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 ($K_{leaf} = \text{the flow rate through the leaf for a given water potential gradient, i.e. } 1/\text{resistance}$) 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 & Andrí 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...
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 & Lauer 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 $K_{\text{leaf}}$ was confirmed for several species using methods other than the HPFM [i.e. the evaporative flux method (EFM) and rehydration kinetics method (RRKM); Cochard et al. 2007; Sellin & Kupper 2007; Scoffoni et al. 2008].

The interaction of $K_{\text{leaf}}$ light and dehydration responses could have important implications for plant water transport. For example, a higher $K_{\text{leaf}}$ under high irradiance may compensate for decline in $K_{\text{leaf}}$ 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 $\Psi_{\text{sat}}$) 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 $K_{\text{leaf}}$ decline with dehydration, and that $K_{\text{leaf}}$ 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 $K_{\text{leaf}}$ decline with dehydration was due mainly to outside-xylem effects, low irradiance-acclimated leaves may be more sensitive.

Our second objective was to clarify the coordination of $K_{\text{leaf}}$ and $g_s$. We expected the light-response of $K_{\text{leaf}}$ might match or exceed that of $g_s$ to maintain $\Psi_{\text{leaf}}$ 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 $K_{\text{leaf}}$ 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 $\Psi_{\text{leaf}}$; (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. 2005). 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, 23.2 and 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 ($\Psi_{\text{leaf}}$) 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, © 2011 Blackwell Publishing Ltd, Plant, Cell and Environment, 35, 857–871).

### 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 $K_{\text{leaf}}$ (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, 23.2 and 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.

### 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 ($\pi_t$), turgor loss point ($\pi_{lp}$), modulus of elasticity ($\epsilon$) and capacitance ($C$)

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

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 $\Psi_{leaf}$. 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 $\Psi_{leaf}$ ($\Psi_{leaf}$; 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 $\Psi_{leaf}$ 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 $\Psi_{leaf}$ ($\Psi_0$) using a pressure chamber. If the difference in the $\Psi_{leaf}$ 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 nm 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 glass 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 $E$, $\Psi_{leaf}$ 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; $\Psi_{final}$ 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 $\Psi_{leaf}$ before reaching steady state transpiration, such that $\Psi_{final}$ is less negative than $\Psi_{o}$ or, alternatively, the transpiration rate may be sufficient for $\Psi_{final}$ to be driven lower than $\Psi_{o}$. To construct vulnerability curves, $K_{leaf}$ (always determined as $E/\Delta \Psi_{leaf}$) was plotted against whichever was lowest, $\Psi_{o}$, or $\Psi_{final}$ (‘$\Psi_{final}$’; 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 $\Psi_{final}$ 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) airspace 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 \( j_{\text{min}} \) 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 \( j_{\text{min}} \) 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 \( K_{\text{leaf}} \) vulnerability curves to determine \( g_s \) responses for the leaves previously dehydrated to a range of \( \Psi_{\text{leaf}} \) 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 (HOB0 Micro Station with Smart Sensors, Onset, Bourne, MA, USA), where mole fraction VPD = \( (1 - (\text{RH} \times \text{VPD}_{\text{sat}})) / 101.3 \text{ kPa} \), and \( \text{VPD}_{\text{sat}} \) 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 \( \Psi_{\text{leaf}} \) corresponding to the steady state leaf transpiration at the end of the EFM measurement (\( \Psi_{\text{leaf}} \)) and also against the lowest \( \Psi_{\text{leaf}} \) achieved either during the dehydration or the steady state measurement (\( \Psi_{\text{lower}} \)).

