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Rapid Communication

Effects of Mercuric Chloride on the Hydraulic Conductivity of Tomato Root Systems[1]

Evidence for a Channel-Mediated Water Pathway

Albino Maggio and Robert J. Joly*

Department of Horticulture, Purdue University, West Lafayette, Indiana 47907–1165

A pressure-flux approach was used to evaluate the effects of $HgCl_2$ on water transport in tomato (*Lycopersicon esculentum*) roots. Addition of $HgCl_2$ to a root-bathing solution caused a large and rapid reduction in pressure-induced root water flux; the inhibition was largely reversible upon addition of β-mercaptoethanol. Root system hydraulic conductivity was reduced by 57%. There was no difference between treatments in the K^+ concentration in xylem exudate. The results are consistent with the presence of a protein-mediated path for transmembrane water flow in tomato roots.

Radial water transport in roots is thought to occur along three parallel pathways, an apoplasmic, a symplasmic (via plasmodesmata), and a transcellular (vacuole to vacuole) path (Weatherley, 1982). Experimental difficulties in separating the symplasmic and the transcellular pathways, however, have limited studies to the apoplasmic and a comprehensive cell-to-cell pathway (Steudle, 1993). Which of these plays the predominant role in radial water movement in roots is not understood, and the literature is still controversial (Moreshet and Huck, 1991).

Despite the lack of knowledge regarding the transcellular pathway, there is increasing evidence to support the idea of a preferential route for water transport across cell membranes. Integral membrane proteins acting as specific water channels, recently referred to as aquaporins (Chrispeels and Maurel, 1994), have been reported to occur in plants at both the plasma membrane (Kammerloher and Schäffner, 1993) and tonoplast (Höfte et al., 1992). These proteins have been identified as members of the major intrinsic protein family and appear to be closely related to the channel-forming integral protein, a 28-kD protein abundant in mammalian red blood cells and renal proximal tubules (Preston et al., 1992). Some aquaporins have been localized exclusively in roots (Yamamoto et al., 1991) or in seeds (Höfte et al., 1992); in either case, they may influence the movement and partitioning of water among neighboring cells.

The use of mercurial sulfhydryl reagents as specific water channel inhibitors has been widely reported (Macey, 1984; Pratz et al., 1986; Meyer and Verkman, 1987), and subsequent addition of an excess of ME has been used to remove Hg from membranes of treated cells or tissues. Such studies have provided strong evidence of a protein-mediated pathway for water movement through membranes of certain mammalian and amphibian cell types (van Hoek et al., 1990; Preston et al., 1992; Maurel et al., 1993). In this report we describe the results of experiments carried out to test the effects of $HgCl_2$, a known inhibitor of water channels (van Heeswijk and van Os, 1986; Ye and Verkman, 1989), on the water permeability of intact tomato (*Lycopersicon esculentum*) root systems. We report that a $HgCl_2$-induced reversible inhibition of root water flux is consistent with the presence of a protein-mediated path for transmembrane water flow in plant roots.

MATERIALS AND METHODS

Tomato seeds (*Lycopersicon esculentum* cv Better Boy) were germinated in February and September 1994 in moist Peat-Lite until the emergence of primary leaves, at which time the seedlings were transferred to a single-basin hydroponic system filled with aerated, half-strength Hoagland solution (Hoagland and Arnon, 1950). The pH of the nutrient medium was maintained between 5.5 and 6.5 until harvest. Plants were grown on a greenhouse bench in the horticulture greenhouse at Purdue University (West Lafayette, IN), under approximately 25°C day maximum and 18°C night minimum temperatures. Average photosynthetic photon flux during this period was approximately 21.6 mol m^{-2} d^{-1}. Two 1000-W high-pressure sodium vapor lamps were positioned approximately 1 m above the plant canopy to supplement natural sunlight and to maintain a PPFD of at least 500 μmol m^{-2} s^{-1} during 12-h photoperiods.

