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The rapid light response of leaf hydraulic conductance: new evidence from two experimental methods

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ABSTRACT

Previous studies have shown a rapid enhancement in leaf hydraulic conductance (K_{leaf}) from low to high irradiance (from <10 to >1000 μmol photons m^{-2} s^{-1}), using the high-pressure flow meter (HPFM), for 7 of 14 tested woody species. However, theoretical suggestions have been made that this response might arise as an artifact of the HPFM. We tested the K_{leaf} light response for six evergreen species using refined versions of the rehydration kinetics method (RKM) and the evaporative flux method (EFM). We found new evidence for a rapid, 60% to 100% increase in K_{leaf} from low to high irradiance for three species. In the RKM, the leaf rehydration time constant declined by up to 70% under high irradiance relative to darkness. In the EFM, under higher irradiance, the flow rate increased disproportionately to the water potential gradient. Combining our data with those of previous studies, we found that heterobaric species, i.e. those with bundle sheath extensions (BSEs) showed a twofold greater K_{leaf} light response on average than homobaric species, i.e. those without BSEs. We suggest further research to characterize this substantial dynamic at the nexus of plant light- and water-relations.

Key-words: bundle sheath extensions; evaporative flux method; heterobaric; homobaric; irradiance; leaf traits; rehydration kinetics method.

INTRODUCTION

The plant hydraulic system is a major determinant of gas exchange rates and drought responses (Tyree & Zimmermann 2002). The ability of the leaf to maintain open stomata at a given transpiration rate and soil water potential depends on a plant hydraulic conductance high enough to keep the leaf from desiccating (Tyree & Zimmermann 2002). Leaves account for a large proportion of the hydraulic resistance of the whole plant (c. 30% on average; Sack *et al.* 2003), but our understanding of the pathways of leaf water transport is still rudimentary. Further, the conductance of these hydraulic pathways can be influenced by internal and external factors, including temperature, leaf water status and light (Nardini, Tyree & Salleo 2001; Sack & Tyree 2005; Sack & Holbrook 2006).

A substantial impact of light on leaf hydraulic conductance (K_{leaf}; the flow rate through the leaf driven by a given leaf water potential gradient) would be of great importance to leaf and plant function. Early experiments showed that plants held in the dark for 1 h or several days had a reduced K_{leaf}, as measured using a pressure-driven flow (Sober 1997; Aasamaa & Sober 2001). A rapid leaf-level response was demonstrated for excised leaves of *Quercus rubra*, switched from low to high irradiance, measured with the high-pressure flow meter (HPFM; Sack *et al.* 2002). Several studies confirmed this rapid response with the HPFM for seven woody species of 14 species tested (Gasco, Nardini & Salleo 2004; Tyree *et al.* 2005; Cochard *et al.* 2007; Voicu *et al.* 2008). Additionally, a light response was found for cell hydraulic conductivity in the vein parenchyma of *Zea mays* (Kim & Steudle 2007). Consistent with these studies, greenhouse and field studies on excised shoots and intact plants found that K_{leaf} increased in parallel with irradiance, diurnally and seasonally (Tsuda & Tyree 2000; Lo Gullo *et al.* 2005; Sellin & Kupper 2007; Sellin, Õunapuu & Kupper 2008), though these responses also correlated with changes in temperature. Detailed work has shown that the HPFM light response may arise from aquaporin expression and/or activation (Nardini, Salleo & Andri 2005; Cochard *et al.* 2007; Voicu *et al.* 2008), as occurs in roots (Henzler *et al.* 1999).

However, several studies have raised the possibility that HPFM may be partly responsible for the rapid light response, because it floods the airspaces, opening new flow pathways (e.g. Sack *et al.* 2002; Tyree *et al.* 2005). A particular concern was that during HPFM measurement, water is forced to flow through stomata, which would not be in the hydraulic pathway *in vivo*; if the stomata shut enough to influence K_{leaf}, these could be responsible for the reduced values under low irradiance. Studies have indicated that the

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stomata do not close tightly enough to cause the effect (when observed by electron microscope; Cochard *et al.* 2007), and that stomatal dynamics in response to light do not match those of K_{leaf} (when measured in air by gas exchange; Tyree *et al.* 2005); further, abscisic acid (ABA) drives stomatal closure without affecting measured K_{leaf} (Tyree *et al.* 2005). However, each paper reporting the K_{leaf} light response has listed these possible artifacts of the HPFM (Sack *et al.* 2002; Tyree *et al.* 2005; Cochard *et al.* 2007; Kim & Steudle 2007; Voicu *et al.* 2008).

We conducted new experiments to test for the existence of a K_{leaf} light response using two of the most common methods other than the HPFM: the rehydration kinetics method (RKM; Brodribb & Holbrook 2003); and the evaporative flux method (EFM, determining transpiration using a potometer; Sack *et al.* 2002). To our knowledge, there have been no published studies of the rapid response of K_{leaf} between low and high irradiance with these methods. Indeed, the RKM and EFM as previously used are not amenable to this test; the EFM cannot be operated in full darkness (Cochard *et al.* 2007), and the RKM requires an equilibration step outside of the light. We modified the methods for these experiments, and designed treatments to test for the maximum K_{leaf} light response that could be assessed with each method. Previous studies with HPFM have found K_{leaf} light responses for deciduous but not evergreen angiosperm species. We focused on six evergreen species, and hypothesized that these experiments would show K_{leaf} light responses for some species. Additionally, we combined our data with those previously published to test for a linkage of the K_{leaf} light response with the presence of bundle sheath extensions (BSEs). BSEs consist of parenchyma or schlerenchyma cells of the vascular bundle sheath surrounding minor veins, which extend to the epidermis (Wylie 1952; McClendon 1992; Kenzo *et al.* 2007). We hypothesized that leaves with BSEs might show a stronger K_{leaf} light response, as BSEs guide light into interior vascular and mesophyll tissues (Karabourniotis, Bornman & Nikolopoulos 2000; Nikolopoulos *et al.* 2002).

METHODS

Plant material

Six evergreen species were selected in and around the campus of University of California, LA, USA, from March to April 2008 (Table 1). For 3 to 10 plants per species, shoots with mature leaves from the most exposed branches were collected the night before measurement, re-cut under filtered water (0.22 μm Thornton 200 CR; MilliPore, Molsheim, France), and rehydrated overnight, covered with plastic.

