Experimental evidence supporting the concept of light-mediated modulation of stem hydraulic conductance

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Summary It is a well-described phenomenon that plant leaves respond to changes in light intensity and duration by adjusting leaf hydraulic efficiency, and there is a general consensus that up- or down-regulation of water channels (aquaporins) in the plasma membrane of the bundle sheath and mesophyll cells play a central role in the underlying mechanisms. Recently, experimental evidence has been provided also for light-mediated changes of stem hydraulic conductance ($K_{stem}$) in field-grown laurel plants. This effect was attributed to differences in potassium ion concentration of xylem sap as a function of light conditions. In the present article, we report evidence obtained in silver birch ($Betula pendula$ Roth), supporting the concept of light-mediated modulation of $K_{stem}$. Both canopy position (long-term effect) and current photosynthetic photon flux density (PPFD; short-term effect) had a significant impact ($P < 0.001$) on $K_{stem}$ measured in shoots taken from the lower (shade shoots) and upper (sun shoots) third of the crowns of ~25-year-old trees growing in a natural forest stand. The shade shoots responded more sensitively to light manipulation: $K_{stem}$ increased by 51% in shade shoots and 26% in sun shoots when PPFD increased from 70 to 330 μmol m$^{-2}$ s$^{-1}$. In 4-year-old trees growing in a dense experimental plantation, $K_{stem}$ specific conductivity of branch-wood ($k_{bw}$) and potassium ion concentration ([K$^+$]) in xylem sap varied in accordance with canopy position ($P < 0.001$). Both $K_{stem}$ and $k_{bw}$ increased considerably with light availability, increasing within the tree crowns from bottom to top; there was a strong relationship between mean values of $K_{stem}$ and [K$^+$] in hydraulically sampled branches.

Keywords: Betula pendula, canopy position, light intensity, potassium ions, silver birch, specific hydraulic conductivity, xylem sap.

Introduction

It is a widely accepted standpoint that plant leaves respond to light in a short time scale by adjusting leaf hydraulic efficiency, and there is a general consensus that up- or down-regulation of water channels in the plasma membrane of leaf cells plays a central role in the underlying mechanisms. Mounting evidence confirms the variability of leaf hydraulic traits depending on light availability (Sack et al. 2005, Sack and Holbrook 2006, Voicu et al. 2009). A study of 11 temperate deciduous tree species revealed that leaf hydraulic conductance ($K_L$) increased with irradiance for six of the species tested (Tyree et al. 2005). Sellin et al. (2008a) indicated that leaves of silver birch ($Betula pendula$ Roth) respond to light by adjusting their hydraulic efficiency depending on both light duration and intensity. Experimental evidence suggests that the light-induced enhancement of $K_L$ is controlled by factors acting on extra-vascular tissues and involves expression or activation of plasma membrane aquaporins (AQPs) in mesophyll or bundle sheath cells (Tyree et al. 2005, Cochrard et al. 2007, Voicu et al. 2008, Heinen et al. 2009). Although some studies (Lee et al. 2009, Voicu et al. 2009) have failed to confirm the role of AQPs in the light-induced enhancement of hydraulic conductance in some species, they do not denounce the concept as a whole. These papers rather indicate that regulation of the AQP-mediated water transport processes is more complicated and cannot always be explained merely by changes in the transcript level. As a rule, hydraulic conductance of sun leaves compared with shade leaves (Sack et al. 2003, Sellin and Kupper 2007a, 2007b, Sellin et al. 2008a), and $K_L$ of sun-dwelling species compared with shade-growing species (Brodribb and Holbrook 2004, Sack et al. 2005, Sack and Frolé 2006), have been found to be significantly higher. Diurnal variation in $K_L$ is also related to temporal patterns of light intensity, confirmed by experiments carried out on both herbaceous (Nardini et al. 2005) and woody plants (Lo Gullo et al. 2005).

