

Impact of light quality on leaf and shoot hydraulic properties: a case study in silver birch (*Betula pendula*)

ARNE SELLIN¹, LAWREN SACK², EELE ÕUNAPUU¹ & ANNIKA KARUSION¹

¹Institute of Ecology and Earth Sciences, University of Tartu, 51005 Tartu, Estonia and ²Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095-1606, USA

ABSTRACT

Responses of leaf and shoot hydraulic conductance to light quality were examined on shoots of silver birch (*Betula pendula*), cut from lower ('shade position') and upper thirds of the crowns ('sun position') of trees growing in a natural temperate forest stand. Hydraulic conductances of leaf blades (K_{lb}), petioles (K_p) and branches (i.e. leafless stem; K_B) were determined using a high pressure flow meter in steady state mode. The shoots were exposed to photosynthetic photon flux density of 200–250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using white, blue or red light. K_{lb} depended significantly on both light quality and canopy position ($P < 0.001$), K_B on canopy position ($P < 0.001$) and exposure time ($P = 0.014$), and none of the three factors had effect on K_p . The highest values of K_{lb} were recorded under the blue light (3.63 and $3.13 \times 10^{-4} \text{ kg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ for the sun and shade leaves, respectively), intermediate values under white light (3.37 and $2.46 \times 10^{-4} \text{ kg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$, respectively) and lowest values under red light (2.83 and $2.02 \times 10^{-4} \text{ kg m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$, respectively). Light quality has an important impact on leaf hydraulic properties, independently of light intensity or of total light energy, and the specific light receptors involved in this response require identification. Given that natural canopy shade depletes blue and red light, K_{lb} may be decreased both by reduced fluence and shifts in light spectra, indicating the need for studies of the natural heterogeneity of K_{lb} within and under canopies, and its impacts on gas exchange.

Key-words: branch hydraulic conductance; forest canopy; leaf hydraulic conductance; light spectrum; liquid-phase resistance; petiole hydraulic conductance; potassium ions; shoot hydraulic conductance; xylem sap.

INTRODUCTION

Leaves account for majority (47–94%) of the hydraulic resistance to water flow through the shoots of woody plants (Yang & Tyree 1994; Nardini & Pitt 1999; Nardini & Salleo 2000; Nardini 2001; Sellin, Õunapuu & Kopper 2008), and a substantial part of the hydraulic resistance in whole plants. For a number of species over a range of life forms, leaves,

including petioles, contribute 30% or more of the plant total liquid-phase resistance (Becker, Tyree & Tsuda 1999; Sack *et al.* 2003; Gyenge *et al.* 2005; Sack & Holbrook 2006; Sellin & Kopper 2007a). Therefore, any changes in leaf hydraulic conductance (K_{leaf}) caused either by environmental or internal stimuli influence the ability of plants to supply water to mesophyll cells, and have consequences on stomatal conductance and photosynthetic capacity (Brodribb & Holbrook 2004, 2005; Franks 2006; Brodribb, Feild & Jordan 2007; Woodruff *et al.* 2007; Domec *et al.* 2009). Hence, leaf hydraulic properties are associated ultimately with productivity, growth rate, competitiveness and survival of plants.

However, our knowledge is still limited with respect to regulation of leaf hydraulic properties in response to ambient conditions because of tremendous diversity of leaf structure and function, species-specific differences in the partitioning of leaf hydraulic resistance ($R_{leaf} = 1/K_{leaf}$), and varying mechanisms controlling water transport capacity of vascular and extravascular compartments depending on environmental variables. There is increasing evidence that confirms the dependence of leaf hydraulic traits on light availability (Sack, Tyree & Holbrook 2005; Sack & Holbrook 2006; Scoffoni *et al.* 2008; Voicu, Cooke & Zwiazek 2009). A study in 11 temperate and tropical tree species revealed that K_{leaf} increased with irradiance for six of the species tested (Tyree *et al.* 2005). Sellin *et al.* (2008) indicated that leaves of silver birch (*Betula pendula* Roth) respond to light by adjusting their hydraulic efficiency to both light duration and intensity. Experimental evidence suggests that the light-induced enhancement of K_{leaf} is controlled by factors acting on extravascular tissues and involves expression or activation of plasma membrane aquaporins (AQPs) in mesophyll and/or bundle sheath cells (Tyree *et al.* 2005; Cochard *et al.* 2007; Voicu, Zwiazek & Tyree 2008; Heinen, Ye & Chaumont 2009; Voicu & Zwiazek 2010). Because of such adjustment, and to differential development of venation properties, the K_{leaf} of sun leaves is higher than for shade leaves (Sack *et al.* 2003; Sellin & Kopper 2007a,b; Sellin *et al.* 2008), and K_{leaf} is on average higher for sun- than shade-establishing species (Brodribb & Holbrook 2004; Sack *et al.* 2005; Sack & Frole 2006). Diurnal variation in K_{leaf} is also related to light availability for both herbaceous (Nardini, Salleo & Andri 2005) and woody plants (Lo Gullo *et al.* 2005). Kim & Steudle (2009) demonstrated that hydraulic conductance of leaf cells is

Correspondence: E. Õunapuu. Phone: +372-7376167; Fax: +372-7376222; e-mail: ounapuu@ut.ee

maximized at a certain light intensity and reduced again by high light, which is probably caused by induction of reactive oxygen species. Recent studies (Nardini *et al.* 2010; Sellin, Öunapuu & Karusion 2010) have presented experimental evidence for fast light-mediated changes also in stem hydraulic conductance.

