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SHOOT HYDRAULIC CONDUCTANCE AND STOMATAL CONDUCTANCE OF SIX TEMPERATE DECIDUOUS TREE SPECIES

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CONTENTS

LIST OF PAPERS

This thesis is based on the following articles, which are referred to by their Roman numerals in the text.

I **Aasamaa K**, Sõber A (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* 44: 65–73.

II **Aasamaa K**, Sõber A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* 28: 765–774.

III **Aasamaa K**, Sõber A, Hartung W, Niinemets Ü (2002) Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiology* 22: 267–276.

IV **Aasamaa K**, Sõber A, Hartung W, Niinemets Ü (2002) Roles of abscisic acid in water-use strategies of deciduous tree species of different layers in temperate forest canopy. Manuscript.

LIST OF SYMBOLS

ABA — abscisic acid
[ABA] — abscisic acid concentration
d — area of guard cell dorsal wall
f — stomatal frequency
g_s — stomatal conductance to water vapour
L — shoot hydraulic conductance
l — length of stomatal pore
l_d — length of guard cell on the dorsal side
P_{max} — maximum (light- and CO_2-saturated) net photosynthetic rate
r^4 — radius (fourth power) of the widest conducting elements of midrib
s_d — stomatal sensitivity to a decrease of leaf water potential
s_i — stomatal sensitivity to an increase of leaf water potential
z — guard cell width
u — area of mesophyll and epidermal cells per unit length of leaf cross-section

1. INTRODUCTION

For optimal rates of metabolic processes in plants, their water content must remain constant. Often the water content of leaves is maintained in conditions when water loss from a plant during the day is much higher than the amount of water in the leaves. This is possible only when the water uptake and water loss of the plant are balanced. Water enters plants through their roots and then flows through the stem to the leaves. Therefore, the water supply of leaves depends on the intensity of water flow through the plant. The intensity of the water flow (transpiration stream) is dependent on the conductance for liquid water — hydraulic conductance, of roots, stem and leaves. Water vapour leaves plants mainly through the stomatal pores of leaves; therefore the water loss of the plants is determined by the stomatal conductance. Consequently, both the hydraulic conductance and the stomatal conductance are important determinants of the water content and water potential of leaves.

There are a lot of "blanks" in the knowledge about the mechanisms that ensure the stable water potential of leaves. Both the hydraulic conductance and the stomatal conductance are probably dependent on the anatomy and the concentrations of regulatory substances of a plant.

1.1. Shoot hydraulic conductance

Water of the transpiration stream moves in stems through the xylem. In leaves, water flows through the xylem and then moves out from the xylem and flows through the parenchymal cells before evaporating in the intercellular spaces (Boyer 1985; Steudle 1997). Therefore, the shoot hydraulic conductance (L) depends on both the hydraulic conductance of the xylem, and on the hydraulic conductance of the leaf parenchymal cells. In most studies the hydraulic conductance of stems (without leaves) has been measured (*e.g.* Sperry *et al.* 1987; Stratton *et al.* 2000; Sellin 2001). The conductance of the shoots with leaves has usually been obtained by the calculations using transpiration rate as the indicator of water flux (*e.g.* Comstock & Mencuccini 1998; Kolb & Stone 2000; Phillips *et al.* 2001). The shoot hydraulic conductance of only a few species has been determined on the basis of measurements of liquid water flow through the shoots with leaves (Kolb *et al.* 1996; Sobrado 1997/98; Tyree *et al.* 1998; Nardini *et al.* 2000, 2001).

In roots the hydraulic conductance is several orders of magnitude higher in the xylem conducting elements than in the parenchymal tissues (Steudle *et al.* 1987; Melchior & Steudle 1993; Steudle & Peterson 1998; Rieger & Litvin 1999). It is not clear, whether the leaf parenchyma has significantly lower hydraulic conductance, compared to the xylem, or not. Hydraulic conductance in xylem correlates positively with the width (*e.g.* Schulte & Gibson 1988;

Huang & Nobel 1993; Villar-Salvador *et al.* 1997) and the number (Zimmermann & Milburn 1982; Legge 1985; Sellin 1994) of the conducting elements. The diameter of the xylem conducting elements is noticeably smaller in the leaves than in the root metaxylem (Esau 1965; Zimmermann 1983). Therefore, the hydraulic conductance of the non-embolised xylem is likely to be lower in shoots than in roots, and not very significantly higher than in the parenchymal tissues of the leaves. Thus, variability of the xylem conductance may be more important in determining the hydraulic conductance in shoots than in roots.

The other important area of study is the relative contribution of apoplastic and symplastic compartments for the hydraulic conductance and the water flow through the foliar parenchyma. The hydraulic conductance is several orders of magnitude higher in nonlignified walls than in the symplast (Rüdinger *et al.* 1994; Steudle & Meshcheryakov 1996; Steudle & Heydt 1997). However, the volume of the cell walls in the leaf is lower than the volume of the symplasts and vacuoles (Esau 1965). Therefore, the hydraulic conductance of these two compartments in the mesophyll may be comparable.

The anatomy of the xylem has so far only been studied in connection with the hydraulic conductance. But the shoot hydraulic conductance includes the foliar conductance, and therefore the anatomy of leaves is also likely to be important. It has not been studied, how the species-specific values of several anatomical characteristics of leaves correlate with the species-specific values of shoot hydraulic conductance, and how the anatomical characteristics and shoot hydraulic conductance differ between individuals (of the same species) grown up in different conditions.

There is some evidence that shoot hydraulic conductance changes rapidly with environmental conditions, *e. g.* the L decreases in the conditions of water stress (Lo Gullo & Salleo 1988; Sperry *et al.* 1993; Stratton *et al.* 2000). One important reason for the decrease is embolism of the xylem conducting elements (Crombie *et al.* 1985; Sperry & Ikeda 1997; Tyree 1997). There is insufficiently data about the other mechanisms by which the shoot hydraulic conductance changes in the changing environment. Abscisic acid concentration ([ABA]) rises in water-stressed plants (Wright 1977; Hartung & Davies 1991; Sauter *et al.* 2001) and it may be that the rapid changes in shoot hydraulic conductance are caused by this important phytohormone. The relations between species-specific [ABA] and the shoot hydraulic conductance of the trees have also not been analysed.

1.2. Stomatal conductance and stomatal sensitivity to changes in leaf water status

Differences of stomatal conductance between species and also between individuals (of the same species) grown in different environments are relatively well documented (*e.g*. Turner & Heichel 1977; Chambers *et al.* 1985; Kolb & Stone 2000; Augé *et al.* 2000). Some data is also available about stomatal response kinetics: It is found that the stomatal conductance is lower and the stomata close faster in response to a decrease in leaf water potential in drought-exposed plants than in well-watered plants (Davies 1977; Ackerson 1980; Heschel & Hausmann 2001). Although there have been less studies on the response of stomata to an increase in leaf water potential, evidence suggests that, compared with well-watered plants, stomatal opening is slower after re-watering in water-limited plants (Ludlow *et al.* 1985; Liu *et al.* 2001). But there are only a few studies (Saliendra *et al.* 1995; Fuchs & Livingston 1996; Liu *et al.* 2001) where both the stomatal sensitivity to a decrease in leaf water potential and the stomatal sensitivity to an increase in leaf water potential have been analysed. Only very little data is also available about interspecific differences in stomatal response kinetics.

It has been proposed that stomatal conductance (g_s) correlates with several anatomical characteristics of the stomata: positively with the length of the stomatal pore (l) and the stomatal frequency (f), and negatively with the guard cell width (z) and the area of the guard cell dorsal wall (d):

$$g_s = k_{gs}\frac{l \times f}{d \times z}, \qquad (1)$$

where k_{gs} is a coefficient incorporating other factors influencing g_s (DeMichele & Sharpe 1973, 1974; Wu *et al.* 1985; Sharpe *et al.* 1987). It is reasonable to presume that the rate of water loss from the leaf can be high when there is a large number of stomata and the stomata are big. Negative correlation of stomatal conductance with the width of the guard cells can be caused by the higher moment of inertia in the wide guard cells. The inverse proportionality between stomatal conductance and the dorsal area of the stomatal guard cells may be caused by the high pressure of the neighbouring cells on the guard cells with an extensive dorsal wall area (see refs of Eq 1). There is insufficient experimental data to confirm this model. In general, research has been limited to stomatal conductance and sensitivity to changes of leaf water status or some anatomical characteristics of the stomata. Only in a few studies, have both the stomatal conductance and some anatomical characteristics of stomata been examined. In herbal species, high stomatal conductance was coupled with high stomatal frequency or long stomatal pores (Rahi 1971; Lawson *et al.* 1998; Tognetti *et al.* 2000). In research on the influence of environmental conditions, stomatal conductance varied in accordance with length of stomatal pore, in some species (Cutler *et al.* 1977; Bradford *et al.* 1983; Franks & Farquhar 2001), but with the stomatal frequency, in other herbal species (Quarrie & Jones 1977; Tichá 1982; Berryman *et al.* 1994; Beerling 1997; Nogués *et al.* 1998; Tognetti *et al.* 2000). Previous research on temperate deciduous trees has been limited to measuring the stomatal frequency as a single anatomical characteristic, in addition to the stomatal conductance, and agreement between these two stomatal characteristics has been found (Wagner *et al.* 1996; Woodward & Bazzaz 1998; Beerling *et al.* 1998). A study incorporating the observations of stomatal conductance and sensitivity and all these anatomical characteristics of stomata (see eq. 1) is missing.

The fact that stomatal conductance is dependent on abscisic acid concentration in leaves is well documented. Stomatal conductance is low in the leaves where the endogenous [ABA] is high (*e.g.* Beardsell & Cohen 1975; Tardieu *et al.* 1996; Ali *et al.* 1999; Peterlunger *et al.* 2000), and stomatal conductance decreases after supply with exogenous ABA (*e.g.* Tal & Imber 1970; Henson *et al.* 1989; Trejo *et al.* 1995; Zhang & Outlaw 2001). There are also some studies (Dörffling *et al.* 1979; Davies *et al.* 1981; Bradford *et al.* 1983; Liu *et al.* 2001) where both the leaf [ABA] and the stomatal sensitivity to the changes in the leaf water potential of one species have been determined. But the interspecific variations of the foliar [ABA] and the dynamics of stomatal response have not been compared so far.

1.3. Relations between hydraulic conductance and stomatal conductance and sensitivity

It is reported in several studies that hydraulic conductance of shoots is positively related to stomatal conductance. Stomatal conductance is high in the species with high hydraulic conductance in shoots (Meinzer *et al.* 1995; Bond & Kavanagh 1999; Nardini & Salleo 2000), both the characteristics are high in the individuals (within the same species) grown in optimal conditions (Comstock 2000), and hydraulic and stomatal conductance often change in parallel when environmental conditions vary (Tyree *et al.* 1994; Sõber 1997; Sparks & Black 1999; Hubbard *et al.* 2001). It is obvious that the water uptake and the water loss must be balanced to maintain optimal water potentials in the plant. Several different mechanisms can exist for the balancing of the hydraulic and stomatal conductances, both in long- and short-term scales.

The correlation of these two conductances between different species and between individuals (within the same species) grown in different conditions can be caused by coordination of the growth and development of different organs and tissues of a plant. It has been found that some anatomical characteristics of stem and leaves are correlated, *e. g.* the cross-sectional area of xylem in the stem is proportional to the area of the foliage (thus, also the number of stomata) served by the stem ("pipe-model") (Shinozaki *et al.* 1964; Osawa 1990; Coyea & Margolis 1992). But most of the anatomical characteristics of the hydraulic architecture of shoots and stomata have not been correlated so far. Therefore, the importance of shoot anatomy in the coupling of hydraulic and stomatal conductance is poorly understood.

In the cases of rapid changes in the plant's water supply, the decrease (or increase) of the hydraulic and stomatal conductance occurs in parallel probably because the water potential changes in all parts of the shoot: in the xylem, in the mesophyll, and in the epidermis. It is possible that mainly the decrease in water potential in leaves, especially in the stomatal district, induces the decrease in the stomatal conductance, and that mainly the decrease in water potential in the xylem induces the decrease of the shoot hydraulic conductance (for example, by the air embolism of the xylem conducting elements). Yet, [ABA] of shoot rises in conditions of water deficit and ABA is able to circulate between different compartments of the shoot (Slovik & Hartung 1992; Sauter *et al.* 2001; Wilkinson & Davies 2002). The significant influence of [ABA] on stomatal conductance is well documented (see the ref-s in chapter 1.2). If [ABA] also has significant influence on the shoot hydraulic conductance, [ABA] can be one factor coupling hydraulic and stomatal conductance. Parallel changes of the [ABA] in the stomatal district and in the rest of the leaf can be the cause of the parallelism of the changes in hydraulic and stomatal conductance in the changing environment.

1.4. Shoot hydraulic conductance and stomatal conductance of tree species with different shade- and drought tolerance

It was found that the drought tolerance, and also the shade-tolerance, of the species studied in this work decreased in the sequence: *Quercus robur*, *Tilia cordata*, *Acer platanoides*, *Padus avium*, *Populus tremula*, *Salix caprea* (Ellenberg 1998). *P. tremula* is common in the highest layer of the canopy, and the species at the beginning of this sequence (especially *T. cordata* and *A. platanoides*) are commonly in the lower layers of the tree canopy in deciduous and also in mixed forests in Estonia (Lõhmus 1984). Preferences of different species for habitats have developed in the course of evolution and are based on their features of anatomy and physiology. Shade-intolerant species grow rapidly (Veenendaal *et al.* 1996; Walters & Reich 2000), probably in order to avoid being shaded by their neighbours. It is probable that hydraulic and stomatal conductance are high in the shade-intolerant species, to enable a good supply of water and CO_2 needed for rapid growth. High hydraulic conductance is also needed to avoid water deficit in the drought sensitive species. Additionally, it can be suggested that the water-use strategy of the species of the overstorey of the forest canopy is probably less conservative than that of the species of the lower layers of the tree canopy. High stomatal conductance during drought (characteristic to the less conservative strategy) can be more beneficial in the overstorey, because of the good light availability for carbon dioxide assimilation. Data about the hydraulic and/or stomatal conductance in temperate deciduous trees (*e.g.* Castro-Diez *et al.* 1998; DeLucia *et al.* 1998; Loewenstein & Pallardy 1998b) is too scanty for the evaluation of the hypotheses on tree species with different shade- and drought tolerance and water-use strategies in the temperate deciduous forest canopy.

2. AIM OF THIS STUDY

The growth, development and productivity of a plant are highly dependent on the plant's ability to maintain the optimal water potentials in its organs. Therefore, knowledge about the characteristics which determine the water potential are very important for understanding the life of plants. It is necessary to study the hydraulic conductance and the stomatal conductance in conjunction, because the water potential in plants is dependent on the balance between these conductances. The study of both the hydraulic and the stomatal conductance, in connection with several other foliar characteristics that can influence these conductances, have been absent so far. Most studies on the shoot water regime have dealt with hydraulic conductance that is influenced by xylem embolism; other aspects of the variation of hydraulic conductance in shoots are less well studied. Therefore, this study deals only with the hydraulic conductance of the shoots with non-embolised xylem.

The main objectives of the present investigation were:

- to determine species-specific values of the characteristics of leaf water relations: shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water potential, of several deciduous tree species, growing in Estonia;
- to relate these species-specific values of the characteristics of leaf water relations to the species-specific values of anatomical characteristics, ABA concentration, and photosynthesis of the leaves;
- to study the influence of several different environmental conditions: long- and short-term water deficit, darkness (causing starvation) for several days, and nitrogen-rich soil, on these characteristics;
- to clarify the role of foliar anatomical characteristics and ABA concentration in the balancing of hydraulic and stomatal conductances in these tree species.

3. MATERIALS AND METHODS

3.1. Materials

The objects of the study were the species: *Acer platanoides* L., *Padus avium* Mill., *Populus tremula* L., *Quercus robur* L., *Salix caprea* L. and *Tilia cordata* Mill.. The saplings of these species were grown in Tartu, Estonia, in a small stand, in full sunlight. In the first series of experiments, the seasonal variations and influence of exogenous ABA were studied (Papers I, III). Two measurement campaigns were conducted during the growing season: fully expanded mature leaves were sampled in July, and old leaves were studied in September. For each experiment (*i. e.* the measurement of foliar gas exchange and hydraulic conductance), a shoot (10–15 cm in length) was cut under water from the tree, and put either in distilled water (control) or in solutions of 1, 10 or 20 μM ABA.

In the second series of experiments, the influence of several growth conditions was studied (Papers I, II). The saplings were grown for 3 years in the stand, and then transplanted to a greenhouse for the year of experiments. Six experimental sets were formed from the saplings of each of the species in the glasshouse: C — control set; N — the trees were fertilised with additional ammonium nitrate solution; W — mild water deficit was maintained in the soil during the whole growth period in the greenhouse; V — the mild water stress of the trees lasted only 2 d before the experiment; T and D — the trees were kept in darkness before the experiment for 4 d or 6 d respectively.

In the third series of experiments, *ca.* 50 years old trees of two species were studied before and in the course of drought (Paper IV). These trees grew near Tartu, in a deciduous mixed forest. *P. tremula* dominated in the overstorey and *T. cordata* in the lower layer of the tree storey. For the experiments of the second and third series, shoots were also cut from the trees.

3.2. Methods

Measurement of the foliar gas exchange characteristics and the shoot hydraulic conductance. In the experiments to observe an increase in the leaf water potential, the cut end of a shoot was driven to a pressure chamber filled with either distilled water or an ABA solution in which the shoot had previously been kept. A part of one intact leaf was hermetically clamped in a leaf chamber (Fig 1 in III). The leaf chamber system enables simultaneous measurement of the time courses of CO_2 uptake and transpiration of one side of the leaf, relative water content (by β-gauge technics), and leaf temperature. At first, the leaf adapted to the conditions of the chamber, until stabilisation of stomatal conductance. Thereafter, the chamber CO_2 concentration was instantly raised to saturation level for 4 min to measure the CO_2- and light-saturated net photosynthetic rate (P_{max}). After a steady-state value of stomatal conductance had been reestablished, the pressure in the pressure chamber was quickly raised to induce infiltration, necessary for the estimation of the shoot hydraulic conductance (Fig 2 in II). In the experiments to observe a decrease in the leaf water content, a leaf of another shoot was clamped in the leaf chamber, the petiole was cut, and the course of the following stomatal closure was observed (Papers I–III). For further details of the measurement system and the experiments see also papers of Rahi (1973), Sõber & Moldau (1977), and Sõber (1996, 1997).

The initial, stabilised (in the laboratory conditions) stomatal conductance and the course of decrease of the stomatal conductance (following the interchanging of the water in the water-vessel of the shoot for ABA solution) in the shoots of the tall trees was detected by steady-state porometer. For estimation of the shoot hydraulic conductance, the leaves of the shoot were infiltrated, using a pressure chamber. Then the shoot was fixed in the pressure chamber so that only the cut end of the shoot was outside the chamber. Pressure was applied and the rate of water flow out of the shoot through the cut end was measured (Paper IV).

Calculations of values of the foliar gas exchange characteristics and the shoot hydraulic conductance. *Stomatal conductance* (g_s) was calculated using the measured values of transpiration rate and the leaf temperature. The value of g_s in the data is the steady-state value, obtained after the initial stabilisation of the stomatal conductance in the leaf chamber (Papers I–III) or in the laboratory (Paper IV).

Stomatal sensitivity to change of leaf water potential (s_i and s_d) was defined as the relative rate of change (increase and decrease, respectively) of the stomatal conductance after the changing of the leaf water potential.

Stomatal sensitivity to exogenous ABA (s_{ABA}) was defined as the relative rate of decrease of the stomatal conductance after the shoot was supplied with exogenous ABA.

Shoot maximum hydraulic conductance (L) was calculated using the maximum rate of increase in the leaf water content (per leaf area) during leaf infiltration, and the pressure difference at the path of the water from the pressure chamber to the intercellular spaces of the leaf. Because the water potential in the intercellular spaces of infiltrating leaves is nearly zero, the pressure difference is equal to the pressure in the pressure chamber (Papers I–III). In the 3-rd series of experiments, the rate of water flow out of the shoot through its cut end, and the pressure in the pressure chamber was used in the calculations of the L (Paper IV).

For further details of the calculation methods see also papers of Mederski (1961), Sõber & Moldau (1977) and Sõber (1992, 1997).

Endogenous abscisic acid concentration. For the analysis of bulk-leaf [ABA], leaf disks were punched between major veins. Shoot xylem sap was forced out from the shoot through the cut end of the shoot by the pressure chamber. The ABA content in the xylem sap and the leaf tissue extract was determined by ELISA, as described by Mertens *et al.* (1985) and Peuke *et al.* (1994) (Papers III, IV).

The anatomical characteristics. The characteristics of the leaf internal architecture were studied by light microscope. Pieces of mature leaf were taken from each of the saplings grown in the greenhouse, embedded in paraffin, cross-sectioned, and permanent slices, stained with safranin O, were made. The anatomical characteristics of stomata were studied by scanning electron microscope (Paper II).

4. RESULTS AND DISCUSSION

4.1. Shoot hydraulic conductance

4.1.1. Shoot hydraulic conductance in different species, ontogenetic stages, and environments

Shoot hydraulic conductance (*L*) was significantly different in saplings of the different species and increased in the sequence: *Acer platanoides* < *Tilia cordata* < *Padus avium* = *Quercus robur* < *Salix caprea* = *Populus tremula* (Fig 1A in I). *L* of *P. tremula* was also higher than the *L* of *T. cordata* in tall trees of the forest canopy (Fig 1E in IV). Thus, the species effect on *L* is significant and already appears in young trees that are equal in height and illumination.

L was higher in July than in September for all the species, but the species ranking was basically the same in July and in September (Fig 1A in I). Thus, the genetic effect on *L* is important throughout the ontogeny of leaves.

L was slightly higher in the trees grown in soil with a higher nitrogen content (compared to the trees of the control set of each species). *L* was lower in the trees grown under mild water stress (both long-term (whole season) and short-term (2 days) stress) for all the species. *L* was the lowest in the trees kept in darkness and was dependent on the duration of darkness (Fig 1A in I). The relative decrease of *L* was about 1/4 after the trees were not watered for 2 days and about 1/2 after keeping the trees in darkness for several days (Fig 2 in I). Thus, *L* is different in different growth conditions and also rapidly responsive to changes in growth conditions. Xylem embolism was evidently eliminated in the experiments by the high pressures of the pressure chamber (Nardini & Pitt 1999; Tyree *et al.* 1999). Therefore, the variation of *L* does not reflect the differences between the shoots in the xylem embolism, but is caused by more complicated mechanisms. The similarity of the relative differences in *L* in the different growth conditions between all the species (Fig 2 in I) refers to the similarity in the regulation of *L* in these species.

4.1.2. Relations of hydraulic conductance with anatomical characteristics of leaves

The species-specific *L* correlated positively with the species-specific radius (fourth power) of the widest conducting elements of midrib (r^4) and with the area of mesophyll and epidermal cells per unit length of leaf cross-section (u) (Fig 3 in II; Tab 1). This result highlights the importance of these anatomical characteristics in the determination of hydraulic conductance.

Table 1. Pearson correlation coefficients (and P-values) between species-specific mean values of shoot hydraulic conductance (L), stomatal conductance (g_s), stomatal sensitivity to an increase in the leaf water potential (s_i), stomatal sensitivity to a decrease in the leaf water potential (s_d), maximum net photosynthetic rate (P_m), carboxylation efficiency (χ), ABA concentration in leaves (a), fourth power of the mean radius of the widest conducting elements in xylem of the leaf midrib (r^4), area of mesophyll and epidermal cells per length of the leaf cross-section (u), length of stomatal pore (l), guard cell width (z) and length of guard cell dorsal side (l_d) of these six tree species.

	L	g_s	s_i	s_d	P_m	χ	a	r^4	u	l	z
g_s	0.97 (0.002)										
s_i	0.95 (0.004)	0.99 (<0.001)									
s_d	–0.83 (0.039)	–0.86 (0.029)	–0.80 (0.058)								
P_m	0.94 (0.005)	0.95 (0.004)	0.91 (0.011)	–0.95 (0.003)							
χ	0.14 (0.792)	0.26 (0.623)	0.17 (0.741)	–0.63 (0.183)	0.47 (0.353)						
a	–0.81 (0.051)	–0.83 (0.043)	–0.82 (0.045)	0.56 (0.252)	–0.77 (0.074)	–0.10 (0.852)					
r^4	0.93 (0.008)	0.95 (0.004)	0.93 (0.007)	–0.75 (0.087)	0.84 (0.037)	0.06 (0.905)	–0.77 (0.073)				
u	0.83 (0.04)	0.94 (0.006)	0.93 (0.07)	–0.86 (0.027)	0.88 (0.021)	0.41 (0.417)	–0.65 (0.167)	0.88 (0.022)			
l	0.86 (0.027)	0.92 (0.01)	0.92 (0.01)	–0.63 (0.176)	0.77 (0.069)	0.03 (0.951)	–0.87 (0.025)	0.96 (0.002)	0.84 (0.037)		
z	–0.85 (0.032)	–0.84 (0.036)	–0.90 (0.015)	0.58 (0.232)	–0.73 (0.097)	0.12 (0.826)	0.67 (0.143)	–0.76 (0.079)	–0.74 (0.09)	–0.73 (0.098)	
l_d	–0.83 (0.043)	–0.82 (0.044)	–0.88 (0.02)	0.59 (0.218)	–0.74 (0.096)	0.05 (0.923)	0.64 (0.172)	–0.71 (0.112)	–0.75 (0.089)	–0.68 (0.136)	0.99 (<0.001)

The high L in the trees grown in nitrogen-rich soil (compared with the control trees of each species) was accompanied by high r^4 and u, and the low L of the shoots grown under water stress was accompanied by low r^4 and u (Fig 1A in I; Figs 2A,C in II). The result suggests that the characteristics r^4 and u may also be important determinants of the variability of shoot hydraulic conductance between individuals of the same species growing in different environmental conditions.

The correlation between the shoot hydraulic conductance and the area of mesophyll and epidermal cells suggests the importance of leaf symplast in the determination of hydraulic conductance in leaves. Larger space for water flow in leaves with higher volume of mesophyll and epidermal cells may be essential for high hydraulic conductance. It is also remarkable that the area of veins per unit length of the leaf cross-section was big in the leaves, where *L* was low: The area of the veins was high in *A. platanoides* and *T. cordata* and in the leaves, which have grown up under water-stress conditions (Fig 1A in I; Fig 2B in II). Thus, the volume of thickened and lignified cell walls of conducting and supporting tissue was likely high in the leaves of these trees. However, the dry mass (that is mainly the mass of the cell walls) per unit volume of leaves of these trees was not higher than that of other trees (data not shown). It is found that the walls of the mesophyll cells are thinner in leaves grown under water-stress conditions compared to those of well-watered leaves (Sweet *et al.* 1990; Passioura *et al.* 1993; Spollen *et al.* 1993). Therefore, it is likely that the dry mass of the leaves grown under water-stress conditions and also of *A. platanoides* and *T. cordata* was not high because the cell walls outside the veins were thin. Due to the low hydraulic conductance in leaves with a small amount of unlignified walls, it is likely that not only the conductance of symplast, but also the conductance of the walls in the mesophyll and in the epidermis is important in determining hydraulic conductance in leaves.

Some other anatomical characteristics with a possible influence on hydraulic conductance (the area of intercellular spaces, the mean area of one epidermal and mesophyll cell, and the proportion of areas of epidermal and mesophyll cells on the leaf cross-section) were also examined. None of these characteristics varied significantly among the trees (all species) grown in different conditions. Between the different species, only the area of the intercellular spaces varied significantly, but it did not correlate with the hydraulic conductance (data not shown).

4.1.3. Relations of hydraulic conductance with abscisic acid concentration

The species-specific *L* correlated negatively with the endogenous abscisic acid concentration ([ABA]) of leaves (Fig 4A in III; Tab 1; Fig 2C in IV; Tab 1 in IV). Therefore it can be supposed, that leaf [ABA] is important in determining the species-specific *L*. The result that in each species the hydraulic conductance was low in the shoots grown under mild water deficit since early spring (Fig 1A in I), is in accordance with previous investigations (Simpson 1981; Mansfield & Atkinson 1990), which demonstrate that [ABA] is high in the plants grown up in conditions of water deficit.

It was also found that although the species-specific foliar [ABA] was lower in September than in July, the species ranking (according to the foliar [ABA]) was basically the same in these different stages of leaf ontogeny (Fig 3A in III). This is in agreement with other studies (Loewenstein & Pallardy 1998a; Augé *et al.* 2000), indicating that species differences in foliar [ABA] are retained throughout leaf ontogeny when the species grow in same conditions. Therefore, the species ranking according to the foliar [ABA] was likely also similar (as in July and September) during the period of intensive growth and development, with the highest leaf [ABA] in *A. platanoides* and the lowest in *P. tremula*. It is found that ABA has a strong influence on the growth and development of plants. Xylem with narrow conducting elements develop in response to high [ABA] in plants (Quarrie & Jones 1977; Pharis *et al.* 1981; Fromm 1997). Strong negative correlation of the species-specific values of leaf [ABA] with the width of the xylem conducting elements and with the volume of foliar epidermal and mesophyll cells was found also in this study (Tab 1). Therefore, the narrow xylem conducting elements and small volume of mesophyll and epidermal cells and walls in *A. platanoides*, and in leaves grown in conditions of water stress, probably have been caused by high [ABA] in the period of intensive growth of young leaves. Thus, the important reason for the correlation between the shoot hydraulic conductance and [ABA] in these mature leaves is probably the earlier influence of ABA on the development of the hydraulic architecture.

It has been found in the last decade that the water channels — aquaporins are located in plant membranes, and the hydraulic conductance of the symplast is highly dependent on the conductance of aquaporins (Maurel *et al.* 1993; Eckert *et al.* 1999; Qiu *et al.* 2000; Maurel & Chrispeels 2001). [ABA] influences the expression of the aquaporin genes in leaves (Kaldenhoff *et al.* 1993, 1996). Therefore, probably species-specific $[\mathrm{ABA}]_{\mathrm{leaf}}$ determines the species-specific level of shoot hydraulic conductance also by affecting the rate of expression of the aquaporin genes. Similarly, the high [ABA] in the leaves grown in conditions of water-stress probably cause the low values of the shoot hydraulic conductance by inhibiting the expression of aquaporins. These conclusions refer to the important role of leaf symplast in determining the shoot hydraulic conductance.

It was also found in this study that the shoot hydraulic conductance did not remain constant throughout the season. The *L* decreased in response to a supply of exogenous ABA already within a few hours (Fig 6A in III). Because [ABA] of plant increases in water-stress conditions (Fig 1F in IV; Wright 1977; Hartung & Davies 1991; Sauter *et al.* 2001) and in darkness (Wang & Kao 1999), ABA probably also caused the decrease in *L* after the exposure of the trees to the short-term water stress and darkness.

It is probable that the shoot hydraulic conductance decreased in response to the short-term water deficit and darkness also due to the decrease in the activity of the aquaporins. Changes of [ABA] induce rapid changes in the concentration of cytosolic Ca^{2+} (Gehring *et al.* 1990; Cousson 1999). Changes in the cytosolic calcium content cause rapid alterations in the conductance of the aquaporins by modifying the activity of protein phosphorylating enzymes (Johansson *et al.* 1996, 1998, 2000). Since keeping aquaporins in working order requires energy (Tyerman *et al.* 1999), the important additional reason for a decrease in the hydraulic conductance of the starving trees (in darkness) can be the lack of energy for the functioning of the aquaporins.

There is also an alternative explanation for the rapid changes in shoot hydraulic conductance. High [ABA] decreases the efflux of ions from the xylem parenchyma into the xylem lumens (Roberts & Snowman 2000) and also from symplast to apoplast in the mesophyll (Sutton *et al.* 2000). Therefore, the ionic concentration of the xylem sap and the walls in the mesophyll likely decreased when the shoots were supplied with exogenous ABA. There are some reports about a rapid decrease in xylem hydraulic conductance in response to a decrease in the ion concentration of the xylem sap (Zimmermann 1978; van Meeteren *et al.* 2000; Zwieniecki *et al.* 2001). The decrease in the xylem hydraulic conductance is explained by the narrowing of the microchannels in the intervessel bordered pit membranes because of the swelling of the pectin matrix of the membranes (Brown 2001; Zwieniecki *et al.* 2001). Therefore, the decrease in hydraulic conductance of the xylem pit membranes (and possibly also in the walls of the leaf parenchymal cells that also contain pectin matrix) may be one reason for the decrease in shoot hydraulic conductance in the shoots supplied with exogenous ABA. However, it is found that the ionic concentration of the apoplast does not change significantly in stressed plants, probably because of the balance between the element release into xylem conduits and element depletion by growing tissues (Berger *et al.* 1994; Schurr & Schulze 1996). Therefore, the decrease in the hydraulic conductance by the modification of the pectin matrix probably did not occur in the trees influenced by short-term water stress or darkness.

On the basis of the result that the relative decrease in L was about 1/3 of that in the control, in response to a high concentration of exogenous ABA (Fig 6A in III), and about 1/4 in short-term water-stress (Fig 2 in I) it was concluded that minimally 1/3 of the shoot hydraulic conductance is potentially and 1/4 is actually adjustable by current ABA concentrations. Because the decrease in L in darkness (about 1/2 of the control (Fig 2 in I)) likely was caused by a decrease in hydraulic conductance of the leaf symplast, the proportion of the symplastic component of the transpiration stream through the mesophyll and epidermis of non-stressed leaves probably was about 50 %, *i.e.* the hydraulic conductances of the symplastic and the apoplastic compartments in the tissues, which are not specialised to water conducting, are about equal in the non-stressed leaves of these tree species.

The high sensitivity of the shoot hydraulic conductance to the conductance of the mesophyll and epidermis of leaves indicates that in shoots (as also in roots) likely the hydraulic resistance is probably significantly higher in the tissues that are not specialised to water conducting, compared to the conductance of (non-embolised) xylem.

4.2. Stomatal conductance and stomatal sensitivity to changes in leaf water status

4.2.1. Stomatal conductance and sensitivity in different species, ontogenetic stages and environments

Both the stomatal conductance (g_s) and stomatal sensitivity (sensitivity to an increase in leaf water potential (s_i) and sensitivity to a decrease in leaf water potential (s_d)) varied between the species. In general, the species-specific g_s and s_i varied in parallel with *L*: they were the lowest in *A. platanoides* and the highest in *P. tremula* and *S. caprea* (Figs 1C–E in I; Figs 1C,E in IV). The species-specific values of g_s correlated positively with the species-specific values of s_i and negatively with s_d (Tab 1). Therefore, s_d changed in the opposite direction compared to g_s and s_i in these species (Figs 1C–E in I). The significant difference in stomatal conductance and sensitivity between the young trees of equal height and in equal growth conditions indicates the importance of genetic factors in the determination of these stomatal characteristics.

The g_s and s_i were higher in July than in September, but the species ranking was basically the same in these two stages of ontogeny (Figs 1C,E in I). Thus, the genetic effect on the stomatal conductance and sensitivity is important throughout the ontogeny of leaves.