The impact of light availability on stem hydraulic conductance ($K_{stem}$) is also well documented; however, it is observable on a longer—developmental—time scale. A number of studies (e.g., Lemoine et al. 2002a, Aasamaa et al. 2004, Jerez et al. 2004, Burgess et al. 2006, Sellin and Kupper 2007a, Sellin et al. 2008a) demonstrate that upper-crown branches, developing under higher radiation load, temperature
and wind, are characterized by higher hydraulic capacity compared with lower-crown branches. All these environmental factors enhance transpirational water loss from the foliage, and therefore a more efficient water transport system is necessary to provide an adequate water supply to sun-exposed leaves, maintain a balance between gas- and liquid-phase conductances and diminish desiccation-induced risk of xylem cavitation. Lemoine et al. (2002b) demonstrated experimentally how vessel diameter and leaf-specific conductivity increased in branches of *Fagus sylvatica* L. in response to increased light availability due to stand thinning.

Until quite recently, xylem—consisting of dead interconnected cells—was treated as a system of inert pipes and a passive pathway for water flow in plants. Hydraulic conductivity of the xylem has been considered to be dependent mainly on the anatomical characteristics of the xylem conduits, whereas the possibility of rapid flow control in xylem has not been considered. More than 30 years ago, Zimmermann (1978) observed that the use of deionized water as a permeation fluid for hydraulic measurements significantly increased the hydraulic resistance of stem segments. However, only during the present decade has it been proved that changes in ionic strength of xylem sap (i.e., the concentration of cations in xylem sap) are responsible for short-term modulation of xylem hydraulic conductance (van Ieperen et al. 2000, Zwieniecki et al. 2001, Gasco et al. 2006, van Ieperen and van Gelder 2006, Nardini et al. 2007, Cochard et al. 2010). The ion-mediated changes in xylem hydraulic conductance could be attributed to the shrinking and swelling of the intervessel pit membrane hydrogels (pectins) in response to the changes in the osmotic strength of the solution perfused (Ryden et al. 2000, Zwieniecki et al. 2001, van Ieperen 2007).

To our knowledge, Nardini et al. (2010) for the first time unequivocally demonstrated that short-term changes in $K_{stem}$ are related to light conditions. More specifically, they recorded significant correlated differences in $K_{stem}$ and xylem sap [$K^+$] in laurel branches with respect to light availability, while [$K^+$] turned out to be a function of light intensity at the time of sampling. Because water flows in the stem through xylem conduits that are inter-connected through a mesh of cellulose microfibrils covered by pectin (in bordered pits), the ionic effect appears to be a likely candidate for explaining short-term changes in $K_{stem}$ induced by light conditions. The present study was not designed specially with the aim of exploring profoundly the mechanisms behind this phenomenon; rather, we just wish to report some evidence, obtained from two different experiments, supporting the concept of light-mediated modulation of stem hydraulic conductance.

**Materials and methods**

**Study area and sample trees**

The studies were performed in silver birch (*B. pendula* Roth), a common broad-leaved tree species in temperate and boreal forests in Europe. The experimental sites were located in Järvselja Experimental Forest, Eastern Estonia; detailed data on the vegetation and climate of the study area are presented in Sellin and Kupper (2005a, 2005b). In the first experiment, birch trees from a naturally regenerated mixed stand located near Liispõllu village (58°16′N, 27°16′E, elevation ~40 m above sea level (ASL)) were sampled. Shoots from four ~25-year-old trees (height 15.5–17.0 m, diameter at breast height 10.3–12.4 cm) were sampled in the lower (shade shoots) and upper (sun shoots) thirds of their crown. The differentiation into sun and shade shoots/foliage was well reflected in leaf morphology, both in specific leaf area and stomatal characteristics, published in our previous studies (Sellin and Kupper 2006, Sellin et al. 2010).

