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ORIGINAL ARTICLE

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# Root water flow and leaf stomatal conductance in aspen (*Populus tremuloides*) seedlings treated with abscisic acid

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**Abstract** Exogenous abscisic acid (ABA) applied to the roots and excised shoots of aspen (*Populus tremuloides* Michx.) inhibited stomatal conductance. However, the effect of ABA on stomatal conductance was more pronounced in the excised shoots compared with the intact seedlings. Approximately 10% of the ABA concentration applied to the roots was found in the xylem exudates of root systems exposed to a hydrostatic pressure of 0.3 MPa. A similar concentration of ABA applied to the excised shoots produced a faster and greater reduction of stomatal conductance. ABA applied to the roots had no effect on root steady-state flow rate over the 5-h experimental period. Moreover, pre-incubating root systems of intact seedlings for 12 h with $5 \times 10^{-5}$ M ABA did not significantly reduce volume flow density. Similarly, ABA had no effect on root hydraulic conductivity and the activation energy of root water flow rates.

**Keywords** Abscisic acid · *Populus* · Root hydraulic conductivity · Stomatal conductance · Water transport

**Abbreviations** ABA: abscisic acid · $E_a$: activation energy · $g_s$: stomatal conductance · $J_v$: volume flow density · $L_p$: root hydraulic conductivity · $Q_v$: steady-state water flow rate of the whole root system

## Introduction

Water balance is largely determined by the rates of root water uptake, its transport through the plant and transpirational water loss. Under the conditions where water is readily available to the roots, root hydraulic conductivity and transpiration are the two key elements controlling water balance. Abscisic acid (ABA) has been proposed to act as an essential mediator in triggering plant response to stress (Leung and Giraudat 1998). Numerous reports have demonstrated a powerful effect of ABA on the stomatal conductance of plants (Kriedemann et al. 1972; Raschke and Hedrich 1985; Mansfield et al. 1990; Trejo et al. 1993). Abscisic acid can also increase (Glinka 1977, 1980; Freundl et al. 1998; Quintero et al. 1999) or decrease (Markhart et al. 1979; Fiscus 1981; Davies et al. 1982) root water flow rates. This variable effect is thought to depend on the rate of root water flow, with an increasing effect at the low flow rates and a decreasing effect when the rates are high (Davies et al. 1982; Fiscus et al. 1982).

A comparative study using intact seedlings, detopped roots and excised shoots could help explain whether ABA regulates stomatal opening in plants through its effects on hydraulic conductivity. Blackman and Davies (1985) and Zhang et al. (1987) found in split-root experiments that the roots could sense soil water status and produce a signal, likely ABA, to trigger the corresponding shoot response. This work has triggered a heated discussion, and studies on the relative importance of roots and shoots as the sensors of water stress (Kramer 1988; Passioura 1988; Boyer 1989). Subsequently, a number of reports showed a close relationship between the concentration of ABA in the xylem sap and the stomatal conductance when the roots were subjected to drying (Wartinger et al. 1990; Zhang and Davies 1990; Tardieu et al. 1992, 1996; Khalil and Grace 1993). However, in addition to ABA, roots also produce other physiologically active substances, including cytokinins, which may affect stomatal opening (Bradford and Hsiao 1982; Davies et al. 1986; Meinzer et al. 1991). The role of these substances may be easily overlooked in the endogenous-ABA studies.

In the present study, we examined the hypothesis that the effect of ABA on stomatal conductance in plants is exerted directly through its effect on stomatal opening rather than indirectly by reducing the supply of water to

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the shoots. Therefore, we applied ABA to the roots of intact plants and excised shoots of aspen seedlings and measured ABA shoot concentrations, root water flow properties and stomatal conductance.

## Materials and methods

### Plant material

Aspen (*Populus tremuloides* Michx.) seedlings were grown in a growth chamber (Controlled Environments Inc., Winnipeg, Manitoba, Canada) from seed collected near Whitecourt, Alberta, Canada. Seeds were germinated in Petri dishes and 1 day after germination the seedlings were transferred to styrofoam containers with sand and grown in the greenhouse for 2 months. After 2 months, the roots of seedlings were gently washed free of sand with tap water and transferred to solution culture containing half-strength modified Hoagland's solution (Epstein 1972). The seedlings were grown in solution culture for 1 month in the growth chamber under the following conditions: 18-h photoperiod with 260 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation at the seedling level, 20/16 °C (day/night) temperature and a constant relative humidity of approximately 65%. The Hoagland's solution was continuously aerated and replaced every 2 weeks.

### Root water flow

Steady-state water flow rates of whole root systems ($Q_v$) were measured using the hydrostatic pressure method as previously described (Wan and Zwiazek 1999). A glass cylinder was inserted into a pressure chamber (PMS Instruments, Corvallis, Ore., USA) and filled with half-strength Hoagland's solution, which was continuously stirred with a magnetic stirrer. The pH of the half-strength Hoagland's solution and that of the xylem sap expressed under pressure from roots was 5.5. The de-topped root system was immediately sealed in the pressure chamber. The whole root system was immersed in the solution and surrounded with a copper coil, which was connected to a circulating cooler system (HAAKE F3, Berlin, Germany) to maintain the desired root temperature (±0.1 °C). The desired pressure was gradually applied with compressed air and maintained during the measurements. A graduated pipette was attached with a short piece of rubber tubing to the stem protruding through the stopper in a pressure chamber. Root flow rates of whole root systems ($Q_v$) were monitored for linearity for at least 30 min and $Q_v$ values are expressed in $m^3$ $s^{-1}$.