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 \( \Psi_{\text{leaf}} \), 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 \( \Psi_{\text{leaf}} \) 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 \( H. \text{annuus} \), 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 (\( g_{\text{min}} \) = minimum epidermal conductance, i.e. when stomata are closed), with the exception of \( H. \text{canariensis} \), which had such a low \( g_{\text{min}} \) 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 \( K_{\text{leaf}} \) and \( g_s \) to \( \Psi_{\text{leaf}} \), outlier tests were conducted for each 0.5 MPa interval (except 0.25 MPa intervals for \( H. \text{annuus} \); 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 \( K_{\text{leaf}} \) or \( g_s \) using maximum likelihood to select among four functions previously used in the literature (Scoffoni et al. 2011b): linear \( (K_{\text{leaf}} \) or \( g_s \) = \( a \Psi_{\text{leaf}} + y_0 \)); sigmoidal \( (K_{\text{leaf}} \) or \( g_s \) = \( a \left[ 1 + \left( \frac{\Psi_{\text{leaf}}}{x_0} \right)^b \right]^{-1} \)); and exponential \( (K_{\text{leaf}} \) or \( g_s \) = \( y_0 + ae^{-b\Psi_{\text{leaf}}} \)). Curves were fitted using the \textit{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 \( K_{\text{leaf}} \) \( (K_{\text{max}}) \) or \( g_s \) \( (g_{\text{max}}) \), and the \( \Psi_{\text{leaf}} \) at which \( K_{\text{leaf}} \) or \( g_s \) had
declined by 80% \( (P_{0.8} K_{leaf} \) or \( g_s \)) were determined using the fitted curve parameters. To test the significance of the dehydation-induced declines of \( g_s \) in response to \( \Psi \) and \( \Psi_{osmotic} \) 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 \( \Psi_{leaf} \) values from full turgor to below turgor loss point \((P_{turgor})\). 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 \( g_{min} \). The mean of the five highest values of \( g_s \) for low irradiance-acclimated leaves were 11- to 47-fold higher than \( g_{min} \).

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 air-space 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 \( \times \) 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 dehydation 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 2). The \( K_{leaf} \) light enhancement averaged for leaves \( \geq 0.5 \) 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 \( P_{min} \) that was less negative on average across species by 1.3 MPa \( \pm 0.6 \) SE \((P = 0.03, \) paired \( t \)-test on log-transformed data). At mid-day operating \( \Psi \), the \( K_{leaf} \) light enhancement was 38% for \( A. magna \),

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

For each species, the five lowest \( g_s \) measurements made for dehydrated leaves under low irradiance were taken as the cuticular conductance \( (g_{min} = \text{minimum epidermal conductance, i.e. when stomata are closed}) \), 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 (Scotfoni 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\(^{-1}\).
74% for *R. indica*, 248% for *H. annuus*, and 307% for *H. canariensis* (estimated from equations in Fig. 1, using \( \Psi_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) for leaves of each species, in both low and high irradiance (Fig. 1); \( K_{\text{leaf}} \) depended not only on transpiration rate (determined by \( g_s \) and laboratory VPD) but also on the leaf water potential during steady state (\( \Psi_{\text{final}} \)). 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 \( \Psi_{\text{leaf}} \), especially for...
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*, 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*\textsubscript{leaf} values experienced were not low enough to cause a complete decline in *g*, for some species, as the goal of the EFM was to sample *K*\textsubscript{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* against the *Ψ*\textsubscript{leaf} established during steady state flow at the end of measurement (*Ψ*\textsubscript{final}) tended to show only a weak correlation (Fig. 2, inset plots). By contrast, the plots of *g* against the lowest *Ψ*\textsubscript{leaf} established during shoot dehydration and the EFM measurement (*Ψ*\textsubscript{lowest}) tended to show strong correlations (Fig. 2, main plots). This pattern was caused by the *Ψ*\textsubscript{lowest} values varying more strongly, as in many cases, the *Ψ*\textsubscript{final} represented leaves that had rehydrated during EFM measurement. Additionally, for many leaves *g*\textsubscript{s} was suppressed by previous dehydration even after *Ψ*\textsubscript{leaf} recovered to *Ψ*\textsubscript{final}. Thus, *g*\textsubscript{s} during steady state transpiration showed a much stronger dependence on *Ψ*\textsubscript{leaf} of dehydration than on final equilibrated *Ψ*\textsubscript{final}.