The g_s and s_i were usually somewhat higher in the trees grown in soil with a higher nitrogen content (than in the trees of the control set of each species) and low in the trees grown under mild water stress, and still more significantly lower in the trees kept in darkness. Mostly the s_d was low in the trees where g_s and s_i were high, and *vice versa*. However, s_d was low in all the trees kept in darkness (similarly with the g_s and s_i of these starved trees) (Figs 1C–E in I). The results indicate that both the stomatal conductance and stomatal sensitivity to changes of leaf water potential are responsive to changes in the growth environment. It is also remarkable that the stomatal conductance and sensitivity were very similar in the trees which had been grown under a mild water deficit since spring and in the trees which suffered the water deficit for only few days. Therefore, the optimal stomatal conductance and sensitivity probably exist for this mild water deficit and these optimal values are achieved rapidly.

4.2.2. Relations of stomatal conductance and sensitivity with anatomical characteristics of leaves

Most anatomical characteristics of stomata given in equation 1 correlated with stomatal conductance and also with the stomatal sensitivity to changes in leaf water potential: The species-specific g_s and s_i correlated positively with length of stomatal pore (l) and negatively with guard cell width (z) and length of guard cell on the dorsal side (l_d) in these species (Figs 4,5A–C in II; Tab 1). Species-specific s_d correlated negatively with l and positively with z and l_d in these species (Figs 5D–F in II; Tab 1). Thus, these three anatomical characteristics (l, z and l_d) probably are important determinants of the inter-specific differences in the stomatal conductance and stomatal sensitivity to changes in leaf water potential.

The length of the stomatal pore was the only anatomical characteristic of stomata that was significantly sensitive to the differences in growth conditions: The values of l were high in the trees grown in nitrogen-rich soil and low in the trees grown under mild water stress (as also the values of g_s and s_i) (Fig 2E in II; Figs 1C,E in I). Therefore, the length of the stomatal pore can also play an important role in the determination of the variability of stomatal conductance and sensitivity among the trees (of the same species) grown with different nitrogen and water supplies.

It is remarkable that the width of the guard cells is not different in the trees (of the same species) grown in different growth conditions (Fig 2F in II). Dimensions of other cells in the leaf epidermis are bigger in plants grown in nutrient-rich soil and smaller in plants grown under water deficit conditions (Spollen *et al.* 1993; Thomas & Howarth 2000). Wide guard cells (with high moment of inertia) probably are suitable for avoiding too high g_s and s_i in conditions of poor water supply. Thus, it can be suggested that the regulation of the width of the guard cells is also important in the adjusting of stomatal conductance and sensitivity to different growth conditions.

It is presumed that the extensive contact area between the guard cells and the neighbouring epidermal cells (in leaves where the dorsal side of guard cells is long) leads to better hydraulic contact of the guard cells with the neighbouring cells, resulting in higher stomatal conductance of well-watered leaves. However, the pressure of the neighbouring cells on the guard cells (which decreases the aperture of the stomatal pore) is likely also high, and the "mechanical advantage" of the neighbouring epidermal cells (Glinka 1971; Edwards *et al.* 1976; Franks *et al.* 1998) may have greater effect on stomatal conductance in the leaves with extensive contact area. It was found that the stomatal conductance was high in the tree species with a short dorsal side (Fig 4C in II; Tab 1). Therefore, it is probable that in the studied tree species, the influence of the hydraulic conductance between guard cells and neighbouring cells is surpassed by the influence of the pressure of the neighbouring epidermal cells.

One member of equation 1 — stomatal frequency (per unit area of the leaf abaxial surface), varied only slightly among most species and among the plants (of the same species) grown in different conditions (Fig 2D in II) and exhibited poor correlation with other stomatal characteristics (data not shown). Therefore, the importance of stomatal frequency in determining the differences in somatal conductance and sensitivity between these tree species, and also between individuals of same species — in trees grown in different conditions, is evidently low.

4.2.3. Relations of stomatal conductance and sensitivity with abscisic acid concentration

The species-specific g_s and s_i correlated negatively and s_d correlated positively with the endogenous species-specific [ABA] of leaves (Figs 4B,C in III; Fig 2A in IV; Tab 1 in IV; Tab I). The g_s and s_i were low and s_d was high in the leaves that had grown up in water stress conditions (Figs 1C–E in I). [ABA] is also known to be high in the leaves that have grown in conditions of water deficit (Simpson 1981; Mansfield & Atkinson 1990).

The species ranking on the basis of the foliar [ABA] (Fig 3 in III) probably was also the same in young, intensively growing leaves (see chapter 4.1.3 above). ABA also has significant influence on growth and development of stomata: Guard cells were small in leaves treated with ABA during the intensive growth stage (Cutler *et al.* 1977; Bradford *et al.* 1983; Franks & Farquhar 2001). In this study the species-specific values of the anatomical characteristics of stomata: length of stomatal pore, guard cell width, and length of guard cell on the dorsal side, correlated with the species-specific values of leaf [ABA] (Tab 1). Therefore, one significant reason for the correlation of the stomatal conductance and sensitivity with the $[ABA]_{leaf}$ is probably the effect of the [ABA] on the development of stomata in young leaves.

It was found in this study that stomatal conductance and sensitivity were the most sensitive characteristics to the exogenous ABA. The g_s and s_i decreased significantly in response to a supply of the lowest used concentrations of exogenous ABA (Figs 6B,C in III). Thus, the current [ABA] in leaves also modifies the stomatal conductance and sensitivity very significantly. Because the [ABA] in plants also increases in conditions of water-stress (Fig 1F in IV; Wright 1977; Hartung & Davies 1991; Sauter *et al.* 2001) and in darkness (Wang & Kao 1999), an important reason for the changed stomatal conductance and sensitivity after exposure of the trees to these conditions was also the increased [ABA].

Increased [ABA] of stomatal district activates several processes, which collectively lead to a reduction in the concentration of the osmotic substances in guard cells, resulting in a corresponding decrease in the guard cell water content, and hence, the decrease in the stomatal conductance (see reviews by

Grabov & Blatt 1998; Assmann & Shimazaki 1999; Schroeder *et al.* 2001). Thus, the low rate of stomatal opening in response to an increase in leaf water potential, and high rate of stomatal closure in response to a decrease in leaf water potential in the plants with high foliar [ABA] were probably both a result of the negative effect of ABA on guard cell water content. Low s_d of the trees kept in darkness was probably caused by the shortage of energy for the hydroactive reactions in stomatal guard cells.

Although the [ABA] of the exogenous solutions was considerably higher than that of severely water-stressed leaves with closed stomata (Davies *et al.* 1981; Tenhunen *et al.* 1994), the stomata still opened when the water potential of the ABA-treated leaf was raised. This demonstrates that the influence of high water potential on the hydroactive reactions overrides the effect of ABA. Stomatal sensitivity to xylem [ABA] decreases in these tree species at high water potentials in leaves.

The results of the experiments with tall forest trees also demonstrate the important role of the variation of stomatal sensitivity to [ABA] in the stomatal regulation of some species in changing environmental conditions. In water-stressed *P. tremula* the shoot xylem [ABA] had increased, but the stomatal sensitivity to ABA (s_{ABA}) had not changed significantly. In water-stressed *T. cordata*, *vice versa*, the shoot xylem [ABA] had not increased, but s_{ABA} was significantly higher than that in non-stressed tree (Figs 1G,H in IV). Thus, the same result — decrease in stomatal conductances in water-stress conditions (Fig 1C in IV) was probably achieved by two basically different mechanisms in these two species.

4.3. Relations between hydraulic conductance and stomatal conductance and sensitivity

The values of shoot hydraulic conductance and stomatal conductance and sensitivity were interrelated: The species-specific L correlated positively with species-specific g_s and s_i, and negatively with s_d (Figs 4–6 in I; Tab 1; Tab 1 in IV). Additionally, both the hydraulic and stomatal conductance (and also s_i) were somewhat higher in the trees grown in nitrogen-rich soil (compared with the values of control trees of each species) and lower in the trees grown under water stress (Figs 1C–E in I).

The species-specific shoot hydraulic conductance and stomatal conductance and sensitivity also correlated with the endogenous foliar [ABA] (Fig 4 in III; Tab 1; Tab 1 in III). Yet, supplying the shoots with low exogenous ABA solutions did not change the hydraulic conductance, but significantly decreased the stomatal conductance and sensitivity. Additionally, the decreases in the shoot hydraulic conductance in response to the higher exogenous ABA solutions and also in response to the short-term water-stress or the keeping of the plant in darkness always were relatively smaller than the decreases of the stomatal conductance and sensitivity (Figs 1A,C–E, 4–6 in I; Figs 6A–C in III). Thus, the leaf current [ABA] probably is not an important generator of the correlation between the hydraulic and stomatal conductance of the shoots with the mature leaves.

The characteristics of leaf hydraulic architecture (r^4 and u) correlated with the anatomical characteristics of stomata (l, z and l_d): (Fig 6 in II; Tab 1): The trees which had wide conducting elements in the xylem and high volume of cells (and probably also a high volume of cell walls) outside the veins also had long stomatal pores, narrow guard cells, and a short dorsal side in guard cells. Therefore, one important explanation for the correlation between shoot hydraulic conductance and stomatal conductance and sensitivity can be the correlation between the values of the anatomical characteristics of the water conducting system and stomata.

As mentioned above, all the anatomical characteristics also correlated with foliar [ABA]. Because of the strong influence of ABA on the leaf development (see the chapters 4.1.3. and 4.2.3.), it may be postulated that an important generator of the correlations between these anatomical characteristics may be [ABA] in young, developing leaves. [ABA] in young leaves probably causes coordinated development of the anatomical characteristics which have an effect on the hydraulic and stomatal conductance and the stomatal sensitivity in leaves. Thus, [ABA] in young leaves may be a more important reason than current [ABA] for the correlation of hydraulic and stomatal conductances of mature leaves.

The L, g_s and also s_i correlated positively with maximum (light- and CO_2-saturated) photosynthetic rate (P_{max}) (Fig 3 in I; Tab 1) and with the CO_2-limited photosynthetic rate (P) (data not shown). It has been found, that the content of chlorophyll and several other components of the photosynthetic apparatus (per area of the leaf) is in accordance with the P_{max} (Farquhar *et al.* 1980; Sõber *et al.* 1999). Therefore, the P was probably also high in the leaves with high P_{max}, because the leaves with high photosynthetic apparatus capacity can also more intensively utilise lower (nonsaturating) quantities of CO_2 and light. Both the aquaporins (and other channels in the membranes of mesophyll cells) and hydroactive processes in stomata need energy. Therefore, one cause of the correlation between the hydraulic and stomatal conductance was probably also the positive correlation of L and g_s with the photosynthetic capacity of the leaves.

ABA probably also affects the development of the photosynthetic apparatus, because thin leaves with low chlorophyll content (per area of leaf) develop for example in conditions of water-stress (Bokhari 1976; Burke & O'Mahony 2001), when [ABA] in the plant is also high (Simpson 1981; Mansfield & Atkinson 1990). It was found in this study that the species-specific $[ABA]_{leaf}$ correlated with the species-specific P_{max} (as also with the L, g_s s_i and s_d) (Figs 5A,4 in III; Tab 1). Additionally, the P_{max} (as also the L, g_s and s_i) was low in the leaves that had grown up in conditions of water deficit (Figs 1A–C in I), when $[ABA]_{leaf}$ was probably high. Thus, one of the factors which coordinates the development of photosynthetic apparatus with the development of leaf hydraulic architecture and stomata can also be the [ABA] of young leaves.

4.4. Shoot hydraulic conductance and stomatal conductance of tree species with different shade- and drought tolerance

The sequences of the species in shade- and drought tolerance (see the chapter 1.4.) or in shoot hydraulic conductance and the stomatal characteristics, and some other characteristics measured in this study (Fig 1 in I; Fig 2 in II; Fig 3 in III), were quite similar. Thus, the hypothesis, set at the beginning of the study was confirmed by the results: In the most shade- and drought intolerant species, the shoot hydraulic conductance, the stomatal conductance, and the stomatal sensitivity to an increase in leaf water potential were the highest, and the stomatal sensitivity to a decrease in leaf water potential and the stomatal sensitivity to exogenous ABA were the lowest. Therefore, the water-use strategy was also the least conservative in *S. caprea* and *P. tremula*. The high hydraulic and stomatal conductance probably permit the intensive growth of these species. It was also found that the capacity of the photosynthetic apparatus was the highest in the species with the highest light demand. High capacity of the photosynthetic apparatus is probably also one reason for the rapid growth, and is beneficial for the utilisation of the intercellular CO_2, the concentration of which is high due to the high stomatal conductance of the species. It can be suggested also that the distribution of these species of *Salicaceae (S. caprea* and *P. tremula*) will decrease in the districts where the air is polluted, because air pollutants are likely to be more harmful for the species with more open stomata. The high hydraulic and stomatal conductance of *Q. robur* (which was determined by Ellenberg to be the most drought- and shade-tolerant among these species) can be related to the fact that Estonia is located near the northern border of the area of distribution of that species. The non-optimal growth conditions may modify species-specific drought- and shade-tolerance.

5. SUMMARY AND CONCLUSIONS

- The shoot hydraulic conductance (L), stomatal conductance (g_s), stomatal sensitivity to an increase in leaf water potential (s_i) and stomatal sensitivity to a decrease in leaf water potential (s_d) differed significantly between different deciduous tree species. Consequently, the genetic factor is very important cause of differences in leaf water relations.
- The L, g_s and s_i were higher in the trees grown in soil with a higher nitrogen content and lower in the trees grown in conditions of mild water deficit (both the water deficit of three months and the water deficit of two days), compared to the trees of the control set of each species. The s_d changed in the opposite direction if compared to the L, g_s and s_i. The L, g_s, s_i and s_d decreased in response to keeping the trees in darkness for some days. Thus, all these important characteristics of leaf water regime are responsive to both the long-term differences and the short-term changes in environmental factors.
- L decreased by about 50% (in all the species) after keeping a sapling in darkness for only a few days. As probably only the conductance of foliar symplast decreased during the starvation in darkness, it was concluded that in the mesophyll and epidermal cells of non-darkened leaves, about a half of the water of the transpiration stream crossed the symplastic compartment. *I.e.*, the hydraulic conductance of the symplastic and the apoplastic compartments is about equal in the non-stressed leaves of all these tree species.
- The high sensitivity of the shoot hydraulic conductance to the conductance of the foliar tissues which are not specialised to water conducting indicates that the hydraulic resistance is significantly higher in the foliar parenchymal tissues, compared to (non-embolised) xylem in the shoots of these species.
- Species-specific L correlated positively with the species-specific radius (fourth power) of the widest conducting elements of leaf midrib (r^4) and the area of mesophyll and epidermal cells on the leaf cross-section (u). r^4 and u were also higher in the trees grown in soil with a higher nitrogen content; r^4 and mostly u were lower in the trees grown under mild water stress (compared with the trees of the control set of each species). Therefore, these two anatomical characteristics are probably important determinants of both the species-specific values of shoot hydraulic conductance and the differences of the L between individuals of the same species — in trees grown in different environments.
- Species-specific g_s and s_i correlated positively with the species-specific length of the stomatal pore (l), and negatively with guard cell width (z) and the length of the guard cell on the dorsal side (l_d). The correlations of the s_d with these anatomical characteristics of stomata were in the opposite direction. Length of the stomatal pore was also higher in the trees grown in

soil with a higher nitrogen content, and lower in the trees grown under mild water stress (compared with the trees of the control set of each species). Consequently, the l, z, and l_d are important determinants of species-specific values of stomatal conductance and stomatal sensitivity to changes in leaf water potential. The l is also important in the adaptation of stomatal conductance and sensitivity to different growth conditions.

- L, g_s and s_i were low and s_d was high in the trees where leaf endogenous abscisic acid concentration ([ABA]) was high. L, g_s and s_i decreased also in response to exogenous ABA. Therefore, the current foliar [ABA] is probably an important determinant of: 1) the species-specific hydraulic and stomatal conductance and the stomatal sensitivity, and 2) the differences of these characteristics within the same species — between the individuals grown in different environments.
- Stomatal sensitivity to (exogenous) ABA decreased in all the species when leaf water potential rose to high values. Stomatal sensitivity to exogenous ABA increased significantly during drought in *Tilia cordata*, but not in the other species analysed — *Populus tremula.* Consequently, variation of the stomatal sensitivity to ABA is an important strategy in the stomatal regulation of trees, especially in some species (*Tilia cordata*).
- L correlated positively with s_i and negatively with s_d. The correlation of shoot hydraulic conductance also with both the stomatal sensitivities (in addition to the correlation with the stomatal conductance) indicates a very tight connection between the regulation of water movement in(to) the leaf and out of the leaf.
- The anatomical characteristics, which potentially have significant influence on hydraulic conductance (r^4 and u) correlated with the anatomical characteristics of stomata (l, z and l_d). Therefore, one reason for the correlation of the hydraulic conductance with the stomatal conductance and sensitivity can be the correlation between the anatomical characteristics of the water conducting system and stomata in these trees.
- The anatomical characteristics (r^4, u l, z and l_d) also correlated with the endogenous [ABA] of the leaves. It was suggested that the specific [ABA] is the coordinator of the development of the anatomical characteristics of the water conducting system and stomata in young leaves, and thereby, indirectly determines the correlation between the hydraulic and stomatal conductance of mature leaves.
- The most shade-intolerant and drought sensitive species also have the highest shoot hydraulic conductance, stomatal conductance, stomatal sensitivity to an increase in leaf water potential, and the maximum photosynthetic apparatus capacity, and the lowest stomatal sensitivity to a decrease in leaf water potential. Therefore, shade- and drought tolerance probably are determined by the species-specific hydraulic and stomatal conductances, the stomatal sensitivity, and the photosynthetic capacity.

REFERENCES

Ackerson RC (1980) Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. *Plant Physiology* 65: 455–459.

Ali M, Jensen CR, Mogensen VO, Andersen MN, Henson IE (1999) Root signalling and osmotic adjustment during intermittent soil drying sustain grain yield of field grown wheat. *Field Crops Research* 62: 35–52.

Assmann SM, Shimazaki KI (1999) The multisensory guard cell. Stomatal responses to blue light and abscisic acid. *Plant Physiology* 119: 809–815.

Augé RM, Green CD, Stodola AJW, Saxton AM, Olinick JB, Evans RM (2000) Correlations of stomatal conductance with hydraulic and chemical factors in several deciduous tree species in a natural habitat. *New Phytologist* 145: 483–500.

Beardsell MF, Cohen D (1975) Relationship between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. *Plant Physiology* 56: 207–212.

Beerling DJ (1997) Carbon isotope discrimination and stomatal responses of mature *Pinus sylvestris* L. trees exposed in situ for three years to elevated CO_2 and temperature. *Acta Oecologia* 18: 697–712.

Beerling DJ, McElwain JC, Osborne CP (1998) Stomatal responses of the "living fossil" *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. *Journal of Experimental Botany* 49: 1603–1607.

Berger A, Oren R, Schulze ED (1994) Element concentrations in the xylem sap of *Picea abies* (L.) Karst. seedlings extracted by various methods under different environmental conditions. *Tree Physiology* 14: 111–128.

Berryman CA, Eamus D, Duff GA (1994) Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. *Journal of Experimental Botany* 45: 539–546.

Bokhari UG (1976) Influence of temperatures, water stress, and nitrogen treatments on chlorophyll and dry matter of western wheatgrass. *Journal of Range Management* 29: 127–131.

Bond BJ, Kavanagh KL (1999) Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiology* 19: 503–510.

Boyer JS (1985) Water transport. *Annual Review of Plant Physiology* 36: 473–516.

Bradford KJ, Sharkey TD, Farquhar GD (1983) Gas exchange, stomatal behavior, and $\delta^{13}C$ values of the *flacca* tomato mutant in relation to abscisic acid. *Plant Physiology* 72: 245–250.

Brown K (2001) Xylem may direct water where it's needed. Science 291: 571–572.

Burke JJ, O'Mahony PJ (2001) Protective Role in acquired thermotolerance of developmentally regulated heat shock proteins in cotton seeds. *Journal of Cotton Science* 5: 174–183.

Castro-Diez P, Puyravaud JP, Cornelissen JHC, Villar-Salvador P (1998) Stem anatomy and relative growth rate in seedlings of a wide range of woody plant species and types. *Oecologia* 116: 57–66.

Chambers JL, Hinckley TM, Cox GS, Metcalf CL, Aslin RG (1985) Boundary-line analysis and models of leaf conductance for four oak-hickory forest species. *Forest Science* 31: 437–450.

Comstock JP (2000) Variation in hydraulic architecture and gas-exchange in two desert sub-shrubs, *Hymenoclea salsola* (T. & G.) and *Ambrosia dumosa* (Payne). *Oecologia* 125: 1–10.

Comstock JP, Mencuccini M (1998) Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T. & G.), a desert subshrub. *Plant, Cell & Environment* 21:1029–1038.

Coyea MR, Margolis HA (1992) Factors affecting the relationship between sapwood area and leaf area of balsam fir. *Canadian Journal of Forest Research* 22: 1684–1693.

Cousson A (1999) Pharmacological study of two potential Ca^{2+} signalling pathways within stomatal closing in response to abscisic acid in *Commelina communis* L.. *Plant Science* 145: 67–74.

Crombie DS, Hipkins MF, Milburn JA (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. *Australian Journal of Plant Physiology* 12: 445–453.

Cutler JM, Rains DW, Loomis RS (1977) The importance of cell size in the water relations of plants. *Physiologia Plantarum* 40: 255–260.

Davies WJ (1977) Stomatal responses to water stress and light in plants grown in controlled environments and in the field. *Crop Science* 17: 735–740.

Davies WJ, Wilson JA, Sharp RE, Osonubi O (1981) Control of stomatal behaviour in water-stressed plants. In: Jarvis PG, Mansfield TA (eds) Stomatal Physiology. pp 163–186. Cambridge University Press.

DeLucia EH, Sipe TW, Herrick J, Maherali H (1998) Sapling biomass allocation and growth in the understory of a deciduous hardwood forest. *American Journal of Botany* 85: 955–963.

DeMichele DW, Sharpe PJH (1973) An analysis of the mechanics of guard cell motion. *Journal Theoretical Biology* 41: 77–96.

DeMichele DW, Sharpe PJH (1974) A parametric analysis of the anatomy and physiology of the stomata. *Agricultural Meteorology* 14: 229–241.

Dörffling K, Tietz D, Streich J, Ludewig M (1979) Studies on the role of abscisic acid in stomatal movements. In: Skoog F (ed) Plant Growth Substances. pp 274–285. Springer Verlag, Berlin, Heidelberg, New York.

Eckert M, Biela A, Siefritz F, Kaldenhoff R (1999) New aspects of plant aquaporin regulation and specificity. *Journal of Experimental Botany* 50: 1541–1545.

Edwards M, Meidner H, Sheriff DW (1976) Direct measurements of turgor pressure potentials of guard cells. *Journal of Experimental Botany* 27: 163–171.

Ellenberg H (1998) Vegetation of Central Europe, including the Alps. Ecology, dynamics, historical aspects. Vol 5. Verlag Eugen Ulmer, Stuttgart. (In German)

Esau K (1965) Plant Anatomy. John Wiley & Sons Inc, New York, London, Sydney.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149: 78–90.

Franks PJ, Cowan IR, Farquhar GD (1998) A study of stomatal mechanics using the cell pressure probe. *Plant, Cell & Environment* 21: 94–100.

Franks PJ, Farquhar GD (2001) The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. *Plant Physiology* 125: 935–942.

Fromm J (1997) Hormonal physiology of wood growth in willow (*Salix viminalis* L.): effects of spermine and abscisic acid. *Wood Science & Technology* 31: 119–130.

Fuchs EE, Livingston NJ (1996) Hydraulic control of stomatal conductance in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Fanco] and alder [*Alnus rubra* (Bong.)] seedlings. *Plant, Cell & Environment* 19: 1091–1098.

Gehring CA, Irving HR, Parish RW (1990) Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. *Proceedings of the National Academy of Sciences*, USA 87: 9645–9649.

Glinka Z (1971) The effect of epidermal cell water potential on stomatal response to illumination of leaf disks of *Vicia faba*. *Physiologia Plantarum* 24: 476–479.

Grabov A, Blatt MR (1998) Co-ordination of signalling elements in guard cell ion channel control. *Journal of Experimental Botany* 49: 351–360.

Hartung W, Davies WJ (1991) Drought-induced changes in physiology and ABA. In: Davies WJ, Jones HG (eds) Abscisic Acid. Physiology and Biochemistry. pp 63–79. BIOS Scientific Publishers Ltd, Oxford.

Henson IE, Jensen CR, Turner NC (1989) Leaf gas exchange and water relations of lupins and wheat. III. Abscisic acid and drought-induced stomatal closure. *Australian Journal of Plant Physiology* 16: 429–442.

Heschel MS, Hausmann NJ (2001) Population differentiation for abscisic acid responsiveness in *Impatiei capensis* (*Balsaminaceae*). *International Journal of Plant Science* 162: 1253–1260.

Huang B, Nobel PS (1993) Hydraulic conductivity and anatomy along lateral roots of cacti: changes with soil water status. *New Phytologist* 123: 499–507.

Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* 24: 113–121.

Johansson I, Larsson C, Ek B, Kjellbom P (1996) The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca^{2+} and apoplastic water potential. *Plant Cell* 8: 1181–1191.

Johansson I, Karlsson M, Shukla VK, Chrispeels J, Larsson C, Kjellbom P (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *Plant Cell* 10: 451–459.

Johansson I, Karlsson M, Johanson U, Larsson C, Kjellbom P (2000) The role of aquaporins in cellular and whole plant water balance. *Biochimica et Biophysica Acta* 1465: 324–342.

Kaldenhoff R, Kölling A, Richter G (1993) A novel blue light- and abscisic acid-inducible gene of *Arabidopsis thaliana* encoding an intrinsic membrane protein. *Plant Molecular Biology* 23:1187–1198.

Kaldenhoff R, Kölling A, Richter G (1996) Regulation of the *Arabidopsis thaliana* aquaporin-gene AthH2 (PIP1b). *Journal of Photochemistry & Photobiology* 36: 351–354.

Kolb KJ, Sperry JS, Lamont BB (1996) A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *Journal of Experimental Botany* 47: 1805–1810.

Kolb TE, Stone JE (2000) Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. *Tree Physiology* 20: 1–12.

Lawson T, James W, Weyers J (1998) A surrogate measure of stomatal aperture. *Journal of Experimental Botany* 49: 1397–1403.

Legge NJ (1985) Anatomical aspects of water movement through stems of mountain ash (*Eucalyptus regnans* F. Muell.). *Australian Journal of Botany* 33: 287–298.

Liu L, McDonald AJS, Stadenberg I, Davies WJ (2001) Abscisic acid in leaves and roots of willow: significance for stomatal conductance. *Tree Physiology* 21: 759–764.

Lo Gullo MA, Salleo S (1988) Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. *New Phytologist* 108: 267–276.

Loewenstein NJ, Pallardy SG (1998a) Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. *Tree Physiology* 18: 421–430.

Loewenstein NJ, Pallardy SG (1998b) Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of canopy trees of three temperate deciduous angiosperms. *Tree Physiology* 18: 431–439.

Lõhmus E (1984) Forest Site Types in Estonia. Estonian Ministry of Forest Management and Nature Preservation, Tallinn. (In Estonian)

Ludlow MM, Fisher MJ, Wilson JR (1985) Stomatal adjustment to water deficits in three tropical grasses and a tropical legume grown in controlled conditions and in the field. *Australian Journal of Plant Physiology* 12: 131–149.

Mansfield TA, Atkinson CJ (1990) Stomatal behaviour in water stressed plants. In: Alscher RG, Cumming JR (eds) Plant Biology Vol **12**. Stress Responses in Plants: Adaptation and Acclimation Mechanisms. pp 241–264. Wiley-Liss Inc, New York.

Maurel C, Reizer J, Schroeder JI, Chrispeels MJ (1993) The vacuolar membrane protein γTIP creates water specific channels in *Xenopus* oocytes. EMBO Journal 12: 2241–2247.

Maurel C, Chrispeels MJ (2001) Aquaporins. A molecilar entry into plant water relations. *Plant Physiology* 125: 135–138.

Mederski HJ (1961) Determination of internal water status of plants by beta ray gauging. *Soil Science* 92: 143–146.

van Meeteren U, van Gelder H, van Ieperen W (2000) Reconsideration of the use of deionized water as vase water in postharvest experiments on cut flowers. *Postharvest Biology & Technology* 18: 169–181.

Meinzer FC, Goldstein G, Jackson P, Holbrook NM, Butierrez MV, Cavelier J (1995) Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic conductance properties. *Oecologia* 101: 514–522.

Melchior W, Steudle E (1993) Water transport in onion (*Allium cepa* L.) roots. Changes of axial and radial hydraulic conductivities during root development. *Plant Physiology* 101: 1305–1315.

Mertens RJ, Deus-Neumann B, Weiler EW (1985) Monoclonal antibodies for the detection and quantitation of the endogenous plant growth regulator abscisic acid. *FEBS Letters* 160: 269–272.

Moldau H, Sõber J, Sõber A (1993) Impact of acute ozone exposure on CO_2 uptake by two cultivars of *Phaseolus vulgaris* L. *Photosynthetica* 28: 133–141.

Nardini A, Pitt F (1999) Drought resistance of *Quercus pubescens* as a function of root hydraulic conductance, xylem embolism and hydraulic architecture. *New Phytologist* 143: 485–493.

Nardini A, Salleo S (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation. *Trees* 15: 14–24.

Nardini A, Salleo S, Tyree MT, Vertovec M (2000) Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annales of Forest Science* 57: 305–312.

Nardini A, Tyree MT, Salleo S (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. *Plant Physiology* 125: 1700–1709.

Nogués S, Allen DJ, Morison JIL, Baker NR (1998) Ultraviolet-B radiation effects on water relations, leaf development and photosynthesis in droughted pea plants. *Plant Physiology* 117: 173–181.

Osawa A (1990) Reconstructed development of stem production and foliage mass and its vertical distribution in Japanese larch. *Tree Physiology* 7: 189–200.

Passioura JB, Condon AG, Richards RA (1993) Water deficits, the development of leaf area and crop productivity. In: Smith JAC, Griffiths H (eds) Water Deficits. pp 253–264. BIOS Scientific Publishers Ltd, Oxford.

Peterlunger E, Buccella A, Iacono F (2000) Physiological changes in grapevine during adjustment to water stress: ABA, leaf gas exchanges and root hydraulic conductivity. *Acta Horticulturae* 526: 201–208.

Peuke AD, Jeschke WD, Hartung W (1994) The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long distance transport of abscisic acid depending on nitrogen nutrition and salt stress. *Journal of Experimental Botany* 45: 741–747.

Pharis RP, Jenkins PA, Aoki H, Sassa T (1981) Hormonal physiology of wood growth in *Pinus radiata* D. Don: effects of gibberellin A4 and the influence of abscisic acid upon (3H) gibberellin A4 metabolism. *Australian Journal of Plant Physiology* 8: 559–570.

Phillips N, Bond BJ, Ryan MG (2001) Gas exchange and hydraulic properties in the crowns of two tree species in a Panamanian moist forest. *Trees* 15: 123–130.

Quarrie SA, Jones HG (1977) Effects of abscisic acid and water stress on development and physiology of wheat. *Journal of Experimental Botany* 28: 192–203.

Qiu Q-S, Wang Z-Z, Zhang N, Cai Q-G, Jiang R-X (2000) Aquaporins in the plasma membrane of leaf callus protoplasts of *Actinidia deliciosa* var. *deliciosa* cv. Hayward. *Australian Journal of Plant Physiology* 27: 71–75.

Rahi M (1971) The characteristics of leaf anatomy and diffusion resistances. *Proceedings of the Estonian Academy of Sciences. Biology* 20: 84–94. [In Russian]

Rahi M (1973) An apparatus for observing components of leaf water potential. *Fiziologia Rastenii* 20: 215–221. [In Russian]

Rieger M, Litvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. *Journal of Experimental Botany* 50: 201–209.

Roberts SK, Snowman BN (2000) The effects of ABA on channel-mediated K^+ transport across higher plants roots. *Journal of Experimental Botany* 51: 1585–1594.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED (1994) Hydraulic and osmotic properties of spruce roots. *Journal of Experimental Botany* 45: 1413–1425.

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance and soil drought in *Betula occidentalis*. *Planta* 196: 357–366.

Sauter A, Davies WJ, Hartung W (2001) The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. *Journal of Experimental Botany* 52: 1991–1997.

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. *Annual Review of Plant Physiology & Plant Molecular Biology* 52: 627–658.

Schulte PJ, Gibson AC (1988) Hydraulic conductance and tracheid anatomy in six species of extant seed plants. *Canadian Journal of Botany* 66: 1073–1079.

Schurr U, Schulze E-D (1996) Effects of drought on nutrient and ABA transport in *Ricinus communis*. *Plant, Cell & Environment* 19: 665–674.

Sellin A (1994) Variation in Hydraulic Architecture of *Picea abies* (L.) Karst. Trees Grown Under Different Environmental Conditions (PhD Thesis). University of Tartu.

Sellin A (2001) Hydraulic and stomatal adjustment of Norway spruce trees to environmental stress. *Tree Physiology* 21: 879–888.

Sharpe PJH, Wu H, Spence RD (1987) Stomatal mechanics. In: Zeiger E, Farquhar D, Cowan IR (eds) Stomatal function. pp 91–114. Stanford University Press.

Shinozaki K, Yoda K, Hozumi K, Kiro T (1964) A quantitative analysis of plant form — the pipe model theory. I. Basic analysis. *Japanese Journal of Ecology* 14: 97–105.

Simpson GM (1981) Water Stress on Plants. Praeger, New York.

Slovik S, Hartung W (1992) Compartmental distribution and redistribution of abscisic acid in intact leaves. III. Analysis of the stress-signal chain. *Planta* 187: 37–47.

Sõber A (1992) Effect of ozone on the rehydration of bean leaves. *Proceedings of the Estonian Academy of Sciences. Biology.* 41: 35–43.

Sõber A (1996) Simultaneous measurement of leaf hydraulic and stomatal conductances by pressure bomb, gas exchange and β-gauge techniques. *Proceedings of the Estonian Academy of Sciences. Biology* 45: 35–43.

Sõber A (1997) Hydraulic conductance, stomatal conductance and maximal photosynthetic rate in bean leaves. *Photosynthetica* 34: 599–603.

Sõber AJ, Moldau HA (1977) An apparatus with separate conditioning of the plant and the leaf for determination of stomatal resistance and leaf water content. *Fiziologia Rastenii* 24: 1301–1307. [In Russian]

Sõber A, Noormets A, Karnosky DF, Isebrands JG, Dickson RE, Sõber J, Kull O (1999) Photosynthetic parameters in aspen grown with interacting elevated CO_2 and tropospheric ozone concentrations. Comparison of data from free air (FACE) and open-top experiments. In: IUFRO Workshop on Canopy Dynamics and Forest Management — a Missing Link? Estonia, Finland and Sweden, August 1–11, Book of Abstracts: p 20.

Sobrado MA (1997/98) Hydraulic conductance and water potential differences inside leaves of tropical evergreen and deciduous species. *Biologia Plantarum* 40: 633–637.

Sparks J, Black R (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiology* 19: 453–459.

Sperry JS, Donnelly JR, Tyree MT (1987) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment* 11: 35–40.