The effect of $HgCl_2$ was studied using two strategies, each based on a pressure-flux approach (Fiscus, 1977; Markhart et al., 1979). In the first set of experiments, the kinetics of inhibition of J_v by $HgCl_2$ and its reversibility by

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* Corresponding author; e-mail joly@sage.cc.purdue.edu; fax 1–317–494–0391.

Abbreviations: J_v, volume flow of water (root water flux); L_p, hydraulic conductivity; ME, β-mercaptoethanol; Q, flow rate.

ME were evaluated. Subsequently, a second set of experiments was carried out to test the effects of $HgCl_2$ on root L_p. It was essential to verify that the observed decrease in water flux was attributable to changes in L_p rather than to changes in either the osmotic component of the driving force for water movement or to imbalances in ion transport. The experimental system for measuring J_v was similar to that of Joly (1989), with some modifications described below.

Inhibition and Recovery of J_v

The effects of $HgCl_2$ on J_v were evaluated in two separate experiments. In the first experiment, six 4-week-old seedlings were selected at random from a population of approximately 100 plants. The $HgCl_2$ treatment was applied in a pairwise manner, such that the response of a single treated root system was compared with an untreated control. The treatment was replicated three times. In a second experiment, we selected eight 6-week-old seedlings and compared two treated root systems simultaneously with two controls in two replications. After we confirmed that the responses of 4- and 6-week-old plants did not differ, percentage inhibition and recovery were calculated on the bulked data for the two experiments. In total, the responses of seven $HgCl_2$-treated plants were compared with those of seven untreated plants.

Upon harvest, stems were severed just below the cotyledonary leaves. Whole-root systems were placed in a two-compartment stainless steel pressure chamber filled with aerated, half-strength Hoagland solution. To avoid compression injury to soft tissue, stems were first inserted through a Parafilm-wrapped brass tube (7 or 9 mm i.d.) before they were sealed into the lid of the chamber through a rubber gasket.

After root systems were sealed in the lid, chamber pressure was gradually increased to 0.3 MPa and held constant until Q became stable. Water expressed from each cut stump was delivered via Tygon tubing to a system of computer-controlled valves and partitioned such that exudate from one root system at a time was weighed by an electronic balance. After Q stabilized, $HgCl_2$ was added to one of the chamber compartments to give a final concentration of 0.5 mM. Because it was essential to evaluate root system response at a constant pressure, a system of syringes was placed inside the chamber that could be activated by pressure from an external supply of compressed air. This system was used to inject $HgCl_2$ as well as ME in an attempt to reverse its effect (Meyer and Verkman, 1987).

After an inhibitory effect was detected and a new steady-state flow was observed, ME was injected into the chamber compartment to provide a final concentration of 0.06 M. Following injection of ME, we continued to measure Q until a new steady state was attained. The evaluation of each root system for initial steady state, inhibition, and recovery occurred during a 2.5- to 3.0-h period. Upon completion of the exudation measurements, root fresh and dry weights were determined; J_v was expressed as mg H_2O g^{-1} fresh weight min^{-1}.

Root L_p

To measure root L_p, pressure-flux curves were determined on twelve 7-week-old seedlings. In each of three replications, two $HgCl_2$-treated root systems were compared with two untreated controls. Treated root systems were in contact with 0.5 mM $HgCl_2$ throughout the duration of the pressure-flux measurement. After root systems were sealed in the lid of the chamber, pressure was gradually increased to 0.20 MPa and held constant until Q became stable. Water expressed from each cut stump was collected as previously described. Five pressures were applied in sequentially increasing order (0.20, 0.26, 0.32, 0.38, and 0.44 MPa). Q values were logged for 45 min at each pressure, allowing a 5-min equilibration period between pressures. Upon completion of the exudation measurements, the total length, l, of each root system was estimated by use of an image analyzer (Decagon Devices, Pullman, WA), after staining with methyl violet. L_p was estimated after making the assumption that the root can be considered, to a first approximation, a cylindrical membrane system with radius r (Joly, 1989). An average root radius of 0.2 mm was obtained by sampling root cross-sections. Root diameter was measured from epidermis to epidermis, and total root surface area was calculated as $2\pi rl$. J_v was computed by transforming Q to reflect total flux on the basis of the root surface area, and L_p was estimated as the slope of the regression of J_v on applied hydrostatic pressure (Dalton et al., 1975; Fiscus, 1977).

