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Combined impacts of irradiance and dehydration on leaf hydraulic conductance: insights into vulnerability and stomatal control

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ABSTRACT

The leaf is a hydraulic bottleneck, accounting for a large part of plant resistance. Thus, the leaf hydraulic conductance (K_{leaf}) is of key importance in determining stomatal conductance (g_s) and rates of gas exchange. Previous studies showed that K_{leaf} is dynamic with leaf water status and irradiance. For four species, we tested the combined impacts of these factors on K_{leaf} and on g_s. We determined responses of K_{leaf} and g_s to declining leaf water potential (Ψ_{leaf}) under low and high irradiance (<6 and >900 μmol photons m^{-2} s^{-1} photosynthetically active radiation, respectively). We hypothesized greater K_{leaf} vulnerability under high irradiance. We also hypothesized that K_{leaf} and g_s would be similar in their responses to either light or dehydration: similar light-responses of K_{leaf} and g_s would stabilize Ψ_{leaf} across irradiances for leaves transpiring at a given vapour pressure deficit, and similar dehydration responses would arise from the control of stomata by Ψ_{leaf} or a correlated signal. For all four species, the K_{leaf} light response declined from full hydration to turgor loss point. The K_{leaf} and g_s differed strongly in their light- and dehydration responses, supporting optimization of hydraulic transport across irradiances, and semi-independent, flexible regulation of liquid and vapour phase water transport with leaf water status.

Key-words: hydraulic resistance; light; soil–plant–atmosphere continuum.

INTRODUCTION

Plant hydraulic resistance is a major constraint on gas exchange and drought responses (Salleo *et al*. 2001; Tyree & Zimmermann 2002; Brodribb & Holbrook 2003; Sack & Holbrook 2006; Brodribb 2009), and the leaf is an important bottleneck, accounting for a large part of plant resistance (30% on average; Sack *et al*. 2003a). The leaf hydraulic conductance (K_{leaf} = the flow rate through the leaf for a given water potential gradient, i.e. 1/resistance) is thus of key importance in determining maximum rates of gas exchange and their declines during drought (Sack & Holbrook 2006). When stomata open for photosynthesis, water is lost via transpiration, and K_{leaf} needs to remain high to prevent the tissue water potential from declining enough to trigger a decline in stomatal conductance (g_s; Tyree & Zimmermann 2002). The K_{leaf} is dynamic in response to internal and external factors, notably including leaf water potential (Ψ_{leaf}) and irradiance (e.g. Nardini, Tyree & Salleo 2001; Sack & Tyree 2005; Sack & Holbrook 2006; Blackman, Brodribb & Jordan 2009; Johnson *et al*. 2011). The aim of this study was to clarify their combined impacts on K_{leaf} and coordination with g_s.

The K_{leaf} is determined by water transport pathways through multiple components: water moves through petiole and vein xylem, and bundle sheath and mesophyll tissue before evaporating through the stomata. The decline of K_{leaf} with Ψ_{leaf} may be caused by losses in conductivity in one or more components (e.g. Kikuta *et al*. 1997; Brodribb & Holbrook 2003; Brodribb & Cochard 2009; Johnson *et al*. 2009a; Scoffoni *et al*. 2011b). The decline is due at least in part to vein xylem cavitation and/or collapse (Milburn 1966; Crombie, Milburn & Hipkins 1985; Kikuta *et al*. 1997; Salleo *et al*. 2000; Cochard *et al*. 2004; Johnson *et al*. 2009a; Scoffoni *et al*. 2011b), and/or to loss of cell turgor and reduced aquaporin activity and potentially by emptying of water-filled cell wall pores in the bundle sheath and the mesophyll (Johansson *et al*. 1998; Koroleva *et al*. 2002; Brodribb & Holbrook 2006; Kim & Steudle 2007; Pieruschka, Huber & Berry 2010; Nardini *et al*. 2010a; Shatil-Cohen, Attia & Moshelion 2011).

The K_{leaf} also responds rapidly to irradiance. Experiments using the high-pressure flow meter (HPFM) showed an up to eightfold light enhancement of K_{leaf} within 30 min in 8 of 16 tested species, caused by an increase in conductance of pathways outside the xylem (Sack *et al*. 2002; Gasco, Nardini & Salleo 2004; Nardini, Salleo & Andri 2005; Tyree *et al*. 2005; Cochard *et al*. 2007; Scoffoni *et al*. 2008; Voicu, Zwiazek & Tyree 2008; Gortan *et al*. 2009; Sellin *et al*. 2011; Voicu & Zwiazek 2011). Several authors proposed that the HPFM may be partly or totally responsible for this response, by opening new liquid flow pathways through the stomata, which open under high irradiance (Sack *et al*. 2002; Tyree *et al*. 2005; Rockwell, Holbrook & Zwieniecki 2011), and by causing anoxia under low irradiance but not

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for light-exposed leaves (Rockwell *et al*. 2011). However, several studies suggested the HPFM light response exists independently of the stomata, and is associated with aquaporin activation and/or expression (Nardini *et al*. 2005; Cochard *et al*. 2007; Voicu *et al*. 2008; Voicu, Cooke & Zwiazek 2009), as occurs in roots (Henzler *et al*. 1999; Almeida-Rodriguez, Hacke & Laur 2011; Sakurai-Ishikawa *et al*. 2011). Further, a light response of hydraulic conductivity was found in vein parenchyma cells of *Zea mays* (Kim & Steudle 2007), and a several-fold light enhancement of K_{leaf} was confirmed for several species using methods other than the HPFM [i.e. the evaporative flux method (EFM) and rehydration kinetics method (RKM); Cochard *et al*. 2007; Sellin & Kupper 2007; Scoffoni *et al*. 2008]

The interaction of K_{leaf} light and dehydration responses could have important implications for plant water transport. For example, a higher K_{leaf} under high irradiance may compensate for decline in K_{leaf} with leaf dehydration, allowing leaf water status and transpiration rate to be maintained with declining soil water status. We determined vulnerability curves (i.e. responses to Ψ_{leaf}) under low and high irradiance allowing tests of whether the response to irradiance varied with hydration, and whether the dehydration response varied with irradiance. Given previous demonstrations that cavitation drives K_{leaf} decline with dehydration, and that K_{leaf} light enhancement occurs in the extra-xylem tissues (Nardini *et al*. 2005), we hypothesized that high light-acclimated leaves, with greater relative allocation of resistance in the xylem, would be more sensitive to cavitation. Conversely, if the K_{leaf} decline with dehydration were due mainly to outside-xylem effects, low irradiance-acclimated leaves may be more sensitive.

Our second objective was to clarify the coordination of K_{leaf} and g_s. We expected the light-response of K_{leaf} might match or exceed that of g_s to maintain Ψ_{leaf} when stomata open under high irradiance, as predicted by Cochard *et al*. 2007). Further, we expected similar coordination of the declines of g_s and K_{leaf} in dehydrating leaves under both high and low irradiance. The decline of g_s in dehydrating leaves has been hypothesized to be driven by: (1) declines of bulk turgor associated with Ψ_{leaf}; (2) an acoustic signal related to xylem cavitation; or (3) the decline of water potential in specific cells, for example, in the epidermis or guard cells (Nardini & Salleo 2000, 2003; Salleo *et al*. 2000; Brodribb & Holbrook 2003; Buckley, Mott & Farquhar 2003; Trifilo *et al*. 2003b; Brodribb & Holbrook 2004a,b; Damour *et al*. 2010; Buckley, Sack & Gilbert 2011). These mechanisms would result in co-regulation of liquid and vapour phase water transport.

MATERIALS AND METHODS

Plant material

The study included four species diverse in phylogeny, origin and in their leaf size, texture and pressure–volume parameters (Table 1). All four species have previously been found to show a light enhancement of K_{leaf} (Nardini *et al*. 2005; Scoffoni *et al*. 2008), and two were found to have high vulnerability to dehydration (*H. canariensis* and *H. annuus*; Trifilo *et al*. 2003a; Scoffoni *et al*. 2011a,b).

Three shrub species (*A. magna*, *H. canariensis* and *R. indica*) were sampled in and around the campus of University of California, Los Angeles, CA, USA, from January to June 2011. Shoots were collected from 3 to 10 plants of each species. Leaves from sunflower (*H. annuus* var Sunspot; Botanical Interests, Broomfield, CO, USA) were collected from greenhouse plants grown from seeds in 3.6 L pots (average minimum, mean and maximum values for temperature on these benches were 21.1, 23.2and 26.0 °C; for humidity, 44, 51 and 59%). Sunflowers were irrigated every 2 d, with 200 to 250 mL L^{-1} 20:20:20 nitrogen : phosphorus : potassium; the irradiance measured at midday on a typical sunny day was up to 550 μmol photons m^{-2} s^{-1} and on average 300 μmol photons m^{-2} s^{-1} (LI-250 light meter; Li-Cor Biosciences, Lincoln, NE, USA). For all experiments, shoots with mature leaves from the most exposed branches, or whole sunflower axial shoots, were collected the night before measurement, re-cut under filtered water (0.22 mm Thornton 200 CR; Millipore, Molsheim, France) and rehydrated overnight.