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *Journal of Experimental Botany* 44: 1075–1082.

Sperry JS, Ikeda T (1997) Xylem cavitation in roots and stems of Douglas-fir and white fir. *Tree Physiology* 17: 275–280.

Spollen WG, Sharp RE, Saab IN, Wu Y (1993) Regulation of cell expansion in roots and shoots at low water potentials. In: Smith JAC, Griffiths, H (eds) Water Deficits.

Plant Responses from Cell to Community. pp. 37–52. BIOS Scientific Publishers Ltd, Oxford.

Steudle E (1997) Water transport across plant tissue: Role of water channels. *Biology of the Cell* 89: 259–273.

Steudle E, Oren R, Schulze ED (1987) Water transport in maize roots. *Plant Physiology* 84: 1220–1232.

Steudle E, Meshcheryakov AB (1996) Hydraulic and osmotic properties of oak roots. *Journal of Experimental Botany* 47: 387–401.

Steudle E, Heydt H (1997) Water transport across tree roots. *Trees* 11: 239–255.

Steudle E, Peterson CA (1998) How does water get through roots? *Journal of Experimental Botany* 49: 775–788.

Stratton L, Goldstein G, Meinzer FC (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. *Plant, Cell & Environment* 23: 99–106.

Sutton F, Paul SS, Wang X-Q, Assmann SM (2000) Distinct abscisic acid signaling pathways for modulation of guard cell versus mesophyll cell potassium channels revealed by expression studies in *Xenopus laevis* oocytes. *Plant Physiology* 124: 223–230.

Sweet WJ, Morrison JC, Labavitch JM, Matthews MA (1990) Altered synthesis and composition of cell wall of grape (*Vitis vinifera* L.) leaves during expansion and growth-limiting water deficits. *Plant & Cell Physiology* 31: 407–414.

Tal M, Imber D (1970) Abnormal stomatal behaviour and hormonal imbalance in *flacca*, a wilty mutant of tomato. II Auxin and abscisic acid-like activity. *Plant Physiology* 46: 373–376.

Tardieu F, Lafarge T, Simonneau TH (1996) Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant, Cell & Environment* 19: 75–84.

Tenhunen JD, Hanano R, Abril M, Weiler EW, Hartung W (1994) Above- and below-ground environmental influences on leaf conductance of *Ceanothus thyrsiflorus* growing in a chaparral environment: drought response and the role of abscisic acid. *Oecologia* 99: 306–314.

Thomas H, Howarth CJ (2000) Five ways to stay green. *Journal of Experimental Botany* 51: 329–337.

Tichá I (1982) Photosynthetic characteristics during ontogenesis of leaves. 7. Stomatal density and sizes. *Photosynthetica* 16: 375–471.

Tognetti R, Minnocci A, Peñuelas J, Raschi A, Jones MB (2000) Comparative field water relations of three Mediterranean shrub species co-occurring at a natural CO_2 vent. *Journal of Experimental Botany* 51: 1135–1146.

Trejo CL, Clephan AL, Davies WJ (1995) How do stomata read abscisic acid signals? *Plant Physiology* 109: 803–811.

Turner NC, Heichel GH (1977) Stomatal development and seasonal changes in diffusive resistance of primary and regrowth foliage of red oak (*Quercus rubra* L.) and red maple (*Acer rubrum* L.). *New Phytologist* 78: 71–81.

Tyerman SD, Bohnert HJ, Maurel C, Steudle E, Smith JAC (1999) Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. *Journal of Experimental Botany* 50: 1055–1071.

Tyree MT (1997) The cohesion-tension theory of sap ascent: current controversies. *Journal of Experimental Botany* 48: 1753–1765.

Tyree MT, Kolb KJ, Rood SB, Patino S (1994) Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiology* 14: 455–466.

Tyree MT, Velez V, Dalling JW (1998) Growth dynamics of root and shoot hydraulic conductance in seedlings of five neotropical tree species: scaling to show possible adaptation to differing light regimes. *Oecologia* 114: 293–298.

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiology* 120: 11–21.

Veenendaal EM, Swaine MD, Lecha RT, Walsh MF, Abebrse IK, Owusu-Afriyie K (1996) Responses of West African forest tree seedlings to irradiance and soil fertility. *Functional Ecology* 10: 501–511.

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus (Fagaceae)* species along a climate gradient in NE Spain. *Trees* 12: 90–96.

Wagner F, Below R, de Klerk P, Dilcher DL, Joosten H, Kürschner WM, Visscher H (1996) A natural experiment on plant acclimation: lifetime stomatal frequency response of an individual tree to annual atmospheric CO_2 increase. *Proceedings of the National Academy of Sciences*, USA 93: 11705–11708.

Walters MB, Reich PB (2000) Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. *Ecology* 81: 1887–1901.

Wang J-W, Kao CH (1999) Endogenous abscisic acid levels are not linked to methyl jasmonate-promoted senescence of detached rice leaves. *Plant Growth Regulation* 28: 17–20.

Wilkinson S, Davies WJ (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell & Environment* 25: 195–210.

Woodward FI, Bazzaz FA (1988) The responses of stomatal density to CO_2 partial pressure. *Journal of Experimental Botany* 39: 1771–1781.

Wright STC (1977) The relationship between leaf water potential (Ψ_{leaf}) and the levels of abscisic acid and ethylene in excised wheat leaves. *Planta* 134: 183–189.

Wu H, Sharpe PJH, Spence RD (1985) Stomatal mechanics. III. Geometric interpretation of the mechanical advantage. *Plant, Cell & Environment* 8: 269–274.

Zhang SQ, Outlaw WHJr (2001) Abscisic acid introduced into the transpiration stream accumulates in the guard-cell apoplast and causes stomatal closure. *Plant, Cell & Environment* 24: 1045–1054.

Zimmermann MH (1978) Hydraulic architecture of some diffuse-porous trees. *Canadian Journal of Botany* 56: 2286–2295.

Zimmermann MH (1983) Xylem Structure and the Ascent of Sap. Springer-Verlag, Berlin.

Zimmemann MH, Milburn JA (1982) Transport and storage of water. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of Plant Physiology. Physiological Plant Ecology II. Vol. 12B. pp. 135–151. Springer-Verlag, Berlin, Heidelberg, New York.

Zwieniecki MA, Melcher PJ, Holbrook NM (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science* 291: 1059–1062.

SUMMARY IN ESTONIAN

Lehtpuuvõrsete hüdrauliline juhtivus ja õhulõhede juhtivus

Käesolevas doktoritöös on põhiliselt analüüsitud kuude Eestis väga arvukalt esindatud liiki kuuluvate puude võrsete veerežiimi olulisimaid karakteristikuid: võrse maksimaalset (ksüleemi juhtelementide kavitatsiooni poolt vähendamata) hüdraulilist juhtivust (L), õhulõhede juhtivust (veeauru suhtes) (g_s), õhulõhede tundlikkust lehe veepotentsiaali suurenemise suhtes (s_i) ja õhulõhede tundlikkust lehe veepotentsiaali vähenemise suhtes (s_d). Uuritavad puuliigid olid harilik vaher (*Acer platanoides* L.), harilik toomingas (*Padus avium* Mill.), harilik haab (*Populus tremula* L.), harilik tamm (*Quercus robur* L.), raagremmelgas (*Salix caprea* L.) ja harilik pärn (*Tilia cordata* Mill.). Töö põhieesmärgiks oli selgitada: 1) nende oluliste veerežiimi karakteristikute liigispetsiifilised väärtused mitmetes Eesti puudes; 2) millised teised võrsete karakteristikud on olulised hüdraulilise juhtivuse ning õhulõhede juhtivuse ja tundlikkuse liigispetsiifiliste väärtuste kindlaksmäärajad; 3) millised karakteristikud tingivad selle, et hüdrauliline juhtivus ning õhulõhede juhtivus ja tundlikkus erinevad sama liiki, aga erinevates kasvutingimustes (erinev kastmis- või valgustamisrežiim või mulla lämmastikusisaldus) kasvanud taimedes; 4) võrse hüdraulilise juhtivuse ja õhulõhede juhtivuse omavahelise balansseerituse mehhanismi: kas hüdraulilise juhtivuse ja õhulõhede juhtivuse positiivset korrelatsiooni põhjustab võrse hüdraulilise arhitektuuri ja õhulõhede ehituse vastavus ja/või abstsiishape.

Leiti, et nii L, g_s, s_i kui ka maksimaalse (küllastavas valguses ja küllastava süsihappegaasisisaldusega õhus) fotosünteesi (P_{max}) liigispetsiifiliste väärtuste suurenemise põhjal (noortes mõneaastastes taimedes) saab need puuliigid reastada järgmiselt: *Acer platanoides* < *Tilia cordata* < *Padus avium* = *Quercus robur* < *Salix caprea* = *Populus tremula*. s_d liigispetsiifilised väärtused vähenesid samas järjekorras. Seega põhjustavad geneetilised erinevused nende karakteristikute erinevusi juba noortes puudes, mille valgustatus jm kasvutingimused on samasugused.

Nii L, g_s, s_i kui P_{max} olid suuremad (võrreldes kontrollrühma puudega) iga liigi neis puudes, mille mulla lämmastikusisaldust oli suurendatud, ja väiksemad neis puudes, mis olid kasvanud mõõduka veedefitsiidi tingimustes. Seejuures ei erinenud nende karakteristikute väärtused enamasti oluliselt puudes, mis olid veedefitsiidi all kannatanud kevadest saadik (*ca* 2 kuud), ja neis puudes, mis olid veedefitsiidi all kannatanud vaid 2 päeva. s_d oli nende erinevate kasvutingimuste mõjul (kõigis liikides) muutunud vastupidises suunas. Mõnepäevane pimedas hoidmine oli esile kutsunud kõigi karakteristikute väärtuste vähenemise. Seega varieerivad kasvutingimused kõiki neid karakteristikuid ka lehtedes, mis ei ole enam intensiivse kasvu staadiumis. Kõige rohkem vähendas nende karakteristikute väärtuseid puude mõnepäevane pimedas hoidmine. Võrse hüdrauliline juhtivus oli kõigis liikides ligikaudu poole võrra vähenenud. Kuna pimedas ilmselt väheneb põhiliselt vaid rakkude sümplasti hüdrauliline juhtivus, järeldati, et hea veevarustuse ja valgustatuse puhul läbib ligikaudu 50% transpiratsioonivoolu veest ka lehtede mesofülli- ja epidermiserakkude sümplasti — lehtede veejuhtimisele mittespetsialiseerunud kudedes on apoplasti ja sümplasti hüdrauliline juhtivus siis ligikaudu võrdsed. See, et ilmselt ainult lehtede parenhüümsete kudede juhtivuse vähenemine põhjustas võrse (summaarse) hüdraulilise juhtivuse ulatusliku vähenemise, viitab sellele, et ka võrsetes (nii nagu ka juurtes) on veejuhtimisele mittespetsialiseerunud kudede hüdrauliline takistus tunduvalt suurem kui takistus (ilma õhuemboliteta) ksüleemis.

Võrse hüdraulilise juhtivuse liigispetsiifilised väärtused olid positiivses korrelatsioonis lehe keskroo suurimate juhtelementide raadiusega (neljandas astmes) (r^4) ning mesofülli- ja epidermiserakkude ruumalaga lehe pinnaühiku kohta (u). Sarnaselt võrse hüdraulilise juhtivusega olid ka r^4 ja u suuremad suurendatud lämmastikusisaldusega mullas kasvanud puude lehtedes ning enamasti väiksemad mõõduka veedefitsiidi tingimustes kasvanud lehtedes (kontrollrühma puudega võrreldes). Järeldati, et ksüleemi juhtelementide läbimõõt ning lehe mesofülli- ja epidermiserakkude ruumala on olulised nii hüdraulilise juhtivuse liigispetsiifiliste väärtuste kui ka erinevates keskkondades kasvavate (sama liiki) puude võrsete hüdraulilise juhtivuse varieeruvuse kujundamisel.

g_s ja s_i liigispetsiifilised väärtused olid positiivses korrelatsioonis õhulõhede õhupilu pikkuse (l) ning negatiivses korrelatsioonis sulgrakkude laiuse (z) ja sulgrakkude väliskülje pikkuse (l_d) liigispetsiifiliste väärtustega. s_d liigispetsiifiliste väärtuste korrelatsioon nende anatoomiliste karakteristikutega oli vastupidine: negatiivne õhupilu pikkuse ja positiivne sulgrakkude laiuse ja väliskülje pikkuse liigispetsiifiliste väärtustega. Seega on need õhulõhede anatoomilised karakteristikud (l, z ja l_d) ilmselt olulised õhulõhede juhtivuse ja tundlikkuse liigispetsiifilise taseme määrajad. Kuna õhupilu pikkus oli (nagu ka g_s ja s_i) suurem lämmastikurikkas mullas kasvanud puude lehtedes ja väiksem veedefitsiidi tingimustes kasvanud lehtedes, järeldati, et õhulõhede juhtivuse ja tundlikkuse varieerumine erinevates keskkondades kasvanud (sama liigi) puudes võib olla olulisel määral tingitud ka õhupilude pikkuse varieerumisest.

L, g_s ja s_i liigispetsiifilised väärtused olid positiivses ja s_d liigispetsiifilised väärtused negatiivses korrelatsioonis abstsiishappe kontsentratsiooniga ([ABA]) lehtedes. Võrse hüdrauliline juhtivus ja õhulõhede juhtivus vähenesid ja lehtede [ABA] suurenes põua ajal ning nii L, g_s kui ka s_i vähenesid, kui võrsetesse lisati abstsiishappelahust. Seega on lehtede [ABA] ilmselt oluline faktor nii võrsete hüdraulilise juhtivuse ning õhulõhede juhtivuse ja tundlikkuse väärtuste liigispetsiifilise taseme kindlaksmääramisel kui ka nende karakteristikute varieeruvuse kujundamisel erinevates keskkondades kasvavatel (sama liiki) puudel.

Kui lehtede veepotentsiaali tunduvalt suurendati, siis õhulõhede tundlikkus ABA suhtes vähenes kõigis liikides, st õhulõhed ei sulgunud pärast suure kontsentratsiooniga ABA-lahuste lisamist võrsetesse. Põua ajal oli õhulõhede tundlikkus [ABA] suhtes oluliselt suurenenud *Tilia cordata* lehtedes, kuid teise uuritud liigi — *Populus tremula,* lehtedes mitte. Järeldati, et õhulõhede ABA-tundlikkuse muutmine on oluline meetod õhulõhede juhtivuse kohandamisel keskkonnatingimustega, aga selle mooduse osakaal on liigiti erinev.

Võrse hüdrauliline juhtivus korreleerus mitte ainult õhulõhede juhtivusega, vaid ka õhulõhede tundlikkusega lehe veepotentsiaali muutumise suhtes. *L* korreleerus positiivselt s_i-ga ja negatiivselt s_d-ga.

Õhulõhede juhtivus ja tundlikkus muutusid oluliselt juba nii väikese kontsentratsiooniga abstsiishappelahuste lisamisel võrsesse, mis hüdraulilist juhtivust veel ei muutnud. Seega ei ole täiskasvanud lehtede [ABA] hetkeväärtused ilmselt hüdraulilise ja õhulõhede juhtivuse vastavuse kujundamisel eriti olulised.

Anatoomilised karakteristikud, mis ilmselt mõjutavad hüdraulilist juhtivust lehes (r^4 ja u), korreleerusid nende anatoomiliste karakteristikutega, mis ilmselt mõjutavad õhulõhede juhtivust (l, z ja l_d). Järeldati, et nende anatoomiliste karakteristikute korrelatsioon võib olla hüdraulilise juhtivuse ning õhulõhede juhtivuse ja tundlikkuse vahelise korrelatsiooni üks olulisi põhjuseid.

Kõik need anatoomilised karakteristikud korreleerusid ka abstsiishappe kontsentratsiooniga lehtedes. Kuna on teada, et ABA avaldab lehtede arengule suurt mõju, oletati, et hüdraulilise juhtivuse ja õhulõhede juhtivuse vahelist korrelatsiooni tingib siiski ka ABA, kuid põhiliselt kaudsemal viisil: [ABA] ilmselt koordineerib noortes, intensiivse kasvu staadiumis olevais lehtedes nende anatoomiliste karakteristikute arengut, mis mõjutavad hüdraulilist juhtivust, õhulõhede juhtivust ja tundlikkust ka täiskasvanud lehtedes.

Põuatundlikumatel ja valgusnõudlikumatel liikidel olid võrse hüdrauliline juhtivus, õhulõhede juhtivus, õhulõhede tundlikkus lehe veesisalduse suurenemise suhtes ja fotosünteesi maksimaalne intensiivsus suuremad ning õhulõhede tundlikkus lehe veesisalduse vähenemise suhtes väiksem kui põua- ja varjutaluvamatel liikidel. Järeldati, et nende karakteristikute väärtuste liigispetsiifiline tase ilmselt määrabki oluliselt liigi põuatundlikkuse või valgusnõudlikkuse.

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PAPERS

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Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species

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Abstract

The relationship between shoot hydraulic conductance (L) and stomatal sensitivity to changes in leaf water status was studied in the saplings of six deciduous tree species. L increased significantly in sequence: *Acer platanoides* < *Tilia cordata* < *Padus avium* = *Quercus robur* < *Salix caprea* = *Populus tremula*. L was higher in the trees grown in soil with a higher nitrogen content and lower in the trees grown under mild water stress or kept in darkness for several days. L was higher in July than in September in all the species. L correlated positively with maximum photosynthesis, stomatal conductance and stomatal sensitivity to an increase in leaf water potential, but negatively with stomatal sensitivity to a decrease in leaf water potential. The correlations between L and any other parameter were approximated by three different curves: data for water-stressed plants fit to the first, data for plants kept in darkness fit to the second and all the other data fit to the third curve. The reasons of the differences of shoot hydraulic conductance in the different experimental sets and the mechanisms which may cause the correlation between L and the other characteristics are discussed.

Additional key words: *Acer platanoides, Padus avium, Populus tremula, Quercus robur, Salix caprea, Tilia cordata.*

Introduction

Shoot hydraulic conductance is an essential characteristic of the plant water regime because it influences the water supply. The hydraulic conductance of the xylem correlates with some of its anatomical parameters (Zimmermann 1983, Calkin *et al.* 1986, Ewers *et al.* 1990) and it changes also during short-term variations in water balance (Cochard 1992, Yang and Tyree 1993, Zotz *et al.* 1994, Kavanagh *et al.* 1999). Usually the hydraulic conductance of stems (without leaves) is directly measured (Sellin 1993, Tognetti *et al.* 1999a,b). The data of conductance in shoots with leaves are usually obtained by calculations using transpiration rates (Comstock and Mencuccini 1998, Bond and Kavanagh 1999). There is meagre data about hydraulic conductance of tree shoots obtained by direct measurements of the liquid water flow through the shoots with leaves (Sobrado 1997/1998, Wei *et al.* 1999a,b).

The path of water through the leaf to evaporation sites in intercellular spaces has been an object of discussions for a long time. The most widely accepted point of view is that water flows through the xylem and then through the parenchymal tissue before evaporation to the intercellular spaces (Boyer 1985, Steudle 1997). The diameters of the xylem conducting elements are relatively small in leaves (Esau 1965), therefore the xylem hydraulic conductance may also be low in the leaves. The hydraulic conductance in the mesophyll may not be much lower than in the xylem of the leaves. Studies which show that tree shoot hydraulic conductance can be limited by the hydraulic conductance in parenchymal cells in leaves are rare.

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Abbreviations: ABA - abscisic acid; E - transpiration rate; g_s - stomatal conductance; I - irradiance; L - hydraulic conductance; p - pressure; P_{max} - maximum photosynthetic rate; s_d - stomatal sensitivity to a decrease in leaf water potential; s_i - stomatal sensitivity to an increase in leaf water potential; v_d - maximum relative rate of stomatal closure; v_i - maximum relative rate of stomatal opening; w - leaf water content per area; x - leaf relative wet mass per area.

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The hydraulic conductance in the cell walls is several orders of magnitude higher than in the symplast (Fiscus 1986, Rüdinger *et al.* 1994, Steudle and Meshcheryakov 1996, Steudle and Heydt 1997). However, the volume of the cell walls in the leaf is lower than the volume of symplasts and vacuoles (Esau 1965). Therefore, the hydraulic conductance of these two compartments in the mesophyll may be comparable.

The rate of water loss from the leaf is dependent on stomatal conductance. A positive correlation exists between hydraulic conductance and stomatal conductance (Reich and Hinckley 1989, Jones and Sutherland 1991, Sõber 1997, Bond and Kavanagh 1999, Tognetti *et al.* 1999a,b). It is resonable to suppose, that the hydraulic conductance may be also correlated with the other essential stomatal parameter: stomatal sensitivity to changes of leaf water potential. Data about the correlation between hydraulic conductance and stomatal sensitivity are available only for the bean (*Phaseolus vulgaris* L.) (Sõber 1996, Sõber 1997). In these studies, a positive correlation between hydraulic conductance and stomatal sensitivity to an increase in the leaf water potential was found.

The main questions asked in the present work were: *1*) Does there exist a correlation between shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes in the leaf water status in deciduous trees? *2*) Can changes which arise in the mesophyll during the water stress or in darkness influence the hydraulic conductance in deciduous tree shoots?

Materials and methods

Plants: The species studied in this work were: *Acer platanoides* L., *Tilia cordata* Mill., *Padus avium* Mill., *Quercus robur* L., *Salix caprea* L. and *Populus tremula* L. In the first series of experiments the trees were studied in the course of seasonal developement. The saplings were grown in Tartu, Estonia, (58°22′ N and 26°44′ E), in a small stand, in clay loam, in full sunlight. All the saplings were 8 - 10 years old. Their terminal branches (about 1 m tall) were cut under the water and were brought (the base of each branch remained submerged) to the laboratory in the late evening of the previous day before the experiment. The experiments were done in July and in September 1997.

In the second series of experiments, the influence of several growth conditions was studied. The pots with 3 year old saplings were brought to a greenhouse. The complex fertilizer was given to all the saplings. The relative content of the elements in the complex fertilizer was: N 10, P 7, K 16, Fe 0.6, Cu 0.4, Mn 0.08, Mo 0.08, Zn 0.07 and B 0.07 parts. Experiments were done in July 1998.

Data from eight experimental sets for each species (two sets from the first and six sets from the second series of experiments) were compared in this study. Some greenhouse-sets of *P. tremula*, *S. caprea* and *Q. robur* are absent because too many plants of these species (characterized by relatively insensitive stomata to a decrease in the leaf water content) did not survive transplanting to the pots.

Apparatus: The initial version of the apparatus is described by Sõber and Moldau (1977). The apparatus enables the simultaneous measurement of transpiration rate (by micropsychrometer), leaf temperature (by infrared termometer), photosynthesis rate (by gas analyzer *LI - 6262*, *Li-Cor*, Lincoln, USA) and the relative changes of leaf water content (by β-gauge technique) of the intact leaf in leaf chamber. The data are registered by computer. The temperature of the leaf chamber was held at 25 °C, the relative air humidity was 50 - 60 %, CO_2 pressure was near the normal ambient (345 μmol mol^{-1}) and the photosynthetic photon flux density was 1020 μmol m^{-2} s^{-1}. The pressure chamber was added to the system and leaf water content was incrased by means of the pressure chamber technique, for details see Rahi (1973).

Experiments: *a*) To observe an increase in the leaf water content a short (10 - 15 cm) shoot was cut under the water from the bigger branch and one leaf was put in the leaf chamber. The shoot was fixed in the water-filled pressure chamber so that the cut end of the shoot was in the water. After the stabilization of stomatal conductance, the CO_2 content of the air in the leaf chamber was quickly raised to 1760 μmol mol^{-1} for 4 min to detect values of maximum (independent of air CO_2 content) photosynthetic rate (P_{max}). When stomatal conductance was stabilized again at natural CO_2 content, the pressure in the pressure chamber was quickly raised from 0 to 0.2 MPa. The experiment was finished when water droplets appeared on the abaxial surface of the leaf. If there was no infiltration after 15 min of the pressure increase, the pressure in chamber was quickly raised to 0.4 MPa and then to 0.7 MPa (if needed), until a dense network of water droplets was seen on the abaxial surface of the leaf. For details see also Sõber (1996) and Sõber (1997).

b) To observe a decrease in the leaf water content the leaf was in the leaf chamber, the end of the shoot was in a water-vessel. After the stabilization of the stomatal conductance, the leaf petiole was cut. The experiment was finished when stomatal conductance reached values near zero.

The leaf part, which had been in the leaf chamber was cut out and its fresh mass was determined in both experiments. The dry mass of the leaf segment was determined after drying it for two days at 80 °C.

Calculations: Stomatal conductance (for water vapour) (g_s) [mol m^{-2} s^{-1}] was calculated using measured values of transpiration rate and leaf temperature, according to Sõber and Moldau (1977).

Stomatal sensitivity to a decrease in leaf water potential (s_d) [% s^{-1}] was defined as a relative rate of stomatal closure:

$$s_d = v_d \times A \tag{1}$$

where v_d is the maximum relative rate of stomatal closure [% s^{-1}] and A is a scaling coefficient which transforms v_d to a common transpiration rate;

$$v_d = [\Delta g_s/(\Delta t \times g_{sa})] \times 100 \tag{2}$$

where Δg_s is the difference of stomatal conductances in the region of maximum rate of stomatal closure; Δt is the time interval [s], corresponding to Δg_s, and g_{sa} is an average stomatal conductance during Δt.

The relative rate of stomatal closure was determined, because stomatal conductance declined after the petiole was cut almost exponentially with time. Coefficient A was determined as:

$$A = E_{aa}/E_a \tag{3}$$

where E_a is the average transpiration rate [mol m^{-2} s^{-1}] during Δt and E_{aa} is the average of E_a for all the experiments (both the experiments of decreasing and increasing the leaf water potential). Coefficient A was defined by transpiration rates, because water potential probably dropped more rapidly after the leaf detachment in the cases of a higher transpiration rate. The maximum relative rate of stomatal closure was in a positive correlation with the transpiration rate (data not shown).

Stomatal sensitivity to an increase in leaf water potential (s_i) [% s^{-1}] was defined as a relative rate of stomatal opening:

$$s_i = v_i \times B \tag{4}$$

where v_i is the maximum relative rate of stomatal opening [% s^{-1}] and B is the coefficient which transforms v_i to a common transpiration rate and the pressure increase in the pressure chamber.

The value of v_i was obtained as:

$$v_i = \{\Delta g_s/[\Delta t \times (g_{smax} - g_{sa})]\} \times 100 \tag{5}$$

where g_{smax} is the maximum value of stomatal conductance after the pressure increase in the pressure chamber. The relative rate of stomatal opening was used because the opening of stomata after the pressure increase was also almost exponential with time.

B was obtained as:

$$B = (E_a \times \Delta p_{ra})/(E_{aa} \times \Delta p_r) \tag{6}$$

where Δp_r is the pressure increase in the pressure chamber [Pa], inducing an increase in leaf water potential; Δp_{ra} is the average of Δp_r for all the experiments. Coefficient B was defined by the transpiration rates and pressure increases, because the rate of water potential increase in the leaf is probably higher when the transpiration rate is lower and when the applied pressure is higher. Several aspects of the method of calculating the stomatal sensitivities were discussed by Sõber (1997) and Sõber and Sild (1999).

The shoot hydraulic conductance (L) [g m^{-2} s^{-1} Pa^{-1}] was calculated by the formula:

$$L = [(\Delta w/\Delta t) + E_a]/\Delta p \tag{7}$$

where Δp is the pressure difference at the path of the water from the pressure chamber to the intercellular spaces of the leaf. It was assumed that the water potential in the leaf intercellular spaces equals zero in the infiltrating leaves, and therefore Δp was equal to the pressure in the pressure chamber. $\Delta w/\Delta t$ is the maximum rate of increase in the leaf water content per leaf area [g m^{-2} s^{-1}] during leaf infiltration. Usually $\Delta w/\Delta t > E_a$ in our experiments. The relative values of leaf wet mass per area (x) were obtained as:

$$x = (\ln I - \ln I_b)/(\ln I_0 - \ln I_b) \tag{8}$$

where I_0 is irradiance in the absence of the leaf in the leaf chamber; I and I_b are current value of irradiance behind the leaf and the irradiance at the beginning of the experiment, respectively. The leaf absolute water content per area [g m^{-2}] was obtained as:

$$w = [(x \times w_{we})/x_e] - w_d \tag{9}$$

where x_e is the leaf relative wet mass per area at the end of the experiment, w_{we} is leaf absolute wet mass per area [g m^{-2}] at the end of the experiment, and w_d is leaf dry mass per area [g m^{-2}]. For other details see Mederski (1961), Sõber (1992), Moldau *et al.* (1993) and Sõber (1997).

Results

Shoot hydraulic conductance (L) increased significantly in sequence: *Acer platanoides* < *Tilia cordata* < *Padus avium* = *Quercus robur* < *Salix caprea* = *Populus tremula* (Fig. 1*A*). L was higher in July than in September for all the species. L was slightly higher in trees grown in soil with a higher nitrogen content for most of the species (*S. caprea* was an exception). L was lower in the trees grown under mild water stress for all the species. L was not very different between the trees which had been grown under a mild water stress since spring and the trees which suffered under the water stress for a only few days. L was the lowest in the trees kept in darkness and it was lower in the trees which were in darkness longer (6 d) compared to the trees which had been in darkness for 4 d (Fig. 1*A*). The relative decrease of L after a short-term mild water deficit or after keeping the trees in darkness was quite similar for the different species (Fig. 2).

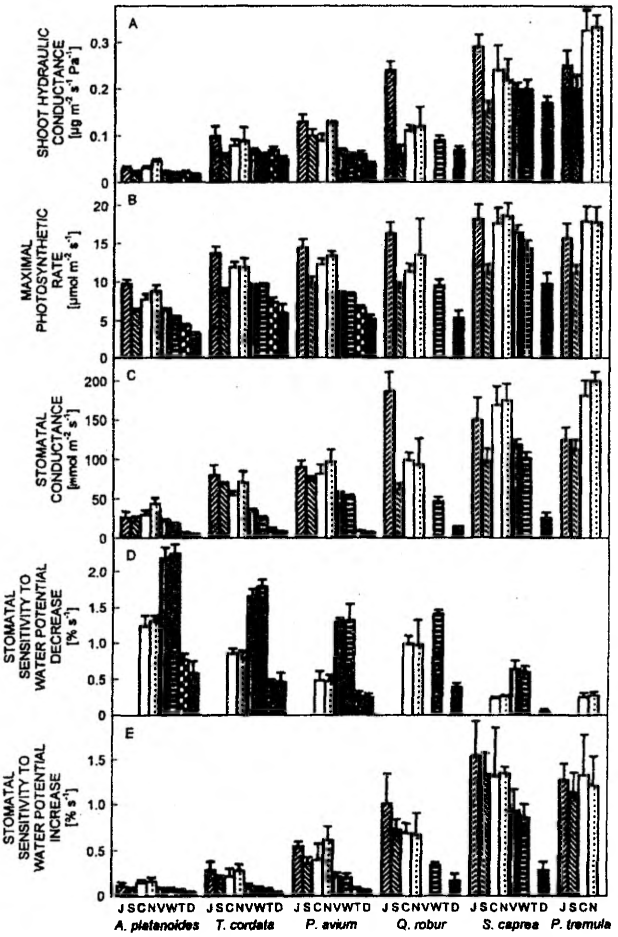


Fig. 1. Shoot hydraulic conductance (*A*), maximum photosynthetic rate (*B*), stomatal conductance (*C*), stomatal sensitivity to water potential decrease (*D*) and stomatal sensitivity to water potential increase (*E*). Means (n = 3 - 7) ± SE. Labelling: J - shoots from stand, July; S - shoots from stand, September; C - shoots from greenhouse, control; N - shoots from greenhouse, nitrogen-fertilized; V - shoots from greenhouse, water-stressed for 2 d; W - shoots from greenhouse, water-stressed since spring; T - shoots from greenhouse, kept in darkness for 4 d; D - shoots from greenhouse, kept in darkness for 6 d.

The maximum photosynthetic rate (at saturating concentration of carbon dioxide) (P_{max}), stomatal conductance at the beginning of the experiment (before changing of the water potential of the leaf) (g_{s0}), stomatal sensitivity to a decrease in leaf water potential (s_d) and stomatal sensitivity to an increase in leaf water potential (s_i) also varied between the species and between the experimental sets within the species (Figs. 1*B*-*E*). In general, P_{max}, g_{s0} and s_i varied in parallel with L: they were the lowest in *A. platanoides* and the highest in *P. tremula* and *S. caprea*. They were higher in July than in September and usually somewhat higher in trees grown in soil with a higher nitrogen content. P_{max}, g_{s0} and s_i were lower in the trees grown under mild water stress and still more significantly lower in the trees kept in darkness (Figs. 1*A*,*B*,*C*,*E*).

In most cases, s_d decreased (Fig. 1*D*) when L and the other parameters increased (Figs. 1*A*,*B*,*C*,*E*): s_d was the lowest in *P. tremula* and *S. caprea* and the highest in *A. platanoides* and higher in the trees grown under mild water stress. But s_d was also lower in all the trees kept in darkness (compared with the trees of the control set) (Fig. 1). The absolute differences in s_d between the experimental sets inside the species were more pronounced in the species characterized with a relatively high s_d in the control set (Fig. 1*D*).

The both sensitivities, s_i and s_d, were almost equal in the well-watered trees of *P. avium* and *Q. robur*. s_d was many times higher than s_i in the species characterized with a low L (*A. platanoides* and *T. cordata*) and s_i was several times higher than s_d in the species characterized with a high L (*S. caprea* and *P. tremula*). The differences between s_d and s_i were the greatest in *A. platanoides*, the species with the lowest L (Figs. 1*A*,*D*,*E*).

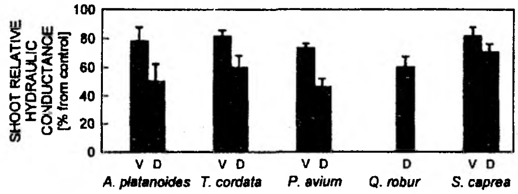
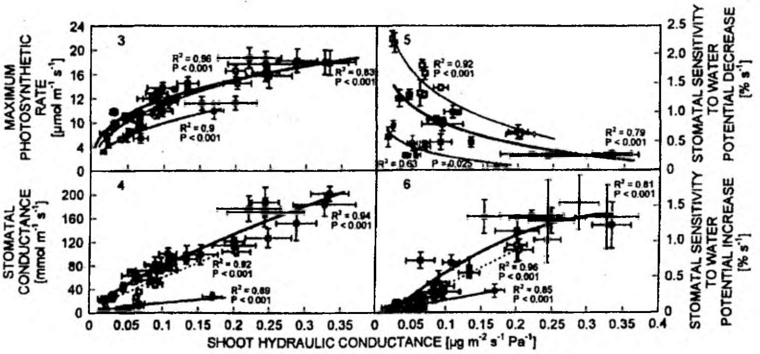


Fig. 2. Shoot relative hydraulic conductance after mild water stress for two days or darkness for six days (compared with shoot hydraulic conductance of control-set of the species) (means (n = 4 - 7) ± SE). Labelling as in Fig. 1.