The second experiment was carried out in a forest plantation equipped with experimental facilities for Free Air Humidity Manipulation (FAHM), situated at Rõka village (58°24′N, 27°29′E, 40–48 m ASL). The experimental area was established on an abandoned agricultural field in 2006–07. The study site is a fenced area of 2.7 ha consisting of nine 14 × 14 m experimental plots planted with hybrid aspen and silver birch plants, surrounded by a buffer zone. The stand density in the buffer zone around the experimental plots is 2,500 trees ha$^{-1}$; the density in experimental plots is 10,000 trees ha$^{-1}$. The computer-operated FAHM system, based on an approach integrating two different technologies—a misting technique to atomize/vaporize water (Adaskaveg et al. 1990) and a FACE-like technology (Hendrey et al. 1999) to mix humidified air inside the plots, enables the relative humidity of the air to be increased up to 18% over the ambient level during humidification treatment. Detailed description of the FAHM site and experimental setup is available on the internet (http://www.lote.ut.ee/FAHM/in-english). The humidification was started in June 2008; hydraulic measurements were performed from the end of July to mid-August in 2009. We sampled destructively six trees from the humidified plots (mean height 3.3 m; leaf area index (LAI) 3.0 m$^2$ m$^{-2}$) and six trees from the control plots (mean height 3.8 m; LAI 3.1 m$^2$ m$^{-2}$). Light availability within a canopy at the FAHM site was estimated using the hemispherical photographic technique (Bréda et al. 2002, Hale and Edwards 2002). Photographs ($n=108$) were taken at different heights in the canopy with a Coolpix digital camera (Nikon Corp., Tokyo, Japan) equipped with a fisheye lens, and the hemispherical images were analysed by applying WinSCANOPY, Vers. 2.1A software (Regent Instruments, Ottawa, Canada). The radiation regime was characterized with a direct site factor (the proportion of direct solar radiation reaching a given location) and indirect site factor (the proportion of diffuse solar radiation reaching a given location). The vertical profiles of light availability are presented in Figure 1. The control and humidified plots did not differ in light regime.
Lab experiment (Experiment I)

In the evening prior to the measurement days, birch shoots (15–35 cm long, bearing at least nine leaves) were cut under water and, if necessary, recut carefully under water and put into beakers with their 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 (T-04-125 ultrasonic-vacuum degasser; Terriss Consolidated Industries, Asbury Park, NJ, USA) water. Two leaves from each shoot were prepared for measurement of water potential of the branch xylem (ΨB). These leaves were enclosed in small minigrip bags, sealed with tape and wrapped in aluminium foil. The shoots were then placed in a dark room and left overnight. Details of the experiment have been published in Sellin et al. (2008a).

In the morning, the shoots were exposed to light (Master SON-T PIA Agro high-pressure sodium lamps, 400 W; Philips, Eindhoven, The Netherlands) for 7 h before the conductance measurements. Both shade (95 shoots in all) and sun (96 shoots in all) shoots were exposed to four different irradiance levels: PPFD was 70, 140, 330 and 610 \text{ mol m}^{-2} \text{s}^{-1}. Irradiance was modified by changing the distance from the lamps and using neutral shading nets. Air above the shoots was agitated with a fan to minimize local temperature gradients. Hydraulic conductance of shoot (KS) and leaves (KL) was determined by the evaporative flux method (Wullschleger et al. 1998, Brodribb and Holbrook 2003) under steady-state conditions and was calculated according to the Ohm’s law analogy:

$$K_{S,L} = \frac{E}{\Delta \Psi},$$

where $E$ is the evaporative flux density and $\Delta \Psi$ is the water potential drop across the corresponding segment (whole shoot or leaf). $E$ was measured at the leaf surface with an LI-1600M steady-state diffusion porometer (Li-Cor Biosciences, Lincoln, NE, USA), sampling three to four leaves per shoot. Leaf temperature ($T_L$) was recorded with fine copper–constantan thermocouples installed in the porometer.

Immediately after the porometric measurements, the bulk leaf water potential (ΨL) in three leaves per shoot was determined by the balancing pressure technique using a Scholander-type pressure chamber. The leaves for porometric and pressure chamber measurements were chosen randomly, from different parts of the shoot. ΨB was estimated by applying the method of bagged leaves (Nardini et al. 2001, Brodribb and Holbrook 2003), sampling two leaves at different heights on the shoot, which had been prepared the previous evening. Water potential of the non-transpiring (bagged) leaves, which was presumed to have equilibrated with the xylem water potential of the branch proximal to the petiole, was taken as a proxy of ΨB. After the pressure chamber measurements, total area of leaf blades was determined with an AM300 digital area meter (ADC BioScientific, Great Amwell, UK). Stem (i.e., leafless branch) hydraulic conductance ($K_{stem}$) was calculated as follows:

$$K_{stem} = \left(K_S^{-1} - K_L^{-1}\right)^{-1},$$

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.