The discoveries that both the plasma membrane and the tonoplast contain AQPs, water channel proteins, have changed our view of how plant cells regulate transmembrane water movement (Chrispeels & Maurel 1994; Maurel 1997; Tyerman, Niemietz & Bramley 2002). AQPs facilitate the rapid exchange of water across cell membranes and are responsible for up to 95% of the water permeability of plasma membranes (Henzler, Ye & Steudle 2004). Plants need to continuously adjust water exchange in response to environmental conditions, and therefore AQPs play an important role in the regulatory processes. Rapid irradiance-mediated increase of K_{leaf} may result from *de novo* expression of AQPs or, more likely, from the up-regulation of pre-existing water channels. Several studies indicate that the post-translational regulation of AQP activity occurs by phosphorylation (Johnson & Chrispeels 1992; Johansson *et al.* 1998; Prak *et al.* 2008). In leaves, where the dominant system involved in energy transduction is photosynthesis, the light response of K_{leaf} has been suggested to involve photophosphorylation-induced opening of AQPs (Tyree *et al.* 2005). To provide co-ordination between gaseous and liquid-phase conductances in heterogeneous radiation field, one can suppose that light quality should have an impact on K_{leaf} . As quantum energy is directly proportional to the frequency of light waves, blue light might have a greater enhancing effect on the hydraulic conductance of the leaf blade than white or red light because of its higher energy. Early studies of the action spectrum of photosynthesis had expected that shorter light wavelengths would stimulate greater activity because of their higher energy, though this pattern was not found, indicating a lower quantum efficiency for blue light than red light (Inada 1976). Further, a recent study has proposed that light energy input determines transpiration rate irrespective of the light colour (Pieruschka, Huber & Berry 2010). Thus, the energy hypothesis for the light quality response of leaf hydraulic conductance required testing. An alternative possibility is that light receptors such as those involved in regulating guard cell turgor for stomatal opening (Chen, Chory & Fankhauser 2004; Shimazaki *et al.* 2007; Briggs 2009) may also be involved in the regulation of AQPs in the hydraulic pathway.

K_{leaf} is determined by the conductance of the xylem and outside-xylem hydraulic pathways, and experiments have pointed to a strong importance of both components. For instance, K_{leaf} responds to temperature, and the slope of the response line is significantly higher than that expected from changes in the dynamic viscosity of water and thus cannot be explained merely by changes in the apoplastic pathway (Matzner & Comstock 2001; Sack, Streeter & Holbrook 2004; Cochard *et al.* 2007; Sellin & Kopper 2007b). Indeed, numerous experiments with application

of metabolic inhibitors confirm involvement of living extravascular tissues in regulation of K_{leaf} (e.g. Nardini *et al.* 2005; Cochard *et al.* 2007; Sellin *et al.* 2008; Voicu *et al.* 2008; Voicu & Zwiazek 2010). Several studies indicate that the extravascular hydraulic resistance constitutes a substantial proportion of leaf total resistance (Trifilò *et al.* 2003; Cochard, Nardini & Coll 2004; Gascó, Nardini & Salleo 2004; Sack *et al.* 2005). Given that extravascular resistances have a strong influence on K_{leaf} , then all factors that enhance AQP expression may facilitate total water flux to sites of evaporation, and importantly constrain leaf gas exchange rates.

Despite many studies on the response of K_{leaf} to irradiance (light quantity), to our knowledge, only one study (Voicu *et al.* 2008) has tested the effects of light colour (light quality) on K_{leaf} . Voicu *et al.* (2008) found a greater hydraulic enhancement in *Quercus macrocarpa* Michx. in response to blue and green light than to visible radiation of longer wavelengths (amber and red). However, the response to white light was greater than to light of any single colour, and the role of wavelength versus total light energy in driving the response was not determined. Our goal was to test experimentally the differential effect of light wavelengths on leaf and shoot hydraulic efficiency and distribution of resistance within the shoots of silver birch (*Betula pendula*). Previous experiments revealed that K_{leaf} of silver birch is sensitive to experimental manipulation of both light intensity and duration (Sellin *et al.* 2008). As different canopy layers in a mixed temperate forest differ substantially in light availability and spectral composition (Smith 1982; Endler 1993; Navrátil *et al.* 2007; Reinhardt, Smith & Carter 2010), the second objective was to estimate the impact of light quality on shoot hydraulic characteristics in relation to canopy position (in sun versus shade shoots). We aimed to broaden the understanding of plant optimization of light and water utilization across the micro-environmental heterogeneity within a forest canopy.

MATERIALS AND METHODS

Plant material

Shoots were sampled from three 25- to 30-year-old trees of *Betula pendula* Roth [height 16.8–19.4 m, diameter at breast height (DBH) 11.6–16.2 cm] growing in Järvselja Experimental Forest (58°16'N, 27°16'E, elevation 40 m ASL), Eastern Estonia, in the lower (shade-exposed shoots) and upper thirds (sun-exposed shoots) of their crown. Detailed data on vegetation and climate of the study area were presented in Sellin & Kopper (2005a,b). In the evening prior to the measurement days, 20–40 cm long shoots were cut under water and put into beakers with the basal ends submerged in water. In the laboratory, the shoots were put into plastic flasks filled with deionized, filtered (Direct-Q3 UV water purification system; Millipore SAS, Molsheim, France) and freshly degassed water (T-04-125 ultrasonic-vacuum degasser; Terriss Consolidated Industries, Asbury Park, NJ, USA), and rehydrated overnight in a dark room.

Measurement of hydraulic conductance

In the morning, the shoots were exposed to light of photosynthetic photon flux density (PPFD) of 200–250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 1, 3 or 5 h before the hydraulic measurements. The PPFD was measured with a LI-190 quantum sensor (Li-Cor Biosciences, Lincoln, NE, USA) and energy flux within the band of photosynthetically active radiation with a SKE 510 energy sensor (Skye Instruments, Llandrindod Wells, UK). The effect of light quality on plant hydraulic properties was investigated by exposing shoots before and during the hydraulic measurements to light from different sources: blue light from metal halide lamps (Art-colour MH-T Blue, 400 W; Philips, Eindhoven, the Netherlands) with spectral emission maximum at 450–460 nm; white light from high pressure sodium lamps (Master SON-T PIA Agro, 400 W; Philips) with wide spectral emission band of 550–710 nm and with additional small peaks in blue band; or red light from the Master SON-T PIA Agro lamps used in combination with 026 Bright Red colour filters (Lee Filters, Andover, UK), which lacked wavelengths below 600 nm. Irradiance was controlled by changing the distance from the lamps. Air above the shoots was agitated with a fan to minimize local temperature gradients.

