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Size-dependent variability of leaf and shoot hydraulic conductance in silver birch

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Abstract Variation in leaf and shoot hydraulic conductance was examined on detached shoots of silver birch (*Betula pendula* Roth), cut from the lower third (shade leaves) and upper third of the crown (sun leaves) of large trees growing in a natural temperate forest stand. Hydraulic conductances of whole shoots (K_S), leaf blades (K_{lb}), petioles (K_P) and branches (i.e. leafless stem; K_B) were determined by water perfusion using a high-pressure flow meter in quasi-steady state mode. The shoots were exposed to irradiance of photosynthetic photon flux density of 200–250 μmol m^{-2} s^{-1}, using different light sources. K_{lb} depended significantly ($P < 0.001$) on light quality, canopy position and leaf blade area (A_L). K_{lb} increased from crown base to tree top, in parallel with vertical patterns of A_L. However, the analysis of data on shade and sun leaves separately revealed an opposite trend: the bigger the A_L the higher K_{lb}. Leaf anatomical study of birch saplings revealed that this trend is attributable to enhanced vascular development with increasing leaf area. Hydraulic traits (K_S, K_B, K_{lb}) of sun shoots were well co-ordinated and more strongly correlated with characteristics of shoot size than those of shade shoots, reflecting their greater evaporative load and need for stricter adjustment of hydraulic capacity with shoot size. K_S increased with increasing xylem cross-sectional area to leaf area ratio (Huber value; $P < 0.01$),
suggesting a preferential investment in water-conducting tissue (sapwood) relative to transpiring tissue (leaves), and most likely contributing to the functional stability of the hydraulic system, essential for fast-growing pioneer species.

Introduction

Leaf hydraulic conductance (K_L) is a characteristic of great functional significance since the majority (47–94%) of the hydraulic resistance to water flow through the shoots of woody plants resides in leaves (Yang and Tyree 1994; Nardini and Pitt 1999; Nardini and Salleo 2000; Nardini 2001; Sellin et al. 2008a). K_L is a measure of water transport capacity through the leaf, expressed per unit of driving force (water potential gradient) for water movement and usually scaled by leaf area (Sack et al. 2005). Therefore, all changes in K_L evoked by environmental conditions, plant internal stimuli or leaf ontogenetic trends influence the ability of plants to transfer water to mesophyll cells, and have consequences for stomatal conductance and photosynthetic capacity (Franks 2006; Brodribb et al. 2007, 2010; Woodruff et al. 2007; Domec et al. 2009). The role of leaf hydraulic function cannot be underestimated also on a global scale: more than 40,000 km^3 year^{-1} of water flows through the hydraulic pathways inside leaves, constituting nearly 70% of terrestrial evapotranspiration (Chapin et al. 2002; Brodribb et al. 2010).

Temperature and light are the most important environmental factors that influence leaf water transport capacity.

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Responses of K_L to both temperature (Matzner and Comstock 2001; Sack et al. 2004; Cochard et al. 2007; Sellin and Kupper 2007b) and light (Tyree et al. 2005; Sack and Holbrook 2006; Scoffoni et al. 2008; Voicu and Zwiazek 2011) have been extensively studied during the last decade. Rapid enhancing effects of increasing temperature and light intensity on leaf hydraulic conductance rely on changes in living extra-vascular tissues. Fast irradiance-mediated increases of K_L involve expression or activation of plasma membrane aquaporins (water channel proteins) in mesophyll or bundle sheath cells (Cochard et al. 2007; Voicu et al. 2008; Heinen et al. 2009; Voicu and Zwiazek 2010).

Long-term effects of light availability are evidenced by systematic differences in the K_L of sun versus shade leaves (Sack et al. 2003; Sellin and Kupper 2007a, b; Sellin et al. 2008a) that develop along the canopy vertical profile or in sun- versus shade-establishing species (Brodribb and Holbrook 2004; Sack et al. 2005; Sack and Frole 2006; Lo Gullo et al. 2010). The long-term impact on leaf hydraulic capacity reflects acclimation of foliage to the spatially heterogeneous light conditions that exist in forest stands, and these responses involve both symplastic and apoplastic pathways in the leaves. Leaves that have developed in a high-irradiance environment possess a more effective vascular system due to greater transpirational water loss and concomitant demand for transport capabilities: higher density of the vein network, veins consist of a larger number of vessels, or conduits are of larger diameter (Eschrich 1997; Nardini et al. 2005a; Sack and Frole 2006; Lo Gullo et al. 2010). K_L increases with individual vein order conductivities and densities; for hierarchical venation systems, the greatest impact is from changes in vein conductivity for lower vein orders and changes in density for higher vein orders (McKown et al. 2010).

