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EELE ÕUNAPUU-PIKAS

Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences

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Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences

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PRESS

Department of Botany, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications which are referred to in the text by Roman numerals:

I Sellin A, **Õunapuu 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.

II Sellin A, Sack L, **Õunapuu E**, Karusion A. 2011. Impact of light quality on leaf and shoot hydraulic properties: a case study in silver birch (*Betula pendula*). *Plant, Cell and Envinonment* **34**: 1079–1087.

III **Õunapuu E**, Sellin A. 2013. Daily dynamics of leaf and soil-to-branch hydraulic conductance in silver birch (*Betula pendula*) measured *in situ*. *Plant Physiology and Biochemistry* **68**: 104–110.

IV Sellin A, Tullus A, Niglas A, **Õunapuu E**, Karusion A, Lõhmus K. 2013. Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecological Research* **28**: 523–535.

V Sellin A, Niglas A, **Õunapuu E**, Karusion A. 2013. Impact of phloem girdling on leaf gas exchange and hydraulic conductance in hybrid aspen. *Biologia Plantarum* **57**: 531–539.

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The participation of the author in preparing the listed publications is as follows:

Paper I – collected and analyzed the data and participated in writing the paper;
Paper II – collected the data and participated in writing the paper;
Paper III – collected and analyzed the data and was leading author in writing the paper;
Paper IV – collected the data and participated in writing the paper;
Paper V – collected the data and participated in writing the paper.

LIST OF ABBREVIATIONS

AED	atmospheric evaporative demand
A_{L}	leaf area (m^2)
AQP	aquaporin
E	transpiration rate ($\mathrm{mmol\ m^{-2}\ s^{-1}}$)
FAHM	Free Air Humidity Manipulation experiment
F_{LA}	sap flux density expressed per unit leaf area ($\mathrm{mmol\ m^{-2}\ s^{-1}}$)
g_{S}	stomatal conductance ($\mathrm{mmol\ m^{-2}\ s^{-1}}$)
K_{B}	hydraulic conductance of leafless branch ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{cap}	hydraulic conductivity of a capillary ($\mathrm{m^4\ s^{-1}\ Pa^{-1}}$)
K_{ex}	hydraulic conductance of leaf extra-vascular pathway ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{L}	leaf hydraulic conductance scaled by leaf area ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
$K_{\mathrm{L\_mass}}$	leaf hydraulic conductance scaled by leaf dry weight ($\mathrm{mmol\ kg^{-1}\ s^{-1}\ MPa^{-1}}$)
K_{lb}	hydraulic conductance of leaf blade ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{P}	petiole hydraulic conductance ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{S}	shoot hydraulic conductance ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{T}	whole-plant hydraulic conductance or total hydraulic conductance of soil-to-leaf pathway ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
K_{x}	hydraulic conductance of leaf vascular compartment ($\mathrm{mmol\ m^{-2}\ s^{-1}\ MPa^{-1}}$)
$[\mathrm{K}^+]$	potassium ion concentration (mM)
L_{p}	hydraulic conductivity of leaf cell membranes ($\mathrm{m\ s^{-1}\ MPa^{-1}}$)
η	dynamic viscosity of water ($\mathrm{Pa\ s^{-1}}$)
P	atmospheric pressure (kPa)
pH	acidity of aqueous solution
P_{50}	leaf water potential inducing 50% loss of maximum K_{L}, i.e. leaf hydraulic vulnerability (MPa)
Q_{P}	photosynthetic photon flux density ($\mathrm{\mu mol\ m^{-2}\ s^{-1}}$)
r	capillary radius (m)
R_{ex}	hydraulic resistance of the leaf extra-vascular compartment ($\mathrm{MPa\ m^2\ s\ mmol^{-1}}$)
RH	air relative humidity (%)
R_{L}	relative contribution of leaves to the whole-shoot/whole-plant hydraulic resistance (%)
R_{x}	hydraulic resistance of the leaf vascular compartment ($\mathrm{MPa\ m^2\ s\ mmol^{-1}}$)
SPAC	soil-plant-atmosphere continuum
t	time (min, h)
T_{A}	air temperature (°C)
T_{L}	leaf temperature (°C)
U	wind speed ($\mathrm{m\ s^{-1}}$)

VPD	water vapour pressure difference between leaf interior and ambient air (kPa)
$\boldsymbol{\Delta\Psi}$	water potential difference between branch xylem and leaves, i.e. the driving force for water flow from branch xylem to leaves (MPa)
$\boldsymbol{\sigma_{sap}}$	electrical conductivity of xylem sap (mS)
$\boldsymbol{\Psi_B}$	branch water potential (MPa)
$\boldsymbol{\Psi_L}$	leaf water potential (MPa)
$\boldsymbol{\Psi_S}$	soil water potential (MPa)

1. INTRODUCTION

1.1. Water transfer in soil-plant-atmosphere continuum

The tissues of plants as of all living organisms are mainly composed of water. Water plays an essential role in the life of plants, as it is the vital medium in which biochemical processes can take place. Water is the most abundant molecule on Earth's surface, but among all the resources that plants need to grow and function, its availability limits plant productivity most strongly on a global scale (Taiz and Zeiger 2010). Water stress is the primary cause of decreased agricultural productivity and water availability, besides temperature, mainly determines the productivity of natural ecosystems (Lambers et al. 2008). Global precipitation gradients lead to the abundant vegetation patterns existing on our planet today. Thus, understanding and manipulating the controls over plant water relations makes it possible to optimize the entire plant performance, including yield and adaptation to environmental constraints, affording direct benefits for mankind by increasing the productivity of agriculture, horticulture and forestry. It also helps to understand plant adaptations to environmental conditions and natural patterns of productivity.

Plants are a central segment of the soil-plant-atmosphere continuum (SPAC), in which water moves from the soil through the plant to the atmosphere along a water potential or hydrostatic pressure gradient (Larcher 2003). As the aboveground parts of plants are exposed to relatively dry environment – the air, they continually lose water by transpiration and thus need a constant water supply from the soil to prevent desiccation of living cells. According to the cohesion-tension theory (Meinzer et al. 2001; Tyree and Zimmermann 2002; Pickard and Melcher 2005), transpiration pulls water from the soil to roots, from roots further to stems and leaves, and creates the water potential gradient through the plant. The steepest water potential drop occurs at the leaf surface, representing the driving force for water movement through plants. At the same time, as water travels across the SPAC, it must overcome substantial resistances, which are arranged in series (root system – stem – foliage) or in parallel (single roots in the root system; branches attached to the stem; single leaves in the foliage; parallel veins in leaf blade).

For plants growing in temporally and spatially changing environments, the ability to regulate efficiently water use and maintain a positive water budget becomes the primary requirement for survival. The basic processes involved in the water balance of a plant are water uptake from the soil, water conduction to all living cells, storing water in internal reserves and transpirational water loss from plant surface. Therefore, balancing the uptake and loss of water represents an important challenge for all land plants (Taiz and Zeiger 2010; Brodribb and McAdam 2011). Even small deviations from equilibrium can cause water deficits and severe malfunctioning of many cellular processes. Although water movement through plants is in principle a passive process, plants have evolved several mechanisms to actively regulate water fluxes in the SPAC. The chief control points for plant water relations are the stomata, which govern the water loss from plants. However, the supply from soil and resistances to water transfer through the plant are equally important control points as they constrain stomatal openness (Larcher 2003).

A plant can be considered a hydraulic system (Cruiziat et al. 2002) acting as an interface between the soil and the atmosphere. Water flow through the plant faces substantial resistances that are not evenly distributed among different organs of the plant. The roots and leaves with high hydraulic resistance are connected with each other by the stem having a relatively low hydraulic resistance. Thus water flow through plants can be treated analogously to the current in an electric circuit and can be described using an analogy to Ohm's Law (Tyree and Ewers 1991; Roderick 2001): the volume flow rate (F; mmol s^{-1}) through the plant or through its separate region is proportional to the hydraulic conductance of this region (K; mmol s^{-1} MPa^{-1}) and the water potential drop across this region ($\Delta\Psi$; MPa). On the basis of this analogy, water transport capacity of plants can be adequately described and measured in terms of conductance to water in the liquid phase:

$$K = \frac{F}{\Delta\Psi} \tag{1}$$

Hydraulic conductance is the inverse of resistance (R). Since resistances are additive in series, whole-plant resistance can be partitioned into components corresponding to different plant organs.

Plant total hydraulic conductance or whole-plant hydraulic conductance, usually expressed per unit leaf area (K_T; mmol m^{-2} s^{-1} MPa^{-1}), quantifies the efficiency of water transport pathway through the whole plant body – from soil-root interface to mesophyll cell walls – in the liquid phase and defines the capacity of a plant for water transfer. Under given atmospheric conditions and soil water availability, the stomatal and boundary-layer conductances determine the transpiration rate, while K determines the leaf water potential at that transpiration rate (Tyree and Zimmermann 2002). Thus K defines how high stomatal conductance a plant may afford without desiccating the leaf, i.e. maximum stomatal conductance. Since plants face the everlasting photosynthesis-transpiration compromise – plants need carbon dioxide for photosynthesis, but the diffusion of CO_2 into the leaf shares a common pathway with the movement of H_2O out of the leaf – the uptake of CO_2 and hence the photosynthetic capacity is constrained by leaf water supply. The existence of a functional relationship between plant hydraulic properties and stomatal conductance or photosynthetic capacity is supported by ample evidence (Sack et al. 2003b; Tyree 2003; Brodribb et al. 2005, 2007; Sack and Holbrook 2006; Brodribb and Jordan 2008; Boyce et al. 2009; Brodribb 2009; Gortan et al. 2009; Meinzer et al. 2009; Savvides et al. 2012; Zhang et al. 2013). Thus, plant hydraulic architecture – the structure and properties of the water transport pathway that govern the balance between water supply and loss – is one of the key determinants of plant productivity (Meinzer et al. 2001). Stomatal regulation provides a means to balance water loss by transpiration with the efficiency of water-conducting tissues to supply water to leaves, while simultaneously avoiding leaf desiccation and maximizing carbon dioxide uptake under given environmental conditions (Sperry 2000; Meinzer 2002).

1.2. Leaves as a major hydraulic bottleneck in plants

Leaves represent a major hydraulic bottleneck for the plant. Although leaves make up less than 1% of the entire water-transport distance inside the tree's body, they alone comprise at least one third of the total hydraulic resistance of the plant (Becker et al. 1999; Sack et al. 2003b; Gyenge et al. 2005; Sack and Holbrook 2006; Sellin and Kupper 2007b). Thus, leaf hydraulic conductance (K_L), generally expressed on a leaf area basis, is a dominant factor in defining whole-plant water transport capacity. 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).