The hydraulic conductance of the shoot (K_S) and its component branch (i.e. leafless stems), petioles, and leaf blades were determined by the water perfusion method using a high pressure flow meter (HPFM; Dynamax, Houston, TX, USA) applied in steady state mode, and by removing leaf blades and petioles in sequence. The hydraulic conductance of leaf blades (K_{lb}) was calculated as

$$K_{lb} = (K_S^{-1} - K_{B+P}^{-1})^{-1}, \quad (1)$$

where K_{B+P} is the hydraulic conductance of a branch together with petioles. K_{lb} is an integral measure for all the transpiration flow paths, from the petiole-leaf junction, to the sites of evaporation, both through apoplast and symplast (Sack *et al.* 2002). The hydraulic conductance of petioles (K_P) was calculated as

$$K_P = (K_{B+P}^{-1} - K_B^{-1})^{-1}, \quad (2)$$

where K_B is the hydraulic conductance of a bare branch. Tyree *et al.* (2005) indicated that stomatal openness does not influence results of the hydraulic measurements using the high pressure flow meter: when stomatal response to light was inhibited by the application of ABA, the K_{leaf} response to light remained unchanged, and other experiments confirmed that stomata did not influence the light-induced alteration of K_{leaf} (Cochard *et al.* 2007; Scoffoni *et al.* 2008; Voicu *et al.* 2008). During the measurements leaf and branch temperatures were recorded and stored with MT2 fast response temperature probes and a DL2e data logger (Delta-T Devices, Burwell, UK). After the hydraulic measurements the number of leaves was counted, and the total area of leaf blades was determined with an AM300 digital area meter (ADC BioScientific,

Great Amwell, UK). The data on hydraulic conductance were corrected for the dynamic viscosity of water at 24 °C and normalized to shoot size by expressing the conductance values per unit area of the leaf blades. Altogether, 108 shoots (3 trees \times 2 canopy positions \times 3 light colours \times 3 exposure times \times 2 replications) were sampled for hydraulic conductance.

Potassium ion concentration of the xylem sap

In order to test for possible light-mediated modification of ionic concentration of the xylem sap and consequent changes in K_B (Zwieniecki, Melcher & Holbrook 2001; Nardini *et al.* 2007; Cochard *et al.* 2010) we determined potassium ion concentration $[(K^+)]$ of the sap. $[K^+]$ was measured simultaneously in pairs of shoots taken from the same tree and canopy layer, including the shoot used for hydraulic measurements, and an additional shoot. The extraction procedure of the xylem sap was based on that of Stark, Spitzner & Essig (1985). Extraction was performed on the leafless stem after ~3 cm of the bark had been removed from the cut end of the shoot to avoid contamination with phloem sap. The cut end of the stem was slipped through a tight-fitting rubber stopper and the stopper was inserted into a pressure chamber (Model 1000; PMS Instrument Company, Albany, OR, USA), with the cut end extending out through the top of the chamber. The stripped end was cleaned with deionized water, wiped with filter paper and fitted with a tight piece of plastic tubing. The pressure was increased slowly and held at 2.0 MPa, and extracted xylem sap was collected into a 1.5 mL Eppendorf tube. A sap sample of 0.1 mL was immediately measured for $[K^+]$ using a C-131 potassium ion meter (Horiba, Kyoto, Japan), based on ion-selective electrodes. Before each measurement time the instrument was calibrated with two standard solutions provided by the manufacturer.

Data analysis

Data analysis was carried out using Statistica, Vers. 7.1 (StatSoft Inc., Tulsa, OK, USA). To analyse the effects of light quality on leaf and shoot hydraulic parameters, an analysis of variance (ANOVA) was performed, with light colour, exposure time and canopy position as fixed factors; type III sums of squares were used in the calculations. Normality and homogeneity of variances were checked using the Kolmogorov–Smirnov D-statistic and the Levene test, respectively (Sokal & Rohlf 1995). When appropriate, logarithmic or root transformations were applied to the data. *Post hoc* mean comparisons were conducted using the Tukey honestly significant difference (HSD) test. Relationships between the studied characteristics and independent variables were assessed by Pearson's correlations or simple linear regressions fitted by least-squares.

Dependent variable	Factor	Statistical significance (<i>P</i>)
Shoot hydraulic conductance, K_S	Canopy position	<0.001
	Light quality	ns
	Exposure time	ns
Leaf blade hydraulic conductance, K_{lb}	Canopy position	<0.001
	Light quality	<0.001
	Exposure time	ns
Branch hydraulic conductance, K_B	Canopy position	<0.001
	Light quality	ns
	Exposure time	0.014
Petiole hydraulic conductance, K_P	Canopy position	ns
	Light quality	ns
	Exposure time	ns

ns, not significant.

RESULTS

Effects of light quality on leaf and shoot hydraulic properties

We found strong impacts of light quality treatments on hydraulic conductances (Table 1). K_S depended only on canopy position, K_{lb} on canopy position and light quality, and K_B on canopy position and exposure time. However, sun leaves did not differ in K_{lb} between the lights of different wavelengths at the 0.05 level of significance (Fig. 1). None of the three factors had statistically significant effect on K_P . Sun shoots exhibited ~1.3 times higher ($P < 0.001$) hydraulic capacity than shade shoots: across all light treatments K_S averaged (\pm SE) 1.73×10^{-4} ($\pm 7.4 \times 10^{-6}$) and 1.33×10^{-4} ($\pm 6.3 \times 10^{-6}$) $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, respectively; K_{lb} was also 1.3 times and K_B 1.4 times higher ($P < 0.001$). K_{lb} increased in the following order – red, white and blue light treatment (Fig. 1), and this was not in accordance with total quantum energy recorded in different treatments. Radiant energy fluxes in the band of PAR ranged in the following order: red (43 W m^{-2}), blue (45 W m^{-2}) and white light (48 W m^{-2}) treatment. Shade leaves demonstrated larger

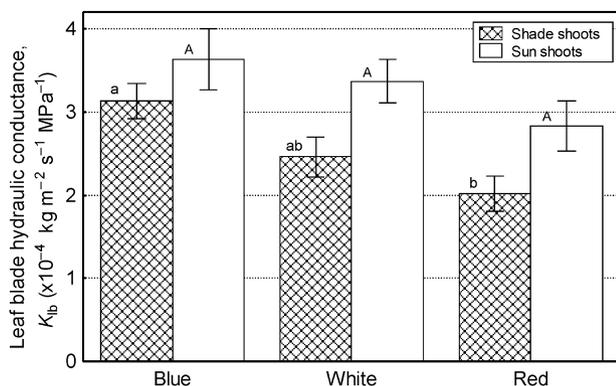


Figure 1. Variation in leaf blade hydraulic conductance (K_{lb}) for *Betula pendula* in response to light colour and canopy position. Different letters denote statistically significant ($P < 0.05$) differences between the treatments.

