

Light response of hydraulic conductance in bur oak (*Quercus macrocarpa*) leaves

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Summary A four- to seven-fold enhancement of leaf hydraulic conductance by light has been reported in three temperate tree species. The enhancement occurs in the liquid-flow pathway between the petiole and the site of water evaporation. The enhancement occurs within 1 h, and dissipates in darkness over a period of 1 to 10 h depending on species. Here we report light-induced enhancement of leaf hydraulic conductance in a fourth species, bur oak (*Quercus macrocarpa* Michx.), the dependence of the effect on light flux and color, its absence in leaves of seedlings, and the impact on the response of leaf vein severance and several metabolic inhibitors. The light response of leaf hydraulic conductance approached saturation at a photosynthetic photon flux of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Hydraulic enhancement was greater in response to blue and green light than to visible radiation of longer wavelengths, although at the same irradiance, the response to white light was greater than to light of any single color. Atrazine (a photosystem II inhibitor), fusicoccin (which stimulates plasma membrane-bound H^+ -ATPase) and HgCl_2 (an aquaporin blocker) reduced the light response of leaf lamina hydraulic conductance. When 2-mercaptoethanol was added following mercury treatment, the light response was totally suppressed. Our results are consistent with the notion that the effect of light on leaf lamina hydraulic conductance is controlled by factors acting outside the leaf veins, possibly through light-induced changes in membrane permeability of either mesophyll or bundle sheath cells, or both.

Keywords: 2-mercaptoethanol, aquaporins, atrazine, fusicoccin, gas exchange, HgCl_2 , irradiance, leaf lamina hydraulic conductance, sodium fluoride, stomatal conductance.

Introduction

Leaf water transport is considered a largely apoplastic process (Westgate and Steudle 1985, Morillon and Chrispeels 2001). This view is supported by studies indicating that 64 to 80% of leaf hydraulic resistance in laurel (*Laurus nobilis* L.), sugar maple (*Acer saccharum* L.) and red oak (*Quercus rubra* L.) leaves is localized in the vascular system (Zwieniecki et al. 2002, Sack et al. 2004). Hydraulic conductance of the apoplast

can be modified by cavitation (Salleo et al. 2001), by temperature-induced changes in water viscosity (Sack et al. 2004) and by changes in cell wall permeability (Zwieniecki 2001). Several recent studies, however, indicate that cell-to-cell water transport may also play a significant role in leaves. It has been estimated that the extra-vascular hydraulic resistance of a leaf can constitute as much as 50–90% of whole-leaf resistance (Trifilò et al. 2003, Cochard et al. 2004, Gascó et al. 2004). Experiments based on dye movement within leaves as well as the temperature and light responses of leaf hydraulic conductance also suggest that at least some water transported by leaves passes through leaf cell membranes (Canny 1990, Cochard et al. 2000, 2007, Tyree et al. 2005).

Water movement across cell membranes is enhanced by aquaporins (Tyerman et al. 2002). In leaves, aquaporins are localized in the mesophyll (Robinson et al. 1996), bundle sheath (Kaldenhoff et al. 1995, Frange et al. 2001) and guard cells (Frayse et al. 2005). Nardini et al. (2005) showed that mercury reversibly inhibits non-vascular leaf water conductance in *Helianthus annuus* L. Leaf hydraulic conductance can be enhanced by light (Söber 1997, Aasamaa and Söber 2001, Tyree et al. 2005). In *Juglans regia* L., a light-induced increase in conductance was correlated with transcript abundance of the PIP2 family of aquaporins (Cochard et al. 2007). Some leaf aquaporins can increase mesophyll conductance to CO_2 , and consequently boost photosynthesis (Flexas et al. 2006).

The link between leaf hydraulic conductance and photosynthesis has become increasingly clear (Sperry 2000, Tyree 2003, Cochard et al. 2007). Whole-plant hydraulic conductance sets an upper limit on stomatal conductance to gaseous diffusion of water and CO_2 , because a decline in xylem and leaf water potentials cannot continue indefinitely, as this would cause runaway cavitation. Therefore, high hydraulic conductivity of plants increases stomatal conductance provided that stomatal conductance is not otherwise limited by factors such as water availability, irradiance or CO_2 concentration. Maximum leaf hydraulic conductance is coordinated with maximum gas exchange rates (Aasamaa et al. 2001, Brodribb and Holbrook 2003, Franks 2006). If stem water conductance limits whole-plant conductance, an increase in vessel abundance and diameter can enhance net assimilation.

In contrast, if non-vascular resistances to water transport dominate whole-plant conductance, then factors that enhance aquaporin expression in roots and leaves (Kaldenhoff et al. 1993, Henzler et al. 1999, Lopez et al. 2003) may enhance net assimilation.

In this study, we demonstrated that bur oak (*Quercus macrocarpa* Michx.) can be added to the small list of species known to modulate leaf hydraulic conductance in response to irradiance. Effects of light flux and color, plant age (juvenile versus adult) and several inhibitors of metabolic processes on this light response are reported.

