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The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response?

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Abstract

This paper examines the dependence of whole leaf hydraulic conductance to liquid water (K_L) on irradiance when measured with a high pressure flowmeter (HPFM). During HPFM measurements, water is perfused into leaves faster than it evaporates hence water infiltrates leaf air spaces and must pass through stomates in the liquid state. Since stomates open and close under high versus low irradiance, respectively, the possibility exists that K_L might change with irradiance if stomates close tightly enough to restrict water movement. However, the dependence of K_L on irradiance could be due to a direct effect of irradiance on the hydraulic properties of other tissues in the leaf. In the present study, K_L increased with irradiance for 6 of the 11 species tested. Whole leaf conductance to water vapour, g_L, was used as a proxy for stomatal aperture and the time-course of changes in K_L and g_L was studied during the transition from low to high irradiance and from high to low irradiance. Experiments showed that in some species K_L changes were not paralleled by g_L changes. Measurements were also done after perfusion of leaves with ABA which inhibited the g_L response to irradiance. These leaves showed the same K_L response to irradiance as control leaves. These experimental results and theoretical calculations suggest that the irradiance dependence of K_L is more consistent with an effect on extravascular (and/or vascular) tissues rather than stomatal aperture. Irradiance-mediated stimulation of aquaporins or hydrogel effects in leaf tracheids may be involved.

Introduction and theory

The high pressure flow meter (HPFM) was first used to measure the hydraulic conductance of whole shoots and its components, i.e. stems, petioles, and leaf blades in *Quercus*, *Acer*, and *Populus* species (Tyree *et al.*, 1993, 1994; Yang and Tyree, 1994). The hydraulic conductance of leaves, K_L, is defined as the water flow rate per unit leaf area divided by the pressure drop driving the flow. The HPFM measures the pressure and the flow rate for the computation of K_L. During HPFM measurements water is forced into leaves faster than it evaporates from leaf air spaces, hence leaf air spaces infiltrate with water, which can be seen emerging from stomatal pores (MT Tyree, personal observation; Wei *et al.*, 1999). Hence the HPFM measures the combined hydraulic conductance of the leaf vascular system, extravascular tissues and stomates in an approximately serial pathway.

Leaf hydraulic conductance (K_L, kg s^{-1} m^{-2} MPa^{-1}) was found to vary from 5×10^{-3} to 2×10^{-5} depending on species (Tyree *et al.*, 1999; Nardini, 2001; references above). Tyree *et al.* (1993) commented on the very low K_L values of *Acer* and *Quercus* and argued that K_L might actually be slightly overestimated by the HPFM (Yang and Tyree, 1994; Tyree *et al.*, 1999) because water flow under positive pressure might follow a higher-conductance, shorter pathway than during evaporation. Yang and Tyree (1994) acknowledged that K_L might be underestimated by

the HPFM if the conductance of liquid water flow through stomates is sufficiently low, but they discounted the option without providing quantitative justification (see theory below). Assuming K_L values for *Acer* and *Quercus* are correct, they concluded that water potential gradients in low-K_L leaves might be quite large during midday transpiration on sunny days, for example, from 0.5 to 1.5 MPa from base of petiole to the evaporative surfaces.

More recently, Sack *et al.* (2002) have demonstrated that K_L values measured by the HPFM are sensitive to irradiance, i.e. values for *Quercus* leaves are $1–3\times10^{-4}$ in high irradiance (>1200 μmol s^{-1} m^{-2}) versus $2–8\times10^{-5}$ in ambient room irradiance (<10 μmol s^{-1} m^{-2}). This observation has been confirmed in some but not in all species in this paper. The results of Sack *et al.* (2002) make it clear that it is advisable to measure K_L in high irradiance if values representative of those in sunlight are desired. Since irradiance has a dramatic effect on stomatal aperture, Sack *et al.* (2002) suggested that the low K_L values measured in low irradiance might be due to stomatal closure. Nevertheless, K_L values measured by the HPFM agree with those measured by other hydraulic methods especially when measurements are all done in high irradiance (Tyree *et al.*, 1994; Yang and Tyree, 1994; Tsuda and Tyree, 2000; Sack *et al.*, 2002).

The observed 'irradiance-effect' could be explained by stomatal closure if the hydraulic conductance through stomates ($K_{L,s}$) is about the same as or less than the conductance of vascular and nonvascular tissues ($K_{L,vt}$). If these conductances can be approximated as conductances in series then

$$K_L = \frac{K_{L,vt}K_{L,s}}{K_{L,vt} + K_{L,s}} \tag{1}$$

It could be argued that both $K_{L,s}$ and $K_{L,vt}$ might decrease with decreasing irradiance. If all values of $K_{L,s}$ are >$K_{L,vt}$ at all irradiance levels, then $K_L \cong K_{L,vt}$ and the irradiance dependence of K_L would have to be ascribed to that of $K_{L,vt}$. In this paper, the theoretical and experimental evidence that the irradiance dependence of K_L is due either to the irradiance dependence of $K_{L,s}$ or $K_{L,vt}$ is examined.