Given the two experiments for determining *g*, responses were broadly similar in their results, we used the porometer experiment to quantify stomatal dynamics independently of the *K*\textsubscript{leaf} experiment, and with the advantage that it did not involve leaves rehydrating, but rather steady state transpiration at the lowest *Ψ*\textsubscript{leaf} experienced, as each experiment began with well-hydrated shoots, and *g*\textsubscript{s} and *Ψ*\textsubscript{leaf} were sampled at a given stage of progressive dehydration. In the porometer experiment, strong declines of *g*\textsubscript{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*\textsubscript{s} against *Ψ*\textsubscript{leaf} (Fig. 3). Determining the maximum *g*\textsubscript{s} by extrapolating the fitted curve, the light enhancement of *g*\textsubscript{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*\textsubscript{s} more rapidly than under high irradiance, and *P*\textsubscript{0} for *g*\textsubscript{s} was less negative by 0.5 MPa. Thus, for semi-dehydrated leaves, *g*\textsubscript{s} was lower in the dark than under high irradiance. For the other three species, the decline of *g*\textsubscript{s} with dehydration was similar under high and low irradiance, as indicated by a similar *P*\textsubscript{0}. Stomatal closure occurred at approximately *Π*\textsubscript{tlp} for *A. magna* and *H. annuus*, but well below *Π*\textsubscript{tlp} for *R. indica* and well above *Π*\textsubscript{tlp} for *H. canariensis* (Fig. 3). For *H. annuus*, stomatal opening was observed at *Ψ*\textsubscript{leaf} values below *Π*\textsubscript{tlp}. Species’ operating *g* values at mid-day *Ψ*\textsubscript{leaf} were within the range determined in the lab for bench-dried shoots under high irradiance at that *Ψ*\textsubscript{leaf} (Fig. 3, see points with star symbols).

### Table 3. Statistical tests of the response of *K*\textsubscript{leaf} to irradiance

<table>
<thead>
<tr>
<th>Species</th>
<th><em>K</em>\textsubscript{leaf} &gt;-0.5 MPa</th>
<th><em>K</em>\textsubscript{leaf} &gt;-1 MPa</th>
<th>All data significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL, HL</td>
<td>LL, HL</td>
<td></td>
</tr>
<tr>
<td><em>Alberta magna</em></td>
<td>6.77 ± 0.504, 11.1 ± 1.05**</td>
<td>4.25 ± 0.356, 5.97 ± 0.549**</td>
<td>*</td>
</tr>
<tr>
<td><em>Hedera canariensis</em></td>
<td>1.20 ± 0.211, 5.97 ± 0.626***</td>
<td>1.05 ± 0.167, 4.59 ± 0.409***</td>
<td>***</td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>3.71 ± 0.536, 14.3 ± 2.60**</td>
<td>3.19 ± 0.341, 10.5 ± 1.85**</td>
<td>**</td>
</tr>
<tr>
<td><em>Raphiolepis indica</em></td>
<td>2.68 ± 0.335, 8.03 ± 1.03**</td>
<td>1.24 ± 0.161, 2.39 ± 0.398**</td>
<td>**</td>
</tr>
</tbody>
</table>

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

### 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*\textsubscript{leaf} and *g*\textsubscript{s} for hydrated leaves (Fig. 4a–c) and *K*\textsubscript{leaf}/*g*\textsubscript{s}. For all species, the increase of *K*\textsubscript{leaf} from low to high irradiance in well-hydrated leaves exceeded that of *g*\textsubscript{s} representing a light-induced increase of hydraulic supply relative to demand. However, in dehydrated leaves the *K*\textsubscript{leaf} initially declined more rapidly than *g*\textsubscript{s}, and in all species the *K*\textsubscript{leaf}/*g*\textsubscript{s} tended to decline during leaf dehydration. Thus, for well-hydrated leaves, the ratio *K*\textsubscript{leaf}/*g*\textsubscript{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*\textsubscript{leaf} in dehydrating leaves under high than low irradiance (Fig. 1) was reflected in their less negative *P*\textsubscript{0} for *K*\textsubscript{leaf} (Fig. 4). By contrast, the decline of *g*\textsubscript{s} in dehydrating leaves was similar under high and low irradiance, except in *H. annuus*, in which the stomata closed more...
Decline of stomatal conductance \((g_s)\) with decreasing leaf water potential \((\Psi_{\text{leaf}})\) determined using the evaporative flux method (EFM) in dehydrating leaves of four species under low and high irradiance (<6 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) and >900 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) photosynthetically active radiation; black and white symbols, respectively). The \(g_s\) was plotted against the lowest \(\Psi_{\text{leaf}}\) experienced by the leaf during bench drying and the EFM measurement (main plots), and against the final \(\Psi_{\text{leaf}}\) 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; \(P = 0.12\) to 0.30). Fitted lines are the best-fit functions selected by maximum likelihood: for low and high irradiance respectively, for \(A. magna\), \(g_s = -3.6 \Psi_{\text{leaf}} + 43 \ (n = 44), \) and \(g_s = 51 + 83e^{-0.05\Psi_{\text{leaf}}} \ (n = 47); \) for \(H. canariensis\), \(g_s = 7.5 + 32e^{-0.05\Psi_{\text{leaf}}} \ (n = 19)\) and \(g_s = 130 [1 + \left(\frac{\Psi_{\text{leaf}}}{1.2}\right)^{1.1}] \ (n = 41); \) for \(H. annuus\), \(g_s = -43 \Psi_{\text{leaf}} + 70 \ (n = 35)\) and \(g_s = -223 \Psi_{\text{leaf}} + 272 \ (n = 40)\); and for \(R. indica\), \(g_s = 63\left[1 + \left(\frac{\Psi_{\text{leaf}}}{2.7}\right)^{1.7}\right] \ (n = 35)\) and \(g_s = 144\left[1 + \left(\frac{\Psi_{\text{leaf}}}{2.3}\right)^{1.5}\right] \ (n = 42).\) \(R^2 = 0.20\)–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 \(\Psi_{\text{leaf}}\) for the remaining partially dehydrated leaves.

