Radial hydraulic conductivity along developing onion roots

David E. Barrowclough¹, Carol A. Peterson¹,3 and Ernst Steudle²

¹Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada
²Lehrstuhl für Pflanzenökologie, Universität Bayreuth, D-95440 Bayreuth, Germany

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Abstract

Although most studies have shown that water uptake varies along the length of a developing root, there is no consistent correlation of this pattern with root anatomy. In the present study, water movement into three zones of onion roots was measured by a series of mini-potometers. Uptake was least in the youngest zone (mean hydraulic conductivity, \( L_p = 1.5 \times 10^{-7} \pm 0.34 \times 10^{-7} \) m MPa\(^{-1}\) s\(^{-1}\); ± SE, \( n = 10 \) roots) in which the endodermis had developed only Casparian bands and the exodermis was immature. Uptake was significantly greater in the middle zone (\( L_p = 2.4 \times 10^{-7} \pm 0.43 \times 10^{-7} \) m MPa\(^{-1}\) s\(^{-1}\); ± SE, \( n = 10 \) roots) which had a mature exodermis with both Casparian bands and suberin lamellae, and continued at this level in the oldest zone in which the endodermis had also developed suberin lamellae (\( L_p = 2.8 \times 10^{-7} \pm 0.30 \times 10^{-7} \) m MPa\(^{-1}\) s\(^{-1}\); ± SE, \( n = 10 \) roots). Measurements of the hydraulic conductivities of individual cells (\( L_p \)) in the outer cortex using a cell pressure probe indicated that this parameter was uniform in all three zones tested (\( L_p = 1.3 \times 10^{-6} \pm 0.01 \times 10^{-6} \) m MPa\(^{-1}\) s\(^{-1}\); ± SE, \( n = 60 \) cells). \( L_p \) of the youngest zone was lowered by mercuric chloride treatment, indicating the involvement of mercury-sensitive water channels (aquaporins). Water flow in the older two root zones measured by mini-potometers was also inhibited by mercuric chloride, despite the demonstrated impermeability of their exodermal layers to this substance. Thus, water channels in the epidermis and/or exodermis of the older regions were especially significant for water flow. The results of this and previous studies are discussed in terms of two models. The first, which describes maize root with an immature exodermis, is the ‘uniform resistance model’ where hydraulic resistances are evenly distributed across the root cylinder. The second, which describes the onion root with a mature exodermis, is the ‘non-uniform resistance model’ where resistances can be variable and are concentrated in a certain layer(s) on the radial path.

Key words: Allium cepa, hydraulic conductivity, water channels, aquaporins, mercuric chloride, roots, exodermis.

Introduction

Water transport within plants can be divided into several discrete steps, one of which is radial flow in roots. Unless the roots are very long or the tracheary elements are largely cavitated during water stress, this is the path of greatest resistance to liquid water flow in the plant (Frensch and Hsiao, 1993; Steudle and Peterson, 1998). Thus, the hydraulic resistance of roots to radial water flow should usually control the water status of the shoot under conditions of uniform stomatal opening. Compared to the wealth of information on regulation of the transpiration stream by the cuticle and guard cell movements in the shoot system, little is known about the regulation of water flow in roots. It has been generally assumed that wall modifications of the cells which are in the radial path play important roles.

On its way from the soil solution to the lumens of the xylem vessels, water passes through a series of different tissues, some of which are anatomically complex. For example, in onion (Allium cepa L.), the plant used in the present investigation, the epidermal walls contain diffuse suberin (Peterson et al., 1978), and the outer layer of the cortex develops into an exodermis (Van Fleet, 1950) containing suberin and lignin in its Casparian bands and suberin in its suberin lamellae (Zeier and Schreiber, 1998).

³To whom correspondence should be addressed. Fax: +1 519 746 0614. E-mail: cpeterso@uwaterloo.ca
This exodermis is dimorphic, i.e. possessing long and short cells with delayed suberum lamella development in the latter (Kamula et al., 1994). Continuing inward on the radial path in onion, the walls of the parenchyma in the central cortex are unmodified, the endodermal cells develop Casparian bands, suberum lamellae and a thickened, tertiary wall, and the pericycle and xylem parenchyma walls remain unmodified. Lastly, the walls of the vessels are thick, lignified and pitted. The wall modifications of the endodermis and exodermis develop at various distances from the root tip (Perumalla and Peterson, 1986), which allows their effects on the root’s hydraulic conductivity ($L_p$) to be tested (Melchior and Steudle, 1993).

Many attempts have been made to correlate root $L_p$, with anatomical features (for an overview, see Steudle and Peterson, 1998). Most investigators have focused on the endodermal and exodermal suberin deposits, since this hydrophobic polymer would be expected to reduce the $L_p$ of the root (Zimmermann et al., 2000). In some cases, the results show the expected correlation (Graham et al., 1974; Melchior and Steudle, 1993; Zimmermann and Steudle, 1998; North and Nobel, 1998; Zimmermann et al., 2000) but in others, they did not (Hodges and Vaadia, 1964; Clarkson et al., 1987). Values of $L_p$ are, in fact, highly variable among species, and can also change along the roots depending on the absolute value of the $L_p$ and the nature of the force applied (Brouwer, 1965). Brouwer’s results can now be explained by the ‘composite transport model’ of water flow in roots, where the $L_p$ varies depending on the source of the gradient (osmotic or hydraulic), and also on root structure (for an overview and references, see Steudle and Peterson, 1998).