Testing the light response of K_{leaf} *with the RKM*

We applied RKM (Brodribb & Holbrook 2003) with several refinements. This method is based on an analogy between the rehydration of desiccated leaves and the

Table 1. Study species, family and origin, and mean values ± standard error for morphological traits, including individual leaf area (*LA*) and leaf mass per area (*LMA*); and pressure volume curve parameters, including saturated water content (*SWC*), osmotic potential at full turgor (π_{ft}) and at zero turgor (π_{tlp}), modulus of elasticity (ε), relative capacitance (C) and absolute capacitance per leaf area (C^*)

Species	Family	Origin	*LA* cm^2	*LMA* $g\ m^{-2}$	*SWC* $g\ g^{-1}$	π_{ft} MPa	π_{tlp} MPa	ε MPa	C MPa^{-1}	C^* $mol\ m^{-2}\ MPa^{-1}$
Alberta magna	Rubiaceae	South Africa	39.1 ± 1.78	144 ± 4.09	2.36 ± 0.07	−1.39 ± 0.05	−1.97 ± 0.07	8.08 ± 0.17	0.086 ± 0.002	1.62 ± 0.05
Eucalyptus erythrocorys	Myrtaceae	Australia	25.5 ± 1.01	268 ± 13.6	1.30 ± 0.20	−1.67 ± 0.06	−2.24 ± 0.10	21.5 ± 2.48	0.040 ± 0.004	0.78 ± 0.04
Hedera canariensis	Araliaceae	Europe	40.0 ± 2.27	88.5 ± 5.5	3.09 ± 0.17	−1.16 ± 0.15	−2.06 ± 0.12	11.7 ± 1.08	0.053 ± 0.002	0.80 ± 0.05
Heteromeles arbutifolia	Rosaceae	California	10.8 ± 0.32	175 ± 4.8	1.62 ± 0.03	−1.89 ± 0.06	−2.34 ± 0.05	17.2 ± 1.19	0.047 ± 0.003	0.75 ± 0.02
Hymenosporum flavum	Pittosporaceae	Australia	21.7 ± 1.19	79.2 ± 1.9	2.38 ± 0.09	−1.38 ± 0.04	−2.06 ± 0.05	5.88 ± 0.48	0.113 ± 0.008	1.18 ± 0.03
Raphiolepis indica	Rosaceae	China	28.8 ± 1.30	192 ± 9.5	1.39 ± 0.12	−1.37 ± 0.07	−2.08 ± 0.11	11.5 ± 0.81	0.055 ± 0.006	0.81 ± 0.04

n = 78–112 for *LA*; 21–28 for *LMA* and C^*; and 6 for pressure–volume curve parameters.

charging of a capacitor in series with a resistor. Briefly, shoots are dehydrated to an initial low water potential (Ψ_o, MPa) and then a leaf from the shoot is rehydrated for a given time period, and the new, higher water potential measured (Ψ_f, MPa). K_{leaf} can be estimated from the rehydration time (t, s), the two water potentials and the relative capacitance determined from the pressure volume curve (C; change in relative water content per change in water potential, MPa^{-1}):

$$\tau = t \Big/ \ln\left(\frac{\Psi_f}{\Psi_o}\right) \quad (1)$$

$$K_{leaf} = \frac{C \times LMA \times SWC}{\tau} \quad (2)$$

where LMA is the leaf dry mass per area ($g\ m^{-2}$), SWC is the saturated water content (mass of water per dry mass in hydrated leaf) and τ is the rehydration time constant (s).

Before desiccating and rehydrating, shoots that included four leaves were acclimated to different light levels. Shoots were acclimated under deionized water for 60 to 90 min under high irradiance [1000–1500 μmol photons $m^{-2}\ s^{-1}$ photosynthetically active radiation (PAR); model 73828 1000 W, 'UV filter'; Sears, Roebuck, Hoffman Estates, IL, USA] or in a darkened room (using a candle as the only light source; <0.1 μmol photons $m^{-2}\ s^{-1}$ PAR); PAR was measured with a Li-Cor 250 light meter (Li-Cor, Lincoln, NE, USA). During acclimation to high irradiance, a Pyrex dish filled with water was set between the lamp and the leaves, and a 1 m^2 fan was on the side so that the temperature was maintained between 23 °C and 27 °C (measured using a thermocouple; Cole-Parmer, Vernon Hills, IL, USA). Subsequently, the shoots were desiccated under same irradiances with a fan. Each leaf on the shoot was then enclosed in a plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI, USA) that has previously been exhaled into, and the shoot with bagged leaves was placed into a larger zipper plastic bag (Ziploc, SC Johnson, Racine, WI, USA) with moist paper towels. Shoots were equilibrated for 10 min, after which time two leaves were excised for determination of Ψ_o. The other two leaves were cut from the shoot under degassed filtered water at 25 °C and were rehydrated for 15–45 s under high irradiance or in darkness. These leaves were then equilibrated in Whirl-Pak bags for determination of Ψ_f. The leaf water potential (Ψ_{leaf}) was measured using a pressure chamber (Plant Moisture Stress Model 1000; PMS Instrument Co, Albany, OR, USA). Measurements for shoots for which the two Ψ_o values differed by more than 0.1 MPa were discarded (Brodribb & Holbrook 2003). Leaf area was measured (Li-Cor 3100 meter) and leaves were weighed for dry mass after over 48 h at 70–80 °C, to allow calculation of LMA. K_{leaf} was calculated according to Eqns 1 and 2. For each species, 9–15 measurements of τ and K_{leaf} were made in each irradiance.

We tested whether epidermal water uptake would occur during leaf rehydration under water. Ten leaves of each species were weighed after being dipped in water and then blotting dry with paper towel; and again after immersing the lamina in water for 1 min and then blotting dry. As in a previous study of 10 species (Zwieniecki, Brodribb & Holbrook 2007), no increase in mass was observed after immersion.

Our objective was to determine K_{leaf} by the RKM for leaves that were nearly fully hydrated. We desiccated shoots to a Ψ_{leaf} of –0.2 to –0.8 MPa. Desiccating shoots to Ψ_{leaf} values above –0.2 MPa led to a high variability in K_{leaf}, including the occurrence of negative values. Such high variability occurred because for mildly dehydrated leaves the recovery of Ψ_{leaf} during short rehydration times was low, often lower than the differences in Ψ_{leaf} among leaves within an equilibrated shoot (see Results). Thus, in some cases, after equilibration, the Ψ_o leaves were those with highest Ψ_{leaf}, and the rehydrated leaves had lower Ψ_{leaf} than the Ψ_o leaves, leading to a negative K_{leaf} value. Desiccating shoots below –0.2 MPa removed the problem of negative K_{leaf} values. Desiccating shoots by less than –0.8 MPa minimized cavitation (Brodribb & Holbrook 2003; Woodruff *et al.* 2007; Hao *et al.* 2008).