In fact, light has a diverse influence on leaf hydraulic capacity. Studies investigating the effect of light on K_L have so far concentrated mainly on the effects of light intensity or duration (e.g., Sack et al. 2002, 2005; Nardini et al. 2005b; Tyree et al. 2005; Cochard et al. 2007; Sellin et al. 2008a; Voicu et al. 2008). A novel issue is the adjustment of leaf hydraulic efficiency depending on light quality (Voicu et al. 2008; Sellin et al. 2011). Our recent experiment in silver birch (*Betula pendula* Roth) indicated that light quality has a significant impact on leaf hydraulic properties and suggests that certain light sensors are likely involved in this response (Sellin et al. 2011). The highest values of leaf blade hydraulic conductance (K_{lb}) were recorded in blue light (3.63 and 3.13 $\times$ 10^{-4} kg m^{-2} MPa^{-1} s^{-1} for the sun and shade leaves, respectively), medium values in white light (3.37 and 2.46 $\times$ 10^{-4} kg m^{-2} MPa^{-1} s^{-1}, respectively) and the lowest values in red light (2.83 and

2.02 $\times$ 10^{-4} kg m^{-2} MPa^{-1} s^{-1}, respectively). However, that study did not consider size effects, i.e. the variation in hydraulic traits related to leaf and shoot dimensions.

Leaf size contributes to optimisation of plant water use and energy balance on both ecological and evolutionary scales (Givnish 1987; Gibson 1998; Nobel 1999; Ackerly 2004; Harrison et al. 2010). However, there is currently little comprehension of the underlying mechanisms that ultimately govern the intra- and interspecific scaling relationships that determine leaf dimensions, physiology and life history (Shipley et al. 2006; Price and Enquist 2007). The construction of leaf vascular networks appears to violate several of the assumptions of the fractal branching model of West et al. (1999). Scoffoni et al. (2011) recently indicated that leaf lamina area is a pertinent issue for the functional stability of the hydraulic system: smaller leaves, which more often have a higher major vein density, have lower hydraulic vulnerability. As we know there are certain vertical trends in leaf size and morphology within a forest canopy; in silver birch, mean leaf area (A_L) decreases from the crown base to tree top (Sellin and Kupper 2006). Hydraulic conductance of leaves and shoots increases in the same direction, i.e. acropetally (Sellin et al. 2008a, 2010b). At first glance, these two trends observed in silver birch are in accordance with that suggested by Zwieniecki et al. (2002)—leaf size is a relevant factor for leaf hydraulic capacity, because longer flow paths within bigger laminae impose greater hydraulic constraints. However, under uniform environmental conditions within a particular canopy layer we have noticed an opposite trend.

The aim of the present study was to test the idea advanced by Zwieniecki et al. (2002) in silver birch; according to that, smaller leaves should have higher hydraulic conductance compared to bigger leaves. In this study, data from two different experiments have been treated together in order to evidence an opposite trend in leaf hydraulic efficiency with respect to leaf blade area. The same experimental data (Sellin et al. 2011) on leaf and shoot hydraulic conductance measured on large forest trees were reanalysed from the size aspect. The results were combined with data on leaf vascular anatomy obtained on birch saplings. As the regulation of leaf hydraulic properties is still poorly understood due to the tremendous diversity of leaf structure and function, this study should broaden the understanding of plants ability to optimise water and light utilisation by responding to micro-environmental heterogeneity within a forest canopy. Information on size-dependent variation in leaf and shoot hydraulic properties might be of interest for researchers scaling up processes observed on leaves and shoots to the canopy level.

Materials and methods

Study area and sample trees

The studies were performed in silver birch (*Betula pendula* Roth), a common broad-leaved tree species in temperate and boreal forests of Europe. The experimental sites were located in Järvselja Experimental Forest, Eastern Estonia; detailed data on the vegetation and climate of the study area has been presented in Sellin and Kupper (2005). In the first experiment (light quality experiment), three 25- to 30-year-old trees (height 16.8–19.4 m, DBH 11.6–16.2 cm) in a naturally regenerated mixed stand located near Liispõllu village (58°16′N, 27°16′E, elevation ~40 m ASL) were sampled from the lower third (shade-exposed shoots) and upper third (sun-exposed shoots) of their crowns.

The second experiment (humidification experiment) was carried out in a forest plantation equipped with facilities for free air humidity manipulation, FAHM (http://www.lote.ut.ee/FAHM/in-english), 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 2006–2007. The study site is a fenced area of 2.7 ha consisting of nine circular experimental plots (diameter 14 m) planted with silver birch and hybrid aspen 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/vaporise 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 (Kupper et al. 2011). The humidification was started in June of 2008, the study was performed in the first half of August in 2009. We sampled leaves from six trees in the humidified plots (mean height 3.3 m; LAI 3.0 m^2 m^{-2}) and from six trees in the control plots (mean height 3.8 m; LAI 3.1 m^2 m^{-2}).

Light quality experiment

In the evening prior to the measurement days, birch shoots (20–40 cm long) were cut under water and transported to the laboratory with their cut ends immersed in water. In the laboratory, the shoots were put into plastic flasks filled with deionised, 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). The sample shoots were placed in a dark room and left overnight. In the morning, the shoots were exposed to light of

different quality at a photosynthetic photon flux density of 200–250 µmol m^{-2} s^{-1}, measured with a LI-190 quantum sensor (Li-Cor Biosciences, Lincoln, NE). Irradiance was controlled by changing the distance from the lamps; the air above the shoots was agitated with a fan to minimise local temperature gradients.

