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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Received March 14, 2001; accepted July 14, 2001; published online February 1, 2002

Summary Correlations between leaf abscisic acid concentration ([ABA]), stomatal conductance (gs), rate of stomatal opening in response to an increase in leaf water potential (s), 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 gs, s, L and light- and CO2-saturated net photosynthesis (Pmax), thus providing strong correlative evidence for a scaling of foliar gas exchange and hydraulic characteristics with leaf endogenous [ABA]. In addition, we suggest that mean gs, s, L and Pmax 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 gs, 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 gs, s, L and Pmax, 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 gs, s, L and Pmax. 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, gs, s, L and Pmax 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 Giraudet 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, Tar-dieu 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 Men-cuccini 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, Salienia 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 pseudosol 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 Söber (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 cm² 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
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 (Medereski 1961). Further details of the measurement system are given by Sőber and Moldau (1977) and Niinemets et al. (1998).

Measurements were started at an ambient chamber CO\(_2\) concentration (\(C_i\)) 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 (\(P_{\text{max}}\)). 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 Sőber and Moldau (1977). Stomatal sensitivity to an increase in leaf water potential (\(s_i\)) was determined from the maximum relative rate of stomatal opening (\(v_i\)):

\[
v_i = v_s B,
\]

where \(B\) is a coefficient normalizing the values of \(v_s\) 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 Sőber 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 (\(v_s\)) was calculated as:

\[
v_s = \frac{100}{\Delta t(g_{\text{max}} - g_{sa})} \Delta g_s,
\]

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

\[
B = \frac{E_s \Delta P_{\text{irr}}}{E_{\text{sat}} \Delta P_r},
\]

where \(E_s\) is mean transpiration rate during \(\Delta t\), \(E_{\text{sat}}\) is mean \(E_s\) for all the experiments, \(\Delta P_r\) is the pressure increase in the pressure chamber, which leads to a corresponding increase in leaf water potential, and \(\Delta P_{\text{irr}}\) is mean \(\Delta P_r\) 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.

**Determining shoot hydraulic conductance**

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

\[
M = \frac{\ln I - \ln I_b}{\ln I_0 - \ln I_b},
\]

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 \(I_b\) is β-irradiance penetrating the leaf at the beginning of the experiment. The leaf absolute water content per area is given by:

\[
w = \frac{M w_\text{sat} - w_d}{M},
\]

where \(M\) is leaf relative fresh mass per area and \(w_\text{sat}\) 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:

\[
L = \frac{\Delta w}{\Delta t} = \frac{E_s}{E_{\text{sat}}} \frac{\Delta w}{\Delta P}.
\]
assumed that water potential in the leaf intercellular space is zero in infiltrated leaves, and thus $\Delta p$ 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 $E_i$ in our experiments.

**Leaf carboxylation efficiency**

In addition to the maximum net assimilation rate at saturating CO$_2$ concentration and quantum flux density ($P_{\text{max}}$), which characterizes the capacity of foliar photosynthetic electron transport (von Caemmerer and Farquhar 1981), we calculated carboxylation efficiency ($\chi$) as an estimate of the initial activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco):

$$\chi = \frac{P}{C_i - \Gamma},$$

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 $\Gamma$ is the CO$_2$ compensation point. A constant value of 40 µmol mol$^{-1}$ was assumed for $\Gamma$.

**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 $-20^\circ$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).

**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 ($g_s$), stomatal sensitivity to leaf water potential increase ($s_i$, Equation 1), maximal light- and CO$_2$-saturated net assimilation rate ($P_{\text{max}}$) and (f) carboxylation efficiency ($\chi$, 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 (2 < 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.

**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 ($P_{\text{max}}$) and (f) carboxylation efficiency ($\chi$, Equation 7) for the control treatments.
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 4d; $r = -0.8$, $P = 0.05$ 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).

Discussion

Species differences in endogenous [ABA]

Foliar [ABA] tends to change during leaf ontogeny; 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
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 vessels 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. platanoideus, 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, Socías 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 Ca²⁺-channels, and the conductance of aquaporines is sensitive to the concentration of Ca²⁺ 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 [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 sto-
mata 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 (Ninemets 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 CO2- and light-saturated rates of net photosynthesis (Pmax) 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 CO2 concentration, especially at low irradiance when photosynthetic rates are limited by the rate of photosynthetic electron transport.

**Acknowledgments**

Financial support was provided by the Estonian Science Foundation (Grants 3780 and 4584) to K.A., A.S. and Ü.N., and K.A. was generously supported by the Deutsche Forschungsgemeinschaft (SFB 251, TP A3). We are grateful to Prof. Dr. Elmar W. Weiler (University of Bochum, Germany) for the generous supply of immunochemicals and to Ms. Barbara Dierich (University of Würzburg, Germany) for expert technical assistance with the analysis of abscisic acid.

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ROLES OF ABA IN LEAVES OF DECIDUOUS TREES 275

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