FAHM experiment (Experiment II)

Sample branches were cut from three heights in the canopy: on average at 53 (mean length 91 cm), 177 (mean length 112 cm) and 227 cm (mean length 91 cm). The cut ends were recut under water and the branches were immediately hydraulically measured under a PPFD of 120–150 \text{ µmol m}^{-2} \text{s}^{-1} (Master SON-T PIA Agro lamps, Philips). Air above the branch was agitated with a fan to avoid spatial temperature differences. Hydraulic conductance of the branches and their parts—leafless stem, petioles, leaf blades—was determined by the water perfusion method using a high-pressure flow meter (HPFM; Dynamax, Houston, TX, USA) applied on a quasi-steady-state mode, and by removing leaf blades and petioles in sequence. Tyree et al. (2005) indicated that stomatal openness does not influence the results of hydraulic measurements using an HPFM. As in most cases the leaf blade cutting (i.e., constant readings for the leafy branch achieved) was reached in 20 min; this time interval was considered too short to cause light-induced changes in xylem sap properties (Sellin et al. 2008a, Nardini et al. 2010). Therefore, the readings of $K_{stem}$ rather reflect a response to the preceding situation, the light conditions included. We concede that dilution of xylem sap

Figure 1. Vertical profiles of light availability within a canopy at the FAHM site in August of 2009. The arrows denote the mean height of the lower (L), medium (M) and upper (U) sample branches.
is inevitable when using deionized water as a permeation fluid.

After achieving stable readings with HPFM for the leafless branch, the distal part of the branch was cut off. The remaining 15- to 20-cm basal segment of the branch, left connected to the pressure coupling, was perfused again to detect the specific hydraulic conductivity of branch-wood ($k_{bw}$), to be calculated as follows:

$$k_{bw} = \frac{K_{seg} \cdot l}{A_X},$$

where $K_{seg}$ is the hydraulic conductance of the branch segment, $l$ is the length of the segment and $A_X$ the cross-sectional area of xylem. The mean pressures applied during the quasi-steady-state measurements were 0.34 ± 0.020 and 0.20 ± 0.016 MPa for determination of $K_{stem}$ and $k_{bw}$, respectively. After the hydraulic measurements, all leaves were collected and the total area of leaf blades was determined with an LI-3100C optical area meter (Li-Cor Biosciences). The data on $K_{stem}$ were corrected for the dynamic viscosity of water at 22 °C and normalized to branch size by expressing the conductance values per unit area of the leaf blades. Altogether, 36 branches (2 treatments × 3 sample plots × 2 trees × 3 canopy positions) were sampled for hydraulic properties.

To test for light-mediated modification of the ionic content of the xylem sap and consequent changes in $K_{stem}$ (Zwieniecki et al. 2001, Nardini et al. 2007, Cochard et al. 2010), we determined potassium ion concentration ([K⁺]) of branches taken from the same tree and height: one of the two branches — a branch used for hydraulic measurements, the other — an unperfused branch closest to the first one. The extraction procedure of the xylem sap followed in main points as described by Stark et al. (1985). Extraction was performed on the leafless branch segment 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 tightly fitting rubber stopper and the stopper was inserted into the 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 tightly fitting piece of plastic tubing. The pressure was increased slowly to 2.0 MPa and the extracted xylem sap was collected in 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.

**Data analysis**

Data analysis was carried out using Statistica, Vers. 7.1 (StatSoft, Inc., USA). To analyse the effects on hydraulic characteristics of light intensity, canopy position and treatment, all treated as fixed factors, an analysis of variance (ANOVA) was applied. 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. When appropriate, logarithmic or root transformations were applied to the data. Post hoc mean comparisons were conducted using the Tukey HSD test. Bivariate relationships between the studied characteristics and independent variables were assessed by Pearson’s correlations or simple linear regressions based on the least-squares method.