Materials and methods

Plant material

Experiments were conducted from July to August 2004, 2005 and 2006. Bur oak shoots were collected from 20–30-year-old trees growing on the University of Alberta campus, Edmonton. Immediately after cutting, branches were placed with their cut ends in distilled water and taken to the laboratory where the shoots were recut under water and kept in water until leaves were assigned to experimental treatments later in the day. We found no significant diurnal or seasonal variation in specific leaf lamina hydraulic conductance (K_{lam} ; data not shown). However, leaves from different trees differed in light responsiveness. For each experiment, therefore, treatment effects were measured in leaves from a single tree.

For experiments involving light responses in seedling leaves, bur oak acorns were collected from mature trees growing in Edmonton, AB. The acorns were germinated and seedlings grown in a controlled-environment growth room for 3 months in pots containing equal volumes of sand and a commercial potting compost. The growth room provided an 18-h photoperiod, a photosynthetic photon flux (PPF) of $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ (at the shoot tip), a day/night temperature of $22/18^\circ\text{C}$ and a relative humidity of 70%. Seedlings were watered daily and fertilized weekly with a 1% (w/v) solution of N,P,K (20,20,20) commercial fertilizer.

Leaf and petiole hydraulic conductance

To measure leaf hydraulic conductance (K_{leaf}), leaf petioles were cut under water and connected to a high pressure flow meter (HPFM, Dynamax, Houston, TX) with compression couplings. Water was forced into the leaf at constant pressure, which varied from leaf to leaf from 350 to 600 kPa. A computer recorded the flow rate (Q , kg s^{-1}) and the applied pressure (P , MPa), calculated K_{leaf} as Q/P every 2 s and saved 60-s mean values. There was no relationship between K_{leaf} and the applied pressure. To buffer temperature fluctuations, leaves in all experiments were immersed in distilled water. Water temperature was recorded with a thermocouple thermometer (HH21, Omega Engineering, Stamford, CT). For each leaf, the measurement of K_{leaf} took 75 to 110 min, depending on the experiment. After measurement of K_{leaf} , the leaf lamina was excised with a razor blade and the hydraulic conductance of the petiole (K_p) recorded. Following excision of the leaf

lamina, the flow usually stabilized within 1 min, and K_p was recorded for another 6 min. We calculated K_p as the mean of six measurements. Petiole hydraulic conductivity (K_{pet}) was calculated as K_p multiplied by petiole length (Sack et al. 2002). Leaf area was determined with an area meter (LI-3100, Li-Cor).

Whole-leaf and leaf lamina hydraulic conductance

Hydraulic conductance of the leaf lamina (K_L) was calculated as: $K_L = 1/(1/K_{leaf} - 1/K_p)$ (Sack et al. 2002). Leaf lamina specific hydraulic conductance (K_{lam}) was also determined on a leaf area basis by dividing K_L by leaf area (Sack et al. 2002). All measurements were performed at ambient temperature. During exposure to high irradiance, the temperature of the water bath in which the leaf was immersed increased by less than 0.8°C , so K_L was uncorrected for temperature. For some experiments, relative values of K_{lam} were used instead of absolute values. For each leaf, a mean was calculated from the last 10 measurements of K_{lam} before turning the light on, and all subsequent values in the K_{lam} series were divided by this mean.

Leaf illumination

To study effects of irradiance on K_{lam} , leaves were exposed to either white light at a PPF of $1000\text{--}2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (high irradiance) or normal laboratory illumination (typically $< 15 \mu\text{mol m}^{-2} \text{s}^{-1}$) (ambient light). Usually, K_{lam} was first determined in ambient light for 30–50 min, then under high irradiance. After another 30 min, the light was turned off and K_{lam} was determined for an additional 30 min. High irradiance white light was from either halogen projector lamps via a mirror and water-filter or LEDs (BL-3000 series, Lamina Ceramic, Westampton, NJ). Irradiance was measured with a Li-Cor LI-185 or LI-250A light meter.

The dependence of the light response of K_{lam} on light quality was investigated by exposing leaves during the HPFM measurements to light from different colored LEDs (BL-3000 series, Lamina Ceramic) with peak wavelengths of 618 nm (red), 589 nm (amber), 525 nm (green) and 470 nm (blue). The response of leaf gas exchange to light quality was also investigated. Freshly excised leaves were supplied with filtered distilled water through a tube attached to the petiole. After 1 h of high irradiance with white light, their gas exchange rates were determined with a Li-Cor LI-1600 infra-red gas analyzer during illumination with light of different colors (at an irradiance of about $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$). The sequence in which a single leaf was exposed to the different colors of light was random. Between successive measurements, the leaves were briefly exposed to high irradiance white light.

The response of K_{leaf} to irradiance with white light was determined by successively exposing single leaves to 0, 10, 20, 30, 40, 50, 100, 150, 200, 500, 1000, 1500 and $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, while K_{lam} was measured with the HPFM. Light from halogen projector lamps was passed through a hot mirror that reflected infrared radiation to limit heating of the leaf. Irradiance was varied with neutral density filters. Each light regime lasted 20–40 min. The relative values of K_{leaf} reported

for the light saturation curve were obtained by dividing K_{leaf} in the light by K_{leaf} in darkness.