As water enters the leaf from the stem, it travels through petiole and vein xylem, crosses the bundle sheath and mesophyll tissues and evaporates in the intercellular spaces. Therefore K_L consists mainly of two components: the vascular and extra-vascular component connected in series. The hydraulic conductance of the leaf vascular compartment depends on the total hydraulic conductivity of each xylem conduit in a leaf. The hydraulic conductivity of a capillary (i.e. xylem conduit – vessel, tracheid; K_{cap}; m^4 s^{-1} Pa^{-1}) is described by the Hagen-Poiseuille equation (Tyree and Zimmermann 2002):

$$K_{cap} = \frac{\pi r^4}{8\eta} \tag{2}$$

where r is the capillary radius (m) and η is the dynamic viscosity of water (Pa s^{-1}). According to Eq. 2, the flow rate through the capillary is proportional to the capillary radius to the fourth power, meaning that a small increase in xylem conduit diameter is accompanied by a substantial increase in conductivity as the resistance to water flow caused by friction along the cell wall decreases dramatically (Tyree and Zimmermann 2002). The hydraulic conductance of leaf vasculature (K_x) is determined also by the architecture of the vein system (McKown et al. 2010; Scoffoni et al. 2011; Sack and Scoffoni 2013), which is highly species-specific, depending on the leaves' developmental stage and subject to variation with growing conditions. Vascular cell and whole vein dimensions, vein hierarchy, vein tapering, vein density, vein topology, free ending veins, bundle seath extensions and accessory transport and support tissues all characterize leaf venation and to a greater or lesser degree affect its water transport capacity (Sack and Scoffoni 2013). Greater number, bigger diameter and length of xylem conduits and higher vein density are primary traits contributing to higher K_x. Higher vein density represents more numerous xylem flow pathways per leaf in parallel, providing also higher dysfunction tolerance of the hydraulic system caused by physical damage or embolism. Some vein branching patterns (with a large number of areoles, loopiness) may provide to a greater extent the same benefits as high vein density than other types of vein branching. Thus, leaf hydraulic efficiency depends significantly also on leaf size – major vein density commonly increases with decreasing leaf blade area, while a strong positive relationship exists between K_x and major vein density (McKown et al. 2010; Sack et al. 2012), although positive correlations between leaf size, vein density and hydraulic conductance have been reported for some species (Price et al. 2012; Sellin et al. 2012).

Recent studies have shown that the xylem hydraulic efficiency depends also on the ionic concentration of xylem sap, especially on the concentration of potassium ions (Zwieniecki et al. 2004; Nardini et al. 2010; Sellin et al. 2010b; Trifiló et al. 2011, 2013). This “ionic effect” is likely based on cation-mediated modifications of the water permeability of interconduit pit membranes (Zwieniecki et al. 2001; Gasco et al. 2006, 2007; Aasamaa and Sõber 2010; Cochard et al. 2010; Gortan et al. 2011; Lee et al. 2012; Nardini et al. 2012a). The exact origin of K^+ ions in the xylem sap is still unclear, although recirculation of ions from the phloem has been reported (Jeschke and Pate 1991; Pate and Jeschke 1995; Zwieniecki et al. 2000, 2004; Salleo et al. 2006). Zwieniecki et al. (2004) first demonstrated that phloem can influence the hydraulic performance of xylem. Thus, phloem girdling, the removal of secondary phloem might disturb the influx of ions to xylem and lead to decreased ion concentration in the xylem sap and a subsequent decline in hydraulic efficiency. Since the vascular pathway (xylem conduits) is an important component of K_L, modifications to K_x by means of phloem girdling should have a substantial impact also on the whole leaf hydraulic conductance. The close functional connection between xylem and phloem needs further investigation, specifically in terms of the importance of phloem in regulation of leaf hydraulic efficiency.

The hydraulic conductance of the leaf extra-vascular compartment (K_{ex}) is determined mainly by water permeability of bundle sheath and mesophyll cell membranes. Ninety-five percent of the water permeation of plant plasma membranes is attributable to aquaporins (AQP) – hydrophilic water channel proteins (Maurel et al. 2008). The regulation of water exchange across cell membranes occurs in two main ways: through AQP abundance (i.e. the level of expression of AQPs) and through AQP activation (i.e. the open/closed state of the hydrophilic channels). Previous studies have shown that AQP expression and activity in leaves is regulated developmentally and sensitive to various environmental factors (Heinen et al. 2009). K_{ex} is also dependent on leaf anatomical structure. To reach the sites of evaporation, water must move out of the vein and cross the mesophyll tissues. Since resistance to water flow through living leaf mesophyll is very high compared to dead xylem, the distance that water must pass through the mesophyll before evaporating has a major impact on leaf hydraulic efficiency. A highly significant negative relationship has been found between K_{L} and mesophyll path length (Brodribb et al. 2007, 2010). In turn, the mesophyll path length depends on the spatial arrangement of veins in the leaf mesophyll (determined by vein density and branching) and the existence of accessory transport tissues (sclereids, transfusion tracheids) (Sack and Scoffoni 2013).

K_{L} integrates all water flow pathways within the leaf, while changing ambient conditions modify the partitioning of hydraulic resistances between the vascular and extra-vascular compartments. Current consensus is that the hydraulic resistances of the vascular and extra-vascular compartments inside the leaf are of the same order of magnitude (Sack and Tyree 2005; Sack and Holbrook 2006). Depending on species specificity or environmental factors, either one can prevail.

Due to the tremendous anatomical and morphological diversity of plant leaves, the species-specific differences in the partitioning of leaf hydraulic resistances and the multiple mechanisms controlling the response of vascular and extra-vascular water transport capacity to environmental conditions, K_{L} shows dramatic variability both among and within species studied to date (Brodribb and Holbrook 2004b; Sack et al. 2005; Sack and Holbrook 2006). Moreover, K_{L} demonstrates dynamic behaviour over a range of time scales, from minutes to months, displaying circadian (Brodribb and Holbrook 2004a; Lo Gullo et al. 2005; Nardini et al. 2005b; Johnson et al. 2009b), seasonal and developmental trends (Aasamaa and Sõber 2005; Brodribb and Holbrook 2005a; Sellin and Kupper 2007b). K_{L} depends on growing conditions and is sensitive to changes in microclimate, being influenced by multiple environmental factors, the most important of which are light (Lo Gullo et al. 2005; Nardini et al. 2005a; Voicu et al. 2008; Savvides et al. 2012), temperature (Sack et al. 2004; Sellin and Kupper 2007b) and water availability (Nardini and Salleo 2005; Brodribb and Holbrook 2006; Gortan et al. 2009; Scoffoni et al. 2012). However, a decline in leaf hydraulic conductance in case of water deficit cannot be treated merely as a passive consequence of decreasing water availability, but down-regulation of K_{L} may be a component of adaptation of plants to drought-prone habitats (Gortan et al. 2009).

1.3. Impact of environmental factors on leaf hydraulic conductance

The primary environmental factor affecting K_L is water availability. On a longer time scale, water shortage can interfere with leaf growth and xylem differentiation, resulting in changes in xylem conduit diameter (Nardini and Salleo 2005) and altered venation properties (i.e. vein density and conduit numbers). In the shorter term, rapid decreases in K_L occur due to water stress-induced vein xylem cavitation and/or collapse (Kikuta et al. 1997; Nardini et al. 2003; Cochard et al. 2004; Brodribb and Holbrook 2005b; Johnson et al. 2009a, 2012). Water stress also influences AQP expression and activity (Galmes et al. 2007; Secchi et al. 2007; Heinen et al. 2009; Parent et al. 2009; Secchi and Zwieniecki 2010; Shatil-Cohen et al. 2011; Pou et al. 2013; Prado and Maurel 2013). Several studies have reported the existence of a trade-off between hydraulic efficiency and vulnerability at the stem level (Meinzer et al. 2010; Barnard et al. 2011), i.e. species characterized by higher water transport capacity are generally more vulnerable to xylem cavitation, and vice-versa. However, leaf hydraulic vulnerability – quantified by P_{50}, the leaf water potential inducing a 50% loss of maximum K_L (Brodribb and Holbrook 2003b) – has been reported to be independent of K_L (Blackman et al. 2010; Nardini et al. 2012b; Scoffoni et al. 2012), reflecting diverse strategies of drought adaptation in plants. Thus, some drought-tolerant species have low K_L, while others have high K_L, depending on their water-use strategy. If leaf hydraulic conductance is expressed on a leaf mass basis (K_{L_mass}), a clear trade-off emerges between leaf hydraulic capacity and functional vulnerability (Nardini et al. 2012b), indicating that drought-resistant leaves are characterized by reduced hydraulic efficiency per unit carbon invested, probably translated into higher costs for leaf construction and reduced growth rate.

Temperature influences K_L, having an effect on both K_x and K_{ex}. The temperature effect on K_x is attributable to changes in water viscosity, while K_{ex} is influenced mainly by cell membrane permeability (Fredeen and Sage 1999; Cochard et al. 2000; Matzner and Comstock 2001; Sack et al. 2004; Sellin and Kupper 2007b). Water viscosity decreases and cell membrane fluidity increases at higher temperatures, both allowing higher hydraulic conductance. Sellin and Kupper (2007b) demonstrated that nearly one third of the temperature effect on K_L in *Tilia cordata* in natural forest conditions is attributable to changes in the dynamic viscosity of water and two thirds to changes in the leaf symplastic compartment.

K_L responds strongly also to light availability across different time scales. For many species, K_L increases with irradiance on a short time scale (Tyree et al. 2005; Scoffoni et al. 2008; Guyot et al. 2012). This light-induced rapid enhancement of K_L has been shown to be related to enhanced expression of AQP in response to irradiance (Cochard et al. 2007; Voicu et al. 2008; Ben Baaziz et al. 2012a, 2012b; Lopez et al. 2013). Light availability has an effect on K_L also on a longer, developmental timescale. K_L is significantly higher in sun-exposed leaves compared to shade leaves (Sack et al. 2003b; Sellin and Kupper 2007a, 2007b) and in sun-adapted species compared to shade-tolerant species (Nardini et al. 2005a; Sack et al. 2005; Sack and Frole 2006), owing mainly to developmental changes in venation properties. In addition, diurnal variation in K_L is related to light availability (Brodribb and Holbrook 2004a; Lo Gullo et al. 2005; Johnson et al. 2009b, 2011; Zhang et al. 2009; Zufferey et al. 2011), although the intrinsic circadian clock of plants also plays a role in this regulation (Nardini et al. 2005b).

The influence of atmospheric humidity on tree performance has so far been scarcely investigated. At northern latitudes a rise in atmospheric humidity and precipitation is predicted as a consequence of global climate change, with a larger increase in the frequency than in the magnitude of precipitation (IPCC 2007). Climate change scenarios for the year 2100 predict an increase in air temperature (by 2.3–4.5°C) and precipitation (by 5–30%) in the Baltic region (Kont et al. 2003). An increase in atmospheric relative humidity (RH), the inevitable result of more frequent rainfall events, and concomitant decline in water vapour pressure difference (VPD) between the air and leaf intercellular spaces ultimately results in altered water flux through the canopy (Fanourakis et al. 2011; Kupper et al. 2011). Artificially raised RH has been shown to decrease the average stem sap flux density per unit leaf area by more than 25% in silver birch trees (Kupper et al. 2011). Changes in water flux within trees affect the structure of leaves and vascular tissues, having an impact on diverse functional traits, including water-use efficiency, Huber values and hydraulic conductance (Mencuccini and Bonosi 2001; Li et al. 2005; Tullus et al. 2012). The impact of increased RH on plant hydraulic traits needs to be elucidated in order to make reliable predictions of the responses of trees and forests to climate change at northern latitudes.

