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Mercuric Chloride Effects on Root Water Transport in Aspen Seedlings[1]

Xianchong Wan and Janusz J. Zwiazek*

Department of Renewable Resources, University of Alberta, 4–42 Earth Sciences Building, Edmonton, Alberta, Canada T6G 2E3

$HgCl_2$ (0.1 mM) reduced pressure-induced water flux and root hydraulic conductivity in the roots of 1-year-old aspen (*Populus tremuloides* Michx.) seedlings by about 50%. The inhibition was reversed with 50 mM mercaptoethanol. Mercurial treatment reduced the activation energy of water transport in the roots from 10.82 ± 0.700 kcal mol^{-1} to 6.67 ± 0.193 kcal mol^{-1} when measured over the 4°C to 25°C temperature range. An increase in rhodamine B concentration in the xylem sap of mercury-treated roots suggested a decrease in the symplastic transport of water. However, the apoplastic pathway in both control and mercury-treated roots constituted only a small fraction of the total root water transport. Electrical conductivity and osmotic potentials of the expressed xylem sap suggested that 0.1 mM $HgCl_2$ and temperature changes over the 4°C to 25°C range did not induce cell membrane leakage. The 0.1 mM $HgCl_2$ solution applied as a root drench severely reduced stomatal conductance in intact plants, and this reduction was partly reversed by 50 mM mercaptoethanol. In excised shoots, 0.1 mM $HgCl_2$ did not affect stomatal conductance, suggesting that the signal that triggered stomatal closure originated in the roots. We suggest that mercury-sensitive processes in aspen roots play a significant role in regulating plant water balance by their effects on root hydraulic conductivity.

Several criteria have been used to infer the presence of water-transporting channels in cell membranes. These include a high ratio of osmotic to diffusional water permeability ($P_f/P_d > 1$), low Arrhenius activation energy ($E_a < 6$ kcal mol^{-1}) for water transport, and its reversible inhibition by mercury sulfhydryl reagents (for reviews, see Chrispeels and Agre, 1994; Verkman et al., 1996; Maurel, 1997). The transport of water through the lipid bilayer has a high E_a, usually above 10 kcal mol^{-1} (Macey, 1984). Water transport can also be via water channel proteins (aquaporins), which have been found in the tonoplasts (Maurel et al., 1993) and plasma membranes (Kammerloher et al., 1994) of plants. It is generally acknowledged that the transport of water via channels is less temperature dependent and has a lower E_a (< 6 kcal mol^{-1}) than transport via the lipid pathway (Finkelstein, 1987; Chrispeels and Agre, 1994). Water transport via aquaporins is characteristically inhibited by mercurial reagents, which react with sulfhydryl groups in the channel proteins and result in closure of the channels. This closure inhibits water transport and increases E_a to the level of that for transport through the lipid pathway (Macey, 1984). An inhibition of water transport by mercury was reported in cell membranes isolated from higher plants (Maurel et al., 1997; Niemietz and Tyerman, 1997) and in whole root systems (Maggio and Joly, 1995; Carvajal et al., 1996). However, the effects of mercury reagents on E_a have not been investigated in intact higher plants.

Based on the composite transport model (Steudle and Frensch, 1996), water transport is via three parallel pathways, apoplastic, symplastic, and transcellular. Both symplastic and transcellular pathways are often referred to as the cell-to-cell pathway (Steudle and Frensch, 1996). In the present study, we use the term symplastic transport to describe the cell-to-cell transport of water involving both the transmembrane transport and that through the plasmodesmata. Due to the cell wall continuum in whole plants, possible effects of $HgCl_2$ on cell walls must be considered. We studied these effects with a fluorescent dye, rhodamine B (RB) that is transported only through the apoplast (Skinner and Radin, 1994).

The importance of root regulation of water flow in plant water relations has received relatively little attention. In the present study, we employed a pressure-flux approach (Markhart et al., 1979a; Rüdinger et al., 1994) to examine the effects of $HgCl_2$ on the properties of water transport and its E_a in the intact root systems of aspen (*Populus tremuloides* Michx.) seedlings grown in solution culture. We also studied the impact of mercury-sensitive root water transport on stomatal conductance (g_s). Since $HgCl_2$ may also act as a general metabolic inhibitor, we also investigated its effect on root oxygen uptake. Based on the results of our study, we suggest that the mercury-sensitive processes of water transport in aspen roots affect plant water balance by regulating root hydraulic conductivity (L_p), which in turn triggers changes in stomatal opening.