Hydraulic conductance of shoots (K_S) and their parts—branches (i.e., leafless stems), petioles, leaf blades—was determined by the water perfusion method using a high-pressure flow meter (HPFM; Dynamax, Houston, TX) applied in quasi-steady state mode, and by removing leaf blades and petioles in sequence. The hydraulic conductance of leaf blades (K_{lb}) was calculated as follows:

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

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 the apoplast and symplast (Sack et al. 2002). The hydraulic conductance of petioles (K_P) was calculated as

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

where K_B is the hydraulic conductance of a bare branch. All functional and structural characteristics measured in the study are listed in Table 1. It is important to separate K_{lb} and K_P, since K_P can undergo changes that are independent of K_{lb} (Voicu and Zwiazek 2011). Tyree et al. (2005) indicated that stomatal openness does not influence results of the hydraulic measurements using a high-pressure flow meter. Rapid light responses of leaf hydraulic conductance have been verified by other independent methods (Scoffoni 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) and the shoot length, with a ruler. Shoot diameter was measured at shoot base under bark in two perpendicular directions with a digital calliper. Huber values (HV) were calculated as cross-sectional area of xylem divided by leaf area supported by the shoot. The data on hydraulic conductance were standardised to 24°C to correct for changes induced by the temperature dependence of water viscosity and normalised by shoot size expressing the conductance values per unit area of the leaf blades. Altogether, 108 shoots (3 trees × 2 canopy positions × 3 light qualities × 6 replications) were sampled for hydraulic conductance.

Table 1 Structural and functional characteristics used in the study

Abbreviations	Explanation	Unit
A_L	Leaf blade area	cm^2
A_{TL}	Total leaf area of shoot	cm^2
A_{xyl}	Xylem cross-sectional area of leaf midrib or shoot	mm^2
D_{sh}	Shoot diameter	mm
D_{ves}	Vessel inner diameter	μm
HV	Huber value (xylem to leaf area ratio)	$m^2\ m^{-2}$
K_B	Area-normalised hydraulic conductance of bare branch	$kg\ m^{-2}\ s^{-1}\ MPa^{-1}$
K_L	Area-normalised leaf hydraulic conductance	$kg\ m^{-2}\ s^{-1}\ MPa^{-1}$
K_{lb}	Area-normalised leaf blade hydraulic conductance	$kg\ m^{-2}\ s^{-1}\ MPa^{-1}$
K_P	Area-normalised petiole hydraulic conductance	$kg\ m^{-2}\ s^{-1}\ MPa^{-1}$
K_S	Area-normalised shoot hydraulic conductance	$kg\ m^{-2}\ s^{-1}\ MPa^{-1}$
K_{TS}	Absolute shoot conductance, unnormalised to leaf area	$kg\ s^{-1}\ MPa^{-1}$
l_{sh}	Shoot length	cm
N_{ves}	Number of vessels in leaf midrib	–
R_{lb}	Relative hydraulic resistance of leaf blades	%
VD	Vein density in leaf blade	$mm\ mm^{-2}$

Humidification experiment

Leaves of silver birch saplings were collected from three heights: from one branch of lower, medium and upper thirds of the crowns. From each branch six leaves were randomly sampled: two leaves for stomatal impressions, two for vascular bundle and mesophyll measurements and two for vein density assessment. Altogether, 216 leaves (2 treatments × 6 trees × 3 canopy positions × 6 leaves) were sampled for anatomical structure. Area of each sample leaf was measured separately with the AM300 area meter. Here we describe merely a preparation of samples for studying leaf vascular anatomy. 1 cm wide pieces of leaf with midrib were cut from middle of the leaf blades, put into test-tubes and fixed in FAE (a mixture of formaldehyde, glacial acetic acid and 70% ethanol) under vacuum as described in Ruzin (1999). Before embedding procedure the samples were dehydrated in an ethanol gradient (70, 80, 90, and 96%), isopropanol/Roti-Histol (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) series and embedded in Roti-Plast paraffin (Ruzin 1999). Cross-sections (thickness 5 μm) of the samples embedded in paraffin were cut with a Leica SM2000 R sliding microtome (Leica Microsystems Nussloch GmbH, Nussloch, Germany) and mounted onto microscope slides previously covered with a mixture of $KCrSO_3$ and gelatine adhesive. After that the slides were dried at 40°C for 24 h and the Roti-Plast was removed in Roti-Histol/isopropanol series. The samples were stained with Fast Green and 1% aqueous safranin, and mounted with Canada balsam. Images of the samples were captured at 40–400× magnification with a Nikon Eclipse 50 light microscope (Nikon Corp., Tokyo, Japan) and photographed with a digital camera (DS-Fi1,

Nikon Corp.). The following vascular traits were measured in midrib using ImageJ image processing software, vers. 1.36b (National Institutes of Health, Bethesda, MD): cross-sectional area of xylem ($μm^2$), minor and major diameters of the vascular bundle (μm), area of the vascular bundle ($μm^2$) and vessel inner diameters in the vascular bundle (μm).