Woody species, especially long-lived tall trees represent a more complex system to study physiological processes governing water relations of plants compared to small-sized herbaceous species. Due to their extensive evaporative surfaces and the long distances water must travel from roots to leaves, tall trees face increasing challenges to maintain the integrity of their water transport system. Tree stems also possess large capacitances (Scholz et al. 2007); their parenchyma and phloem tissues act as water storage to buffer daily transpirational water loss (Zweifel et al. 2000; Sevanto et al. 2011). In addition, forest canopies are characterized by substantial within-canopy environmental gradients and the environmental conditions change during stand development. Therefore, leaves located at different positions within the crown are exposed to different environments, with the most pronounced effect on the light regime. Hence woody species need to develop foliage that is both physiologically and morphologically adapted to the spatially and temporally changing micro-environmental conditions existing within forest canopies (Niinemets et al. 1998; Sellin 2001). Such adaptations should be also expressed in vertical patterns of leaf hydraulic properties within the crown. Whole-plant hydraulic conductance has been shown to increase from basal to top branches (Sellin and Kupper 2004, 2005a, 2005b, 2007a). Such spatial patterns of K_T within a crown are largely associated with the upward increase in hydraulic efficiency of the foliage.

1.4. Aims of the thesis

Most studies on leaf hydraulics have been carried out on herbaceous plants or tree seedlings grown in controlled environments (Nardini et al. 2000, 2005b; Tsuda and Tyree 2000; Matzner and Comstock 2001; Trifiló et al. 2003; Nardini and Salleo 2005; Holloway-Phillips and Brodribb 2011; Savvides et al. 2012). Only a few studies have focused on measuring K_L on trees in forest stands *in situ* (Brodribb and Holbrook 2003a; Sack et al. 2003a; Aranda et al. 2005; Sellin and Kupper 2007b; Johnson et al. 2009b), therefore data on leaf hydraulic conductance measured on large forest trees in their natural environment are scarce. The main objective of the thesis was to investigate the variability of leaf hydraulic conductance both spatially and temporally within a tree crown with respect to acclimation to environmental factors. It is necessary to understand optimization of plant water use across the micro-environmental heterogeneity within a forest canopy and the co-ordination between liquid- and gaseous phase conductances. Hydraulic architecture of trees has considerable implications for forest conditions and productivity over longer periods (McDowell et al. 2008; Nardini et al. 2013; Zeppel et al. 2013). In light of global climate change, such knowledge will give us the opportunity to predict the responses of the plant water conducting system and contribute to elaboration of forest management measures to improve plant growth and alleviate possible adverse consequences on tree performance under changing conditions (Reyer et al. 2013).

This thesis aimed at the following objectives:

- To estimate the impact of diverse light properties – intensity, quality and duration of exposure – on K_L (**I** and **II**).
- To determine the vertical patterns of K_L within a crown and the distribution of resistances among different parts of the trees growing in natural forest stands (**I–IV**).
- To determine the daily dynamics of K_L with respect to main environmental variables under natural conditions (**III**).
- To quantify changes in leaf hydraulic traits in response to artificially elevated air humidity to mimic predicted long-term trends in atmospheric humidity (**IV**).
- To test K_L for expected impact of phloem girdling, i.e. to assess the impact of disruption of phloem transport by means of secondary phloem removal (**V**).

The key hypotheses set up and tested in the thesis:

1. The response of K_L to irradiance depends on the spectral light composition: blue light has a greater enhancing effect on K_L than white or red light due to differencial sensing by specific light receptors.
2. Leaf hydraulic conductance increases with increasing light intensity and duration of exposure, determining principally the daily pattern of K_L.
3. Leaf hydraulic conductance decreases in response to declined transpirational flux due to increased atmospheric relative humidity.
4. Leaf hydraulic conductance decreases as a consequence of phloem severing due to disturbed potassium ion recirculation between the phloem and xylem.

2. MATERIALS AND METHODS

2.1. Study sites and species

Fieldwork was conducted at two study sites in Eastern Estonia, representing a hemiboreal vegetation zone, and on two deciduous tree species: silver birch (*Betula pendula* Roth.) and hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.). The long-term average annual precipitation in the region is 650 mm; the mean temperature in July is 17.0 °C and in January – 6.7 °C. The total annual global short-wave radiation in the region averages 3518 MJ m^{-2}, and the annual radiation budget, 2552 MJ m^{-2}. The vegetation period lasts on average 175–180 days, from mid-April to October.

Site I

The first study site is located near Liispõllu village in Järvselja Experimental Forest (58°16’N, 27°16’E, 40 m a.s.l.) in a mixed naturally regenerated forest stand (**I**, **II**, **III**). The stand is dominated by silver birch (*B. pendula*) and Norway spruce [*Picea abies* (L.) Karst.] growing in the transition zone of the *Oxalis* boreal and *Filipendula* paludifying forest site types. The soil is a gleyed pseudopodsol formed on a loamy till, and characterized by a large water storage capacity (Niinemets and Kull 2001).

Site II

The second study site is located at Rõka Village (58°14’N, 27°17’E, 40–48 m a.s.l.) in an experimental forest plantation at the Free Air Humidity Manipulation (FAHM) site designed to investigate the effect of elevated air relative humidity (RH) on forest ecosystems (**IV** and **V**). The FAHM site consists of nine 14×14 m experimental plots planted with silver birch and hybrid aspen (stand density 1 tree m^{-2}) and surrounded by a buffer zone (stand density 0.25 trees m^{-2}). A detailed description of the FAHM site and technical setup is presented in Kupper et al. (2011). The soil is a fertile Endogenic Mollic Planosol (WRB 2007) with an A-horizon thickness of 27 cm. Total nitrogen content is 0.11–0.14%, C/N ratio is 11.4, and pH is 5.7–6.3.

2.2. Experimental set-up

Papers I and II

Shoots from four ~25-year-old (height 15.5–17.0 m; **I**) or three 25- to 30-year-old (height 16.8–19.4 m; **II**) trees of silver birch growing at Järvselja Experimental Forest were sampled from the upper (sun foliage) and lower (shade foliage) thirds of the tree crowns the evening before the measurement day. Shoots 15–35 cm (**I**) or 20–40 cm (**II**) long were cut under water with a razor blade and put into test tubes with the cut 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, USA), and rehydrated overnight in a dark room. Prior to and during the conductance measurements, the shoots were exposed to light at a certain photosynthetic photon flux density (Q_P = 610 or 180 µmol m^{-2} s^{-1}, for sun and shade foliage respectively) for different time periods (t = 1–11 h; "*Time experiment*" in **I**) or to different irradiance levels (Q_P = 70, 140, 330 or 610 µmol m^{-2} s^{-1}) for a certain time period (t = 7 h; "*Light experiment*" in **I**) or to different wavelengths of light (white, blue and red) at constant irradiance (Q_P of 200–250 µmol m^{-2} s^{-1}) for different time periods (t = 1, 3 or 5 h; **II**). Air above the shoots was agitated with a fan to minimize local temperature gradients. The shoots were measured for transpiration rate (E; mmol m^{-2} s^{-1}) by using LI-1600M steady-state porometer (Li-Cor, USA) and leaf (Ψ_L; MPa) and branch water potentials (Ψ_B; MPa) by using a Scholander-type pressure chamber at 2-hour intervals throughout the measurement days ("*Time experiment*" in **I**) or at noon ("*Light experiment*" in **I**). Ψ_B was estimated by applying the method of bagged leaves (Nardini et al. 2001; Brodribb and Holbrook 2003a), each time sampling two leaves that were enclosed in small minigrip bags and wrapped in aluminium foil the previous evening. Water potential of the non-trinspiring (bagged) leaves was taken as a proxy of Ψ_B. These data were used to calculate leaf hydraulic conductance (K_L) according to the evaporative flux method based on leaf-level transpiration rates (porometric method). In **II** K_L was determined by the water perfusion method using a high pressure flow meter (HPFM; Dynamax Inc., Houston, USA) applied on quasi-steady state mode.

Paper III

Branches of three 20- to 30-year-old silver birch trees (height 16.8–19.4 m) growing at Järvselja Experimental Forest were chosen from the basal (lower canopy) and top thirds (upper canopy) of the crowns. Sap flow was recorded at 1-min intervals with the stem heat balance method (Sakuratani 1981; Smith and Allen 1996) using Dynagage sensors (Dynamax Inc.) installed at the base of the sample branches and expressed as sap flux density per unit leaf area (F_{LA}; mmol m^{-2} s^{-1}). F_{LA} (10-min averages), leaf (Ψ_L; MPa) and branch water potentials (Ψ_B; MPa) were measured on the sample branches at 2-hour intervals throughout the measurement days. All major environmental variables affecting plant water use were monitored continuously: photosynthetic photon flux density (Q_P; µmol m^{-2} s^{-1}), air temperature (T_A; °C) and relative humiditiy (RH; %), wind speed (U; m s^{-1}), soil water potential (Ψ_S; MPa). Plant hydraulic properties were estimated by the evaporative flux method based on sap flux density measured at the stem and branch bases (sap flow method) or on leaf-level transpiration rates (porometric method).

Paper IV

The experiment was conducted on 4-year-old saplings of silver birch growing at the FAHM site. Three sample plots with non-manipulated environmental conditions were used as control areas (**C** treatment), while artificial air humidification was applied in three plots (**H** treatment). Two trees per plot were sampled, i.e. all together six from **C** plots (height 3.8 m) and six from **H** plots (height 3.3 m). Three branches from each sample tree cut from the lower, middle and upper layer of the crown were measured hydraulically under Q_P of 120–150 µmol m^{-2} s^{-1}. Hydraulic conductance of whole shoots (K_S) and their parts – whole leaves (K_L), leafless branch (K_B), petioles (K_P), leaf blades (K_{lb}) – was determined by the water perfusion method using a high pressure flow meter (HPFM).

Paper V

The experiment was conducted on hybrid aspen saplings growing in the buffer zone of the FAHM site. Ten 5-year-old aspen trees (height 4.0 m) were sampled for gas exchange and hydraulics. Two neighbouring branches of equal size were chosen from each tree: on one branch, phloem was girdled at the branch base; the second branch was left untreated and served as a control. Measurements were taken immediately before and 1, 2, and 3 days after the phloem girdling. Hydraulic measurements were performed using the evaporative flux method based on leaf-level transpiration rates (LI-1600M steady-state porometer; Li-Cor, USA). After the final hydraulic measurements all leaves were removed from the sample branches and xylem sap was extracted according to (Stark et al. 1985). The sap was extracted from branch segments at a pressure of 2.0 MPa generated by a pressure chamber (Model 1000; PMS Instrument Company, Albany, USA). The potassium ion concentration ($[K^+]$), electrical conductivity (σ_{sap}) and acidity of the sap were determined using a Cardy C-131 potassium ion meter (Horiba, Kyoto, Japan), electric conductometer (Twin Cond B-173; Horiba) and pH meter (Twin B-121; Horiba), respectively.