Theoretical consideration

The purpose of this section is to quantify the theoretical effect of stomatal aperture on conductance of stomates to liquid-water ($K_{L,s}$) and water-vapour (g_s). The following questions will be answered: (i) How tightly do stomates have to close before $K_{L,s} \cong K_L$, so it could be expected that stomates will dominate K_L measurements with an HPFM? (ii) What will the g_s values be when $K_{L,s} \cong K_L$ and can this be confirmed by measurements of whole leaf conductance to water vapour? (iii) Can stomatal width be confirmed when $K_{L,s} \cong K_L$ with a light microscope? To begin with, a simple geometry for stomatal pores will be assumed.

Stomatal pores are closely approximated by an elongated ellipse with a fixed width of the major axis and variable width of the minor axis (Parlange and Waggoner, 1970; Willmer and Fricker, 1996). *Quercus rubra* leaves have about 150 million stomates per m^2 (=N) and stomatal pores with a major axis of about 10.7 μm (=a). If the pores of 15 μm length (=L) are approximated by uniform bores with 'parallel' walls, then both hydraulic conductance and water-vapour conductance of stomates can be computed from this simple geometry.

The equation for laminar flow of liquid water through N pipes m^{-2} with an elliptical cross-section is:

$$K_{L,s} = N \frac{\pi(a^3 b^3)}{64\eta(a^2 + b^2)L} 10^9 \tag{2}$$

where a and b equal the major and minor axis widths, η is the viscosity of water (0.001 Pa s at 20 °C), and 10^9 is the conversion factor between $K_{L,s}$ in m^3 s^{-1} Pa^{-1} m^{-2} and kg s^{-1} MPa^{-1} m^{-2} (Tyree and Zimmermann, 2002).

For the stomatal conductance to water vapour, from Fick's Law and the fact that pores are conductances in series that are additive, it is found that:

$$g_s = 4.155\times10^4 \frac{DN\pi ab}{4L} \tag{3}$$

where D is the diffusion coefficient of water vapour in air (2.4×10^{-5} m s^{-1} at 20 °C and 101.3 kPa), and 4.155×10^4 is the conversion factor between g_s in units of m s^{-1} and units of mmol s^{-1} m^{-2} (Nobel, 1991).

Equations (2) and (3) are plotted in Fig. 1A and B, respectively. Figure 1A clearly shows that $K_{L,s}$ increases with the pore width (b) to nearly the third power (slope=2.97), and that for most pore widths the value of $K_{L,s}$ is orders of magnitude more than K_L of *Quercus*. From Fig. 1A it can be concluded that K_L from equation (1) is due to vessel and tissue properties ($K_{L,vt}$) for most stomatal apertures. The dashed line in Fig. 1A represents the theoretical value of K_L versus pore width assuming $K_{L,vt}$ is constant at 2.5×10^{-4} kg s^{-1} MPa^{-1} m^{-2} (see below). $K_{L,s}$ has no significant impact on K_L until pore width falls below 0.08 μm and does not equal the low-irradiance values observed until width falls to <0.02 μm. Since the resolution of light microscopy is limited to a measurement discrimination of about 0.4 μm, a microscope cannot be used for visual confirmation of the hypothesis that the irradiance effect on K_L is due solely to the stomatal closure.

Could confirmation of the hypothesis be obtained from measurements of whole leaf conductance to water vapour as a proxy for stomatal aperture? In Fig. 1B it can be seen that g_s varies linearly with pore width that might be concluded from equation (3) with a as a constant. However, the whole leaf conductance to water vapour equals the sum of the cuticular conductance, g_c, and the stomatal conductance, g_s, in parallel. Since a probable value of g_c=2–8 mmol s^{-1} m^{-2} in tree leaves (Nobel, 1991; Sack *et al.*, 2003), the

whole leaf conductance will approach a constant value (dashed or solid line in Fig. 1B) about when or a little before pore width begins to limit K_L. Given the measurement uncertainty of whole leaf conductance to water vapour, $g_L = g_c + g_s$, a pore width of less than about 0.02–0.1 μm from g_L measurements depending on g_c cannot be inferred.

Would these conclusions change if a more realistic pore profile was used? In reality the width profile of stomatal pores are uneven, i.e. sometimes narrower near the middle or at the extreme end depending on species. In a more realistic pore shape, the minimum width that would limit K_L would be even smaller, hence visual observations with the aid of a microscope would be harder. Confirmations by measurement of g_L would still be limited by g_c in many species.