Table 1. Results of analysis of variance (ANOVA) for the effects of canopy position, light quality and exposure time on hydraulic conductance of shoots and their components ($n = 108$)

dynamics of K_{lb} in response to the light treatments than the sun leaves (increased 55% versus 28% under blue compared with red light, respectively), although the effect of canopy position \times light quality interaction was not significant in the ANOVA model (Table 2).

The changes in K_{lb} induced by light quality brought about a considerable redistribution of the liquid-phase resistance within the sample shoots. Both in sun and shade shoots the contribution of leaf blades to the whole-shoot resistance increased and that of bare branch decreased in the order – blue, white and red light (Fig. 2). Under white light, about 11% of the leaf resistance was located in the petiole, being independent of the canopy position. In white light, the whole-leaf conductance (K_{leaf}) averaged 2.95 ± 0.21 and $2.17 \pm 0.21 \times 10^{-4} \text{ kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ for sun and shade foliage, respectively.

Effect of light exposure time on shoot hydraulic properties

K_S , K_{lb} and K_P did not depend on light exposure time (Table 1), but K_B varied with both canopy position ($P < 0.001$) and exposure time ($P = 0.014$). K_B of the sun shoots decreased considerably over time independently of the light quality (Fig. 3). K_B decreased 23% for sun shoots, by contrast with 10% (statistically insignificant) for shade shoots over 5 h of exposure.

Table 2. Results of analysis of variance (ANOVA) for the effects of canopy position and light quality on leaf blade hydraulic conductance ($\ln K_{lb}$); $n = 108$

Factor	SS	df	MS	F	<i>P</i>
Canopy position	1.923	1	1.923	11.68	<0.001
Light quality	2.577	2	1.288	7.83	<0.001
Canopy position \times light quality	0.379	2	0.189	1.15	0.321
Error	16.785	102	0.165	–	–

SS, sum of squares; MS, mean square; F, *F*-ratio; *P*, statistical significance.

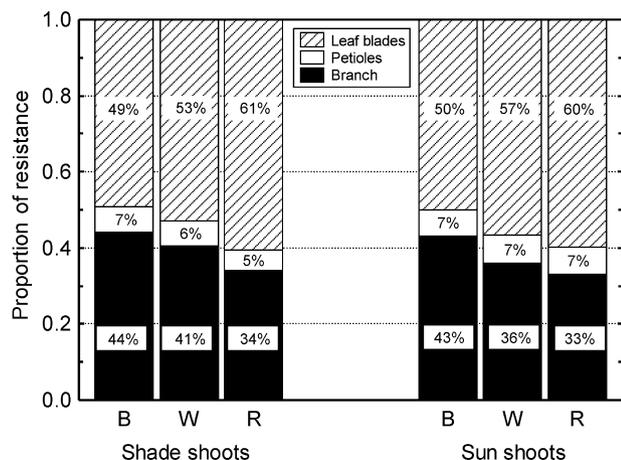


Figure 2. The proportion of liquid-phase resistance to branch, petiole and leaf blade within shoots of *Betula pendula* exposed to blue (B), white (W) or red light (R).

We did not find significant differences in the potassium ion concentration of the xylem sap $[(K^+)]$ between the light treatments. Only canopy position had an effect ($P < 0.05$) on $[K^+]$; values averaged 16.6 ± 1.61 and 24.3 ± 3.03 p.p.m. for the lower- and upper-canopy unperfused shoots, respectively. In spite of high variation, K_B showed a rising trend ($R^2 = 0.281$, $P < 0.05$) with increasing potassium ion concentration in the xylem sap across all treatments.

DISCUSSION

Light quality

Light is one of the most important environmental factors that influence leaf water transport capacity. Studies investigating the effect of light on leaf hydraulic conductance have so far concentrated mainly on the effects of light intensity or duration (e.g. Sack *et al.* 2002, 2005; Nardini *et al.* 2005; Tyree *et al.* 2005; Cochard *et al.* 2007; Sellin *et al.* 2008; Voicu *et al.* 2008). But a novel and fascinating issue is the effect of light quality on hydraulic properties. We found a strong effect of light quality on leaf hydraulic efficiency in *B. pendula*. Our experiment revealed that K_{lb} was highest in blue light, followed by white light and lowest in red light (Fig. 1). The K_{lb} of leaves under blue light was on average 1.2 times higher than under white and 1.4 times higher than under red light. The short-term (time scale in hours) variation in K_{lb} with respect to light spectral traits would relate to changes in the conductance of the tissues outside the xylem, and likely reflected changes in the water permeability of plasma membranes of leaf cells. The results confirm our hypothesis that blue light has greater enhancing effect on leaf hydraulic conductance than white or red light. However, the response of K_{lb} cannot be attributed to differences in total quantum energy and, thus, our results do not support the idea of energy balance-dependent photophosphorylation-induced opening of plasma membrane AQPs. Indeed, experiments performed in *Populus*

tremuloides Michx. suggested that on a shorter time scale (minutes), photosynthetic electron transport was not directly involved in the control of K_{leaf} (Voicu & Zwiazek 2010).