**Results**

**Experiment I**

The ANOVA revealed that the effects of both canopy position and PPFD on stem hydraulic conductance ($K_{stem}$) measured in the shoots of the trees growing in a natural forest stand were highly significant ($P<0.001$; Table 1). The interaction of canopy position and PPFD also proved to be relevant and is expressed by different patterns of $K_{stem}$ in shade versus sun shoots with increasing irradiance (Figure 2). In shade shoots, $K_{stem}$ increased consistently with irradiance rising from 70 to 330 μmol m⁻² s⁻¹, and decreased again at a PPFD of 610 μmol m⁻² s⁻¹. In sun shoots, $K_{stem}$ did not start to increase until a PPFD of 140 μmol m⁻² s⁻¹ was reached and did not show a decrease at PPFD >330 μmol m⁻² s⁻¹. The shade shoots responded more sensitively to the light manipulation: $K_{stem}$ increased by 51% in shade and 26% in sun shoots if PPFD increased by 51% in shade and 26% in sun shoots if PPFD increased from 70 to 330 μmol m⁻² s⁻¹. Across all light intensities, $K_{stem}$ averaged 15.5 ± 0.43 and 11.1 ± 0.29 × 10⁻² kg m⁻² s⁻¹ MPa⁻¹ for sun and shade shoots, respectively.

**Experiment II**

Canopy position had a significant effect on stem hydraulic properties and potassium ion concentration in xylem sap of the branches in young birch trees growing at the FAHM site (Table 2). As we did not establish statistically significant changes in any of the four characteristics with respect to the humidification, the data on both treatments were further

**Table 1. Results of ANOVA for the effects of canopy position and light intensity on stem hydraulic conductance (ln $K_{stem}$) measured in shoots of trees growing in a natural forest stand (n = 756).**

<table>
<thead>
<tr>
<th>Factor</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy position</td>
<td>18.41</td>
<td>1</td>
<td>18.41</td>
<td>76.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPFD</td>
<td>11.57</td>
<td>3</td>
<td>3.86</td>
<td>16.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Can. pos. × PPFD</td>
<td>2.86</td>
<td>3</td>
<td>0.95</td>
<td>3.97</td>
<td>0.008</td>
</tr>
<tr>
<td>Error</td>
<td>179.79</td>
<td>748</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS, sum of squares; MS, mean square; F, F-ratio; P, statistical significance.
analysed together. $K_{stem}$ increased within the tree crowns from bottom to top: medium branches exhibited on average 1.2 times and top branches 1.8 times higher $K_{stem}$ when compared with the lower-crown branches (Figure 3). Specific hydraulic conductivity of branch-wood ($k_{bw}$) followed a similar trend, although the within-crown range of $k_{bw}$ was smaller (1.5-fold). Similarly, [K+] of xylem sap in both perfused and unperfused branches increased acropetally within the canopy (Figure 4). Water perfusion of hydraulically sampled branches brought about dilution of the xylem sap and caused [K+] to decrease roughly by half ($P < 0.001$). In spite of the large scatter of the data and the dilution effect, there was a strong relationship ($R^2 = 0.917$, $P = 0.003$) between the mean values of $K_{stem}$ and [K+] in hydraulically sampled branches (Figure 5).

Table 2. Results of ANOVA for the effects of canopy position and humidity treatment on stem hydraulic properties and potassium ion concentration of xylem sap ([K+]) in branches of trees at the FAHM site ($n = 36$).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Factor</th>
<th>Statistical significance ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem hydraulic conductance, $K_{stem}$</td>
<td>Canopy position</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>ns</td>
</tr>
<tr>
<td>Specific conductivity of branch-wood, $k_{bw}$</td>
<td>Canopy position</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>ns</td>
</tr>
<tr>
<td>[K+] in perfused branches</td>
<td>Canopy position</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>ns</td>
</tr>
<tr>
<td>[K+] in unperfused branches</td>
<td>Canopy position</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, not significant.

Figure 2. Variation in stem hydraulic conductance ($K_{stem}$) depending on light intensity. Shoots were illuminated for 7 h before the conductance measurements. The bars indicate SE of the mean; different letters denote statistically significant ($P < 0.05$) differences.

Figure 3. Vertical variation in stem hydraulic conductance ($K_{stem}$) and specific conductivity of branch-wood ($k_{bw}$) within a canopy. The bars indicate SE of the mean; different letters denote statistically significant ($P < 0.05$) differences. Note, the scales for $K_{stem}$ (left) and $k_{bw}$ (right) are different.