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

**DISCUSSION**

**An interaction of the \(K_{\text{leaf}}\) light and dehydration responses**

This study provided new resolution of dynamic responses of \(K_{\text{leaf}}\) to the environment. For well-hydrated leaves in each of
the four species, \( K_{\text{leaf}} \) showed a light enhancement greater in magnitude than that of \( g_s \). Further, the \( K_{\text{leaf}} \) light enhancement depended on leaf water status. Each of the four species showed a light response of \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) to dehydration was stronger in high- than low-irradiance acclimated leaves. Comparative magnitudes and trajectories of responses of \( K_{\text{leaf}} \) and \( g_s \) to irradiance and dehydration indicated semi-independent responses to \( Y_{\text{leaf}} \). These findings provide insights into the regulation of liquid and vapour phase water transport, with implications for whole plant function.

Confirming the \( K_{\text{leaf}} \) light effect and its importance in the EFM

The findings of this study further confirm a \( K_{\text{leaf}} \) light response that can be resolved using the EFM. Recently Rockwell et al. (2011) proposed that this \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) observed for light-acclimated leaves would not indicate a true light enhancement of the pathways of transpiration, but actually a change of...
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 \( g_{\text{min}} \), 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 \( J_{\text{area}} \). 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 \( K_{\text{leaf}} \) light effect for partially dehydrated leaves was also consistent with a true light effect in the transpiration pathways.

**Insights into the mechanisms of the \( K_{\text{leaf}} \) light and dehydration responses**

The experiments in this study focused on characterizing the combined \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \), its effects would be exacerbated under high irradiance, both because: (1) there would be a more negative xylem water potential at any \( \Psi_{\text{leaf}} \), 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 \( K_{\text{leaf}} \) (as discussed by Meinzer 2002; Scoffoni et al. 2008). Thus, the stronger \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) light effect in the plant water transport system**

The \( K_{\text{leaf}} \) light effect was not only strong in fully hydrated leaves. For leaves operating at their mid-day \( \Psi_{\text{leaf}} \), the \( K_{\text{leaf}} \)
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 ($K_{plnt}$) by 17% (±1.08). 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 $K_{plnt}$ with high irradiance have been shown in greenhouse and field studies (Tsuda & Tyrree 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 $\Psi_{leaf}$ at levels moderate enough to avoid further embolism and stomatal closure (Cochard et al. 2007). In illuminated leaves operating at their mid-day $\Psi_{leaf}$ 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 $\Psi_{leaf}$, 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 (Brodbribb & Holbrook 2004a; Brodbribb & 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 of or 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 $\Psi_{leaf}$ 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 (Brodbribb & 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 $\Psi_{leaf}$.