To determine the effect of ABA on $Q_v$, de-topped root systems were sealed in the pressure chamber and gradually pressurized to a constant pressure of 0.3 MPa. When a stable $Q_v$ was reached, ABA solution (*cis-trans* isomers; Sigma-Aldrich Canada, Oakville, Ontario, Canada) in 0.1% ethanol was injected with a syringe into the chamber to reach a final concentration of $5\times10^{-5}$ M. $Q_v$ was monitored for the next 5 h. A control experiment was run in a similar manner, except that 0.1% ethanol solution was injected in place of ABA solution. Another group of intact seedlings was pre-incubated for twelve hours with $5\times10^{-5}$ M ABA or 0.1% ethanol (control). The seedlings were excised and the root systems were used for $Q_v$ measurements. Roots were assumed to be cylindrical and root surface area was calculated by multiplying the projected area, measured following computer scanning (Sigma Scan 3.0; Jandel Scientific, San Rafael, Calif., USA), by $\pi$. The volume flow density ($J_v$) was calculated as a steady-state flow rate per unit root surface area and expressed as $m^3$ $m^{-2}$ $s^{-1}$.

### Hydraulic conductivity

Roots were immersed in half-strength Hoagland's solution (with 0.1% ethanol for control; with 0.1% ethanol and ABA for treatment) in a pressure chamber. The pressure increased every 40 min from 0.1, 0.2, 0.3, 0.4 and 0.5 MPa. The volume flow density ($J_v$) was calculated as $Q_v$ per whole root and plotted against hydrostatic pressures. To minimize variability, the $Q_v$ at 0.3 MPa in half-strength Hoagland's solution was measured for 30 min and then the similar $Q_v$ values were paired, one used for control and another for treatment. Therefore, the data were analyzed with paired *t*- test. Root hydraulic conductivity ($L_p$) was calculated from the slope of the curve between 0.1 and 0.5 MPa where the relationship between pressure and $J_v$ ($mm^3$ $s^{-1}$ $root^{-1}$) was linear, and it is expressed in $mm^3$ $s^{-1}$ $MPa^{-1}$ $root^{-1}$.

### Arrhenius plots

Root systems were immersed in half-strength Hoagland's solution (with 0.1% ethanol for control; with 0.1% ethanol and ABA for treatment) and held in a constant pressure of 0.3 MPa with the temperature changing from 25 °C to 4 °C in 3 °C steps for Arrhenius plot determinations. The temperature was monitored using a microprocessor thermometer with a fine-wire-type J-K-T thermocouple sealed into the pressure chamber through the rubber stopper. The compressed air was used for applying pressure in the chamber and the solution was continuously stirred with a magnetic stirrer. The Arrhenius plots were obtained by plotting the logarithm of $Q_v$ against the reciprocal of the absolute temperature and the activation energy ($E_a$) was calculated from the slope of the whole curve of the plot.

### Root anatomy

Cross-sections of primary roots of hydroponically grown aspen were made with a razor blade at 20, 40, 60, 80 and 100 mm from the root tips. The sections were stained for 1 h with 0.1% berberine hemisulfate followed by 0.5% (w/v) toluidine blue O as described by Freundl et al. (2000). After being rinsed with distilled water to eliminate the background color, the sections were observed under an epifluorescence microscope (Nikon, Japan) using excitation/emission wavelengths of 365/395 nm.

### Stomatal conductance ($g_s$)

Intact seedlings were transferred to 600-ml tall beakers containing aerated half-strength Hoagland's solution in a growth chamber under the environmental conditions described for growth. In another experiment, excised shoots were used instead of intact seedlings. The leaves were removed from the lower part of the stem and 2 days later the shoots were immersed in half-strength Hoagland's solution and excised at the root collar. The cut end of the shoot was immersed in a vessel containing the nutrient solution and immediately placed in the growth chamber. A blank measurement of $g_s$ was conducted before adding ABA. The ABA was dissolved in 95% ethanol and diluted with distilled water to a required final concentration. For the control, 0.1% ethanol was used in place of the ABA. Measurements of $g_s$ were conducted on the uppermost fully expanded leaves with a steady-state porometer (LI-1600; Li-Cor, Lincoln, Neb., USA). The measurements started 3 h after the onset of the light period. Since seedlings had different $g_s$ values before ABA treatments, ABA inhibition of $g_s$ was analyzed for each group separately as percent of the initial value (before treatment).

### ABA analysis

Roots were immersed in half-strength Hoagland's solution (with $5\times10^{-5}$ M ABA in 0.1% ethanol for treated plants and 0.1% ethanol for control plants) in a pressure chamber. The pressure increased gradually to 0.3 MPa and was held for 3 h. Then, samples of xylem exudates were collected to measure ABA concentration.