The osmolality of both the Hoagland solution in contact with the roots and the expressed root exudate were measured with a Wescor model 5100C vapor pressure osmometer (Wescor, Inc., Logan, UT). In addition, aliquots of expressed sap were collected from each root system and bulked over all pressures for later analysis of K^+ content using inductively coupled plasma atomic emission spectrometry. K^+ flux into the xylem was calculated as the product of the sap flux and concentration of K^+ in the sap (Jackson and Weatherley, 1962).

RESULTS

A representative comparison of a single $HgCl_2$-treated root system and its corresponding untreated control, measured simultaneously, is shown in Figure 1. J_v from the untreated root system remained virtually constant throughout an approximately 2.4-h measurement period. Upon injection of $HgCl_2$, however, sap flux decreased rapidly, reaching a minimum value of 11 mg H_2O g^{-1} fresh weight min^{-1} in approximately 16 min. This represented a reduction of approximately 82% from pretreatment flux. The kinetics of flux reduction could be very well described by a power function of the form: Flux = $aT^b + c$, where a, b, and c are coefficients from a nonlinear regression and T denotes time elapsed after $HgCl_2$ injection. The coefficient of determination, r^2, for this relation was 0.999. Following a 32-min period of stable flux, ME was injected into the bathing solution. This caused a very rapid response, with an increase being observed within 4.8 min. Sap flux increased in an exponential manner, reaching a generally

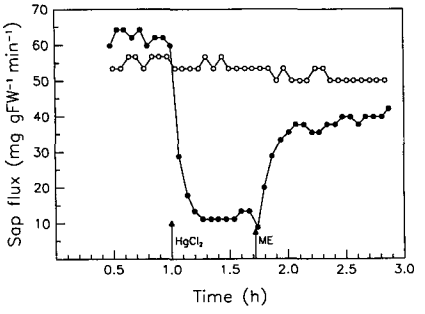

Figure 1. Pressure-induced water flux through a single $HgCl_2$-treated tomato root system (●) and its corresponding untreated control (o), measured simultaneously. Times of injection of $HgCl_2$ and ME are indicated by arrows. FW, Fresh weight.

constant value of 37 mg g^{-1} fresh weight min^{-1} in about 20 min. The kinetics of flux recovery were well described by an asymptotic exponential function of the form: Flux = $a(1 - \exp^{-bT+cT^2})$, where T denotes time elapsed after ME injection; the r^2 for the relation was 0.98.

The percentages of J_v inhibition and subsequent recovery were computed for each individual root system, and mean values and SDs were calculated over all seven replicates. $HgCl_2$ caused a mean reduction of 71.5 ± 11.4% from the initial preinjection value, and subsequent addition of ME permitted recovery to 79.8 ± 16.0% of the initial value.

A representative comparison of pressure-flux curves for two $HgCl_2$-treated root systems and their corresponding untreated controls, measured simultaneously, is shown in Figure 2. Linear regressions of J_v on hydrostatic pressure were performed on the combined data (two root systems per treatment). In addition, L_p, evaluated as the slope of the regression line, was estimated singly for each of six $HgCl_2$-treated and six control root systems, and mean values were calculated. Mean L_p was 2.01 ± 0.05 × 10^{-7} and 4.64 ± 0.17 × 10^{-7} m s^{-1} MPa^{-1}, respectively, for $HgCl_2$-treated and control plants (Table I). r^2 values for individual root systems varied between 0.91 and 0.98, with the exception of a value of 0.84 for one of the treated plants.