Even if g_L or pore width could be measured with fairly high resolution, it still could not be proved that $K_{L,s}$ limits K_L if allowance is made for patchy stomatal closure. For example, assume that just 0.1% of the stomates remain open in low irradiance and retain a width of 1 μm while the rest have closed to ≤0.02 μm width. In this case $g_s = 2.5$ mmol s^{-1} m^{-2} with patchy closure versus 1.7 mmol s^{-1} m^{-2} with uniform closure and g_L would equal about 11.5 versus 10.7 mmol s^{-1} m^{-2} (with $g_c = 8$ mmol s^{-1} m^{-2}), a difference that would be difficult to resolve experimentally. By contrast, the impact of patchy closure would be much smaller on K_L than on g_s in the above example. $K_{L,s}$ would be 5.2×10^{-3} kg s^{-1} MPa^{-1} m^{-2} with just 0.1% of the stomates open at 1 μm and the rest at ≤0.02 μm width and hence would have a negligible impact on K_L, i.e. 2.39×10^{-4} kg s^{-1} MPa^{-1} m^{-2} with 0.1% of the stomates open at 1 μm width versus 2.50×10^{-4} kg s^{-1} MPa^{-1} m^{-2} with all stomates open ≥1 μm.

On the other hand, there is some value in measuring g_L during measurements of K_L, because if it can be proved that K_L values change in transition from high- to low-irradiance before g_L closely approaches g_c then this would support the hypothesis that the irradiance dependence of K_L is caused by the irradiance dependence of $K_{L,vt}$. The purpose of this paper is to search for evidence that the irradiance dependence of K_L is due to $K_{L,vt}$. The theoretical relationship between K_L and g_L is given in Fig. 2; quite low values of g_L must be reached before K_L declines, i.e. around 10–15 mmol s^{-1} m^{-2} in this model. Can irradiance-induced changes in K_L be demonstrated at stomatal apertures that yield higher values of g_L? Alternatively, can irradiance-induced changes in K_L be demonstrated while g_L is at low and constant value?

Materials and methods

All measurements were performed on mature current-year leaves of several temperate deciduous trees: *Cercis siliquastrum* L. (Fabaceae), *Acer pseudoplatanus* L. (Sapindaceae), and *Juglans regia* L. (Juglandaceae) in Trieste, Italy; *A. saccharum* Marsh. and *Quercus rubra* L. (Fagaceae) in Burlington, Vermont (nomenclature follows Gleason and Carlquist, 1991; Tutin *et al.*, 1964; Judd *et al.*,
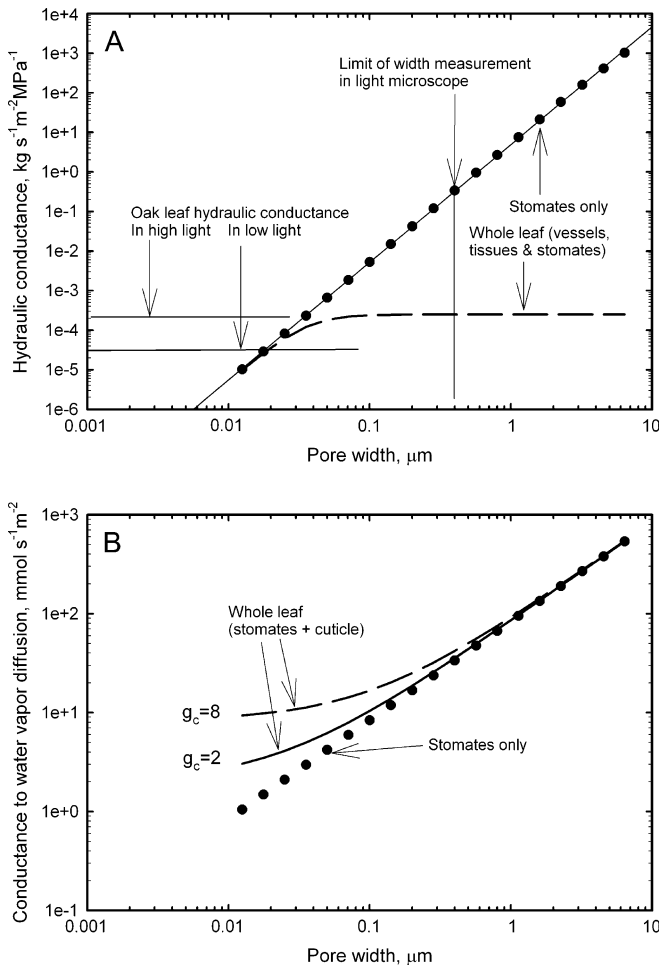