ABA analysis followed the methodology used by Markhart (1982). The xylem exudates were filtered through a 0.45-μm Millipore filter before injecting into a high-pressure liquid chromatograph (HPLC) equipped with a 254-nm UV detector. ABA was separated by an LC-18 column (Supelcosil) using a methanol:$H_2O$:acetic acid (50:50:1, by vol.) solvent system and a flow rate of 0.8 ml $min^{-1}$. Standard curves, with the treating ABA solution, of peak area versus concentrations were linear between $5\times10^{-5}$ M and $5\times10^{-7}$ M, and were used to determine ABA concentration in xylem exudates. The peak areas were calculated with a Hewlett-Packard HP 3396A integrator.

### Water potential measurements

Shoot water potentials were measured in intact seedlings and excised shoots using a Scholander pressure chamber as described previously (Wan et al. 1999).

### Reagents

All reagents were of the highest available grade and were purchased from Sigma.

### Statistical analysis

The data are presented as the means of at least six replicates (seedlings). The results were analyzed by ANOVA and with Duncan multiple comparison, *t*-test or paired *t*-test using SAS 6.12 software package (SAS Institute Inc., Cary, N.C., USA). All statistically significant differences were tested at the $P=0.05$ level.

## Results

### Anatomy

Aspen roots possessed a tri-arch xylem in the primary roots. In most roots, lateral branching took place at a distance greater than 100 mm from the root tip and originated in the pericycle opposite xylem ridges. There was no exodermis present in the roots. The roots developed a primary endodermis with Casparian bands in the radial cell walls at a distance greater than 20 mm from the root tip (Fig. 1A, B). At distances greater than approximately 60 mm, fluorescent deposits covered whole outer surfaces of the cell walls in the endodermis, with the exception of small groups of cells found opposite xylem arms. However, the radial walls of these passage cells also contained small amounts of fluorescent material (Fig. 1C).

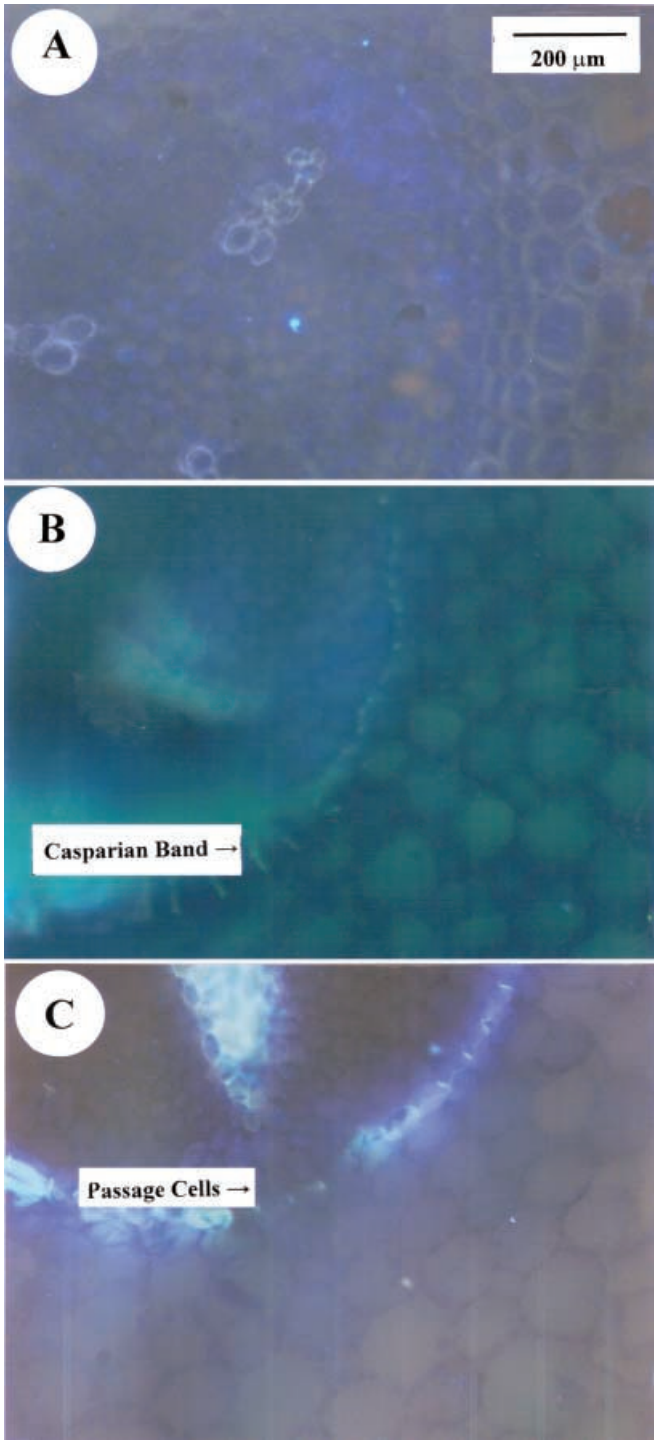


**Fig. 1A–C** Cross-sections of aspen (*Populus tremuloides*) primary roots at various stages of development. The fresh sections were taken 20 mm (**A**), 40 mm (**B**), and 80 mm (**C**) from the root tip, stained with berberin hemisulfate and counterstained with toluidine blue O