Measurement of mid-day water status and stomatal conductance

Measurements were made of mid-day leaf water potential (Ψ_{leaf}) and stomatal conductance (g_s), on two sunny days for each species. On each measurement day, five to six leaves were measured from three to six plants of each species. The g_s was measured using a porometer (Delta-T Devices,

Table 1. Study species, family, location of origin and mean ± standard error values for leaf traits indicating their diversity in form and physiology, leaf area (*LA*), leaf mass per area (*LMA*), and pressure volume parameters, osmotic potential at full turgor (π_{ft}), turgor loss point (π_{tlp}), modulus of elasticity (ε) and capacitance (C)

Species	Family	Origin	*LA* (cm^2)	*LMA* ($g\ m^{-2}$)	π_{ft} (MPa)	π_{tlp} (MPa)	ε (MPa)	C (MPa^{-1})
Alberta magna	Rubiaceae	S. Africa	46.5 ± 1.25	144 ± 4.09	–1.39 ± 0.05	–1.97 ± 0.07	8.08 ± 0.17	0.086 ± 0.002
Hedera canariensis	Araliaceae	Europe	47.9 ± 2.77	88.5 ± 5.5	–1.16 ± 0.15	–2.06 ± 0.12	11.7 ± 1.08	0.053 ± 0.002
Helianthus annuus	Asteraceae	N. America	106 ± 3.08	56.2 ± 6.98	–0.88 ± 0.12	–1.09 ± 0.12	5.49 ± 0.79	0.16 ± 0.02
Raphiolepis indica	Rosaceae	China	14.8 ± 0.33	192 ± 9.5	–1.37 ± 0.07	–2.08 ± 0.11	11.5 ± 0.81	0.055 ± 0.006

Data for *LA* are from this study, and data for *LMA* and pressure volume parameters for these species were taken from Scoffoni *et al*. 2008, 2011b (n = 78–112 for *LA*; 21–28 for *LMA*, and 6 for pressure–volume curve parameters).

Cambridge, UK), and then the leaf was sampled for measurement of Ψ_{leaf}. The leaf was placed in a sealable bag (Whirl-Pak, Nasco, Fort Atkinson, WI, USA), which was previously exhaled into, such that high CO_2 and humidity would render transpiration negligible, and that bag was placed within a second bag with wet paper towel, and brought to the lab, for measurement with a pressure chamber (Plant Moisture Stress, Model 1000, Albany, OR, USA).

Measuring the light and dehydration responses of K_{leaf} with the EFM

K_{leaf} was determined using the EFM, as the ratio of steady-state transpirational flow rate (E, mmol m^{-2} s^{-1}) to the water potential driving force ($\Delta\Psi_{leaf}$, MPa), which was determined at the end of the measurement as the difference between the water at atmospheric pressure entering the petiole (i.e. 0 MPa relative pressure) and the steady-state Ψ_{leaf} (Ψ_{final}; Sack *et al.* 2002). K_{leaf} was determined for dehydrated leaves to produce vulnerability curves (Scoffoni *et al.* 2011a). Shoots were cut into segments with at least four leaves under deionized water and then bench-dehydrated to a range of Ψ_{leaf} values, using a fan when necessary. Dehydrated shoots were placed into a sealable bag as described earlier for leaves sampled for water potential. Shoots were allowed to equilibrate at least 20 min before two leaves were excised and measured for initial Ψ_{leaf} (Ψ_o) using a pressure chamber. If the difference in the Ψ_{leaf} of those two leaves was greater than 0.2 MPa, the shoot was discarded; for very dehydrated shoots, this range was extended to 0.3 MPa. The two other leaves (typically the middle leaves) were measured for K_{leaf} using the EFM under high and low irradiance.

Leaves were cut from the shoot with a fresh razor blade under ultrapure water (0.22 mm Thornton 200 CR; Millipore). The petiole was then rapidly connected to silicone tubing under water to prevent air entering the system. The tubing connected the leaf to a water source on a balance (models XS205 and AB265, ±10 μg; Mettler Toledo, Columbus, OH, USA) that logged data every 30 s to a computer for the calculation of flow rate through the leaf (E). Leaves were held adaxial surface upwards in wood frames strung with fishing line above a large box fan (Lakewood Engineering & Manufacturing, Chicago, IL, USA). 'High irradiance' leaves were illuminated with >900 μmol photons m^{-2} s^{-1} photosynthetically active radiation at the leaf surface by floodlights (model 73828 1000 W, 'UV filter'; Sears Roebuck, Hoffman Estates, IL, USA) suspended above a Pyrex container (Corning Incorporated, Corning, NY, USA) filled with water to absorb the heat of the lamp. The 'low irradiance' leaves received only ambient irradiance (<6 μmol photons m^{-2} s^{-1}).

Leaves were allowed to transpire on the apparatus for at least 30 min and until flow rate stabilized, with no upward or downward trend, and with a coefficient of variation <5% for at least ten measurements made at 30 s flow intervals. When flow rate was low (<40 μg s^{-1}), stability was determined with the same criterion, but across ten running averages of the last five 30 s intervals. Previous studies found these criteria to be sufficient for stabilization of E, Ψ_{leaf} and K_{leaf}; tests with seven species (including three from this study) of a wide range of leaf capacitance showed no relationship of K_{leaf} with measurement time after stable flow was established for any given species (Scoffoni *et al.* 2008; Pasquet-Kok, Creese & Sack 2010). Measurements were discarded if the flow rate failed to stabilize, or suddenly changed, either because of strong stomatal closure, leakage from the seal or blockage in the system by particles or air bubbles. Following stabilization of the flow rate, leaf temperature was recorded with a thermocouple (Cole-Parmer, Vernon Hills, IL, USA), and with few exceptions varied from 22 to 28 °C during the experiments. The final 10 flow rate measurements were averaged. The leaf was quickly removed from the tubing, the petiole was dabbed dry, and the leaf was collected for water potential measurement as described earlier; Ψ_{final} was determined following at least 20 min equilibration in the bag. K_{leaf} was calculated as $E/\Delta\Psi_{leaf}$ (where $\Delta\Psi_{leaf} = 0$ MPa $- \Psi_{final}$) and further normalized by leaf area measured with a leaf area meter (Li-Cor 3100 meter). To correct for changes induced by the temperature dependence of water viscosity, K_{leaf} values were standardized to 25 °C (Weast 1974; Yang & Tyree 1993; Sack *et al.* 2002).

Notably, when leaves are measured with the EFM, the stomata open (see the Results section), and dehydrated leaves may recover in Ψ_{leaf} before reaching steady state transpiration, such that Ψ_{final} is less negative than Ψ_o, or, alternatively, the transpiration rate may be sufficient for Ψ_{final} to be driven lower than Ψ_o. To construct vulnerability curves, K_{leaf} (always determined as as $E/-\Psi_{final}$) was plotted against whichever was lowest, Ψ_o or Ψ_{final} ('Ψ_{lowest}'), that is, the Ψ_{leaf} associated with the strongest dehydration experienced during the experiment, and each leaf was considered as a data point ('Ψ_{lowest}'; see Scoffoni *et al.* 2011a). For each species, at least five to six K_{leaf} values were obtained for each 0.5 MPa interval of Ψ_{lowest} from full hydration to strong dehydration (0.25 MPa intervals for *H. annuus*, which had a steeper vulnerability response).

Determining the rate of water uptake into leaf cells and/or airspaces of a hydrated leaf

We conducted additional experiments to validate the EFM for investigating the K_{leaf} light response. Rockwell *et al.* (2011) speculated that well-hydrated, low irradiance-acclimated leaves may not in fact transpire in the EFM but instead may take up water by infiltration into their airspaces and/or into turgid cells with expansible walls. Such uptake pathways would perhaps have a low conductivity, and result in a low K_{leaf}, whereas, by contrast, light-acclimated leaves would have open stomata, and would transpire at a high rate. According to this view, the light enhancement of K_{leaf} could in actuality thus represent a change of flow pathways, as an artefact of the method. To test this possibility, we determined for low irradiance-acclimated, well-hydrated leaves in the EFM whether: (1) stomatal conductance exceeded cuticular conductance, which would indicate that stomata were open (measured as described in following section); and (2) air-space or cell infiltration rather than transpiration could account for observed flows in the EFM. For five to nine leaves per species, we measured the rate of water uptake per leaf area for low irradiance-acclimated, well-hydrated, non-transpiring leaves (J_{area}) by connecting a leaf cut-off a rehydrated shoot to tubing running to a graduated cylinder of ultrapure water on a balance, and placing the leaf under water in a Pyrex dish under lab irradiance. The leaf in its water bath was raised 2 cm above the meniscus of the water in the graduated cylinder, as in the EFM, to ensure that the uptake was caused by capillarity or an osmotic driving force within the leaf rather than a positive pressure-driven flow as would have occurred if the water level were above the leaf. During measurement, the water bath temperature was measured each 3 min with a thermometer and maintained between 20 and 25 °C. Leaves were maintained on the system, making 30 s flow measurements for at least 30 min and until flow rate stabilized, with no upward or downward trend, and with a coefficient of variation of the measurements or of the running average of the last 10 measurements of <5%. Flow rates were normalized by leaf area, measured using a leaf area meter, and standardized to 25 °C as for leaves in the EFM. In case the J_{area} measurements might have been influenced by the leaves being submerged (e.g. by anoxia), a second set of measurements were performed on four to six leaves per species wrapped in moist paper towel within a sealable bag that had been previously exhaled in.