Before rehydration, desiccated shoots that had been acclimated to high irradiance were left to equilibrate in their clear bags under ambient irradiance (2–5 μmol photons $m^{-2}\ s^{-1}$); shoots acclimated to darkness were equilibrated in darkness. We found that shoots in bags under high irradiance failed to equilibrate in Ψ_{leaf} even if placed under water to prevent overheating; the leaves absorbed light, heated the air in their bags and transpired at varying rates. Thus, we aimed for a short equilibration time outside of high irradiance. For each species we tested if 10 min was a sufficient equilibration time. We allowed desiccated four-leaf shoots to equilibrate in bags (leaves in Whirl-Pak bags, and the shoot in a Ziploc bag with a moist paper towel) for 10, 15 or 20 min, and then we measured Ψ_{leaf} for the four leaves (n = 4 to 6 shoots per equilibration time), and calculated the maximum difference in Ψ_{leaf} among the leaves on the shoot. We repeated this experiment for equilibration times of 10, 60 and 120 min (n = 5 to 9 shoots).

We determined pressure-volume curves for six leaves sampled from three to six plants of each species (Tyree & Hammel 1972). Leaves from shoots rehydrated the previous night were desiccated slowly and measured repeatedly for mass and Ψ_{leaf}. Pressure–volume curve parameters were calculated: SWC, osmotic potential at full and zero turgor (π_{ft} and π_{tlp}, respectively), modulus of elasticity (ε), C and absolute capacitance per leaf area ($C^* = C \times SWC \times LMA$).

Testing the light response of K_{leaf} with the EFM

In the EFM (Sack *et al.* 2003), K_{leaf} is calculated as the steady-state transpirational flow rate (E, mmol $m^{-2}\ s^{-1}$) divided by the water potential driving force ($\Delta\Psi_{leaf}$, MPa). Leaves were excised under the perfusing solution (filtered water, degassed overnight with a vacuum pump). The petiole was wrapped in parafilm and rapidly connected to tubing containing solution and running to a plastic

cylinder on a balance that logged data every 30 s to a computer for the calculation of flow rate into the petiole (E). Leaves were supported abaxial surface down above a box fan using a wood frame strung with fishing line. Leaves were either illuminated by ambient irradiance (<3 μmol photons m^{-2} s^{-1} PAR), or by a light source suspended above a Pyrex container filled with water (1000–1500 μmol photons m^{-2} s^{-1} PAR). For each species, 10–16 leaves were measured at each irradiance. The leaf temperature was maintained between 23 and 27 °C. Typically, leaves were left to transpire at least 30 min and until flow rate stabilized with a coefficient of variation <5% for at least 3–5 min (times ranged up to 2 h). The average stable flow rate was recorded, and within 5 s the leaf was removed from the tubing, the petiole was dabbed dry and the leaf was placed into a Whirl-Pak bag that had been previously exhaled in, and Ψ_{leaf} was measured after at least 10 min equilibration. K_{leaf} was calculated as $E/\Delta\Psi_{leaf}$ (where $\Delta\Psi_{leaf} = -\Psi_{leaf}$), normalized by leaf area and standardized to 25 °C to correct for changes induced by the temperature dependence of water viscosity (Weast 1974; Yang & Tyree 1993; Sack *et al.* 2002). Leaves from *Heteromeles arbutifolia* reached stability within 15 min on average, and were taken out of the EFM just after stabilization. To confirm that the leaves had sufficient light acclimation time during that shorter period, and to test whether flow time might affect K_{leaf}, measurements were made of K_{leaf} after 15 and 30 min in the EFM for *H. arbutifolia*; no significant difference was found (t-test, $P = 0.86$; $n = 8$). Further, no relationship was found between flow time and K_{leaf} for any species ($r_s = -0.18$ to 0.46; $P > 0.20$; $n = 11$–14).

Statistical analysis

To test potential time-related differences in Ψ_{leaf} equilibration within a shoot for all species combined, two-way analyses of variance (ANOVAS) were applied, with factors species and time [all statistics performed using Minitab Release 15 (Minitab, State College, PA, USA)]; and for individual species, one-way ANOVAS and Tukey pairwise comparisons were used (Sokal & Rohlf 1995). To test the impact of species and light on τ and K_{leaf}, two-way ANOVAS were used for all species combined, and t-tests were used for each species.

Differences in K_{leaf} values from the two methods were evaluated by three-way ANOVA, with factors species, method and light treatment. To test whether K_{leaf} values from the two methods were correlated across species, we determined Spearman and Pearson coefficients (r_s and r_p; Sokal & Rohlf 1995).

To test whether heterobaric and homobaric species differed in their K_{leaf} light response, we calculated a response index, K_{leaf} in high/low irradiance. We tested whether the light response was significant for each leaf type using t-tests (testing difference from 0 after log transformation), and we tested whether the leaf types differed using one-way ANOVA.

To improve normality and heteroscedasticity, data were log-transformed for ANOVAS, and for τ data assessed with t-tests (Sokal & Rohlf 1995).

RESULTS

Tests of sufficient equilibration time for the RKM

We modified the RKM such that during equilibration partially desiccated shoots de-acclimated a short time outside of high irradiance, and we tested whether such a short equilibration time would be sufficient. In the first experiment, dehydrated shoots of six species were equilibrated for 10, 15 and 20 min; the average Ψ_{leaf} for the equilibrated shoots ranged across species from –0.34 to –0.09 MPa, and the difference between the highest and the lowest Ψ_{leaf} on a shoot ($\Delta\Psi_{max}$) ranged from 0.07 to 0.14 MPa. We found no effect of equilibration time on $\Delta\Psi_{max}$ (two-way ANOVA for all species combined; species, $P = 0.03$; time, $P = 0.15$; species × time, $P = 0.62$; one-way ANOVA for each species, $P = 0.14$–0.94). In the second experiment, dehydrated shoots of the six species were equilibrated for 10, 60 and 120 min; the average Ψ_{leaf} for the equilibrated shoots ranged across species from –0.37 to –0.14 MPa, and $\Delta\Psi_{max}$ ranged from 0.07 to 0.15 MPa. The effect of equilibration time on $\Delta\Psi_{max}$ was not significant for all species considered together (two-way ANOVA; species, $P < 0.001$; time, $P = 0.06$; species × time, $P = 0.45$; Fig. 1). When testing each species individually, only *Raphiolepis indica* showed a slight difference between the treatments for $\Delta\Psi_{max}$ (one-way ANOVA; $P = 0.03$; Fig. 1), whereas the five other species did not ($P = 0.25$–0.998). Furthermore, for *R. indica* only the 10 and 120 min treatments were significantly different at $P = 0.024$ (Tukey tests); no significant differences were found between the 10 and 60 min treatments or between the 60 and 120 min treatments ($P = 0.23$–0.53). We concluded that the variation in Ψ_{leaf} among leaves on shoots equilibrated for 10 min after dehydration was typically the same as that for longer equilibration times and moderate enough to allow assessment of the effect of irradiance on K_{leaf}.