To investigate whether the response of K_{leaf} to irradiance with white light is dependent on the incident light to which leaves have been exposed, attached leaves were covered with aluminum foil for 2 weeks before branch excision and the light response of K_{lam} determined.

Leaf vein severance

To investigate (1) whether the main resistance to water flow was located in the vascular or non-vascular compartments of the leaf and (2) which resistance component was responsive to light, most of the tertiary veins of several leaves were severed with a dissecting blade at 0.5 cm from the secondary vein and the light response of K_{lam} determined.

Metabolic inhibitors

Sodium fluoride To investigate the role of metabolism in the light response of K_{lam} , shoots were placed with their cut ends in either filtered distilled water (control) or a 1 mM solution of the general metabolic inhibitor, NaF, and kept in ambient light for 24 h. Stomatal conductance (g_s) was measured with a steady-state porometer (LI-1600, Li-Cor) at 0, 1, 3, 5 and 24 h after the start of treatment. Leaves were then excised and the light response of K_{lam} assessed. The same parameters were measured for an additional set of leaves that were exposed to 1 mM NaF for 5 h.

Mercuric chloride To examine a possible involvement of aquaporins in the light response of K_{lam} , leaves were infiltrated for 100 min under pressure (0.5 MPa) and high irradiance with 0.1 mM $HgCl_2$ (an aquaporin blocker). Following 90 min treatment with $HgCl_2$, one set of leaves was pressure-infiltrated for 60 min high irradiance with a solution of 15 mM 2-mercaptoethanol with the objective of restoring aquaporin function after mercury blocking (Wan and Zwiazek 1999). Leaf lamina hydraulic conductance of the treated leaves was then determined during 30 min of high irradiance followed by 30 min in ambient light.

Atrazine and fusicoccin Excised leaves were placed with their petioles in a solution of 0.5 mM atrazine (a PSII inhibitor; Hall et al. 1999) or 1.4 μ M fusicoccin (which induces stomatal opening; De Boer 1997) for 60 and 120 min, respectively, under high irradiance with white light, and leaf gas exchange measured. The effect of high irradiance on K_{lam} was then determined. The experiment with atrazine was performed with both mature and senescing leaves.

Statistical analysis

Effects of light quality on K_{lam} , g_s , and net CO_2 exchange were evaluated by repeated measures analysis of variance. Other treatment effects on K_{lam} were analyzed by a paired *t*-test. For every treatment, five to six replicates were used.

Results

Light response of lamina conductance in mature leaves

Exposing leaves of mature trees to high irradiance increased K_{lam} (Figure 1A) about threefold over 30 min, reaching a value of 189 ± 7 versus an original value of only $69 \pm 7 \text{ mg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$. Irradiance had no effect on K_{pet} (Table 1).

Lamina conductance, stomatal conductance and sodium fluoride

Lamina conductance of leaves treated for 5 h with 1 mM NaF increased nearly threefold during a 30-min exposure to high irradiance (Figure 1B), but became insensitive to light after 24 h of treatment (Figure 1C). Neither irradiance nor NaF treatment affected K_{pet} (Table 1).

In NaF-treated leaves, g_s was significantly reduced compared with control leaves after 5 h ($P = 0.03$) (Figure 1E). There were no significant differences in g_s after 24 h of NaF treatment (Figure 1E). Some leaves exposed to NaF developed necrotic spots.

Light response of lamina conductance in seedling leaves

Lamina conductance of leaves of bur oak seedlings raised in the growth room was $217 \pm 19 \text{ mg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ in ambient light and showed no significant increase when leaves were exposed to 30 min of high irradiance (Figure 1D).

Lamina conductance in covered leaves and leaves with severed veins

When exposed to high irradiance, K_{lam} of control leaves increased about threefold (Figure 2A). Lamina conductance of leaves covered for 2 weeks increased slightly less, from 48 ± 9 to $102 \pm 12 \text{ mg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$, in response to high irradiance, but the effect of leaf covering was not statistically significant.

When most minor leaf veins were severed, K_{lam} was about 23 times higher than in intact leaves, but did not respond to a 30-min exposure to high irradiance (Figure 2A). During perfusion of leaves with severed tertiary veins, water penetrated the mesophyll to the points of vein severance.

The light response curve of lamina conductance

Leaf hydraulic conductance increased with increasing irradiance, the response saturating at a PPF of about $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Figure 2B).

Light quality and lamina conductance

After a 30-min exposure to high irradiance, K_{lam} increased by about 163% in white light, 100% in blue and green light, and less than 30% in red and amber light (Figure 2C). There were significant treatment differences ($P < 0.05$), with the effects of green and blue light differing from those of white, amber and red light, and the effects of amber and red light differing significantly from that of white light. Light color had no effect on K_{pet} (Table 1.)