For vein density ($mm\ mm^{-2}$) assessment entire leaf blades were held in 5% solution of NaOH, which was changed several times. After clearing the leaves were stained with 0.05% toluidine blue in 96% ethanol (Ruzin 1999) and the images of vein networks were taken at 100× magnification using a digital camera attached the Nikon Eclipse 50 microscope. The images were analysed using the ImageJ.

Data analysis

Data analysis was carried out using Statistica, Vers. 7.1 (StatSoft Inc., USA). To analyse the effects of different factors on leaf and shoot hydraulic parameters, an analysis of covariance (ANCOVA) was performed, with light quality and canopy position as fixed factors and mean leaf blade area as a covariate; type III sums of squares were used in the calculations. The variation of leaf vascular traits was analysed with respect to the treatment, canopy position and leaf blade area. Normality and homogeneity of variances were checked using the Kolmogorov–Smirnov D-statistic and the Levene test, respectively (Sokal and Rohlf 1995). When appropriate, logarithmic or root transformations were applied to the data. Post hoc mean comparisons were conducted using the Tukey HSD test. Relationships between the studied characteristics and

independent variables were assessed by Pearson's correlations and simple linear or nonlinear regressions fitted by least-squares. Bivariate relationships among two groups of data (sun vs. shade shoots) were analysed by applying standardised major axis (SMA) technique (Warton et al. 2006) using a SMATR, Vers. 2.0 freeware (http://www.bio.mq.edu.au/ecology/SMATR/).

Results

Responses of hydraulic conductance to light

The experiment revealed a strong impact of light quality treatments on leaf blade hydraulic conductance and its relative resistance, i.e. contribution of leaf blades to the whole-shoot resistance, R_{lb} (Table 2). K_S depended on canopy position and mean leaf blade area (A_L), K_{lb} on canopy position, light quality and A_L, and R_{lb} on light quality and A_L. Sun-exposed shoots exhibited $\sim$1.3 times higher ($P < 0.001$) values of both K_S and K_{lb} than shade shoots (Table 3). K_{lb} increased and R_{lb} decreased in the following order: red, white and blue light treatment (see for details Sellin et al. 2011).

Sun and shade shoots differed significantly ($P < 0.001$) regarding A_L, averaging (mean $\pm$ SE) 10.6 ± 0.34 and 12.4 ± 0.26 cm^2, respectively; the respective medians were 10.8 and 12.5 cm^2. K_{lb} depended significantly on mean leaf blade area, analysed either across the whole dataset ($P < 0.001$; Table 2) or for sun ($P = 0.022$) and shade shoots (marginally significant effect; $P = 0.057$) separately: in all data subsets—three light qualities combined with two canopy layers—larger leaves ($A_L \geq$ median for given canopy layer) tended to have higher lamina hydraulic conductance compared to smaller leaves (Fig. 1). The size effects were more pronounced in sun foliage, in which K_{lb} was on average 1.5 times (1.25 times in shade foliage) higher for the large leaves group compared to small leaves.

Relationships between hydraulic and size characteristics

Absolute shoot conductance (K_{TS}), i.e. the values not normalised by leaf area, increased with shoot size: K_{TS} correlated with total leaf area (A_{TL}), mean leaf blade area (A_L), shoot diameter measured at the base (D_{sh}), shoot length (l_{sh}), xylem cross-sectional area (A_{xyl}) and Huber value (Table 4). We analysed the K_{TS} versus A_{TL} and D_{sh} relationships for possible differentiation between the sun and shade shoots: the corresponding lines (Fig. 2) differed neither in slope nor intercept (SMA, $P > 0.05$). The unnormalized values of hydraulic conductance of all components of the sun shoot (bare branch, petioles, leaf blades) were coupled ($R^2 = 0.20$–0.31, $P < 0.001$) to each other across the treatments; in shade shoots, only the hydraulic conductances of the bare branches and leaf blades were correlated ($R^2 = 0.37$, $P < 0.001$).

We also found that hydraulic characteristics expressed per unit leaf area (K_S, K_B, K_{lb}) increased with increasing shoot size. In sun shoots, K_P was also positively correlated with shoot length ($r = 0.28$, $P < 0.05$), shoot diameter ($r = 0.31$, $P < 0.05$), xylem cross-sectional area ($r = 0.32$, $P < 0.05$) and Huber value ($r = 0.387$, $P < 0.01$). The increasing trends of K_S with increasing HV were highly significant ($P < 0.01$; Fig. 3); the slopes did not differ (SMA, $P = 0.279$) between the sun and shade shoots, whilst there was a significant (SMA, $P = 0.009$) elevation shift between the groups. The HV versus K_S relationship was independent of shoot size. In shade shoots, HV did not depend on shoot size, i.e., neither on A_{TL} nor l_{sh} ($P = 0.24$). In sun shoots, HV correlated with l_{sh} ($r = 0.44$, $P = 0.001$), but K_S was positively correlated also with the residuals of the HV $= f(l_{sh})$ regression ($R^2 = 0.11$, $P = 0.012$), indicating that the relationship of K_S with HV was independent of shoot size. The Huber values in the upper- versus lower-canopy shoots (averaging 1.32 and 1.24×10^{-4} m^2 m^{-2}, respectively) were not statistically distinguishable.