Measuring the light and dehydration responses of stomatal conductance

We determined the light and dehydration responses of stomatal conductance (g_s) with two different experiments on dehydrating shoots that had been rehydrated overnight (after Salleo *et al.* 2000). First, we used the flow data from the EFM K_{leaf} vulnerability curves to determine g_s responses for the leaves previously dehydrated to a range of Ψ_{leaf} and either rehydrated or further dehydrated while transpiring on the EFM. To determine the g_s, the final E was divided by the mole fraction vapour pressure deficit (VPD), derived from temperature and relative humidity (RH) measurements in the lab from a weather station that logged measurements each 5 min (HOBO Micro Station with Smart Sensors, Onset, Bourne, MA, USA), where mole fraction VPD = (1 − (RH × VP_{sat}))/101.3 kPa, and VP_{sat} is saturation vapour pressure determined using the Arden-Buck equation (Buck 1981). Across all measurement days, the mean of daily mean VPD and its standard deviation were 0.0203 and 0.00101 mol mol^{-1}, respectively. We plotted g_s against both the Ψ_{leaf} corresponding to the steady state leaf transpiration at the end of the EFM measurement (Ψ_{final}) and also against the lowest Ψ_{leaf} achieved either during the dehydration or the steady state measurement (Ψ_{lowest}).

As a second method, to determine g_s responses in dehydrating leaves without the impact of dehydration and rehydration, we conducted similar experiments using porometer measurements on bench-drying shoots (after Salleo *et al.* 2000). To achieve values for g_s at high Ψ_{leaf}, first, the end of a shoot that had been rehydrated overnight was sealed with cable ties into a water-filled bag (Whirl-Pak, Nasco). Shoots were held by a clamp on an aluminium-foil covered box, when necessary fixing leaves adaxial surfaces upwards with small pieces of lab tape. High and low irradiance treatments were applied as in the EFM, with leaf temperature maintained with few exceptions at 22–28 °C, assisted by a large box fan placed on the side circulating air around the shoot. Shoots were acclimated for at least 30 min before leaves were measured with a porometer (Delta-T Devices) or cut from the shoot with a fresh razor blade and dehydrated on the box to a range of Ψ_{leaf} values before measurement. When three to five consecutive porometer g_s values were the same, values were taken of g_s, temperature and irradiance. The leaf was then sampled for leaf water potential determination as described earlier, with measurement made following at least 20 min equilibration. For each species, at least 5–6 g_s values were obtained for each 0.5 MPa interval from full hydration to strong dehydration (0.25 MPa intervals for *H. annuus*, which had a steeper response). For each species, the five lowest g_s measurements made for dehydrated leaves under low irradiance were taken as the cuticular conductance (g_{min} = minimum epidermal conductance, i.e. when stomata are closed), with the exception of *H. canariensis*, which had such a low g_{min} as to be assigned 0 values by the porometer; for this species, the value was taken from gravimetric measurement previously published for the same plants (Scoffoni *et al.* 2011b).

Statistics

For the responses of K_{leaf} and g_s to Ψ_{leaf}, outlier tests were conducted for each 0.5 MPa interval (except 0.25 MPa intervals for *H. annuus*; Dixon test; Sokal & Rohlf 1995); zero to four outliers were removed from each curve (i.e. from a total of 27 to 68 points in given curves). For each species, we determined the functional response of K_{leaf} or g_s using maximum likelihood to select among four functions previously used in the literature (Scoffoni *et al.* 2011b): linear (K_{leaf} or $g_s = a\ \Psi_{leaf} + y_o$); sigmoidal $\left[K_{leaf} \text{ or } g_s = \frac{a}{1+e^{-\left(\frac{\Psi_{leaf}-x_0}{b}\right)}}\right]$; logistic $\left\{K_{leaf} \text{ or } g_s = a/\left[1+\left(\frac{\Psi_{leaf}}{x_0}\right)^b\right]\right\}$; and exponential ($K_{leaf}$ or $g_s = y_0 + ae^{-b\Psi_{leaf}}$). Curves were fitted using the *optim* function in R.2.9.2 (http://www.r-project.org; Burnham & Anderson 2002; Scoffoni *et al.* 2011a; our scripts are available on request). The maximum K_{leaf} (K_{max}) or g_s (g_{max}), and the Ψ_{leaf} at which K_{leaf} or g_s had declined by 80% (P_{80} of K_{leaf} or g_s) were determined using the fitted curve parameters. To test the significance of the dehydration-induced declines of g_s in response to Ψ_{final} and Ψ_{lowest} we determined Spearman correlation coefficients (r_s), which do not depend on the shape of the decline (Sokal & Rohlf 1995).

We tested for species- and treatment effects on the uptake of water into hydrated non-transpiring leaves (J_{area}) using analyses of variance (Minitab Release 15, State College, PA, USA; Sokal & Rohlf 1995).We tested the significance of the K_{leaf} light enhancement using *t*-tests.

RESULTS

Validation tests of the EFM for measurements of K_{leaf}

The EFM was used to quantify K_{leaf} for high- and low-irradiance acclimated leaves that had been dehydrated to a range of Ψ_{leaf} values from full turgor to below turgor loss point (π_{tlp}). Virtually all leaves had their stomata open and had established steady state transpiration during the EFM, as evidenced by the g_s exceeding the g_{min}, even for low irradiance-acclimated, well-hydrated leaves. For all species, the mean of the five highest values of g_s for low irradiance-acclimated leaves were 11- to 47-fold higher than g_{min}. Even previously dehydrated leaves tended to have open stomata on the EFM; for each species, the mean of the lowest values of g_s under low irradiance (i.e. for dehydrated leaves) were 1.6 to sixfold higher than g_{min} (Table 2). Only one to three severely dehydrated leaves of *A. magna* and *H. annuus* under low- and high irradiance had stomata closed during measurement (of $n = 23$ to 64).

Further, for well-hydrated leaves in the EFM the flow rates far exceeded J_{area}, that is, the flow rate determined for hydrated leaves placed under water or in a moist bag and taking up water through their petioles into lamina airspace or cells. In these experiments, J_{area} tended to decline, increase or oscillate before stabilizing after 12–72 min, and continuing uptake at a similar or decreased rate for at least 2 h. The J_{area} averaged for 30–35 min or for the 5 min after the first steady state were typically similar, with non-significant increases of 2–8% on average ($P > 0.05$). The J_{area} measured for bagged leaves did not differ from that measured for submerged leaves for either interval (analyses of variance; for species effect, $P < 0.001$ to 0.007; for treatment effect and species × treatment effect, $P = 0.11$–0.83). The J_{area} would not have impacted on flow measurements in the low irradiance-acclimated, well-hydrated leaves; the J_{area} for submerged or bagged leaves after 30–35 min were 2–5% of the E_{area} values for well-hydrated leaves for under low irradiance (Table 2). Thus, any uptake by well-hydrated leaves by capillarity into airspaces or by osmosis into expansible cells, even if it could be maintained during transpiration, would not affect the EFM measurement of flow rate.

The K_{leaf} response to dehydration in low versus high irradiance

For all four species, K_{leaf} decreased strongly with dehydration under low and high irradiance (Fig. 1). For all species, the logistic model best fitted the vulnerability curves under high and low irradiance except for *H. annuus* and *H. canariensis* under low irradiance, for which the linear model was selected over logistic by maximum likelihood (Fig. 1). The light enhancement of K_{leaf} was significant in all four species whether considering K_{leaf} data above –0.5 MPa, or above –1 MPa, or considering all the K_{leaf} data in the vulnerability curves (Table 3). The K_{leaf} light enhancement averaged for leaves >–0.5 MPa varied across species: 1.6-fold for *A. magna*, threefold for *R. indica,* 3.8-fold for *H. annuus*, and fivefold for *H. canariensis* (Table 3). In all species the light response was highest for well-hydrated leaves, and disappeared by turgor loss point (Fig. 1). In all species, the decline of K_{leaf} with dehydration was steeper under high irradiance, as indicated by a P_{80} that was less negative on average across species by 1.3 MPa ± 0.6 SE ($P = 0.03$, paired *t*-test on log-transformed data). At mid-day operating Ψ_{leaf}, the K_{leaf} light enhancement was 38% for *A. magna*,

Table 2. Data to test the effectiveness of the evaporative flux method, including low, mean and high values, separated by dashes (where low and high values are means for the five highest and five lowest values, i.e. for well-hydrated and previously-dehydrated leaves respectively) for flow rates per leaf area (E_{area}) under low and high irradiance (LL and HL, respectively), and stomatal conductance (g_s) determined for those leaves by dividing by the mole fraction vapour pressure deficit and mean ± standard error

Species	E_{area} LL	E_{area} HL	g_s LL	g_s HL	g_{min}	$J_{area\ (water)}$	$J_{area\ (bag)}$
Alberta magna	0.23–0.75–1.56	0.40–1.48–2.82	11.3–36.9–76.8	19.7–72.9–139	7.04 ± 0.32	0.042 ± 0.005	0.033 ± 0.002
Hedera canariensis	0.05–0.18–0.42	0.09–1.70–4.12	2.46–8.87–20.7	4.43–83.7–203	0.44 ± 0.03	0.018 ± 0.002	0.028 ± 0.003
Helianthus annuus	0.36–0.95–1.98	0.48–2.68–8.31	17.7–46.8–97.5	23.6–132–409	8.36 ± 0.55	0.024 ± 0.004	0.034 ± 0.006
Raphiolepis indica	0.21–0.92–2.11	0.3–2.01–3.70	10.3–45.3–104	16.2–99.0–182	5.78 ± 0.54	0.053 ± 0.014	0.055 ± 0.009

For each species, the five lowest g_s measurements made for dehydrated leaves under low irradiance were taken as the cuticular conductance (g_{min} = minimum epidermal conductance, i.e. when stomata are closed), with the exception of *H. canariensis*, which had such a low g_{min} as to be assigned 0 values by the porometer; for this species, the value was taken from gravimetric measurement previously published for the same plants (Scoffoni *et al.* 2011b). Values for the passive water uptake rate per leaf area by airspace infiltration or cell uptake for hydrated leaves after 30–35 min under water or in a bag (J_{area}). All units of mmol m^{-2} s^{-1}.