In freshly collected leaves, net CO_2 exchange rates were lower in blue light than in light of other colors at a similar irradiance (Table 2), whereas photosynthetic rates were simi-

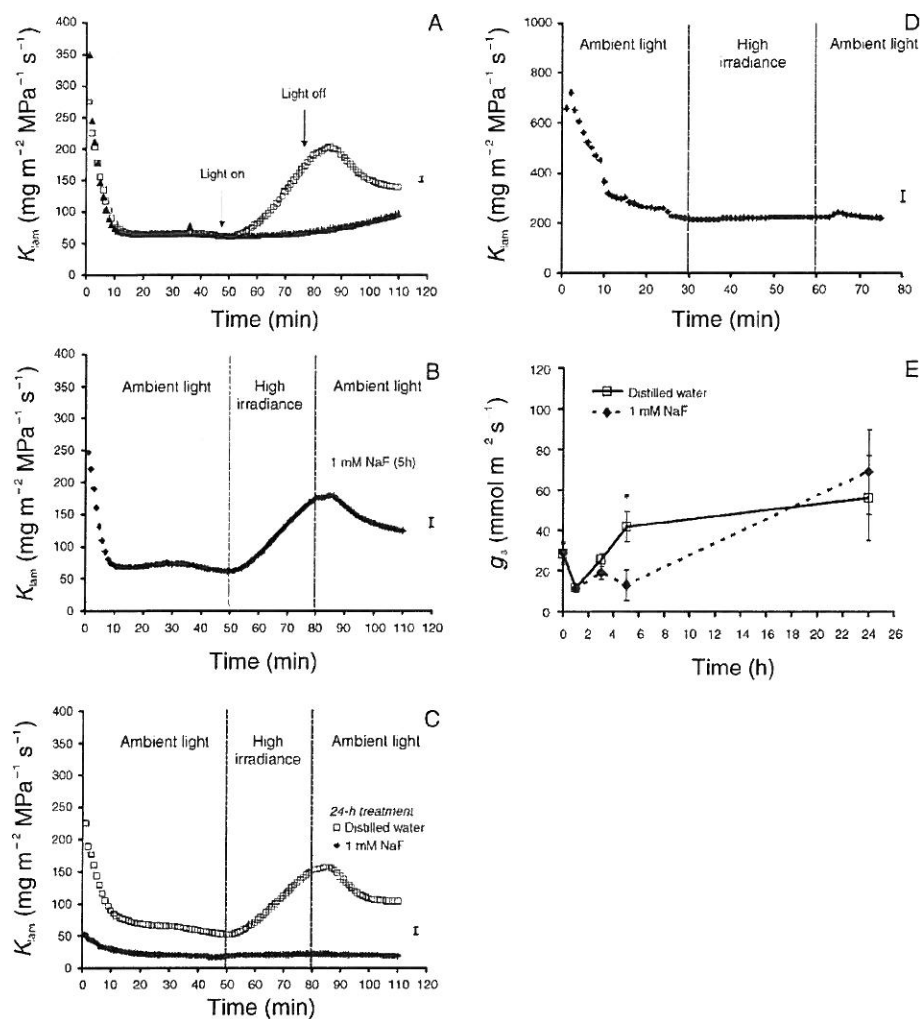


Figure 1. Effects of high irradiance with white light and NaF treatment on leaf lamina hydraulic conductance (K_{lam}) of bur oak (*Quercus macrocarpa*) leaves: (A) leaves measured in ambient light (\blacktriangle) and leaves exposed to high irradiance (\square , between the arrows); (B) leaves exposed to 1 mM NaF for 5 h; (C) leaves exposed to either 1 mM NaF or distilled water for 24 h; and (D) leaves of seedlings. (E) time course of stomatal conductance (g_s) of leaves from C during pre-treatment. An asterisk (*) indicates a significant difference from the control ($P = 0.05$). Values are least square means or means (B, D) \pm SE ($n = 6$). Mean standard error bars are also shown.

lar in white, green, amber and red light. Transpiration and leaf temperature were unaffected by color of light to which leaves were exposed (Table 2).

Mercuric chloride, 2-mercaptoethanol and lamina conductance

Compared with water-treated leaves, K_{lam} of $HgCl_2$ -treated leaves was significantly reduced ($P < 0.05$ for all measurement times; Figure 3A). Following exposure to high irradiance, 30 min of ambient light significantly decreased K_{lam} of $HgCl_2$ -treated leaves by approximately two-thirds ($P < 0.0001$) (Table 1). In contrast, $HgCl_2$ had no effect on K_{pet} (Table 1).

Treatment with 2-mercaptoethanol (ME) after $HgCl_2$ treatment significantly reduced K_{lam} throughout the measurement period ($P < 0.05$ for all measurements times; Figure 3B). During 30 min in ambient light following exposure to high irradiance, K_{lam} of leaves treated with $HgCl_2$ + ME did not change significantly. In contrast, K_{lam} of control (water treated) leaves significantly decreased from 295 ± 20 to 193 ± 20 mg m⁻² MPa⁻¹ s⁻¹ ($P < 0.0001$). There were significant differences in K_{pet} between the treatments (Table 1).