Table 2 Results of ANCOVA for the effects of canopy position, light quality and mean leaf area on hydraulic conductance of shoots and leaf blades, and on leaf blade relative resistance ($N = 108$)

Dependent variable	Factor	Statistical significance (P)
Shoot hydraulic conductance (K_S)	Canopy position	<0.001
	Light quality	ns
	Leaf area	<0.001
Hydraulic conductance of leaf blades (ln K_{lb})	Canopy position	<0.001
	Light quality	<0.001
	Leaf area	<0.001
Leaf blade relative resistance (R_{lb})	Canopy position	ns
	Light quality	<0.001
	Leaf area	0.011

ns not significant

Table 3 Mean values ± SE of shoot and leaf hydraulic conductance (kg m^{-2} MPa^{-1} s^{-1}) in three light treatments combined with two canopy layers ($N = 108$)

Characteristic	Light quality	Canopy layer	
		Lower	Upper
Shoot hydraulic conductance, K_S ($\times 10^{-4}$)	Blue	1.52 ± 0.10	1.75 ± 0.14
	White	1.29 ± 0.11	1.86 ± 0.13[**]
	Red	1.18 ± 0.10	1.60 ± 0.12[*]
Hydraulic conductance of bare branch, K_B ($\times 10^{-4}$)	Blue	3.73 ± 0.44	4.40 ± 0.45
	White	3.45 ± 0.38	5.31 ± 0.40[**]
	Red	3.59 ± 0.29	5.19 ± 0.37[**]
Hydraulic conductance of leaf blades, K_{lb} ($\times 10^{-4}$)	Blue	3.13 ± 0.21	3.63 ± 0.37
	White	2.46 ± 0.24	3.37 ± 0.26[*]
	Red	2.02 ± 0.21	2.83 ± 0.30[*]
Hydraulic conductance of petioles, K_P ($\times 10^{-3}$)	Blue	2.48 ± 0.20	2.62 ± 0.21
	White	2.30 ± 0.28	2.79 ± 0.20
	Red	2.77 ± 0.38	2.33 ± 0.10

Statistical significance for the difference between the canopy layers: [*] $P < 0.05$, [**] $P < 0.01$
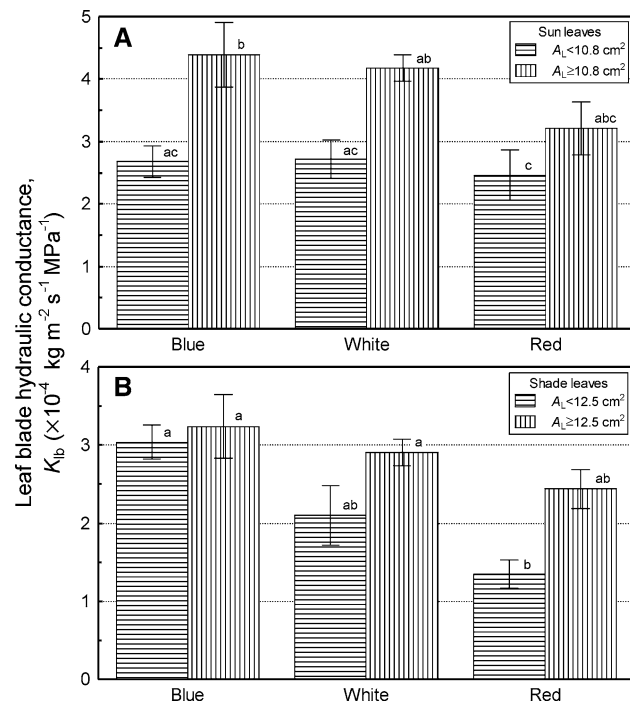

Fig. 1 Variation in leaf blade hydraulic conductance (K_{lb}) depending on light quality and leaf size. The data on both shade and sun foliage were divided into two groups according to the mean leaf blade area (A_L)—below and above median

In order to test whether K_{lb} relates to leaf area independently of shoot size, we developed K_{lb} regressions from shoot length and total leaf area, and then performed a residual analysis. The residuals of the K_{lb} regressions from both l_{sh} ($P < 0.001$; Fig. 4) and A_{TL} ($P = 0.005$) increased with mean leaf blade area (A_L) in sun foliage, but this pattern was not found for the shade foliage. The relationship depicted in Fig. 4 remained highly significant

($P < 0.001$) also after removal of the divergent data point with great leaf area ($A_L = 19.1$ cm^2). As a matter of fact, this divergent data point fits well in the common range of A_L in mature silver birch trees (i.e. 6–25 cm^2; see Fig. 4a in Sellin and Kupper 2006). In fast-growing saplings, the range of A_L is substantially wider (Fig. 6).

Leaf blade area versus vascular traits

Data on the effects of humidification treatment, canopy position and leaf blade area on leaf vascular traits measured on saplings of *B. pendula* is presented in Table 5. Only mean diameter of vessels in midrib (D_{ves}) depended significantly on the treatment, while all the studied characteristics varied with A_L. All four characteristics—xylem cross-sectional area in midrib ($R^2 = 0.429$, $P < 0.001$), vessel number in midrib ($R^2 = 0.541$, $P < 0.001$), vessel diameter in midrib ($R^2 = 0.216$, $P < 0.001$) and vein density ($R^2 = 0.371$, $P < 0.001$)—increased with increasing leaf blade area (Figs. 5, 6).