Fig. 1. The theoretical dependence of the conductance to liquid water (A, equation 2) and water vapour (B, equation 3) of stomates as a function of stomatal aperture (pore width) based on elliptical geometry. See text for more details.
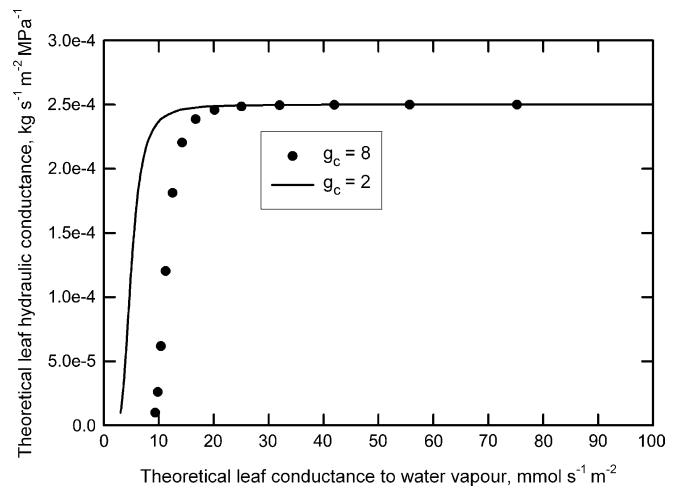
Fig. 2. Theoretical dependence of whole leaf hydraulic conductance to liquid water on whole leaf conductance to water vapour. See text for details.

2002). Leaves of tropical trees were also tested: *Schefflera arboricola* (Hayata) Merr. (Apiaceae) in a greenhouse in Burlington and *Calophyllum longifolium* (Clusiaceae), *Cordia alliodora* (Bignoniaceae), *Dendropanax arboreus* (Apiaceae), *Lindackeria laurina* (Achariaceae), and *Miconia argentea* (Melastomataceae) in tropical lowland rainforest, Barro Colorado Island, Panama (Smithsonian Tropical Research Institute; nomenclature follows Croat, 1978; Judd *et al.*, 2002).

Leaves were harvested early in the morning from adult trees or shrubs and transported to the laboratory while keeping the petiole immersed in a 10 mM KCl solution. Leaves were then connected to the HPFM (Tyree *et al.*, 1995) via the petiole using compression fittings. Leaves were immersed in distilled water to stop transpiration and to prevent leaf overheating upon illumination (see below). Leaves were perfused at 0.3–0.5 MPa with a 10 mM KCl solution for 50 min. Leaf hydraulic conductance (K_L) was measured every 2 s and saved as means every 30 to 60 s under ambient laboratory irradiance (PAR <6 μmol m^{-2} s^{-1}). The temperature of the water bath (T_w) was also recorded at the same time interval using a thermocouple thermometer (Digi-Sense model 91100–40, Cole-Parmer Instrument Co., Vernon Hills, IL, USA). Leaves were then illuminated at a PAR of 1000–1200 μmol m^{-2} s^{-1} using a slide projector or floodlight (as used in Panama). In order to avoid excessive heating of the water bath, irradiance from the projector was reflected by a mirror before striking the leaves. This way, water temperature never increased more than 2 °C during the following 30 min. During the high irradiance period, both K_L and T_w were recorded every 60 s. Then, irradiance was returned to ambient and both K_L and T_w were measured for 30 min more. Overall, each experiment lasted 115 min. Control experiments were performed where K_L was measured on leaves connected to the HPFM for 115 min and never illuminated. At the end of each experiment, leaf surface area (A_L) was measured for K_L calculation using a leaf area meter (LI-3000A, Li-Cor Inc., Lincoln, NE, USA) and K_L was scaled by A_L. All K_L measurements were corrected for eventual temperature changes to account for changes in water viscosity.

Because Sack *et al.* (2002) had suggested that the irradiance response of K_L might be due to stomatal opening, changes in stomatal aperture were monitored during the dark and high irradiance periods. Leaves were connected to the HPFM and pressurized at 0.02 MPa, in order to prevent leaf desiccation while avoiding substantial infiltration of leaf air spaces. Leaves were not immersed in water but kept in air. The time-course of the experiment was the same as for K_L measurements but, in this case, leaf conductance to water vapour (g_L) was measured every 2 min, using a steady-state porometer (LI-1600, Li-Cor Inc.) in Trieste or with a LI-6400 (Li-Cor Inc.) in Burlington. It was confirmed that both porometers gave values of g_L near zero when leaves were replaced by plastic sheets, i.e. 0.1±0.2 mmol s^{-1} m^{-2} for the LI-1600 and 1.4±2.3 mmol s^{-1} m^{-2} for the LI-6400 (mean ±sd).

To investigate the generality of the irradiance response of K_L, less detailed experiments of a confirmatory nature were performed for tropical species. K_L values were compared either for given leaves before and after changing from low to high irradiance, or for different leaves sampled from the same branch held constantly at low versus high irradiance. Leaves of tropical species were not tested for g_L responses.

In all cases, differences between K_L measured in the dark or after illumination were tested for statistical significance using One-way-ANOVA.