MATERIALS AND METHODS

One-year-old aspen (*Populus tremuloides* Michx.) seedlings were grown in the greenhouse from seed collected at Drayton Valley, Alberta, Canada. The plants were grown in plastic containers containing garden soil and were set dormant before being transferred to solution culture. The roots of dormant seedlings were gently washed free of soil with

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* Corresponding author; e-mail janusz.zwiazek@ualberta.ca; fax 780–492–1767.

cold tap water and the seedlings were transferred to solution culture containing one-half-strength modified Hoagland solution (Epstein, 1972). The plants were grown for another 1.5 months in a growth chamber (Controlled Environments, Winnipeg, Mannitoba, Canada) set at a 16-h photoperiod with 260 μmol m^{-2} s^{-1} PPFD at the seedling level, 22°C/18°C (day/night) temperature, and a constant RH of approximately 65%. The Hoagland solution was continuously aerated and replaced every 2 weeks.

The steady-state flow rate (Q_v) was measured using the hydrostatic pressure method (Markhart et al., 1979a; Rüdinger et al., 1994) with some modifications. A glass cylinder was inserted into a pressure chamber (PMS Instruments, Corvallis, OR) and filled with one-half-strength Hoagland solution, which was continuously stirred with a magnetic stirrer. The detopped 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 (F3, HAAKE, Berlin) to maintain the desired root temperature (±0.1°C). A 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 were monitored for linearity for at least 30 min. and Q_v values are expressed in cubic meters per second. The volume flow density (J_v) was calculated as Q_v per unit root surface area and expressed as cubic meters per meter per second. 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, CA) by π. In a previous study (Wan et al., 1999), we found that Q_v in aspen was closely related to new root growth. Therefore, in the present study, J_v values are based on the new root surface area.

Dose Response and Time Course for $HgCl_2$

Root systems were gradually pressurized to a constant pressure of 0.3 MPa. A stable Q_v was maintained for at least 30 min followed by injection of $HgCl_2$ with a syringe into the chamber to reach the desired concentration. The Q_v was monitored during the following 2 h. Distilled water was injected in place of $HgCl_2$ as a control. The stable mean Q_v values measured over the 30-min period before $HgCl_2$ injection were used to normalize the treatment values.

Root Respiration

Respiration was measured as oxygen uptake using a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH). Intact roots were transferred to an airtight cuvette containing aerated one-half-strength Hoagland solution that was continuously stirred with a magnetic stirrer and aerated every 30 min. The mean values from the first 30 min before injecting $HgCl_2$ were used to normalize the respiration rates following the treatments. Distilled water or different concentrations of $HgCl_2$ were added and oxygen uptake measured every 2 min.

The Kinetics of Water Flow Inhibition by $HgCl_2$ and Its Reversibility by 2-Mercaptoethanol (ME)

Root systems were gradually pressurized to a constant pressure of 0.3 MPa. When a stable Q_v was reached, $HgCl_2$ was injected with a syringe into the chamber to reach a final concentration of 0.1 mM. Q_v was monitored until a new stable flow rate was attained, and then ME was injected into the chamber to provide a final concentration of 50 mM. The measurements of Q_v continued until another stable Q_v was reached. A control experiment was run in a similar manner, except that distilled water was injected in place of the $HgCl_2$ solution.

Measurements of E_a

Root systems were immersed in one-half-strength Hoagland solution and held at a constant pressure of 0.3 MPa with the temperature changing from 25°C to 4°C (descend) and back to 25°C (ascend) 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. After the descending temperature series, the pressure chamber was opened and the solution aerated before continuing with the ascending temperature series.

The Arrhenius plots were obtained by plotting the logarithm of Q_v against the reciprocal of the absolute temperature, and E_a was calculated from the slope of the whole curve of the descending plot. Before $HgCl_2$ addition, a Q_v value was measured at 25°C and used as a blank. Then, $HgCl_2$ was added into the solution to a final concentration of 0.1 mM, and the temperature was changed to 4°C and back to 25°C in 3°C steps.

The exudates were collected when the measurement temperature was at 25°C. For control group, there were two 25°C points, one at the beginning of the descending temperature series and the other at the end of the ascending temperature series. These are referred to as descending sap and ascending sap, respectively. For the $HgCl_2$-treated group, before $HgCl_2$ addition, a Q_v value was measured at 25°C as a blank reference. Thereafter, $HgCl_2$ was added to the root medium and the temperature was changed as for the control group, i.e. from 25°C to 4°C and back to 25°C. Therefore, there are three 25°C points in the treatment group, which are referred to as the reference sap, descending sap, and ascending sap, respectively. The xylem saps were collected for osmotic potential and electrical conductivity determinations. Osmotic potential was measured with a thermocouple psychrometer (HR-33T, 5112, Wescor, Logan, UT) and a C52 sensor in the dew point mode, and the electrical conductivities were determined with a conductivity meter (model C33, Fisher Scientific, Nepean, Ontario, Canada).