Evidence for light response of leaf hydraulic conductance from the RKM

Three species – *Alberta magna, Hedera canariensis* and *R. indica* – showed a strong light response in the RKM experiments. These species showed significantly higher τ in the dark relative to high irradiance (two-way ANOVA, testing impact of species and light treatment; species effect, $P < 0.001$; light, $P < 0.001$; interaction, $P = 0.07$). The light-response of τ ranged from twofold for *R. indica* to threefold for *A. magna* (t-tests; $P < 0.001$ to $P = 0.03$; Fig. 2). For the other three species – *Eucalyptus erythrocorys, H. arbutifolia* and *Hymenosporum flavum* – we found no significant differences in τ between dark and high irradiance (t-tests, $P = 0.33$ to 0.84; $n = 8$–15; Fig. 2). Associated with the light response of τ, three of the species showed a significant K_{leaf} light response in the RKM (Fig. 3; two-way ANOVA, species, $P < 0.001$; light, $P = 0.004$; interaction, $P = 0.03$). *A. magna, H. canariensis* and *R. indica* showed an increased K_{leaf} in high irradiance relative to darkness, by 1.7-fold, 1.5-fold and

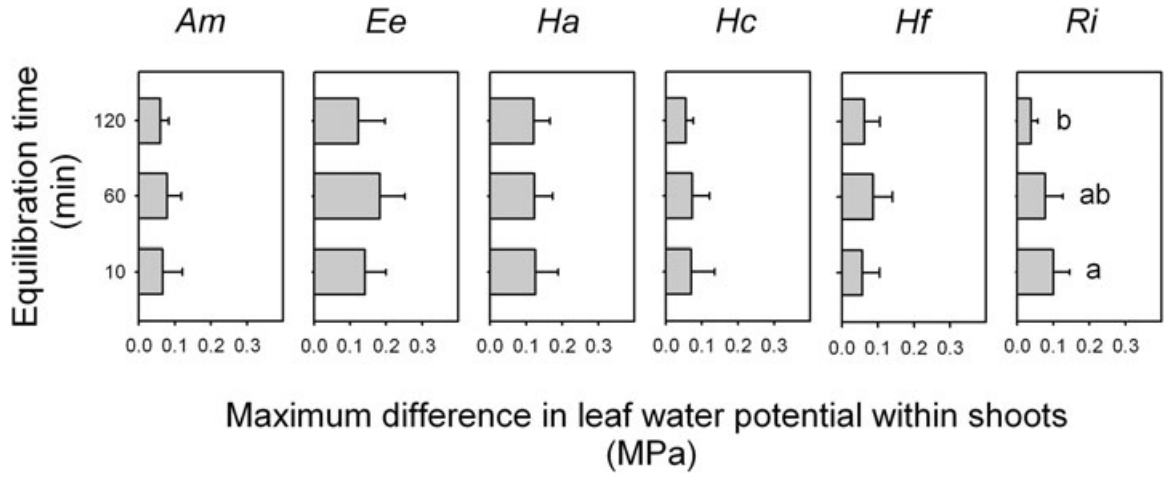

Figure 1. Maximum difference between leaves in water potential on partially dehydrated four-leaf shoots equilibrated for different times ($n = 5$–9). Different letters indicate $P < 0.05$ (Tukey pairwise contrasts). *Am*, *Alberta magna*; *Hc*, *Hedera canariensis*; *Ri*, *Raphiolepis indica*; *Hf*, *Hymenosporum flavum*; *Ha Heteromeles arbutifolia*; *Ee*, *Eucalyptus erythrocorys*.

2.5-fold, respectively (*t*-tests, $P = 0.003$ to 0.04; $n = 10$–12), whereas the other species showed no significant differences ($P = 0.49$–0.94; $n = 8$–15).

Evidence for light response of leaf hydraulic conductance from the EFM

When tested using the EFM, all six species showed higher flow rates (E) under high versus low irradiance: under low irradiance, species ranged 0.4 to 3 mmol m^{-2} s^{-1}, and under high irradiance 1.5 to 5 mmol m^{-2} s^{-1} (Table 2; two-way ANOVA; species $P < 0.001$; light, $P < 0.001$; species × light, $P = 0.01$). Similarly, the six species had higher $\Delta\Psi_{leaf}$ under high versus low irradiance; under low irradiance, species ranged 0.06 to 0.26 MPa, and under high irradiance, 0.11 to 0.55 MPa (Table 2; two-way ANOVA; species $P < 0.001$; light, $P < 0.001$; species × light, $P = 0.009$). Three species showed a strongly significant K_{leaf} light response (Fig. 3; two-way

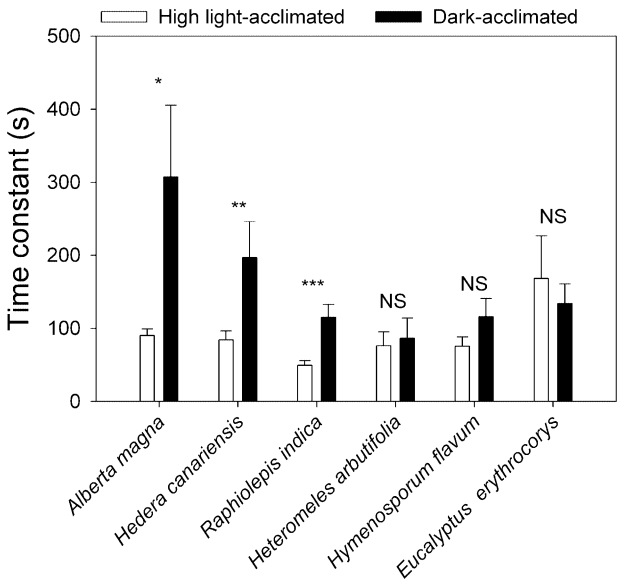

Figure 2. Mean ± standard error for rehydration time constants of leaves acclimated to high irradiance and darkness, for six evergreen species ($n = 9$–15). *0.05 > $P \geq 0.01$; **0.01 > $P \geq 0.001$; ***$P < 0.001$; NS, $P > 0.05$.