2.3. Hydraulic measurements

K_L was determined mainly by two different methods. In **I**, **III** and **V** the evaporative flux method was applied (Brodribb and Holbrook 2003a) and K_L was calculated according to the following formula:

$$K_L = \frac{F}{(\Psi_B - \Psi_L)} \tag{3}$$

where F is the leaf-level transpiration rate (E; mmol m^{-2} s^{-1}; in **I** and **V**) or sap flux density (F_{LA}; mmol m^{-2} s^{-1}; in **III**) expressed per unit leaf area and (Ψ_B –

Ψ_L) is the water potential drop from the branch xylem to leaves. E was measured with a LI-1600M steady-state diffusion porometer (in **I** and **V**) or calculated from stomatal conductance (g_S; mmol m^{-2} s^{-1}) and relative VPD [VPD/P; P is the atmospheric pressure (kPa)]; F_{LA} was recorded using Dynagage sensors. Leaf water potentsial (Ψ_L; MPa) was measured destructively with a Scholander-type pressure chamber. Xylem water potential of the branch (Ψ_B; MPa) was estimated by applying the method of bagged leaves (Nardini et al. 2001; Brodribb and Holbrook 2003a). Water potential of the non-transpiring leaves (enclosed in minigrip bags and covered with aluminium foil the previous evening) was taken as an estimate of Ψ_B.

In **II** and **IV** the water perfusion method using a high-pressure flow meter (HPFM) applied in a quasi-steady-state mode was used. K_L was calculated as follows:

$$K_L = (K_S^{-1} - K_B^{-1})^{-1} \tag{4}$$

where K_B is the hydraulic conductance of the branch (i.e. leafless stem) and K_S is the hydraulic conductance of the whole shoot. Total leaf area (A_L; m^2) of the shoots in all experiments was determined with an AM300 digital area meter (ADC BioScientific, Great Amwell, UK) or with a LI-3100C optical area meter (Li-Cor Biosciences, Lincoln, USA) for larger samples and all K_L values were scaled by leaf area. K_L data was standardized for the dynamic viscosity of water at a certain temperature.

2.4. Data analysis

Statistical data analysis was performed using Statistica, Version 7.1 (StatSoft Inc., USA). To analyze the effects of fixed factors (light duration, light intensity, light colour, canopy position, time of day, treatment) and environmental variables (Ψ_S, Q_P, T_A, RH, VPD and U) on leaf hydraulic conductance, an analysis of variance/covariance was performed using the General Linear Models module. Type III (for balanced data sets) or type IV sums of squares (for unbalanced data sets) were used in the calculations. Normality and homogeneity of variances were checked using the Kolmogorov-Smirnov D statistic and the Levene test, respectively. To fulfil the assumptions for analysis of variance (ANOVA), logarithmic or complex transformations were applied to the data when necessary. During the analysis, the ANCOVA models were modified by removing insignificant covariates step-by-step from the model. *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 and simple linear or nonlinear least-squares regressions. See more details about statistical analysis in **I**–**V**.

3. RESULTS AND DISCUSSION

3.1. Impact of light properties

Light intensity

Leaf hydraulic conductance of *B. pendula* varied significantly ($P<0.001$) with light intensity: K_L increased with irradiance and achieved highest values at Q_P of 330 µmol m^{-2} s^{-1} (Fig. 2A in **I**). Similar patterns of K_L with respect to irradiance have now been reported for several plant species (Sack et al. 2002; Nardini et al. 2005b; Tyree et al. 2005; Scoffoni et al. 2008; Voicu et al. 2008, 2009; Rockwell et al. 2011; Aasamaa and Sõber 2012; Guyot et al. 2012). The study performed by Tyree et al. (2005) on temperate and tropical trees revealed that K_L increased significantly with irradiance for 6 of 11 species. Aasamaa and Sõber (2012) showed light sensitivity for all five tested temperate tree species. Additionally, a light response was found for cell hydraulic conductivity in the leaf vein parenchyma of *Zea mays* L. (Kim and Steudle 2007, 2009).

The light response of K_L was previously thought to be associated mainly with the regulation of stomatal aperture (Sack et al. 2002; Rockwell et al. 2011), i.e. directly governed by gaseous phase conductance, but experiments have shown that the stimulation of K_L by irradiance is independent of stomatal openness (Tyree et al. 2005; Cochard et al. 2007). Recent physiological and molecular work suggests that the response of K_L to light intensity on a short time scale (hours, as in the "*Light experiment*" in **I**) arises from the extra-vascular compartment of leaves and is most likely associated with the regulation of the plasma membrane aquaporin abundance leading to changes in the water permeability of the transcellular water pathway (Cochard et al. 2007; Ben Baaziz et al. 2012a, 2012b). There is unequivocal evidence pointing to aquaporin-dependent pathways, for example, the application of $HgCl_2$, an aquaporin inhibitor, has been shown to reduce K_L and its light response (Nardini et al. 2005b; Voicu et al. 2008; Lopez et al. 2013). In shade leaves of silver birch K_L declined from 6.32 to 4.84 mmol m^{-2} s^{-1} MPa^{-1} and in sun leaves from 7.96 to 6.28 mmol m^{-2} s^{-1} MPa^{-1} when the shoots were fed a water solution of $HgCl_2$ (Table 3 in **I**). The stimulation of K_L by irradiance has been shown to parallel closely with the increase in aquaporin transcript abundance (Cochard et al. 2007; Ben Baaziz et al. 2012a, 2012b).

The K_L light response has a clear ecophysiological meaning – to enhance plant water transport capacity under conditions of great water demand, as high irradiance is accompanied by higher leaf temperature, evaporation rates and water losses. It is especially important point for forest trees, the leaves of which are exposed to spatially highly heterogenous and temporally dynamic radiation fields. Moreover, experimental data suggest that stem hydraulic conductance may also vary substantially with incident Q_P, supporting the concept of light-mediated modulation of stem hydraulic conductance (Nardini et al. 2010; Sellin et al. 2010b), the phenomenon that can substantially contribute to the control of water fluxes within trees to optimize leaf water supply in the changeable light environment.

The precise contribution of aquaporins to the regulation of leaf water transport is not well understood. Little is known about the mechanisms through which irradiance can control aquaporin expression, and the relationship between light-induced K_L changes and aquaporin transcript levels seems not to extend to all species tested to date. For example, an absence of a link between the light response of K_L and aquaporin expression has been reported for *Quercus macrocarpa* Michx. and *Populus tremula* L. (Voicu et al. 2009; Ben Baaziz et al. 2012b). However, since there are numerous leaf-expressed aquaporin genes, it is possible that aquaporins other than those tested in these two species contribute to the light effect of K_L. In addition, not only the expression, but also activity of aquaporins involved in regulating K_L may be controlled, while the expression and activity of aquaporins may be species specific (Voicu et al. 2009). Thus, further studies are needed to clarify the role of aquaporins in leaf water transport.

Although current research has shown that the light sensitivity of hydraulic conductance is a widespread characteristic, it appears to be highly species specific. K_L stimulation by light varies from absence of light response to several-fold increases (Ben Baaziz et al. 2012b). The reasons for such a large variation remain unclear. Most likely they arise from the relative contribution of the vascular versus extra-vascular leaf compartments to the distribution of liquid-phase resistance. Although the current consensus is that hydraulic resistances of the vascular (R_x) and extra-vascular compartments (R_{ex}) inside the leaf are of the same order of magnitude (Sack and Holbrook 2006), depending on the species or environmental factors, either one can prevail. Therefore, if R_x is of greater magnitude, changes in R_{ex} through aquaporin abundance would have a minor effect on the total K_L.

Light enhancement of K_L depends also on leaf water status. Guyot et al. (2012) showed that the light response of K_L for four studied species was strongest for fully hydrated leaves, but declined during leaf desiccation and became insignificant at turgor loss point. Therefore, the differences in K_L light sensitivity among species studied so far may be because the leaves in these experiments may have been at different levels of hydration.

Research suggests that the light response of K_L has an optimum under given conditions. At higher light intensities ($Q_P > 600$ µmol m^{-2} s^{-1}), K_L of silver birch started to decrease again (Fig. 2A in **I**). There is no explicit explanation for this. This response might result from very high water flux under experimental conditions (Fig. 2B in **I**), as experiments with cell pressure probes have suggested that a high flow rate in the aquaporin channel may cause a change in the channel structure and thereby its closure (Wan et al. 2004). The mean transpiration rates (E) recorded at 330 µmol m^{-2} s^{-1} exceeded 1.5–2.0 times the daily maximum values of E occurring within natural birch canopies (Sellin and Kupper 2005b). Another explanation is that high light intensities inhibit aquaporins. Kim and

Steudle (2009) found that the hydraulic conductivity of leaf cell membranes (L_p) was enhanced under low light conditions (Q_P < 200 µmol m^{-2} s^{-1}), but again reduced under high-light conditions (Q_P > 800 µmol m^{-2} s^{-1}). They propose that high light intensities inhibit aquaporins through oxidative gating, since the treatment of the plant tissue with oxidants (H_2O_2 and -OH) had a decreasing effect on L_p similarly to high light intensity, and the presence of antioxidants prevented the inhibiton by high light (Kim and Steudle 2009).

Besides aquaporin expression, there may be other mechanisms responsible for the light response of K_L. For instance, irradiance-driven changes in K_L may also result from the vascular compartment of leaves, i.e. leaf vein network, and mediated by modifications of xylem sap composition (Zwieniecki et al. 2004; Nardini et al. 2010; Sellin et al. 2010b).

Light quality

Besides intensity, light may exert its effect on plant hydraulic capacity also through its quality – spectral properties. The experimental results confirm the first hypothesis that light quality affects leaf hydraulic efficiency in *B. pendula*, while blue spectral band (emission maximum at 450–460 nm) has a greater enhancing effect on leaf hydraulic conductance than white or red light. Thus, K_L was highest in blue light, intermediate in white light and lowest in red light (Fig. 1; Fig. 1 in **II**).

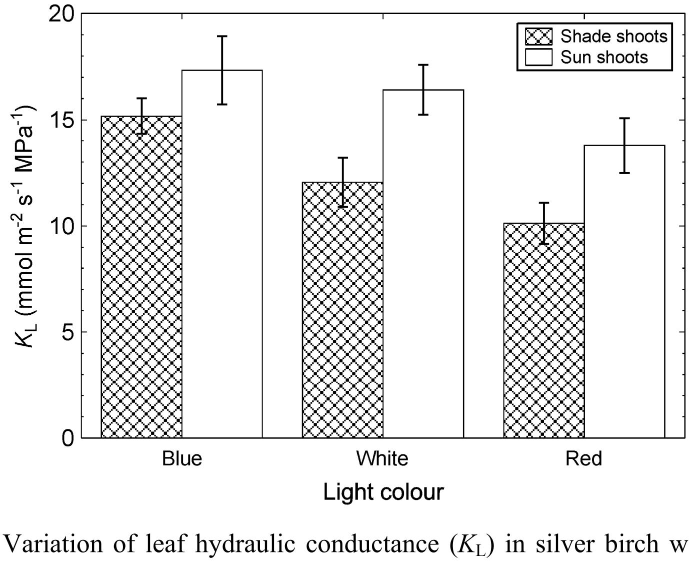

Figure 1. Variation of leaf hydraulic conductance (K_L) in silver birch with respect to light colour and canopy position; treatment effect P = 0.002. Bars indicate ±SE of the means.

Although there are very few works on the effects of light of different wavelengths on leaf hydraulics, our findings on silver birch (Paper **II**; Sellin et al. 2012) are in accordance with the data currently available for other species (Voicu et al. 2008; Ben Baaziz et al. 2012b; Savvides et al. 2012). Voicu et al. (2008) reported a greater hydraulic enhancement in leaves of *Quercus macrocarpa* Michx. under blue and green light than under longer visible wavelengths. The experiment performed by Baaziz et al. (2012b) also supports our findings: when leaves of *Juglans regia* L. were exposed to blue-free light using light filters, K_L was 65% lower than under control conditions, i.e. under white light. Furthermore, they reported that aquaporin transcript accumulation was significantly lower in the absence of blue light. These results suggest that blue light contributes largely to light-induced upregulation of K_L and that K_L depends on spectrum-sensitive light receptors that are involved in the regulation of AQP gene expression.