Determinations of L_p

Roots were immersed for 30 min in aerated one-half-strength Hoagland solution in a pressure chamber at 22°C. Water or $HgCl_2$ was added as previously described, and the pressure increased every 30 min from 0 to 0.025, 0.05, 0.075, 0.10, 0.125, 0.15, 0.2, 0.3, 0.4, and 0.5 MPa. J_v was calculated as Q_v per unit root surface area and plotted against hydrostatic pressures. L_p was calculated from the slope of the curve between 0.15 and 0.5 MPa, where the relationship between pressure and J_v was linear, and is expressed in meters per second per megapascal.

Symplastic and Apoplastic Pathways

RB was used to trace root water transport and to detect the effect of $HgCl_2$ on the symplastic and apoplastic flux. RB is a fluorescent dye believed to be transported only through the apoplast (Skinner and Radin, 1994). A root system was sealed in a pressure chamber filled with a one-half-strength Hoagland solution and the chamber pressurized to 0.3 MPa. The Q_v value was measured and RB was added to a final concentration of 20 μg mL^{-1}. The Q_v value was measured again for 1 h and $HgCl_2$ was added for another 1 h and Q_v measured again over the 1-h incubation period. The first 30-min xylem exudates were discarded and the rest collected to measure RB concentration, electrical conductivity, and osmotic potential. The concentration of RB was measured using a fluorometer (Sequoia-Turner model 450, Apple Scientific, Chesterland, OH). The excitation and emission wavelengths were 520 and 605 nm, respectively. A standard curve of known RB concentrations was established to calculate RB in xylem exudates. The apoplastic flow was estimated by dividing the tracer concentration in the expressed xylem exudate by its concentration in the root incubation solution.

Measurements of g_s

For g_s measurements in intact plants, the seedlings were grown in aerated one-half-strength Hoagland solution in a growth chamber maintained under identical environmental conditions as those described earlier. Measurements of g_s were conducted on the sixth expanded leaf with a steady-state porometer (LI-1600, LI-COR, Lincoln, NE). In the control (untreated) group, g_s was measured in 30-min intervals for 4 h and after 16 h. In treated plants, g_s was measured before and after $HgCl_2$ was added to the incubation solution to a final concentration of 0.1 mM. The measurements were conducted in 30-min intervals for 3 h. Subsequently, ME was added to a concentration of 50 mM and g_s was measured after 30 min, 1 h, and 3 h. All measurements were conducted during the light period.

In the second experiment, excised shoots were used instead of intact seedlings. The seedlings were placed in a one-half-strength Hoagland solution in the dark growth chamber for 4 h and the shoots were excised at the root collar under the solution. Excised shoots were immersed in one-half-strength Hoagland solution and exposed to light in the growth chamber. For $HgCl_2$ treatment, the shoots were placed in one-half-strength Hoagland solution containing 0.1 mM $HgCl_2$ and g_s was measured at the same times as in the controls.

Reagents

All reagents were of the highest available grade and were purchased from Sigma (St. Louis).

Statistical Analysis

The data are presented as the means of at least four replicates (seedlings). The results were analyzed by ANOVA and with Duncan multiple comparison or t test using the SAS 6.12 software package (SAS Institute, Cary, NC). All statistically significant differences were tested at the $P \leq 0.05$ level.

RESULTS

The concentrations of $HgCl_2$ ranging from 0.05 to 0.25 mM resulted in a similar level of inhibition of Q_v within 60 min from application (Fig. 1). The higher 0.5 mM concentration inhibited Q_v more rapidly than the lower concentrations and the lower 0.025 mM concentration acted rela-

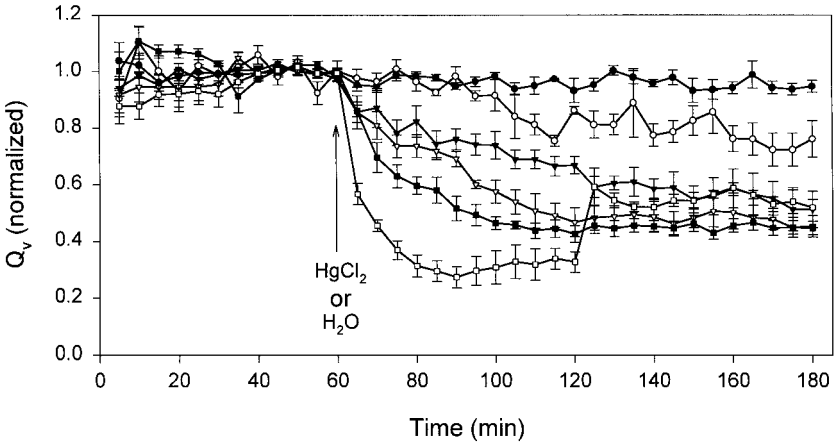

Figure 1. Dose response and time course of Q_v inhibition by $HgCl_2$. Q_v was normalized to the mean rate over the initial 30 min before $HgCl_2$ injection. The time of injection of $HgCl_2$ (H_2O for controls) is indicated by the arrows. Means ± SE are shown ($n = 4$). ●, Control; ○, 0.025 mM; ▼, 0.050 mM; ▽, 0.100 mM; ■, 0.250 mM; □, 0.500 mM.