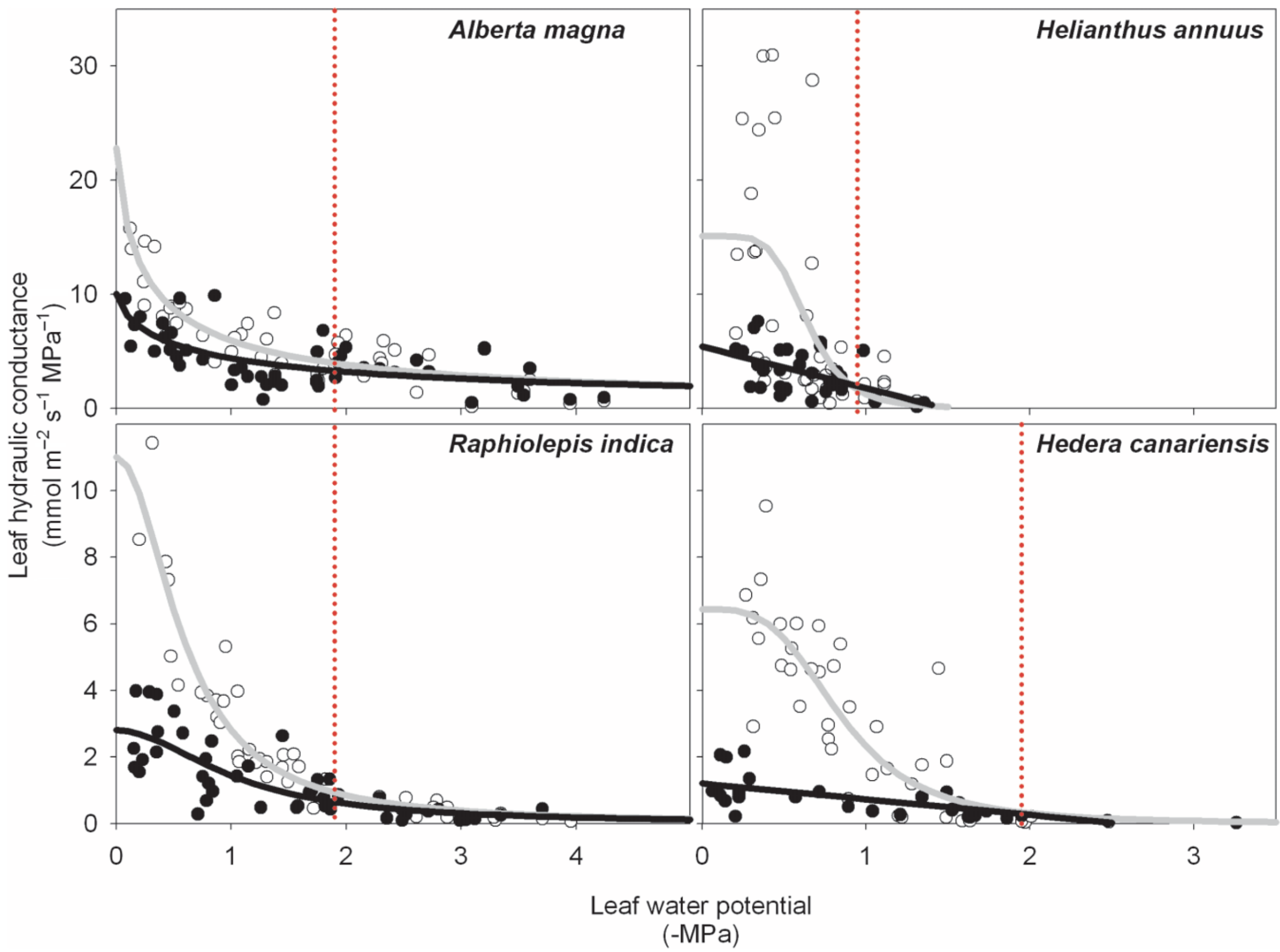

Figure 1. Decline of leaf hydraulic conductance (K_{leaf}) with decreasing leaf water potential (Ψ_{leaf}) in dehydrating leaves of four species under low and high irradiance (<6 μmol m^{-2} s^{-1} and >900 μmol m^{-2} s^{-1} photosynthetically active radiation; black and white symbols and black and grey lines, respectively). The K_{leaf} was determined as transpiration divided by the final leaf water potential in the evaporative flux method (EFM; see the Methods section). To present vulnerability responses, K_{leaf} was plotted against the lowest Ψ_{leaf} experienced by the leaf during bench drying and the EFM measurement. Fitted lines are the best-fit functions selected by maximum likelihood: for low and high irradiance respectively, for *A. magna*, $K_{leaf} = 10.1/\left[1+\left(\frac{\Psi_{leaf}}{0.71}\right)^{0.72}\right]$ ($n = 44$), and $K_{leaf} = 22.8/\left[1+\left(\frac{\Psi_{leaf}}{0.27}\right)^{0.8}\right]$ ($n = 47$); for *H. canariensis*, $K_{leaf} = -0.47\ \Psi_{leaf} + 1.2$ ($n = 27$) and $K_{leaf} = 6.44/\left[1+\left(\frac{\Psi_{leaf}}{0.85}\right)^{3.5}\right]$ ($n = 41$); for *H. annuus*, $K_{leaf} = -3.6\ \Psi_{leaf} + 5.4$ ($n = 35$) and $K_{leaf} = 15.1/\left[1+\left(\frac{\Psi_{leaf}}{0.64}\right)^{5.4}\right]$ ($n = 40$); and for *R. indica*, $K_{leaf} = 2.8/\left[1+\left(\frac{\Psi_{leaf}}{1.03}\right)^{1.96}\right]$ ($n = 47$) and $K_{leaf} = 11/\left[1+\left(\frac{\Psi_{leaf}}{0.59}\right)^{2.03}\right]$ ($n = 42$). $R^2 = 0.31$–0.91 ($P < 0.001$). The vertical red dotted line represents the turgor loss point (Table 1).

74% for *R. indica*, 248% for *H. annuus*, and 307% for *H. canariensis* (estimated from equations in Fig. 1, using Ψ_{leaf} data presented in Fig. 3).

The response of stomatal conductance to dehydration in low versus high irradiance

For all four species, g_s decreased strongly with dehydration in low and high irradiance, as assessed both from leaves from the EFM and from the separate porometry experiments on dehydrating shoots (Figs 2 & 3). Notably, the response of g_s to leaf dehydration in the EFM differed from that of K_{leaf} for leaves of each species, in both low and high irradiance (Fig. 1); K_{leaf} depended not only on transpiration rate (determined by g_s and laboratory VPD) but also on the leaf water potential during steady state (Ψ_{final}). The two experiments on stomatal responses (Figs 2 & 3) gave comparable results for the vulnerability of g_s to dehydration in high and low irradiance, with some differences as expected given their contrasting treatments. Thus, the two experiments were similar in showing that the g_s for each species was highly variable even at a given Ψ_{leaf}, especially for

Table 3. Statistical tests of the response of K_{leaf} to irradiance

Species	K_{leaf}, >–0.5 MPa LL, HL	K_{leaf}, >–1 MPa LL, HL	All data significance
Alberta magna	6.77 ± 0.504, 11.1 ± 1.05**	4.25 ± 0.356, 5.97 ± 0.549**	*
Hedera canariensis	1.20 ± 0.211, 5.97 ± 0.626***	1.05 ± 0.167, 4.59 ± 0.409***	***
Helianthus annuus	3.71 ± 0.536, 14.3 ± 2.60**	3.19 ± 0.341, 10.5 ± 1.85**	**
Raphiolepis indica	2.68 ± 0.335, 8.03 ± 1.03**	1.24 ± 0.161, 2.39 ± 0.398**	**

Mean ± standard error values for low versus high irradiance and *t*-tests, applied to data for $\Psi_{leaf} > -0.5$ MPa and for $\Psi_{leaf} > -1$ MPa, and significance of *t*-tests calculated across all data in the vulnerability curves. *$0.05 > P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$. We tested the data for low- versus high-irradiance acclimated leaves from the vulnerability curve: (1) for K_{leaf} data corresponding to Ψ_{lowest} values > –0.5 MPa; (2) for K_{leaf} data corresponding to Ψ_{lowest} values > –1.0 MPa; and (3) for all K_{leaf} data on the vulnerability curve.

well-hydrated leaves, for which stomata ranged from virtually shut to open at their maximum conductance, with a similar range of values in both experiments. Notably, for *H. canariensis* in low irradiance, most well-hydrated leaves (<–0.1 MPa) all had stomata very minimally open in both experiments, but by –0.5 MPa the stomata had opened, and subsequently g_s declined as leaves dehydrated. In the porometer experiment (Fig. 3), many well-hydrated leaves of *H. canariensis* had stomata totally shut; this result was not observed in the EFM experiment, because such leaves would have been abandoned after failing to establish a steady state rate of uptake. Further, in the EFM experiment, the Ψ_{leaf} values experienced were not low enough to cause a complete decline in g_s for some species, as the goal of the EFM was to sample K_{leaf} to its decline (Fig. 1) rather than a zero flow, which again would have led to abandonment of the leaf.