Figs. 3 - 6. Relationships between shoot hydraulic conductance and maximum photosynthetic rate (Fig. 3), stomatal conductance (Fig. 4), stomatal sensitivity to water potential decrease (Fig. 5) and stomatal sensitivity to water potential increase (Fig. 6). Means ± SE; n = 3 - 7). Figs. 3, 4, 6: upper curve - well-watered trees, not kept in darkness (C-, N-, J- and S-set); middle curve - water-stressed trees (V- and W-set); lower curve - trees, kept in darkness (T- and D-set). Fig. 5: middle curve - well-watered trees; upper curve - water-stressed trees; lower curve - trees kept in darkness.

L was positively correlated with P_{max} (Fig. 3), g_{s0} (Fig. 4) and s_i (Fig. 6), and negatively with s_d (Fig. 5). Keeping in darkness for several days and the mild water deficit changed the correlation curves. Keeping in darkness lowered P_{max}, g_{s0}, s_i, and s_d significantly more than L (Figs. 3 - 6)). Mild water stress affected s_d and s_i differently: s_d (compared at some equal L values) was significantly increased (Fig. 5), but s_i was slightly decreased (Fig. 6).

Discussion

As shoot hydraulic conductance was variable between different tree species grown in the same conditions, the role of genetical factors in its determination may be strong. One important reason for the differences in L may be the different structure of the xylem in the trees. It is found that the largest conducting elements in the xylem are considerably smaller in maple, than in aspen (Holdheide 1951, Carlquist 1988). Also, the xylem conducting elements are smaller in the plants grown under water stress (Doley and Leyton 1968, Bissing 1982, Arnold and Mauseth 1999) and wider in plants grown in nutrient-rich soil (Shan and Mehta 1978). Hydraulic conductance in the xylem vessels is in a positive correlation with the diameter of the vessels (Calkin *et al.* 1986). Thus, it is probable that the differences of L between the species and experimental sets were caused partially by the different hydraulic conductance of their xylem.

We found that L in the tree shoots changed significantly during only a few days of mild water stress or starvation in darkness. Cavitation in the xylem vessels (Sperry *et al.* 1994, Zwienieckin and Holbrook 1998, Magnani *et al.* 2000) can be one important reason for the decrease of L in water-stressed plants and at the end of the growing season. But cavitation is unprobable in the well-watered trees in darkness and there are no data about other such rapid changes in the xylem vessels during such a short time. It is known that several detrimental changes take place in the other tissues of leaves during starvation (Neumann *et al.* 1989, Pell and Dann 1991). Thus, it is possible that the decrease of L was caused by the changes of the parenchymal tissues in leaves. Consequently, it was probable that hydraulic conductance in leaf parenchymal tissues affected the values of shoot hydraulic conductance in trees in some cases.

What may be the mechanism of change of hydraulic conductance in the mesophyll in darkness? There are no data about such rapid changes of anatomy in the parenchymal tissues of full-grown leaves, but several changes can take place in physiology. Changes in hydraulic conductance in leaves accompany variations in apoplastic pH (Sõber and Aasamaa 1998). The polysacharide cell wall is known to be a sparse structure, with large holes between chains of the polymers (Brett and Waldron 1996). In light of the present knowledge of cell wall physiology, it is unprobable that rapid changes in water permeability of such a sparse, well water-permeable structure take place. Variations in apoplastic pH have a rapid influence on the structures of the plasmalemma (Michelet and Boutry 1996). The revolutionary discoveries of the last decade have shown that rapid changes in hydraulic conductance in the symplast can be induced by changing the conductance of aquaporines - proteins, that form channels for water in plasmalemma and tonoplast (Fray *et al.* 1994, Maggio and Joly 1995, Steudle and Henzler 1995, Steudle and Frensch 1996, Maurel 1997, Schütz and Tyerman 1997, Steudle 1997, Eckert *et al.* 1999). The functioning of the water channels needs energy (Johansson *et al.* 1998, Tyerman *et al.* 1999). Therefore, the reason for a decrease in L may be the lack of energy to keep aquaporins in working order in the starving plants.

Thus, it is probable that the rapid changes in shoot hydraulic conductance were caused by changes in the symplasts of the leaf mesophyll during light deprivation. Therefore, the cell-to-cell pathway may be important in the leaves of deciduous trees.

We found that the relative decrease of L during darkness was similar in the different species. If the hydraulic conductance of symplast was close to zero after six days in darkness (about 50 % of control) reflects the hydraulic conductance in the leaf apoplast. It can be concluded that the proportion of symplastic and apoplastic components of transpirational flow is about 1:1 in the non-stressed leaves and this proportion is not very different in the tree species.

The positive correlation between L and the stomatal characteristics g_{s0} and s_i and the negative correlation between L and s_d occurred in most cases. One reason for the correlation between L and s_i may be that the water flows more rapidly in the shoots which have a higher L and the leaf water potential rises also more rapidly, and therefore the stomatal opening is more rapid. One of the reasons for the negative correlation between L and s_d may be similar: in the shoots which have higher L, the water flows to the stomatal region more rapidly and the water potential of the epidermis is lowering at a lower rate. For this reason, the stomatal closure proceed at a lower rate.

In addition, the correlation between L and the stomatal characteristics may be caused by some other physiological mechanisms. The abscisic acid (ABA) content increases in leaves when a water deficit arises in the soil and/or the leaf water potential decreases (Simpson 1981, Mansfield and Atkinson 1990, Jarvis and Davies 1997, Wilkinson and Davies 1997, Niinemets *et al.* 1999). The ABA content also increases in leaves in the course of senescence during the growing season (Laurière 1983, Nooden 1988, Pell and Dann 1991, Smart 1994, Wingler *et al.* 1998). The decrease in stomatal conductance is induced by the addition of ABA, even for a well-watered leaf (Cousson 1999, Leymarie *et al.* 1999). ABA is responsible for changing the activity of membrane channels (Owen 1988, Hahn 1996, Du *et al.* 1997, Roberts 1998, Barkla *et al.* 1999, Blatt 1999, Cousson 1999, Frank *et al.* 2000, Li *et al.* 2000). The addition of ABA induces increase of water permeability of roots (Cram and Pitman 1972, Tardieu and Davies 1993), it is possible, that ABA rises the water permeability of membranes in roots (Pitman and Wellfare 1978). But many effects of ABA are contrary in leaves and roots (Hsiao and Jing 1987, Saab *et al.* 1990, Hetherington and Quatrano 1991, Jackson 1991, Spollen *et al.* 1993). Therefore, we hypothesize that probably L, g_{s0}, and s_i are low and s_d is high in the shoots characterized with a high content of ABA.

The trees kept in darkness for several days had very low values of all the measured parameters. The reactions for changing and maintaining stomatal conductance in leaves are energy-consuming (MacRobbie 1981, Hedrich and Schroeder 1989, Armstrong and Blatt 1995). Probably s_d and s_i fell in the leaves because there was not enough energy and also the shortage of sugars as osmoticum. The starvation stress exerts a notably stronger influence on the stomata and photosynthesis than to L. We suppose, that the reason for this difference may be that one part of the L - the apoplastic component of water flow did not decrease in darkness; apoplastic conductivity is probably rather insensitive to physiological conditions in the leaf.

References

Armstrong, F., Blatt, M.R.: Evidence for K^+ channel control in *Vicia* guard cells coupled by G-proteins to a 7TMS receptor mimetic. - Plant J. **8**: 187-198, 1995.

Arnold, D.H., Mauseth, J.D.: Effects of environmental factors on development of wood. - Amer. J. Bot. **86**: 367-371, 1999.

Barkla, B.J., Vera-Estrella, R., Maldonado-Gama, M., Pantoja, O.: Abscisic acid induction of vacuolar H^+-ATPase activity in *Mesembryanthemum crystallinum* is developmentally regulated. - Plant Physiol. **120**: 811-820, 1999.

Bissing, D.R.: Variation in qualitative anatomical features of the xylem of selected dicotyledonous woods in relation to water availability. - Bull. Torrey bot. Club **109**: 371-384, 1982.

Blatt, M.R.: Reassessing roles for Ca^{2+} in guard cell signalling. - J. exp. Bot. **50**: 989-999, 1999.

Bond, B.J., Kavanagh, K.L.: Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. - Tree Physiol. **19**: 503-510, 1999.

Boyer, J.S.: Water transport. - Annu. Rev. Plant Physiol. **36**: 473-516, 1985.

Brett, C.T., Waldron, K.W.: Physiology and Biochemistry of Plant Cell Walls. - Chapman & Hall, London 1996.

Calkin, H.W., Gibson, A.C., Nobel, P.S.: Biophysical model of xylem conductance in tracheids of the fern *Pteris vittata*. - J. exp. Bot. **37**: 1054-1064, 1986.

Carlquist, S.: Comparative Wood Anatomy. - Springer-Verlag, Berlin 1988.

Cochard, H.: Vulnerability of several conifers to air embolism. - Tree Physiol. **11**: 73-83, 1992.

Comstock, J., Mencuccini, M.: Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T. & G.), a desert subshrub. - Plant Cell Environ. **21**: 1029-1038, 1998.

Cousson, A.: Pharmacological study of two potential Ca^{2+} signalling pathways within stomatal closing in response to abscisic acid in *Commelina communis* L.. - Plant Sci. **145**: 67-74, 1999.

Cram, W.J., Pitman, M.G.: The action of abscisic acid on ion uptake and water flow in plant roots. - Aust. J. biol. Sci. **25**: 1125-1132, 1972.

Doley, D., Leyton, L.: Effects of growth regulating substances and water potential on the development of secondary xylem in *Fraxinus*. - New Phytol. **67**: 579-594, 1968.

Du, Z., Aghora, K., Outlaw, W.H., Jr.: *In vivo* phosphorylation of phosphoenylpyruvate carboxylase in guard cells of *Vicia faba* L. is enhanced by fusiococcin and suppressed by abscisic acid. - Arch. Biochem. Biophys. **337**: 345-350, 1997.

Eckert, M., Biela, A., Siefritz, F., Kaldenhoff, R.: New aspects of plant aquaporin regulation and specificity. - J. exp. Bot. **50**: 1541-1545, 1999.

Esau, K.: Plant Anatomy. - John Wiley & Sons Inc., New York - London - Sydney 1965.

Ewers, F.W., Fisher, J.B., Chiu, S.-T.: A survey of vessel dimensions in stems of tropical lianas and other growth forms. - Oecologia **84**: 544-552, 1990.

Fiscus, E.L.: Diurnal changes in volume and solute transport coefficients of *Phaseolus* roots. - Plant Physiol. **80**: 752-759, 1986.

Frank, W., Munnik, T., Kerkmann, K., Salamini, F., Bartels, D.: Water deficit triggers phospholipase D activity in the resurrection plant *Craterostigma plantagineum*. - Plant Cell **12**: 111-124, 2000.

Fray, R.G., Wallace, A., Grierson, D., Lycett, G.W.: Nucleotide sequence and expression of a ripening and water stress-related cDNA from tomato with homology to the MIP class of membrane channel proteins. - Plant mol. Biol. **24**: 539-543, 1994.

Hahn, M.G.: Signal perception at the plasma membrane: binding proteins and receptors. - In: Smallwood, M., Knox, J.P., Bowles, D.J. (ed.): Membranes: Specialized Functions in Plants. Pp. 103-136. BIOS Scientific Publishers, Oxford 1996.

Hedrich, R., Schroeder, J.J.: The physiology of ion channels and electrogenic pumps in higher plants. - Annu. Rev. Plant Physiol. **40**: 539-569, 1989.

Hetherington, A.M., Quatrano, R.S.: Mechanisms of action of abscisic acid at the cellular level. - New Phytol. **119**: 9-32, 1991.

Holdheide, W.: Anatomie mitteleuropäischer Gehölzrinden (mit mikrophotographischen Atlas). - In: Freund, H. (ed.): Handbuch der Mikroskopie in der Technik **5**. Pp. 193-367. Umschau Verlag, Frankfurt am Main 1951.

Hsiao, T.C., Jing, J.: Leaf and root expansive growth in response to water deficits. - In: Cosgrove, D.J., Knievel, D.P. (ed.): Physiology of Cell Expansion During Plant Growth. Pp. 180-192. American Society of Plant Physiologists, Rockville 1987.

Jackson, M.B.: Regulation of water relationships in flooded plants by ABA from leaves, roots and xylem sap. - In: Davies, W.J., Jones, H.G. (ed.): Abscisic Acid Physiology and Biochemistry. Pp. 217-226. BIOS Scientific Publishers, Oxford 1991.

Jarvis, A.J., Davies, W.J.: Whole-plant ABA flux and the regulation of water loss in *Cedrella odorata*. - Plant Cell

Environ. **20**: 521-527, 1997.

Johansson, I., Karlsson, M., Shukla, V.K., Chrispeels, J., Larsson, C., Kjellbom, P.: Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. - Plant Cell **10**: 451-459, 1998.

Jones, H.G., Sutherland, R.A.: Stomatal control of xylem embolism. - Plant Cell Environ. **14**: 607-612, 1991.

Kavanagh, K.L., Bond, B.J., Aitken, S.N., Gartner, B.L., Knowe, S.: Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. - Tree Physiol. **19**: 31-37, 1999.

Laurière, C.: Enzymes and leaf senescence. - Physiol. vég. **21**: 1159-1177, 1983.

Leymarie, J., Lascève, G., Vavasseur, A.: Elevated CO_2 enhances stomatal responses to osmotic stress and abscisic acid in *Arabidopsis thaliana*. - Plant Cell Environ. **22**: 301-308, 1999.

Li, J., Wang, X.-Q., Watson, M.B., Assmann, S.M.: Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. - Science **287**: 300-303, 2000.

MacRobbie, E.A.C.: Ionic relations in guard cells. - In: Jarvis, P.G., Mansfield, T.A. (ed.): Stomatal Physiology. Pp. 51-70. Cambridge University Press, Cambridge 1981.

Maggio, A., Joly, R.J.: Effects of mercuric chloride on the hydraulic conductivity of tomato root systems: evidence for a channel-mediated pathway. - Plant Physiol. **109**: 331-335, 1995.

Magnani, F., Mencuccini, M., Grace, J.: Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. - Plant Cell Environ. **23**: 251-263, 2000.

Mansfield, T.A., Atkinson, C.J.: Stomatal behaviour in water stressed plants. - In: Alscher, R.G., Cumming, J.R. (ed.): Plant Biology. Vol 12: Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Pp. 241-264. Wiley-Liss, New York 1990.

Maurel, C.: Aquaporins and water permeability of plant membranes. - Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 399-429, 1997.

Mederski, H.J.: Determination of internal water status of plants by beta ray gauging. - Soil Sci. **92**: 143-146, 1961.

Michelet, B., Boutry, M.: Proton-translocating ATPases of the plasma membrane: biological functions, biochemistry and molecular genetics. - In: Smallwood, M., Knox, J.P., Bowles, D.J. (ed.): Membranes: Specialized Functions in Plants. Pp. 261-279. BIOS Scientific Publishers, Oxford 1996.

Moldau, H., Sõber, J., Sõber, A.: Impact of acute ozone exposure on CO_2 uptake by two cultivars of *Phaseolus vulgaris* L.. - Photosynthetica **28**: 133-141, 1993.

Neumann, D., Nover, L., Parthier, B., Rieger, R., Scharf, K.-D., Wollgiehn, R., Nieden, U.: Heat shock and other stress response systems of plants. - Biol. Zentralbl. **108**: 505-661, 1989.

Niinemets, Ü., Sõber, A., Kull, O., Hartung, W., Tenhunen, J.D.: Apparent controls on leaf conductance by soil water availability and via light-acclimation of foliage structural and physiological properties in a mixed deciduous, temperate forest. - Int. J. Plant Sci. **160**: 707-721, 1999.

Nooden, L.D.: Whole-plant senescence. - In: Nooden, L.D., Leopold, A.C. (ed.): Senescence and Aging in Plants. Pp. 391-439. Academic Press, New York 1988.

Owen, J.H.: Role of abscisic acid in a Ca^{2+} second messenger system. - Physiol. Plant. **72**: 637-641, 1988.

Pell, E.J., Dann, M.S.: Multiple stress-induced foliar senescence and implications for whole-plant longevity. - In: Mooney, H.A., Winner, W.E., Pell, E.J. (ed.): Responses of Plants to Multiple Stresses. Pp. 189-204. Academic Press, New York 1991.

Pitman, M.G., Wellfare, D.: Inhibition of ion transport in excised barley roots by abscisic acid: relation to water permeabilty of the roots. - J. exp. Bot. **29**: 1125-1138, 1978.

Rahi, M.: [An apparatus for observing components of leaf water potential.] - Fiziol. Rast. **20**: 215-221, 1973. [In Russ.]

Reich, P.B., Hinckley, T.M.: Influence of predawn water potential and soil-to-leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. - Funct. Ecol. **3**: 719-726, 1989.

Roberts, S.K.: Regulation of K^+ channels in maize roots by water stress and abscisic acid. - Plant Physiol. **116**: 145-153, 1998.

Rüdinger, M., Hallgren, S.W., Steudle, E., Schulze, E.-D.: Hydraulic and osmotic properties of spruce roots. - J. exp. Bot. **45**: 1413-1425, 1994.

Saab, I.N., Sharp, R.E., Pritchard, J., Voetberg, G.: Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. - Plant Physiol. **93**: 1329-1336, 1990.

Schütz, K., Tyerman, S.D.: Water channels in *Chara corallina*. - J. exp. Bot. **48**: 1511-1518, 1997.

Sellin, A.: Resistance to water flow in xylem of *Picea abies* (L.) Karst. trees grown under contrasting light conditions. - Trees 7: 220-226, 1993.

Shan, R.R., Mehta, A.R.: Effect of nitrogen nutrition of growth and phenolic accumulation in *Crotalaria* tissues cultured in vitro. - Indian J. exp. Bot. **16**: 1144-1147, 1978.

Simpson, G.M.: Water Stress on Plants. - Praeger, New York 1981.

Smart, C.M.: Gene expression during leaf senescence. - New Phytol. **126**: 419-448, 1994.

Sõber, A.: Effect of ozone on the rehydration of bean leaves. - Proc. Estonian Acad. Sci. Biol. **41**: 35-43, 1992.

Sõber, A.: Simultaneous measurement of leaf hydraulic and stomatal conductances by pressure bomb, gas exchange and β-gauge techniques. - Proc. Estonian Acad. Sci. Biol. **45**: 35-43, 1996.

Sõber, A.: Hydraulic conductance, stomatal conductance and maximal photosynthetic rate in bean leaves. - Photosynthetica **34**: 599-603, 1997.

Sõber, A., Aasamaa, K.: Hydraulic conductance between leaf petiole and evaporation sites depends on light conditions and on water stress. - In: Abstracts for Plant Biology **98**. The Annual Meeting of the American Society of Plant Physiologists. P. 59. Amer. Soc. Plant Physiologists, Madison 1998.

Sõber, A.J., Moldau, H.A.: [An apparatus with separate conditioning of the plant and the leaf for determination of stomatal resistance and leaf water content.] - Fiziol. Rast. **24**: 1301-1307, 1977. [In Russ.]

Sõber, A., Sild, E.: Stomatal closure during tobacco leaf desiccation as affected by ozone. - Biol. Plant. **42**: 267-272, 1999.

Sobrado, M.A.: Hydraulic conductance and water potential differences inside leaves of tropical evergreen and deciduous species. - Biol. Plant. **40**: 633-637, 1997/1998.

Sperry, J.S., Nichols, K.L., Sullivan, J.E.M., Eastlack, S.E.: Xylem embolism in ring-porous, diffuse-porous and coniferous trees of northern Utah and interior Alaska. - Ecology **75**: 1736-1752, 1994.

Spollen, W.G., Sharp, R.E., Saab, I.N., Wu, Y.: Regulation of cell expansion in roots and shoots at low water potentials. - In: Smith, J.A.C., Griffiths, H. (ed.): Water Deficits. Pp. 37-52. BIOS Scientific Publishers, Oxford 1993.

Steudle, E.: Water transport across plant tissue: Role of water channels. - Biol. Cell **89**: 259-273, 1997.

Steudle, E., Frensch, J.: Water transport in plants: role of apoplast. - Plant Soil **187**: 67-79, 1996.

Steudle, E., Henzler, T.: Water channels in plants: do basic principles of water transport change? - J. exp. Bot. **46**: 1067-1076, 1995.

Steudle, E., Heydt, H.: Water transport across tree roots. - Trees **11**: 239-255, 1997.

Steudle, E., Meshcheryakov, A.B.: Hydraulic and osmotic properties of oak roots. - J. exp. Bot. **47**: 387-401, 1996.

Tardieu, F., Davies, W.J.: Root-shoot communication and whole-plant ragulation of water flux. - In: Smith, J.A.C., Griffiths, H. (ed.): Water Deficits. Pp. 147-162. BIOS Scientific Publishers, Oxford 1993.

Tognetti, R., Longobucco, A., Miglietta, F., Raschi, A.: Water relations, stomatal response and transpiration of *Quercus pubescens* trees during summer in a Mediterranean carbon dioxide spring. - Tree Physiol. **19**: 261-270, 1999a.

Tognetti, R., Longobucco, A., Raschi, A.: Seasonal embolism and xylem vulnerability in deciduous and evergreen Mediterranean trees influenced by proximity to a carbon dioxide spring. - Tree Physiol. **19**: 271-277, 1999b.

Tyerman, S.D., Bohnert, H.J., Maurel, C., Steudle, E., Smith, J.A.C.: Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. - J. exp. Bot. **50**: 1055-1071, 1999.

Wei, C., Steudle, E., Tyree, M.T.: Water ascent in plants: do ongoing controversies have a sound basis. - Trends in Plant Sci. **4**: 372-375, 1999a.

Wei, C., Tyree, M.T., Steudle, W.: Direct measurement of xylem pressure in leaves of intact maize plants. A test of the cohesion-tension theory taking hydraulic architecture into consideration. - Plant Physiol. **121**: 1191-1205, 1999b.

Wilkinson, S., Davies, W.J.: Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. - Plant Physiol. **113**: 559-573, 1997.

Wingler, A., von Schaewen, A., Leegood, R.C., Lea, P.J., Quick, W.P.: Regulation of leaf senescence by cytokinin, sugars and light. - Plant Physiol. **116**: 329-335, 1998.

Yang, S., Tyree, M.T.: Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. - Tree Physiol. **12**: 231-242, 1993.

Zimmermann, M.H: Xylem structure and the ascent of sap. - Springer, Berlin 1983.

Zotz, G., Tyree, M.T., Cochard, H.: Hydraulic architecture, water relations and vulnerability to cavitation of *Clusia uvitana* Pittier: a C3-CAM tropical hemiepiphyte. - New Phytol. **127**: 287-296, 1994.

Zwienieckin, M.A., Holbrook, M.: Diurnal variation in xylem hydraulic conductivity in white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). - Plant Cell Environ. **21**: 1173-1180, 1998.

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Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees

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Abstract. Some anatomical characteristics in leaves relating to hydraulic conductance and stomatal conductance were examined in six temperate deciduous tree species. The fourth power of the radius of the conducting elements in xylem (r^4) and the area of mesophyll and epidermal cells per unit length of leaf cross-section (u) were high in leaves with high hydraulic conductance (L). Stomatal conductance (g_s) and stomatal sensitivity to an increase in leaf water potential (s_i) correlated positively with the length of stomatal pore (l), but negatively with the guard cell width (z) and the length of the dorsal side of the guard cells (l_d). Stomatal sensitivity to a decrease in leaf water potential (s_d) correlated negatively with l and positively with z and l_d. The anatomical characteristics associated with hydraulic conductance (r^4 and u) and those associated with stomatal conductance and sensitivity to changes of leaf water potential (l, z and l_d) were correlated. We conclude that hydraulic conductance may depend on anatomical characteristics of xylem, mesophyll and epidermis, and stomatal conductance and its sensitivity to changing water potential may depend on anatomical characteristics of stomata. The correlation of shoot hydraulic conductance with stomatal conductance and its sensitivity may be based largely on the correlation between the anatomical characteristics of the water conducting system and stomata in these trees.

Keywords: *Acer platanoides* L., anatomical characteristics of leaf, hydraulic conductance, *Padus avium* Mill., *Populus tremula* L., *Quercus robur* L., *Salix caprea* L., stomatal conductance, stomatal sensitivity to changing water potential, *Tilia cordata* Mill.

Introduction

The water status of leaves is determined by both the hydraulic conductance and the stomatal conductance in the plant. Hydraulic conductance in xylem (L_y) correlates positively with the width (Schulte and Gibson 1988; Ewers *et al.* 1990; Huang and Nobel 1993; Sellin 1994; Steudle and Heydt 1997; Villar-Salvador *et al.* 1997) and the number (Zimmermann and Milburn 1982; Legge 1985; Sellin 1994) of the conducting elements:

$$L_y = k_{Ly}\, r^4\, n \qquad (1)$$

where r is the radius and n is the number of the conducting elements in the xylem and k_{Ly} is a coefficient incorporating other influences on L_y. The hydraulic conductance of xylem conducting elements in roots is several orders of magnitude higher than that of the root parenchymal tissues (Steudle *et al.* 1987; Frensch and Steudle 1989; Melchior and Steudle 1993; Steudle and Peterson 1998; Rieger and Litvin 1999). In leaves the diameter of the xylem conducting elements is noticeably smaller than that in the root metaxylem (Esau 1965; Zimmermann and Brown 1971; Zimmermann 1983). Therefore, the hydraulic conductance of non-embolized xylem may be significantly lower in shoots than in roots.

Water flows through the parenchymal cells in leaves before being evaporated in the intercellular spaces (Boyer 1985; Steudle 1997). Therefore, the hydraulic conductance may depend not only on the hydraulic conductance of xylem, but also on the hydraulic conductance of the leaf

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Abbreviations used: d, area of guard cell dorsal wall; E, transpiration rate; e, air humidity; f, stomatal frequency; g_s, stomatal conductance; I, intensity of β-irradiance; L, shoot hydraulic conductance; l, length of stomatal pore; l_d, length of guard cell dorsal side; n, number of conducting elements in xylem; p, pressure; R, resistance to water vapour; r, radius of conducting elements in xylem; s_d, stomatal sensitivity to a decrease in leaf water potential; s_i, stomatal sensitivity to an increase in leaf water potential; u, area of mesophyll and epidermal cells per unit length of the leaf cross-section; v_d, maximum relative rate of stomatal closure; v_i, maximum relative rate of stomatal opening; w, leaf (absolute) water content per unit area; x, leaf (relative) wet mass per unit area; z, guard cell width.

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parenchymal cells. The relationships between anatomical characteristics of mesophyll and epidermis and hydraulic conductance in deciduous tree shoots remain unresolved.

It has been proposed that stomatal conductance is correlated positively with the length of the stomatal pore (l) and the stomatal frequency (f) and negatively with the guard cell width (z) and the area of the guard cell dorsal wall (l_d):

$$g_s = k_{gs} \frac{lf}{l_d z} \tag{2}$$

where k_{gs} is a coefficient incorporating other factors influencing g_s (DeMichele and Sharpe 1973, 1974; Wu *et al.* 1985; Sharpe *et al.* 1987). There are few experimental data confirming this model. in general, research has been limited to the anatomical characteristics of stomata (Singh *et al.* 1974; Wilkinson and Beard 1975; Spence *et al.* 1986; Bettarini *et al.* 1998) or stomatal conductance and sensitivity to changes of leaf water status (Davies *et al.* 1981; Mansfield and Atkinson 1990; Saliendra *et al.* 1995; Sõber 1996, 1997; Crocker *et al.* 1998; Oren *et al.* 1999). In a few studies, both the stomatal conductance and some of the anatomical characteristics of stomata have been examined; some herbal species with high stomatal conductance also have relatively long stomatal pores, although some species have high stomatal frequency (Rahi 1971; Lawson *et al.* 1998; Tognetti *et al.* 2000). In research on the influence of environmental conditions, stomatal frequency varied in accordance with stomatal conductance (Quarrie and Jones 1977; Tichá 1982; Berryman *et al.* 1994; Wagner *et al.* 1996; Beerling 1997; Nogués *et al.* 1998; Tognetti *et al.* 2000). Previous research of temperate deciduous trees has been limited to measuring stomatal frequency as a single anatomical characteristic in addition to stomatal conductance, and agreement between these two stomatal characteristics has been found (Woodward and Bazzaz 1988; Beerling *et al.* 1998).

Our earlier study (Aasamaa and Sõber 2001) showed that hydraulic conductance (determining the rate of leaf water uptake) and stomatal conductance and sensitivity (determining the rate of leaf water loss) are associated; stomatal conductance and the stomatal sensitivity to an increase in leaf water potential were high and the stomatal sensitivity to a decrease in leaf water potential was low in trees with high hydraulic conductance in shoots. We suggest, that the correlation between hydraulic conductance and stomatal conductance may be based on the correlation between anatomical characteristics of the water conducting system and stomata.

In this study, several anatomical characteristics of leaves of temperate deciduous tree saplings were determined and compared with the hydraulic conductance and stomatal conductance characteristics estimated in our previous study (Aasamaa and Sõber 2001). The main objective was to determine which anatomical characteristics of leaves of these deciduous trees influence the shoot hydraulic conductance, the stomatal conductance and stomatal sensitivity to changes in leaf water potential, and how these anatomical characteristics are correlated.

Materials and methods

Plants

The species studied in this work were *Acer platanoides* L., *Tilia cordata* Mill., *Padus avium* Mill., *Quercus robur* L., *Salix caprea* L. and *Populus tremula* L. The saplings of all the species were grown for 3 years in Tartu (58°25′ N and 26°58′ E), Estonia, in a stand, in clay loam, in full sunlight, after which they were transplanted in autumn into pots (height 0.16 m, diameter 0.14 m), containing the soil from the growing site. In the following spring, just before the budding out of the leaves, the pots were taken into a glasshouse and placed under three different growth conditions. A complex fertilizer (ratio of elements (parts): N 10, P 7, K 16, Fe 0.6, Cu 0.4, Mn 0.08, Mo 0.08, Zn 0.07 and B 0.07) was given to all the saplings. Each pot was fertilized with 0.5 L 0.25% solution of the fertilizer four times beginning on the first day in the glasshouse. Data from three experimental sets for each of the species were compared: C, control set, water content in the soil was maintained at 70% of the maximal water capacity during the growth period in glasshouse; N, each pot was fertilized with ammonium nitrate solution (0.6 mM nitrogen) four times (in addition to the complex fertilizer); W, water content in the soil was maintained at 40% of the maximal water capacity during the growth period in glasshouse. The W set of aspen is absent because too few saplings survived transplanting.

Anatomical characteristics

Six leaves were taken from each of the sets in the middle of the growing season. A piece (approx 100 mm^2) was detached from the middle of each leaf for light microscopic analysis. The pieces were fixed in a mixture of 50% ethanol, glacial acetic acid and formaldehyde (18 : 1 : 1, v/v), embedded in paraffin and permanent slices, stained with safranin O (Sigma Ltd, St Louis, MO, USA) of 4-μm thick cross-sections were made (for details see Lacey 1991; Mader 1991). Magnifications of 350–875 times were used in the analysis with the light microscope (PZO; Warsaw, Poland). The diameter of the three widest conducting elements in the xylem of the central vein were measured by an ocular micrometer (MOB-1; Moscow, Soviet Union). The length of the cross-section, the leaf thickness, the area of the veins, mesophyll cells, intercellular spaces and epidermal cells were measured by a grid ocular vernier. Dry mass per leaf area was determined after drying each piece of leaf at 80°C for 2 days. The dry mass per leaf volume was calculated by dividing the dry mass per area and the leaf thickness.

Pieces (approx. 50 mm^2) were taken from the middle of each leaf to observe the abaxial surface by electron microscopy. The pieces were fixed in glutaraldehyde, critical point-dried in CO_2, mounted on sample stub with conductive tape and sputter-coated with gold (ВУП-4; Moscow, Soviet Union) (for details on standard methods see Hall and Hawes 1991). Leaves were viewed and photographed by scanning electron microscope (Tesla BS 301; Prague, Czech Republic). The stomatal frequency per unit area on the abaxial surface of the leaves was examined under 790–1170 times magnification and the characteristics of the stomatal size were examined under 5000 times magnification. The stomatal characteristics were measured from magnified images of the photo negatives. The characteristics of guard cells are shown in Fig. 1. Measurement of the dorsal area of the guard cell (d in equation 2) is methodically difficult, therefore the length of the dorsal side of the closed guard cell (l_d) was measured and deemed equivalent to the characteristic d.

Shoot hydraulic conductance and sensitivity of stomatal conductance to water potential changes

Apparatus and experiments

Short shoots (100–150 mm) were cut from the saplings while the stems were submerged in water. The cut end of a shoot was driven through the rubber seal of a pressure bomb. Thereafter, the cut end of the stem was immersed in water in the bomb and re-cut under water. The bomb was closed and a 962-mm^2 portion of one intact leaf of the shoot was hermetically enclosed in the leaf chamber for the duration of the experiment. The leaf chamber system enabled simultaneous measurement of the time courses of leaf temperature (by infrared thermometer), relative water content (by β-gauge technics) and CO_2 uptake and transpiration of one side of the leaf. In the gas system of the chamber the humidity and CO_2 were both scrubbed out of the ingoing air and then added back to controlled, constant level. Outgoing humidity was measured by a micropsychrometer and outgoing CO_2 concentration was measured by a gas analyser (LI-COR, LI-6262; Lincoln, NE, USA). The temperature of the leaf chamber was 25°C, irradiance was saturating (1020 μmol m^{-2} s^{-1}), CO_2 concentration of the ingoing air was 350 μmol mol^{-1} and relative humidity was 50–60%. The leaves of all the tree species are amphistomatous, but the stomatal frequency is low on the adaxial surface. Therefore the gas exchange of the abaxial side was observed in our experiments. Stomatal conductance of all these species increased after the leaf was enclosed in the leaf chamber. The stomatal opening indicates that the influence of exposure of the leaf to higher vapour pressure difference and wind speed is surpassed by the influence of high light intensity in all these species. After the stomatal conductance had stabilized on this higher level, the steady-state value of g_s was obtained. Then the pressure in the pressure chamber was raised to determine the shoot hydraulic conductance and stomatal sensitivity to an increase of leaf water potential. At first, the pressure in the pressure chamber was raised quickly from 0 to 0.2 MPa. If there was no infiltration with 15 min of the pressure increase, the chamber pressure was raised quickly to 0.4 MPa and then to 0.7 MPa (if necessary), until the leaf was fully infiltrated. Our method for the determination of hydraulic conductance differed from the balancing pressure method (e.g. Passioura 1980; Passioura and Munns 1984). Our aim was to induce infiltration of water to intercellular spaces; we assumed the water potential in the infiltrating intercellular spaces to be zero (for details see also Sõber 1996, 1997). To observe a decrease in the leaf water content, the leaf of another shoot was enclosed in the leaf chamber. After stabilization of stomatal conductance and obtaining its steady-state value, the petiole was cut. The experiment was terminated when stomatal conductance reached values near zero. After both experiments the portion of the leaf that had been in the leaf chamber was cut out and its fresh mass was determined immediately. The dry mass of the leaf segment was determined after drying for 2 days at 80°C.