Atrazine, fusicoccin and lamina leaf conductances and gas exchange

In mature leaves, atrazine significantly ($P < 0.05$) reduced the light response of K_{lam} , but had no effect on K_{pet} (Figure 3C, Table 2). Atrazine significantly reduced the rates of net CO₂ exchange and transpiration (Table 2). Similar results were obtained with senescing leaves (Figure 3D). Atrazine had no effect on K_{pet} , net CO₂ exchange or transpiration in senescing leaves (Tables 1 and 2).

Lamina conductance of fusicoccin-treated leaves was less responsive to high irradiance than that of control leaves ($P < 0.05$; Figure 3E). Fusicoccin had no effect on K_{pet} (Table 1), net CO₂ exchange or transpiration (Table 2).

Discussion

In most previous studies, the light-induced enhancement of leaf conductance was computed for the whole leaf, (i.e., lamina plus petiole resistance in series). There is evidence that increases in K_L in response to light are the result of changes in

Table 1. Petiole hydraulic conductivity (K_{pet} ; $10^{-2} \text{ mg m MPa}^{-1} \text{ s}^{-1}$) of bur oak (*Quercus macrocarpa*) leaves as measured in different experiments, and ANOVA probability values (P) for treatment effects on K_{pet} . The corresponding values for K_{lam} , which were determined after the measurement of K_{pet} , are shown in Figures 1–3, as indicated. Values are least square means \pm SE ($n = 5-6$).

Treatment	K_{pet}	P
<i>Figures 1A–C</i>		
Ambient irradiance	5.28 ± 0.39	0.6464
High irradiance	5.29 ± 0.39	
NaF, 1 mM for 5 h	5.17 ± 0.39	
Distilled water for 24 h	4.80 ± 0.39	
NaF, 1 mM for 24 h	4.60 ± 0.39	
<i>Figure 1D</i>		
Seedling	0.93 ± 0.09	
<i>Figure 2A</i>		
Control	5.16 ± 0.47	0.5347
Veins severed	5.64 ± 0.42	
Leaves covered before treatment	4.95 ± 0.70	
<i>Figure 2C</i>		
Amber light	5.59 ± 0.24	0.1765
Blue light	4.79 ± 0.23	
Green light	5.38 ± 0.23	
Red light	5.04 ± 0.23	
White light	5.28 ± 0.24	
<i>Figure 3A</i>		
Distilled water	154 ± 24	0.0972
HgCl ₂ , 0.1 mM	90 ± 25	
<i>Figure 3B</i>		
Distilled water	167 ± 10 a	0.0011
HgCl ₂ , 0.1 mM and 2-mercaptoethanol, 15 mM	101 ± 10 b	
<i>Figure 3C: mature leaves</i>		
Distilled water	100 ± 14	0.4507
Atrazine, 0.5 mM	114 ± 14	
<i>Figure 3D: senescing leaves</i>		
Distilled water	64.0 ± 9.5	0.8410
Atrazine, 0.5 mM	61.3 ± 9.5	
<i>Figure 3E</i>		
Distilled water	108 ± 21	0.8983
Fusicoccin, 1.4 μM	105 ± 21	

the non-vascular pathways (Cochard et al. 2007). We therefore subtracted petiole resistance from whole-leaf resistances and computed K_{lam} from the inverse of the result. Many species show a slight ($< 1.5\times$) enhancement in K_L in response to irradiance (Sack et al. 2002, Tyree et al. 2005), but only four species so far, including bur oak, as reported in this study, exhibit such a large (2- to 7-fold) increase.

This paper is the first to report effects of irradiance and light quality on the enhancement of K_{lam} . The light response saturated at relatively low irradiances and was greater in blue and green light than in orange and red light. We demonstrated sup-

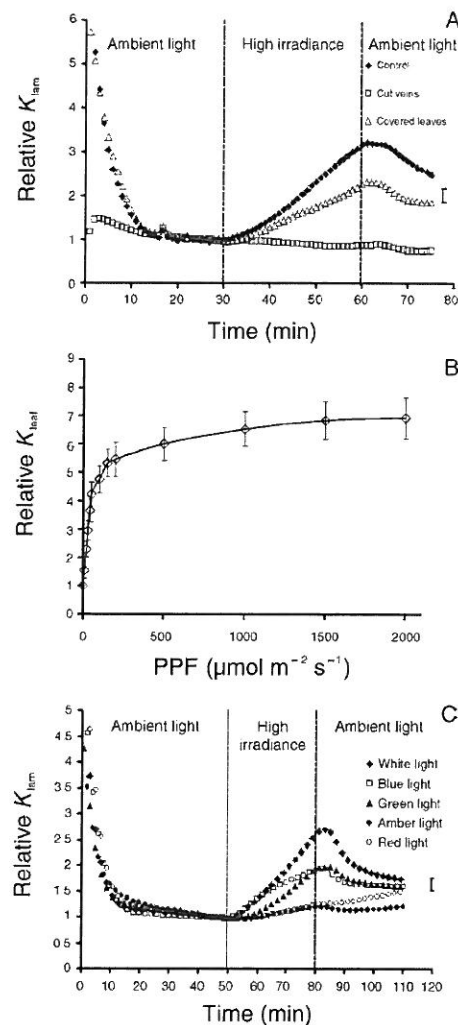


Figure 2. (A) Effects of tertiary vein severance and 2 weeks of leaf covering on the light response of relative leaf lamina hydraulic conductance (K_{lam}) in bur oak (*Quercus macrocarpa*) leaves. (B) Effect of irradiance on relative leaf hydraulic conductance (K_{lam}). (C) Relative K_{lam} in response to high irradiance with LED light of different peak wavelengths (about $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF): white, blue (470 nm), green (525 nm), amber (589 nm) and red (619 nm). Values of K_{lam} are relative to ambient light values (A,C) or the value in darkness (B). Vertical bars equal ± 1 standard error (B) or the mean standard error (A,C).