### HPLC analysis of ABA

Figure 2 shows an HPLC separation of $5\times10^{-5}$ M *cis-trans* ABA standard solution (A), and root exudates with (B) and without (C) ABA added to the roots. ABA concentrations in exudates of roots treated with $5\times10^{-5}$ M ABA averaged $5.16\times10^{-6}\pm0.658\times10^{-6}$ M ($n=4$), that equals 10.3% of the *cis-trans* ABA concentration present in the root solution. An ABA peak was absent from the control root exudates (Fig. 2 C) analyzed by HPLC.

### Effects of ABA on $g_s$

ABA added to the nutrient solution reduced $g_s$ in intact aspen seedlings and excised shoots (Fig. 3). In intact seedlings treated with $5\times10^{-5}$ M and $10^{-4}$ M ABA, $g_s$

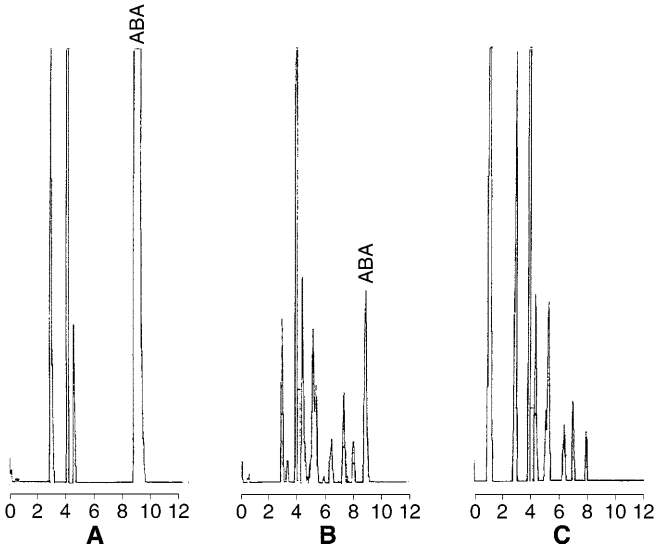


**Fig. 2** HPLC separation of $5\times10^{-5}$ M *cis-trans* ABA treatment solution (**A**) and xylem exudates of aspen roots treated (**B**) or not treated (**C**, control) with ABA

declined by approximately 15% after 4 h (Fig. 3A). After 24 h, $g_s$ decreased by 29% and 36% in $10^{-4}$ M and $5\times10^{-5}$ M ABA treatments, respectively. Seedlings in the $10^{-5}$ M ABA had lower $g_s$ values than control after 12 h of treatment. However, there was no significant decline in $g_s$ values over time as a result of this ABA treatment ($P=0.726$) since before treatment, this group of seedlings had lower $g_s$ values compared with plants from other experimental groups (Fig. 3A). In seedlings treated with $10^{-5}$ M ABA, a significant ($P<0.05$) decline in $g_s$ was measured after 24 h following the treatment (Fig. 3A).

The concentrations of exogenous ABA needed to reduce $g_s$ were considerably lower in the excised shoots compared with the intact seedlings (Fig. 3). In plants treated with $5\times10^{-6}$ M and $10^{-5}$ M ABA, $g_s$ declined in 90 min. by about 20% and 34%, respectively (Fig. 3B). After 24 h, $g_s$ decreased by 41% in $5\times10^{-6}$ M and 54% in $10^{-5}$ M ABA compared with the untreated controls. The lower, $10^{-6}$ M ABA concentration had little effect on $g_s$ over time (Fig. 3B).