ABA experiments

In order to investigate the response to irradiance of leaves whose stomatal irradiance response had been inhibited, leaves of *J. regia* were collected early in the morning and transported to the laboratory

as described above. Here, they were rehydrated for 2 h with either 10 mM KCl solution (controls) or with a solution of 10 mM KCl+0.5 mM abscisic acid solution (ABA, Sigma-Aldrich S.r.l.). The plant hormone ABA is well known for its effects on stomatal movements. In particular, ABA is known to inhibit the irradiance-induced stomatal opening at low concentrations (10^{-3} mM, Trejo *et al.*, 1993). Control and ABA-treated samples were then measured for K_L changes under low or high irradiance conditions as described above. Preliminary experiments were performed to assess the effect of ABA on stomatal aperture; leaf conductance to water vapour of control or ABA-treated leaves was measured under conditions similar to experiments described above. In particular, leaves rehydrated with either KCl or KCl+ABA solutions were connected to the HPFM and pressurized at 0.02 MPa, in order to avoid substantial infiltration of air spaces. Leaves were immersed in water and kept for 15 min under normal laboratory irradiance (see above). Leaves were taken out of water, carefully dried with filter paper and their leaf conductance to water vapour (g_L) was measured using a steady-state porometer (LI-1600, Li-Cor Inc.). Leaves were immersed again in water and illuminated as described above. After 30 min, leaf surface was dried again and leaves were re-measured for g_L.

Results

Sack *et al.* (2002) measured the irradiance response of K_L on only two species (*Quercus rubra* and *Hedera helix*). In the former they found a large irradiance response when measured with the HPFM, but no significant effect on the latter when measured with the vacuum chamber method. The results of Sack *et al.* (2002) were replicated on *Q. rubra* and also on *Acer saccharum*. *A. saccharum* (n=12) had a smaller effect than *Q. rubra* (n=29). After 50 min in ambient irradiance *A. saccharum* and *Q. rubra* had K_L values that did not differ significantly, i.e. 3.66±0.46×10^{-5} (mean± sem) and 3.57±0.31×10^{-5}, respectively (Fig. 3); but K_L increased after 33 min in high irradiance by 64% and 392% for *A. saccharum* and *Q. rubra*, respectively. K_L values in high irradiance were lower by about a 30–50% than those shown in Fig. 2 of Sack *et al.* (2002). The differences may be genetic or environmental in origin or may be due to the time of year (measurements were made in May–June versus August in Sack *et al.*, 2002). In both studies the leaves were exposed to high irradiance for about the same time period (20–35 min) prior to recording K_L values.

Two out of the three species studied in Trieste showed an irradiance-induced increase of K_L. In particular, K_L of *Cercis siliquastrum* did not respond to irradiance level, but *Acer pseudoplatanus* and *Juglans regia* showed marked responses to illumination in that their K_L increased by about 150% and 300% (for *J. regia* and *A. pseudoplatanus*, respectively, Fig. 4). In the latter two species K_L increased gradually upon illumination, but remained relatively constant after lights were turned off in contrast to the behaviour of *A. saccharum* and *Q. rubra* where K_L fell slowly after returning to ambient irradiance. In the case of *C. siliquastrum*, K_L did not differ in control or illuminated leaves, thus suggesting that irradiance had no effect on K_L in this species. The analysis of stomatal kinetics (Fig. 4, triangles) showed that
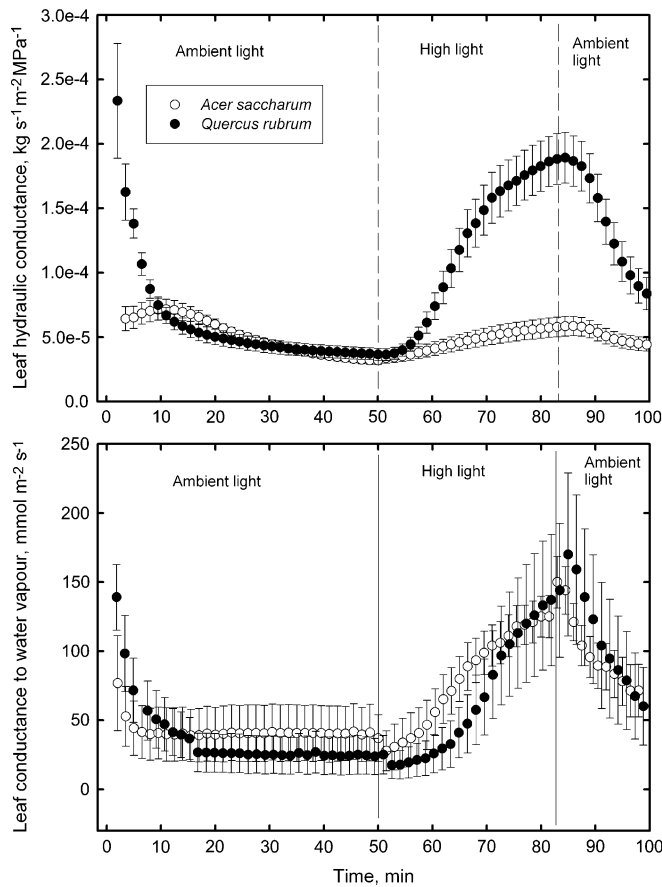
Fig. 3. Hydraulic conductance and leaf conductance to water vapour measurements on *Acer saccharum* and *Quercus rubra* leaves. Upper panel: whole leaf hydraulic conductance to liquid water versus time. For the first 50 min irradiance was <10 mmol s^{-1} m^{-2} (ambient irradiance), from 50–83 min irradiance was >1000 mmol s^{-1} m^{-2}, and after 83 min irradiance was again ambient. Lower panel: whole leaf conductance to water vapour versus time. Timing of irradiance is the same as in the upper panel. Means are reported ±SEM (standard error of the mean).