The osmotic potential of the nutrient solution in contact with the roots was −0.14 MPa for both $HgCl_2$-treated and untreated plants; the addition of only 0.5 mM $HgCl_2$ did not significantly change the osmolality of the Hoagland solution (Table I). Furthermore, the osmotic potential of root system exudate of $HgCl_2$-treated roots (−0.14 MPa) was not significantly different from that of untreated controls (−0.13 MPa). Finally, the flux of K^+ into the xylem was not significantly affected by the presence of $HgCl_2$ in the nutrient solution; it ranged between 54 and 56 mg min^{-1} (Table I), values similar to those reported by Jackson and Weatherly (1962).

DISCUSSION

We used a pressure-flux approach (Fiscus, 1977; Markhart et al., 1979) to characterize the effects of $HgCl_2$ on water transport in tomato root systems and to evaluate whether a facilitated pathway for water movement exists in intact roots. A known inhibitor of membrane water channels was used to test whether the kinetics of inhibition and recovery were consistent with the presence of specialized water-selective channels in plant roots. Three conclusions emerge from our results. First, $HgCl_2$ markedly depresses water flux through tomato roots, and the reduction is rapidly reversible with ME. Second, this reduction in water flux is associated with a large (56.7%) and statistically significant reduction in root L_p (Table I). Third, a transcellular pathway is involved in the radial movement of water across the root to the xylem, and this path is likely protein mediated.

The sensitivity of aquaporins to $HgCl_2$ has recently been demonstrated in the red blood cell channel-forming integral protein molecule by Preston et al. (1993) and in *Arabidopsis thaliana* tonoplasts by Daniels et al. (1994). Both reports suggest that mercurial sulfhydryl reagents inhibit osmotic water permeability through aquaporin channels by physically blocking water from passing through the channel. In the present study, both the osmolality of the nutrient solution and the applied hydrostatic pressure were essentially constant throughout any given inhibition-recovery experiment. Hg^{2+} ions probably reacted with free sulfhydryl groups of proteins associated with root membrane water channels and resulted in their closure.

The kinetics of flux inhibition observed upon treatment with $HgCl_2$ and its reversal with ME conformed well to highly defined nonlinear functions. Inhibition and subsequent recovery occurred over time periods of approximately 16 and 20 min, respectively. The curvilinear nature of the responses may be related to the time required for $HgCl_2$ or ME to reach reactive sites within putative water channels and/or to the kinetics of Hg^{2+} interaction with these proteins.

The rapid inhibition of J_v by 0.5 mM $HgCl_2$ and its subsequent reversal by ME provide evidence of a protein-mediated path for water transport in tomato roots. This is supported by measurements of large reductions in L_p in $HgCl_2$-treated roots (Table I), relative to untreated controls. We note that these data can be interpreted only in terms of a whole-root-system response. The pressure-flux technique used in this study yields an average L_p for the entire root surface in contact with solution, and spatial heterogeneity

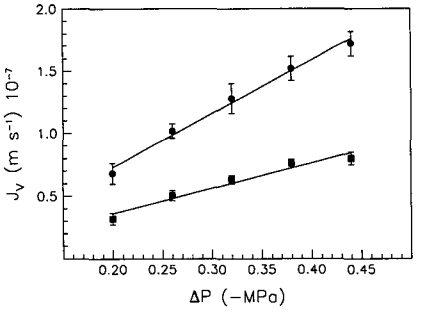

Figure 2. Relation between J_v and applied hydrostatic pressure for root systems treated with 0.5 mM $HgCl_2$ (■) and untreated controls (●), measured simultaneously. Regression lines were estimated from the responses of two root systems per treatment. Points are means ± 1 SD (n = 14 or 16). ΔP, Hydrostatic pressure.

Table I. *Effect of 0.5 mM $HgCl_2$ applied in a root-bathing nutrient solution on the L_p and osmotic relations of tomato root systems*

L_p was evaluated from the linear portion of pressure-flux curves. The osmotic potential of both the Hoagland solution in contact with the roots and the expressed root exudate were measured by vapor pressure osmometry. K^+ flux into the xylem was calculated as the product of the sap flux and concentration of K^+ in the sap. Values are means ± 1 SD (n = 6).