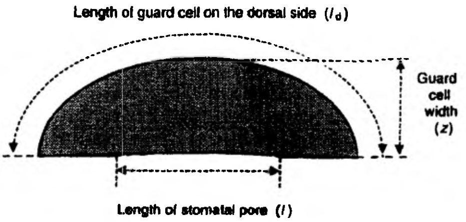

Fig. 1. Diagram of the surface view of a stomatal guard cell showing the characteristics measured.

Calculations

Stomatal conductance (g_s) was calculated using the measured values of transpiration rate and leaf temperature:

$$R_l = \alpha \frac{(e_l - e)}{E} - R_a \tag{3}$$

and

$$g_s = \frac{R_c - R_l}{R_c R_l} \tag{4}$$

where E is the transpiration rate, e_l is the saturating humidity at leaf temperature, e is the humidity in the leaf chamber, R_a, R_c and R_l are boundary layer, cuticular and leaf (whole) resistance, respectively, and α is the mass-flow correction. R_a of only rarely hairy abaxial surfaces of the leaves of all these species in the leaf chamber was determined to be 0.5 m^{-2} s^{-1} mol, using wet filter paper 'leaf' as an evaporating surface. For other details of the determination of stomatal conductance see Sõber and Moldau (1977) and Sõber (1992). Steady-state values of g_s were obtained after stabilization of g_s in the leaf chamber (before the pressure rise or leaf cutting).

Stomatal sensitivity to change in leaf water potential (s) was defined as the relative rate of changing of the stomatal conductance:

$$s = \nu A \tag{5}$$

where ν is the maximum relative rate of stomatal opening or closure and A is a scaling coefficient which transforms ν of different leaves to a common rate of water potential change. ν was determined from:

$$\nu = \frac{\Delta g_s}{\Delta t\,(g_{se} - g_{sa})} 100\% \tag{6}$$

where Δg_s is the difference of stomatal conductances in the region of the maximum rate of changing of stomatal conductance, Δt is the time interval, corresponding to Δg_s, g_{sa} is an average stomatal conductance during the time interval and g_{se} is stomatal conductance at the end of the experiment. g_{se} was zero in the experiments determining the rate of stomatal closure.

The relative rate of changing of stomatal conductance was determined because the stomatal conductance changed almost exponentially with time both after the leaf was cut and after the pressure rise in the pressure chamber.

The maximum relative rate of stomatal closure correlated positively with the transpiration rate (data not shown). It is likely that the leaf water potential decreases more rapidly in intensively transpiring leaves. Therefore, in the calculations of the stomatal sensitivity to a decrease in leaf water potential (s_d) the scaling coefficient A_d was determined from:

$$A_d = \frac{E_{aa}}{E_a} \tag{7}$$

where E_a is the average transpiration rate during the time interval and E_{aa} is the average of E_a for all the experiments (both the experiments of decreasing and increasing leaf water potential). We compared stomatal opening and closing rates at the same transpiration (not at the same water vapour pressure difference) because it is repeatedly shown that stomata respond to the transpiration rate (which changes water potential in the leaf) rather than directly to the air humidity gradient (Sõber 1980; Mott and Parkhurst 1991; Monteith 1995). The increase of leaf water potential likely depends also on the pressure rise applied in the pressure chamber. Therefore, the scaling coefficient (A_i) for calculating the stomatal sensitivity to an increase in leaf water potential (s_i) additionally included the pressure increase in the pressure chamber (Δp_r) inducing an infiltration:

$$A_i = \frac{E_a \Delta p_{ra}}{E_{aa} \Delta p_r}. \tag{8}$$

where Δp_{ra} is the average of Δp_r for all experiments.

Shoot hydraulic conductance (L) was calculated from:

$$L = \frac{(\Delta w / \Delta t) + E_a}{\Delta p} \qquad (9)$$

where Δp is the pressure difference at the path of water from the pressure chamber to the intercellular spaces of the leaf. It was assumed that the water potential in the leaf intercellular spaces in the leaves being infiltrated was zero, and therefore Δp was equal to the pressure in the pressure chamber. $\Delta w / \Delta t$ is the maximum rate of increase in the leaf water content per leaf area during leaf infiltration. Usually, $\Delta w / \Delta t >> E_a$ in our experiments. The relative value of leaf wet mass per unit area (x) was determined from:

$$x = \frac{\ln I - \ln I_b}{\ln I_0 - \ln I_b} \qquad (10)$$

where I_0 is the intensity of irradiance of β-particles (β-irradiance) with no leaf in the leaf chamber, I and I_b are the values of β-irradiance behind the leaf at that moment and at the beginning of the experiment, respectively. The leaf absolute water content per area was determined from:

$$w = \left(\frac{x\, w_{we}}{x_e}\right) - w_d \qquad (11)$$

where x_e is the relative wet mass per area at the end of the experiment, w_{we} is leaf absolute wet mass per unit area at the end of the experiment and w_d is dry mass per unit area. For other details of the method see Mederski (1961), Sõber (1992, 1997) and Moldau *et al.* (1993).

Statistical analysis

Single factor analysis of variance was used to compute statistically significant differences between the means of the data sets (at the level $P < 0.1$). Correlation coefficients (r) between the characteristics and P values of these coefficients were computed as a linear (Pearson) correlation matrix.

Results

Several anatomical characteristics differed significantly among the tree species and among the different growth conditions. The fourth power of the mean radius (r^4) of the three widest conducting elements of xylem of the leaf central vein was different in different species and increased in the order (Fig. 2*a*): *A. platanoides* = *T. cordata* < *P. avium* < *Q. robur* < *S. caprea* < *P. tremula*. Usually, r^4 was higher in the trees grown in nitrogen-rich soil and lower in the trees grown under mild water stress (compared with the trees of the control set of each species; Fig. 2*a*); r^4 correlated positively with hydraulic conductance of the shoot (L) ($r = 0.89$, $P < 0.001$; Fig. 3*a*). Some other anatomical characteristics of the leaf that might affect shoot hydraulic conductance were also measured. The area of mesophyll cells and epidermal cells per unit length of leaf cross-section (u) increased in almost the same order of species. The characteristic u was usually higher in trees grown in nitrogen-rich soil and lower in trees grown under mild water stress, compared with the control trees for each species (Fig. 2*c*). The characteristic u and the shoot hydraulic conductance were strongly positively correlated ($r = 0.83$, $P < 0.001$; Fig. 3*b*). Strong positive correlation was also exhibited between the characteristics r^4 and u ($r = 0.88$, $P < 0.001$), and between $r^4 \times u$ and the shoot hydraulic conductance ($r = 0.93$, $P < 0.001$). The area of veins per unit length of leaf cross-section was higher in *A. platanoides* and *T. cordata* than in the other species and also higher in the trees grown under mild water stress than in the well-watered trees (Fig. 2*b*).

Other anatomical characteristics with a possible influence on hydraulic conductance were the proportion of areas of epidermal and mesophyll cells, the area of the intercellular spaces and the mean area of one epidermal and mesophyll cell on the cross-section of the leaves, and leaf dry mass per volume. None of these characteristics varied significantly among the trees (all species) grown in different conditions. Only the area of the intercellular spaces varied among species, but did not correlate with the hydraulic conductance (data not shown).

The anatomical characteristics of stomata: the width of the guard cell of closed stomata (z) and the length of guard cell dorsal side (l_d) decreased (Figs 2*f*, *g*) and the length of the stomatal pore (l) increased (Fig. 2*e*) in almost the same species order as r^4 and u. Steady-state stomatal conductance (g_s) correlated positively with the length of stomatal pore ($r = 0.86$, $P < 0.001$; Fig. 4*a*) and negatively with guard cell width ($r = -0.76$, $P < 0.001$; Fig. 4*b*) and the length of the guard cell dorsal side ($r = -0.71$, $P = 0.001$; Fig. 4*c*). Thus, these three anatomical characteristics of stomata (l, z and l_d) were in accordance with equation 2. Additionally, stomatal sensitivity to an increase of leaf water potential (s_i) was positively correlated with the length of the stomatal pore ($r = 0.89$, $P < 0.001$; Fig. 5*a*) and negatively with guard cell width ($r = -0.82$, $P < 0.001$; Fig. 5*b*) and the contact area between guard cells and their neighbouring cells ($r = -0.80$, $P < 0.001$; Fig. 5*c*). Stomatal sensitivity to a decrease in the leaf water potential (s_d) correlated negatively with the length of stomatal pore ($r = -0.66$, $P = 0.004$; Fig. 5*d*) and positively with the guard cell width ($r = 0.5$, $P = 0.041$; Fig. 5*e*) and with the contact area between guard cells and their neighbouring cells ($r = 0.47$, $P = 0.06$; Fig. 5*f*). The correlation between these anatomical characteristics of the stomata and s_d was noticeably weaker than the correlation between the anatomical characteristics and s_i. The length of the stomatal pore was the only anatomical characteristic that was significantly sensitive to differences in growth conditions: it was high in trees grown in nitrogen-rich soil and low in trees grown under mild water stress (Fig. 2*e*). Therefore g_s, s_i and s_d exhibited stronger correlation with the length of the stomatal pore than with the guard cell width and the length of guard cell dorsal side which were insensitive to growth conditions (data for water-stressed plants are fitted to separate curves in Figs 4*b*, *c*, 5*b*, *c*, *e*, *f*). The length of the stomatal pore was negatively correlated with the guard cell width ($r = -0.71$, $P = 0.001$) and with the length of guard cell dorsal side ($r = -0.67$, $P = 0.003$).

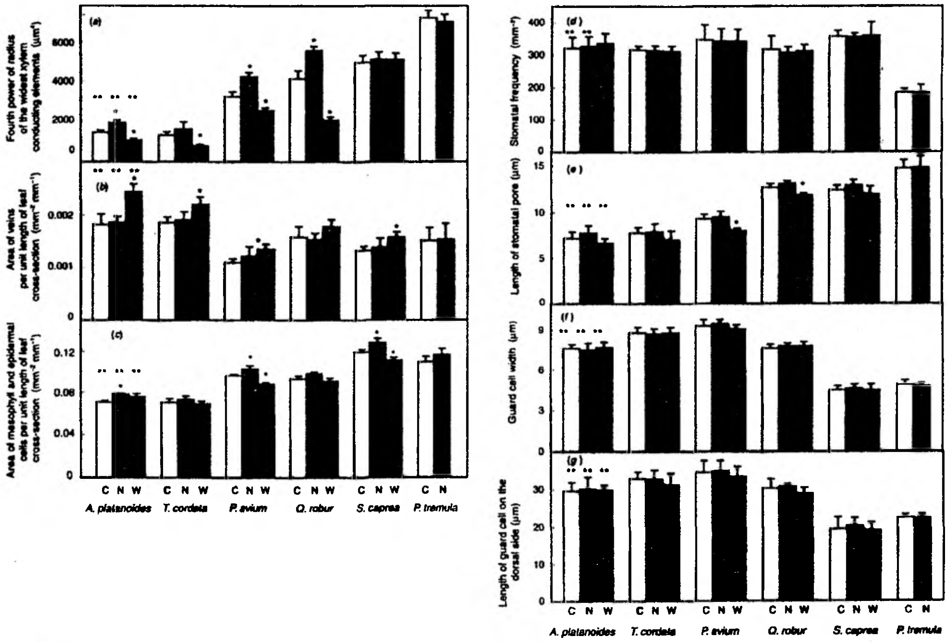

Fig. 2. The fourth power of the radius of the widest conducting elements in xylem of the leaf central vein (*a*), area of veins per unit length of the leaf cross-section (*b*), area of the mesophyll and epidermal cells per unit length of the leaf cross-section (*c*), stomatal frequency per unit area of leaf abaxial surface (*d*), length of stomatal pore (*e*), guard cell width (*f*), and length of guard cell dorsal side (*g*). Data are means ± s.e., $n = 8$–12. □ C, control; ■ N, additional nitrogen-fertilization; ■ W, water-stressed. *$P < 0.1$ indicates statistically significant difference compared with the control set of the species; **$P < 0.1$ indicates significant differences of control, nitrogen-fertilized or water-stressed sets accordingly between all the different species.

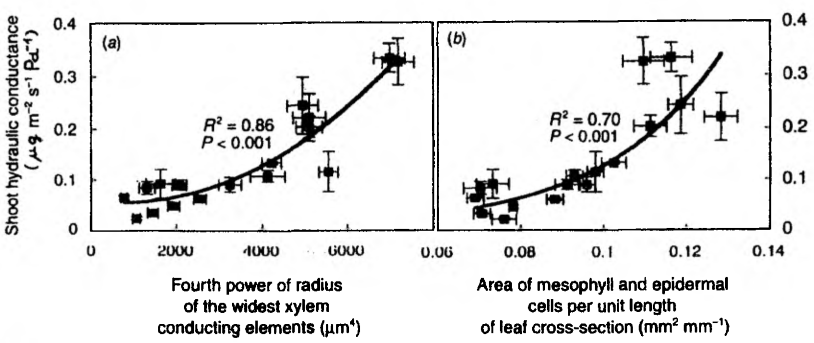

Fig. 3. Relationship between average shoot hydraulic conductance and the fourth power of the radius of the widest conducting elements in xylem of the leaf central vein (*a*) and area of mesophyll and epidermal cells per unit length of the leaf cross-section (*b*).

Stomatal frequency (per unit area of the leaf abaxial surface) varied only slightly among most species and exhibited poor correlation with other stomatal characteristics (data not shown).

The anatomical characteristics of the xylem (r^4) and the mesophyll and epidermal cells (u) correlated with the anatomical characteristics of the stomata. The r^4 of the leaf central vein was positively correlated with the length of the stomatal pore ($r = 0.92$, $P < 0.001$; Fig. 6*a*) and negatively correlated with the guard cell width ($r = -0.69$, $P = 0.002$) and with the length of the guard cell dorsal side ($r = -0.64$, $P = 0.006$). The area of the mesophyll and epidermal cells per unit length of the leaf cross-section also correlated positively with l ($r = 0.84$, $P < 0.001$; Fig. 6*b*) and negatively with z ($r = -0.74$, $P = 0.001$) and l_d ($r = -0.73$, $P = 0.001$). Characteristics r^4 and u correlated with the length of the stomatal pore better than with the other anatomical characteristics of the stomata.

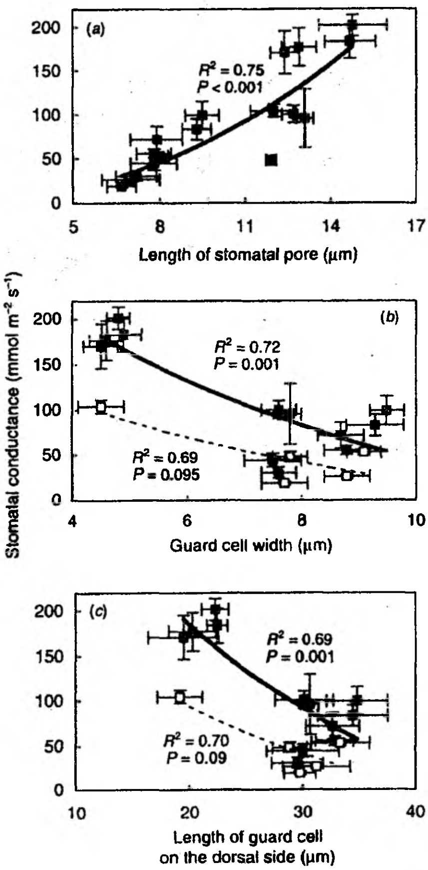

Fig. 4. Relationship between average stomatal conductance and the length of stomatal pore (*a*), guard cell width (*b*) and length of guard cell dorsal side (*c*). Open symbols represent trees grown with mild water stress; closed symbols represent all other trees.

Discussion

The width of the xylem conducting elements correlated strongly with the shoot hydraulic conductance (Fig. 3*a*). Thus, r^4 fitted the model of equation 1 and this result is consistent with existing data showing a correlation between the width of conducting elements and hydraulic conductance in xylem (Schulte and Gibson 1988; Ewers *et al.* 1990; Huang and Nobel 1993; Sellin 1994; Steudle and Heydt 1997; Villar-Salvador *et al.* 1997). We conclude that the width of the xylem conducting elements is important in determining the leaf hydraulic conductance in these deciduous tree species.

The strong positive correlation between hydraulic conductance and the amount of mesophyll and epidermal cells (Fig. 3*b*) highlights the importance of these cells in the determination of hydraulic conductance. Larger space for water flow outside the veins in leaves with higher amounts of mesophyll and epidermal cells may be essential for high hydraulic conductance.

The magnitude of the part of water moving in cell walls and the part of water moving in the symplast through the mesophyll and epidermis of leaves remains unknown. Some of our results unexpectedly show the importance of walls for water flow through the mesophyll and epidermis. The area of veins (per length of the leaf cross-section) was high in *A. platanoides* and *T. cordata* and in trees grown under water stress conditions (Fig. 2*b*). Therefore, the amount of thickened and lignified cell walls of conducting and supporting tissue was likely higher in the leaves of these trees. However, the dry mass (that is mainly the mass of the cell walls) per unit volume of the leaves of these trees was not higher than in the other leaves. We suggest that the dry mass of the leaves of *A. platanoides* and *T. cordata* and in the trees grown under water stress conditions was not high, because the cell walls outside the veins were thin. Our opinion is supported by data from several species showing that the walls of the mesophyll cells are thinner in leaves grown under water stress conditions compared with those in well-watered leaves (Sweet *et al.* 1990; Passioura *et al.* 1993; Spollen *et al.* 1993). Because the hydraulic conductance was low in the leaves with likely a small amount of the walls outside the veins, we suggest that the walls of the mesophyll and the epidermis may be an important flow-path for water in the leaves of these trees.

Most anatomical characteristics of stomata given in equation 2 correlated with stomatal conductance and also with the stomatal sensitivity to changes in leaf water potential. The correlation of the stomatal conductance and sensitivity to changes in leaf water potential was the highest for the length of the stomatal pore and the lowest for stomatal frequency (Figs 4, 5). Therefore, we conclude that the length of the stomatal pore may be the anatomical characteristic that plays the most important role in the determination of the variability of stomatal conductance and sensitivity to changes in leaf water potential among these deciduous tree species, and among the trees (of the same species) grown with different nitrogen and water supplies. The influence of stomatal frequency on transpiration is evidently low in these trees.

Negative correlation between the stomatal conductance and the width of the guard cells is caused by the higher moment of inertia in the wide guard cells (DeMichele and Sharpe 1974). On the basis of our results (Figs 4*b*, 5*b*, *e*), we conclude that the width of the guard cells may be essential to determine differences of stomatal conductance and sensitivity to changes in leaf water potential between these species. All three dimensions of other cells in the leaf epidermis or inside the plant organs were smaller in the plants grown under water deficit conditions and larger in the plants grown in nutrient-rich soil (Spollen *et al.* 1993; Thomas and Howarth 2000). However, the guard cells exhibit special features in their reaction to changes in the

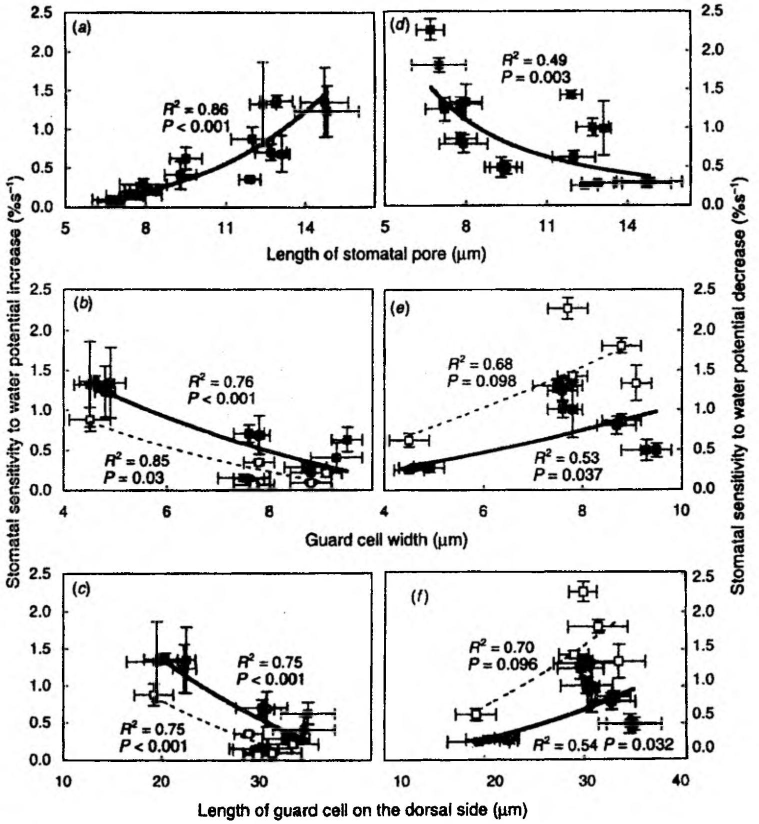

Fig. 5. Relationship between average stomatal sensitivity to an increase in leaf water potential and length of stomatal pore (*a*), guard cell width (*b*), and length of guard cell dorsal side (*c*); relationship between average stomatal sensitivity to a decrease in leaf water potential and length of stomatal pore (*d*), guard cell width (*e*), and length of guard cell dorsal side (*f*). Labelling as in Fig. 4.

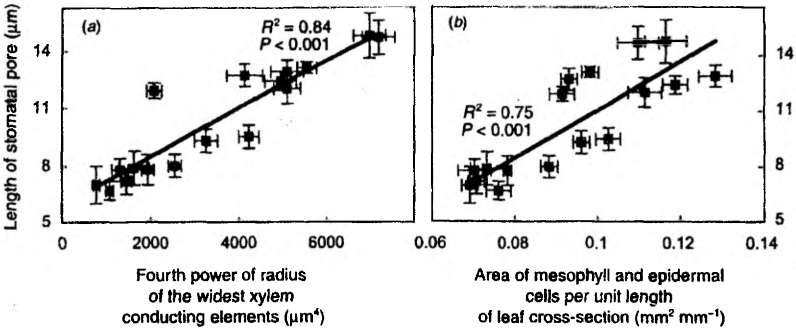

Fig. 6. Relationship between average length of stomatal pore and the fourth power of the radius of the widest conducting elements in xylem of the leaf central vein (*a*) and area of mesophyll and epidermal cells per unit length of the leaf cross-section (*b*).

environment (Davies *et al.* 1981; Mansfield and Atkinson 1990; Assmann and Shimazaki 1999). We suggest that the insensitivity of at least one dimension of guard cells, the width (likely also the thickness), to differences in the plant growth conditions may also represent one of the special characteristics of guard cells. Guard cells with insignificantly different width possibly develop under different growth conditions, because of the essential role of this anatomical characteristic in the determination of stomatal conductance. The high moment of inertia of the stomata of the water-stressed trees (due to their wide guard cells) may be relevant for avoiding too high g_s and s_i in conditions of poor water supply.

It is presumed that the extensive contact area between the guard cells and the neighbouring epidermal cells (in leaves where the dorsal side of guard cells is long) leads to better hydraulic contact of the guard cells with the neighbouring cells, resulting in higher stomatal conductance. However, the pressure of the neighbouring cells on the guard cells (which decreases the aperture of the stomatal pore) is likely also to be high, and the mechanical advantage of the neighbouring epidermal cells (Glinka 1971; Edwards *et al.* 1976; Spence *et al.* 1983, 1986) may have greater effect than stomatal conductance in leaves with extensive contact area. We found that the stomatal conductance was high in species with a short dorsal side (Fig. 4*c*). Therefore, it is probable that the influence of the hydraulic conductance between the guard cells and neighbouring cells is surpassed by the influence of the pressure of the neighbouring epidermal cells in the temperate deciduous tree species. The degree of the contact area may play an important role in determining the differences in stomatal conductance and sensitivity between the tree species. The guard cell width and the length of the guard cell dorsal side were noticeably shorter in *S. caprea* and *P. tremula* than in the other species. Therefore, the low moment of inertia of the guard cells and low mechanical advantage of the neighbouring epidermal cells may be especially important determinants of the high g_s and s_i, and low s_d, detected in these water-stress sensitive riparian tree species.

Higher stomatal sensitivity to an increase in leaf water potential enables a more rapid rise in photosynthetic intensity (and assimilation) in conditions of improved plant water supply (Jarvis and Morison 1981). However, rapid stomatal closure is needed to avoid deleterious effects of low water potential on the plant (Jarvis and Morison 1981; Lo Gullo and Salleo 1988; Augé *et al.* 1998). Thus, it is likely that stomatal sensitivity to decreases of the leaf water potential is more important for plant survival than features of stomatal sensitivity to increases in leaf water potential, especially in trees. The anatomical characteristics formed in young leaves remain relatively constant throughout the life of the leaves (Palevitz 1981; Sack 1987). It is likely that the influence of the anatomical characteristics to stomatal sensitivity is also rather invariable with age. We presume that s_i can be influenced significantly by the constant anatomical characteristics of stomata in the trees. However, the correlation between these anatomical characteristics and s_d was poorer, because of the greater importance for the plant survival process of stomatal closure, likely must have more subtle physiological regulation.

Trees which had wide conducting elements in the xylem and a high volume of cells (and likely also a high volume of cell walls) outside the veins also had long stomatal pores, narrow guard cells and a short dorsal side in the guard cells. Thus, the features of the anatomical characteristics that likely influence hydraulic conductance are inter-related with each other and also with the characteristics that influence stomatal conductance and sensitivity to changes in leaf water potential. The formation of all leaf parts, influenced by several environmental and genetical factors, takes place simultaneously in developing leaves (Palevitz 1981; Nobel 1991; Pyke *et al.* 1991). Therefore, the features of mesophyll or epidermis (including the stomata) are probably not caused by the characteristics of xylem and *vice versa.* However, one important explanation for the correlation between shoot hydraulic conductance and stomatal conductance and sensitivity in these deciduous trees, is probably the correlation between the values of these anatomical characteristics of the water conducting system and stomata.

Acknowledgment

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References

Aasamaa K, Sõber A (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* **44**, 65–73.

Assmann SM, Shimazaki KI (1999) The multisensory guard cell. Stomatal responses to blue light and abscisic acid. *Plant Physiology* **119**, 809–815.

Augé RM, Duan X, Croker JL, Witte WT, Green CD (1998) Foliar dehydration tolerance of twelve deciduous tree species. *Journal of Experimental Botany* **49**, 753–759.

Beerling DJ (1997) Carbon isotope discrimination and stomatal responses of mature *Pinus sylvestris* L. trees exposed in situ for three years to elevated CO_2 and temperature. *Acta Oecologia* **18**, 697–712.

Beerling DJ, McElwain JC, Osborne CP (1998) Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. *Journal of Experimental Botany* **49**, 1603–1607.

Berryman CA, Eamus D, Duff GA (1994) Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. *Journal of Experimental Botany* **45**, 539–546.

Bettarini I, Vaccari FP, Miglietta F (1998) Elevated CO_2 concentrations and stomatal density: observations from 17 plant species growing in a CO_2 spring in central Italy. *Global Change Biology* **4**, 17–22.

Boyer JS (1985) Water transport. *Annual Review of Plant Physiology* **36**, 473–516.

Croker JL, Witte WT, Augé RM (1998) Stomatal sensitivity of six temperate, deciduous tree species to non-hydraulic root-to-shoot signalling of partial soil drying. *Journal of Experimental Botany* **49**, 761–774.

Davies WJ, Wilson JA, Sharp RE, Osonubi O (1981) Control of stomatal behaviour in water-stressed plants. In 'Stomatal physiology'. (Eds PG Jarvis and TA Mansfield) pp. 163–186. (Cambridge University Press: Cambridge, UK)

DeMichele DW, Sharpe PJH (1973) An analysis of the mechanics of guard cell motion. *Journal of Theoretical Biology* **41**, 77–96.

DeMichele DW, Sharpe PJH (1974) A parametric analysis of the anatomy and physiology of the stomata. *Agricultural Meteorology* **14**, 229–241.

Edwards M, Meidner H, Sheriff DW (1976) Direct measurements of turgor pressure potentials of guard cells. *Journal of Experimental Botany* **27**, 163–171.

Esau K (1965) 'Plant anatomy'. (John Wiley & Sons Inc.: New York)

Ewers FW, Fisher JB, Chiu S-T (1990) A survey of vessel dimensions in stems of tropical lianas and other growth forms. *Oecologia* **84**, 544–552.

Frensch J, Steudle E (1989) Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). *Plant Physiology* **91**, 719–726.

Glinka Z (1971) The effect of epidermal cell water potential on stomatal response to illumination of leaf disks of *Vicia faba*. *Physiologia Plantarum* **24**, 476–479.

Hall JL, Hawes C (1991) 'Electron microscopy of plant cells.' (Academic Press Inc.: San Diego)

Huang B, Nobel PS (1993) Hydraulic conductivity and anatomy along lateral roots of cacti: changes with soil water status. *New Phytologist* **123**, 499–507.

Jarvis PG, Morison JIL (1981) Stomatal control of transpiration and photosynthesis. In 'Stomatal physiology'. (Eds PG Jarvis and TA Mansfield) pp. 247–280. (Cambridge University Press: Cambridge, UK)

Lacey AJ (1991) 'Light microscopy in biology: a practical approach.' (Oxford University Press: Oxford)

Lawson T, James W, Weyers J (1998) A surrogate measure of stomatal aperture. *Journal of Experimental Botany* **49**, 1397–1403.

Legge NJ (1985) Anatomical aspects of water movement through stems of mountain ash (*Eucalyptus regnans* F. Muell.). *Australian Journal of Botany* **33**, 287–298.

Lo Gullo MA, Salleo S (1988) Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. *New Phytologist* **108**, 267–276.

Mader SS (1991) 'Inquiry into life. Laboratory manual.' (W.C. Brown Publisher: Dubuque, Iowa)

Mansfield TA, Atkinson CJ (1990) Stomatal behaviour in water stressed plants. In 'Plant biology. Vol 12. Stress responses in plants: adaptation and acclimation mechanisms'. (Eds RG Alscher and JR Cumming) pp. 241–264. (Wiley-Liss Inc.: New York)

Mederski HJ (1961) Determination of internal water status of plants by beta ray gauging. *Soil Science* **92**, 143–146.

Melchior W, Steudle E (1993) Water transport in onion (*Allium cepa* L.) roots. Changes of axial and radial hydraulic conductivities during root development. *Plant Physiology* **101**, 1305–1315.

Moldau H, Sõber J, Sõber A (1993) Impact of acute ozone exposure on CO_2 uptake by two cultivars of *Phaseolus vulgaris* L. *Photosynthetica* **28**, 133–141.

Monteith JL (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment* **18**, 357–364.

Mott KA, Parkhurst DF (1991) Stomatal response to humidity in air and helox. *Plant, Cell and Environment* **14**, 509–515.

Nobel PS (1991) 'Physicochemical and environmental plant physiology.' (Academic Press Inc.: San Diego)

Nogués S, Allen DJ, Morison JIL, Baker NR (1998) Ultraviolet-B radiation effects on water relations, leaf development and photosynthesis in droughted pea plants. *Plant Physiology* **117**, 173–181.

Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schäfer KVR (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment* **22**, 1515–1526.

Palevitz BA (1981) The structure and development of stomatal cells. In 'Stomatal physiology'. (Eds PG Jarvis and TA Mansfield) pp. 1–23. (Cambridge University Press: Cambridge, UK)

Passioura JB (1980) The transport of water from soil to shoot in wheat seedlings. *Journal of Experimental Botany* **31**, 333–345.

Passioura JB, Munns R (1984) Hydraulic resistance of plants. II. Effects of rooting medium, and time of day, in barley and lupin. *Australian Journal of Plant Physiology* **11**, 341–350.

Passioura JB, Condon AG, Richards RA (1993) Water deficits, the development of leaf area and crop productivity. In 'Water deficits'. (Eds JAC Smith and H Griffiths) pp. 253–264. (BIOS Scientific Publishers: Oxford)

Pyke KA, Morrison JL, Leech RM (1991) Temporal and spatial development of the cells of the expanding first leaf of *Arabidopsis thaliana* (L.) Heynh. *Journal of Experimental Botany* **42**, 1407–1416.

Qarrie SA, Jones HG (1977) Effects of abscisic acid and water stress on development and physiology of wheat. *Journal of Experimental Botany* **28**, 192–203.

Rahi M (1971) The characteristics of leaf anatomy and diffusion resistances. *Proceedings of Estonian Academy of Sciences. Biology* **20**, 84–94.

Rieger M, Litvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. *Journal of Experimental Botany* **50**, 201–209.

Sack FD (1987) The development and structure of stomata. In 'Stomatal function'. (Eds E Zeiger, GD Farquhar and IR Cowan) pp. 59–89. (Stanford University Press: Stanford)

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance and soil drought in *Betula occidentalis*. *Planta* **196**, 357–366.

Schulte PJ, Gibson AC (1988) Hydraulic conductance and tracheid anatomy in six species of extant seed plants. *Canadian Journal of Botany* **66**, 1073–1079.

Sharpe PJH, Wu H, Spence RD (1987) Stomatal mechanics. In 'Stomatal function'. (Eds E Zeiger, GD Farquhar and IR Cowan) pp. 91–114. (Stanford University Press: Stanford)

Sellin A (1994) Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different environmental conditions. PhD thesis, University of Tartu, Estonia.

Singh M, Ogren WL, Wildholm JM (1974) Photosynthetic characteristics of several C_3 and C_4 plant species grown under different light intensities. *Crop Science* **14**, 563–566.

Sõber A (1980) Stomatal responses to changes of air humidity and participation of CO_2 in these reactions. *Soviet Plant Physiology* **27**, 383–391.

Sõber A (1992) Effect of ozone on the rehydration of bean leaves. *Proceedings of Estonian Academy of Sciences. Biology* **41**, 35–43.

Sõber A (1996) Simultaneous measurement of leaf hydraulic and stomatal conductances by pressure bomb, gas exchange and β-gauge techniques. *Proceedings of Estonian Academy of Sciences. Biology* **45**, 35–43.

Sõber A (1997) Hydraulic conductance, stomatal conductance and maximal photosynthetic rate in bean leaves. *Photosynthetica* **34**, 599–603.

Sõber A, Moldau H (1977) An apparatus with separate conditioning of the plant and the leaf for determination of stomatal resistance and leaf water content. *Fiziologia Rastenii* **24**, 1301–1307.

Spence RD, Sharpe PJH, Powell RD, Rogers CA (1983) Epidermal and guard cell interactions on stomatal aperture in epidermal strips and intact leaves. *Annals of Botany* **52**, 1–12.

Spence RD, Wu H, Sharpe PJH, Clark KG (1986) Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells. *Plant, Cell and Environment* **9**, 197–202.

Spollen WG, Sharp RE, Saab IN, Wu Y (1993) Regulation of cell expansion in roots and shoots at low water potentials. In 'Water deficits'. (Eds JAC Smith and H Griffiths) pp. 37–52. (BIOS Scientific Publishers: Oxford)

Steudle E (1997) Water transport across plant tissue: role of water channels. *Biology of the Cell* **89**, 259–273.

Steudle E, Heydt H (1997) Water transport across tree roots. In 'Trees — contributions to modern tree physiology'. (Eds H Rennenberg, W Eschrich and H Ziegler) pp. 239–255. (Backhuys Publishers: Leiden)

Steudle E, Peterson CA (1998) How does water get through roots? *Journal of Experimental Botany* **49**, 775–788.