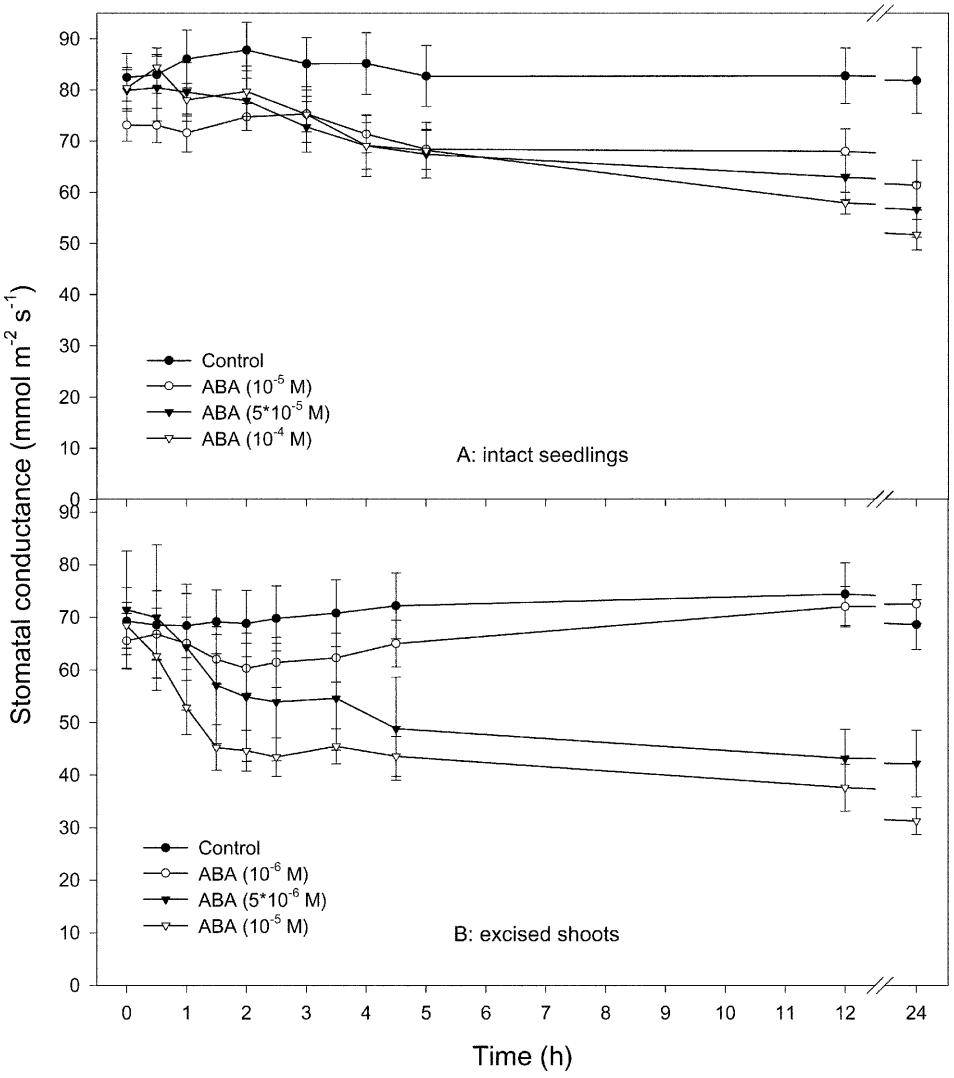


**Fig. 3** Effects of ABA on leaf stomatal conductance ($g_s$) in intact aspen seedlings (**A**) and excised shoots (**B**). Means ± SE ($n=6$) are shown

### Effects of ABA on root water flow

ABA treatment did not have an effect on $Q_v$ during the 5-h experimental period (Fig. 4). Moreover, pre-incubating root systems of intact seedlings for 12 h with $5\times10^{-5}$ M ABA did not significantly affect $J_v$. The control and ABA-treated roots had $J_v$ values of $5.87\times10^{-8}\pm0.401\times10^{-8}$ $m^3$ $m^{-2}$ $s^{-1}$ and $5.02\times10^{-8}\pm0.303\times10^{-8}$ $m^3$ $m^{-2}$ $s^{-1}$ ($n=6$, mean ± SE), respectively. The pH of the xylem sap exuded at 0.3 MPa pressure was 5.5, a similar value to that of the half-strength Hoagland's solution.

Pressure-flux curves showed a linear relationship between 0.1 and 0.5 MPa in both control and ABA-treated roots (Fig. 5). There was also no difference in $L_p$ values, which measured $1.88\pm0.569$ and $1.89\pm0.572$ $mm^3$ $s^{-1}$ $MPa^{-1}$ $root^{-1}$ ($n=6$, mean ± SE) in ABA-treated and control roots, respectively.

Both control and treated roots had linear Arrhenius plots for $Q_v$ (Fig. 6). There existed variation in $Q_v$ between control and treatment; however, their activation energies ($E_a$) were similar and measured $8.73\pm0.430$ and $8.31\pm0.249$ kcal $mol^{-1}$ ($n=6$, mean ± SE) in ABA-treated and control roots, respectively.

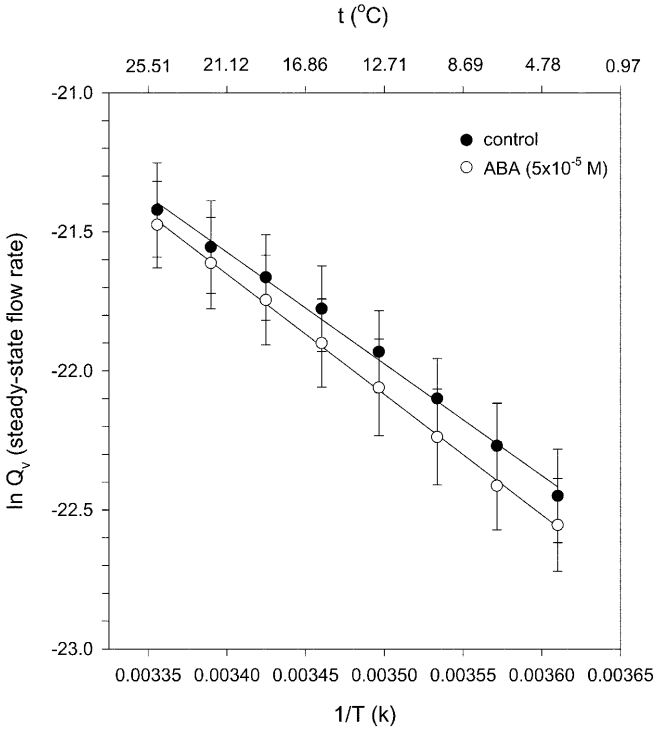


**Fig. 5** Pressure-flow relationship in control roots and aspen roots treated with $5\times10^{-5}$ M ABA. The ABA-treated and control root samples of similar $Q_v$ values at 0.3 MPa hydrostatic pressure were paired and analyzed by the paired *t*-test. Means ± SE are shown ($n=6$)