Figure 4. Differences in potassium ion concentration ([K+]) of xylem sap in perfused and unperfused branches with respect to canopy position. The bars indicate SE of the mean; different letters denote statistically significant ($P < 0.05$) differences.

Discussion

Shoots of *B. pendula*, sampled by the evaporative flux method in lab conditions (Experiment I), exhibited substantial differences in stem hydraulic conductance depending on the incident PPFD (Figure 2). $K_{stem}$ of sun shoots increased with increasing light intensity across the whole range of PPFD applied in the experiment, that of shade shoots, up to 330 μmol m$^{-2}$ s$^{-1}$, although the shade shoots responded more sensitively to the light manipulation. The results suggest that light can induce short-term (time scale in hours) changes in the water transport capacity of the xylem, enhancing hydraulic efficiency at higher irradiance. Our data are consistent with the concept of light-mediated modulation of stem hydraulic conductance advanced by Nardini et al. (2010). Their experiment unequivocally demonstrated that light-driven changes in $K_{stem}$ are associated with differences in [K+] of xylem sap, i.e., it is a consequence of
enhanced uptake and/or loading of potassium into the xylem and interaction of cations with the pectic matrix of pit membranes (the ionic effect). Macduff et al. (1997) have suggested that $K^+$ uptake exhibits a pronounced diurnal variation—increasing during the light period and decreasing during darkness, and a dependence on actual irradiance. In continuous light under experimental conditions, diurnal variations in solute concentrations of xylem sap vanish (Herdel et al. 2001). $[K^+]$ of xylem sap in hydroponically grown poplar (Populus tremula L. × Populus alba L.) plants has been reported to increase for 4 h after the light has been switched on, and then to start to decrease as a result of increasing transpiration rate, obviously in part due to the dilution of the sap (Siebrecht et al. 2003). Although $K^+$ has been reported to be released into the stem xylem by the phloem via the rays (De Boer and Volkov 2003) or directly by vessel-associated cells (Salleo et al. 2004; Zwieniecki et al. 2004), the exact origin of ions loaded into the xylem still remains unclear. The ecophysiological implication of the phenomenon is probably to provide a regulatory mechanism of shoot hydraulics based on the ionic effect, which would facilitate water flow towards fully illuminated branches, thus possibly reducing hydraulic resistance and water potential drop, maximizing in this way stomatal aperture and photosynthesis (Nardini et al. 2010). Other studies demonstrating fast changes in $K_{stem}$ driven by light conditions are practically absent; short-term dynamics is usually associated with cavitation of xylem conduits. Only Tsuda and Tyree (2000) have reported that $K_{stem}$ of sunflower changed diurnally, being substantially higher in the light period.

At all levels of irradiance, $K_{stem}$ in birch shoots taken from the sun-exposed part of the canopy was consistently higher, on average by 40%, than in shoots taken from the shaded canopy layer (Figure 2). This difference reflects the consequences of the long-term influence experienced by branches growing for years in different environments (light regime in particular) formed along the canopy vertical profile. This distinction in $K_{stem}$ can be attributed to variation in xylem anatomical structure, primarily in diameter and density of vessels/tracheids (Protz et al. 2000, Lemoine et al. 2002b, Sellin et al. 2008b, Zach et al. 2010). In order to provide sufficient water transfer to the upper, better-insolated foliage, which is the photosynthetically more efficient part of the canopy, the hydraulic capacity of branches should acropetally increase (vessels should widen according to Hagen–Poiseuille’s law) to overcome the resistance resulting from longer flow pathways and increasing gravitational forces.