Steudle E, Oren R, Schulze ED (1987) Water transport in maize roots. *Plant Physiology* **84**, 1220–1232.

Sweet WJ, Morrison JC, Labavitch JM, Matthews MA (1990) Altered synthesis and composition of cell wall of grape (*Vitis vinifera* L.) leaves during expansion and growth-limiting water deficits. *Plant and Cell Physiology* **31**, 407–414.

Thomas H, Howarth CJ (2000) Five ways to stay green. *Journal of Experimental Botany* **51**, 329–337.

Tichá I (1982) Photosynthetic characteristics during ontogenesis of leaves. 7. Stomatal density and sizes. *Photosynthetica* **16**, 375–471.

Tognetti R, Minnocci A, Peñuelas J, Raschi A, Jones MB (2000) Comparative field water relations of three Mediterranean shrub species co-occurring at a natural CO_2 vent. *Journal of Experimental Botany* **51**, 1135–1146.

Villar-Salvador P, Castro-Diez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (*Fagaceae*) species along a climate gradient in NE Spain. *Trees* **12**, 90–96.

Wagner F, Below R, de Klerk P, Dilcher DL, Joosten H, Kürschner WM, Visscher H (1996) A natural experiment on plant acclimation: lifetime stomatal frequency response of an individual tree to annual atmospheric CO_2 increase. *Proceedings of the National Academy of Sciences USA* **93**, 11705–11708.

Wilkinson JF, Beard JB (1975) Anatomical responses of "Merion" Kentucky bluegrass and 'Pennlawn' red pescul at reduced light intensities. *Crop Science* **15**, 189–194.

Woodward FI, Bazzaz FA (1988) The responses of stomatal density to CO_2 partial pressure. *Journal of Experimental Botany* **39**, 1771–1781.

Wu H, Sharpe PJH, Spence RD (1985) Stomatal mechanics. III Geometric interpretation of the mechanical advantage. *Plant, Cell and Environment* **8**, 269–274.

Zimmermann MH (1983) 'Xylem structure and the ascent of sap.' (Springer-Verlag: Berlin)

Zimmermann MH, Brown CL (1971) (Eds) 'Trees: structure and function.' (Springer-Verlag: Berlin)

Zimmemann MH, Milburn JA (1982) Transport and storage of water. In 'Encyclopedia of plant physiology. Physiological plant ecology II. Vol. 12B'. (Eds OL Lange, PS Nobel, CB Osmond and H Ziegler) pp. 135–151. (Springer-Verlag: Berlin)

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III

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Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees

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Summary Correlations between leaf abscisic acid concentration ([ABA]), stomatal conductance (g_s), rate of stomatal opening in response to an increase in leaf water potential (s_i), shoot hydraulic conductance (L) and photosynthetic characteristics were examined in saplings of six temperate deciduous tree species: *Acer platanoides* L., *Padus avium* Mill., *Populus tremula* L., *Quercus robur* L., *Salix caprea* L. and *Tilia cordata* Mill. Species-specific values of foliar [ABA] were negatively related to the mean values of g_s, s_i, L and light- and CO_2-saturated net photosynthesis (P_{max}), thus providing strong correlative evidence of a scaling of foliar gas exchange and hydraulic characteristics with leaf endogenous [ABA]. In addition, we suggest that mean g_s, s_i, L and P_{max} for mature leaves may partly be determined by the species-specific [ABA] during leaf growth. The most drought-intolerant species had the lowest [ABA] and the highest g_s, suggesting that interspecific differences in [ABA] may be linked to differences in species-specific water-use efficiency. Application of high concentrations of exogenous ABA led to large decreases in g_s, s_i and P_{max}, further underscoring the direct role of ABA in regulating stomatal opening and photosynthetic rate. Exogenous ABA also decreased L, but the decreases were considerably smaller than the decreases in g_s, s_i and P_{max}. Thus, exogenous ABA predominantly affected the stomata directly, but modification of L by ABA may also be an important mechanism of ABA action. We conclude that interspecific variability in endogenous [ABA] during foliage growth and in mature leaves provides an important factor explaining observed differences in L, g_s, s_i and P_{max} among temperate deciduous tree species.

Keywords: Acer platanoides, drought tolerance, interspecific variability, Padus avium, Populus tremula, Quercus robur, Salix caprea, stomatal sensitivity, Tilia cordata.

Introduction

Abscisic acid (ABA) is a phytohormone regulating several important plant processes of varying time response (Zeevaart et al. 1991, Mäntylä et al. 1995, Leung and Giraudat 1998), especially plant adjustment to water stress. Abscisic acid concentration ([ABA]) is higher and stomatal conductance is lower in water-stressed plants than in well-watered plants (Blake and Ferrell 1977, McMichael and Hanny 1977, Radin 1984, Tardieu et al. 1996). Also, stomata generally close faster after a decrease in leaf water potential in drought-exposed plants than in well-watered plants (Ackerson 1980, Hartung and Davies 1991, Chandler and Robertson 1994, Comstock and Mencuccini 1998). Although there have been few studies on the response of stomata to an increase in leaf water potential, evidence suggests that, compared with well-watered plants, stomatal opening is slower after re-watering in water-limited plants (Cowan and Farquhar 1977, Ludlow et al. 1985, Saliendra et al. 1995, Fang et al. 1996). Differences among species in stomatal conductance (e.g., Körner et al. 1979, Körner 1994) and stomatal response kinetics (Woods and Turner 1971, Davies and Kozlowski 1975) are well documented. However, because most studies of ABA have been carried out with drought-exposed and well-watered plants of the same species, the relationship between interspecific variation in foliar [ABA] and species differences in maximum stomatal conductance and the dynamics of stomatal response has not been clarified (Henson et al. 1989, Liang et al. 1996). Quarrie (1991) compared several lines of the same species and reported that stomatal conductance was lower in the variety with the highest leaf [ABA]. Understanding the determinants of the rate of stomatal opening is important when simulating foliar carbon gain in fluctuating environmental conditions, especially in fluctuating light environments. Because the activation of the biochemical reactions of photosynthesis is faster than stomatal opening, foliar lightfleck-use efficiency primarily depends on the kinetics of stomatal movements (Kirschbaum et al. 1988, Tinoco-Ojanguren and Pearcy 1993, Pearcy 1994).

Shoot hydraulic conductance is mainly determined by the conductance of the xylem. Xylem hydraulic conductance is a function of conduit diameter (Schulte and Gibson 1988, Ewers et al. 1990), but may also depend on the length of the conduits (Pothier et al. 1989). For the six species studied here, we have previously observed that the diameter of large vessels of leaf xylem increased in the sequence: *Tilia cordata* Mill. = *Acer platanoides* L. < *Padus avium* Mill. < *Quercus robur* L. < *Salix caprea* L.< *Populus tremula* L. (Aasamaa et al. 2001). This ranking may have resulted from interspecific variability in [ABA] because there is evidence that the development of xylem depends on ABA. For example, leaves supplied with ABA during their growth are more xeromorphic than control leaves (Fromm 1997). Therefore, a demonstration that species-specific leaf [ABA] of these six species decreases in the same sequence would provide strong evidence that differences in vessel diameter and hydraulic conductance result from differences in [ABA] in the growing shoots.

We determined relationships between foliar [ABA], stomatal conductance and stomatal sensitivity to an increase in water potential for six temperate deciduous tree species. Long-term observations (Ellenberg 1996) of these species suggest that they rank in decreasing drought tolerance: *Quercus robur* > *Tilia cordata* > *Acer platanoides* = *Padus avium* = *Populus tremula* > *Salix caprea*. We demonstrate that leaf [ABA] is correlated with species-specific values of stomatal conductance, stomatal sensitivity to an increase in leaf water potential, shoot hydraulic conductance and photosynthesis. To gain further insight into the relationship between endogenous leaf [ABA] and foliar gas exchange and hydraulic characteristics, the influence of exogenously supplied ABA on these values was also examined. We suggest that the observed interspecific variability in foliar [ABA] provides an explanation for inter-specific differences in stomatal conductance, kinetics of stomatal opening and sensitivity to water stress.

Materials and methods

Study site and foliage sampling

Saplings of all species were sampled from a mixed deciduous stand on a clay loam pseudopodsol near Tartu, Estonia (58°22′ N, 26°44′ E, elevation about 65 m a.s.l.). The stand was 17–19 m high and about 50 years old, and the overstory was dominated by *Tilia cordata* and *Acer platanoides*. Saplings 2–3 m high and 8–10 years old were sampled in a large gap where all sampled trees received more than 75% of above-canopy daily integrated quantum flux density. Two measurement campaigns were conducted during the growing season of 1997. Because all saplings produced only one leaf flush during the growing season, fully expanded mature leaves were sampled in July, and leaves in early stages of senescence were studied in September.

We sampled non-transpiring plants in the morning. Several hours before the experiments, the terminal portion (about 1 m) of each sapling was enclosed in a plastic bag, cut under water and brought immediately to the laboratory. The cut ends were kept in water during transportation and recut in the laboratory. For the experiments, four short (100–150 mm) shoots with 3–4 leaves were cut under water from the main branch, and put either in distilled water (control) or in solutions of 1, 10 or 20 µM ABA (Sigma, St. Louis, MO). In addition, one shoot was kept in distilled water and used to determine the initial endogenous [ABA] in leaves. Before measurement of foliar gas exchange and hydraulic conductance, and sampling for ABA analyses, the shoots were conditioned in the growth chamber at a photosynthetic quantum flux density of 450 µmol m^{-2} s^{-1} and an air temperature of 23–26 °C for 2 h.

Foliar gas exchange and hydraulic conductance measurements

Foliar gas exchange and hydraulic characteristics were measured as described by Sõber (1996, 1997). For simultaneous determinations of foliar stomatal and hydraulic conductances and net photosynthetic rates, we used a measurement system that integrated a pressure chamber and an open gas-exchange system with an infrared gas analyzer (Model LI-6262, Li-Cor, Lincoln, NE). A circular portion of leaf with an area of 9.6 cm^2 was hermetically clamped in a thermostatically controlled leaf chamber, while the cut shoot end was maintained inside the pressure chamber filled with either distilled water (control) or an ABA solution in which the shoot had previously been kept (Figure 1). During all gas exchange measurements, leaf temperature was maintained at 25 ± 1 °C, and relative humidity in the chamber at 55 ± 5%. A photosynthetic photon flux density of 1020 µmol m^{-2} s^{-1} from a halogen lamp was saturating for all leaves investigated. The leaf chamber was equipped with a

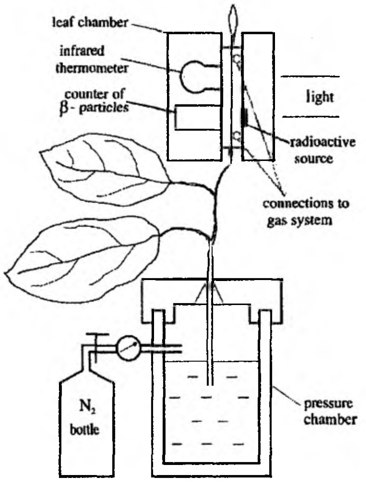

Figure 1. Configuration of the measurement system combining the pressure chamber and the gas-exchange apparatus for simultaneous determinations of foliar transpiration and photosynthetic rate, temperature, water content and hydraulic conductance (for further details see Sõber and Moldau 1977, Sõber 1992, 1996).

β-gauge (comprising a $Ba^{14}CO_3$ emitter on one side of the leaf and a Geiger-Müller counter on the other side) for continuous monitoring of relative leaf water content (Mederski 1961). Further details of the measurement system are given by Sõber and Moldau (1977) and Niinemets et al. (1998).

Measurements were started at an ambient chamber CO_2 concentration (C_a) of 350 μmol mol^{-1}, which was maintained until steady-state rates of net assimilation (P) and stomatal conductance (g_s) were observed. Thereafter, ambient chamber CO_2 concentration was instantly raised to 1760 μmol mol^{-1} for 4 min to measure the CO_2- and light-saturated net photosynthetic rate (P_{max}). Chamber CO_2 concentration was then restored to 350 μmol mol^{-1}, and foliar gas exchange allowed to stabilize. After steady-state values of net assimilation and stomatal conductance had been reestablished, the pressure in the pressure chamber was quickly (within 1 s) raised from 0 to 0.2 MPa. If there was no infiltration within 15 min, the pressure was increased again to 0.4 MPa and then to 0.7 MPa, if needed. The experiment was terminated when leaf infiltration occurred. The part of the leaf enclosed in the cuvette was excised and weighed. The leaf section was then dried at 80 °C for 48 h and reweighed.

Estimating the relative rate of stomatal opening

Stomatal conductance to water vapor (g_s) was calculated according to Sõber and Moldau (1977). Stomatal sensitivity to an increase in leaf water potential (s_i) was determined from the maximum relative rate of stomatal opening (v_i):

$$s_i = v_i B, \tag{1}$$

where B is a coefficient normalizing the values of v_i from different experiments to a common transpiration rate and pressure increase in the pressure chamber. Because the opening rate after the pressure change was almost exponential with time (Figure 2, see also Sõber and Sild 1999), we used the relative rate of stomatal opening, which is the rate constant of stomatal opening. The (maximum) relative rate of stomatal opening (v_i) was calculated as:

$$v_i = 100\frac{\Delta g_s}{\Delta t(g_{smax} - g_{sa})}, \tag{2}$$

where Δg_s is the difference in stomatal conductances close to the maximum rate of stomatal opening (Figure 2), Δt is the time interval corresponding to Δg_s, g_{sa} is mean stomatal conductance during Δt, and g_{smax} is the maximum value of stomatal conductance after the pressure increase. Coefficient B was obtained as:

$$B = \frac{E_a \Delta p_m}{E_{aa} \Delta p_r}, \tag{3}$$

where E_a is mean transpiration rate during Δt, E_{aa} is mean E_a for all the experiments, Δp_r is the pressure increase in the pressure chamber, which leads to a corresponding increase in leaf water potential, and Δp_m is mean Δp_r for all experiments. The coefficient B standardizes all values of the rate of stomatal opening to account for the potentially higher rate of increase in leaf water potential for leaves with lower transpiration rates, and for greater applied pressures.

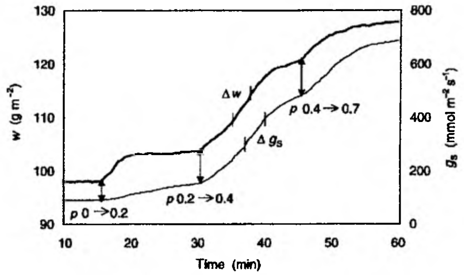

Figure 2. Representative time courses of leaf water content (w) and stomatal conductance (g_s) to determine the maximum rate of stomatal opening (s_i, Equation 2), and shoot hydraulic conductance (L, Equation 6). The timing of the pressure (p) increases in the pressure chamber are indicated by arrows, and the occurrence of maximum stomatal opening rate and shoot hydraulic conductance are bounded by the vertical lines.

Determining shoot hydraulic conductance

The relative values of leaf fresh mass per area (M) were estimated from measurements of β-ray transmission (Mederski 1961, Sõber 1992, 1997, Moldau et al. 1993) as:

$$M = \frac{\ln I - \ln I_h}{\ln I_0 - \ln I_h}, \tag{4}$$

where I_0 is β-irradiance in the absence of the leaf in the leaf chamber, I is the instantaneous estimate of β-irradiance penetrating the leaf, and I_h is β-irradiance penetrating the leaf at the beginning of the experiment. The leaf absolute water content per area is given by:

$$w = \frac{M w_{fe}}{M_e} - w_d, \tag{5}$$

where M_e is leaf relative fresh mass per area and w_{fe} is leaf absolute fresh mass per area at the end of the experiment, and w_d is leaf dry mass per area. Having determined w, shoot hydraulic conductance (L) was calculated as:

$$L = \frac{\frac{\Delta w}{\Delta t} + E_a}{\Delta p}, \tag{6}$$

where Δp is the pressure difference between water in the pressure chamber and that in the leaf intercellular air space. We assumed that water potential in the leaf intercellular space is zero in infiltrated leaves, and thus Δp equals the pressure in the pressure chamber. The term $\Delta w/\Delta t$ is the maximum rate of increase in leaf water content per leaf area (Figure 2) during leaf infiltration. Usually, $\Delta w/\Delta t$ was greater than E_a in our experiments.

Leaf carboxylation efficiency

In addition to the maximum net assimilation rate at saturating CO_2 concentration and quantum flux density (P_{max}), which characterizes the capacity of foliar photosynthetic electron transport (von Caemmerer and Farquhar 1981), we calculated carboxylation efficiency (χ) as an estimate of the initial activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco):

$$\chi = \frac{P}{C_i - \Gamma}, \qquad (7)$$

where P is steady-state net photosynthetic rate measured at an ambient CO_2 concentration of 350 μmol mol^{-1}, C_i is the corresponding intercellular CO_2 concentration, and Γ is the CO_2 compensation point. A constant value of 40 μmol mol^{-1} was assumed for Γ.

Leaf endogenous ABA content

Leaf disks (~50–300 mg) were punched between major leaf veins from another set of conditioned shoots. The disks were enclosed in Eppendorf cups, immediately frozen in liquid nitrogen and stored at −20 °C until extraction of ABA. The frozen tissue samples were homogenized and extracted in 80% methanol. The extracts were passed through a Sep-Pak C_{18} cartridge. Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in Tris buffer, and the ABA content of the solution determined by ELISA as described by Mertens et al. (1985) and Peuke et al. (1994).

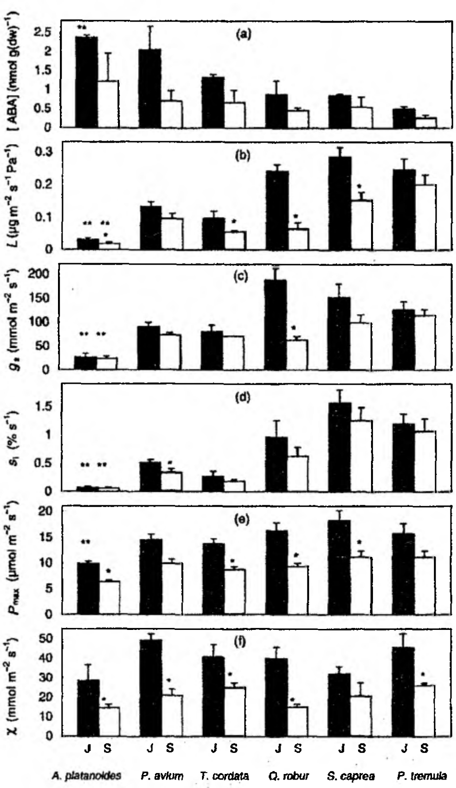

Figure 3. (a) Foliar ABA concentrations ([ABA]), (b) shoot hydraulic conductance (L, Equation 6), (c) stomatal conductance (g_s), (d) stomatal sensitivity to leaf water potential increase (s_i, Equation 1), (e) maximal light- and CO_2-saturated net assimilation rate (P_{max}) and (f) carboxylation efficiency (χ, Equation 7) for the control treatments. Values are means of three to seven shoots, and the bars represent + SE. The solid bars (J) denote the July measurements, and open bars (S) denote the September measurements. One asterisk indicates significant differences ($P < 0.1$ according to a one-way ANOVA) within the species, between the means of July and September. Double asterisks indicate significant differences of either July or September data or both between all species.

Results

Species differences in leaf [ABA], shoot hydraulic conductance and gas exchange parameters

Leaf [ABA] differed significantly among tree species (Figure 3). Species ranking based on leaf [ABA] was *Acer platanoides* > *Padus avium* > *Tilia cordata* > *Quercus robur* = *Salix caprea* > *Populus tremula*. For the control treatments, shoot hydraulic conductance (L, Equation 6), stomatal conductance (g_s) at the beginning of the experiment and rate of stomatal opening in response to an increase in leaf water potential (s_i, Equation 1) also differed significantly between species (Figures 3b–d). Compared with leaf [ABA], species ranking was reversed for shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to an increase in leaf water potential (Figures 3b–d), indicating that leaf [ABA] is negatively correlated with these characteristics.

Light- and CO_2-saturated net photosynthetic rates of the species increased in almost the same sequence as shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to an increase in leaf water potential (Figure 3e); however, carboxylation efficiency (χ, Equation 7) did not differ significantly among species (Figure 3f).

Seasonal changes in leaf [ABA], shoot hydraulic conductance and gas exchange characteristics

Foliar [ABA] was two- to threefold lower and the differences between species were smaller in September than in July. Nevertheless, species ranking based on leaf [ABA] remained practically the same at different stages of development (Figure 3a). Similar patterns were also observed for other characteristics. For the September data, the species effect was still significant for shoot hydraulic conductance (Figure 3b), stomatal conductance (Figure 3c) and the rate of stomatal opening (Figure 3d).

Scaling shoot hydraulic conductance and gas exchange characteristics with leaf [ABA] for the control treatments

Mean foliar [ABA] of a species correlated negatively with mean shoot hydraulic conductance (Figure 4a; Pearson linear correlation coefficient: $r = -0.87$, $P = 0.02$ for July and $r = -0.78$, $P = 0.07$ for September), with mean stomatal conductance (Figure 4b; $r = -0.81$, $P = 0.05$ for July and $r = -0.88$, $P = 0.02$ for September), with mean stomatal sensitivity to leaf water potential increase (Figure 4c; $r = -0.82$, $P = 0.05$ for July and $r = -0.74$, $P = 0.09$ for September) and with mean maximum photosynthetic rate of the species (Figure 5a; $r = -0.8$, $P = 0.05$ for July and $r = -0.89$, $P = 0.02$ for September). Within species, there was no significant correlation between mean foliar [ABA] and mean carboxylation efficiency (Figure 5b).

Effect of exogenous ABA

In all species, shoot hydraulic conductance, stomatal conductance, stomatal sensitivity to leaf water potential increase and maximum net photosynthetic rate decreased in response to exogenous ABA (Figure 6). The rate of stomatal opening and stomatal conductance changed more than fivefold in response to the exogenous [ABA] tested in July. Shoot hydraulic conductance was less sensitive to exogenous ABA than stomatal characteristics. Although shoot hydraulic conductance responded significantly to exogenous ABA, it never decreased more than 30%. The relative decrease in all four parameters in response to exogenous ABA was smaller in September than in July. Carboxylation efficiency showed a negligible response to exogenous ABA (Figure 6).

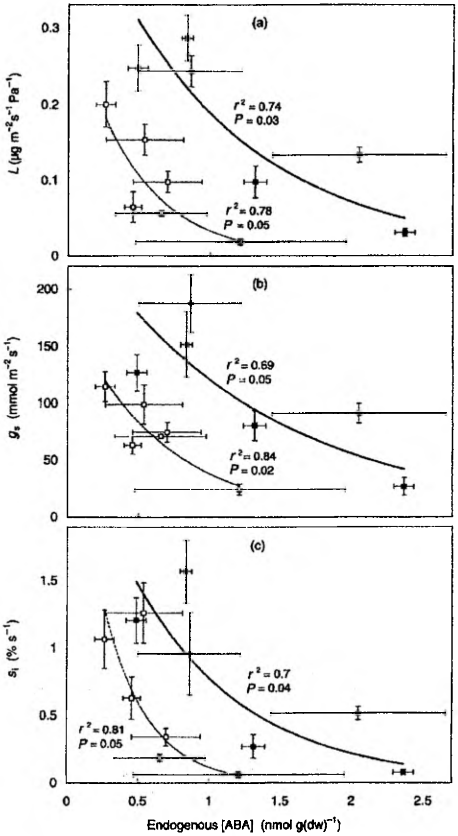

Figure 4. Relationships between foliar endogenous ABA concentration and (a) shoot hydraulic conductance (L), (b) stomatal conductance (g_s) and (c) stomatal sensitivity to leaf water potential increase (s_i) in July (■, thick line) and September (□, thin line) for the control treatments. Each value is the mean ± SE for the particular species studied. Nonlinear regression analysis was used to fit the data to exponential functions (r^2 = coefficient of determination).

Discussion

Species differences in endogenous [ABA]

Foliar [ABA] tends to change during leaf ontogenesis; for example, [ABA] increases in the early stages of leaf senescence and decreases in the later stages (Becker and Apel 1993, Philosoph-Hadas et al. 1993). Although foliar [ABA] was lower in September than in July (Figure 3a), species ranking according to foliar [ABA] was essentially the same and thus likely independent of leaf ontogenetic stage. This agrees with the results of other studies, and indicates that species differences in foliar [ABA] are retained throughout leaf ontogeny (Loewenstein and Pallardy 1998). It seems likely therefore, that the species ranking based on foliar [ABA] observed for July and September (Figure 3) was similar during the period of intensive shoot growth, with the highest leaf [ABA] in *A. platanoides* and the lowest in *P. tremula*.

Relationships between endogenous [ABA] and stomatal conductance

Taking the results of this study in conjunction with our previous observations demonstrates that mean foliar [ABA] for each species (Figure 3a) is related to mean guard cell width and inversely related to mean stomatal pore length (Aasamaa et al. 2001). Because these anatomical characteristics are

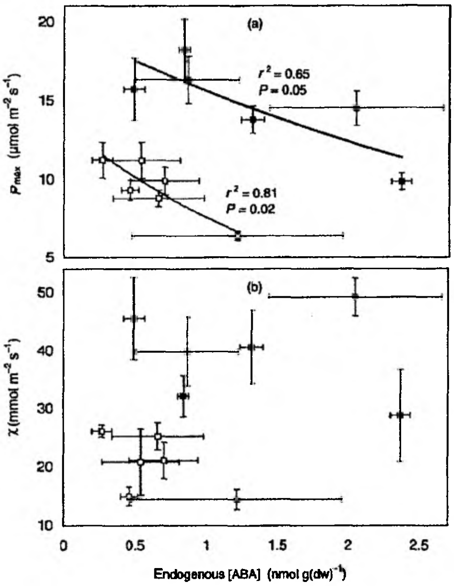

Figure 5. Relationships between foliar endogenous ABA concentration and (a) maximum net assimilation rate (P_{max}), and (b) carboxylation efficiency (χ) in July (■, thick line) and September (□, thin line) for the control treatment. Each value is the mean ± SE for the particular species studied. Nonlinear regression analysis was used to fit the data to exponential functions (r^2 = coefficient of determination).

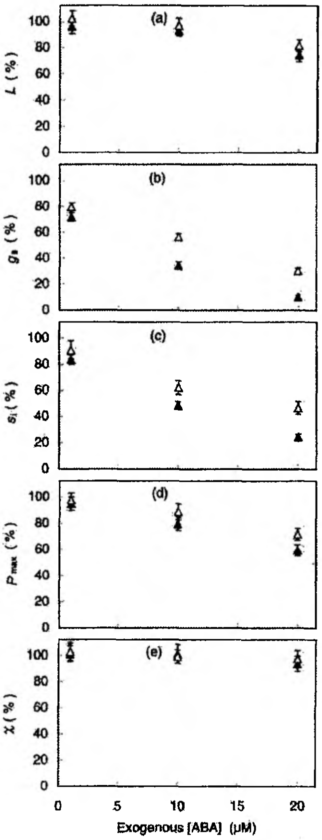

Figure 6. Relationships between the exogenous [ABA] and (a) shoot hydraulic conductance (L), (b) stomatal conductance (g_s), (c) stomatal sensitivity to leaf water potential increase (s_i), (d) maximum net assimilation rate (P_{max}), and (e) carboxylation efficiency (χ) of the six studied species in July (▲) and September (△). The values were averaged for all species for each ABA concentration, and the data are expressed as a percent of the values for control shoots in distilled water (see Figure 3 for the absolute values of the control treatments).

strongly correlated with stomatal conductance and with stomatal sensitivity to changes in leaf water potential, we hypothesize that the species-specific stomatal anatomical characteristics, stomatal conductance and stomatal sensitivity are determined largely by [ABA] during leaf growth and development.

It has been demonstrated repeatedly that [ABA] has a strong influence on stomatal aperture (e.g., Gowing et al. 1993, Davies et al. 1994). Thus, even though species differences in stomatal anatomy may partly explain interspecific variability in stomatal conductance, variability in foliar [ABA] (Figure 3a) may provide an alternative explanation for interspecific variation in stomatal conductance.

Throughout the season, the lowest values of leaf [ABA] and the highest values of stomatal conductance were found in the two species of the family *Salicaceae*: *Populus tremula* and *Salix caprea* (Figure 3a), which are also considered the most drought-intolerant of the six study species (Ellenberg 1996). Although many plant traits including leaf-level characteristics, phenology, rooting and biomass partition patterns are responsible for drought tolerance, our study suggests that high foliar [ABA] and low stomatal conductance may be important adaptive characteristics contributing to water conservation in drought-tolerant trees.

The rate of change in stomatal aperture in response to changes in environmental conditions is an important parameter that may account for differences among species in light-use efficiency in highly variable light environments (Pearcy 1994, Küppers et al. 1996, Stegemann et al. 1999). However, a mechanistic explanation for the interspecific variability in the rate of stomatal opening is lacking. We found that, within a species, mean foliar [ABA] was strongly correlated with rate of stomatal opening in response to changes in leaf water status (Figure 4c). This relationship also indicates that differences in foliar [ABA] may explain interspecific variability in carbon gain.

Relationships between endogenous [ABA] and shoot hydraulic conductance

There was a strong negative correlation between mean foliar [ABA] and shoot hydraulic conductance in the six study species (Figure 4a). Many studies have shown that narrow xylem vessel elements develop in response to an increase in endogenous [ABA] (Little and Wareing 1981, Pharis et al. 1981, Fromm 1997). Our study demonstrates that species ranking based on decreasing leaf [ABA] (Figure 3a) is the same as that based on increasing radius of the xylem vessels of leaves (Aasamaa et al. 2001). Therefore, because of the essential role of ABA in xylem development, we conclude that species differences in [ABA] during the period of intensive foliage growth may partly account for the interspecific variability in the diameter of xylem vessels. Hydraulic conductance of vessels strongly depends on their diameter (Schulte and Gibson 1988). Thus, among the study species, hydraulic conductance may have been lowest in *A. platanoides*, because foliar [ABA] was highest during the growth period.

Effect of exogenous ABA on shoot hydraulic conductance

Aasamaa and Sõber (2001) found that hydraulic conductance in mature leaves decreased significantly after only a few days of water stress (embolism was eliminated in the experiments) or darkness. The ABA content of leaves increases both in response to water stress (Cowan and Farquhar 1977, Jones 1996, Socias et al. 1997) and darkness (Gowing et al. 1993, Williams et al. 1994, Weatherwax et al. 1996). Therefore, short-term changes in shoot hydraulic conductance may be associated with changes in foliar [ABA].

It appears that exogenously supplied ABA is not immediately metabolized in leaves, because the [ABA] in mesophyll cells increases at least half as much as the [ABA] in xylem in response to a supply of exogenous ABA (Jia et al. 1996, Wilkinson and Davies 1997). Therefore, we postulate that mesophyll [ABA] increased several fold when we fed the xylem with a high [ABA]. However, there were only minor changes in hydraulic conductance in response to the application of exogenous ABA (Figure 6a). This finding implies that in vivo changes in mesophyll [ABA] of mature leaves, for instance in response to diurnal changes in leaf water status, should have no significant influence on hydraulic conductance. Because the diameters of xylem vessels cannot be changed after cessation of growth, hydraulic conductance in mature leaves should be more sensitive to [ABA] during the period of growth than subsequently.

Nevertheless, shoot hydraulic conductance decreased significantly in response to a high concentration of exogenous ABA (Figure 6a). Because there is no evidence of a rapid change in hydraulic conductance in response to modification of leaf apoplast hydraulic characteristics in well-watered plants, we suggest that the decrease in shoot hydraulic conductance in response to exogenous ABA was the result of ABA action on hydraulic conductance of the leaf symplast. There is a growing consensus that rapid and effective regulation of the hydraulic conductance of plant membranes takes place by means of aquaporines (Eckert et al. 1999, Tyerman et al. 1999, Netting 2000). Abscisic acid likely acts on aquaporines indirectly: ABA acts on membrane Ca^{2+}-channels, and the conductance of aquaporines is sensitive to the concentration of Ca^{2+} in the cells (Quintero et al. 1998, 1999). Therefore, we hypothesize that high concentrations of exogenous ABA decreased shoot hydraulic conductance by changing the conductance of the aquaporines. The relatively moderate influence of exogenous ABA on hydraulic conductance may be explained by the movement of water through the apoplast (Steudle 1997, 2000), where rapid changes in hydraulic conductance likely do not occur.

Effect of exogenous ABA on stomatal conductance

We observed that both stomatal conductance and the rate of stomatal opening decreased in response to exogenous ABA (Figures 6b and 6c). This further strengthens the suggestion that leaf endogenous [ABA] modifies these stomatal variables (Figures 4b and 4c), and that species-specific differences in leaf [ABA] partly explain species differences in stomatal responses.

When the xylem is supplied with exogenous ABA, there is an increase in leaf epidermal [ABA] (Jia et al. 1996, Wilkinson and Davies 1997, Zhang et al. 1997, Hartung et al. 1998). Increased [ABA] activates several processes, which collectively lead to a reduction in the concentration of the osmotic compounds in guard cells, resulting in a corresponding decrease in guard cell water content, and hence, stomatal closure (see reviews by Grabov and Blatt 1998, Assmann and Shimazaki 1999, Netting 2000). Therefore, the low rate of stomatal opening in species with high endogenous leaf [ABA] (Figure 4c) and the decrease in the rate of stomatal opening in response to exogenous ABA (Figure 6c) are probably both a result of the negative effect of ABA on guard cell water content.

Although the [ABA] of well-watered ABA-treated leaves was considerably higher than that of water-stressed leaves with closed stomata (Davies et al. 1981, Tenhunen et al. 1994), the stomata still opened when the water potential of the ABA-treated leaf was raised. This shows that the influence of high leaf water potentials on the hydroactive reactions necessary for stomatal opening overrides the effect of ABA. When water potential in these deciduous trees is high, either stomatal sensitivity to ABA decreases or ABA is isolated from its site of action.

Possible indirect effect of ABA on stomata

There may be an indirect effect of ABA on stomata resulting from changes in water flow in the leaf, because high [ABA] decreases shoot hydraulic conductance (Figure 6a). However, the decrease in stomatal conductance (Figure 6b) and stomatal sensitivity (Figure 6c) in response to a rapid increase in [ABA] were considerably larger than the decrease in shoot hydraulic conductance (Figure 6a). Therefore, we conclude that rapid changes in leaf [ABA] influence stomata in the mature leaves mostly directly, whereas the indirect influence of ABA on stomata via changes in foliar hydraulic conductance is of minor significance.

Role of ABA in older leaves

We found that leaf [ABA] was low in September (Figure 3a) but the other variables also exhibited reductions in values between July and September (Figures 3b–f) such that similar values of these variables were observed at a lower [ABA] in September than in July (Figure 4). This suggests that older leaves had greater sensitivity to [ABA] than younger leaves. However, the sensitivity of these characteristics to exogenous [ABA] was lower in September than in July (Figure 6). The discrepancy suggests that decreased hydraulic and stomatal conductance of older leaves (Figures 3b and 3c) led to decreased ABA fluxes to the leaves. Alternatively, leaf [ABA] may be less important in determining stomatal conductance and the rate of stomatal opening at later stages of leaf ontogeny. Older leaves are characterized by a high degree of vessel cavitation and clogging, immobilization of nitrogen and other limiting elements (Stoddart and Thomas 1982, Celikel and van Doorn 1995) and greater concentrations of osmotically active substances (Niinemets et al. 1999). Thus, it seems likely that metabolic reorganization accounts for the low [ABA] and the low values of the other measured variables in older leaves in September.