Spectral sensitivity of K_L in silver birch as an early-successional species suits well with its general life strategy. 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 the far-red band in the inner canopy and understorey (Smith 1982; Urban et al. 2007). Exposure of leaves to direct radiation (relatively rich in blue light) in sun flecks enable enhancement of K_L, thus allowing high stomatal conductance and carbon gain, and supporting higher growth rates. On the other hand, a lower K_L in plants/leaves growing under chronic shade (depleted by blue light) reduces energy expenditure associated with AQP expression. *B. pendula* 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 et al. 2001), thus reflecting a shade-avoidance strategy that would contribute to fitness in dense plant communities. Probably spectral responses of K_L join other plant processes responding to light quality as part of shade-avoidance mechanisms.

Light quality exerts a considerable influence on leaf hydraulics also on a long time scale, i.e. during leaf development. Savvides et al. (2012) found that K_L was much lower in leaves of cucumber (*Cucumis sativus* L. cv. Hoffmann giganta) developed without blue light and suggested that light quality during leaf development influences K_L through anatomical and morphological changes. It is still unclear whether the hydraulic effects are due to alterations in the vascular or extra-vascular pathway.

Light duration

Light duration *per se* had a small but significant ($P = 0.030$) effect on leaf hydraulic conductance in silver birch. K_L achieved maximum values after 9 h of illumination (Fig. 1A in **I**). This is most likely associated with an irradiance-mediated effect on extra-vascular tissues involving expression of cell membrane aquaporins (see section “Light intensity”). Thus, the results obtained from *B. pendula* under experimental conditions support the second hypothesis – leaf hydraulic conductance increases both with increasing light intensity and duration of exposure. However, data analysis revealed that the dynamics of K_L in "*Time experiment*" was also affected by changes in leaf temperature (T_L; °C), which increased consistently during the experiment (Fig. 1D in **I**). Temperature has been shown previously to have a significant effect on K_L (Sack and Holbrook 2006), while the increase in K_L with rising temperature is much greater than would be expected from changes in the water viscosity alone (Cochard et al. 2000; Matzner and Comstock 2001; Sack et al. 2004). In *Tilia cordata* growing in natural forest conditions, about a third of the temperature effect on K_L is attributable to changes in water viscosity, and two thirds to changes in cell membrane permeability (Sellin and Kupper 2007b).

3.2. Vertical variation within a tree crown and distribution of resistances

Mean leaf hydraulic conductance in both silver birch and hybrid aspen varied significantly along the canopy vertical profile. The upper-canopy foliage had 1.25–1.7 times higher K_L than the lower-canopy foliage (Figs. 1, 3 and 4; Figs. 1A and 2A in **I**; Fig. 1C in **III**; Fig. 5 in **IV**). Such a spatial pattern of K_L is induced primarily by a vertical gradient of irradiance occurring within naural forest canopies. Mean Q_P in the lower layers of natural silver birch canopies extend only to 140 µmol m^{-2} s^{-1} in the midday period, whereas in the upper layers of the canopy Q_P reaches close to 800 µmol m^{-2} s^{-1} (Table 2 in **III**). Thus, sun leaves located in the upper part of the canopy are acclimated to higher irradiance and atmospheric evaporative demand (AED) and are hydraulically less constrained than shade leaves located in the lower part of the canopy. Similar differences in K_L have been observed in many plant species growing in sun-exposed versus shade conditions (Sack et al. 2003b; Sellin and Kupper 2007b) and between sun- and shade-establishing species (Brodribb and Holbrook 2004a, 2004b; Nardini et al. 2005a; Sack et al. 2005; Lo Gullo et al. 2010).

The greater light availability in the upper canopy compared to the lower canopy has consequences for both leaf vascular and extravascular compartments. As discussed above, in the short term, light intensity has a substantial enhancing effect on K_L involving changes in the permeability of the symplastic pathway through expression of AQPs in the bundle sheath and mesophyll cells (see section "Light intensity"). In the long term, a high radiation load accompanied by large transpirational water loss influences the development of the leaf venous system. A study of 10 tropical tree species varying in their regeneration irradiance revealed that sun-establishing species, having a higher K_L compared to shade-establishing species, also possess higher vein density and veins with more numerous and larger-diameter xylem conduits, i.e. leaves of sun-establishing species possess a more efficient vascular system (Sack and Frole 2006). Vertical trends of leaf hydraulic properties with respect to leaf size and anatomical traits in *B. pendula* indicated that the upward increase in 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* (Sellin et al. 2012). Thus, the data obtained in silver birch do not support the idea advanced by Zwieniecki et al. (2002) that smaller leaves (as are sun leaves) have higher hydraulic conductance compared to bigger leaves (as are shade leaves) because shorter flow paths outside the vascular bundles within smaller laminae impose smaller hydraulic constraints. In birch, enhanced vascular development – bigger xylem cross-sectional area and number of vessels in leaf midrib, higher vein density – was observed with increasing leaf blade area. The greater K_L in sun leaves contributes to compensation for potentially greater hydraulic stress caused by the longer pathway from soil to the uppermost shoots (Yoshimura 2011); it also sustains greater stomatal conductance and photosynthesis per unit leaf area and thus higher relative growth rate compared with shade shoots (Sack et al. 2005; Sellin et al. 2010a). Since lower-canopy leaves experience less water loss due to lower AED and photosynthesis is constrained by low light availability rather than by water supply, shade leaves have a lower need for investment of carbon into producing a more effective venous system. At the same time, the associated vascular construction costs in sun leaves would be repaid in a relatively short period of time (Sack et al. 2005).

Although leaves represent a very small fraction of the total length of the liquid water transport path through the plant, their hydraulic properties set a disproportionate limit on plant water relations because the leaf represents a significant hydraulic bottleneck in plants. In 20 m tall trees of silver birch, ~ 40% of the total hydraulic resistance of the tree resides in leaves (R_L) in the mid-day period (Fig. 3 in **III**). In birch saplings of 4 m height, R_L is of the comparable magnitude – 32–44% (Sellin et al. 2014). These results coincide with that reported for little-leaf linden (*Tilia cordata* Mill.), a shade-tolerant temperate tree species (Sellin and Kupper 2007a), and falls into the range (30–98%) published for a variety of plant life forms (Sack and Holbrook 2006). The distribution of hydraulic resistance among segments of the water transport pathway can vary significantly intraspecifically with time of day, water supply, irradiance, temperature and plant age, as does the hydraulic conductance, and thus can be the dominant factor in determining whole-plant water transport efficiency (Sack and Holbrook 2006; Sellin et al. 2014). Due to substantially higher soil-to-branch hydraulic conductance for the upper canopy (Fig. 2 in **III**), the contribution of leaves to the total liquid-phase resistance is mostly greater for the sun foliage as observed in *B. pendula* (Fig. 3 in **III**) and *T. cordata* (Sellin and Kupper 2007a).

Leaves contribute 53–95% to the whole-shoot hydraulic resistance (Fig. 3 in **I**; Fig. 2 in **II**; Fig. 6 in **IV**) in silver birch, but substantial shifts occur in the distribution of resistance within branches in response to variation in leaf water status, air relative humidity, light quality and canopy position. On a short time scale, the relative contribution of leaves to the whole-shoot hydraulic resistance increases with falling leaf water potential (Fig. 2). On the other hand, leaf development under increased RH results in increased R_L (i.e. long-term effect; Fig. 6 in **IV**). This could be a critical point under more frequently occurring climate extremes (severe drought periods, heat waves) in the future, since the leaf water supply under such conditions may become limited by stomatal openness and might represent a potential threat to the functional integrity of the water transport system itself. In response to changes in light spectrum, R_L increased from 57% under blue light to 67% under red light (Fig. 2 in **II**), reflecting an acclimation capacity of foliage/canopy to spectral variability within the forest stands. The shift in the distribution of the resistance between the branch and leaves probably has implications for the adjustment of the hydraulic system in response to other environmental variables, for instance soil water availability and atmospheric evaporative demand.

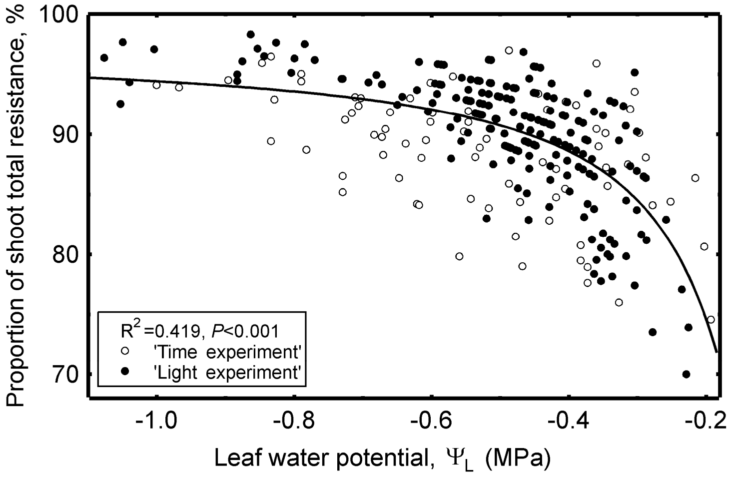

Figure 2. Leaves' share of shoot total resistance (R_L) in silver birch depending on leaf water status for the datasets obtained from the '*Time*' and '*Light experiments*' (paper **I**).

3.3. Diurnal variation

Previous research (Nardini et al. 2005b; Sack and Holbrook 2006) has shown that K_L is diurnally variable and its temporal pattern involves an endogenous circadian rhythm as well as simultaneous responses to multiple environmental factors (light, temperature, water availability). In this study, we measured daily dynamics of K_L in *Betula pendula* by applying two different methods in forest conditions *in situ* and the results confirmed that K_L varies significantly with time of day. In its daily dynamics recorded from 0700 to 1700 h, K_L based on evaporative flux determined by the sap flow method showed a gradual rise throughout the day (Fig. 1c in **III**), achieving maximum values by 1700 h (4.75

and 3.38 mmol m^{-2} s^{-1} MPa^{-1} for the upper and lower canopy, respectively). The porometric method used to estimate the evaporative flux gave somewhat different results (Fig. 3): K_L started to increase in the morning and the maximum in lower-canopy leaves was reached at 1700 h (4.05 mmol m^{-2} s^{-1} MPa^{-1}), although it was not statistically different from the values at 1100–1500 h. In upper-canopy leaves K_L attained maximum values at 1300 h (4.66 mmol m^{-2} s^{-1} MPa^{-1}). Thus, the results revealed that K_L is lowest in the morning and rises gradually until early evening or reaches a flat peak already around noon.

K_L calculated from the porometric data was slightly greater than that obtained by the sap flow method. This is due to the fact that the porometric method tends to overestimate transpiration, since it ignores canopy aerodynamic resistance to water vapour diffusion (Sack et al. 2005; Sellin and Lubenets 2010). Therefore, the differences between the two estimates proved to be greater (37 versus 22%) for the lower-canopy data, as these leaves are located deep within the forest canopy. The substantial rise in K_L by 1700 h measured by sap flow method can be explained by disproportionate changes in F_{LA} and $\Delta\Psi$ – the water potential drop decreased more rapidly than the sap flux density in the afternoon. This might be caused by lags between the processes (stomatal responses versus sap flow) in relation to the tissue capacitance effect.