As we observed small differences in radiant energy fluxes driving substantial differences in K_{lb} between the light treatments, the results imply that leaf hydraulic conductance probably depends on differential sensing by specific light receptors. Blue light acts as a signal for stomatal opening in a number of C_3 and C_4 plants, and on a quantum basis, it is several times more effective than red light in opening stomata (Sharkey & Ogawa 1987; Zeiger *et al.* 2002; Inada *et al.* 2004; Shimazaki *et al.* 2007; Inoue *et al.* 2008). Based on the present results, we propose that blue light acts also as a signal to up-regulate AQPs in order to maintain functional balance between gaseous and liquid-phase conductances at high stomatal conductance. Additional mechanistic studies are needed to test this hypothesis. Plants use a range of blue light receptors (including cryptochromes and phototropins) to modulate a variety of responses, and these interact with circadian oscillators (Demarsy & Fankhauser 2009). Further, the transduction of the light signal to the physiological response requires investigation. A fruitful area of research will be the stimulation of AQPs by light quality. We note at least one demonstration of an AQP promoter (*AthH2* in *Arabidopsis*) that is stimulated by blue and white light more than red light (Kaldenhoff, Kölling & Richter 1996). The intermediate enhancing effect of the white light on K_{lb} observed in the present study can be explained by some comprisal of blue band (small emission peaks), while the red light totally lacked wavelengths below 600 nm.

The spectrum-dependent responses of K_{lb} in silver birch resulted in a considerable shift of resistance within the whole shoot: the contribution of leaf blades to the total shoot hydraulic resistance increased from 49–50% under blue light to 60–61% under red light (Fig. 2). Under white light, on average 11% of the leaf hydraulic resistance was

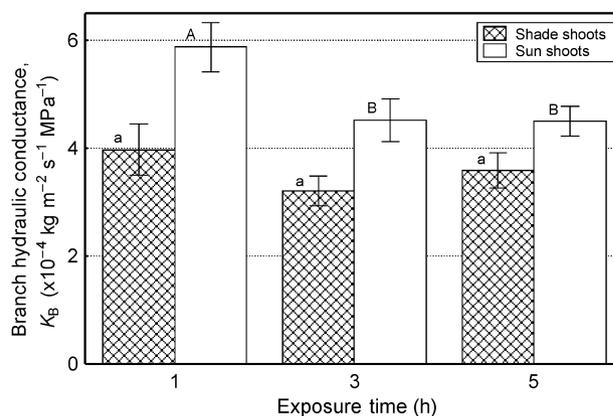


Figure 3. Variation in hydraulic conductance of bare branches (K_B) of *Betula pendula* in response to light exposure time. Different letters denote statistically significant ($P < 0.05$) differences between the treatments.

located in a petiole, coinciding with that found for *Betula papyrifera* Marsh. (~10%), a congeneric species (Sack *et al.* 2003). As compared with other species, it is less than for shade-tolerant *Acer saccharum* Marsh. (14%) and greater than for shade-intolerant *Quercus rubra* L. leaves (4%), and matches with that published for the light-demanding tropical tree species (Sack & Tyree 2005; Sack *et al.* 2005). Sun foliage exhibited ~1.4 times higher whole-leaf hydraulic conductance (K_{leaf}) relative to shade foliage under white light, coinciding with the ratio obtained on silver birch using evaporative flux method (Sellin *et al.* 2008). However, the values of K_{leaf} were 47–52% higher in this study, which may have arisen from inter-annual variability, or from differences between the measurement techniques; the high-pressure method removes all emboli from xylem conduits and fills intercellular spaces of the lamina. The present data on K_{leaf} (2.95 and $2.17 \times 10^{-4} \text{ kg m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ for sun and shade foliage, respectively) match well with those published for *B. papyrifera* – 2.5 to $2.7 \times 10^{-4} \text{ kg m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ (leaves taken from exposed part of the crown; Sack *et al.* 2002). Our findings are in accordance also with those obtained for the effects of light of different wavelengths on leaves of *Quercus macrocarpa* (Voicu *et al.* 2008). That study reported a greater hydraulic enhancement under blue and green light than under longer visible wavelengths. In that study, treatment of *Q. macrocarpa* leaves with fusicoccin (to induce stomatal opening) had no effect on transpiration or on the low-light value of K_{ib} , but decreased the high-light value of K_{ib} , indicating that stomata were not involved in the light response of K_{ib} .

B. pendula, an early-successional species, has been reported to be morphologically very sensitive to changes in the ratio of red to far-red light relative to several other temperate broad-leaved tree species (Gilbert, Jarvis & Smith 2001). The high morphogenetic responsiveness reflects a shade-avoidance strategy that would contribute to fitness in dense plant communities. Probably, K_{ib} joins other plant processes known to respond to light quality, as part of the shade-avoidance mechanisms. On the one hand, exposure of leaves to direct radiation (relatively rich in blue light) in sun flecks enable enhancement of K_{leaf} , thus allowing high stomatal conductance and carbon gain, and supporting higher growth rate. On the other hand, a lower K_{leaf} in plants/leaves growing under chronic shade (depleted by blue light) reduces energy expenditure associated with AQP expression.

Green canopies strongly reduce not only total irradiance, but especially reduce blue and red light, leading to a spectrum with a small peak in green and a large peak in far-red band in the inner canopy and understorey (Smith 1982; Urban *et al.* 2007). Those findings in combination with the effects on K_{ib} shown in this study result in a greater K_{ib} for sun-exposed leaves than for the inner canopy and understorey, given suppression by reduced irradiance as well as shifting light quality. Notably, cloud cover causes the spectra within and under canopies to shift to 'white', and thus could reduce the relative inhibition of K_{ib} for shaded leaves under canopies (Endler 1993; Navrátil *et al.* 2007). The variation in

fluence rate and spectra within canopies would produce strong natural heterogeneity of K_{ib} within and under canopies that would be dynamic with cloud cover, with potentially major impacts on gas exchange during overcast days.