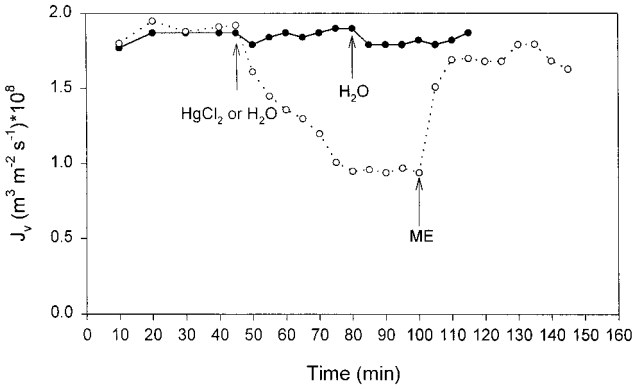

Figure 2. J_v in aspen roots treated with 0.1 mM $HgCl_2$ and 50 mM ME. Treatment (○) is the mean of three seedlings; control (●) is the mean of two seedlings. Times of injections of $HgCl_2$ (water for controls) and ME (water for controls) are indicated by arrows.

tively slowly on Q_v and was less effective than the higher concentration treatments (Fig. 1).

When the roots were held at the constant pressure of 0.3 MPa, 0.1 mM $HgCl_2$ caused a rapid decrease in J_v (Fig. 2). Within 10 min following injection of ME into the solution, this inhibition was almost completely reversed (Fig. 2). The results calculated from eight replicates indicated that $HgCl_2$ inhibited J_v by 47% (± 3.17%) and that J_v returned to 91% (± 3.36%) of the original values after adding ME. There was no significant difference in J_v of the control roots over the 2-h measurement period.

Pressure-flux curves from $HgCl_2$-treated and control roots ($n = 6$) showed a linear relationship between 0.15 and 0.5 MPa (Fig. 3). The L_P values calculated over this range were $9.71 \pm 0.836 \times 10^{-8}$ m s^{-1} MPa^{-1} and $4.88 \pm 0.263 \times$

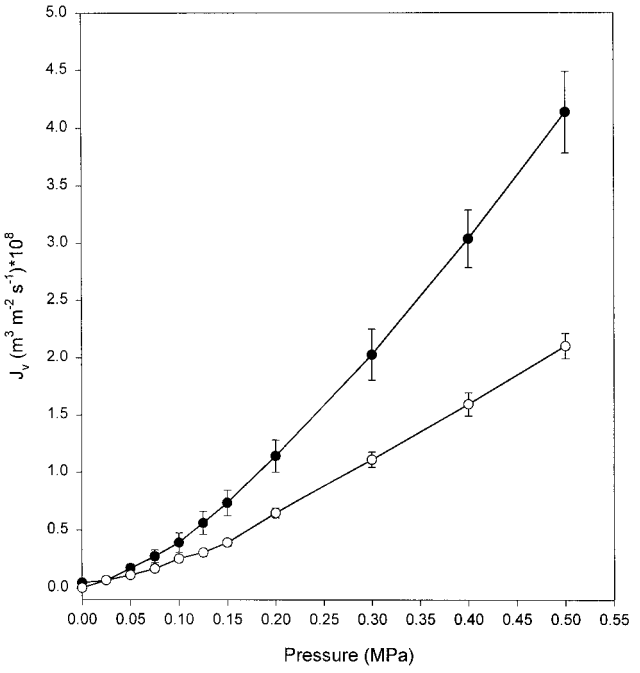

Figure 3. Pressure-flow relationship in control roots (●) and roots treated with 0.1 mM $HgCl_2$ (○). Means ± SE are shown ($n = 6$).