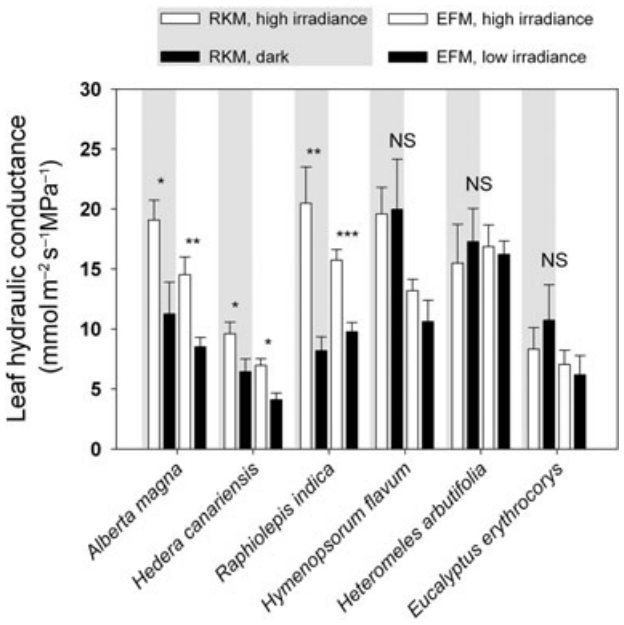

Figure 3. Mean ± standard error for leaf hydraulic conductance for six evergreen species acclimated to dark versus high irradiance using the rehydration kinetics method (RKM; gray background; $n = 9$–15; <0.1 versus 1000–1500 μmol photons m^{-2} s^{-1} photosynthetically active radiation; PAR) and in low versus high irradiance using the evaporative flux method (EFM; $n = 10$–16; <3 versus >1000 μmol photons m^{-2} s^{-1} PAR). Open bars represent high irradiance whereas filled bars represent low irradiance. *0.05 > $P \geq 0.01$; **0.01 > $P \geq 0.001$; ***$P < 0.001$; NS, $P > 0.05$.

Table 2. Mean parameters for measurements of leaf hydraulic conductance by the evaporative flux method, for leaves in low versus high irradiance (<3 versus >1000 μmol photons m^{-2} s^{-1} photosynthetically active radiation): flow rate (E) and water potential gradient force ($\Delta\Psi_{leaf}$); n = 10–16

Species	E in low irradiance (mmol m^{-2} s^{-1})	E in high irradiance (mmol m^{-2} s^{-1})	$\Delta\Psi_{leaf}$ in low irradiance (MPa)	$\Delta\Psi_{leaf}$ in high irradiance (MPa)
Alberta magna	0.68 ± 0.09	1.50 ± 0.12	0.09 ± 0.01	0.11 ± 0.01
Eucalyptus erythrocorys	0.42 ± 0.08	1.46 ± 0.54	0.11 ± 0.02	0.22 ± 0.05
Hedera canariensis	0.89 ± 0.13	3.55 ± 0.22	0.26 ± 0.05	0.55 ± 0.07
Heteromeles arbutifolia	3.02 ± 0.30	4.93 ± 0.25	0.22 ± 0.04	0.32 ± 0.91
Hymenosporum flavum	0.51 ± 0.05	1.69 ± 0.27	0.06 ± 0.01	0.15 ± 0.03
Raphiolepis indica	1.57 ± 0.21	3.93 ± 0.33	0.16 ± 0.02	0.26 ± 0.03

ANOVA; species, $P < 0.001$; light, $P < 0.001$; interaction, $P = 0.39$); *A. magna, H. canariensis* and *R. indica* showed a nearly twofold light response in K_{leaf} ($= E/\Delta\Psi_{leaf}$; t-tests, $P < 0.001$ to $P = 0.002$; n = 10–11), as they did in the RKM experiment. Although all six species showed increased E under high versus low irradiance (Table 2), in these three species, the difference in E was not matched by a proportional difference in $\Delta\Psi_{leaf}$ (Table 2). The other three species, *E. erythrocorys, H. arbutifolia* and *H. flavum*, showed no significant K_{leaf} light-response (t-tests, $P = 0.58$ to 0.78; n = 12 to 16).

Comparability of leaf hydraulic conductance and its light response across two methods

The two methods gave slightly different values for K_{leaf} (Fig. 3), but values were correlated under high irradiance ($r_s = 0.54$, $P = 0.27$; $r_p = 0.85$; $P = 0.03$) and under low irradiance ($r_s = 0.77$, $P = 0.07$; $r_p = 0.72$; $P = 0.11$). Across species, the RKM measurement of K_{leaf} under low irradiance was on average (±SE) 40 ± 16% higher than the EFM measurement, and 26 ± 8% higher under high irradiance. The methods did not significantly differ in their measured light responses (three-way ANOVA: species, $P < 0.001$; light, $P < 0.001$; method, $P = 0.02$; species × light, $P = 0.006$; species × method, $P = 0.08$; light × method, $P = 0.88$; species × light × method, $P = 0.55$).

Correlation of K_{leaf} light response with the presence of bundle sheath extensions

We found a linkage of the K_{leaf} light response with the presence of BSEs. We analysed the data from the literature, for which K_{leaf} light responses were determined at <10 and at >1000 μmol photons m^{-2} s^{-1} using the HPFM for 13 woody species (see legend of Fig. 4), and by the vacuum method for one species (*Hedera helix*). This analysis showed that heterobaric species had on average a twofold higher K_{leaf} light response than homobaric species ($P = 0.024$; t-test on log-transformed data; n = 9 and 5, respectively). We combined those published data with our new data for six species, resulting in a combined dataset for 20 species of temperate and tropical origin, with leaves of a range of sizes and textures. This combined data set achieved a greater and more even replication of the leaf types, and heterobaric species again had on average a twofold higher K_{leaf} light response than homobaric species ($P = 0.016$; n = 11 and 9, respectively; Fig. 4). We note that there were non-responsive heterobaric species, as well as responsive homobaric species; on average both groups showed a