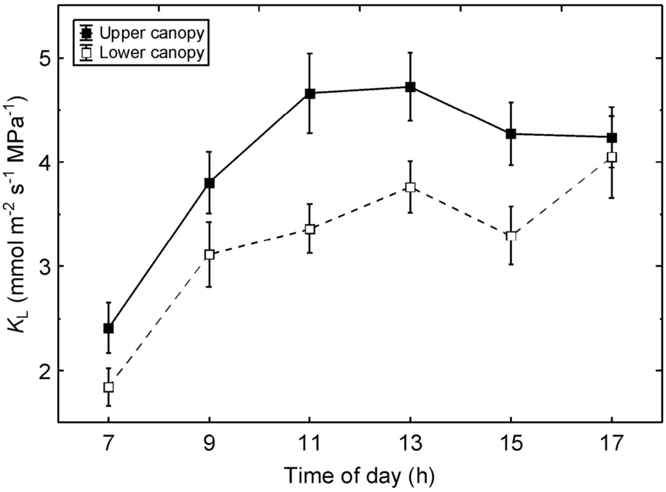

Figure 3. Daily patterns of leaf hydraulic conductance (K_L) in the upper and lower canopy of silver birch trees sampled in natural forest stand *in situ*. Evaporative flux density was determined by porometric method. Bars indicate ±SE of the means.

Various trends have been reported for the diurnal pattern of K_L. Most often a decline around midday with a recovery of K_L in the afternoon has been observed (Brodribb and Holbrook 2004a; Johnson et al. 2009b, 2011). Diurnal depression of hydraulic conductance has been reported also for petioles (Bucci et al. 2003; Zufferey et al. 2011). However, daily trajectories, where K_L achieves maximum at noon and decreases thereafter or where K_L is maximal in the morning and lowest in the evening, have been reported (Lo Gullo et al. 2005; Voicu and Zwiazek 2011; Yang et al. 2012). Consistent with the results in this study, K_L has also been shown to rise gradually throughout the day, reaching maximum values in the evening (Johnson et al. 2011). Such a vast variation of daily patterns must be taken into account when K_L is compared among different species, because their values of K_L may differ significantly or coincide depending on the time the measurements were taken.

Diurnal down-regulation of leaf hydraulic conductance is of great adaptive significance for tree daily water use under conditions of water deficit. Midday depression of K_L has been suggested to be the direct result of cavitation in leaf veins (Johnson et al. 2009b) and it could be a hydraulic signal involved in normal daily stomatal regulation to amplify the signal of evaporative demand to the stomata in order to trigger a stomatal response preventing further water loss and embolism in stems (Brodribb and Holbrook 2004a; Yang et al. 2012). Our recent experiment in *B. pendula* revealed that leaf gas exchange parameters, including stomatal conductance, showed a substantially stronger association with Ψ_B than with Ψ_L (Sellin et al. 2014). Thus, the gas exchange and stomatal conductance of silver birch are determined by direct water availability to the leaf, estimated by Ψ_B in the petiole insertion point, rather than by the current leaf water status (Ψ_L) itself. Meinzer et al. (2009) proposed that stomatal openness is regulated in a way to prevent primarily dysfunction of stem xylem. This idea has recently been confirmed by experimental data obtained in silver birch (Sellin et al. 2014) and in a number of subtropical tree species (Zhang et al. 2013).

Leaves have been shown consistently to be hydraulically more vulnerable than stems (Johnson et al. 2011; Bucci et al. 2012) and they constitute a substantial part of the whole-plant hydraulic resistance (see section 3.2.). Under prolonged drought, such hydraulic segmentation between the foliage and stem enables protection of the more costly and less replaceable woody portion of the hydraulic pathway at the expence of the more easily disposable and less expensive leaves. Thus, leaf cavitation performs as a “hydraulic fuse”, isolating the upstream xylem, limiting leaf transpiration and preventing runaway embolism and possible stem dieback, thereby protecting the integrity of other organs (shoots and roots) during water stress (Johnson et al. 2011; Zufferey et al. 2011).

The birch trees growing in a natural mixed forest stand and sampled for daily dynamics of K_L (paper **III**) did not experience significant water stress. The daily trends of K_L in this study reflect response to changes in atmospheric factors during the course of the day. In the morning K_L starts to increase along with rising irradiance and temperature and with decreasing air relative humidity, representing growing atmospheric evaporative demand (AED) imposed on the plant. There is mounting evidence that K_L (symplastic component) is upregulated with increasing temperature (Matzner and Comstock 2001; Sack et al. 2004; Sellin and Kupper 2007b) and light intensity and duration (see section 3.1.). Environmental factors significantly affecting K_L of silver birch in natural forest conditions were Q_P, RH and T_A (Table 3 in **III**). Thus, the results obtained from intact foliage measured *in situ* (Fig. 1c in **III**) in general confirm the trends observed in lab conditions with respect to increasing irradiance and exposition time, providing additional support to the second hypothesis. Although irradiance has proved to be the primary environmental variable controlling K_L, in our case RH and T_A had stronger effect on K_L. This can be explained by the fact that this study was conducted under ambient conditions in the natural forest environment and the impact of light in such conditions might be masked by several other environmental factors varying as well. Air temperature and relative humidity are stable environmental factors compared to the temporally and spatially highly heterogeneous light field existing in forest canopies because of permanently changing sunflake pattern (due to wind and daily sun course) and cloudiness.

Midday depression of K_L did not occur in silver birch, since there were sufficient soil water reserves and AED was not severe enough to induce substantial cavitation in xylem (exceeding light-induced enhancing effect on hydraulic conductance). Guyot et al. (2012) demonstrated clearly simultaneous counter effects of high irradiance (impact on extravascular compartment) and water deficit (impact primarily on vascular compartment) on K_L. The absence of significant cavitation effects in our case was confirmed by total independence of K_L on leaf water potential. If plants experience water deficit while maintaining a high K_L throughout the day, this might have negative consequences to the integrity of their water conducting systems. Therefore, it is important to know how trees would adjust their K_L in response to water stress and what its impact could be on plant water status. Internal cues also play a role in the diurnal trend of K_L. Nardini et al. (2005b) demonstrated that when plants were subjected to continuous darkness, K_L continued to oscillate between light and dark values synchronously with the preceding photoperiod, indicating that changes in K_L are also controlled by a circadian clock.

3.4. Impact of elevated air relative humidity

The air humidity manipulation induced a substantial change in leaf hydraulic efficiency of silver birch (Fig. 4): mean K_L across all canopy positions decreased from 19.6 ± 1.6 in control plots (**C** treatment) to 15.9 ± 1.1 mmol m^{-2} s^{-1} MPa^{-1} in humidified plots (**H** treatment). Data analysis revealed a significant effect of both treatment (P = 0.030) and branch position (P = 0.007) on K_L. There was even greater change in hydraulic conductance of leaf blades (K_{lb}) under humidification (Fig. 5 in **IV**): mean K_{lb} decreased from 23.1 ± 2.0 in **C** plots to 18.5 ± 1.3 mmol m^{-2} s^{-1} MPa^{-1} in **H** plots (P = 0.023). Thus, the results support the third hypothesis that leaf hydraulic conductance decreases as a result of declined transpirational flux due to increased atmospheric relative humidity.

However, different segments of the soil-to-leaf water transport pathway in *B. pendula* responded differently to artificially elevated atmospheric humidity. The treatment had no effect on the hydraulic conductance of other parts of the shoots – K_S, K_B and K_P (Table 5 in **IV**), while hydraulic conductance of the root system increased in response to the air humidity manipulation (Fig. 8 in **IV**).

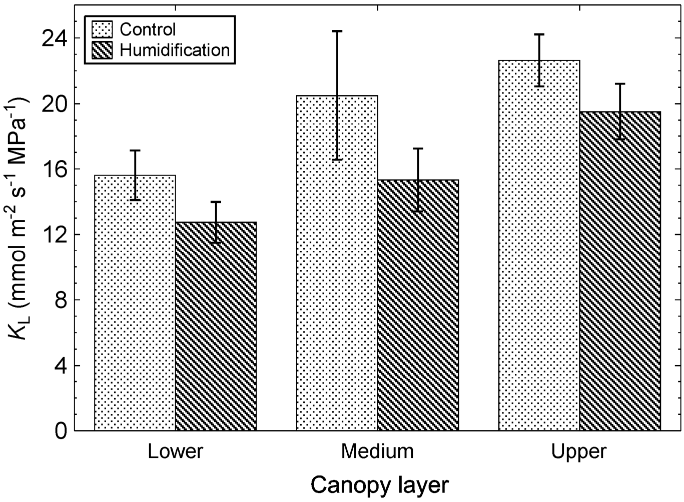

Figure 4. Variation of leaf hydraulic conductance (K_L) in silver birch depending on air humidity manipulation and branch height above the ground. Treatment effect $P = 0.030$. Bars indicate ±SE of the means.

The decrease in K_L under the humidification treatment resulted from both physiological and morphological processes. The contribution of separate leaf compartments to the K_L decline was as follows (Sellin and Alber 2013): petiole 14%, lamina vascular network 66%, extravascular compartment 20%. The decline in K_L is attributable primarily to changes in lamina vasculature, supported by anatomical measurements: mean vessel diameter (11.8→10.7 µm; P=0.025), hydraulic diameter (16.3→14.5 µm; P=0.018) and xylem theoretical conductivity (1.53→1.11 kg m^{-1} s^{-1} MPa^{-1}; P=0.006) in midvein and vein frequency (9.87→9.40 mm mm^{-2}; P=0.064) decreased under the humidification treatment. Under higher RH the need for development of efficient water transport system is diminished because the AED is lower and the transpirational flux through plants is decreased. The average increase in RH at fhe FAHM experimental site was 7% over the whole experimental period, resulting in a 24.8% decrease in stem sap flux density per unit leaf area (F_{LA}) in silver birch (Kupper et al. 2011).

On the other hand, the humidity-induced changes in leaf hydraulic efficiency may depend also on aquaporin expression, which influences the conductance of the leaf extra-vascular compartment. AQP expression has been shown to be up-regulated by low air humidity in both roots and leaves (Kuwagata et al. 2012;

Laur and Hacke 2013) and this transpiration-triggered up-regulation occurs not only after a short-term exposure of plants to low humidity, but also in plants developed in dry air. Furthermore, root AQP mRNA expression has been reported to be substantially reduced by overhumidification of plant aerial parts (Sakurai-Ishikawa et al. 2011).

To compensate for large water losses from foliage while keeping stomata open in a dry environment, trees develop a highly efficient water transport system that is costly in terms of recource usage; more recources are allocated into developing the leaf venous system and more energy is spent on AQP synthesis. Trees growing in more humid conditions experience smaller water loss and, thus, do not need such an effective system for water supply. They are able to hold the balance between water uptake and loss having a lower leaf hydraulic conductance. Both the development of less efficient leaf vascular system and the down-regulation on leaf AQP genes under high RH serve this purpose. However, the decreased K_L in combination with weak stomatal control over water losses (Fanourakis et al. 2010, 2011) could be a crucial point if abrupt weather fluctuations (severe drought, disastrous heat wave) become more frequent in the future, although on average the humidity will increase at high latitudes. Trees grown under high atmospheric humidity might be more susceptible to cavitation-induced embolism during high AED under climatic extremes. At the same time, weakened stomatal control amplifies the risk of desiccation-induced hydraulic dysfunction and dehydration of leaves.