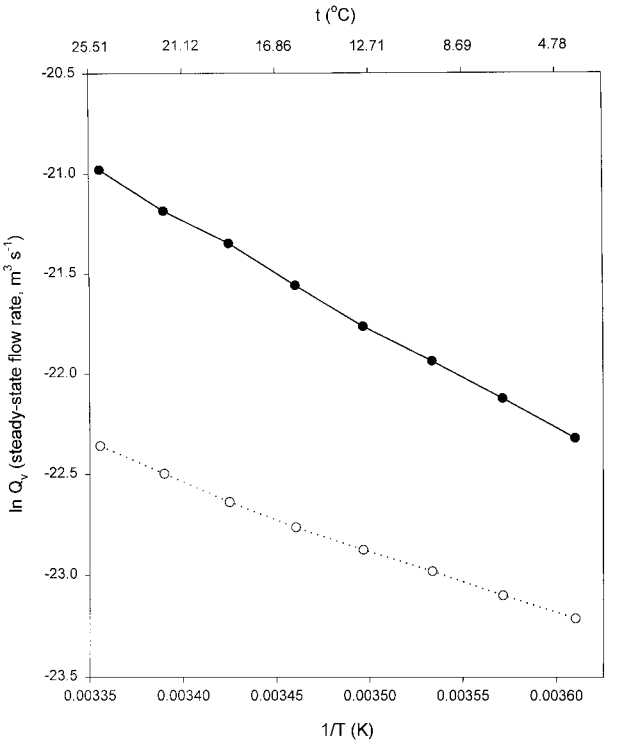

Figure 4. Temperature effects on water flow through aspen roots at constant hydrostatic pressure of 0.3 MPa and decreasing temperatures. Each curve is the mean of six seedlings from six repeated experiments. ●, Control; ○, $HgCl_2$ treated.

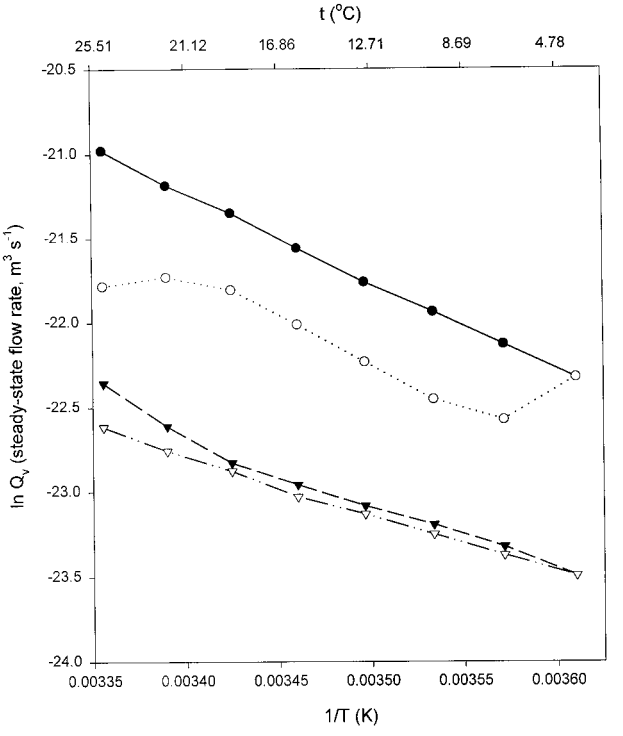

Figure 5. Temperature and $HgCl_2$ (0.1 mM) effects on water flow through aspen roots. Q_v was continually measured in temperatures descending to 4°C followed by ascending temperatures to 25°C. ●, Control descending; ○, control ascending; ▼, $HgCl_2$ descending; ▼, $HgCl_2$ ascending.

Table I. *Effect of 0.1 mM $HgCl_2$ and temperature on the properties of xylem exudates*

Sap was collected only when the measurement temperature was 25°C. Descending and ascending refer to decreasing and increasing temperatures, as explained in the text. The reference xylem sap was collected at 25°C before $HgCl_2$ was added. Means ± SE (n = 6) followed by different letters are significantly different at the 0.05 level.

Sap		Reference	Descending	Ascending
Q_v at 25°C ($[m^3\ s^{-1}] * 10^{10}$)	Control	–	7.944 ± 0.801 a	3.665 ± 0.294 b
	$HgCl_2$	4.085 ± 0.902 a	2.205 ± 0.441 b	1.730 ± 0.385 b
Electrical conductivity (μS)	Control	–	670.83 ± 42.387 b	746.67 ± 65.167 a
	$HgCl_2$	660.33 ± 21.833 b	825.07 ± 38.224 a	800.33 ± 33.482 a
Osmotic potential (MPa)	Control	–	−0.053 ± 0.0060 a	−0.059 ± 0.0129 a
	$HgCl_2$	−0.058 ± 0.0014 a	−0.056 ± 0.0014 a	−0.057 ± 0.0023 a

10^{-8} m s^{-1} MPa^{-1} for the control and $HgCl_2$-treated roots, respectively, and the difference was statistically significant.

Both control and treated roots had linear Arrhenius plots for Q_v (Fig. 4). The treatment with $HgCl_2$ reduced not only Q_v but also E_a. The E_a value was 10.82 ± 0.7 and 6.67 ± 0.193 kcal mol^{-1} for the control and treated roots, respectively, and the difference was highly significant. In control roots but not $HgCl_2$-treated roots the Arrhenius plots were not linear in higher temperatures when measured for ascending temperatures following temperature decrease to 4°C (Fig. 5). Neither $HgCl_2$ nor temperature changed the osmotic potentials of the xylem exudates (Table I). However, $HgCl_2$ treatment increased the electrical conductivity of the expressed sap (Table I). In control roots, the electrical conductivity increased after the temperature was decreased to 4°C and then increased back to 25°C.