The shift in distribution of the resistance between branch, petioles and leaf blades could also have implications for the dynamics of the hydraulic system in response to other environmental variables. K_{leaf} is known to decline as leaves dehydrate because of reductions of conductivity in the xylem caused by cavitation, and in the extra-xylem tissues caused by losses of turgor. The impact of cavitation on K_{leaf} will be stronger when more resistance is in the xylem, whereas the impact of losses of turgor may be stronger when more resistance is located outside the xylem (Brodribb & Holbrook 2006; Scoffoni *et al.* 2008). Thus, light quality and quantity effects may alter the response of K_{leaf} to dehydration by modulating the partitioning of resistance between sym- and apoplast (including the xylem of branch, petiole and venation). The same principle may apply to temperature variation, as K_{leaf} shows a small response of xylem conductivity to temperature caused by changes in viscosity of water, and a greater response in the tissues outside the xylem because of changes in membrane permeability; the greater the resistance of the extra-xylem pathway, the greater the temperature response (Sack *et al.* 2004).

Our experiment revealed that the shade leaves of silver birch exhibited greater physiological dynamics in response to spectral changes than sun leaves. The spectral sensitivity of K_{leaf} may be important for leaves deep within the canopy that experience a highly variable light regime and rely on sun flecks for photosynthesis. In large trees, the rapid adjustment of stomatal aperture and water fluxes, and consequent effects on leaf cooling, could be beneficial as a response to sun fleck patterns in lower canopy layers. The greater physiological responsiveness of shade foliage in response to light treatments in our study (Fig. 1) supports this idea. Our previous study (Sellin & Lubenets 2010) indicated that the partitioning of transpirational fluxes in the canopy depended largely on soil-to-leaf hydraulic resistance, that is, on distribution of the resistance within a tree crown. The regulation of K_{leaf} by light quality provides an additional mechanism for the adjustment of plant water transport capacity to variation in the spectral distribution within vegetation, diurnally and seasonally. This regulation may represent a mechanism for fine-tuning of leaf hydraulic properties in the heterogeneous light environment within a forest canopy.

Light exposure time

Experimental evidence suggests that K_{leaf} depends on the duration of exposure to light (Cochard *et al.* 2007; Sellin *et al.* 2008). Our previous study in silver birch (Sellin *et al.* 2008) revealed that K_{leaf} increased over 9 h of illumination and then showed a decrease. The current experiment did not indicate a significant trend in K_{ib} over 5 h of exposure (Table 1). This discrepancy between the two studies could

be caused by the different experimental setups and measurement techniques: the methods rely on different driving forces, the high-pressure method fills embolized xylem conduits, and different photosynthetic photon flux densities and exposure times were applied.

We found that the K_B of sun shoots decreased with increasing exposure time (Fig. 3). Until quite recently, xylem was considered as a system of inert pipes and a passive pathway for water flow. The possibility of rapid flow control in xylem was not taken under consideration until the past decade, with the demonstration that changes in ionic concentration of xylem sap are responsible for short-term modulation of xylem hydraulic conductance (Zwieniecki *et al.* 2001; Gascó *et al.* 2006; Nardini *et al.* 2007; Cochard *et al.* 2010). The ion-mediated changes of xylem hydraulic conductance could arise from the shrinking and swelling of the inter-vessel pit membrane hydrogels in response to the osmotic concentration of the solution perfused (Ryden *et al.* 2000; Zwieniecki *et al.* 2001). As we kept the shoots in deionized water during the experiment, the decrease of K_B may have arisen from the dilution of xylem sap over time and loss of cations from the intervessel pit membranes. The observation that K_B showed a rising trend with increasing potassium ion concentration in the xylem sap of the shoots across all treatments supports this idea. Additionally, a longer-term effect of light availability on potassium ion concentration with respect to canopy position was also observable: mean $[K^+]$ was significantly higher in the upper-canopy shoots independently of the experimental treatments, in accordance with higher K_B . Our recent experiment (Sellin *et al.* 2010) revealed that significant differences in $[K^+]$ remained in branches of silver birch, taken from different heights, after a 20 min perfusion of stems with deionized water under high pressure, suggesting that living tissues continue to enrich the xylem sap with potassium even after shoot/branch cutting (Metzner *et al.* 2010).

Overall our experiments indicated that light quality had a significant impact on leaf hydraulic properties, in a way suggesting that spectrum-sensitive light receptors are involved in the regulation of AQPs in the hydraulic pathway. The changes in K_{leaf} with light spectral properties resulted in a redistribution of hydraulic resistance within the shoots. These results support the idea that light-mediated regulation of K_{leaf} involves differential opening of the plasma membrane AQPs in mesophyll or bundle sheath cells and points to a need to determine the light receptors important in this process.

ACKNOWLEDGMENT

This study was supported by Grant no. 8333 from the Estonian Science Foundation.

REFERENCES

Becker P., Tyree M.T. & Tsuda M. (1999) Hydraulic conductances of angiosperms versus conifers: similar transport sufficiency at the whole-plant level. *Tree Physiology* **19**, 445–452.