Treatment	L_p	Osmotic Potential		K^+ Flux into the Xylem
		Hoagland solution	Expressed root sap	
	$m\ s^{-1}\ MPa^{-1} \times 10^{-7}$	MPa	MPa	$\mu g\ min^{-1}$
Control	4.64 ± 0.17	−0.139 ± 0.004	−0.134 ± 0.005	54 ± 8
$HgCl_2$	2.01 ± 0.05	−0.142 ± 0.004	−0.141 ± 0.004	56 ± 16

in either local L_p or reflection coefficients within the root system cannot be resolved by this approach. Nevertheless, given these limitations on interpretation, root system L_p was markedly reduced upon exposure to $HgCl_2$. Furthermore, the relation between J_v and applied pressure was highly linear, suggesting that the Hg treatment did not cause broad-scale deleterious changes in root function during the time course of the pressure-flux procedure. The measured values of J_v were similar to those reported by Jackson and Weatherley (1962), Lopushinsky (1964), and Perry and Greenway (1973). Our estimates of L_p for untreated controls were slightly higher than those reported by Salim and Pitman (1984), although growth conditions used in their study differed greatly from those utilized here, especially with respect to PPFD. A very slight underestimate of the effective root radius also could have contributed to higher values of L_p .

Two additional observations support the validity of the pressure-induced flow analysis used in both the flux-kinetics experiments and in the estimation of root L_p. First, analysis of xylem solute potential demonstrates that J_v was not significantly affected by osmotic forces in these experiments. Neither the osmolality of the root exudate nor that of the root-bathing solution differed between $HgCl_2$-treated and untreated roots. Second, there was no significant difference between treatments in the K^+ concentration in xylem exudate delivered through whole-root systems (Table I). The latter observation is important in light of the report by Welch et al. (1993) that the regulation of sulfhydryl groups by a plasma membrane reductase system can affect the transport of osmotically important cations, especially K^+, across the root-cell plasmalemma. $HgCl_2$ is reactive with many sulfhydryl-containing substances, and we do not expect that it would interact exclusively with water channel proteins. Nevertheless, these data demonstrate that the Hg^{2+} concentration and exposure durations used here did not poison root cells in a manner that caused them to become leaky to ions. Thus, the effect of Hg^{2+} on the osmotic relations of the root tissues analyzed here appears to be minimal. We also note that the concentration of $HgCl_2$ used here was lower than that used in recent studies of mercurial effects on water channel function (Pratz et al., 1986; Preston et al., 1992; Daniels et al., 1994).

The large reductions in both J_v (Fig. 1) and L_p (Table I) indicate that water crosses at least one membrane with Hg-sensitive channels during its transit across the root radius. The marked inhibition of symplastic water transport observed here is consistent with results obtained from studies in which the kinetics of H_2O-D_2O exchange across the root radius were evaluated as well from studies in which the pressure probe was utilized to assess cell-specific L_p values. Using proton NMR, for example, Bačić and Ratković (1987) observed that a significant fraction of radial water flux in maize roots occurred through cells, not through intercellular spaces. The authors suggested that 80 to 90% of radial flux could occur via the cell-to-cell pathway, an estimation in substantial agreement with the results shown here for roots treated with $HgCl_2$. Utilizing the pressure probe technique, Steudle and Jeschke (1983) and Steudle and Brinckmann (1989) each concluded that the cell-to-cell pathway rather than the apoplastic route is predominant in young roots of *Hordeum distichon* and *Phaseolus coccineus*, respectively.

Recent evidence for water channels in plants appears to open new avenues for exploring plant water transport, especially as it relates to water uptake and cell expansion. Accurate spatial and temporal localization of these structures in the plant may prove critical in understanding phenomena like the variable radial resistance to water movement, for which there is currently no clear explanation (Passioura, 1988). Despite the current lack of information about regulatory mechanisms or patterns of expression of water channels, the results presented here suggest that such structures might be intimately involved in the regulation of plant water status.

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