Recent data obtained in silver birch support this apprehension, revealing disproportionate changes in sensitivity of stomatal versus leaf hydraulic conductance to water deficit in trees grown under elevated atmospheric humidity (Sellin et al. 2014). K_L declined by a factor of 2.3 ($P<0.01$) and leaf gaseous phase conductance by a factor of 1.4 ($P<0.05$) faster in humidity-treated trees compared to the control with decreasing Ψ_B. Despite the mitigating impact of increased air humidity under moderate drought (A. Niglas, unpubl. data), a future rise in atmospheric humidity at high latitudes may be disadvantageous to trees during weather extremes and represents a potential threat in hemiboreal forest ecosystems in the future. Findings by Nardini et al. (2012b, 2014) highlight the role of regulation of K_L in plant acclimation to climate change, suggesting that leaf resistance to drought-induced hydraulic dysfunction is a key to plant survival and competition even over limited geographical ranges.

3.5. Impact of phloem girdling

Phloem girdling had a significant effect on leaf hydraulic conductance: mean K_L decreased from 6.64 to 3.78 mmol m^{-2} s^{-1} MPa^{-1} (by 43%) during the three days following the phloem severing in saplings of hybrid aspen (Fig. 5). Domec and Pruyn (2008) also reported decreased K_L after stem girdling for ponderosa pine (*Pinus ponderosa* Dougl. ex P. Laws.). Zwieniecki *et al.* (2004) reported a decline in branch hydraulic conductance for two *Acer* species after phloem girdling at the branch base. They proposed that since phloem girdling disrupts the ion exchange between phloem and xylem, it will lead to a decrease of ion concentration in xylem sap. Decreases in the ionic concentration of the xylem sap are thought to reduce xylem conductance because of the swelling of hydrogels in bordered pit membranes (Zwieniecki et al. 2001; Nardini et al. 2010; Sellin et al. 2010b). Our results do not support the fourth hypothesis that the decrease in K_L due to phloem severing is a result of the disturbed ion recirculation between the xylem and the phloem. Phloem severance at the branch base had no impact on the measured physico-chemical properties of xylem sap (Table 1) during the following three days and, therefore, the variation in K_L was unrelated to [K^+], σ_{sap} or pH of xylem sap. Consequently, girdling at the branch base did not affect ion recycling from phloem to xylem in the aspen trees.

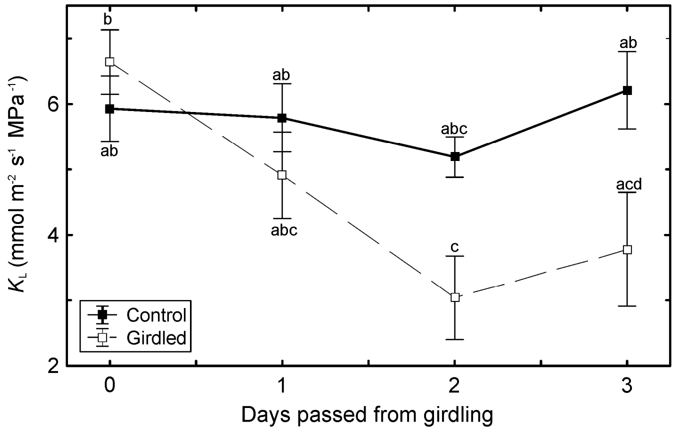

Figure 5. Changes in leaf hydraulic conductance (K_L) of hybrid aspen in response to phloem girdling. Day 0 – measured just before girdling. Error bars represent ±SE, different letters denote statistically significant ($P<0.05$) differences between means.

There were no differences in branch water potential (Table 1) between the control and girdled branches of aspen trees, indicating that the girdling manipulation did not produce higher levels of cavitation and embolism, and thus it cannot be the cause of lower K_L. In fact, phloem girdling resulted in more favourable leaf water status of the girdled branches compared with the control (Fig. 4 in **V**). The girdled branches had less negative Ψ_L than the control branches at all measurement times after phloem girdling. On the third day after treatment, girdled branches had 0.52 MPa higher Ψ_L than control branches (Table 1). Thus, phloem girdling had a significant impact on leaf water status of aspen trees. Higher Ψ_L in girdled branches was the result of significantly lower transpirational water loss. Phloem girdling induced a considerable decrease in transpiration rate (E), declining from 4.66 to 0.61 mmol m^{-2} s^{-1} (by 87%) over three days (Fig. 5A in **V**). Higher leaf water potential due to decreased transpiration resulting from phloem girdling has also been shown for grape vine (*Vitis vinifera* L.; Williams et al. 2000) and ponderosa pine (*Pinus ponderosa* Dougl. ex P. Laws.; Domec and Pruyn 2008). The decrease in E after phloem girdling is the result of significantly lower stomatal conductance to water vapour (g_S; mmol m^{-2} s^{-1}) of girdled branches (Fig. 1A in **V**). Phloem girdling represents an experimental manipulation of source-sink relationships leading to an accumulation of assimilates in leaves and stems above the girdle and a consequent decrease in photosynthetic rate through end-product inhibition (Myers et al. 1999; Murakami et al. 2008). The decreased photosynthetic rate in turn leads to stomatal narrowing.

Table 1. Mean (±SE) values of the physico-chemical parameters of xylem sap and hydraulic parameters of aspen branches before and three days after phloem girdling. Ψ_L – leaf water potential; Ψ_B – branch water potential; K_L – leaf hydraulic conductance scaled by leaf area; [K^+] – potassium ion concentration; σ_{sap} – electrical conductivity; pH – acidity.

Variable	Mean		*P* value
	Control	Girdled	
Ψ_L (MPa)	–1.19	–0.67	0.002
Ψ_B (MPa)	–0.47	–0.45	0.900
K_L (mmol m^{-2} s^{-1} MPa^{-1})	6.64	3.78	0.006
[K^+] (mM)	0.44	0.65	0.180
σ_{sap} (mS cm^{-1})	0.59	0.64	0.557
pH	6.3	6.5	0.185

The response of leaf hydraulic conductance to phloem girdling may result from changes in transpiration rate (E) and/or driving forces ($\Delta\Psi$). The dynamics of K_L in the girdled aspen branches was primarily determined by decreasing E, as the mean values varied by a factor of 7.6 versus 3.4 for E and $\Delta\Psi$, respectively (Figs. 5A and 5B in **V**). Moreover, changes in leaf symplastic compartment cannot be ruled out, as changes in transpirational flux induce coordinated up/down-regulation of many aquaporin genes in leaves (Levin et al. 2007; Kuwagata et al. 2012). The mechanism by which girdling affects leaf hydraulic conductance still remains unclear and needs further investigation.

Although the phloem girdling experiment performed in hybrid aspen did not confirm the idea that the K_L decline is associated with disturbance of cation exchange between phloem and xylem, the results do not refute the probable role of living tissues (phloem, parenchyma) in regulation of xylem transport. Application of $HgCl_2$, a metabolic inhibitor, decreased substantially hydraulic conductance of both leaves (by 21–23%) and branches (by 26–68%) in silver birch (Table 3 in **I**), implying that shoot living tissues are very likely involved in these responses. The decrease in K_L is attributable primarily to the inhibition of aquaporin-based transport channels in bundle sheath and mesophyll cell membranes by mercuric ions (Nardini et al. 2005b; Cochard et al. 2007). The drastic impact of Hg^{2+} in branches can be accounted for by two different mechanisms. First, the decline in K_B might result from changes in polysaccharides of the intervessel pit membranes, because of a direct cation-mediated effect on hydrogel behaviour of pit membranes (Zwieniecki et al. 2001; Nardini et al. 2007). Second, inhibition of phloem metabolism could also decrease lateral ion transport into the xylem (Zwieniecki et al. 2004), i.e. due to disturbance of the functional link between phloem and xylem hydraulic systems.

4. CONCLUSIONS

1. Leaf hydraulic conductance is significantly affected by different aspects of light as electromagnetic radiation – by its intensity, spectral properties and duration of exposure. Light intensity influences both leaf vascular and extra-vascular compartments through the development of the vein network and expression/activation of aquaporins. High light intensity promotes the development of a hydraulically efficient leaf venous system and triggers aquaporin expression, thus lowering the resistance to water movement through leaf vascular and extravascular tissues. Light quality exerts its influence through spectrum-sensitive light receptors that are involved in the regulation of AQP gene expression. The impact of light duration on K_L is realized mainly indirectly through the cumulative expression of AQPs, but also via the direct temperature effect on water viscosity and cell membrane properties.

2. Leaf hydraulic conductance varies along the canopy vertical profile. Sun leaves exhibit higher hydraulic efficiency than shade leaves, contributing in this way to sufficient water supply to the sun foliage, photosynthetically the most important part of the canopy. The higher K_L in sun leaves compensates for potentially greater hydraulic stress caused by the longer pathway from soil to the uppermost shoots and sustains greater transpiration rate and carbon gain compared with shade leaves. Under long-term exposure to higher atmospheric evaporative demand, sun leaves develop a more efficient vascular system. In the short term, higher light intensity perceived by the sun leaves has a substantial enhancing effect on K_L involving changes in the permeability of the symplastic pathway through expression of AQPs in the bundle sheath and mesophyll cells.

3. Leaves, constituting a major part of the total liquid-phase resistance in trees, represent a significant hydraulic bottleneck for plants, because small changes in leaf hydraulic properties may have a substantial impact on whole-plant water relations. Leaves are also hydraulically more constrained and functionally more vulnerable than branches. Such hydraulic segmentation between foliage and branches is of adaptive significance because most peripheral organs would be damaged by cavitation, whereas stem and branches (i.e. central segments of the water conducting system), which are costly in terms of carbon investment and critical for integrity of the hydraulic system, would be conserved.

4. Leaf hydraulic conductance is diurnally variable. Without experiencing significant water stress, K_L of naturally growing birch trees is lowest in the morning and rises gradually until evening or reaches a flat peak already at noon. Atmospheric factors driving K_L daily dynamics are photosynthetic photon flux density, relative air humidity and air temperature. Adjusting their leaf hydraulic capacity sensitively to atmospheric conditions changing during the day enables plants to maximize their carbon gain under given soil water availability. In

natural forest stands, the daily dynamics of K_L depends more on air temperature and relative humidity than on irradiance.

5. Under increasing atmospheric humidity, the climate trend predicted for northern Europe on a longer time scale, leaf hydraulic conductance decreases as a result of declined transpirational flux. The decrease in K_L is attributable to alterations in both leaf vascular and extravascular compartments. This might have considerable implications for drought tolerance of trees under extreme weather conditions, making plants more susceptible to desiccation-induced hydraulic dysfunction and representing a potential threat in hemiboreal forest ecosystems in the future.

6. Disruption of phloem transport by means of phloem girdling causes a decrease in leaf hydraulic conductance. The girdling at branch base does not disturb lateral ion transfer to xylem in hybrid aspen, and the decrease in leaf hydraulic conductance in response to the manipulation is not caused by changes in potassium ion or total cationic concentrations of the xylem sap. The mechanism by which girdling affects leaf hydraulic conductance remains unclear and needs further investigation.