- Briggs W.R. (2009) The ever widening world of plant photoreceptors: what they are and what they do. *Annual Review of Plant Biology* **60**. DOI:10.1146/annurev.arplant.59.092409.100001.
- Brodribb T.J. & Holbrook N.M. (2004) Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. *New Phytologist* **162**, 663–670.
- Brodribb T.J. & Holbrook N.M. (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *New Phytologist* **165**, 839–846.
- Brodribb T.J. & Holbrook N.M. (2006) Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. *Plant, Cell & Environment* **29**, 2205–2215.
- Brodribb T.J., Feild T.S. & Jordan G.J. (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**, 1890–1898.
- Chen M., Chory J. & Fankhauser C. (2004) Light signal transduction in higher plants. *Annual Review of Genetics* **38**, 87–117.
- Chrispeels M.J. & Maurel C. (1994) Aquaporins: the molecular basis of facilitated water movement through living plant cells? *Plant Physiology* **105**, 9–13.
- Cochard H., Nardini A. & Coll L. (2004) Hydraulic architecture of leaf blades: where is the main resistance? *Plant, Cell & Environment* **27**, 1257–1267.
- Cochard H., Venisse J.-S., Barigah T.S., Brunel N., Herbette S., Guilliot A., Tyree M.T. & Sakr S. (2007) Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiology* **143**, 122–133.
- Cochard H., Herbette S., Hernández E., Hölttä T. & Mencuccini M. (2010) The effects of sap ionic composition on xylem vulnerability to cavitation. *Journal of Experimental Botany* **61**, 275–285.
- Demarsy E. & Fankhauser C. (2009) Higher plants use LOV to perceive blue light. *Current Opinion in Plant Biology* **12**, 69–74.
- Domec J.-C., Palmroth S., Ward E., Maier C.A., Thérézien M. & Oren R. (2009) Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO₂ (free-air CO₂ enrichment) and N-fertilization. *Plant, Cell & Environment* **32**, 1500–1512.
- Endler J.A. (1993) The color of light in forests and its implications. *Ecological Monographs* **63**, 1–27.
- Franks P.J. (2006) Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients. *Plant, Cell & Environment* **29**, 584–592.
- Gascó A., Nardini A. & Salleo S. (2004) Resistance to water flow through leaves of *Coffea arabica* is dominated by extra-vascular tissues. *Functional Plant Biology* **31**, 1161–1168.
- Gascó A., Nardini A., Gortan E. & Salleo S. (2006) Ion-mediated increase in the hydraulic conductivity of Laurel stems: role of pits and consequences for the impact of cavitation on water transport. *Plant, Cell & Environment* **29**, 1946–1955.
- Gilbert I.R., Jarvis P.G. & Smith H. (2001) Proximity signal and shade avoidance differences between early and late successional trees. *Nature* **411**, 792–795.
- Gyenge J.E., Fernández M.E., Dalla Salda G. & Schlichter T. (2005) Leaf and whole-plant water relations of the Patagonian conifer *Austrocedrus chilensis* (D. Don) Pic. Ser. et Bizzarri: implications on its drought resistance capacity. *Annals of Forest Science* **62**, 297–302.
- Heinen R.B., Ye Q. & Chaumont F. (2009) Role of aquaporins in leaf physiology. *Journal of Experimental Botany* **60**, 2971–2985.
- Henzler T., Ye Q. & Steudle E. (2004) Oxidative gating of water channels (aquaporins) in *Chara* by hydroxyl radicals. *Plant, Cell & Environment* **27**, 1184–1195.
- Inada K. (1976) Action spectra for photosynthesis in higher plants. *Plant and Cell Physiology* **17**, 355–365.

- Inada S., Ohgishi M., Mayama T., Okada K. & Sakai T. (2004) RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phototropin 1 in *Arabidopsis thaliana*. *The Plant Cell* **16**, 887–896.
- Inoue S.-I., Kinoshita T., Matsumoto M., Nakayama K.I., Doi M. & Shimazaki K.-I. (2008) Blue light-induced autophosphorylation of phototropin is a primary step for signaling. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 5626–5631.
- Johansson I., Karlsson M., Shukla V.K., Chrispeels M.J., Larsson C. & Kjellbom P. (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *The Plant Cell* **10**, 451–459.
- Johnson K.D. & Chrispeels M.J. (1992) Tonoplast-bound protein kinase phosphorylates tonoplast intrinsic protein. *Plant Physiology* **100**, 1787–1795.
- Kaldenhoff R., Kölling A. & Richter G. (1996) Regulation of the *Arabidopsis thaliana* aquaporin gene AthH2 (PIP1b). *Journal of Photochemistry and Photobiology B: Biology* **36**, 351–354.
- Kim Y.X. & Steudle E. (2009) Gating of aquaporins by light and reactive oxygen species in leaf parenchyma cells of the midrib of *Zea mays*. *Journal of Experimental Botany* **60**, 547–556.
- Lo Gullo M.A., Nardini A., Trifilò P. & Salleo S. (2005) Diurnal and seasonal variations in leaf hydraulic conductance in evergreen and deciduous trees. *Tree Physiology* **25**, 505–512.
- Matzner S. & Comstock J. (2001) The temperature dependence of shoot hydraulic resistance: implications for stomatal behaviour and hydraulic limitation. *Plant, Cell & Environment* **24**, 1299–1307.
- Maurel C. (1997) Aquaporins and water permeability of plant membranes. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 399–429.
- Metzner R., Thorpe M.R., Breuer U., Blümner P., Schurr U., Schneider H.U. & Schroeder W.H. (2010) Contrasting dynamics of water and mineral nutrients in stems shown by stable isotope tracers and cryo-SIMS. *Plant, Cell & Environment* **33**, 1393–1407.
- Nardini A. (2001) Are sclerophylls and malacophylls hydraulically different? *Biologia Plantarum* **44**, 239–245.
- Nardini A. & Pitt F. (1999) Drought resistance of *Quercus pubescens* as a function of root hydraulic conductance, xylem embolism and hydraulic architecture. *New Phytologist* **143**, 485–493.
- Nardini A. & Salleo S. (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees* **15**, 14–24.
- Nardini A., Salleo S. & Andri S. (2005) Circadian regulation of leaf hydraulic conductance in sunflower (*Helianthus annuus* L. cv Margot). *Plant, Cell & Environment* **28**, 750–759.
- Nardini A., Gasco A., Trifilo P., Lo Gullo M.A. & Salleo S. (2007) Ion-mediated enhancement of xylem hydraulic conductivity is not always suppressed by the presence of Ca²⁺ in the sap. *Journal of Experimental Botany* **58**, 2609–2615.
- Nardini A., Grego F., Trifilò P. & Salleo S. (2010) Changes of xylem sap ionic content and stem hydraulics in response to irradiance in *Laurus nobilis*. *Tree Physiology* **30**, 628–635.
- Navrátil M., Špunda V., Marková I. & Janouš D. (2007) Spectral composition of photosynthetically active radiation penetrating into a Norway spruce canopy: the opposite dynamics of the blue/red spectral ratio during clear and overcast days. *Trees* **21**, 311–320.
- Pieruschka R., Huber G. & Berry J.A. (2010) Control of transpiration by radiation. *Proceedings of the National Academy of Sciences of United States of America* **107**, 13372–13377.
- Prak S., Hem S., Boudet J., Viennois G., Sommerer N., Rossignol M., Maurel C. & Santoni V. (2008) Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins. *Molecular & Cellular Proteomics* **7**, 1019–1030.
- Reinhardt K., Smith W.K. & Carter G.A. (2010) Clouds and cloud immersion alter photosynthetic light quality in a temperate mountain cloud forest. *Botany* **88**, 462–470.
- Ryden P., MacDougall A.J., Tibbits C.W. & Ring S.G. (2000) Hydration of pectic polysaccharides. *Biopolymers* **54**, 398–405.
- Sack L. & Frole K. (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* **87**, 483–491.
- Sack L. & Holbrook N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology* **57**, 361–381.
- Sack L. & Tyree M.T. (2005) Leaf hydraulics and its implications in plant structure and functions. In *Vascular Transport in Plants* (eds N.M. Holbrook & M.A. Zwieniecki) pp. 93–114. Elsevier Academic Press, Burlington, VT, USA.
- Sack L., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* **53**, 2177–2184.
- Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* **26**, 1343–1356.
- Sack L., Streeter C.M. & Holbrook N.M. (2004) Hydraulic analysis of water flow through leaves of sugar maple and red oak. *Plant Physiology* **134**, 1824–1833.
- Sack L., Tyree M.T. & Holbrook N.M. (2005) Leaf hydraulic architecture correlates with regeneration irradiance in tropical rain-forest trees. *New Phytologist* **167**, 403–413.
- Scoffoni C., Pou A., Aasamaa K. & Sack L. (2008) The rapid light response of leaf hydraulic conductance: new evidence from two experimental methods. *Plant Cell & Environment* **31**, 1803–1812.
- Sellin A. & Kupper P. (2005a) Effects of light availability versus hydraulic constraints on stomatal responses within a crown of silver birch. *Oecologia* **142**, 388–397.
- Sellin A. & Kupper P. (2005b) Variation in leaf conductance of silver birch: effects of irradiance, vapour pressure deficit, leaf water status and position within a crown. *Forest Ecology and Management* **206**, 153–166.
- Sellin A. & Kupper P. (2007a) Effects of enhanced hydraulic supply for foliage on stomatal responses in little-leaf linden (*Tilia cordata* Mill.). *European Journal of Forest Research* **126**, 241–251.
- Sellin A. & Kupper P. (2007b) Temperature, light and leaf hydraulic conductance of little-leaf linden (*Tilia cordata*) in a mixed forest canopy. *Tree Physiology* **27**, 679–688.
- Sellin A. & Lubenets K. (2010) Variation of transpiration within a canopy of silver birch: effect of canopy position and daily versus nightly water loss. *Ecophysiology* **3**, 467–477.
- Sellin A., Öunapuu E. & Kupper P. (2008) Effects of light intensity and duration on leaf hydraulic conductance and distribution of resistance in shoots of silver birch (*Betula pendula*). *Physiologia Plantarum* **134**, 412–420.
- Sellin A., Öunapuu E. & Karusion A. (2010) Experimental evidence supporting the concept of light-mediated modulation of stem hydraulic conductance. *Tree Physiology* **30**, 1528–1535.
- Sharkey T.D. & Ogawa T. (1987) Stomatal responses to light. In *Stomatal Function* (eds E. Zeiger, G. Farquhar & I. Cowan) pp. 195–208. Stanford University Press, Stanford, CA, USA.
- Shimazaki K., Doi M., Assmann S.M. & Kinoshita T. (2007) Light regulation of stomatal movement. *Annual Review of Plant Biology* **58**, 219–247.
- Smith H. (1982) Light quality, photoperception, and plant strategy. *Annual Review of Plant Physiology* **33**, 481–518.