This study indicated that stomatal responses were partially related to $\Psi_{leaf}$, and mechanistically independent. In both the EFM and porometer experiments, the $g_s$ response to $\Psi_{leaf}$ was noisy, indicating that $g_s$ was sensitive to other factors than $\Psi_{leaf}$. Indeed, the EFM experiment showed that $g_s$ was not determined by the steady-state $\Psi_{leaf}$, but rather had a ‘memory’ of previous dehydration – $g_s$ was better correlated with $\Psi_{least}$. These findings did not support the ideas that $g_s$ declines in dehydrating leaves because of: (1) changes in bulk $\Psi_{leaf}$; (2) low $\Psi_{leaf}$ precipitated by $K_{leaf}$ decline; or (3) declines in the turgor of leaf cells. Such a dependence of $g_s$ on $\Psi_{leaf}$ 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 $\Psi_{leaf}$ (Salleo et al. 2000). When shoots were dehydrated, $g_s$ declined slowly with $\Psi_{leaf}$ 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 $P_{crit}$ 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 $\Psi_{leaf}$ 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 $\Psi_{laf}$, but rather is influenced by the water potential at or near the guard cells or epidermis ($\Psi^e$), 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 $\Psi^e$ would decline with transpiration rate, but depending on the hydraulic conductance to the sensor site at or near the epidermis ($K^e$). Notably, the $K^e$ would not necessarily be equivalent to $K_{laf}$, 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 $\Psi_{laf}$ during steady state, but to the lowest $\Psi_{laf}$ during dehydration. Just as the dehydration caused $K_{laf}$ to decline, it would have caused a decline in $K^e$, driving declines in $\Psi^e$ and $g_s$ that may persist even after recovery of $\Psi_{laf}$. 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 $K^e$ 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 $\Psi_{laf}$ and of the trajectory of $K_{laf}$. 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 $K_{laf}$ and $g_s$ arose from their independent responses to irradiance, and their different irradiance × dehydration interactions. For $K_{laf}$ 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 $\Psi_{laf}$. Only for *H. annuus* did stomata close at a less negative $\Psi_{laf}$ under low irradiance.

These studies also indicated the importance of species-variation in the coordination of the responses of $g_s$ and $K_{laf}$ to dehydration, and how this shifts from low to high irradiance. Previous work has emphasized that both $g_s$ and $K_{laf}$ tend to decline strongly by turgor loss point ($\pi_{tlp}$; e.g. Brodribb & Holbrook 2003; Brodribb et al. 2003; Blackman et al. 2009). In this study, $K_{laf}$ declined strongly in all species in both irradiances at close to $\pi_{tlp}$, but this was not necessarily true of $g_s$. Although two species (*A. magna* and *H. annuus*) shut their stomata near $\pi_{tlp}$, the other two did not, with *H. canariensis* shutting its stomata well in advance of $\pi_{tlp}$, and *R. indica* shutting stomata at lower $\Psi_{laf}$ than $\pi_{tlp}$. This discrepancy between the $g_s$ and $K_{laf}$ 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 $K_{laf}$ 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 $\sim1$ MPa, well before $\pi_{tlp}$, though it maintains a substantial $K_{laf}$ both under low and high irradiance. This mechanism would contribute to the drought tolerance of this species despite its relatively high vulnerability in $K_{laf}$ (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 $K_{laf}$ and $g_s$, both becoming negligible by $\pi_{tlp}$; this species shows the ability to recover in $K_{laf}$ with rehydration (Trifilo et al. 2003a; Scoffoni et al. 2011a). By contrast, *A. magna* and *R. indica* both maintain open stomata even as $K_{laf}$ declines to a low level, as reported to be the case for many woody species in a compilation of data of $K_{laf}$ decline and mid-day operating $g_s$ (Johnson et al. 2009b). This pattern may indicate hydraulic redundancy in well-hydrated leaves, such that $K_{laf}$ 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 $K_{laf}$ 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 $K_{laf}$ 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 $K_{laf}$ light and dehydration effects**

This study presented new discoveries of a stronger $K_{laf}$ dehydration effect under high than low irradiance, and a diminishing light effect from full turgor to $\pi_{tlp}$. These responses indicated a strong role for loss of xylem
conductivity in the dehydration response. Further, the $K_{\text{leaf}}$ and $g_*$ 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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