In the EFM experiment (Fig. 2), the plots of g_s against the Ψ_{leaf} established during steady state flow at the end of measurement (Ψ_{final}) tended to show only a weak correlation (Fig. 2, inset plots). By contrast, the plots of g_s against the lowest Ψ_{leaf} established during shoot dehydration and the EFM measurement (Ψ_{lowest}) tended to show strong correlations (Fig. 2, main plots). This pattern was caused by the Ψ_{lowest} values varying more strongly, as in many cases, the Ψ_{final} represented leaves that had rehydrated during EFM measurement. Additionally, for many leaves g_s was suppressed by previous dehydration even after Ψ_{leaf} recovered to Ψ_{final}. Thus, g_s during steady state transpiration showed a much stronger dependence on Ψ_{leaf} of dehydration than on final equilibrated Ψ_{final}.

Given the two experiments for determining g_s responses were broadly similar in their results, we used the porometer experiment to quantify stomatal dynamics independently of the K_{leaf} experiment, and with the advantage that it did not involve leaves rehydrating, but rather steady state transpiration at the lowest Ψ_{leaf} experienced, as each experiment began with well-hydrated shoots, and g_s and Ψ_{leaf} were sampled at a given stage of progressive dehydration. In the porometer experiment, strong declines of g_s were observed down to stomatal closure. The logistic model was the best-fit function for all species except for *H. annuus* in high irradiance, for which a linear model was selected by maximum likelihood for g_s against Ψ_{leaf} (Fig. 3). Determining the maximum g_s by extrapolating the fitted curve, the light enhancement of g_s for fully hydrated leaves ranged from nonsignificant for *H. annuus* to 91% for *A. magna*. Notably, for *H. annuus*, leaves in the dark declined in g_s more rapidly than under high irradiance, and P_{80} for g_s was less negative by 0.5 MPa. Thus, for semi-dehydrated leaves, g_s was lower in the dark than under high irradiance. For the other three species, the decline of g_s with dehydration was similar under high and low irradiance, as indicated by a similar P_{80}. Stomatal closure occurred at approximately π_{tlp} for *A. magna* and *H. annuus*, but well below π_{tlp} for *R. indica* and well above π_{tlp} for *H. canariensis* (Fig. 3). For *H. annuus*, stomatal opening was observed at Ψ_{leaf} values below π_{tlp}. Species' operating g_s values at mid-day Ψ_{leaf} were within the range determined in the lab for bench-dried shoots under high irradiance at that Ψ_{leaf} (Fig. 3, see points with star symbols).

Summarizing the effects of irradiance on hydraulic and stomatal parameters

Irradiance had strong effects on all variables measured for the four study species (Fig. 4). Thus, higher irradiance led to substantial increases in K_{leaf} and g_s for hydrated leaves (Fig. 4a–c) and K_{leaf}/g_s. For all species, the increase of K_{leaf} from low to high irradiance in well-hydrated leaves exceeded that of g_s, representing a light-induced increase of hydraulic supply relative to demand. However, in dehydrated leaves the K_{leaf} initially declined more rapidly than g_s, and in all species the K_{leaf}/g_s tended to decline during leaf dehydration. Thus, for well-hydrated leaves, the ratio K_{leaf}/g_s in high irradiance exceeded that in low irradiance, by 161% for *H. annuus*, by 217% for *H. canariensis*, and by 114% for *R. indica* (Figs 1, 2 & 4).

The stronger decline of K_{leaf} in dehydrating leaves under high than low irradiance (Fig. 1) was reflected in their less negative P_{80} for K_{leaf} (Fig. 4). By contrast, the decline of g_s in dehydrating leaves was similar under high and low irradiance, except in *H. annuus*, in which the stomata closed more

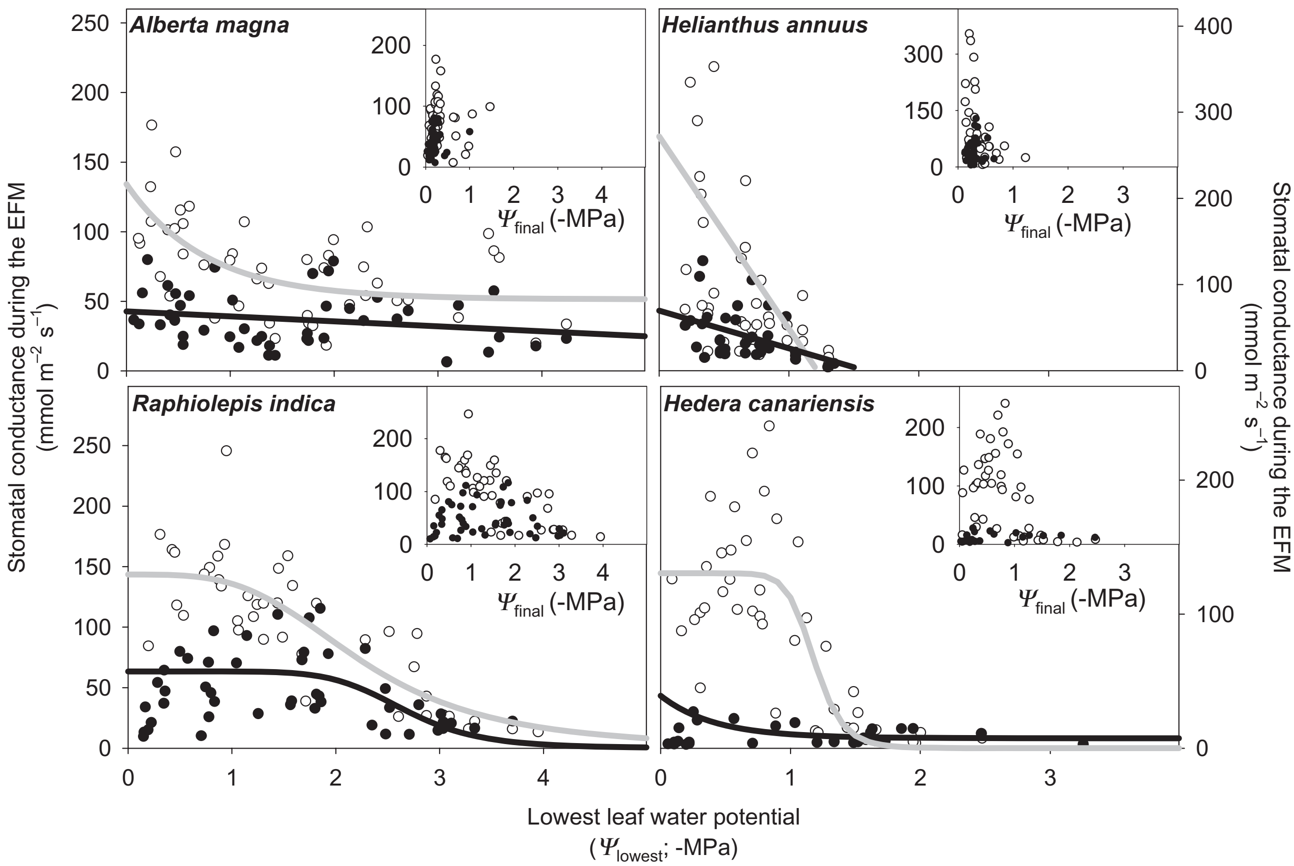

Figure 2. Decline of stomatal conductance (g_s) with decreasing leaf water potential (Ψ_{leaf}) determined using the evaporative flux method (EFM) in dehydrating leaves of four species under low and high irradiance (<6 μmol m^{-2} s^{-1} and >900 μmol m^{-2} s^{-1} photosynthetically active radiation; black and white symbols, respectively). The g_s was plotted against the lowest Ψ_{leaf} experienced by the leaf during bench drying and the EFM measurement (main plots), and against the final Ψ_{leaf} during steady state at the end of the EFM measurement (inset plots). While correlations in the inset plots were weak (Spearman coefficients significantly negative only for *H. canariensis* and *R. indica* in high irradiance; $r_s = -0.46$ to -0.72, $P < 0.001$ to 0.004), the correlations in the main plots were strong ($r_s = -0.44$ to -0.85, $P < 0.001$ to 0.008), with the exception of *A. magna* and *H. canariensis* and *R. indica* under low irradiance ($r_s = -0.22$ to 0.21; $P = 0.12$–0.30). Fitted lines are the best-fit functions selected by maximum likelihood: for low and high irradiance respectively, for *A. magna*, $g_s = -3.6\ \Psi_{leaf} + 43$ ($n = 44$), and $g_s = 51 + 83^{(-1.3\times\Psi_{leaf})}$ ($n = 47$); for *H. canariensis*, $g_s = 7.5 + 32^{(-2.4\times\Psi_{leaf})}$ ($n = 19$) and $g_s = 130/\left[1+\left(\frac{\Psi_{leaf}}{1.2}\right)^{11}\right]$ ($n = 41$); for *H. annuus*, $g_s = -43\ \Psi_{leaf} + 70$ ($n = 35$) and $g_s = -223\ \Psi_{leaf} + 272$ ($n = 40$); and for *R. indica*, $g_s = 63/\left[1+\left(\frac{\Psi_{leaf}}{2.7}\right)^{7.3}\right]$ ($n = 35$) and $g_s = 144/\left[1+\left(\frac{\Psi_{leaf}}{2.3}\right)^{3.5}\right]$ ($n = 42$). $R^2 = 0.20$–0.68 ($P < 0.005$) for all species except *A. magna* ($R^2 = 0.04$; $P > 0.05$) For *H. canariensis* and *R. indica* lines were fitted excluding points above −0.25 and −0.5 MPa respectively, because well-hydrated leaves tended to have minimally open stomata; excluding those points resulted in a significant response of g_s to Ψ_{leaf} for the remaining partially dehydrated leaves.