Discussion

Leaf size versus leaf blade hydraulic conductance

Light is one of the most important environmental factors influencing plant water relations: on the one hand, transpirational water loss largely depends on photon flux density incident on the leaf surface, on the other hand, light availability controls leaf water transport capacity. We found that both leaf size and leaf position in the crown modulated responses of leaf hydraulic efficiency to light quality (Tables 2, 3). As the spectral effects on leaf hydraulic

Table 4 Correlations between absolute shoot hydraulic conductance (K_{TS}) and shoot variables in lower and upper canopy layers

Shoot variable	Pearson's correlation coefficient (r)	
	Lower canopy	Upper canopy
Total leaf area (A_{TL})	0.641[***]	0.734[***]
Mean leaf blade area (A_L)	0.225	0.608[***]
Shoot length (l_{sh})	0.235	0.464[***]
Mean shoot diameter (D_{sh})	0.619[***]	0.715[***]
Xylem cross-sectional area (A_{xyl})	0.615[***]	0.700[***]
Huber value (HV)	0.225	0.330[*]

Statistical significance: [*] $P < 0.05$, [***] $P < 0.001$
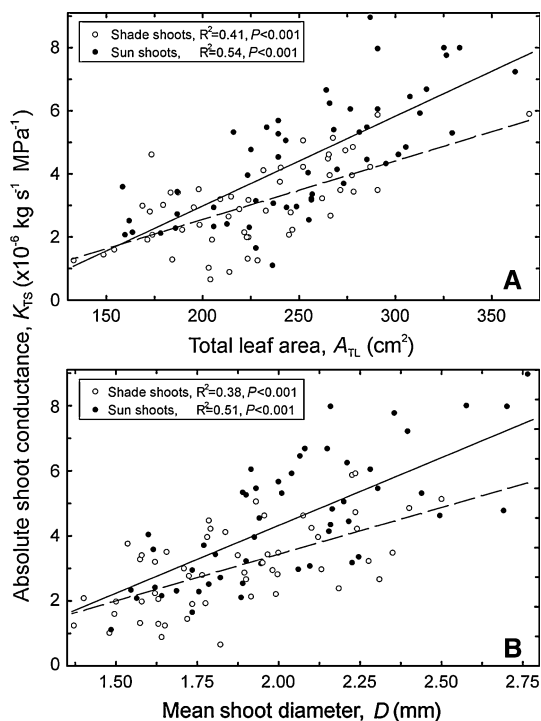
Fig. 2 Absolute shoot hydraulic conductance (K_{TS}) plotted against total leaf area (**a**) and shoot diameter measured under bark (**b**) for shoots of *Betula pendula*
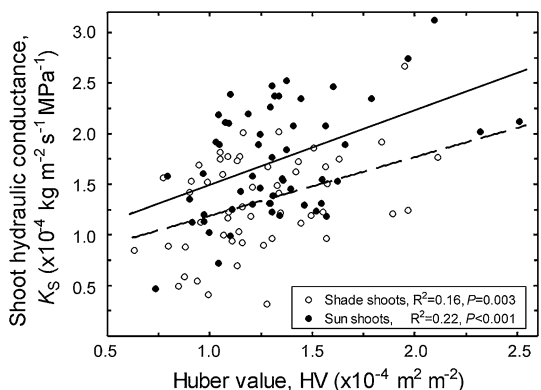

Fig. 3 Shoot hydraulic conductance (K_S) plotted against the Huber values (HV) for shoots of *Betula pendula*
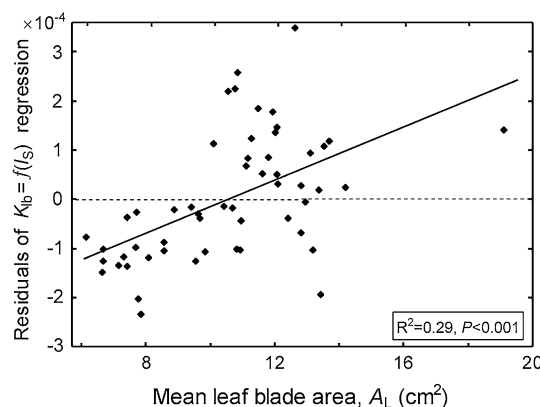

Fig. 4 Residuals of the $K_{lb} = f(l_{sh})$ regression plotted against the mean leaf blade area (A_L) in sun shoots of *Betula pendula*

capacity and its ecophysiological implications have been thoroughly discussed in our previous article (Sellin et al. 2011), we will not consider this issue further here.