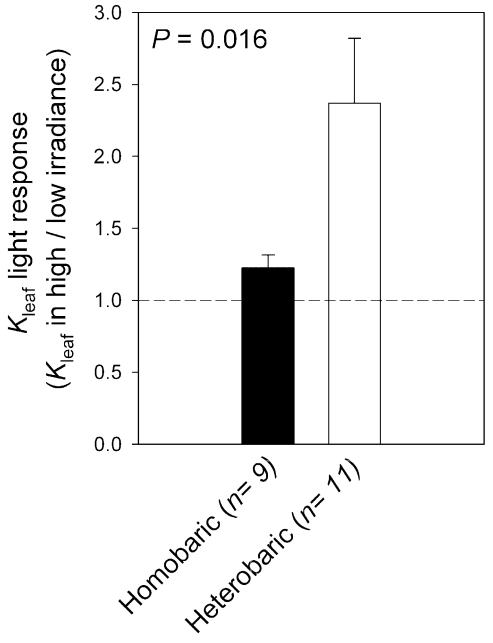

Figure 4. Mean ± standard error for K_{leaf} light response of woody species showing a twofold greater response for heterobaric than homobaric leaves (analysis of variance); the dotted line indicates zero response. Homobaric: *Hedera helix* (Sack *et al.* 2002), *Coffea arabica* (Gasco *et al.* 2004), *Dendropanax arboreus, Lindackeria laurina, Schefflera arboricola* (Tyree *et al.* 2005), *Alberta magna*, *Eucalyptus erythrocorys*, *Hedera canariensis* and *Hymenosporum flavum* (this study); heterobaric: *Quercus rubra* (Sack *et al.* 2002), *Acer pseudoplatanus, Acer saccharum, Calophyllum longifolium, Cercis siliquastrum, Cordia alliodora, Juglans regia, Miconia argentea, Q. rubra* (Tyree *et al.* 2005), *J. regia* (Cochard *et al.* 2007), *Q. macrocarpa* (Voicu *et al.* 2008), *Heteromeles arbutifolia* and *Raphiolepis indica* (this study). Data were averaged for species measured in multiple studies. Values were determined with high-pressure flow meter, except for *H. helix* (vacuum method) and species from this study (averaged across two methods).

significant light response of K_{leaf} ($P = 0.002$ and $P = 0.028$, respectively; one-sample *t*-tests). However, three times more heterobaric species than homobaric species showed a significant light-response of K_{leaf} (8 of 11 versus two of nine species; $P = 0.009$; confidence interval test).

DISCUSSION

We found new evidence demonstrating a rapid light response for K_{leaf}, in experiments with two of the most commonly used approaches for leaf hydraulics measurements, the EFM and RKM. Our work supports the K_{leaf} light effect as existing apart from any artifacts of the HPFM. This study increases the number of woody species tested by 50% (from 14 to 20), and as well as the number showing a strong (approximately twofold or higher) K_{leaf} light response (from four to seven). This broader demonstration of a strong, rapid light response of K_{leaf} has implications for measurement practices and for the pathways of water movement in the leaf and their relationship to leaf structure and to whole-plant performance.

The K_{leaf} light response as assessed using two measurement methods

The experiments showed an enhancement in K_{leaf} of 60% to 100% for three species. The light responses measured by the RKM and EFM were statistically similar, despite the differences in the low and high irradiance treatments. For the low irradiance treatment, we used darkness in the RKM and ambient light in the EFM. For the high irradiance treatment the RKM involved a de-acclimation period; the EFM did not. Our findings suggest that the K_{leaf} light response de-acclimates slowly from high to low irradiance, as shown by previous HPFM studies (Tyree *et al.* 2005; Cochard *et al.* 2007).

We note that our objective was to test for the K_{leaf} light response using additional methods to the HPFM. There is a need for future studies to compare quantitatively the response measured with the HPFM with that assessed by other methods (cf. Rockwell, Holbrook & Zwieniecki 2006). Precise comparisons of methods should best be conducted on the same days and ideally for leaves from the same individuals, to avoid temporal and plant-level variation in K_{leaf} (Lo Gullo *et al.* 2005; Voicu *et al.* 2008). Equally, precise comparison of methods would apply the same irradiance treatments across methods, which is complicated by the fact that the RKM requires a period of de-acclimation during leaf equilibration, and the EFM cannot be carried out in darkness. Our study included modifications to the RKM that can be applied to future methods comparisons.

We found that K_{leaf} values in low and high irradiance were comparable in the RKM and EFM, though slightly higher for the RKM, confirming previous research (Brodribb & Holbrook 2006). We note that because our study was designed to test for the maximum K_{leaf} light response that could be assessed with each method separately; it did not provide for an ideal comparison of the RKM and EFM, which would have used identical light treatments. Previous work has shown that the HPFM and EFM (as well as vacuum method) produce similar K_{leaf} values (within 10% to 30%) for given species, especially when leaves from given individuals were measured under the same conditions (Tsuda & Tyree 2000; Nardini *et al.* 2001, 2005; Sack *et al.* 2002; Trifilo *et al.* 2003; Cochard *et al.* 2007). By contrast with the HPFM (reviewed in Sack & Tyree 2005), no study to our knowledge has theoretically considered the differences in flow pathways between EFM and RKM, or how those might impact on measured K_{leaf}. One study has shown that rehydration pathways measured in the RKM may differ across species according to leaf anatomy. In 'uncompartmentalized leaves', water moves beyond the bundle sheath through the whole of the mesophyll, whereas in 'compartmentalized leaves', water flows principally through only certain tissues of the leaf (e.g. BSEs; Zwieniecki *et al.* 2007). For an uncompartmentalized leaf in which water evaporates evenly in the leaf airspaces, then the EFM and RKM may both measure transport along similar pathways to most leaf mesophyll cells and thereby produce similar K_{leaf} values. Furthermore, even for compartmentalized leaves, the RKM and EFM might theoretically yield similar values, with the K_{leaf} representing a flow rate disproportionate to the apparent $\Delta\Psi_{leaf}$ driving force. In the EFM, the $\Delta\Psi_{leaf}$ used to calculate K_{leaf} is lower than the true driving force, as it is based on the bulk Ψ_{leaf}, a volume-weighted average for the relatively few cells of low water potential in the compartment in which water is moving and evaporating, and for the more numerous cells of high water potential in the compartment isolated from the transpiration stream. In the RKM, the K_{leaf} could also represent a flow rate disproportionate to the true driving force, as the leaf is equilibrated before rehydration, and thus water flows to all cells of the leaf, i.e. many more than those in which water would move and evaporate during transpiration.