### Shoot water potentials

The shoot water potentials measured $-0.63\pm0.036$ MPa ($n=6$, mean ± SE) in intact plants and $-0.41\pm0.015$ MPa ($n=6$, mean ± SE) in excised shoots.

## Discussion

To study the effects of ABA on root water flow properties, we used concentrations of ABA which, in our experimental conditions, were effective in inducing stomatal closure when added to nutrient solution. The presence of ABA in the root xylem exudates demonstrates that, in our study, substantial quantities of ABA added to the root medium were carried with the transpiration stream to the shoot. Similar results have been reported previously (Markhart 1982; Freundl et al. 1998). The concentration of ABA in the xylem sap is believed to modulate stomatal conductance (Tardieu and Davies 1992; Liang et al. 1996) and transpiration rate (Jarvis and Davies 1997). In the $5\times10^{-5}$ M ABA treatment, the concentration of ABA in the xylem sap was $5.16\times10^{-6}\pm0.658\times10^{-6}$ M, similar to the concentration used for the excised shoots effective in reducing stomatal conductance (Fig. 3). ABA transport out of the root systems is a function of volume flux (Fiscus et al. 1982). The uptake of ABA from the medium by solvent

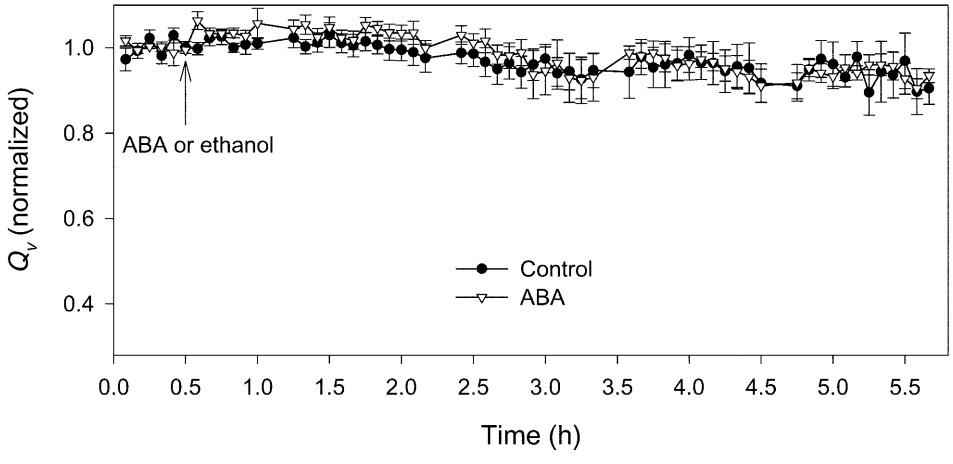


**Fig. 4** Water flow rate ($Q_v$) in aspen roots treated with $5\times10^{-5}$ M ABA. $Q_v$ was normalized to the mean rate over the initial 30 min before ABA or ethanol was added to treated and control roots, respectively. *Arrow* indicates time of addition of ABA (and ethanol for controls). Means ± SE are shown ($n=6$)

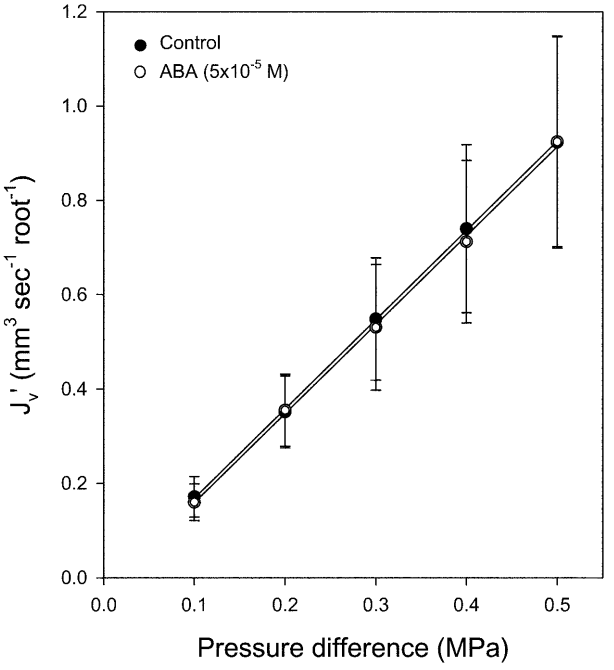


**Fig. 6** Temperature effects on water flow through aspen roots at a constant hydrostatic pressure of 0.3 MPa and decreasing temperatures. Means ± SE are shown ($n = 6$)

drag may increase the concentration of ABA in the xylem by water uptake (Freundl et al. 1998). In our study, the water potential difference between the shoots and root medium was about –0.6 MPa. Therefore, it is possible that the concentration of ABA in the xylem sap of intact seedlings treated with ABA was even higher than that measured in the root exudates expressed at 0.3 MPa pressure.