stomata opened in response to high irradiance stimulus and closed in low irradiance, as expected for all species. However, g_L changes did not parallel K_L changes. In fact, in the case of *A. pseudoplatanus* and *J. regia*, stomatal opening followed K_L increase upon illumination with a delay of 5–10 min. Moreover, after irradiance was turned off, g_L markedly decreased to its previous levels in low irradiance but K_L did not. In the case of *C. siliquastrum*, g_L increased after illumination of the leaf but K_L remained constant.

Both control and ABA-rehydrated leaves of *J. regia* showed similar dark values of g_L (about 8–10 mmol m^{-2} s^{-1}, Fig. 5B). In high irradiance, g_L of control leaves increased to about 55 mmol m^{-2} s^{-1} while that of ABA-treated leaves remained at the same values as measured in the dark. The kinetics of K_L response to illumination was the same for control and ABA-treated leaves (Fig. 5A).

Two of the six tropical species in this study changed K_L in response to irradiance. Table 1 gives K_L values in low and high irradiance conditions.
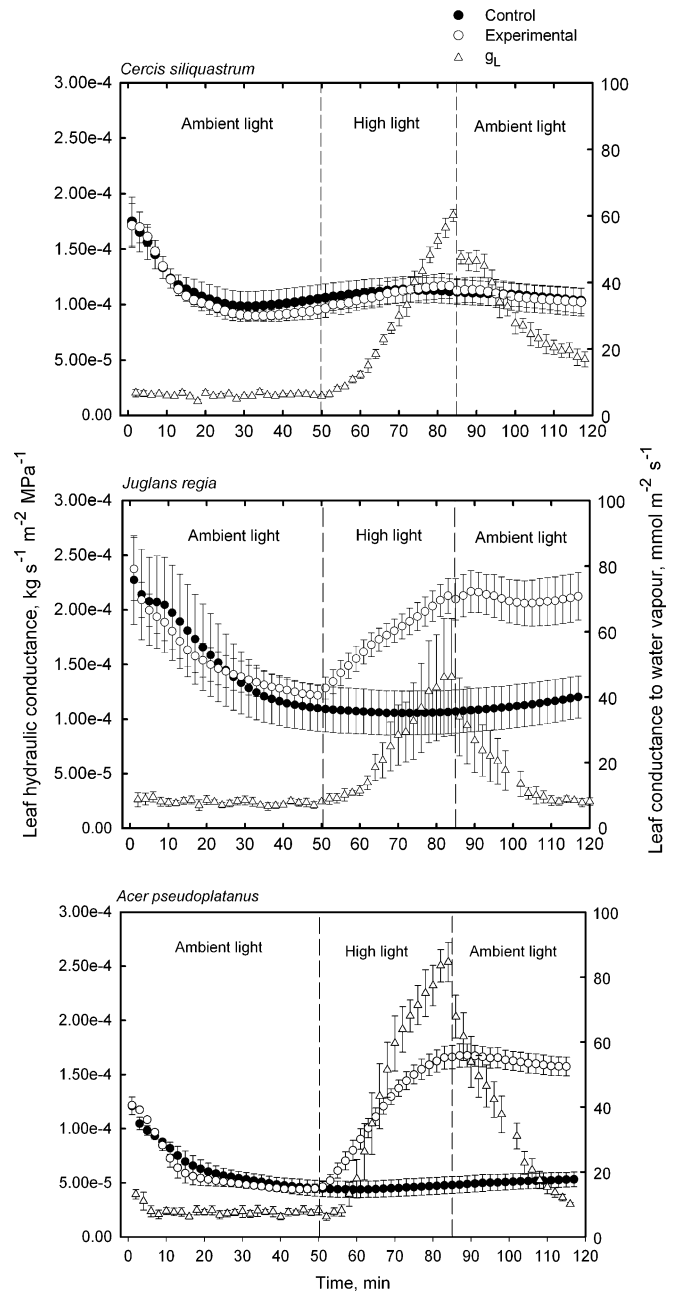

Fig. 4. Hydraulic conductance and leaf conductance to water vapour measurements on *Cercis siliquastrum*, *Juglans regia*, *and Acer pseudoplatanus* leaves versus time and irradiance. Control leaves were constantly in ambient irradiance (<6 mol s^{-1} m^{-2}). Means are reported ±SEM (standard error of the mean).