In the light of the aims of the present study, it is meaningful that leaf hydraulic efficiency depended significantly ($P < 0.001$; Table 2) on mean leaf blade area (A_L): larger leaves exhibited higher K_{lb} independently of specific canopy layers (Fig. 1). The increase of K_L from crown base to tree top observed in silver birch results from long-term acclimation to vertical light gradient formed along a canopy profile (Sellin et al. 2008a), probably involving changes both in the symplastic and apoplastic compartments of the leaves. However, this trend is not related to vertical within-canopy patterns of leaf lamina area: when analysing the data on two canopy layers separately, an opposite trend becomes evident—bigger leaves exhibit higher hydraulic efficiency. Thus, the enhancing effect of high irradiance in silver birch appears to override the size-related effects on K_{lb}.

The residual analysis excluded the possibility of an artefact that could ensue from shoot size variation (Fig. 4). Higher K_{lb} observed in bigger leaves is obviously attributable to enhanced vascular development with increasing A_L. In birch saplings, cross-sectional area of xylem, number of vessels and mean vessel diameter in midrib as well as vein density rise with increasing lamina area

Table 5 Results of ANCOVA for the effects of humidification treatment, canopy position and leaf blade area on leaf vascular traits ($N = 53–67$)

| Dependent variable	Factor	Statistical significance (P)
Xylem cross-sectional area in midrib (A_{xyl})	Treatment	ns
	Canopy position	ns
	Leaf area	0.001
Vessel number in midrib (N_{ves})	Treatment	ns
	Canopy position	0.002
	Leaf area	0.005
Vessel diameter in midrib (D_{ves})	Treatment	0.044
	Canopy position	ns
	Leaf area	0.016
Vein density (VD)	Treatment	ns
	Canopy position	0.005
	Leaf area	0.040

ns not significant
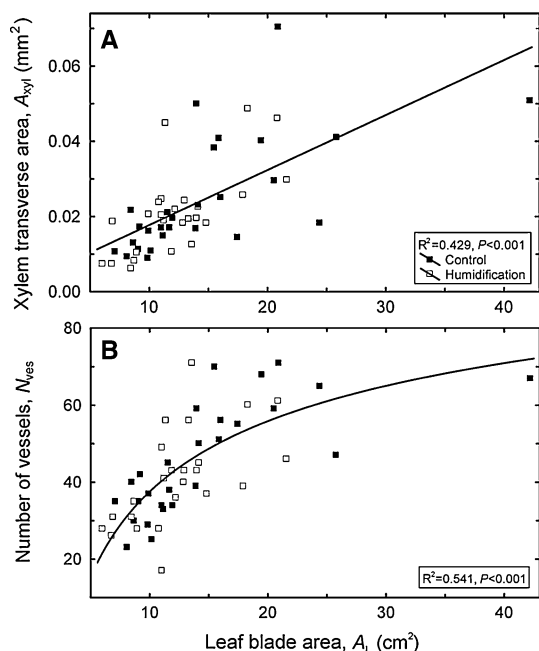
Fig. 5 Relationships between leaf blade area (A_L) and xylem cross-sectional area (A_{xyl} **a**) and number of vessels in leaf midrib (N_{ves} **b**) of silver birch saplings across the control and humidification treatments

Fig. 6 Leaf blade area (A_L) versus vein density (VD) in saplings of silver birch across the control and humidification treatments

independently of the humidity treatment (Figs. 5, 6). Both greater number and bigger diameter of vessels contribute to higher hydraulic conductance of the leaf vascular network. Higher venation density results in smaller distance between the veins (Uhl and Mosbrugger 1999), i.e. shorter flow paths through the mesophyll apoplast to evaporation sites. Therefore, K_L is expected to scale with total venation density (Niinemets and Sack 2006; Brodribb and Feild 2010; Brodribb and Jordan 2011). Uhl and Mosbrugger (1999) have demonstrated that leaf venation density may or may not vary with leaf size, depending on the plant species. The data on *B. pendula* coincide with those published for
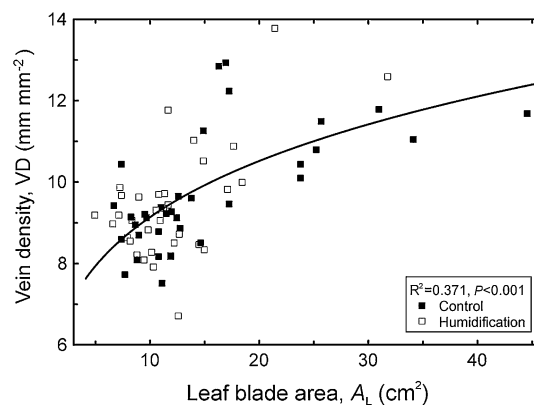
Plantago species by Dunbar-Co et al. (2009). They found that vein conduit numbers and diameters were greater in larger leaves, leading to a greater vein hydraulic conductivity. In other broad-leaved tree species (different poplar clones, *Liriodendron tulipifera* L.), the fraction of leaf biomass in major veins has been reported to increase with increasing leaf size, which is the optimal strategy to maximise light harvesting and water supply to the mesophyll for a given biomass investment in mechanical support (Niinemets et al. 2004; Niinemets and Sack 2006).