Water flow in aspen roots was more dependent on temperature than a viscous flow of water across a porous system (Fig. 6). This, and a relatively high reflection coefficient that we measured according to Freundl et al. (1998) suggest that symplastic and (or) cell-to-cell water flow predominate in aspen roots. The composite transport model, proposed by Steudle (1994), provides a good description of water and solute radial movement through roots. In aspen, water and solutes could freely enter the roots by the apoplastic pathway before reaching the endodermis, which is believed to act as a barrier to water and solutes. However, the endodermis may not completely block water and solute transport. The formation of Casparian bands is a dynamic process and, as shown in our study, younger parts of aspen roots may not have a functional endodermis. The origin of lateral roots from the pericycle also creates gaps that provide a bypassing route for water and solutes. There is growing evidence that the chemical composition of Casparian strips does not make them truly impermeable to water and ions. Smaller molecules, such as ABA, may cross through the bands (Freundl et al. 1998, 2000; Schreiber et al. 1999). ABA is an endogenous substance present in all higher plants that has been proposed to act as a messenger from roots to shoots (Davies et al. 1994). Transport of ABA through the cell membranes is thought to be mediated by carriers (Milborrow and Rubery 1985). ABA may also be present and absorbed by roots from the rhizosphere (Hartung et al. 1996). Therefore, it is conceivable that roots may have evolved the capacity to absorb and transport ABA via both apoplastic and symplastic routes. This could explain the relatively high concentrations of ABA that we measured in the xylem sap of intact plants.

In the present experiments, ABA application to roots did not alter the rate of water flow as reported for sunflower (Glinka 1977) and soybean (Markhart et al. 1979). ABA can have different effects on root water flow depending on hydrostatic pressure gradients (Markhart et al. 1979). ABA increased root water flow rate when the rate was initially low (Davies et al. 1982; Markhart 1984) due to its stimulating effect on ion accumulation (Fiscus 1981). In our study, ABA had no effect on root water flow at both low and high flow rates, as evidenced by the lack of effect on $L_p$ and on Arrhenius plots (Figs. 5, 6). Additionally, ABA did not significantly affect $Q_v$, $J_v$, $L_p$ and $E_a$ of aspen roots. The results suggest that ABA applied to the roots affects stomatal conductance directly by triggering stomatal closure rather than indirectly by an inhibition of water flow like that reported for cold soils (Wan et al. 1999).

It appears that the differences in stomatal response of excised shoots and intact seedlings may not be due to ABA concentration differences. Similarly, the different stomatal responses were not likely due to the pH differences of the xylem sap since the pH of the xylem sap expressed from the roots was similar to that of the mineral solution applied to the excised shoots. The higher xylem concentrations of ABA applied to the rooted seedlings exerted less effect on $g_s$ than in the excised shoots. The sensitivity of stomata to ABA could also be due to the differences in plant water status (Tardieu and Davies 1992). We cannot completely exclude the possibility that shoot excision made the stomata more responsive to ABA treatments. However, in the intact aspen seedlings, shoot water potential was significantly lower than that in the excised shoots. Therefore, we expected the stomata in the intact plants to be more sensitive to ABA than those in the excised shoots (Tardieu and Davies 1992). Our results suggest that the presence of roots modulates stomatal response. It is evident from this and other studies (Zhang et al. 1987; Davies and Zhang 1991; Davies et al. 1994) that ABA can act as a signal substance to regulate stomatal aperture. However, the differential effects of ABA on stomatal conductance between the intact seedlings and excised shoots suggests the possibility that other signals which originate in the roots may modify stomatal response to ABA. It has been proposed that the interactions between ABA and cytokinins control stomatal movement in plants (Bradford and Hsiao 1982). It is possible that the higher sensitivity of stomata to ABA in excised aspen shoots compared with that in rooted

plants could be explained by the absence of the source of cytokinins.

*In summary*, our results showed that exogenous ABA applied to the roots inhibited stomatal conductance in aspen seedlings but had no effect on the root water flow. The concentration of ABA needed to reduce stomatal conductance in rooted plants was significantly higher compared with excised shoots. These results suggest that the roots modulate the stomatal response to ABA.