rapidly under low irradiance (Fig. 4), reflected in a less negative P_{80} of g_s (Fig. 4e). As a consequence of these differences, species varied in the trajectories of decline of K_{leaf} and g_s, and thus the way that hydraulic and stomatal conductance were coordinated between low and high irradiance. Under high irradiance, the P_{80} for K_{leaf} was close to that for g_s in *A. magna* and *H. annuus*, but in *H. canariensis* the P_{80} for g_s was less negative than that for K_{leaf}, and in *R. indica* the opposite pattern was observed, in which P_{80} for g_s was more negative than that for K_{leaf}. Under low irradiance, P_{80} for g_s was less negative than that for K_{leaf} for all species but *R. indica*, in which again P_{80} for g_s was more negative.

DISCUSSION

An interaction of the K_{leaf} light and dehydration responses

This study provided new resolution of dynamic responses of K_{leaf} to the environment. For well-hydrated leaves in each of

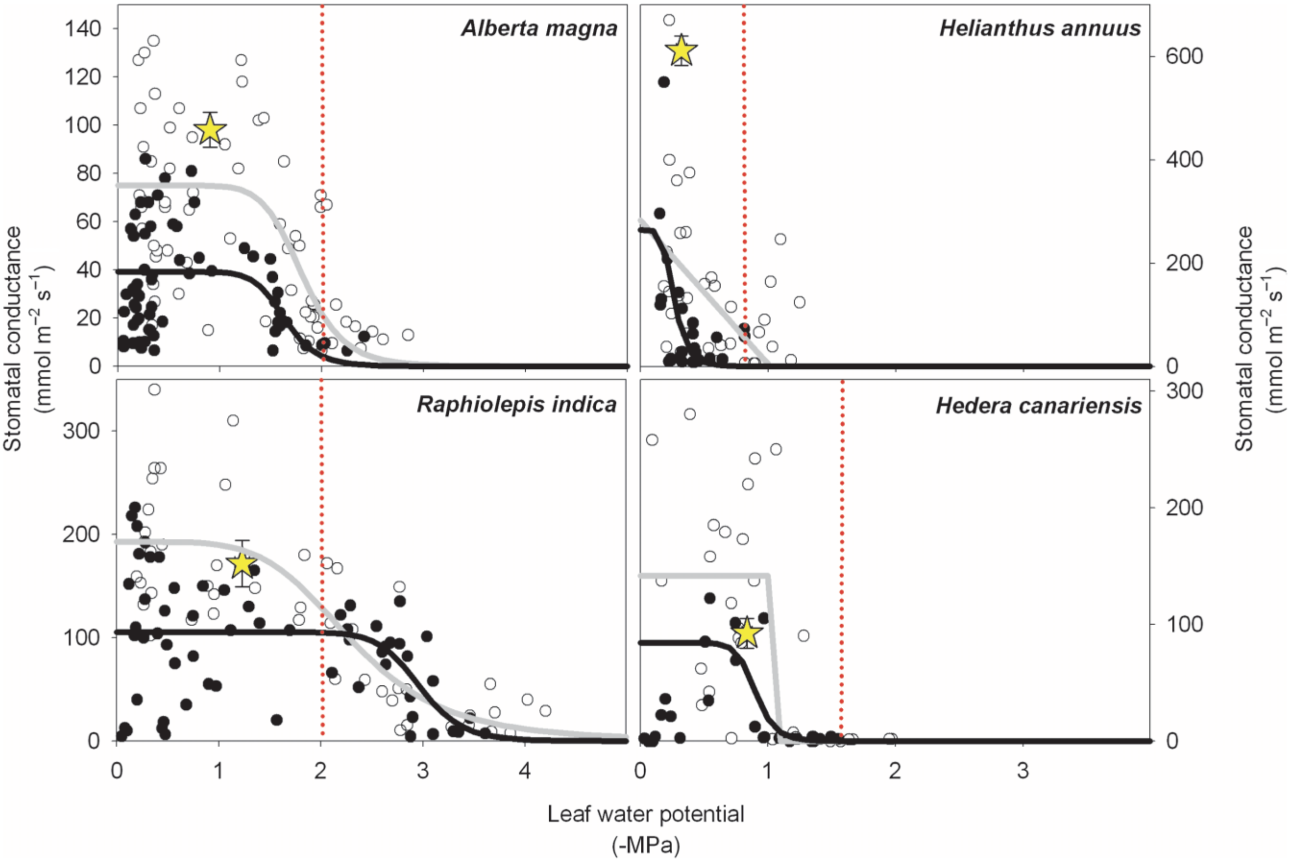

Figure 3. Decline of stomatal conductance (g_s) with decreasing leaf water potential (Ψ_{leaf}) as measured with a porometer in dehydrating leaves of four species under low and high irradiance (<6 μmol m^{-2} s^{-1} and >900 μmol m^{-2} s^{-1} photosynthetically active radiation; black and white symbols, respectively). Fitted lines are the best-fit functions selected by maximum likelihood: for low and high light respectively, for *A. magna* $g_s = 39.2/\left[1+\left(\frac{\Psi_{leaf}}{1.6}\right)^{10.5}\right]$ (n = 64) and $g_s = 75.1/\left[1+\left(\frac{\Psi_{leaf}}{1.8}\right)^{9}\right]$ (n = 40); for *H. canariensis* $g_s = 84.1/\left[1+\left(\frac{\Psi_{leaf}}{0.9}\right)^{11.8}\right]$ (n = 25) and $g_s = 142/\left[1+\left(\frac{\Psi_{leaf}}{1.04}\right)^{226}\right]$ (n = 32); for *H. annuus* $g_s = 264/\left[1+\left(\frac{\Psi_{leaf}}{0.26}\right)^{5.3}\right]$ (n = 26) and $g_s = -278\ \Psi_{leaf} + 283$ (n = 32); and for *R. indica* $g_s = 105/\left[1+\left(\frac{\Psi_{leaf}}{3.0}\right)^{14}\right]$ (n = 61) and $g_s = 193/\left[1+\left(\frac{\Psi_{leaf}}{2.3}\right)^{5}\right]$ (n = 47). R^2 = 0.15–0.66 (P < 0.002). The vertical red dotted line represents the turgor loss point (Table 1). Yellow stars indicate mean ± standard error values for Ψ_{leaf} and g_s measured on plants at mid-day on sunny days. For *H. canariensis* under LL, the line was fitted excluding points above –0.5 MPa, because well-hydrated leaves tended to have minimally open stomata; excluding those points resulted in a significant response of g_s to Ψ_{leaf} for the remaining partially dehydrated leaves.

the four species, K_{leaf} showed a light enhancement greater in magnitude than that of g_s. Further, the K_{leaf} light enhancement depended on leaf water status. Each of the four species showed a light response of K_{leaf} that was strongest for fully hydrated leaves but that declined during leaf dehydration, being negligible at turgor loss point. Put another way, the response of K_{leaf} to dehydration was stronger in high- than low-irradiance acclimated leaves. Comparative magnitudes and trajectories of responses of K_{leaf} and g_s to irradiance and dehydration indicated semi-independent responses to Ψ_{leaf}. These findings provide insights into the regulation of liquid and vapour phase water transport, with implications for whole plant function.