Discussion

In all species K_L decreased with time after the onset of perfusion with the HPFM. This initial decrease may be of no functional significance since it is probably due to non-steady-state conditions that will exist until leaves, initially dehydrated, become fully hydrated. Not all species showed a statistically significant response in leaf hydraulic conductance to irradiance when measured with the HPFM; five

species did not respond to irradiance and six species did. This is consistent with Sack *et al.* (2002) who reported an irradiance response in one of two species during measurements of K_L with the HPFM or vacuum chamber. Irradiance effects on K_L were not replicated as much in some species as others, hence the statistical power to resolve changes were unequal in these studies. Therefore, the effects of ir-
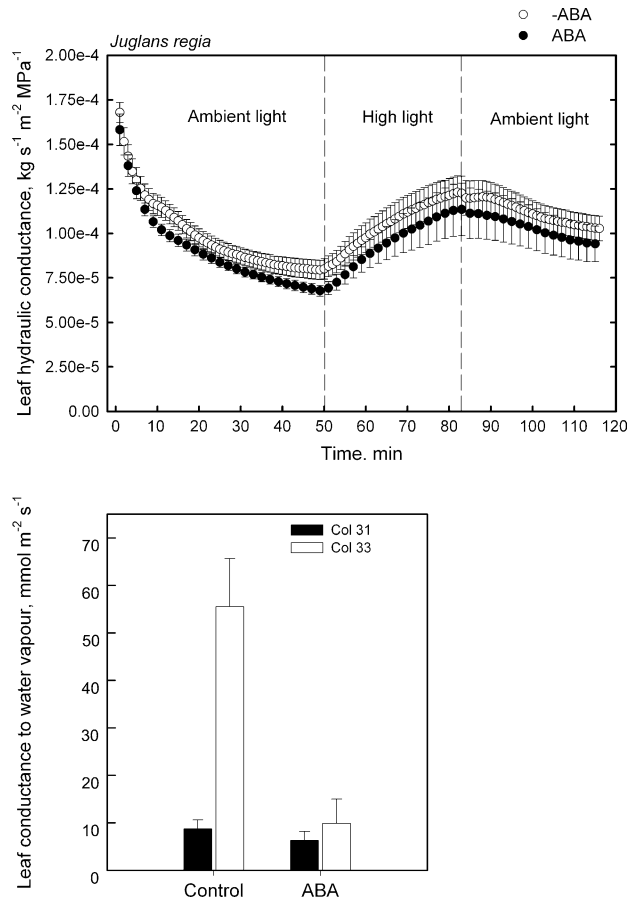

Fig. 5. Upper: whole leaf hydraulic conductance versus time and irradiance. Solid symbols, leaves perfused with 10 mM KCl plus 0.5 mM ABA. Open symbols, leaves perfused with 10 mM KCl. Lower: whole leaf conductance to water vapour versus irradiance in the presence of ABA or without ABA (control). Means are reported ±SEM (standard error of the mean).

radiance on leaf hydraulic properties might be more ubiquituous.

It is tentatively concluded that these data are most consistent with the hypothesis that the irradiance dependence of K_L is due more to a dependence of the leaf blade tissues accounted for in the vessel+tissue component ($K_{L,vt}$) than in the stomatal component ($K_{L,s}$). When stomatal response to irradiance is inhibited by the application of ABA, the irradiance response to K_L remains unchanged (Fig. 5). The ABA-response might still be consistent with the hypothesis that stomates control K_L in low irradiance if it is assumed that ABA does not cause complete stomatal closure and prevents further response of stomates to irradiance. In *A. pseudoplatanus* and *J. regia* the kinetics of the irradiance response of g_L and K_L (Fig. 4) are not what is expected from the theory (Figs 1, 2) if stomatal conductance were driving the K_L response. Theory would predict a rapid increase in K_L to a maximum value with a negligible change in g_L immediately after the lights are turned on (Fig. 2). What is found is a gradual increase in K_L as g_L increases gradually. In addition, it would be expected that the decrease in K_L would mirror the decrease in g_L when the lights are turned off. Instead, it was found that K_L remained relatively constant while g_L falls back almost to the original, dark-adapted value in *A. pseudoplatanus* and *J. regia* (Fig. 4). Furthermore, no irradiance response is observed in *C. siliquastrum* even though g_L is varying over the same range as the other two species.

These results might still be consistent with the irradiance dependence of K_L being caused by irradiance dependence of $K_{L,s}$ but it would have to be supposed that there are differences in the kinetics of stomatal response to irradiance in the HPFM experiments versus the gas exchange experiments. It would probably be necessary to invoke hysteresis in the kinetics of g_L changes upon change in irradiance and it might have to be supposed that g_L changes over different ranges of values in the HPFM and gas exchange experiments. These possibilities cannot totally be discounted, especially if it is taken into account that experimental conditions during K_L measurements (leaf immersed in water) and g_L measurements (leaf kept in air) were not the same.

Table 1. *Leaf hydraulic conductance (K_L, kg s^{-1} m^{-2} MPa^{-1}) of tropical species as measured under ambient or high irradiance conditions*

One-tailed *t*-test significance: * P <0.05; ** P <0.01; ns, not significant. SEM is the standard error of the mean and *n* is the number of samples.