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## References


Blackman PG, Davies WJ (1985) Root to shoot communication in maize plants of effects of soil drying. J Exp Bot 36:39–48

Boyer JS (1989) Water potential and plant metabolism: comments on Dr P. J. Kramer's article, "Changing concepts regarding plant water relations", Volume 11, pp. 565–568, and Dr J. B. Passioura's Response, pp. 569–571. Plant Cell Environ 12:213–216

Bradford KJ, Hsiao TC (1982) Physiological responses to moderate water stress. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology II. Water relations and carbon assimilation. Encyclopedia of Plant Physiology NS, vol 12B, Springer, Berlin Heidelberg New York, pp 264–324

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Physiol Plant Mol Biol 42:55–76

Davies WJ, Rodriguez JL, Fiscus EL (1982) Stomatal behaviour and water movement through roots of wheat plants treated with abscisic acid. Plant Cell Environ 5:485–493

Davies WJ, Metcalfe J, Lodge TA, DaCosta AR (1986) Plant growth substances and the regulation of growth under drought. Aust J Plant Physiol 13:105–125

Davies WJ, Tardieu F, Trejo CL (1994) How do chemical signals work in plants that grow in drying soil? Plant Physiol 104:309–314

Epstein E (1972) Mineral nutrition of plants: principles and perspectives. Wiley, London

Fiscus EL (1981) Effects of abscisic acid on the hydraulic conductance of and the total ion transport through *Phaseolus* root systems. Plant Physiol 68:169–174

Fiscus EL, Stermitz FR, Amoros LG (1982) Abscisic acid transport coefficients of *Phaseolus* root systems. Plant Physiol 69:1351–1355

Freundl E, Steudle E, Hartung W (1998) Water uptake by roots of maize and sunflower affects the radial transport of abscisic acid and its concentration in the xylem. Planta 207:8–19

Freundl E, Steudle E, Hartung W (2000) Apoplastic transport of abscisic acid through roots of maize: effect of the exodermis. Planta 210:222–231

Glinka Z (1977) Effects of abscisic acid and of hydrostatic pressure gradient on water movement through excised sunflower roots. Plant Physiol 59:933–935

Glinka Z (1980) Abscisic acid promotes both volume flow and ion release to the xylem in sunflower roots. Plant Physiol 65:537–540

Hartung W, Sauter A, Turner NC, Fillery I, Heilmeier H (1996) Abscisic acid in soils: What is its function and which mechanisms influence its concentration? Plant Soil 184:105–110

Jarvis AJ, Davies WJ (1997) Whole-plant ABA flux and the regulation of water loss in *Cedrella odorata*. Plant Cell Environ 20:521–527

Khalil AAM, 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

Kramer PJ (1988) Changing concepts regarding plant water relations. Plant Cell Environ 11:565–568

Kriedemann PE, Loveys BR, Fuller GL, Leopold AC (1972) Abscisic acid and stomatal regulation. Plant Physiol 49:842–847

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

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

Mansfield TA, Hetherington AM, Atkinson CJ (1990) Some current aspects of stomatal physiology. Annu Rev Plant Physiol Mol Bio 41:55–75

Markhart AH (1982) Penetration of soybean root systems by abscisic acid isomers. Plant Physiol 69:1350–1352

Markhart AH (1984) Amelioration of chilling-induced water stress by abscisic acid-induced changes in root hydraulic conductance. Plant Physiol 74:81–83

Markhart AH, Fiscus EL, Naylor AW, Kramer PJ (1979) Effects of abscisic acid on root hydraulic conductivity. Plant Physiol 64:611–614

Meinzer FC, Grantz DA, Smit B (1991) Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. Aust J Plant Physiol 18:329–338

Milborrow BW, Rubery PH (1985) The specificity of the carrier-mediated uptake of ABA by root segments of *Phaseolus coccineus* L. J Exp Bot 36:807–822

Passioura JB (1988) Response to Dr P. J. Kramer's article, 'Changing concepts regarding plant water relations', Volume 11, Number 7, pp. 565–568. Plant Cell Environ 11:569–571

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

Raschke K, Hedrich R (1985) Simultaneous and independent effects of abscisic acid on stomata and the photosynthetic apparatus in whole leaves. Planta 163:108–118

Schreiber L, Hartmann K, Skrabs M, Zeier J (1999) Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. J Exp Bot 50:1267–1280

Steudle, E (1994) Water transport across roots. Plant Soil 167:79–90

Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol 98:540–545

Tardieu F, Zhang J, Katerji N, Bethenod O, Palmer SJ, Davies WJ (1992) Xylem ABA controls the stomatal conductance or soil drying. Plant Cell Environ 15:185–191

Tardieu F, Lafarge T, Simonneau T (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

Trejo CL, Davies WJ, Ruiz LMP (1993) Sensitivity of stomata to abscisic acid: an effect of the mesophyll. Plant Physiol 102:497–502

Wan X, Zwiazek JJ (1999) Mercuric chloride effects on root water transport in aspen seedlings. Plant Physiol 121:939–946

Wan X, Landhausser SM, Zwiazek JJ, Lieffers VJ (1999) Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. Tree Physiol 19:879–884

Wartinger A, Heilmeier H, Hartung W, Schulze E-D (1990) Daily and seasonal courses of leaf conductance and abscisic acid in the xylem sap of almond trees (*Prunus dulcis* M.) under desert conditions. New Phytol 116:581–587

Zhang J, Davies WJ (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant Cell Environ 13:271–285

Zhang J, Schurr U, Davies WJ (1987) Control of stomatal behaviour by abscisic acid which apparently originates in the roots. J Exp Bot 38:1174–1181