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SUMMARY IN ESTONIAN

Lehe hüdraulilise juhtivuse ajalis-ruumiline varieerumine puit-taimedel: ökofüsioloogilised tagajärjed

Vesi etendab taimede elus ülimalt olulist rolli, olles biokeemiliste ja molekulaarsete protsesside toimumiskeskkonnaks. Kuna taimed kaotavad evaporatsiooni tõttu pidevalt vett, vajavad nad konstantset veevarustust mullakeskkonnast. Maismaataimede jaoks, kes kasvavad ajas ja ruumis pidevalt muutuvas keskkonnas, on võime efektiivselt reguleerida oma veekasutust ning säilitada positiivne veebilanss esmatähtsaks ellujäämise eelduseks.

Lehed kujutavad endast puittaimede jaoks hüdraulika seisukohalt olulist „pudelikaela”, moodustades üle 30% kogu taime hüdraulilisest takistusest. Seetõttu on lehe hüdrauliline juhtivus (K_L) oluline füsioloogiline parameeter, mis väljendab taimesisest veetranspordi efektiivsust. Kuna taime hüdrauliline juhtivus on positiivselt seotud õhulõhede juhtivuse ja fotosünteesivõimega, mõjutab K_L olulisel määral taimede produktiivsust, kasvukiirust ning elumust.

Puude suurtest mõõtmetest tulenevalt iseloomustab puistus võrastiku sisemust keskkonnatingimuste tohutu ruumiline varieeruvus. Paljudest keskkonna parameetritest varieerub enim valguse kättesaadavus ja seda eeskätt vertikaalses plaanis. Seetõttu on lehed, olles võra erinevates kihtides eksponeeritud erinevatele keskkonnatingimustele, kohanenud nii füsioloogiliselt kui morfoloogiliselt konkreetsete mikrokeskkonna tingimustega. Käesoleva töö peamiseks eesmärgiks oli uurida lehe hüdraulilise juhtivuse varieeruvust puuvõras nii ruumilises kui ka ajalises skaalas sõltuvalt keskkonnafaktoritest. Selleks viidi eksperimentaalne töö läbi kahel puuliigil: arukask (*Betula pendula* Roth.) ning hübriidhaab (*Populus tremula* L. × *P. tremuloides* Michx.). Proovialadeks olid looduslikult uuenenud segamets ja eksperimentaalne metsaökosüsteemi katseala FAHM (*Free Air Humidity Manipulation*), mis loodi suureneva atmosfääri suhtelise õhuniiskuse mõju uurimiseks metsaökosüsteemidele.

Töö kitsamad eesmärgid olid:

- Hinnata valguse erinevate tahkude – intensiivsuse, spektraalse koostise ja kestuse – mõju lehe hüdraulilisele juhtivusele.
- Teha kindlaks lehe hüdraulilise juhtivuse varieerumine võrastiku vertikaalprofiilil ning hüdraulilise takistuse jaotumine puu erinevate osade vahel.
- Teha kindlaks lehe hüdraulilise juhtivuse päevane dünaamika looduslikes tingimustes ja tuvastada seda kontrollivad keskkonnafaktorid.
- Hinnata suurenenud atmosfääri suhtelise õhuniiskuse mõju lehe hüdraulilisele juhtivusele.
- Kontrollida floeemi sälkamise oodatavat mõju lehe hüdraulilisele juhtivusele.

Töös kontrolliti järgmisi hüpoteese:

1. Lehe hüdraulilise juhtivuse reaktsioon valgusele sõltub selle spektraalsest koostisest: vahendatuna spektritundlikest valgusretseptoritest suureneb K_{L} sinises valguses rohkem kui valges või punases valguses.
2. Lehe hüdrauliline juhtivus suureneb koos valguse intensiivsuse tõusu ning valguse ekspositsiooni aja pikenemisega, mis suurel määral determineerivad lehe hüdraulilise juhtivuse ööpäevase dünaamika.
3. Suureneva suhtelise õhuniiskuse tingimustes lehe hüdrauliline juhtivus väheneb tulenevalt aeglustunud transpiratsioonivoost läbi taimede.
4. Floeemitranspordi katkestamine floeemi sälkamise teel vähendab lehe hüdraulilist juhtivust kaaliumioonide retsirkulatiooni häirumise tõttu floeemi ja ksüleemi vahel.

Doktoritöö tulemused näitasid, et lehe hüdrauliline juhtivus sõltub oluliselt nii valguse intensiivsusest, kvaliteedist kui ka kestusest, kinnitades töös püstitatud esimest ja teist hüpoteesi. Valguse intensiivsus mõjutab nii lehe vaskulaarset (ksüleem leheroodudes ja -rootsus) kui ka ekstravaskulaarset (kimbuümbrise ning mesofülli rakud) komponenti läbi juhtkudede arenemise ja hüdrofiilsete transportvalkude – akvaporiinide (AQP) – ekspressiooni rakumembraanidel. Kõrgem valguse intensiivsus soosib efektiivse veetranspordisüsteemi arenemist ning aktiveerib akvaporiinide ekspressiooni, alandades sellega lehe mõlema kompartmendi takistust vee liikumisele. Valguse kvaliteet avaldab oma mõju läbi spektritundlike valgusretseptorite, mis osalevad akvaporiinide geeniekspressiooni regulatsioonis. Valguse kestuse mõju lehe hüdraulilisele juhtivusele realiseerub kaudselt läbi AQP kumuleeruva ekspressiooni või otseselt temperatuuri mõjuna vee viskoossusele ning rakumembraani omadustele.

Vertikaalsetest keskkonnagradientidest tulenevalt varieerub lehe hüdrauliline juhtivus sõltuvalt asukohast võras. Võra ülemises osas asuvad lehed (valguslehed) on veetranspordi seisukohalt oluliselt efektiivsemad kui võra alumises osas asuvad lehed (varjulehed). Olles pikaajaliselt eksponeeritud kõrgemale atmosfääri evaporatiivsele nõudlusele, areneb valguslehtedes efektiivsem juhtkimpude võrgustik. Lühemas ajaskaalas vaadatuna suurendab tugevam valguse intensiivsus võra ülemises kihis lehe hüdraulilist juhtivust läbi rakumembraanide läbilaskvuse muutuste akvaporiinide ekspressiooni teel. Kõrgem lehe hüdrauliline juhtivus kompenseerib tugevamat survet valguslehtede veevarustusele, mis on tingitud pikemast veetranspordi teekonnast mullast ülemiste oksteni ja suurematest veekadudest hästi eksponeeritud lehtedest, ning võimaldab kõrgemat transpiratsiooni intensiivsust ja süsinikusaagist võrreldes varjulehtedega.

Lehed, hõlmates suure osa (>30%) kogu-puu hüdraulilisest takistusest, kujutavad endast veetranspordi seisukohalt taime jaoks olulist ”pudelikaela”, mistõttu juba väikesed muutused lehe hüdraulilistes omadustes võivad avaldada märkimisväärset mõju kogu puu veevahetusele. Lehed on suurema hüdraulilise takistusega ning veestressile tundlikumad kui oksad. Selline hüdrauliline segmentatsioon lehestiku ja okste vahel on teatud adaptatiivse tähtsusega taime jaoks. Näiteks põua tingimustes kahjustuksid kavitatsiooni tõttu eeskätt lehed kui kõige perifeersemad ning lihtsamini asendatavad organid, samas kui veetranspordisüsteemi kesksed osad – tüvi ja oksad, mis on süsiniku investeerimise mõttes kulukad ning aeglaselt asendatavad, oleksid kaitstud.

Lehe hüdraulilist juhtivust iseloomustab ööpäevane dünaamika. Kui puud parasjagu olulise veestressi all ei kannata, siis looduslikes puistutes kasvavatel arukaskedel on lehe hüdrauliline juhtivus madalaim hommikul ning tõuseb järkjärgult keskkonnafaktorite mõjul päeva jooksul kuni õhtuni või saavutab platoo juba peale keskpäeva. Atmosfäärifaktorid, mis lehe hüdraulilist juhtivust looduslikes tingimustes oluliselt mõjutavad, on valguse intensiivsus, suhteline õhuniiskus ning õhu temperatuur, kusjuures temperatuuri ja õhuniiskuse mõju ületab valguse oma. Hüdraulilise efektiivsuse kiire reguleerimine vastavalt päeva jooksul muutuvatele keskkonnatingimustele võimaldab taimedel maksimeerida süsiniku omastamist antud mullavee kättesaadavuse tingimustes.

Töös püstitatud kolmas hüpotees leidis samuti kinnitust – suureneva suhtelise õhuniiskuse tingimustes (s.o. kliimatrend, mida ennustatakse pikemas ajaskaalas Põhja-Euroopale) lehe hüdrauliline juhtivus väheneb tulenevalt vähenenud transpiratsioonivoost läbi taimede. Sellel võivad olla olulised tagajärjed puude põuataluvusele ekstreemsete ilmastikuolude (karmimad põuaperioodid, kuumalained) tingimustes, suurendades kavitatsioonist põhjustatud ksüleemi düsfunktsiooni riski ja kujutades seega potentsiaalset ohtu hemiboreaalsetes metsaökosüsteemides.

Doktoritöö tulemused kinnitasid neljandat hüpoteesi vaid osaliselt – floeemitranspordi katkestamine floeemi sälkamise teel küll vähendab lehe hüdraulilist juhtivust, aga vastupidiselt oletustele ei mõjuta hübriidhaaval floeemi sälkamine oksa alusel lateraalset ioonide vahetust floeemi ja ksüleemi vahel. Seega ei saa väita, et lehe hüdraulilise juhtivuse vähenemine floeemi sälkmise tõttu oleks tingitud kaaliumioonide kontsentratsiooni või summaarse ioonide sisalduse muutustest ksüleemimahlas. Mehhanism, mille kaudu sälkamine lehe hüdraulilist juhtivust mõjutab, jääb seni selgusetuks ning vajab edasisi uuringuid.

Puude kui suuremõõtmeliste ja pikaealiste taimede globaalsete kliimamuutustega kohanemise potentsiaali mõistmiseks on oluline teada, kuidas muutuvates keskkonnatingimustes ning puistus esineva mikrokeskkonna heterogeensuse tingimustes taimed optimeerivad oma veekasutust ning saavutavad tasakaalu gaasilise ja vedela faasi juhtivuste vahel. Puude veetranspordisüsteemi ehitus ja omadused mõjutavad oluliselt puude seisundit ning seeläbi metsa produktiivsust pikemas ajaskaalas. Teadmised sellest, kuidas puude veevahetus toimub ning millised on selle regulatsioonimehhanismid, annavad meile võimaluse prognoosida taimede hüdraulilise arhitektuuri tõenäolisi muutusi tulevikus ning globaalsete kliimamuutuste valguses välja töötada metsanduslikke meetmeid, et säilitada metsade produktiivsust ning leevendada kliimamuutuste võimalikke ebasoodsaid tagajärgi metsale.

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Õunapuu, E, Sellin, A. Effect of light duration, intensity and quality on leaf hydraulic conductance in silver birch. Poster. Conference “Next generation insights into geosciences and ecology”, Tartu, Estonia, 12–13 May, 2011.

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