The RB concentration in the xylem sap of the control roots was about 0.01% of that in the incubation solution. In $HgCl_2$-treated roots, the decrease in Q_v was accompanied by an increase in the concentration of RB and in the electrical conductivity of the expressed xylem sap (Table II). However, there was no difference in osmotic potentials of the control and $HgCl_2$-treated exudates (Table II).

$HgCl_2$ significantly inhibited g_s in intact seedlings. After 3 h of incubation in $HgCl_2$, the g_s rates declined from about 23 mmol m^{-2} s^{-1} to less than 7 mmol m^{-2} s^{-1} (Fig. 6A). The inhibition of g_s was only partly reversed by 50 mM ME. After 1 h, ME resulted in a significant (P = 0.013) increase in g_s to above 10 mmol m^{-2} s^{-1} (Fig. 6A). Over the experimental period, no significant changes in g_s were detected in control seedlings (P = 0.318).

In excised shoots, g_s rates remained stable in the first 12 h and thereafter declined with time in both control and $HgCl_2$-treated plants. However, there was no significant difference in g_s between the control and treated shoots (Fig. 6B).

Treatment with 0.1 mM $HgCl_2$ did not significantly reduce root respiration in the 1st h (Fig. 7). However after 4 h of treatment, 0.1 mM $HgCl_2$ caused a reduction in oxygen uptake by 17%, while that in 0.5 mM $HgCl_2$ was reduced by 30% to 43% (Fig. 7).

DISCUSSION

Mercury reversibly inhibits the bulk water transport across membranes in animal cells (Pratz et al., 1986; Meyer and Verkman, 1987) and plant cells (Maggio and Joly, 1995; Carvajal et al., 1996; Maurel et al., 1997; Niemietz and Tyerman, 1997). This reversible inhibition is used to demonstrate the existence of proteinaceous water channels (Chrispeels and Maurel, 1994). Our experiment followed the methodology used by Maggio and Joly (1995), which employs the whole root system and the pressure-flux approach. The result of the kinetics of reversible mercurial inhibition of water flow suggested the presence of water channels in aspen roots. $HgCl_2$ inhibited root water flow in aspen by decreasing L_p. This suggests that root water channels play an important role in regulating plant water relations. The pressure-flux curves in untreated controls and in $HgCl_2$-treated roots were consistent with this theory (Fiscus, 1975) and previous observations (Lopushinsky, 1964; Markhart et al., 1979b; Jackson et al., 1996). The relationship between J_v and applied pressure was highly linear in pressures above 0.15 MPa. Below this point, the curve was not linear and did not cross at zero J_v, especially for controls, in which water flows were observed at 0 MPa of pressure due to root pressure described by Fiscus (1975). The values of J_v and L_p observed in this experiment were low compared with those in tomato (Maggio and Joly, 1995), soybean (Fiscus, 1977), bean (Fiscus, 1981), and maize (Zhu and Steudle, 1991). This is in agreement with earlier observations that the roots of woody plants have

Table II. *Properties of xylem sap collected from control roots and from roots treated with $HgCl_2$ incubated in solutions containing RB*

Means ± SE (n = 6) followed by different letters are significantly different at the 0.05 level.

Treatment	Q_v	Electrical Conductivity	Osmotic Potential	RB Concentration	C_e/C_b[a]
	$(m^3\ s^{-1}) * 10^{10}$	μS	MPa	$(\mu g\ mL^{-1}) * 10^3$	$(\%) * 10^2$
Control	6.12 ± 1.263 a	538.56 ± 22.076 b	−0.064 ± 0.0037 a	2.43 ± 0.205 b	1.22 ± 0.101 b
$HgCl_2$	2.87 ± 0.610 b	797.87 ± 54.658 a	−0.062 ± 0.0054 a	5.00 ± 1.106 a	2.50 ± 0.563 a

[a] C_e denotes RB concentration in the xylem sap, and C_b is RB concentration in the incubation solution.