Species	K_L (ambient irr.)	SEM	*n*	K_L (high irr.)	SEM	*n*	*t*-test	Irradiance response
Lindackeria laurina	1.96E-4	7.96E-5	5	2.73E-4	3.80E-5	5	ns	No
Miconia argentea	1.01E-4	8.13E-6	4	2.99E-4	1.70E-5	7	**	Yes (196%)
Cordia alliodora	1.73E-4	3.24E-5	7	2.10E-4	3.39E-5	8	*	Yes (21%)
Calophyllum longifolium	1.25E-4	6.98E-5	2	1.85E-4	3.24E-5	5	ns	No
Dendropanax arboreus	1.46E-4	2.38E-5	4	1.60E-4	2.97E-5	8	ns	No
Schefflera arboricola	1.61E-4	3.93E-5	20	1.65E-4	3.10E-5	20	ns	No

Because these results are less consistent with the hypothesis that stomatal opening is responsible for the irradiance-mediated increase of K_L than with the hypothesis that there is an irradiance-mediated response of tissues ($K_{L,vt}$), possible alternative explanations have to be sought. Several studies suggest that leaf hydraulic resistance is dominated by the extravascular component (Tyree and Cheung, 1977; Tyree *et al.*, 2001; Salleo *et al.*, 2003; Trifilò *et al.*, 2003). Water flow across cell membranes is known to be enhanced (by over three times) by the presence of aquaporins (Maurel, 1997). Hence, it is conceivable that rapid irradiance-mediated increase of leaf hydraulic conductance is due to the *de novo* expression of aquaporins or, more likely, to the up-regulation of pre-existing water channels. Irradiance-mediated circadian regulation of aquaporin expression has been shown to be involved in the regulation of root water permeability (Henzler *et al.*, 1999; Lopez *et al.*, 2003) and in the pulvinar movements of *Samanea saman* (Moshelion *et al.*, 2002). Post-translational regulation of aquaporin activity through phosphorylation has also been reported (Maurel *et al.*, 1995; Johnsson *et al.*, 1998, 2000; Eckert *et al.*, 1999; Chaumont *et al.*, 2000; Baiges *et al.*, 2002). It is therefore conceivable that the increase of K_L observed in the present study might be due to irradiance-mediated activity of specific kinases which, in turn, would lead to up-regulation of aquaporins and consequent enhanced water transport through the bundle sheath and/or mesophyll. Although there is no experimental evidence supporting this mechanism, it is felt that this hypothesis represents an interesting starting point for future studies addressed at elucidating the role of aquaporin regulation in mediating the response of K_L to environmental stimuli.

Some recent studies (Zwieniecki *et al.*, 2002; Sack *et al.*, 2004) reported a dominance of vascular hydraulic resistance on K_L in some species. If this were the case, then irradiance-induced changes of K_L might be mediated by changes in the hydraulic properties of the leaf venation system as well. In turn, these changes might be caused by modifications of xylem sap composition (Zwieniecki *et al.*, 2001) possibly mediated by the phloem (Zwieniecki *et al.*, 2004). However, previous work on *A. saccharum* and *Q. rubra* has shown K_L to be invariant with large changes in the ion composition of the flow solution (i.e. K_L was the same when measured with deionized filtered water as with 10 mM KCl; see 'Materials and methods' in Sack *et al.*, 2004). For such species, at least, it is suggested that the irradiance response is localized in the extravascular portion of the water flow pathway.

It has to be pointed out that all results presented in this study and related discussion might only apply to leaves when measured with the HPFM. Eventual irradiance-mediated K_L changes in the field were not measured and it is felt that methods to measure K_L directly would be difficult to devise on intact plants. However, it is considered that such changes might be of adaptive value if K_L increases

during the central part of the day, when PAR and evaporative demand is highest, thus allowing plants to sustain high transpiration rates while buffering leaf water potential above critical values inducing xylem cavitation (Bond and Kavanagh, 1999; Nardini and Salleo, 2000). Moreover, the relative magnitude of K_L response to irradiance might depend on several factors like the relative contribution of the vascular and extravascular compartments to leaf hydraulics or the abundance of aquaporins in leaf tissues. Aquaporins would only influence the hydraulic conductance of the extravascular tissues, and not the xylem. Thus, if aquaporins are involved in the irradiance response, a stronger irradiance effect would be expected for leaves in which the extravascular tissue is a more important determinant of K_L (Tyree *et al.*, 2001; Salleo *et al.*, 2003). Apparently, the response of K_L to irradiance is highly variable across species, and it is also possible that the exact mechanisms may vary among species. However, because the irradiance effect is common and substantial, it is recommended that K_L measurements be conducted under the irradiance most relevant to the biological application. Clearly, the functional and ecological implications of the phenomenon described in this paper invite further studies.

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