pression of the light response by several inhibitors that affect various physiological processes including NaF (a general metabolic inhibitor) atrazine (which inhibits photosynthetic electron transport), HgCl₂ (an aquaporin blocker), fusicoccin (which stimulates plasma membrane-bound H⁺-ATPase) and 2-mercaptoethanol (a reducing agent that disrupts quaternary protein structure).

Following perfusion with HPFM, K_{lam} abruptly decreased before reaching a steady-state (Figures 1A–C). It has been suggested that the initial decrease is due to leaf rehydration (Tyree et al. 2005), the capacitance of tubing and the leaf (Sack

Table 2. Gas exchange rate, transpiration rate and temperature of bur oak (*Quercus macrocarpa*) leaves of trees in different light and chemical treatments. ANOVA probability values (P) for treatment differences are given. In most experiments, following gas exchange measurement, the light response of K_{lam} was determined, and the data are shown in Figure 3, as indicated. Values are least square means \pm SE ($n = 6$). Values followed by different letters are significantly different.

Treatment	Net CO ₂ exchange ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<i>P</i>	Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)	<i>P</i>	Leaf temperature ($^{\circ}\text{C}$)	<i>P</i>
<i>Light treatment</i>						
Amber light	5.82 ± 0.19 a	< 0.0001	1.095 ± 0.017	0.5855	26.51 ± 0.48	0.3745
Blue light	4.00 ± 0.19 b		1.114 ± 0.024		26.73 ± 0.48	
Green light	5.44 ± 0.19 a		1.099 ± 0.017		26.69 ± 0.48	
Red light	5.80 ± 0.19 a		1.098 ± 0.021		26.77 ± 0.48	
White light	5.72 ± 0.19 a		1.172 ± 0.047		26.75 ± 0.48	
<i>Figure 3C: mature leaves</i>						
Distilled water	5.45 ± 0.33 a	0.0002	0.82 ± 0.07 a	0.0064	26.62 ± 0.48	0.3144
Atrazine, 0.5 mM	1.79 ± 0.33 b		0.43 ± 0.07 b		27.37 ± 0.48	
<i>Figure 3D: senescing leaves</i>						
Distilled water	0.20 ± 0.14	0.9068	0.06 ± 0.02	0.1816	27.47 ± 0.65	0.6058
Atrazine, 0.5 mM	0.17 ± 0.14		0.10 ± 0.02		26.96 ± 0.65	
<i>Figure 3E</i>						
Distilled water	5.90 ± 0.72	0.8885	0.70 ± 0.08	0.8343	25.71 ± 0.09 a	0.0013
Fusicoccin, 1.4 μM	5.75 ± 0.72		0.67 ± 0.08		25.00 ± 0.09 b	