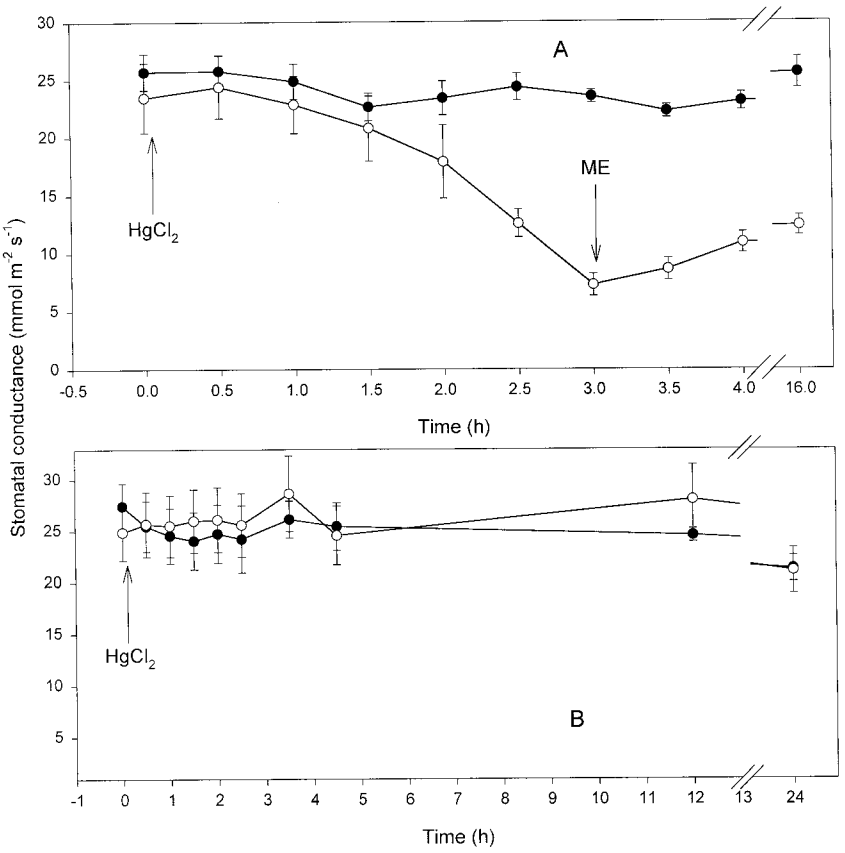

Figure 6. Effects of 0.1 mM $HgCl_2$ and 50 mM ME on leaf g_s in intact seedlings (A) and excised shoots (B). Arrows indicate the times when $HgCl_2$ and ME were added. Means ± SE (n = 6) are shown. ●, Control; ○, $HgCl_2$ treated.

lower permeability to water compared with herbaceous species (Steudle and Meshcheryakov, 1996).

A low E_a (<6 kcal mol^{-1}) for water transport is among the typical features of membranes with water-transporting pores (Chrispeels and Agre, 1994; Verkman et al., 1996; Maurel et al., 1997; Niemietz and Tyerman, 1997), while transport through the membrane lipid bilayer is associated with high Arrhenius E_a values. Mercurials can increase the E_a of water permeation facilitated by water channels (Macey, 1984; Meyer and Verkman, 1987; van Hoek et al., 1990). However, in our study, $HgCl_2$ significantly reduced E_a values for Q_v in roots and more studies will be required for a proper explanation of these results. The proportion of apoplastic flow increased in the $HgCl_2$-treated roots. However, this increase may not necessarily be the reason for E_a reduction.

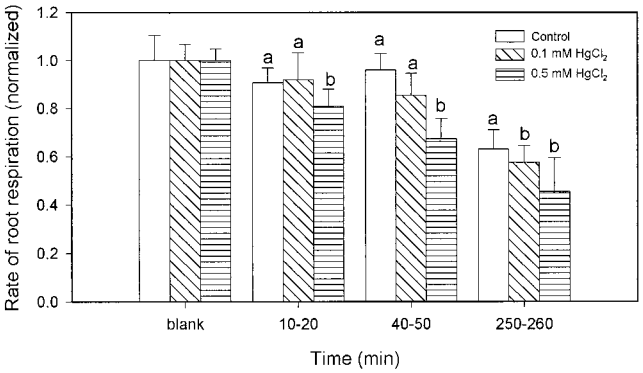

Figure 7. Effect of $HgCl_2$ on root respiration. The mean values from the first 30 min before the $HgCl_2$ injection were used to normalize the respiration rates following the treatments. Bars with the different letters in the same group are significantly different at the 0.05 level. Means ± SE are shown (n = 4).

We assumed that the mercuric inhibition of Q_v was due to blocking of the water channels. It is commonly accepted that water channels have a low temperature sensitivity. The Q_{10} value for water transport through an aqueous pore is essentially the same as that for the viscosity of water, about 1.25 (Finkelstein, 1987). From this point of view, the apoplast is similar to the water channels. Therefore, the inhibition of the temperature-insensitive processes should not be expected to reduce temperature sensitivity for the whole water transport. At the present time, we cannot conclude with any certainty that the water channels in aspen are sensitive to temperature; nevertheless, the results suggest that the mercury-sensitive processes are also temperature sensitive. If the effect of $HgCl_2$ is mainly on water channels, as reported for individual cells and isolated membrane vesicles (Macey, 1984; Meyer and Verkman, 1987; Pratz et al., 1986; Maurel et al., 1997; Niemietz and Tyerman, 1997), then the channels may indeed be temperature sensitive. However, we cannot exclude the possibility that other, temperature-sensitive processes involved in root water transport are affected by mercury resulting in this effect. The increased sensitivity of root water flow to low temperatures that we found in aspen could be an

adaptive feature if present in other perennial plants that are exposed to seasonal low temperatures. This increased sensitivity could allow the plants to regulate root water flow at the low temperatures to prevent xylem cavitation at the end of the growth season and prepare for winter rest.