Confirming the K_{leaf} light effect and its importance in the EFM

The findings of this study further confirm a K_{leaf} light response that can be resolved using the EFM. Recently Rockwell *et al.* (2011) proposed that this K_{leaf} light response might be artifactual. If low irradiance-acclimated, well-hydrated leaves measured in the EFM had stomata closed and were instead taking up water by infiltration into their airspaces or into cells, via pathways with low hydraulic conductance, then the high K_{leaf} observed for light-acclimated leaves would not indicate a true light enhancement of the pathways of transpiration, but actually a change of

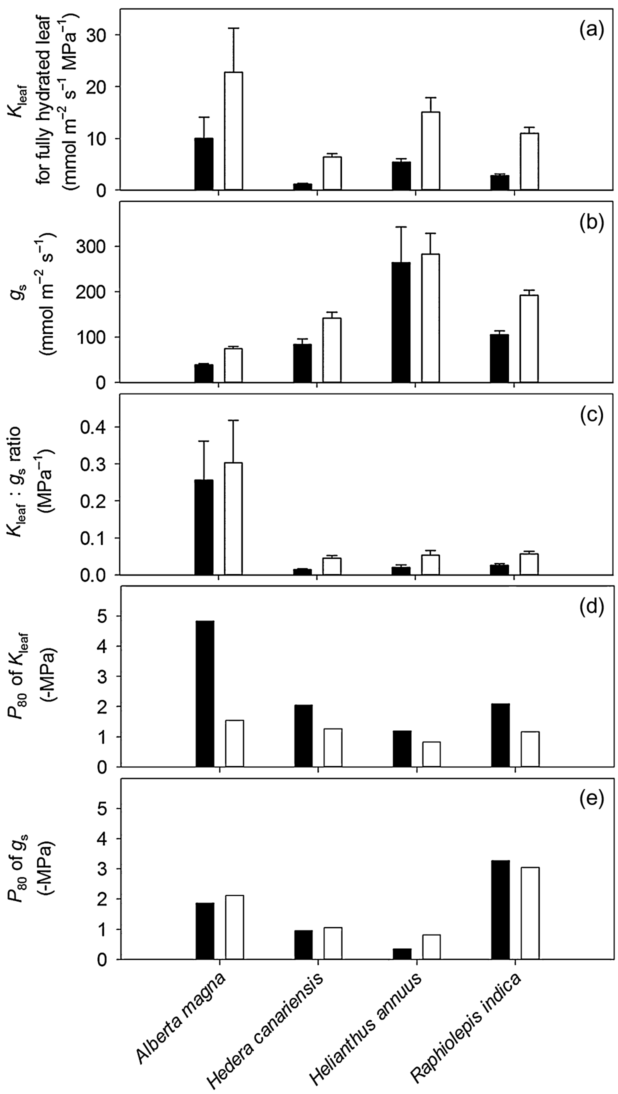

Figure 4. Mean ± standard error values for parameters of hydraulic and stomatal responses to dehydration for four species, for leaves acclimated to ambient laboratory irradiance (<6 mmol m^{-2} s^{-1} photosynthetically active radiation; black bars) and high irradiance (grey bars; >900 mmol m^{-2} s^{-1}): (a) leaf hydraulic conductance (K_{leaf}) for fully hydrated leaves (i.e. K_{max}); (b) stomatal conductance (g_s) for fully hydrated leaves; (c) the ratio of K_{leaf} to g_s for fully hydrated leaves; (d) the leaf water potential at which K_{leaf} had declined by 80% (P_{80} of K_{leaf}); and (e) the leaf water potential at which g_s had declined by 80% (P_{80} of g_s).

pathways, with water now evaporating from mesophyll tissue. However, for the study species, leaves acclimated to both low and high irradiance were in fact transpiring in the EFM, with g_s far exceeding g_{min}, and further, any uptake of water into fully hydrated leaves caused by infiltration of cells and airspaces made a negligible contribution to the flow rate, as determined by direct measurement of J_{area}. Notably, such uptake could only occur in well-hydrated leaves; over-expansion of cells could not occur below full turgor, and dehydrated leaves with stomata open would transpire away liquid water infiltrating the airspaces. Thus, the finding of a K_{leaf} light effect for partially dehydrated leaves was also consistent with a true light effect in the transpiration pathways.

Insights into the mechanisms of the K_{leaf} light and dehydration responses

The experiments in this study focused on characterizing the combined K_{leaf} responses to irradiance and leaf water status, rather than probing mechanisms, but the findings provided insights into the basis for these responses. The demonstration that the light enhancement of K_{leaf} was strongest for well-hydrated leaves but disappeared by turgor loss point, mirrors the findings of probe studies that indicated that cell membrane permeabilities outside the xylem increased with irradiance and declined with loss of turgor (Kim & Steudle 2007). Such parallel leaf- and cell-level responses to light and turgor further implicate aquaporins in the extra-xylem flow pathways (Luu & Maurel 2005). Both these cell and whole-leaf level findings are consistent with the model proposed by Cochard *et al.* (2007) for the K_{leaf} light response, that is, that in low irradiance-acclimated leaves, water movement is mainly via low-conductance apoplastic pathways, but in high irradiance-acclimated leaves, water moves across the bundle sheath and/or mesophyll membranes easily because of the expression of aquaporins, increasing the numbers of high conductance pathways.

The greater hydraulic vulnerability of high irradiance-acclimated leaves also provided insight into the mechanisms for K_{leaf} decline with dehydration. Given that the light enhancement occurs in the extra-xylem tissues, high irradiance-acclimated leaves would have a greater relative importance of xylem hydraulic resistance. Consequently, if cavitation is responsible for declines of K_{leaf}, its effects would be exacerbated under high irradiance, both because: (1) there would be a more negative xylem water potential at any Ψ_{leaf}, and thus, air seeding may be stronger, driving more substantial cavitation; and (2) declines in xylem resistance would scale up to a stronger impact on overall K_{leaf} (as discussed by Meinzer 2002; Scoffoni *et al.* 2008). Thus, the stronger K_{leaf} decline in high-irradiance acclimated leaves of all species supported a strong role for xylem cavitation or collapse during dehydration. These findings do not exclude a role of decline in conductivity outside the xylem. Indeed, recent work shows that aquaporin sensitivity in the bundle sheath cells can lead to declines in outside-xylem conductance (Shatil-Cohen *et al.* 2011). Additional mechanistic work, comprehensively examining declines in conductivity within the xylem and outside-xylem compartments under low and high irradiance are necessary in future studies.

Importance of the K_{leaf} light effect in the plant water transport system

The K_{leaf} light effect was not only strong in fully hydrated leaves. For leaves operating at their mid-day Ψ_{leaf}, the K_{leaf}

light enhancement ranged from 41 to 179%, as estimated from their vulnerability curves. As the leaf is a major component of plant resistance, this response would have impacts at whole-plant level. For example, if the leaf accounts for 30% of whole-plant resistance, then a 100% increase of K_{leaf} would reduce plant resistance by 15%, or increase whole-plant hydraulic conductance (K_{plant}) by 17% (=1/0.85). The increase of K_{leaf} with high irradiance would coincide with increases in root hydraulic conductance under high irradiance and transpiration (Henzler *et al.* 1999; Sakurai-Ishikawa *et al.* 2011) and possibly with increases in stem conductivity caused by increases in xylem sap ion concentration under high irradiance, widening nanopores in the pectins within pit membranes (Nardini *et al.* 2010b; Sellin, Ounapuu & Karusion 2010). Such increases would also act synergistically with increases in hydraulic conductance with increased temperature because of lower viscosity and greater membrane permeabilities throughout the plant (Sack, Streeter & Holbrook 2004; Sellin & Kupper 2007). Consistent with these effects, diurnal increases of K_{leaf} and K_{plant} with high irradiance have been shown in greenhouse and field studies (Tsuda & Tyree 2000; Lo Gullo *et al.* 2005; Sellin & Kupper 2007; Sellin, Ounapuu & Kupper 2008).

One clear benefit of this effect would be to allow higher transpiration rates while maintaining xylem tensions and Ψ_{leaf} at levels moderate enough to avoid further embolism and stomatal closure (Cochard *et al.* 2007). In illuminated leaves operating at their mid-day Ψ_{leaf} a higher K_{leaf} is achieved even despite the greater decline of K_{leaf} associated with high irradiance. Notably, the light enhancement of K_{leaf} was greatest for hydrated leaves, declined with decreasing Ψ_{leaf}, and remains important when the leaf is mildly dehydrated.

A second potential advantage of the light enhancement of K_{leaf} indicated in this study arises from its greater magnitude than that of g_s. The ratio of hydraulic supply to demand (K_{leaf}/g_s) was greater under higher irradiance. Such a greater K_{leaf} than necessary to balance transpiration, at a given VPD, implies the possibility of added benefits. For example, a higher K_{leaf}/g_s may render the plant more capable of tolerating transiently high VPD or mild soil drought without shutting stomata (Brodribb & Holbrook 2004a; Brodribb & Jordan 2008). Notably, the K_{leaf}/g_s depended on particular irradiance and leaf water status, and future modelling should determine the impact of the K_{leaf} light response on the ability to withstand high VPD or soil drought.

A third potential benefit of the K_{leaf} light response relates to the economics of metabolism. The reduced expression or deactivation or of aquaporins may save energy (Netting 2002), and would be especially advantageous in low irradiance. The potential adaptive importance across natural resource gradients merits further investigation.

Implications for stomatal control and for hydraulic-stomatal coordination

The g_s responses provide new insights into the mechanism of stomatal dynamics and coordination within the integrated hydraulic-stomatal system. Our experiments followed previous studies of hydraulic-stomatal coordination in detached shoots (Salleo *et al.* 2000; Brodribb & Holbrook 2004b). By controlling shoot dehydration for both the g_s and K_{leaf} measurements, their relative responses at given Ψ_{leaf} could be assessed. Although experiments on detached shoots might differ in their results from those on droughted plants, because of, for example, drought signals from drying roots (Comstock 2002; Holbrook *et al.* 2002), we note that previous work has shown similar K_{leaf} and/or g_s declines in droughted plants and dehydrated shoots (Brodribb & Holbrook 2004a; Blackman *et al.* 2009; Pasquet-Kok *et al.* 2010), and mid-day g_s for leaves on transpiring plants were similar to those on detached shoots with the same Ψ_{leaf}.

