,** 498–499.

James S.A., Smith W.K. & Vogelmann T.C. (1999) Ontogenetic differences in mesophyll structure and chlorophyll distribution in *Eucalyptus globulus* ssp. *globulus* (Myrtaceae). *American Journal of Botany* **86,** 198–207.

Jones C.S. (1995) Does shade prolong juvenile development: a morphological analysis of leaf shape changes in *Cucurbita argyrosperma* ssp. *sororia* (Cucurbitaceae). *American Journal of Botany* **82,** 346–359.

Jurik T.W. (1986) Temporal and spatial patterns of specific leaf weight in successional northern hardwood tree species. *American Journal of Botany* **73,** 1083–1092.

Kerstiens G. (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* **47,** 1813–1832.

Kikuta S.B., LoGullo M.A., Nardini A., Richter H. & Salleo S. (1997) Ultrasound acoustic emissions from dehydrating leaves of deciduous and evergreen trees. *Plant, Cell and Environment* **20,** 1381–1390.

Koide R.T., Robichaux R.H., Morse S.R. & Smith C.M. (2000) Plant water status, hydraulic resistance and capacitance. In *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J.R. Ehleringer, H.A. Mooney & P.W. Rundel), pp. 161–183. Kluwer, Dordrecht, The Netherlands.

Koike T. (1988) Leaf structure and photosynthetic performance as related to the forest succession of deciduous broad-leaved trees. *Plant Species Biology* **3,** 77–87.

Kolb K.J., Sperry J.S. & Lamont B.B. (1996) A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *Journal of Experimental Botany* **47,** 1805–1810.

Kubiske M.E. & Abrams M.D. (1991) Rehydration effects on pressure-volume relationships in four temperate woody species: variability with site, time of season and drought conditions. *Oecologia* **85,** 537–542.

Kuppers M. (1984) Carbon relations and competition between woody species in a central European hedgerow. 2. Stomatal responses, water-use, and hydraulic conductivity in the root-leaf pathway. *Oecologia* **64,** 344–354.

Lamont B.B. & Lamont H.C. (2000) Utilizable water in leaves of 8 arid species as derived from pressure-volume curves and chlorophyll fluorescence. *Physiologia Plantarum* **110,** 64–71.

Lichtenthaler H.K. (1985) Differences in morphology and chemical composition of leaves grown at different light intensities and qualities. In *Control of Leaf Growth* eds N.R. Baker, W.J. Davies & C.K. Ong), pp. 201–221. Cambridge University Press, Cambridge, UK.

Martre P., Cochard H. & Durand J.L. (2001) Hydraulic architecture and water flow in growing grass tillers (*Festuca arundinacea* Schreb.). *Plant, Cell and Environment* **24,** 65–76.

Maximov N.A. (1931) The physiological significance of the xeromorphic structure of plants. *Journal of Ecology* **19,** 279–282.

McLellan T. & Endler J.A. (1998) The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology* **47,** 264–281.

Meinzer F.C. (2002) Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell and Environment* **25,** 265–274.

Meinzer F.C. & Grantz D.A. (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant, Cell and Environment* **13,** 383–388.

Nardini A. (2001) Are sclerophylls and malacophylls hydraulically different? *Biologia Plantarum* **44,** 239–245.

Nardini A. & Salleo S. (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees – Structure and Function* **15,** 14–24.

Nardini A. & Tyree M.T. (1999) Root and shoot hydraulic conductance of seven *Quercus* species. *Annals of Forest Science* **56,** 371–377.

Nardini A., Salleo S., Tyree M.T. & Vertovec M. (2000) Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annals of Forest Science* **57,** 305–312.

Nardini A., Tyree M.T. & Salleo S. (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. *Plant Physiology* **125,** 1700–1709.

Niinemets U. (1999) Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* **144,** 35–47.

Niinemets U. (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* **82,** 453–469.

Niinemets U. & Kull K. (1994) Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *Forest Ecology and Management* **70,** 1–10.

Niklas K.J. (1994) *Plant Allometry.* University of Chicago Press, Chicago, IL, USA.