It is often assumed that the water-transporting pores are rigid and rarely change their shape or size with changing temperature, while the water permeability of the phospholipid bilayer is temperature dependent (Chrispeels and Agre, 1994). However, protein pores do not have to be rigid. E_a depends on the nature of the rate-limiting barrier for water movement and on the energetics of the water-pore interactions (Verkman et al., 1996). Moreover, Arrhenius plots of water movement in soybean (Markhart et al., 1979a) and in renal proximal tubule cell membranes (Meyer and Verkman, 1987) were found to be nonlinear. In our experiment, when the temperature was decreased from 25°C to 4°C and then increased back to 25°C, control roots did not yield a linear Arrhenius plot (Fig. 5). This did not appear to be due to membrane damage by the low temperatures. The ascending sap had a higher electrical conductivity than the descending one (Table I); however, the increased conductivity was likely due to the relative increase in the apoplastic transport after the symplastic transport was inhibited by the low temperatures. Unlike in control seedlings, the $HgCl_2$-treated roots had fully reversible linear plots (Fig. 5). This suggests that following mercuric treatment, the roots lost their sensitivity to low temperature.

In our experiment, the concentration of RB in the xylem sap expressed from the control roots was only 0.012% of that in the incubation solution, suggesting that only a very small fraction of water was transported in the roots through the apoplast. The concentration of RB in the xylem sap of $HgCl_2$-treated roots increased to 0.025% of that in the solution. This indicates some increase in the apoplastic water transport, but also suggests the shift from bulk to diffusional water transport across the membranes, since the concentration of RB was only a small fraction of that present in the incubation solution. However, fluorescent tracer results for water movement must be interpreted with caution. The molecule mass of RB is 479 D, higher than that of the water molecule. The rates of transport of fluorescent tracers and water through the apoplast may be different due to their different molecular sizes (Hanson et al., 1985; Yeo et al., 1987). Therefore, the concentration of RB in the xylem sap gives an indication rather than a precise estimate of the ratio of symplastic to apoplastic water transport.

Electrical conductivity increased along with an increase in RB concentration in the xylem sap of $HgCl_2$-treated roots and in those exposed to low temperatures (Tables I and II). These results confirm the increase in the apoplastic transport of the treated roots, because the apoplastic transport does not selectively filter out ions present in the root medium (Peterson et al., 1981; Yeo et al., 1987). It is interesting that the increase in the electrical conductivity of the xylem sap was not reflected by a decrease in osmotic potentials. This suggests a change in the solute composition of the xylem sap that resulted from the change in the water transport pathway.

$HgCl_2$-treated plants with intact roots closed their stomata and showed signs of wilting within 2 to 3 h following treatment. This stomatal closure was partly reversed with 50 mM ME (Fig. 6A) and was triggered by $HgCl_2$ effects on roots, since we did not observe any effect of $HgCl_2$ in excised shoots (Fig. 6B). Low soil temperatures are known to inhibit L_p and induce stomatal closure in aspen (Wan et al., 1999). It is possible that, like low root temperature (Chen et al., 1983; Lee et al., 1993) and drought (Zhang et al., 1987; Lång et al., 1994), $HgCl_2$ treatment triggered ABA synthesis, which directly caused stomatal closure. This could explain the slow recovery of stomatal opening following ME treatment.

The respiration experiment showed that 0.1 mM $HgCl_2$ did not significantly reduce root respiration during the initial hour, the time when the root water flow rate was significantly reduced (Figs. 1 and 2). This suggests that the mercuric inhibition of root water flow was not due to metabolic inhibition. However, higher concentrations of $HgCl_2$ and longer treatments reduced root oxygen uptake (Fig. 7). The 0.1 mM $HgCl_2$ concentration used in our study caused a reduction of root respiration over time. However, the reduction in respiration rates was not paralleled by the reduction in the water flow rates. Additional evidence suggesting that the reduction of root water flow by mercury was not due to the inhibition of metabolism comes from the experiments designed to measure the E_a for root water flow, in which the ascending plot for the 0.1 mM $HgCl_2$ treatment almost exactly overlapped with the descending one (Fig. 5). Also, the same concentration of $HgCl_2$ had no effect on the g_s in the excised shoots for at least 12 h of the treatment (Fig. 6b). Our results suggest that mercury-sensitive processes, likely those involving water channels, play an important role in regulating L_p and, in effect, water relations in aspen. The observed low-temperature sensitivity of water transport in roots may be an important factor in the adaptation to winter conditions.

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