In Experiment I, cation concentrations in xylem sap were not determined, and we cannot therefore prove that the light impact on $K_{stem}$ in birch was exactly mediated by changes in sap ionic strength. However, these results can be complemented by data from Experiment II. $K_{stem}$, $k_{low}$ and $[K^+]$ measured in branches of young B. pendula trees exhibited the same spatial pattern along the vertical canopy profile—increased from bottom to top (Figures 3 and 4), and this trend is consistent with the light availability gradient formed in the canopy (Figure 1). The acropetal increase in the $[K^+]$ of xylem sap within a crown revealed in our study is in agreement with the findings obtained in forest-grown trees of Acer platanoides L. (Schill et al. 1996). $[K^+]$ in sun-exposed branches of laurel trees was $\approx$3.6 times higher than that in shade-exposed ones (Nardini et al. 2010). In contrast, in small poplar plants grown in a homogeneous light field, $[K^+]$ of the xylem sap did not change along the plant axis (Siebrecht et al. 2003). One can suppose that the ionic content of xylem sap in silver birch was actively increased (up-regulated) in upper branches in response to higher irradiance (Figure 1), to support substantially higher sap flow rates ($\approx$1.8-fold greater in large trees) in the upper canopy compared with the lower canopy in the daytime (Sellin and Lubenets 2010). Otherwise, the $[K^+]$ of well-illuminated branches would have been expected to decrease compared with that of shaded branches as a result of increased transpiration rate and dilution of sap, as recorded in some species (Herdel et al. 2001, Siebrecht et al. 2003). Herdel et al. (2001) concluded from their experiment that diurnal variations of xylem sap composition are due to plant internal regulation and transpiration rate, whereas nutrient availability merely affects the amplitude of the diurnal patterns.

On the one hand, the differences in hydraulic characteristics observed in silver birch undoubtedly express long-term effects of light availability with respect to branch height in the canopy. On the other hand, the branches were sampled using the high-pressure method immediately after being cut, and the readings obtained should also reflect adjustment of the hydraulic system to the environmental situation existing prior to sampling. The time interval from branch cutting to getting readings was too short to induce significant light-mediated adjustment of xylem hydraulic properties or sap content (besides the dilution effect in perfused branches). The good agreement between the patterns of sap $[K^+]$ and

Figure 5. Potassium ion concentration ($[K^+]$) of xylem sap measured in perfused and unperfused branches versus stem hydraulic conductance ($K_{stem}$). Each data point ($n=6$) in the figure denotes the mean value of a particular treatment and canopy layer.
branch hydraulic traits (Figures 3 and 4) suggests that potassium ions were involved in the regulation of stem hydraulic efficiency in relation to light availability. There was a strong relationship ($R^2 = 0.917$, $P = 0.003$) between the mean values of $K_{stem}$ and [K+] in the hydraulically sampled branches (Figure 5). Without doubt, potassium plays a central role in the ion-mediated effect, as K+ constitutes the largest share of the total inorganic solutes of the xylem sap: in poplar, 46.1% of total ions (Siebrecht et al. 2003), in *Ricinus communis* L., 70% of night-time and 80% of daytime total cations (Herdel et al. 2001). Furthermore, it has been shown for various tree species that K+ represents a mandatory factor at xylem formation (Fromm 2010).

It is intriguing that significant differences in [K+] remained even after water perfusion treatment among birch branches previously exposed to different light conditions (Figure 4). A 20-min perfusion of stems with deionized water under high pressure should fill completely the xylem conduits with deionized water and, hence, mask any light-mediated changes in both [K+] and $K_{stem}$. This suggests that living tissues were probably enriching the xylem sap with potassium ions even during HPFM measurements. In a recent paper, Metzner et al. (2010) provide evidence for substantial up-regulation of potassium in the sap of detached bean shoots immersed in a dilute potassium solution. [K+] was found to be maintained at a level higher than the input solution for at least 4 h after shoot cutting. The large amount of potassium delivered from the stem tissues—required to maintain enhanced concentration in the vessels—supports the idea of potassium recirculation via the phloem, responsible for the observed increase in [K+] in the xylem sap (Metzner et al. 2010).

To summarize, the results obtained from the two experiments revealed that stem hydraulic conductance may vary substantially with incident PPFD, and changes in potassium ion concentration in xylem sap and xylem hydraulic traits with respect to light availability within a tree crown are co-ordinated in silver birch. The results suggest that potassium is involved in the regulation of xylem hydraulic efficiency. Our data support the concept of light-mediated modulation of stem hydraulic conductance, the phenomenon that can substantially contribute to the control of water fluxes within trees to optimize leaf water supply in the heterogeneous light environment existing in forest canopies.

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