Nobel P.S. (1999) *Physicochemical and Environmental Plant Physiology*, 2nd edn. Academic Press, San Diego, CA, USA.

Nobel P.S. & Jordan P.W. (1983) Transpiration stream of desert species: resistances and capacitances for a $C_3$, a $C_4$, and a CAM plant. *Journal of Experimental Botany* **34,** 1379–1391.

Nobel P.S., Zaragoza L.J. & Smith W.K. (1975) Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. *Plant Physiology* **55,** 1067–1070.

Pearcy R.W., Schulze E.-D. & Zimmermann R. (2000) Measurement of transpiration and leaf conductance. In *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J.R. Ehleringer, H.A. Mooney & P.W. Rundel), pp. 137–160. Kluwer Academic, Dordrecht, The Netherlands.

Philpott J. (1953) A blade tissue study of leaves of 47 species of *Ficus*. *Botanical Gazette* **115,** 15–35.

Poole I., Weyers J.D.B., Lawson T. & Raven J.A. (1996) Variations in stomatal density and index: implications for palaeoclimatic reconstructions. *Plant, Cell and Environment* **19,** 705–712.

Poorter H. & Van der Werf A. (1998) Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In *Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences* (eds H. Lambers, H. Poorter & M.M.I. Van Vuuren), pp. 309–336. Backhuys, Leiden, The Netherlands.

Powers H.O. (1967) A blade tissue study of 47 species and varieties of Aceraceae. *American Midland Naturalist* **78,** 301–323.

Reich P.B., Ellsworth D.S. & Walters M.B. (1998) Leaf structure (specific leaf area) modulates photosynthesis – nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology* **12,** 948–958.

Reich P.B., Ellsworth D.S., Walters M.B., Vose J.M., Gresham C., Volin J.C. & Bowman W.D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology* **80,** 1955–1969.

Reich P.B., Walters M.B. & Ellsworth D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the USA* **94,** 13730–13734.

Roderick M.L., Berry S.L., Noble I.R. & Farquhar G.D. (1999a) A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology* **13,** 683–695.

Roderick M.L., Berry S.L., Saunders A.R. & Noble I.R. (1999b) On the relationship between the composition, morphology and function of leaves. *Functional Ecology* **13,** 696–710.

Rosen R. (1967) *Optimality Principles in Biology.* Plenum, London, UK.

Sack L. (2000) Plant Responses to Deep Shade plus Drought. PhD Dissertation. University of Cambridge, Cambridge, UK.

Sack L., Cowan P.D. & Holbrook N.M. (2003) The major veins of mesomorphic leaves revisited: testing for conductive overload in *Acer saccharum* (Aceraceae) and *Quercus rubra* (Fagaceae). *American Journal of Botany* **90,** 32–39.

Sack L., Marañón T. & Grubb P.J. (2003) The functional morphology of plant juveniles tolerant of deep shade plus drought in three mediterranean-climate mixed forests of southern Spain. *Plant Ecology* **168,** in press.

Sack L., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* **53,** 2177–2184.

Salisbury E.J. (1927) On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London Series B-Biology Sciences* **216,** 1–65.

Salleo S. & Nardini A. (2000) Sclerophylly: evolutionary advantage or mere epiphenomenon? *Plant Biosystems* **134,** 247–259.

Salleo S., Lo Gullo M.A., Raimondo F. & Nardini A. (2001) Vulnerability to cavitation of leaf minor veins: any impact on leaf gas exchange? *Plant, Cell and Environment* **24,** 851–859.

Schreiber L., Skrabs M., Hartmann K.D., Diamantopoulos P., Simanova E. & Santrucek J. (2001) Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. *Planta* **214,** 274–282.

Schultz H.R. & Matthews M.A. (1993) Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): evidence that low light uncouples water transport capacity from leaf area. *Planta* **190,** 393–406.

Sheriff D.W. & Meidner H. (1974) Water pathways in leaves of *Hedera helix* L. and *Tradescantia virginiana* L. *Journal of Experimental Botany* **25,** 1147–1156.