Beyond this theoretical discussion, the RKM may yield a slightly higher K_{leaf} than the EFM for a practical reason. The RKM is based on measurement of rehydration time t – the time the petiole is held under water; however, rehydration of cells continues after the leaf is removed from water and equilibrated. The underestimation of t would lead to an overestimation of K_{leaf} (Eqns 1 and 2) for the RKM relative to the EFM values, even if the flow pathways were the same.

Implications for future measurements of K_{leaf} and its responses to external factors

Our finding that the EFM and RKM are both sensitive to irradiance should inform future measurements. Previous studies using the RKM have at most reported acclimating leaves to high irradiance only during rehydration itself (<2 min), which had a negligible effect for 11 species (Brodribb & Holbrook 2003; Woodruff *et al.* 2007; Zwieniecki *et al.* 2007; Hao *et al.* 2008). However, we speculate that such a short acclimation might not drive the full light enhancement. For the EFM and RKM, as previously

recommended for the HPFM (Sack *et al.* 2002; Tyree *et al.* 2005), K_{leaf} should be studied in leaves acclimated to the appropriate irradiance, until a light response can be excluded for a given species. The need to consider irradiance extends to studies of leaf temperature responses (Cochard *et al.* 2007) and desiccation responses. A light response could theoretically influence the decline of K_{leaf} during desiccation, for two reasons. Part of the decline of K_{leaf} during desiccation may arise due to a linear loss of conductance in the extra-xylem tissues with turgor loss (Brodribb & Holbrook 2006; Kim & Steudle 2007). Because under high irradiance leaf parenchyma cells increase in hydraulic conductivity at a given turgor (Kim & Steudle 2007), the linear decline of K_{leaf} with turgor loss (Brodribb & Holbrook 2006) would be shifted upwards; light-acclimated leaves would thus show lower vulnerability (as assessed, for example, by an increase of Ψ_{leaf} corresponding to a 50% loss of K_{leaf}). On the other hand, part of the decline of K_{leaf} during desiccation may be caused by xylem cavitation. Because the K_{leaf} light response is located in the extra-xylem pathways, leaves acclimated to high irradiance, with a lower extra-vascular resistance (Nardini *et al.* 2005; Voicu *et al.* 2008), would show a relatively greater loss of K_{leaf} from a given episode of xylem cavitation; light-acclimated leaves would thus show a greater vulnerability. The two mechanisms for K_{leaf} declines during desiccation – turgor-loss and cavitation-induced declines – may thus be affected in opposite directions by light acclimation. Such interactions of K_{leaf} light and desiccation responses require explicit study, as they would suggest important and complex impacts in the field, given high variability in leaf-level irradiance, water supply and evaporative load.

Species-differences in the K_{leaf} light response

Three out of six studied species showed a significant K_{leaf} light response. This finding is consistent with seven of 14 tested woody species being responsive in HPFM studies, plus sunflower (Gasco *et al.* 2004; Nardini *et al.* 2005; Tyree *et al.* 2005; Cochard *et al.* 2007; Voicu *et al.* 2008). The K_{leaf} light response has been reported so far in 11 species of eight genera and eight families. In three families (Araliaceae, Rosaceae and Rubiaceae) and even in one genus (*Hedera*), there were cases of one species having a light response and another not; the light response may be evolutionarily labile. Further work is needed to determine the causes of species differences. Previous work showed that the K_{leaf} light response is located in the extra-xylem compartment, as cutting leaf minor veins during HPFM measurements – such that water avoids the extra-xylem pathways – removed the light response (Nardini *et al.* 2005; Voicu *et al.* 2008). The bundle sheath cells may be the locus for the light responses of K_{leaf}, and thus the response may involve increasing the conductivity of the bundle sheath and/or the downstream mesophyll via aquaporin expression (Sack & Holbrook 2006; Kim & Steudle 2007). Further, the light response may involve changes in the flow pathways outside the bundle sheath themselves, i.e. from apoplastic flow under low irradiance to cell-to-cell flow under high irradiance (Cochard *et al.* 2007). Species differences may thus arise from variation in the partitioning of resistance in xylem and extra-xylem pathways, and additionally may correlate with anatomical differences as well as differences in aquaporin activity (see e.g. Nardini *et al.* 2005; Tyree *et al.* 2005; Brodribb, Feild & Jordan 2007; Cochard *et al.* 2007; Zwieniecki *et al.* 2007).

Stronger K_{leaf} light response in heterobaric than homobaric leaves

In our combined dataset of K_{leaf} light responses for 20 woody species we found that twice as many heterobaric as homobaric species showed a significant response, and – on average – heterobaric species showed a twofold higher response. Along with their other functions in physiology and mechanical support, BSEs guide visible light to the mesophyll (Karabourniotis *et al.* 2000; Nikolopoulos *et al.* 2002; Kenzo *et al.* 2007), and possibly, also to the locus of the K_{leaf} light response. Alternatively, BSEs and the light response may be functionally rather than structurally linked. In heterobaric leaves, the BSEs may be an important route for water to flow from minor veins to mesophyll and/or epidermis evaporation sites (Wylie 1952; Zwieniecki *et al.* 2007). Such leaves may have stomata more closely equilibrated with the xylem during transpiration and thus could operate at close to critical xylem pressures for dysfunction; these leaves may especially benefit by modulating flow according to irradiance. By contrast, leaves without BSEs would have their epidermis relatively hydraulically isolated from the xylem, and would tend to close stomata well before xylem pressures reach a critical stage (Zwieniecki *et al.* 2007); an extra-xylem light response would destabilize such a protective mechanism. We note that heterobaric leaves are found typically among temperate deciduous species, as well as among tropical evergreen forest canopy trees, whereas homobaric leaves are common for tropical evergreen forest understory species (McClendon 1992; Kenzo *et al.* 2007). Tests are needed of whether these functional groups also differ in their K_{leaf} light response.