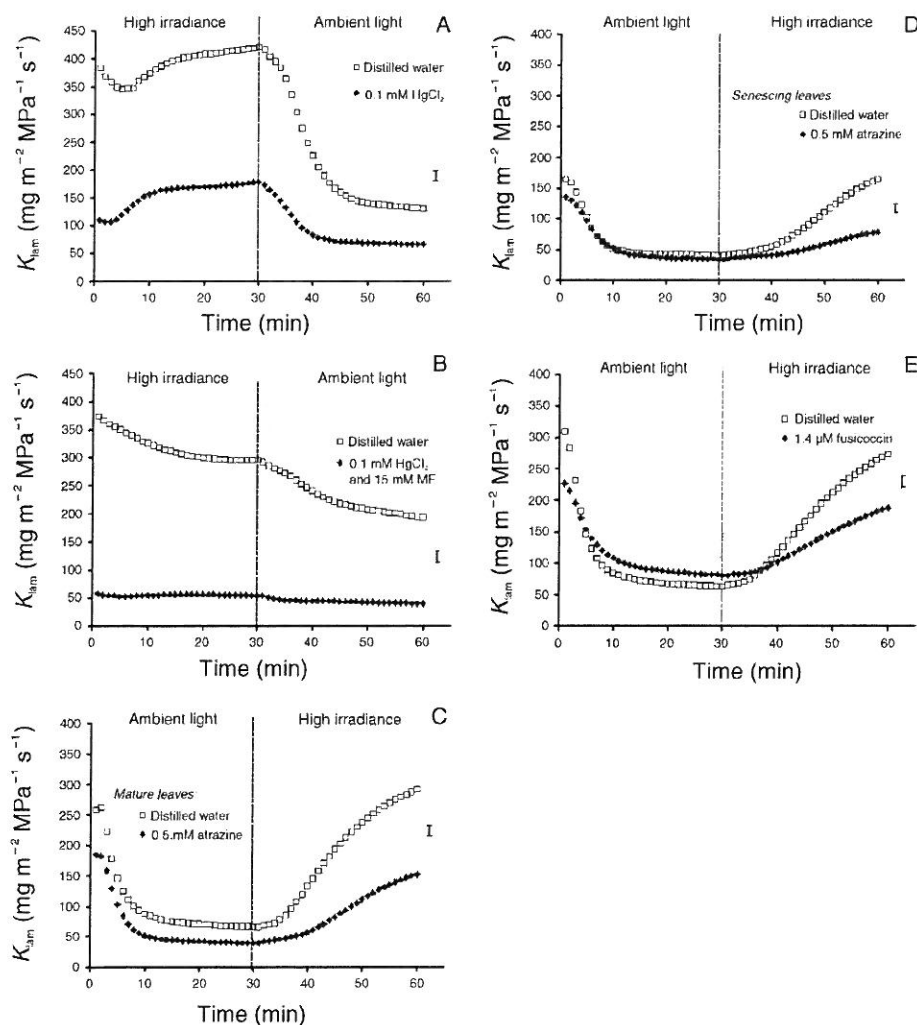


Figure 3. Response of leaf lamina hydraulic conductance (K_{lam}) of bur oak (*Quercus macrocarpa*) leaves to high irradiance with white light as influenced by pre-treatment with different chemicals. Leaves pretreated with distilled water (\pm ethanol) served as controls. Time zero represents the end of the pretreatment and the beginning of the HPFM measurements. (A) Mature leaves pretreated with 0.1 mM HgCl_2 for 100 min. (B) Mature leaves pretreated with 0.1 mM HgCl_2 followed by 15 mM 2-mercaptoethanol (ME) for 90 and 60 min, respectively. (C) Mature and (D) senescing leaves pretreated with 0.5 mM atrazine for 1 h. (E) Leaves pretreated with 1.4 μM fusicoccin for 2 h. Values are least square means \pm SE ($n = 6$). Mean standard error bars are also shown.

et al. 2002), or to dilution of the solutes in the apoplast (Gasco et al. 2004). However, these suggestions have never been put to a quantitative test. Therefore, it is possible that the initial decrease of K_{lam} is determined by elements situated outside of the leaf main vein system, possibly in the cell membranes.

In mature bur oak, K_{lam} increased about 4–7-fold in response to high irradiance. The light response of K_{lam} may be due to changes in the apoplastic, symplastic or cell-to-cell pathways of water flow (Sperry et al. 2003, Cochard et al. 2004, 2007). To investigate the contribution of the main vein network to the K_{lam} light responsiveness, we cut tertiary veins in leaves at about 0.5 cm from the secondary veins. Cutting tertiary veins will open a low resistance pathway for water flow if the main resistance is in the living cells. Leaves with severed veins had a more than 10-fold increased hydraulic conductance and showed no light response, suggesting that hydraulic properties of leaf veins were not involved in irradiance-induced changes in K_{lam} (Figure 2A).

The effects of various chemical treatments on the light response of K_{lam} suggest metabolic regulation. Treatment of leaves for 5 h with 1 mM NaF decreased g_s about 3-fold (Figure 1E), but had little effect on the light response of K_{lam} (Figure 1B). Fluoride is a metabolic inhibitor that alters the activity of protein phosphatases and plasma membrane H^+ -ATPase (Zwiazek and Shay 1988, Struglics et al. 2000). It also strongly inhibits root aquaporin activity, and this inhibition precedes stomatal closure (Kamaluddin and Zwiazek 2003).

Treatment with 0.1 mM HgCl_2 reduced K_{lam} and its light response to high irradiance (Figure 3A). Mercury is a commonly used aquaporin blocker and, in higher concentrations, it inhibits respiration and metabolic processes (Tyerman et al. 1999, Wan and Zwiazek 1999). We tried to restore the light response of K_{lam} following HgCl_2 treatment by adding 15 mM 2-mercaptoethanol, but this treatment entirely eliminated the light response of K_{lam} (Figure 3B). Although reducing agents are often used to restore water transport following mercury treatment of roots or leaves (Wan and Zwiazek 1999, Nardini et al. 2005), further reduction of root hydraulic conductance by 2-mercaptoethanol following mercury treatment has also been observed (Siemens and Zwiazek, University of Alberta, unpublished data, Bramley 2006). 2-Mercaptoethanol is a thiol reducing agent affecting disulfide bonds and can interfere with the redox status of cells (Schürmann and Jacquot 2000, Foyer and Noctor 2005) and with the formation of aquaporin tetramers (Ampilogova et al. 2006). However, in the present study, 2-mercaptoethanol and mercury may have interacted in an unknown manner causing a further decline in K_{lam} .