- Sokal R.R. & Rohlf F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* p. 887. W. H. Freeman and Co., New York, NY, USA.
- Stark N., Spitzner C. & Essig D. (1985) Xylem sap analysis for determining the nutritional status of trees: *Pseudotsuga menziesii*. *Canadian Journal of Forest Research* **15**, 429–437.
- Trifilò P., Nardini A., Lo Gullo M.A. & Salleo S. (2003) Vein cavitation and stomatal behaviour of sunflower (*Helianthus annuus*) leaves under water limitation. *Physiologia Plantarum* **119**, 409–417.
- Tyerman S.D., Niemietz C.M. & Bramley H. (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. *Plant, Cell & Environment* **25**, 173–194.
- Tyree M.T., Nardini A., Salleo S., Sack L. & El Omari B. (2005) The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response? *Journal of Experimental Botany* **56**, 737–744.
- Urban O., Janouš D., Acosta M., *et al.* (2007) Ecophysiological controls over the net ecosystem exchange of mountain spruce stand. Comparison of the response in direct versus diffuse solar radiation. *Global Change Biology* **13**, 157–168.
- Voicu M.C. & Zwiazek J.J. (2010) Inhibitor studies of leaf lamina hydraulic conductance in trembling aspen (*Populus tremuloides* Michx.) leaves. *Tree Physiology* **30**, 193–204.
- Voicu M.C., Zwiazek J.J. & Tyree M.T. (2008) Light response of hydraulic conductance in bur oak (*Quercus macrocarpa*) leaves. *Tree Physiology* **28**, 1007–1015.
- Voicu M.C., Cooke J.E.K. & Zwiazek J.J. (2009) Aquaporin gene expression and apoplastic water flow in bur oak (*Quercus macrocarpa*) leaves in relation to the light response of leaf hydraulic conductance. *Journal of Experimental Botany* **60**, 4063–4075.
- Woodruff D.R., McCulloh K.A., Warren J.M., Meinzer F.C. & Lachenbruch B. (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell & Environment* **30**, 559–569.
- Yang S.D. & Tyree M.T. (1994) Hydraulic architecture of *Acer saccharum* and *A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. *Journal of Experimental Botany* **45**, 179–186.
- Zeiger E., Talbott L.D., Frechilla S., Srivastava A. & Zhu J. (2002) The guard cell chloroplast: a perspective for the twenty-first century. *New Phytologist* **153**, 415–424.
- Zwieniecki M.A., Melcher P.J. & Holbrook N.M. (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science* **291**, 1059–1062.

Received 19 October 2010; received in revised form 1 February 2011; accepted for publication 3 February 2011