This study indicated that stomatal responses were partially related to Ψ_{leaf}, and mechanistically independent. In both the EFM and porometer experiments, the g_s response to Ψ_{leaf} was noisy, indicating that g_s was sensitive to other factors than Ψ_{leaf}. Indeed, the EFM experiment showed that g_s was not determined by the steady-state Ψ_{leaf}, but rather had a 'memory' of previous dehydration – g_s was better correlated with Ψ_{lowest}. These findings did not support the ideas that g_s declines in dehydrating leaves because of: (1) changes in bulk Ψ_{leaf}; (2) low Ψ_{leaf} precipitated by K_{leaf} decline; or (3) declines in the turgor of leaf cells. Such a dependence of g_s on Ψ_{leaf} has been assumed in certain models (reviewed in Damour *et al.* 2010). Notably, a previous study on *Laurus nobilis* also found that g_s was unlinked from Ψ_{leaf} (Salleo *et al.* 2000). When shoots were dehydrated, g_s declined slowly with Ψ_{leaf} until a certain threshold at which strong cavitation was indicated by acoustic emissions, and then g_s declined strongly. That finding had led to the proposal that xylem cavitation might directly trigger g_s decline via a hydraulic or hormonal signal. However, our results did not support in any clear way a direct trigger for g_s decline by cavitation, as across species and irradiance treatments, the decline of g_s rarely matched that of K_{leaf}. Rather, the decline of K_{leaf} began immediately with dehydration, whereas that of g_s began only after substantial turgor loss, and the P_{80} for both responses were not aligned. Previous work has shown that cavitation was certainly responsible for a major portion of K_{leaf} decline (see Introduction), though effects in the extra-xylem pathways might also be involved, and thus the differences in trajectories of K_{leaf} decline and of stomatal closure did not implicate cavitation itself as a key signal for stomatal closure.

The sensitivity of g_s to leaf dehydration and its independence of steady-state Ψ_{leaf} and of the trajectory of K_{leaf}, can be explained by one or more of three possible mechanisms. Firstly, the decline of g_s in dehydrating leaves may be in part related to synthesis or apoplastic redistribution of ABA and/or ethylene, or increased tissue sensitivity to hormones, in response to the strongest leaf dehydration experienced. Such signals could be provoked by osmosensing cells given reduced cellular volume, or plasmalemma tension (Tardieu & Davies 1993; Comstock 2002; Jia *et al.* 2002; Jia & Zhang 2008). Indeed, previous studies of excised leaves, which had been dehydrated with or without subsequent rehydration, showed that g_s declined with increasing ABA concentration (Wright 1977; Lin, Sucoff & Brenner 1986; Liu *et al.* 2001). Another candidate explanation is the hydraulic-mechanical hypothesis for stomatal control, proposed based on models and on experiments directly on the turgor of guard cells and epidermis, and measurements of stomatal responses to VPD (e.g. Franks 2004; Buckley *et al.* 2011). Here, g_s is not directly dependent on bulk Ψ_{leaf}, but rather is influenced by the water potential at or near the guard cells or epidermis (Ψ^*), with stomatal opening determined by the guard cell turgor against the pressure of surrounding epidermal cells. A 'mechanical advantage' of the epidermis was apparent in the low g_s for very well-hydrated leaves, in which the epidermis would have exerted pressure on the guard cells. Conversely, in *H. annuus*, open stomata were observed in strongly dehydrated leaves, consistent with flaccid epidermal cells no longer exerting pressure against the guard cells, as shown in previous work (Franks, Cowan & Farquhar 1998; Tang & Boyer 2007). Notably, the Ψ^* would decline with transpiration rate, but depending on the hydraulic conductance to the sensor site at or near the epidermis (K^*). Notably, the K^* would not necessarily be equivalent to K_{leaf}, as the pathways to the sensing site would not be those of transpired water, which, for example, may evaporate throughout the mesophyll, though they may share a component, for example, the vein xylem pathways and bundle sheath, and part of the routes of water flow through the mesophyll. This model is consistent with the fact that in the EFM experiment, g_s did not relate to Ψ_{leaf} during steady state, but to the lowest Ψ_{leaf} during dehydration. Just as the dehydration caused K_{leaf} to decline, it would have caused a decline in K^*, driving declines in Ψ^* and g_s that may persist even after recovery of Ψ_{leaf}. This explanation is consistent with previous demonstrations of the independence of the g_s response to VPD on either side of a well-hydrated, transpiring amphistomatous leaf (Mott 2007), given that the K^* is dominated by an extra-xylem component, distinct for each epidermis, as found with probe work on the epidermis (Ye, Holbrook & Zwieniecki 2008). A third possible explanation for the sensitivity of g_s to leaf dehydration is a vapour-phase control of g_s (Peak & Mott 2011). Here too, the g_s would be independent of Ψ_{leaf} and of the trajectory of K_{leaf}. However, why a vapour-phase signal should lead to a suppression of g_s in dehydrated and rehydrated leaves is not clear, and requires further investigation.

Further evidence for mechanistic independence of K_{leaf} and g_s arose from their independent responses to irradiance, and their different irradiance × dehydration interactions. For K_{leaf}, all species showed a decline with dehydration that was stronger under high than low irradiance, whereas for g_s, the light and dehydration responses were apparently independent for three species, that is, the response to light was proportional in low and high irradiance, with stomatal closure apparently occurring at the same Ψ_{leaf}. Only for *H. annuus* did stomata close at a less negative Ψ_{leaf} under low irradiance.

These studies also indicated the importance of species-variation in the coordination of the responses of g_s and K_{leaf} to dehydration, and how this shifts from low to high irradiance. Previous work has emphasized that both g_s and K_{leaf} tend to decline strongly by turgor loss point (π_{tlp}; e.g. Brodribb & Holbrook 2003; Brodribb *et al.* 2003; Blackman *et al.* 2009). In this study, K_{leaf} declined strongly in all species in both irradiances at close to π_{tlp}, but this was not necessarily true of g_s. Although two species (*A. magna* and *H. annuus*) did shut their stomata near π_{tlp}, the other two did not, with *H. canariensis* shutting its stomata well in advance of π_{tlp}, and *R. indica* shutting stomata at lower Ψ_{leaf} than π_{tlp}. This discrepancy between the g_s and K_{leaf} responses is consistent with the mechanistic decoupling described earlier, and also supports the possibility that variation in stomatal and hydraulic coordination is ecologically important. Such a possibility was previously proposed with respect to low-light adapted ferns relative to high-light adapted angiosperms; the ferns closed their stomata before K_{leaf} declined substantially (Brodribb & Holbrook 2004b). We here extend this finding among angiosperms. Thus, *H. canariensis*, a species tolerant of drought in high and low irradiance, shuts its stomata at –1 MPa, well before π_{tlp}, though it maintains a substantial K_{leaf}, both under low and high irradiance. This mechanism would contribute to the drought tolerance of this species despite its relatively high vulnerability in K_{leaf} (Scoffoni *et al.* 2011a); early stomatal closure would provide a benefit for leaf survival given its low g_{min} and high water storage capacitance (Sack, Grubb & Marañón 2003b; Metcalfe 2005; Scoffoni *et al.* 2011b). *H. annuus* shows a simultaneous decline of K_{leaf} and g_s, both becoming negligible by π_{tlp}; this species shows the ability to recover in K_{leaf} with rehydration (Trifilo *et al.* 2003a; Scoffoni *et al.* 2011a). By contrast, *A. magna* and *R. indica* both maintain open stomata even as K_{leaf} declines to a low level, as reported to be the case for many woody species in a compilation of data of K_{leaf} decline and mid-day operating g_s (Johnson *et al.* 2009b). This pattern may indicate hydraulic redundancy in well-hydrated leaves, such that K_{leaf} loss does not impact on g_s, as shown previously for *Acacia koa* during drought (Pasquet-Kok *et al.* 2010). Notably, the light response may interact with these patterns. Thus, the strong K_{leaf} light response of *R. indica* would allow high irradiance to partially compensate for lower leaf water status, and greater hydration to compensate for lower irradiance, an interaction that could potentially contribute to maintaining leaf water status and functional gas exchange under a wider range of environmental conditions. The demonstration that K_{leaf} is influenced by specific combinations of irradiance and water status implies a potential role for the magnitude and trajectories of these responses in explaining substantial species-variation in whole plant hydraulic behaviour and gas exchange.

Conclusions and future work on the K_{leaf} light and dehydration effects

This study presented new discoveries of a stronger K_{leaf} dehydration effect under high than low irradiance, and a diminishing light effect from full turgor to π_{tlp}. These responses indicated a strong role for loss of xylem conductivity in the dehydration response. Further, the K_{leaf} and g_s responses to irradiance and dehydration were found to be semi-independent, with implications for the coordination of liquid and vapour phase transport with dynamics of environmental factors. More work is needed on the mechanistic bases of both light and dehydration responses and of the implications of plastic and adaptive differences within and among species across gradients of irradiance and water supply. These responses of leaf water transport capacity have strong potential to impact processes across scales from the cell to the leaf function to ecological distributions.

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