We used fusicoccin, which is reported to induce stomatal opening, in an attempt to assess the role of stomata in the light response of K_{lam} . Treatment with this substance had no effect on transpiration in the light (Table 2), but decreased the light response of K_{lam} (Figure 3E), indicating that stomata are not involved in the response. Fusicoccin exerts its action by binding to 14-3-3 proteins, which in turn can bind to phosphorylated proteins that further modulate a large number of signaling and regulatory pathways (De Boer 1997, Roberts 2003).

Our results show that the light response of K_{lam} is metaboli-

cally dependent and not located within the leaf veins. Outside the veins, water can follow the apoplastic or the cell-to-cell pathways. Most of the inhibitors used did not significantly affect K_{lam} in darkness, supporting the hypothesis that K_{lam} in darkness is largely determined by the apoplastic flow, whereas the light response of K_{lam} is determined by the cell-to-cell pathway (Cochard et al. 2007). Therefore, the decrease in K_{lam} in bur oak that was brought about by the inhibitors likely reflected a decrease in plasma membrane water permeability.

The permeability of biological membranes to water can be enhanced by aquaporins (Tyerman et al. 2002), which are present in leaves (Kaldenhoff et al. 1995, Frange et al. 2001). In roots, short-term changes in water transport can be modulated by the effects of cellular respiration (phosphorylation) on the activity of aquaporins (Zhang and Tyerman 1999, Kamaluddin and Zwiazek 2001). However, in leaves, the dominant system involved in energy transduction is photosynthesis, and it has been suggested that the high irradiance light response of K_{leaf} involves photophosphorylation-induced opening of the aquaporins (Tyree et al. 2005).

When leaves were exposed to light of different colors but at the same PPF, K_{lam} followed a different pattern than net CO_2 uptake. Net CO_2 uptake of leaves was much lower in blue light than in light of the other tested colors (Table 1), whereas white, green and blue light were more effective in increasing K_{lam} than orange and red light (Figure 2C). Because CO_2 diffusion in water is limited and leaf air spaces are flooded during K_{lam} measurements, it is unlikely that CO_2 uptake was a driving force behind the light response of K_{lam} . However, photosynthetic electron transport, mitochondrial respiration and photorespiration may have been unaffected by leaf flooding (Feild et al. 2005).

To test the hypothesis that photosynthetic electron transport is involved in the light response of K_{lam} , we used a PSII inhibitor, atrazine, in mature and senescing leaves. Atrazine decreased the light response of K_{lam} in both mature and senescing leaves (Figures 3C and 3D). In mature leaves, there was a large decrease in net CO_2 uptake and transpiration following atrazine application (Table 2). Atrazine acts by physically blocking the high energy electron transfer between Photosystem II (PSII) and plastoquinone (Hall et al. 1999, Oettmeier 1999). Although we cannot exclude the possibility of oxidative damage to non-photosynthetic processes by atrazine (Hall et al. 1999, Rutherford and Krieger-Liszka 2001), the rapidity of the response suggests that the primary effect was on photosynthetic electron transport.

Green light was almost as effective as blue light in increasing K_{lam} , and both wavelengths were significantly more effective than red light (Figure 2C). Red light, which efficiently drives net CO_2 uptake, had a minimal effect on K_{lam} . In white light, the response of K_{leaf} saturated at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Figure 2B). White light was also more effective in enhancing K_{lam} (Figure 2C) than light of similar PPF from any of the colored LEDs, suggesting light of different wavelengths may act independently.

The light response of K_{lam} was observed in senescing leaves in which photosynthetic capacity was reduced, suggesting that

mechanisms other than, or in addition to, photosynthesis mediate the response. Some green light responses of higher plants have been documented, and it has been suggested that these are mediated by blue light photoreceptors including an isomer of zeaxanthin (Talbot et al. 2002, Folta 2004) and phototropin (Casal 2000). The response of K_{lam} to green light may be important for leaves deep within the canopy that rely heavily on sunflecks for photosynthesis. However, the mechanisms for K_{leaf} enhancement by light may vary with species. In walnut leaves, the response of K_{leaf} to light saturated at about 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Cochard et al. 2005).

Leaf conductance in bur oak seedlings raised in a growth room was insensitive to changes in irradiance and was generally higher than in leaves of mature trees (Figure 1D). Higher K_{lam} of seedlings could be triggered by growth conditions such as water and nutrient supply (Aasamaa et al. 2001). In larger trees, there may be adaptation of leaf conductance to ambient conditions. However, we found that if leaves of mature trees were held in darkness for two weeks they did not lose the ability to respond to high irradiance by increasing K_{lam} (Figure 2A). This suggests that the potential for this response in leaves is present regardless of their recent history of exposure to light. In larger trees, the fine tuning of water flow could be beneficial because of the lower water demand of severely shaded branches.

In conclusion, the response of K_{lam} in bur oak to light is likely localized in the non-vascular pathway and requires requires an irradiance that is only a fraction of full sunlight to induce near maximal enhancement of K_{lam} . Our results on the differential response of K_{lam} to light color will require follow-up studies to help characterize and identify photoreceptors that are involved in this response. Although photosynthetic electron transport appears to be among the processes involved in the light response, the processes are likely complex and involve other receptors. Future work in this lab will focus on the connection between the light response and the putative role of aquaporins.

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