Effects of [ABA] on foliar carbon gain characteristics

There is evidence that the photosynthetic rate decreases in response to an increase in either endogenous foliar [ABA] (Loveys and Düring 1984, Liang et al. 1997) or exogenous ABA (Cornic and Miginiac 1983, Meyer and Genty 1999, Horváth et al. 2000). Usually, the decrease in photosynthetic rate is not entirely attributable to decreases in stomatal conductance, as was observed also in our study (Figures 5a and 6d).

Carboxylation efficiency (χ, Equation 7) is essentially an indicator of Rubisco activity, and the CO_2- and light-saturated rates of net photosynthesis (P_{max}) indicate the capacity for photosynthetic electron transport (Farquhar et al. 1980). The finding that species-specific endogenous [ABA] correlated with light-saturated photosynthetic rate but not with carboxylation efficiency (Figure 5), as was also found for exogenous [ABA] (Figures 6d and 6e), suggests that the non-stomatal influence of ABA may be stronger on electron transport than on Rubisco-mediated reactions in these species. The sensitivity of the light reactions of photosynthesis to foliar [ABA] has been noted previously. The efficiency of excitation energy capture decreases in leaves with high [ABA] in several plant species (Bunce 1987, Lu and Zhang 1998, 1999, Escalona et al. 1999). The non-stomatal influence of ABA on foliar photosynthetic characteristics (Figures 5a and 6d) was considerably less than on stomatal conductance (Figures 4b and 5b) and the rate of stomatal opening (Figures 4c and 5c). However, decreases in electron transport rate may be significant in vivo at present-day ambient CO_2 concentration, especially at low irradiance when photosynthetic rates are limited by the rate of photosynthetic electron transport.

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References

Aasamaa, K. and A. Sõber. 2001. Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. Biol. Plant. 44:65–73.

Aasamaa, K., A. Sõber and M. Rahi. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. Aust. J. Plant Physiol. 28:765–774.

Ackerson, R.C. 1980. Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. Plant Physiol. 65: 455–459.

Assmann, S.M. and K.-I. Shimazaki. 1999. The multisensory guard cell. Stomatal responses to blue light and abscisic acid. Plant Physiol. 119:809–816.

Becker, W. and K. Apel. 1993. Differences in gene expression between natural and artificially induced senescence. Planta 189: 74–79.

Blake, J. and W.K. Ferrell. 1977. The association between soil and xylem water potential, leaf resistance, and abscisic acid content in droughted seedlings of Douglas-fir (*Pseudotsuga menziesii*). Physiol. Plant. 39:106–109.

Bunce, J.A. 1987. Species-specific responses to water stress of gas exchange parameters mimicked by applied abscisic acid. Can. J. Bot. 65:103–106.

Celikel, F.G. and W.G. van Doorn. 1995. Solute leakage, lipid peroxidation and protein degradation during the senescence of *Iris* sepals. Physiol. Plant. 94:515–521.

Chandler, P.M. and M. Robertson. 1994. Gene expression regulated by abscisic acid and its regulation to stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:113–141.

Comstock, J. and M. Mencuccini. 1998. Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T. & G.), a desert subshrub. Plant Cell Environ. 21:1029–1038.

Cornic, G. and E. Miginiac. 1983. Nonstomatal inhibition of net CO_2 uptake by (±) abscisic acid in *Pharbitis nil*. Plant Physiol. 73: 529–533.

Cowan, I.R. and G.D. Farquhar. 1977. Stomatal function in relation to leaf metabolism and environment. *In* Integration of Activity in the Higher Plant. Ed. D.H. Jennings. Symp. Soc. Exp. Biol. 31. Cambridge University Press, pp 471–505.

Davies, W.J. and T.T. Kozlowski. 1975. Stomatal responses to changes in light intensity as influenced by plant water stress. For. Sci. 21:129–133.

Davies, W.J., J.A. Wilson, R.E. Sharp and O. Osonubi. 1981. Control of stomatal behaviour in water-stressed plants. *In* Stomatal Physiology. Eds. P.G. Jarvis and T.A. Mansfield. Cambridge University Press, pp 163–186.

Davies, W.J., F. Tardieu and C.L. Trejo. 1994. How do chemical signals work in plants that grow in drying soil. Plant Physiol. 104: 309–314.

Eckert, M., A. Biela, F. Siefritz and R. Kaldenhoff. 1999. New aspects of plant aquaporin regulation and specificity. J. Exp. Bot. 50: 1541–1545.

Ellenberg, H. 1996. Vegetation of central Europe with the Alps in ecological, dynamic and historical view. Vol. 5. Verlag Eugen Ulmer, Stuttgart, 1096 p. In German.

Escalona, J.M., J. Flexas and H. Medrano. 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. Aust. J. Plant Physiol. 26:421–433.

Ewers, F.W., J.B. Fisher and S.T. Chiu. 1990. A survey of vessel dimensions in stems of tropical lianas and other growth forms. Oecologia 84:544–552.

Fang, C., R.K. Monson and E.B. Cowling. 1996. Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. Tree Physiol. 16:441–446.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90.

Fromm, J. 1997. Hormonal physiology of wood growth in willow (*Salix viminalis* L.): effects of spermine and abscisic acid. Wood Sci. Technol. 31:119–130.

Gowing, D.J.G., W.J. Davies, C.L. Trejo and H.G. Jones. 1993. Xylem-transported chemical signals and the regulation of plant growth and physiology. Phil. Trans. R. Soc. Lond. B 341:41–47.

Grabov, A. and M.R. Blatt. 1998. Co-ordination of signaling elements in guard cell ion channel control. J. Exp. Bot. 49:351–360.

Hartung, W. and W.J. Davies. 1991. Drought-induced changes in physiology and ABA. *In* Abscisic Acid: Physiology and Biochemistry. Eds. W.J. Davies and H.G. Jones. BIOS Scientific Publishers, Oxford, pp 63–79.

Hartung, W., S. Wilkinson and W.J. Davies. 1998. Factors that regulate abscisic acid concentrations at the primary site of action at the guard cell. J. Exp. Bot. 49:361–367.

Henson, I.E., C.R. Jensen and N.C. Turner. 1989. Leaf gas exchange and water relations of lupins and wheat. III. Abscisic acid and drought-induced stomatal closure. Aust. J. Plant Physiol. 16: 429–442.

Horváth, E.M., S.O. Peter, T. Joët, et al. 2000. Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. Plant Physiol. 123:1337–1350.

Jia, W.S., J.H. Zhang and D.P. Zhang. 1996. Metabolism of xylem-delivered ABA in relation to ABA flux and concentration in leaves of maize and *Commelina communis*. J. Exp. Bot. 47:1085–1091.

Jones, H.G. 1996. Drought tolerance and water-use efficiency. *In* Water Deficits. Plant Responses from Cell to Community. Eds. J.A.C. Smith and H. Griffiths. BIOS Scientific Publishers, Oxford, pp 193–203.

Kirschbaum, M.U.F., L.J. Gross and R.W. Pearcy. 1988. Observed and modelled stomatal responses to dynamic light environments in the shade plant *Alocasia macrorrhiza*. Plant Cell Environ. 11: 111–121.

Körner, C. 1994. Leaf diffusive conductances in the major vegetation types of the globe. *In* Ecophysiology of Photosynthesis. Eds. E.-D. Schulze and M.M. Caldwell. Springer-Verlag, Berlin, pp 463–490.

Körner, C., J.A. Scheel and H. Bauer. 1979. Maximum leaf diffusive conductance in vascular plants. Photosynthetica 13:45–82.

Küppers, M., H. Timm, F. Orth, J. Stegemann, R. Stober, H. Schneider, K. Paliwal, K.S.T.K. Karunaichamy and R. Ortiz. 1996. Effects of light environment and successional status on lightfleck use by understory trees of temperate and tropical forests. Tree Physiol. 16:69–80.

Leung, J. and J. Giraudat. 1998. Abscisic acid signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:199–222.

Liang, J., J. Zhang and M.H. Wong. 1996. Stomatal conductance in relation to xylem sap abscisic acid concentrations in two tropical trees, *Acacia confusa* and *Litsea glutinosa*. Plant Cell Environ. 19:93–100.

Liang, J., J. Zhang and M.H. Wong. 1997. Can stomatal closure caused by xylem ABA explain the inhibition of leaf photosynthesis under soil drying? Photosynth. Res. 51:149–159.

Little, C.H.A. and P.F. Wareing. 1981. Control of cambial activity and dormancy in *Picea sithensis* by indol-3-acetic and abscisic acids. Can. J. Bot. 59:1480–1493.

Loewenstein, N.J. and S.G. Pallardy. 1998. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of canopy trees of three temperate deciduous angiosperms. Tree Physiol. 18:431–439.

Loveys, B.R. and H. Düring. 1984. Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars. II. Abscisic acid changes under semi-arid conditions. New Phytol. 97:37–47.

Lu, C. and J. Zhang. 1998. Effects of water stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants. Aust. J. Plant Physiol. 25:883–892.

Lu, C. and J. Zhang. 1999. Effects of water stress on Photosystem II photochemistry and its thermostability in wheat plants. J. Exp. Bot. 50:1199–1206.

Ludlow, M.M., M.J. Fisher and J.R. Wilson. 1985. Stomatal adjustment to water deficits in three tropical grasses and a tropical legume grown in controlled conditions and in the field. Aust. J. Plant Physiol. 12:131–149.

Mäntylä, E., V. Lang and E.T. Palva. 1995. Role of abscisic acid in drought-induced freezing tolerance, cold acclimation and accumulation of LTI78 and RAB18 proteins in *Arabidopsis thaliana*. Plant Physiol. 107:141–148.

McMichael, B.L. and B.W. Hanny. 1977. Endogenous levels of abscisic acid in water-stressed cotton leaves. Agron. J. 69: 979–982.

Mederski, H.J. 1961. Determination of internal water status of plants by beta ray gauging. Soil Sci. 92:143–146.

Mertens, R.J., B. Deus-Neumann and E.W. Weiler. 1985. Monoclonal antibodies for the detection and quantitation of the endogenous plant growth regulator, abscisic acid. FEBS Lett. 160: 269–272.

Meyer, S. and B. Genty. 1999. Heterogeneous inhibition of photosynthesis over the leaf surface of *Rosa rubiginosa* L. during water stress and abscisic acid treatment: induction of a metabolic component by limitation of CO_2 diffusion. Planta 210:126–131.

Moldau, H., J. Sõber and A. Sõber. 1993. Impact of acute ozone exposure on CO_2 uptake by two cultivars of *Phaseolus vulgaris*. Photosynthetica 28:133–141.

Netting, A.G. 2000. pH, abscisic acid and the integration of metabolism in plants under stressed and non-stressed conditions: cellular responses to stress and their implications for plant water relations. J. Exp. Bot. 51:147–158.

Niinemets, Ü., O. Kull and J.D. Tenhunen. 1998. An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. Tree Physiol. 18:681–696.

Niinemets, Ü., A. Sõber, O. Kull, W. Hartung and J.D. Tenhunen. 1999. Apparent controls on leaf conductance by soil water availability and via light-acclimation of foliage structural and physiological properties in a mixed deciduous, temperate forest. Int. J. Plant Sci. 160:707–721.

Pearcy, R.W. 1994. Photosynthetic response to sunflecks and light gaps: mechanisms and constraints. *In* Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Eds. N.R. Baker and J.R. Bowyer. BIOS Scientific Publishers, Oxford, pp 255–271.

Peuke, A.D., W.D. Jeschke and W. Hartung. 1994. The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long distance transport of abscisic acid depending on nitrogen nutrition and salt stress. J. Exp. Bot. 45:741–747.

Pharis, R.P., P.A. Jenkins, H. Aoki and T. Sassa. 1981. Hormonal physiology of wood growth in *Pinus radiata* D. Don: effects of gibberellin A_4 and the influence of abscisic acid upon (^{3}H) gibberellin A_4 metabolism. Aust. J. Plant Physiol. 8:559–570.

Philosoph-Hadas, S., E. Hadas and N. Aharoni. 1993. Characterization and use in ELISA of a new monoclonal antibody for quantitation of abscisic acid in senescing rice leaves. Plant Growth Regul. 12:71–78.

Pothier, D., H.A. Margolis, J. Poliquin and R.H. Waring. 1989. Relation between the permeability and the anatomy of jack pine sapwood with stand develompent. Can. J. For. Res. 19:1564–1570.

Quarrie, S.A. 1991. Implication of genetic differences in ABA accumulation from crop production. *In* Abscisic Acid. Physiology and Biochemistry. Eds. W.J. Davies and A.G. Jones. BIOS Scientific Publishers, Oxford, pp 227–243.

Quintero, J.M., J.M. Fournier, J. Ramos and M. Benlloch. 1998. K^+ status and ABA affect both exudation rate and hydraulic conductivity in sunflower roots. Physiol. Plant. 102:279–284.

Quintero, J.M., J.M. Fournier and M. Benlloch. 1999. Water transport in sunflower root systems: effects of ABA, Ca^{2+} status and $HgCl_2$. J. Exp. Bot. 50:1607–1612.

Radin, J.W. 1984. Stomatal responses to water stress and to abscisic acid in phosphorous deficient cotton plants. Plant Physiol. 76: 392–394.

Saliendra, N.Z., J.S. Sperry and J.P. Comstock. 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta 196: 357–366.

Schulte, P.J. and A.C. Gibson. 1988. Hydraulic conductance and tracheid anatomy in six species of extant seed plants. Can. J. Bot. 66:1073–1079.

Sõber, A. 1992. Effect of ozone on the rehydration of bean leaves. Proc. Est. Acad. Sci. Biol. 41:35–43.

Sõber, A. 1996. Simultaneous measurement of leaf hydraulic and stomatal conductances by pressure bomb, gas exchange and β-gauge techniques. Proc. Est. Acad. Sci. Biol. 45:35–43.

Sõber, A. 1997. Hydraulic conductance, stomatal conductance, and maximal photosynthetic rate in bean leaves. Photosynthetica 34: 599–603.

Sõber, A.J. and H.A. Moldau. 1977. An apparatus with separate conditioning of the plant and the leaf for the determination of stomatal resistance and leaf water content. Fiziol. Rast. 24:1301–1307. In Russian.

Sõber, A. and E. Sild. 1999. Stomatal closure during tobacco leaf desiccation as affected by ozone. Biol. Plant. 42:267–272.

Socias, X., M.J. Correia, M. Chaves and H. Medrano. 1997. The role of abscisic acid and water relations in drought responses of subterranean clover. J. Exp. Bot. 48:1281–1288.

Stegemann, J., H.-C. Timm and M. Küppers. 1999. Simulation of photosynthetic plasticity in response to highly fluctuating light: an empirical model integrating dynamic photosynthetic induction and capacity. Trees 14:145–160.

Steudle, E. 1997. Water transport across plant tissue: role of water channels. Biol. Cell 89:259–273.

Steudle, E. 2000. Water uptake by roots: effect of water deficit. J. Exp. Bot. 51:1531–1542.

Stoddart, L. and H. Thomas. 1982. Leaf senescence. *In* Encyclopedia of Plant Physiology, New Series. Vol. 14A. Eds. D. Boulter and B. Parthier. Springer-Verlag, Berlin, pp 135–151.

Tardieu, F., T. Lafarge and T. Simonneau. 1996. Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. Plant Cell Environ. 19:75–84.

Tenhunen, J.D., R. Hanano, M. Abril, E.W. Weiler and W. Hartung. 1994. Above- and below-ground environmental influences on leaf conductance of *Ceanothus thyrsiflorus* growing in a chaparral environment: drought response and the role of abscisic acid. Oecologia 99:306–314.

Tinoco-Ojanguren, C. and R.W. Pearcy. 1993. Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. II. Stomatal versus biochemical limitations during photosynthetic induction. Oecologia 94:395–402.

Tyerman, S.D., H.J. Bohnert, C. Maurel, E. Steudle and J.A.C. Smith. 1999. Plant aquaporines: their molecular biology, biophysics and significance for plant water relations. J. Exp. Bot. 50:1055–1071.

von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387.

Weatherwax, S.C., M.S. Ong, J. Degenhardt, E.A. Bray and E.M. Tobin. 1996. The interaction of light and abscisic acid in the regulation of plant gene expression. Plant Physiol. 111:363–370.

Wilkinson, S. and W.J. Davies. 1997. Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol. 113:559–573.

Williams, S.A., S.C. Weatherwax, E.A. Bray and E.M. Tobin. 1994. NPR genes, which are negatively regulated by phytochrome action in *Lemna gibba* L. G-3, can also be positively regulated by abscisic acid. Plant Physiol. 105:949–954.

Woods, D.B. and N.C. Turner. 1971. Stomatal responses to changing light intensity by four tree species of varying shade tolerance. New Phytol. 70:77–84.

Zeevaart, J.A.D., C.D. Rock, F. Fantauzzo, T.G. Heath and D.A. Gage. 1991. Metabolism of ABA and its physiological implications. *In* Abscisic Acid. Physiology and Biochemistry. Eds. W.J. Davies and H.G. Jones. BIOS Scientific Publishers, Oxford, pp 39–52.

Zhang, J., W. Jia and D.-P. Zhang. 1997. Re-export and metabolism of xylem-delivered ABA in attached maize leaves under different transpirational fluxes and xylem ABA concentrations. J. Exp. Bot. 48:1557–1564.

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ROLES OF ABSCISIC ACID IN WATER-USE STRATEGIES OF DECIDUOUS TREE SPECIES OF DIFFERENT LAYERS IN TEMPERATE FOREST CANOPY

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ABSTRACT

Water-use strategies of *Populus tremula* and *Tilia cordata*, and especially the role of abscisic acid in these strategies were analysed. *P. tremula* dominated in the overstorey and *T. cordata* in the lower layer of the tree storey of the temperate deciduous forest canopy. Shoot water potential (Ψ), bulk-leaf abscisic acid concentration ($[\text{ABA}]_{\text{leaf}}$), abscisic acid concentration in xylem sap ($[\text{ABA}]_{\text{xyl}}$), and stomatal sensitivity to exogenous ABA (s_{ABA}) were lower, and the foliar water content per area (w), stomatal conductance (g_s), content of the leaf osmotica (c) and the shoot hydraulic conductance (L) were higher in non-stressed *P. tremula* than in non-stressed *T. cordata*. In drought conditions, the Ψ, w, g_s and L decreased, and c, $[\text{ABA}]_{\text{leaf}}$ and s_{ABA} increased in both the species. The change of L and $[\text{ABA}]_{\text{leaf}}$ was similar in both the species. Yet, the effect of water deficit was more pronounced on the g_s and s_{ABA} in *T. cordata*, and on the Ψ, w and c in *P. tremula*. $[\text{ABA}]_{\text{xyl}}$ increased only in *P. tremula* in the drought conditions. Thus, the regulation of water relations is basically different in these two species in drought conditions. Probably the stomatal conductance varies due to changes of $[\text{ABA}]_{\text{xyl}}$ in *P. tremula*, but due to changes in stomatal sensitivity to ABA in *T. cordata*. The role of ABA in the regulation of the shoot hydraulic conductance and the osmotic adjustment in response to drought is also discussed.

Keywords: Abscisic acid, *Populus tremula*, Shoot hydraulic conductance, Stomatal conductance, *Tilia cordata*

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List of symbols: ABA — abscisic acid; $[\mathrm{ABA}]_{\mathrm{leaf}}$ — bulk-leaf abscisic acid concentration (nmol $\mathrm{g(dw)}^{-1}$); $[\mathrm{ABA}]_{\mathrm{xyl}}$ — abscisic acid concentration in xylem sap (μM); c — content of leaf osmotica (mmol m^{-2}); E — transpiration rate (mmol $\mathrm{m}^{-2}\mathrm{s}^{-1}$); Ψ — shoot water potential (MPa); g_s — stomatal conductance (mmol $\mathrm{m}^{-2}\mathrm{s}^{-1}$); L — shoot hydraulic conductance (μg $\mathrm{m}^{-2}\mathrm{s}^{-1}\mathrm{Pa}^{-1}$); s_{ABA} — stomatal sensitivity to exogenous ABA (s^{-1}); v — maximum relative rate of the decrease of the stomatal conductance (% s^{-1}); w — leaf water content per area (g m^{-2})

INTRODUCTION

Plants with conservative water-use strategy promptly slow down their water use, as water becomes scarce. In drought conditions, these plants decrease stomatal openness significantly to avoid a decrease in water potentials in shoot. Plants with a prodigal water-use strategy continue with intensive water use in conditions of water deficit, until the soil water reserves are almost completely exhausted. These plants retain high stomatal openness, and the metabolism of the plants is relatively tolerant to low water potentials (Ludlow 1989; Donovan *et al.* 2000). The hydraulic conductance of shoots usually decreases in conditions of water deficit, and a smaller decrease has been measured in plants with more conservative water-use strategy (Nardini *et al.* 1999; Donovan *et al.* 2000; Cinnirella *et al.* 2002). In studies of the water use of tree species, juvenile trees of equal height and equal light availability have been observed in most cases (*e.g.* Augé *et al.* 1998; Croker *et al.* 1998; Loewenstein and Pallardy 1998a). The regulation of water relations in trees changes with age (Jones *et al.* 1983; Whitehead 1998). There is less comparative data (Bahari *et al.* 1985; Loewenstein and Pallardy 1998b) about the water use of mature trees of different canopy layers. Knowledge about the traits of water-use strategy in different species and in different aged plants is important for modelling the changes in the pattern of species in a changing environment. For example, a rise in the atmospheric CO_2 concentration probably gives an advantage to the plants with a conservative strategy, because of the decrease in the growth limitation by CO_2 concentration. Air pollution is probably more harmful to the plants with a less conservative strategy, because larger amounts of pollutants can enter the leaves through the stomata.

The physiological mechanisms applied in realising the different water-use strategies also are incompletely documented. It is known that stomatal conductance is dependent on abscisic acid concentration ([ABA]): Increased [ABA] activates several processes, which collectively lead to a reduction in the concentration of the osmotic compounds in the stomatal guard cells, resulting in a corresponding decrease in the guard cell water content, and hence, a decrease in stomatal openness (see reviews by Grabov and Blatt 1998; Assmann and Shimazaki 1999; Schroeder *et al.* 2001). The increase of [ABA] in droughted plants is repeatedly observed (*e.g.* Wright 1977; Hartung and Davies 1991; Liu *et al.* 2001; Sauter *et al.* 2001). However, the compartmentalisation pattern of ABA in leaves is incompletely documented. Only [ABA] of xylem sap ($[\mathrm{ABA}]_{\mathrm{xyl}}$) or only [ABA] of bulk leaves ($[\mathrm{ABA}]_{\mathrm{leaf}}$) have been measured, in addition to the stomatal conductance, in most studies. In the studies detecting both these concentrations, better accordance of stomatal conductance with the $[\mathrm{ABA}]_{\mathrm{xyl}}$ than with the $[\mathrm{ABA}]_{\mathrm{leaf}}$ have been found in most cases, but these patterns differed significantly in different species (Zhang and Davies 1989; Khalil and Grace 1993; Liang *et al.* 1996; Zhang *et al.* 1997; Zdunek and Lips 2001). There is insufficient data about the relations between stomatal conductance and the concentrations of ABA in different compartments of shoots of mature temperate deciduous trees. The relations between the specific features of stomatal regulation by ABA and the water-use strategy have also not been analysed.

It has been shown repeatedly that osmotic adjustment is higher in the leaves, which accumulate more ABA in response to drought (Düring 1986; Stewart *et al.* 1986; LaRosa *et al.* 1987; Chandler and Robertson 1994). Additionally, osmotic adjustment of mesophyll cells is induced by an exogenous supply of ABA. ABA decreases the efflux of inorganic ions from symplast to apoplast (Roberts and Snowman 2000; Sutton *et al.* 2000) and enhances the synthesis of small organic molecules in the mesophyll cells (Pesci 1992; Gosti *et al.* 1994; Savoure *et al.* 1997). Yet, the relations between the osmotic adjustment and the compartmentalisation pattern of ABA in leaves of the temperate deciduous trees have not been analysed.

Shoot hydraulic conductance (*L*) is also an important characteristic of leaf water relations, because the leaf water supply depends on the *L*. There is only very little data about the mechanisms by which the shoot hydraulic conductance changes. One important reason for the decrease of the *L* in drought conditions is embolism of the xylem conducting elements (Crombie *et al.* 1985; Sperry and Ikeda 1997; Tyree 1997). We eliminated xylem embolism in our previous experiments. Yet, the shoot hydraulic conductance was significantly lower in the saplings subjected to short-term water stress than in well-watered young trees (Aasamaa and Sõber 2001). We also found that the *L* of well-watered saplings significantly decreased in response to exogenous ABA (Aasamaa *et al.*

2002). Therefore, the decrease of shoot hydraulic conductance in water-stressed trees is caused not only by the embolism of xylem vessels, but by some additional mechanisms. One important modifier of the shoot hydraulic conductance of young trees of *P. tremula* and *T. cordata* is probably ABA. Yet, gene expression varies in the course of ontogeny and the sensitivity of several processes to ABA also changes (Willmer *et al.* 1988; Brenac *et al.* 1997; Fan *et al.* 1997). Thus, the importance of ABA in regulation of the shoot hydraulic conductance can differ between the saplings and old trees of the same species. Additionally, the relations between shoot hydraulic conductance and the [ABA] of different compartments of the shoot have not been analysed.

Two tree species were analysed in the present study: *Populus tremula* — dominating in the overstorey and *Tilia cordata* – dominating in the lower layer of the tree storey of the temperate deciduous forest canopy. The main objectives of the present work were: 1) To determine the characteristics of water-use strategy of these trees; 2) To clarify the role of ABA in these strategies: To determine the compartmental pattern of ABA (bulk-leaf [ABA] and xylem sap [ABA]) in non-stressed and droughted shoots of these two species and to relate these concentrations of ABA to the stomatal conductance, the osmotic adjustment, and the shoot hydraulic conductance.

MATERIALS AND METHODS

Plants

The trees grew in Järvselja, Estonia (58°16'N, 27°20'E, elevation ~40 m a.s.l.), in temperate mixed forest, on podzolic soils, the trees were *ca.* 50 years old. *Populus tremula* L. dominated in the overstorey (19–25 m) and *Tilia cordata* Mill. in the lower layer of the tree storey (5–18 m) of the forest canopy. For further details of the canopy see the paper of Niinemets *et al.* (1998). Shoots (10–15 cm in length) from the top and base of the foliages of both the species were cut from the trees for the analysis. The shoots were sampled in the morning, about two hours after sunrise; thus the daily maximum values of stomatal conductance were observed.

Non-stressed trees with fully expanded mature leaves were analysed at the beginning of July. At the end of July, after three weeks of drought with unusually high temperatures for the Estonian climate (daily mean temperatures ~25°C, maximum ~30°C) the same trees were analysed again.

Measurements of stomatal conductance (g_s) and stomatal sensitivity to ABA (s_{ABA})

The shoots were enclosed in a plastic bag, cut under (distilled) water, brought (the base of each shoot remained submerged) to the laboratory, and placed under halogen lamps (saturating irradiance: 1410 μmol $m^{-2}s^{-1}$). The values of g_s in the results of this paper (Fig. 1c) are the stabilised values of the abaxial stomatal conductance observed after each shoot had been in the laboratory conditions ~15 min. Then the distilled water of the water-vessel of the shoot was interchanged for a solution of 5 or 10 μM ABA and the following decrease of the stomatal conductance was observed (during ~1.5–2 h). The stomatal conductances and also transpiration rates were measured by steady-state porometer LI-1600 (LI-COR; Lincoln, NE, USA).

Calculation of the stomatal sensitivity to ABA

s_{ABA} was defined as the relative rate of decrease of the stomatal conductance after the shoot was supplied with the exogenous ABA:

$$s_{ABA} = v\ A \qquad (1)$$

where v is the maximum relative rate of decrease of the stomatal conductance, and A is a scaling coefficient which transforms the v of different leaves to a common rate of water potential change. v was determined from:

$$v = \frac{\Delta g_s}{\Delta t\ (g_{se} - g_{sa})} 100\% \qquad (2)$$

where Δg_s is the difference of the stomatal conductances in the region of the maximum rate of the decrease of stomatal conductance; Δt is the time interval corresponding to Δg_s; g_{sa} is the average stomatal conductance during the time interval, and g_{se} is the stomatal conductance at the end of the experiment. The relative rate of the change of the stomatal conductance was determined because the decrease of stomatal conductance was almost exponential with time. It is likely that the influence of the exogenous ABA is greater in the shoots with intensively transpiring leaves, because larger amounts of the exogenous ABA enter the leaves. Therefore, the scaling coefficient was determined from:

$$A = \frac{E_{0a}}{E_0} \qquad (3)$$

where E_0 is the initial, stabilised transpiration rate in the beginning of each experiment, and E_{0a} is the average of E_0 for all the experiments. For the calculation methods see also the paper of Aasamaa and Sõber (2001).

Determination of shoot water potential (Ψ) and hydraulic conductance (L)

The shoots were enclosed in a plastic bag, cut from the tree, and the Ψ was measured immediately by Scholander pressure chamber. Then the pressure chamber was filled with water and the shoot was fixed in the chamber so that the cut end of the shoot was in the water (and the remainder of the shoot was outside the chamber). The pressure ~1 MPa was applied in the chamber until the leaves of the shoot were fully infiltrated. The high pressure of the pressure chamber evidently eliminated xylem embolism in the shoots (Nardini and Pitt 1999; Tyree *et al.* 1999). Then the water was poured out from the pressure chamber and the shoot was fixed in the chamber so that only the cut end of the shoot was outside the chamber. A pressure of ~0.2 MPa was applied in the chamber, and the water that flowed out from the shoot through the cut end during 1 min was collected and weighed. L was calculated from:

$$L = \frac{\Delta m}{S\,\Delta t\,p} \tag{4}$$

where Δm is the mass of the water collected during the 1 min period (Δt); S is the summarised area of all the leaves of the shoot, and p is the pressure in the pressure chamber. Circumferences of the leaves of the shoot were traced with a computer digitizer QD-1212 (QTronix; Taiwan) and area of the leaves was calculated with self-developed computer software.

Determination of the endogenous concentration of abscisic acid ([ABA]), leaf osmotica (c), and leaf water content (w)

Leaf disks (~50–300 mg FW) were punched between major leaf veins from other shoots (brought to the laboratory in plastic bags). Shoot xylem sap was pressurised out from the shoots through the cut end of the shoot by the pressure chamber technique. The leaf disks and the xylem sap were enclosed in Eppendorf cups, immediately frozen in liquid nitrogen and stored at –20°C until the ABA analysis. For extraction of ABA from the leaf disks, the frozen tissue samples were homogenised and extracted in 80% methanol. The extracts and xylem sap were passed through a Sep-Pak C_{18}-cartridge. Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3. The ethyl acetate of the combined organic fractions was removed under reduced pressure and the residue was taken up in Tris buffer. The ABA content in the buffer solution and in the xylem sap was determined by ELISA as described by Mertens *et al.* (1985) and Peuke *et al.* (1994).

For the measurement of leaf osmotica, leaf disks (~50–100 mg FW) were also punched from the leaves, enclosed in Eppendorf cups, immediately frozen in liquid nitrogen and stored at –20°C until the analysis. The c was measured cryoscopically as in the study of Niinemets *et al.* (1999). For the determination of leaf water content (per area of the leaf), the leaf disks were weighed immediately after being punched from the leaves, and again after drying at 80°C for 48 h.

RESULTS

There was a vertical gradient of shoot water potential (Ψ), leaf water content per area (w), stomatal conductance (g_s), content of leaf osmotica (c), and shoot hydraulic conductance (L) through the tree storey; Ψ was the lowest in the top of the foliage of *P. tremula* and the highest in the base of the foliage of *T. cordata*. The values of w, g_s, c and L increased acropetally in the canopy. The values of Ψ, w, g_s and L decreased in the drought conditions. The relative decreases of Ψ and w were bigger and the relative decrease of g_s was slightly smaller in *P. tremula* than in *T. cordata*. The decrease of L was similar in the different species and in different positions in the foliage. Content of leaf osmotica (c) increased in the drought conditions (osmotic adjustment). The increase in the c was larger in *P. tremula*, than in *T. cordata* (Fig. 1a–e).

The bulk-leaf abscisic acid concentration ($[\mathrm{ABA}]_{\mathrm{leaf}}$) also changed gradually through the tree storey: $[\mathrm{ABA}]_{\mathrm{leaf}}$ was the lowest in the top of the foliage of *P. tremula* and the highest in the base of the foliage of *T. cordata*. $[\mathrm{ABA}]_{\mathrm{leaf}}$ had increased in both these species in drought conditions (Fig. 1f). The abscisic acid concentration in the shoot xylem ($[\mathrm{ABA}]_{\mathrm{xyl}}$) was higher in *T. cordata* than in *P. tremula* in non-stressed trees. Yet, the $[\mathrm{ABA}]_{\mathrm{xyl}}$ had increased several times in droughted *P. tremula*, but not in droughted *T. cordata* (Fig. 1g). The stomatal sensitivity to exogenous ABA (s_{ABA}) decreased gradually towards the top of the tree storey: s_{ABA} was the highest in the base of the foliage of *T. cordata* and the lowest in the top of the foliage of *P. tremula*, both in the non-stressed and in the droughted trees. Yet, in the drought conditions the increase in s_{ABA} was greater in *T. cordata* than in *P. tremula* (Fig. 1h).

The stomatal conductance, the stomatal sensitivity to exogenous ABA and the shoot hydraulic conductance correlated strongly with the $[\mathrm{ABA}]_{\mathrm{leaf}}$ (Fig. 2; Table 1), but not with the $[\mathrm{ABA}]_{\mathrm{xyl}}$ (data not shown).

DISCUSSION

Water-use strategies in *P. tremula* and in *T. cordata*

The stomatal conductance, the shoot hydraulic conductance, the shoot water potential, and the leaf water content decreased, and osmotic adjustment was induced in both species in response to a several week-long drought (Fig. 1a–d). Therefore, in both the species, the water-use strategy is not extremely conservative or extremely prodigal. Yet, only the decrease in the shoot hydraulic conductance was similar in both the species. The change in all the other characteristics was different in these two species: The decrease in the stomatal conductance was relatively greater, the osmotic adjustment was significantly lower, and the decrease in the shoot water potential and the leaf water content were smaller in *T. cordata*, than in *P. tremula* (Fig. 1a–d). Therefore, the water-use strategy in *T. cordata* is probably more conservative than in *P. tremula*. We also have found in our previous study that the maximum (CO_2- and light-saturated) photosynthetic rate (maximum photosynthetic capacity) is lower in *T. cordata* than in *P. tremula* already in saplings (Aasamaa and Sõber 2001). The choice of water-use strategy may be related to light availability and the characteristics of the photosynthetic apparatus. A less conservative strategy associated with high photosynthetic capacity is probably more suitable for the trees of the upper canopy layers, due to the sufficient availability of light for CO_2 assimilation. In lower layers of the tree storey a more conservative strategy and a low maximal photosynthetic capacity can be more suitable, because of the lack of light energy for high rates of CO_2 assimilation.