Shipley B. (1995) Structured interspecific determinants of specific leaf area in 34 species of herbaceous angiosperms. *Functional Ecology* **9,** 312–319.

Shipley B. (2002) Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. *Functional Ecology* **16,** 682–689.

Shipley B. & Lechowicz M.J. (2000) The functional co-ordination of leaf morphology, nitrogen concentration, and gas exchange in 40 wetland species. *Ecoscience* **7,** 183–194.

Sisó S., Camarero J.J. & Gil-Pelegrín E. (2001) Relationship between hydraulic resistance and leaf morphology in broadleaf *Quercus* species: a new interpretation of leaf lobation. *Trees – Structure and Function* **15,** 341–345.

Slaton M.R., Hunt E.R. & Smith W.K. (2001) Estimating near-infrared leaf reflectance from leaf structural characteristics. *American Journal of Botany* **88,** 278–284.

Sobrado M.A. (1998) Hydraulic conductance and water potential differences inside leaves of tropical evergreen and deciduous species. *Biologia Plantarum* **40,** 633–637.

Sokal R.R. & Rohlf F.J. (1995) *Biometry*, 3rd edn. W.H. Freeman, New York, USA.

Talbert C.M. & Holch A.E. (1957) A study of the lobing of sun and shade leaves. *Ecology* **38,** 655–658.

Thoday D. (1931) The significance of reduction in the size of leaves. *Journal of Ecology* **19,** 297–303.

Tsuda M. & Tyree M.T. (2000) Plant hydraulic conductance measured by the high pressure flow meter in crop plants. *Journal of Experimental Botany* **51,** 823–828.

Turrell F.M. (1936) The area of the internal exposed surface of dicotyledon leaves. *American Journal of Botany* **23,** 255–264.

Turrell F.M. (1944) Correlation between internal surface and transpiration rate in mesomorphic and xeromorphic leaves grown under artificial light. *Botanical Gazette* **105,** 413–425.

Tyree M.T. & Zimmermann M.H. (2002) *Xylem Structure and the Ascent of Sap.* Springer, Berlin, Germany.

Tyree M.T., Sobrado M.A., Stratton L.J. & Becker P. (1999) Diversity of hydraulic conductance in leaves of temperate and tropical species: possible causes and consequences. *Journal of Tropical Forest Science* **11,** 47–60.

Vendramini F., Diaz S., Gurvich D.E., Wilson P.J., Thompson K. & Hodgson J.G. (2002) Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytologist* **154,** 147–157.

Vogel S. (1968) Sun leaves and shade leaves differences in convective heat dissipation. *Ecology* **49,** 1203–1204.

Vogel S. (1970) Convective cooling at low airspeeds and shapes of broad leaves. *Journal of Experimental Botany* **21,** 91–&.

Witkowski E.T.F. & Lamont B.B. (1991) Leaf specific mass confounds leaf density and thickness. *Oecologia* **88,** 486–493.

Wood J.G. (1934) The physiology of xerophytism in Australian plants: the stomatal frequencies, transpiration and osmotic pressures of sclerophyll and tomentose-succulent leaved plants. *Journal of Ecology* **22,** 69–87.

Wright I.J. & Westoby M. (2002) Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. *New Phytologist* **155,** 403–416.

Wright I.J., Reich P.B. & Westoby M. (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology* **15,** 423–434.

Wylie R.B. (1943) The leaf organization of *Hedera helix*. *Proceedings of the Iowa Academy of Sciences* **50,** 199–207.

Wylie R.B. (1951) Principles of foliar organization shown by sun-shade leaves from ten species of deciduous dicotyledonous trees. *American Journal of Botany* **38,** 355–361.

Yang S.D. & Tyree M.T. (1994) Hydraulic architecture of *Acer saccharum* and *A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. *Journal of Experimental Botany* **45,** 179–186.

Zar J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Upper Saddle River, NJ, USA.

Zwieniecki M.A., Melcher P.J., Boyce C.K., Sack L. & Holbrook N.M. (2002) The hydraulic architecture of the leaf venation in *Laurus nobilis* L. *Plant, Cell and Environment* **25,** 1445–1450.

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