Our results do not support the idea proposed by Zwieniecki et al. (2002), according to which bigger leaves are hydraulically less efficient as longer flow paths within bigger laminae impose greater hydraulic constraints, while smaller leaves have all areas of mesophyll closer to major veins, supplied by shorter flow paths through minor veins and mesophyll. In a similar way, deep leaf lobation has been considered to be associated with higher K_L, as leaf lobation can be a means of reducing mesophyll area and allowing a better water supply to mesophyll cells and

evaporation sites (Sisó et al. 2001). Nevertheless, K_L has been reported to correlate negatively with leaf size for exposed leaves in only three of eight diverse woody species tested (reviewed by McKown et al. 2010). Analyses across different species suggest that area-normalised K_L is independent of leaf blade area because of tremendous phylogenetic and developmental diversity of vascular architecture (Sack et al. 2003; Sack and Frole 2006).

Size-related variation in other hydraulic traits

The absolute shoot conductance (K_{TS}), as well as the area-normalised hydraulic characteristics (K_S, K_B, K_{lb}) increased with shoot size in silver birch (Table 4, Fig. 2). To provide adequate water transfer to mesophyll cells, the hydraulic capacity of shoots and their components must increase with increasing shoot size. Larger shoots sustain larger transpiring area and afford higher water flux. Therefore, a positive relationship between branch or stem diameter and water transport capacity is a universal principle recognised in dozens of species from different systematic groups (Yang and Tyree 1994; Patiňo et al. 1995; Mencuccini and Grace 1996; Maherali and DeLucia 2000; Tyree and Zimmermann 2002). This relationship holds also across different species, as demonstrated for a number of angiosperm and coniferous tree species by Lusk et al. (2007). Anatomical analysis of birch branch-wood (Sellin et al. 2008b) revealed a strong relationship between branch diameter and values of both specific ($R^2 = 0.51$, $P < 0.001$) and leaf-specific conductivity of xylem ($R^2 = 0.62$, $P < 0.001$). Additionally, K_S increased with higher HV ($P < 0.001$), suggesting a disproportionate advantage in hydraulic efficiency of increasing sapwood allocation relative to leaf area (Fig. 3). At the same time, branch contribution to the shoot total liquid-phase resistance, measured in white light, was substantially smaller (36–41%) compared to that of leaf laminae (53–57%; Sellin et al. 2011). Some explanation may be hidden in fundamental differences in branching architecture between vascular system of the leaf and the xylem network within the stem (Price and Enquist 2007). The form and function of stem vasculature appears to have been selected to minimise hydraulic resistance so as to efficiently deliver water and nutrients to terminal sinks (leaves). Minimization of energy or hydraulic resistance within leaf venous networks likely does not hold, because water and nutrient exchange with living cells apparently demands slower rates of liquid transport and an increase in total hydraulic resistance.

In addition, contrary to leaves, the branch/stem water-transport system is of more inert and rigid nature; the possibilities for rapid regulation of xylem (consisting of dead xylem conduits) hydraulic conductivity are limited (Nardini et al. 2010; Sellin et al. 2010b). Hence, for functional safety (to provide wider safety margin) of the hydraulic system in a highly fluctuating environment, it is probably beneficial to increase investment in water-conducting tissue (sapwood) relative to transpiring tissue (leaves). This may be a crucial point in conditions of water shortage, as *B. pendula* is a woody species with the highest vulnerability to cavitation among a number of temperate broad-leaved tree species (Barigah et al. 2006). High xylem functional vulnerability is a charge that supports high whole-plant leaf-specific hydraulic conductance and the highest possible root hydraulic efficiency characteristics in fast-growing and pioneer species. The experiments on saplings of silver birch confirmed the relevance of HV in stomatal regulation: higher HV provides potentially better water supply to leaves, which allows stomata to remain more open at the same leaf water potential and leads to a higher ratio of intercellular to ambient CO_2 concentrations, C_i/C_a (A. Sellin, unpublished data).

The present study showed that the hydraulic traits (K_S, K_B, K_{lb}) were functionally well co-ordinated and more strongly correlated with shoot size for sun than for shade shoots. This likely reflects their greater evaporative load, and thus a greater need in sun shoots for the adjustment of hydraulic capacity with shoot size, to sustain higher sap flow rates (Sellin and Lubenets 2010) and photosynthesis per unit leaf area (Sellin et al. 2010a), compared with shade shoots. This co-ordination expresses a matching of transport capability of different segments linked in series along the water-conduction pathway.

In conclusion, our study indicates that responses of leaf hydraulic properties to light quality are significantly modulated by leaf position in the crown and by lamina size. The data obtained in silver birch does not support the idea proposed by Zwieniecki et al. (2002), because larger leaves exhibit higher hydraulic conductance. The upward increase of K_L within a canopy results from long-term acclimation to higher irradiance incident to leaves in the upper crown rather than from the vertical trends in leaf area per se. Shoot hydraulic conductance increases with increasing HV, suggesting a disproportionate advantage in hydraulic efficiency of increasing sapwood allocation relative to leaf area. This allocation pattern contributes to higher functional stability of the water-conducting system in a highly dynamic and spatially heterogeneous environment, allowing better exploitation of rapid changes in irradiance (sunflecks) to support the strategy of a fast-growing pioneer species.

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