Relations of [ABA] with stomatal conductance in the two species

Though the [ABA] of the xylem sap entering the leaf is changed by the symplast of mesophyll or epidermis cells (Gowing *et al.* 1993; Trejo *et al.* 1995; Jia *et al.* 1996; Hartung *et al.* 1998), the $[ABA]_{xyl}$ is probably in accordance with the [ABA] in the apoplast surrounding the stomatal guard cells (Tardieu and Simonneau 1998). It has repeatedly been shown that guard cells are sensitive to [ABA] of an external medium and therefore it is supposed that important receptors for ABA are localised in the external surface of the plasmalemma of guard cells (Hartung 1983; Hornberg and Weiler 1984; Wilkinson and Davies 1997). Thus, our result that the decrease of g_s was associated with the increase of $[ABA]_{xyl}$ in the droughted *P. tremula*, was in accordance with expectations. We suppose that the varying of the apoplastic [ABA] is an important mechanism in the regulation of the stomatal conductance of *P. tremula*. The increase in the $[ABA]_{xyl}$ of tree shoots probably is the result of the increased release of ABA from roots, but also the increased production and export of ABA out of the foliar mesophyll cells through the plasmalemma (Jeschke *et al.*

1997; Zhang *et al.* 1997; Zhang *et al.* 2001). Therefore, the special characteristic of *P. tremula* in its resistance to water deficit is probably the high responsiveness of physiological processes in the mesophyll to the water conditions. The concentrations of the exogenous ABA were several times higher than the $[\mathrm{ABA}]_{\mathrm{xyl}}$ found in trees (Liang *et al.* 1996; Loewenstein and Pallardy 1998a,b; Niinemets *et al.* 1999; Augé *et al.* 2000). Therefore, the sequestration to symplast of mesophyll and other epidermal cells probably did not decrease the exogenous [ABA] significantly, and the [ABA] in the vicinity of guard cells also increased in the leaves supplied with the exogenous ABA. Thus, the lack of significant change in stomatal sensitivity to the exogenous ABA in *P. tremula* during drought was probably not just apparent, but was real. The modifying of stomatal sensitivity to ABA is not an important method in the water-use strategy of *P. tremula*.

The decrease in the stomatal conductance in droughted *T. cordata* was not accompanied with an increase in $[\mathrm{ABA}]_{\mathrm{xyl}}$. However, the decrease in the g_s was accompanied by an increase in the stomatal sensitivity to (exogenous) ABA (Fig. 1c,g,h). The plasmalemma of the guard cells probably is highly responsive to the water deficit in *T. cordata*.

Therefore, we postulate that the decrease in stomatal conductance in response to water deficit was achieved by two principally different mechanisms in these two species: In *P. tremula* the membranes of mesophyll cells are highly responsive to water stress and therefore more ABA exits from the mesophyll, resulting in higher concentrations of ABA reaching the vicinity of guard cells. In *T. cordata* the membranes of guard cells are more responsive to drought: the guard cells become more sensitive to the ABA.

The strong correlation between the $[\mathrm{ABA}]_{\mathrm{leaf}}$ and g_s through the tree storey (Fig. 2a) and also the increase of $[\mathrm{ABA}]_{\mathrm{leaf}}$ (associated with the decrease of g_s) at all heights in drought conditions (Fig. 1f,c) leads us to believe that the relationship between $[\mathrm{ABA}]_{\mathrm{leaf}}$ and g_s may be causal. It was found in our previous study, that the stomatal conductance was higher and $[\mathrm{ABA}]_{\mathrm{leaf}}$ was lower also in saplings of *P. tremula*, compared to saplings of *T. cordata* (Aasamaa *et al.* 2002). We also found that the anatomical characteristics of the stomata probably favoured the stomatal conductances to be higher in *P. tremula* than in *T. cordata*: the stomatal pores were longer, and the width of the guard cells and length of the dorsal side of the guard cells were smaller in *P. tremula*, than in *T. cordata* (Aasamaa *et al.* 2001). Additionally, it has been found that ABA has a very significant influence on the development of young leaves (Cutler *et al.* 1977; Bradford *et al.* 1983; Franks and Farquhar 2001) and the species differences in $[\mathrm{ABA}]_{\mathrm{leaf}}$ are retained throughout leaf ontogeny, when the trees grow in the same environment (Loewenstein and Pallardy 1998b; Augé *et al.* 2000). Therefore, the relatively indirect influence of foliar [ABA] can explain the correlation between the $[\mathrm{ABA}]_{\mathrm{leaf}}$ and g_s through the tree storey: [ABA] in young leaves probably determines the development of the anatomical characteristics that have an influence on stomatal conductance also in mature leaves.

It has been found that in the leaves with high $[ABA]_{leaf}$, the [ABA] is also high in guard cells (Weiler *et al.* 1982; Harris *et al.* 1988; Harris and Outlaw 1991). There is also some data showing that the stomatal openness changes in accordance with the changes of [ABA] in the guard cells, and it is supposed that internal receptors for ABA also exist in the guard cells (Allan and Trewavas 1994; Allan *et al.* 1994; Assmann *et al.* 1994; Schwartz *et al.* 1994; Wang *et al.* 1998). Our result that stomatal conductance and also stomatal sensitivity to the exogenous ABA were strongly correlated with $[ABA]_{leaf}$ (and likely also with the [ABA] in the guard cells) through the canopy, can, alternatively, indicate that both these tree species have internal ABA receptors in the guard cells, and the stomatal conductance and sensitivity of these species may be significantly influenced by the action of ABA inside the guard cells.

Relations of [ABA] with osmotic adjustment and shoot hydraulic conductance

We found that the osmotic adjustment was higher in *P. tremula*, where $[ABA]_{xyl}$ also increased significantly, whereas the increase of $[ABA]_{leaf}$ was not higher in *P. tremula* than in *T. cordata* in response to drought (Fig. 1d, f, g). Therefore, the osmotic adjustment in these species may be more influenced by the [ABA] outside the plasmalemma of the mesophyll cells (than by the [ABA] inside the cells), or, alternatively, the factors that induce higher compartmentalisation of ABA in the apoplast of the shoot can additionally favour osmotic adjustment.

Shoot hydraulic conductance depends on the hydraulic conductance in both the xylem and in the leaf parenchymal cells, because the water of the transpiration stream moves through the xylem in the stem, petiole, and leaves, and then moves out from the xylem and flows through the parenchymal cells of the leaves before being evaporated in the intercellular spaces (Boyer 1985; Steudle 1997). It is possible that the decrease in the shoot hydraulic conductance during the drought was also mediated by ABA. The increase in ionic concentration in the symplast, which is induced by high $[ABA]_{xyl}$, is accompanied by a decrease in the ionic content in the apoplastic fraction of the shoot (Roberts and Snowman 2000; Sutton *et al.* 2000). Some recent papers demonstrate that the xylem hydraulic conductance rapidly falls in response to a decrease in the ionic concentration of the xylem sap (van Meeteren *et al.* 2000; Brown 2001; Zwieniecki *et al.* 2001). Yet, we found that the decrease in the shoot hydraulic conductance was not greater in *P. tremula* (which is characterised by a big rise of $[ABA]_{xyl}$) than in *T. cordata* during the drought (Figs. 1e,g). Therefore, an important reason for the decrease in the L of these species in the drought conditions was probably not the decrease in the hydraulic conductance in the xylem and in the apoplast of the foliar parenchymal cells.

However, the relatively equal decrease in the shoot hydraulic conductance was accompanied by the relatively equal increase in $[ABA]_{leaf}$ at all heights of the tree storey (Fig. 1e,f). Therefore, we suppose that an important reason for the decrease in L could be the decrease in hydraulic conductance of the leaf symplast. Effective regulation of the hydraulic conductance of the plant symplast takes place by changing the conductance of aquaporins in membranes (Eckert *et al.* 1999; Kjellbom *et al.* 1999; Qiu *et al.* 2000; Maurel and Chrispeels 2001). Thus, it is reliable to suppose that an important cause of the decrease in the shoot hydraulic conductance in the drought conditions was the decrease in the conductance of the aquaporins of the mesophyll cells due to a rise in [ABA] in the leaves. The correlation of the L with the $[ABA]_{leaf}$, but not with $[ABA]_{xyl}$, through the whole tree storey (both in non-stressed and in droughted trees) suggests that the sites where ABA affects the aquaporins can be located on the internal side of the plasmalemma in both the species.

Similarly to the correlation between the $[ABA]_{leaf}$ and g_s, the correlation of the $[ABA]_{leaf}$ with the L through the whole tree storey can, alternatively, be explained by the influence of the species- and position- (in the foliage) specific [ABA] on the development of foliar hydraulic architecture in young, intensively developing leaves.

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REFERENCES

Aasamaa K, Sõber A (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. Biol Plant 44:65–73

Aasamaa K, Sõber A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. Aust J Plant Physiol 28:765–774

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (2002) Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. Tree Physiol 22:267–276

Allan AC, Fricker MD, Ward JL, Beale MH, Trewavas AJ (1994) Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. Plant Cell 6:1319–1328

Allan AC, Trewavas AJ (1994) Abscisic acid and gibberellin perception: inside or out? Plant Physiol 104:1107–1108

Assmann SM (1994) Ins and outs of guard cell ABA receptors. Plant Cell 6:1187–1190

Assmann SM, Shimazaki KI (1999) The multisensory guard cell. Stomatal responses to blue light and abscisic acid. Plant Physiol 119:809–815

Augé RM, Duan X, Croker JL, Witte WT, Green CD (1998) Foliar dehydration tolerance of twelve deciduous tree species. J Exp Bot 49:753–759

Augé RM, Green CD, Stodola AJW, Saxton AM, Olinick JB, Evans RM (2000) Correlations of stomatal conductance with hydraulic and chemical factors in several deciduous tree species in a natural habitat. New Phytol 145:483–500

Bahari ZA, Pallardy SG, Parker WC (1985) Photosynthesis, water relations, and drought adaptation in six woody species of oak-hickory forests in central Missouri. Forest Sci 31:557–569

Boyer JS (1985) Water transport. Annu Rev Plant Physiol 36:473–516

Bradford KJ, Sharkey TD, Farquhar GD (1983) Gas exchange, stomatal behaviour, and $\delta^{13}C$ values of the *flacca* tomato mutant in relation to abscisic acid. Plant Physiol 72:245–250

Brenac P, Smith ME, Obendorf RL (1997) Raffinose accumulation in maize embryos in the absence of a fully functional *Vp1* gene product. Planta 203:222–228

Brown K (2001) Xylem may direct water where it's needed. Sci 291:571–572

Chandler PM, Robertson M (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 45:113–141

Cinnirella S, Magnani F, Saracino A, Borghetti M (2002) Response of a mature *Pinus laricio* plantation to a three-year restriction of water supply: structural and functional acclimation to drought. Tree Physiol 22:21–30

Croker JL, Witte WT, Augé RM (1998) Stomatal sensitivity of six temperate, deciduous tree species to non-hydraulic root-to-shoot signalling of partial soil drying. J Exp Bot 49:761–774

Crombie DS, Hipkins MF, Milburn JA (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. Austr J Plant Physiol 12:445–453

Cutler JM, Rains DW, Loomis RS (1977) The importance of cell size in the water relations of plants. Physiol Plant 40:255–260

Donovan LA, West JB, McLeod KW (2000) *Quercus* species differ in water and nutrient characteristics in a resource-limited fall-line sandhill habitat. Tree Physiol 20:929–936

Düring H (1986) ABA and water stress in grapevines. Acta Hort 179:413–420

Eckert M, Biela A, Siefritz F, Kaldenhoff R (1999) New aspects of plant aquaporin regulation and specificity. J Exp Bot 50:1541–1545

Fan L, Zheng S, Wang X (1997) Antisense suppression of phospholipase D alpha retards abscisic acid- and ethylene-promoted senescence of postharvest *Arabidopsis* leaves. Plant Cell 9:2183–2196

Fort C, Fauveau ML, Muller F, Label P, Granier A, Dreyer E (1997) Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. Tree Physiol 17:281–289

Franks PJ, Farquhar GD (2001) The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. Plant Physiol 125:935–942

Gosti F, Bertauche N, Vartanian N, Giraudat J (1995) Abscisic acid-dependent and -independent regulation of gene expression by progressive drought in *Arabidopsis thaliana*. Mol Gen Genet 246:10–18

Gowing DJG, Jones HG, Davies WJ (1993) Xylem-transported abscisic acid — the relative importance of its mass and its concentration in the control of stomatal aperture. Plant Cell Environ 16:453–459

Grabov A, Blatt MR (1998) Co-ordination of signalling elements in guard cell ion channel control. J Exp Bot 49:351–360

Harris MJ, Outlaw WHJr, Mertens R, Weiler EW (1988) Water-stress-induced changes in the abscisic acid content of guard cells and other cells of *Vicia faba* L. leaves as determined by enzyme-amplified immunoassay. Proc Nat Acad Sci USA 85:2584–2588

Harris MJ, Outlaw WHJr (1991) Rapid adjustment of guard cell abscisic acid levels to current leaf water status. Plant Physiol 95:171–173

Hartung W (1983) The site of action of abscisic acid at the guard cell plasmalemma of *Valerianella locusta*. Plant Cell Environ 6:427–428

Hartung W, Davies WJ (1991) Drought-induced changes in physiology and ABA. In: Davies WJ, Jones HG (eds) Abscisic Acid. Physiology and Biochemistry. BIOS Scientific Publ Ltd, Oxford, pp 63–79

Hornberg C, Weiler EW (1984) High affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells. Nature 310:321–324

Jeschke WD, Peuke AD, Pate JS, Hartung W (1997) Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. J Exp Bot 48:1737–1747

Jia W, Zhang J, Zhang DP (1996) Metabolism of xylem-delivered ABA in relation to ABA flux and concentration in leaves of maize and *Commelina communis*. J Exp Bot 47:1085–1091

Jones RJ, Luton MT, Higgs KH, Hamer PJC (1983) Experimental control of water status in an apple orchard. J Hort Sci 58:301–316

Khalil AM, Grace J (1993) Does xylem sap ABA control the stomatal behaviour of water stressed sycamore (*Acer pseudoplatanus* L.) seedlings? J Exp Bot 44:1127–1134

Kjellbom P, Larsson C, Johannson I, Karlsson M, Johanson U (1999) Aquaporins and water homeostasis in plants. Trends Plant Sci 4:1360–1385

LaRosa PC, Hasegawa PM, Rhodes D, Clithero JM, Watad A-EA, Bressan RA (1987) Abscisic acid stimulated osmotic adjustment and its involvement in adaptation of tobacco cells to NaCl. Plant Physiol 85:174–181

Liang J, Zhang J, Wong MH (1996) Stomatal conductance in relation to xylem sap abscisic acid concentrations in two tropical trees, *Acacia confusa* and *Litsea glutinosa*. Plant Cell Environ 19:93–100

Liu L, McDonald AJS, Stadenberg I, Davies WJ (2001) Abscisic acid in leaves and roots of willow: significance for stomatal conductance. Tree Physiol 21:759–764

Lo Gullo MA, Salleo S (1988) Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. New Phytol 108:267–276

Loewenstein NJ, Pallardy SG (1998a) Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. Tree Physiol 18:421–430

Loewenstein NJ, Pallardy SG (1998b) Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of canopy trees of three temperate deciduous angiosperms. Tree Physiol 18:431–439

Ludlow MM (1989) Strategies in response to water stress. In: Kreeb HK, Richter H, Hinckley TM (eds) Structural and Functional Responses to Environmental Stresses: Water Shortage. SPB Academic Press, The Hague, pp 269–281

Maurel C, Chrispeels MJ: Aquaporins. A molecular entry into plant water relations. Plant Physiol 125:135–138 (2001)

Meeteren U van, Gelder H van, Ieperen W van (2000) Reconsideration of the use of deionized water as vase water in postharvest experiments on cut flowers. Postharvest Biol Technol 18:169–181

Mertens RJ, Deus-Neumann B, Weiler EW (1985) Monoclonal antibodies for the detection and quantitation of the endogenous plant growth regulator abscisic acid. FEBS Lett 160:269–272

Nardini A, Lo Gullo MA, Salleo S (1999) Competitive strategies for water availability in two Mediterranean *Quercus* species. Plant Cell Environ 22:109–116

Nardini A, Pitt F (1999) Drought resistance of *Quercus pubescens* as a function of root hydraulic conductance, xylem embolism and hydraulic architecture. New Phytol 143:485–493

Niinemets Ü, Sõber A, Kull O, Hartung W, Tenhunen JD (1999) Apparent controls on leaf conductance by soil water availability and via light-acclimation of foliage structural and physiological properties in a mixed deciduous, temperate forest. Int J Plant Sci 160:707–721

Pesci P (1992) Effect of light on abscisic acid-induced proline accumulation in leaves: comparison between barley and wheat. Physiol Plant 86:209–214

Peuke AD, Jeschke WD, Hartung W (1994) The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long distance transport of abscisic acid depending on nitrogen nutrition and salt stress. J Exp Bot 45:741–747

Qiu Q-S, Wang Z-Z, Zhang N, Cai Q-G, Jiang R-X (2000) Aquaporins in the plasma membrane of leaf callus protoplasts of *Actinidia deliciosa* var. *deliciosa* cv. Hayward. Aust J Plant Physiol 27:71–75

Roberts SK, Snowman BN (2000) The effects of ABA on channel-mediated K^+ transport across higher plants roots. J Exp Bot 51:1585–1594

Sauter A, Davies WJ, Hartung W (2001) The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. J Exp Bot 52:1991–1997

Savoure A, Hua X-J, Bertauche N, Van Montagu M, Verbruggen N (1997) Abscisic acid-independent and abscisic acid-dependent regulation of proline biosynthesis following cold and osmotic stresses in *Arabidopsis thaliana*. Mol Gen Genet 254:104–109

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D: Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol 52:627–658 (2001)

Schwartz A, Wu W-H, Tucker EB, Assmann SM (1994) Inhibition of inward K^+ channels and stomatal response by abscisic acid: an intracellular locus of phytohormone action. Proc Nat Acad Sci USA 91:4019–4023

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. J Exp Bot 44:1075–1082

Sperry JS, Ikeda T (1997) Xylem cavitation in roots and stems of Douglas-fir and white fir. Tree Physiol 17:275–280

Steudle E (1997) Water transport across plant tissue: Role of water channels. Biol Cell 89:259–273

Stewart CR, Voetberg G, Rayapati PJ (1986) The effects of benzyladenine, cycloheximide, and cordycepin on wilting-induced abscisic acid and proline accumulations and abscisic acid- and salt-induced proline accumulation in barley leaves. Plant Physiol 82:703–707

Stratton L, Goldstein G, Meinzer FC (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. Plant Cell Environ 23:99–106

Sutton F, Paul SS, Wang X-Q, Assmann SM (2000) Distinct abscisic acid signaling pathways for modulation of guard cell versus mesophyll cell potassium channels revealed by expression studies in *Xenopus laevis* oocytes. Plant Physiol 124:223–230

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49:419–432

Trejo CL, Clephan AL, Davies WJ (1995) How do stomata read abscisic acid signals? Plant Physiol 109:803–811

Tyree MT (1997) The cohesion-tension theory of sap ascent: current controversies. J Exp Bot 48:1753–1765

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? Plant Physiol 120:11–21

Wang X-Q, Wu W-H, Assmann SM (1998) Differential responses of abaxial and adaxial guard cells of broad bean to abscisic acid and calcium. Plant Physiol 118:1421–1429

Weiler EW, Schnabl H, Hornberg C (1982) Stress related levels of abscisic acid in guard cell protoplasts of *Vicia faba* L. Planta 154:24–28

Whitehead D (1998) Regulation of stomatal conductance and transpiration in forest canopies. Tree Physiol 18:633–644

Wilkinson S, Davies WJ (1997) Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol 113:559–573

Willmer CM, Wilson AB, Jones HG (1988) Changing responses of stomata to abscisic acid and carbon dioxide as leaves and plants age. J Exp Bot 39:401–410

Wright STC (1977) The relationship between leaf water potential (Ψ_{leaf}) and the levels of abscisic acid and ethylene in excised wheat leaves. Planta 134:183–189

Zdunek E, Lips SH (2001) Transport and accumulation rates of abscisic acid and aldehyde oxidase activity in *Pisum sativum* L. in response to suboptimal growth conditions. J Exp Bot 52:1269–1276

Zhang J, Davies WJ (1989) Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. New Phytol 113:167–174

Zhang J, Jia W, Zhang D-P (1997) Re-export and metabolism of xylem-delivered ABA in attached maize leaves under different transpirational fluxes and xylem ABA concentrations. J Exp Bot 48:1557–1564

Zhang SQ, Outlaw WHJr, Aghoram K (2001) Relationship between changes in the guard cell abscisic acid content and other stress-related physiological parameters in intact plants. J Exp Bot 52:301–308

Zwieniecki MA, Melcher PJ, Holbrook NM (2001) Hydrogel control of xylem hydraulic resistance in plants. Sci 291:1059–1062

Table 1. Pearson correlation coefficients (and P-values) between the stomatal conductance of non-stressed trees (g_{sN}), shoot hydraulic conductance of non-stressed trees (L_N), bulk-leaf ABA concentration of non-stressed trees (a_N), stomatal conductance of droughted trees (g_{sD}), shoot hydraulic conductance of droughted trees (L_D) and bulk-leaf ABA concentration of droughted trees (a_D). The analysis accounts mean values of these characteristics in top and base of foliages of *P. tremula* and *T. cordata*.

	g_{sN}	L_N	a_N	g_{sD}	L_D
L_N	0.88 (0.117)				
a_N	−0.85 (0.147)	−0.74 (0.262)			
g_{sD}	0.99 (0.008)	0.93 (0.072)	−0.82 (0.185)		
L_D	0.82 (0.178)	0.99 (0.007)	−0.69 (0.314)	0.88 (0.121)	
a_D	−0.92 (0.077)	−0.81 (0.193)	0.99 (0.012)	−0.89 (0.107)	−0.75 (0.248)

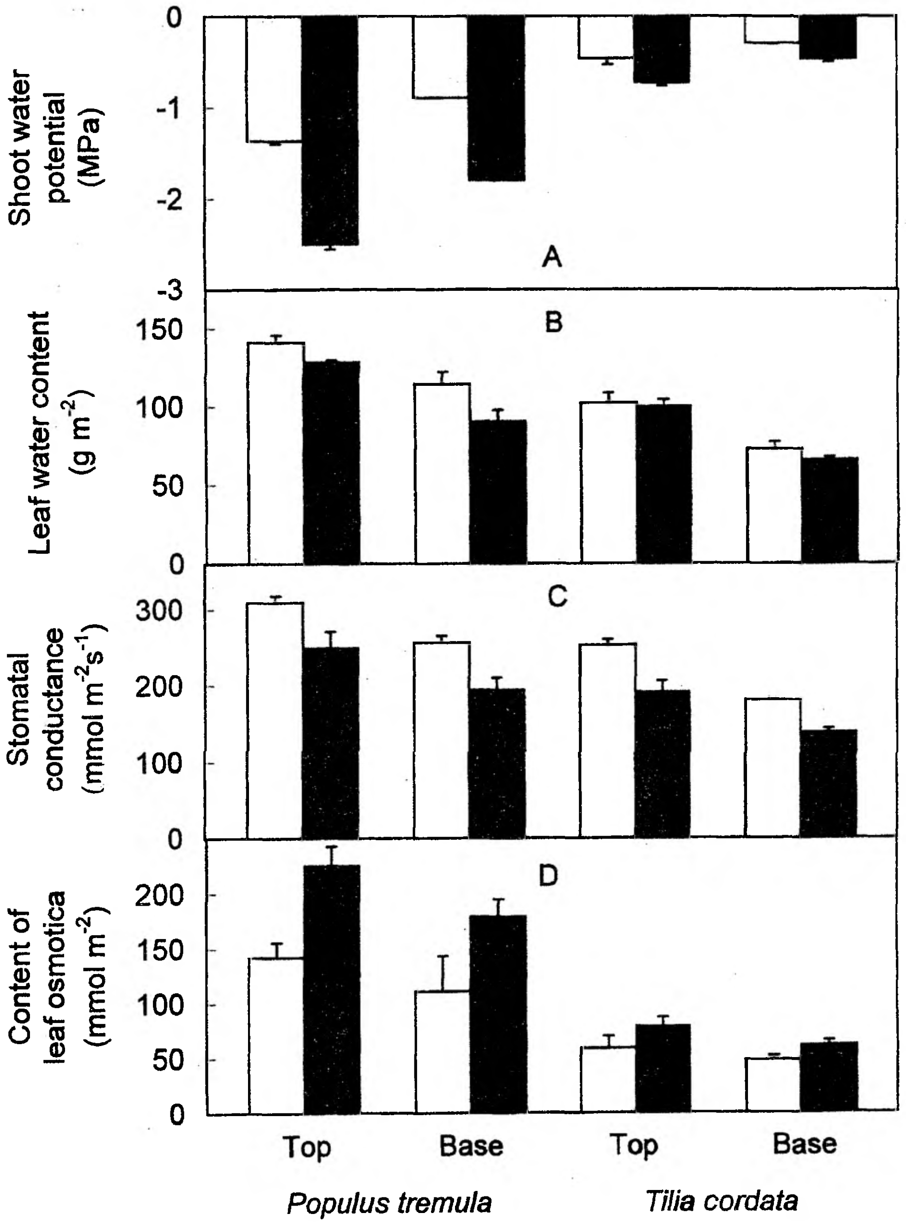
Shoot water potential (MPa)
0
-1
-2
-3
A
Leaf water content (g m^{-2})
150
100
50
0
B
Stomatal conductance (mmol m^{-2}s^{-1})
300
200
100
0
C
Content of leaf osmotica (mmol m^{-2})
200
150
100
50
0
D
Top
Base
Top
Base
Populus tremula
Tilia cordata

Figure 1

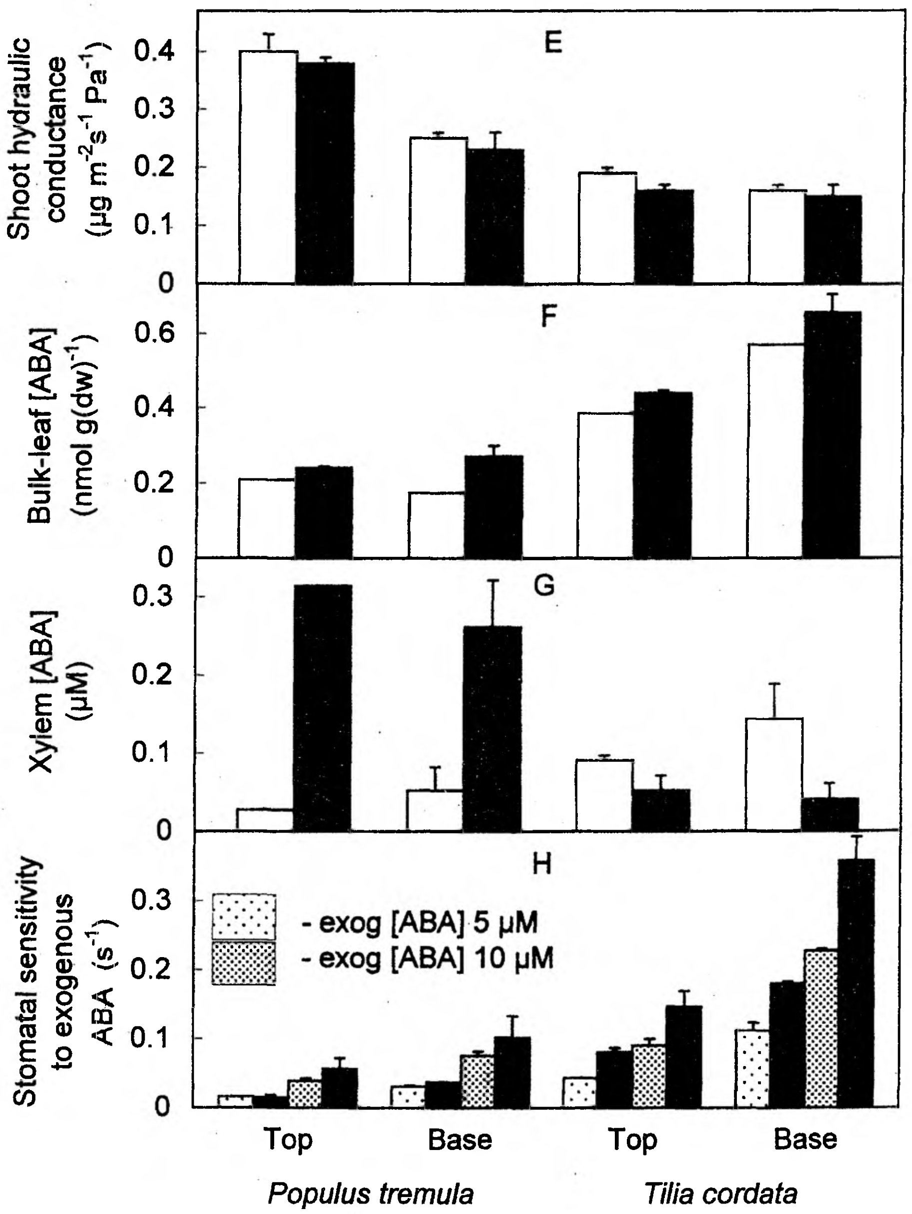

Figure 1. Shoot water potential (**a**); leaf water content (**b**); stomatal conductance (**c**); content of leaf osmotica (**d**); shoot hydraulic conductance (**e**); bulk-leaf ABA concentration (**f**); shoot xylem ABA concentration (**g**) and stomatal sensitivity to exogenous ABA (**h**) in top and base of foliage of non-stressed (white bars) and water-stressed (grey bars) *P. tremula* and *T. cordata* (means ±SE).

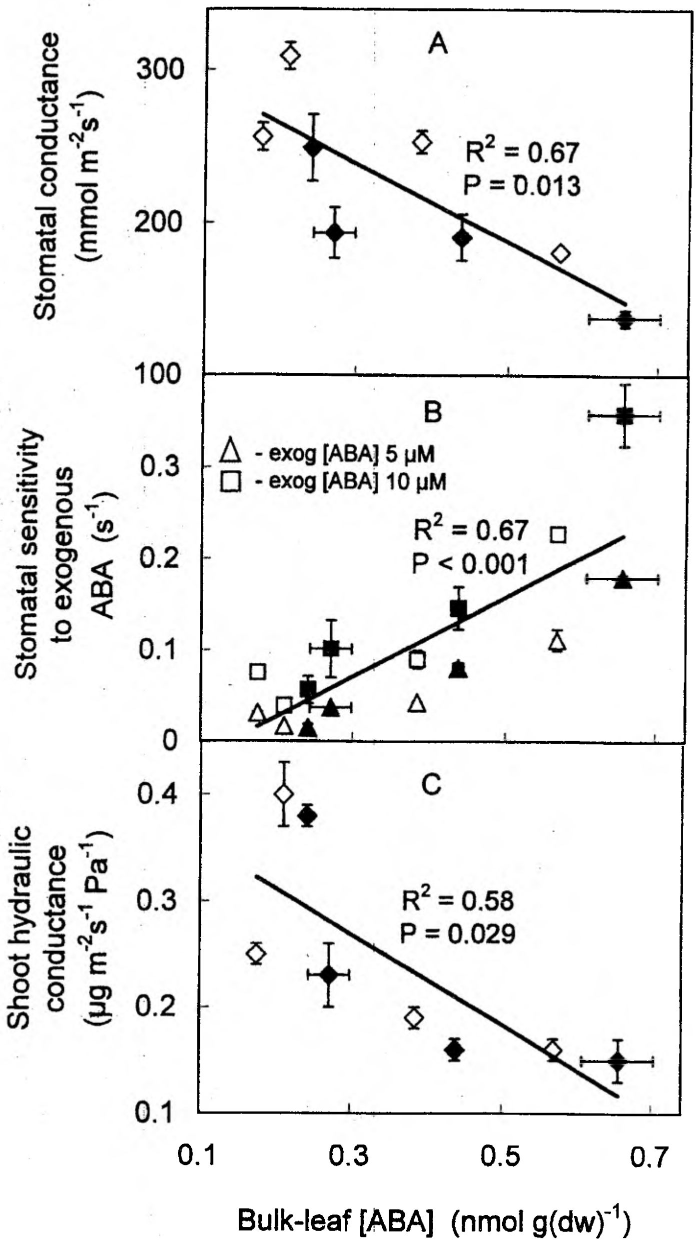

Figure 2. Relationships between bulk-leaf ABA concentration and (**a**) stomatal conductance; (**b**) stomatal sensitivity to exogenous ABA and (**c**) shoot hydraulic conductance, in top and base of foliage of non-stressed (white symbols) and droughted (grey symbols) *P. tremula* and *T. cordata*. Each value is the mean ±SE for the particular species and position (in the foliage) studied. Linear regression analysis was used to fit the data (R^2 — coefficient of determination).

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no. 4585 "Efficiency of light interception and use in natural plant communities: acclimation potential of leaf canopies to availabilities of light and water in trees, shrubs, herbs and mosses";
no. 5305 "Ontogenetic development of structural and functional characteristics of water relations and photosynthesis of leaves in temperate deciduous tree canopy".

Conference thesis:

Sõber A, **Aasamaa K** (1998) Hydraulic conductance between leaf petiole and evaporation sites depends on light conditions and on water stress. In: The Annual Meeting of the American Society of Plant Physiologists, Madison, United States of America, June 27–28, Abstracts for Plant Biology 98: p 59.

Sõber A, **Aasamaa K** (2001) Hydraulic conductance of deciduous tree leaves in relation to stomatal conductance: effects of both leaf structure and physiology. In: Workshop in Hyytiälä Forest Station: Water Transport in Woody Plants and Linkages to Plant Structure and Productivity, Finland, March 14–16, Book of Abstracts: p 11.

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (2001) Xylem and bulk-leaf abscisic acid concentration as related to stomatal conductance of two tree species of deciduous temperate forest. In: New Phytologist Symposium: Stomata 2001, Birmingham, United Kingdom, July 26–28, Book of Poster Abstracts: p 1.

Publications:

Aasamaa K, Sõber A (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* 44: 65–73.

Aasamaa K, Sõber A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* 28: 765–774.

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (2002) Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiology* 22: 267–276.

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Sõber A, **Aasamaa K** (2001) Hydraulic conductance of deciduous tree leaves in relation to stomatal conductance: effects of both leaf structure and physiology. In: Workshop in Hyytiälä Forest Station: Water Transport in Woody Plants and Linkages to Plant Structure and Productivity, Finland, March 14–16, Book of Abstracts: p 11.

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (2001) Xylem and bulk-leaf abscisic acid concentration as related to stomatal conductance of two tree species of deciduous temperate forest. In: New Phytologist Symposium: Stomata 2001, Birmingham, United Kingdom, July 26–28, Book of Poster Abstracts: p 1.

Publikatsioonid: **Aasamaa K**, Sõber A (2001) Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* 44: 65–73.

Aasamaa K, Sõber A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* 28: 765–774.

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (2002) Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiology* 22: 267–276.

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