Need for future studies

Given the demonstration of a K_{leaf} light response using multiple measurement methods, future work should increase the elucidation of its characteristics, including parameterizing light-response curves, measurement of the action spectrum across wavelengths and the time course of K_{leaf} light/dark transitions; HPFM studies show that de-acclimation in darkness is typically slower than acclimation to high irradiance (Nardini *et al.* 2005; Tyree *et al.* 2005; Cochard *et al.* 2007; Voicu *et al.* 2008). Studies are also needed of the functional implications of the K_{leaf} light response. The response could improve water supply to the mesophyll of illuminated foliage, allowing increased stomatal aperture and leaf gas exchange, at the same time

minimizing the decline in Ψ_{leaf} (Sack & Holbrook 2006; Cochard *et al.* 2007; Voicu *et al.* 2008). However, the advantage of the K_{leaf} light response may be counteracted by the decline of K_{leaf} with desiccation under high transpiration loads. This interaction may be responsible for the fact that some species show midday increases in K_{leaf} (Tsuda & Tyree 2000; Lo Gullo *et al.* 2005) and others show declines (Nardini, Salleo & Raimondo 2003; Brodribb & Holbrook 2004; Sack & Holbrook 2006). This dynamic at the nexus of plant light and water relations merits further study.

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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.

Brodribb T.J. & Holbrook N.M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132,** 2166–2173.

Brodribb T.J. & Holbrook N.M. (2004) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant, Cell & Environment* **27,** 820–827.

Brodribb T.J. & Holbrook N.M. (2006) Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. *Plant, Cell & Environment* **29,** 2205–2215.

Brodribb T.J., Feild T.S. & Jordan G.J. (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144,** 1890–1898.

Cochard H., Venisse J.S., Barigah T.S., Brunel N., Herbette S., Guilliot A., Tyree M.T. & Sakr S. (2007) Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiology* **143,** 122–133.

Gasco A., Nardini A. & Salleo S. (2004) Resistance to water flow through leaves of *Coffea arabica* is dominated by extra-vascular tissues. *Functional Plant Biology* **31,** 1161–1168.

Hao G.Y., Hoffmann W.A., Scholz F.G., Bucci S.J., Meinzer F.C., Franco A.C., Cao K.F. & Goldstein G. (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* **155,** 405–415.

Henzler T., Waterhouse R.N., Smyth A.J., Carvajal M., Cooke D.T., Schaffner A.R., Steudle E. & Clarkson D.T. (1999) Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* **210,** 50–60.

Karabourniotis G., Bornman J.F. & Nikolopoulos D. (2000) A possible optical role of the bundle sheath extensions of the heterobaric leaves of *Vitis vinifera* and *Quercus coccifera*. *Plant, Cell & Environment* **23,** 423–430.

Kenzo T., Ichie T., Watanabe Y. & Hiromi T. (2007) Ecological distribution of homobaric and heterobaric leaves in tree species of Malaysian lowland tropical rainforest. *American Journal of Botany* **94,** 764–775.

Kim Y.X. & Steudle E. (2007) Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of Zea mays. *Journal of Experimental Botany* **58,** 4119–4129.

Lo Gullo M.A., Nardini A., Trifilo P. & Salleo S. (2005) Diurnal and seasonal variations in leaf hydraulic conductance in evergreen and deciduous trees. *Tree Physiology* **25,** 505–512.

McClendon J.H. (1992) Photographic survey of the occurrence of bundle sheath extensions in deciduous dicots. *Plant Physiology* **99,** 1677–1679.

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.

Nardini A., Salleo S. & Raimondo F. (2003) Changes in leaf hydraulic conductance correlate with leaf vein embolism in *Cercis siliquastrum* L. *Trees-Structure and Function* **17,** 529–534.

Nardini A., Salleo S. & Andri S. (2005) Circadian regulation of leaf hydraulic conductance in sunflower (*Helianthus annuus* L. cv Margot). *Plant, Cell & Environment* **28,** 750–759.

Nikolopoulos D., Liakopoulos G., Drossopoulos I. & Karabourniotis G. (2002) The relationship between anatomy and photosynthetic performance of heterobaric leaves. *Plant Physiology* **129,** 235–243.

Rockwell F.E., Holbrook N.M. & Zwieniecki M.A. (2006) The hydraulics of red oak leaves: is there room for a light response? Abstract from *Biology of Transpiration* meeting of the American Association of Plant Biologists. http://www.aspb.org/meetings/Transpiration06/justabstracts.cfm

Sack L. & Holbrook N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology* **57,** 361–381.

Sack L. & Tyree M.T. (2005) Leaf hydraulics and its implications in plant structure and function. In *Vascular Transport in Plants* (eds N.M. Holbrook & M.A. Zweiniecki), pp. 93–114. Elsevier/Academic Press, Oxford, UK.

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.

Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* **26,** 1343–1356.

Sellin A. & Kupper P. (2007) Temperature, light and leaf hydraulic conductance of little-leaf linden (*Tilia cordata*) in a mixed forest canopy. *Tree Physiology* **27,** 679–688.

Sellin A., Õunapuu E. & Kupper P. (2008) Effects of light intensity and duration on leaf hydraulic conductance and distribution of resistqance in shoots of silver birch (*Betula pendula*). *Physiologia Plantarum* (in press).

Sober A. (1997) Hydraulic conductance, stomatal conductance, and maximal photosynthetic rate in bean leaves. *Photosynthetica* **34,** 599–603.

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

Trifilo P., Gasco A., Raimondo F., Nardini A. & Salleo S. (2003) Kinetics of recovery of leaf hydraulic conductance and vein functionality from cavitation-induced embolism in sunflower. *Journal of Experimental Botany* **54,** 2323–2330.

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.

Tyree M.T. & Hammel H.T. (1972) Measurement of turgor pressure and water relations of plants by pressure bomb technique. *Journal of Experimental Botany* **23,** 267.

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

Tyree M.T., Nardini A., Salleo S., Sack L. & El Omari B. (2005) The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response? *Journal of Experimental Botany* **56,** 737–744.

Voicu M.C.Z., Zwiazek J.J. & Tyree M.T. (2008) Light response of hydraulic conductance in bur oak (*Quercus macrocarpa*) leaves. *Tree Physiology* **28,** 1007–1015.

Weast R.C., ed. (1974) *Handbook of Chemistry and Physics* 54th edn, CRC Press, Cleveland, OH, USA.

Woodruff D.R., McCulloh K.A., Warren J.M., Meinzer F.C. & Lachenbruch B. (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas fir. *Plant, Cell & Environment* **30,** 559–569.

Wylie R.B. (1952) The bundle sheath extension in leaves of dicotyledons. *American Journal of Botany* **39,** 645–651.

Yang S.D. & Tyree M.T. (1993) Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. *Tree Physiology* **12,** 231–242.

Zwieniecki M.A., Brodribb T.J. & Holbrook N.M. (2007) Hydraulic design of leaves: insights from rehydration kinetics. *Plant, Cell & Environment* **30,** 910–921.

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