The effect that various wall modifications in the root will have on water flow depends on the relative importance of the pathways used. Water flow through living, parenchymatic cells may occur through the walls (apoplast), the interconnected cytoplasms (symplast), or across whole cells through membranes (transcellular). A combination of the latter two is known as the cell-to-cell pathway, and its hydraulic conductivity ($L_p$) can be measured in individual cells using a cell pressure probe (Steudle, 1993). Casparian bands are in a position to hinder radial, apoplastic water flow, and suberin lamellae are in a position to inhibit cell-to-cell flow, all the more so if they disrupt plasmodesmata. In some species, a predominantly cell-to-cell pathway is preferred, while in others, the apoplastic pathway is pre-eminent as indicated by detailed measurements of root cell $L_p$ and overall root $L_p$ (e.g. Zhu and Steudle, 1991; Steudle and Peterson, 1998). In the latter type, when root membranes were affected by stresses such as salinity or anoxia, the effects at the cell level ($L_p$) were much more pronounced than those of $L_p$, indicating substantial use of the apoplastic pathway (Birner and Steudle, 1993; Azaizeh et al., 1992; Zhang and Tyerman, 1999).

It is evident that the only alternative way for water flow across roots to be influenced by factors other than apoplastic wall modifications is by changes in the cell-to-cell path. One likely candidate for such a modification would be water channels which increase membrane $L_p$. In cases where they have been examined from a molecular standpoint, water channels proved to be aquaporins (for references, see Maurel, 1997; Schäßner, 1998). These are transmembrane proteins (MW about 30 kDa) which allow passive movement of single files of water molecules through plasmalemma and tonoplast membranes. It has been estimated that as much as 70–90% of the water moving into or out of a cell passes through these pores (Henzler and Steudle, 1995; Schütz and Tyerman, 1997; Tazawa et al., 1997; Zhang and Tyerman, 1999).

Aquaporins occur in a wide variety of plants and plant parts (Maurel, 1997). To date, the only indication of the presence of aquaporins in onion is that a homologue was detected in bulb scale (McCallum JA, Lancaster JE, 1996; Bulb onion STSs, unpublished data, accession number U58207). Several aquaporins may occur in one root (Weig et al., 1997; Higuchi et al., 1998). In maize, the mRNA coding for aquaporin synthesis seems to be most prevalent in the endodermis and xylem parenchyma in mature areas of the root (Barriè et al., 1998).

An indication of the activity of water channels can be obtained by treatment with mercurials. Mercury is thought to bind to sulphhydryl groups at the mouths of the pores, physically blocking the channels and reducing their hydraulic conductivity (Tyerman et al., 1999). Results from experiments with mercurials must be viewed with caution because mercury also blocks the pores of other transmembrane proteins, some of which are involved in ion transport (Maurel, 1997; Schütz and Tyerman, 1997; Tyerman et al., 1999). Thus, mercury treatment may disturb the ionic balance in cells, which will eventually lead to changes in water flow due to osmotic effects. If there is a substantial water flow across ion channels, their closure may also directly contribute to water flow. However, it has been shown for *Xenopus* egg, Chara and cells of wheat roots that this contribution is small (Maurel et al., 1993; Schütz and Tyerman, 1997; Zhang and Tyerman, 1999). A potential problem with mercurials is that these substances may be toxic to the tissue, either by affecting the cytoplasmic activity or the integrity of the plasma membranes. Thus, dosage is critical (Henzler and Steudle, 1995; Zhang and Tyerman, 1999). To avoid non-specific effects, concentrations have to be as low as possible and times of exposure as short as possible. One good indicator of lack of toxicity is the reversal of mercury inhibition by the scavenger 2-mercaptoethanol. Evidence for the presence of water
channels in tomato roots has been obtained by using mercury inhibition and its reversal by 2-mercaptoethanol (Maggio and Joly, 1995; Peyrano et al., 1997), although the concentration of the mercurial used (500 μM) was fairly high which would tend to cause other effects besides the closure of water channels and would eventually kill the roots (Henzler and Steudle, 1995; Zhang and Tyerman, 1999). It was found that the hydraulic conductivity of barley roots could be reduced by treatment with 100 μM HgCl₂ (Tazawa et al., 1997), but this was not reversed by subsequent incubation in 2-mercaptoethanol. The hydraulic conductivity of barley roots was reduced with only 50 μM HgCl₂, an effect which was reversed by dithiothreitol (Carvajal et al., 1996).

The present paper provides the first analysis of water channel activity along the length of a root. Both root Lp, and cell Lp in three anatomically distinct zones of onion adventitious roots were measured. Root Lp, was obtained with mini-potometers, and Lp of individual cortical cells with a cell pressure probe. The involvement of water channels in both root zones and individual cells was tested by application of mercuric chloride (HgCl₂). Onion roots provide a useful model system for a study of this type since their anatomy has been well characterized; young, growing roots lack laterals which can disrupt the continuity of the endodermis and exodermis, and the usual absence of root hairs allows the roots to be removed from solid growing media without injury to the epidermis.

Materials and methods

Plant material

Adventitious roots of onion (Allium cepa L. cv. Ebeneezer) from the same crop were used for cell pressure probe experiments in Bayreuth, Germany, and for mini-potometer experiments in Waterloo, Canada. The outer scales were removed from the bulbs prior to planting in 300 mm deep pots containing vermiculite saturated with tap water. Pots were watered to saturation with tap water every second day. In Bayreuth, the growth chamber had 16 h light (intensity 1260 μmol photons m⁻² s⁻¹) at 20 °C, and 8 h darkness at 18 °C. In Bayreuth, the growth chamber had 16 h light (intensity 1260 μmol photons m⁻² s⁻¹) at 21 °C, and 8 h darkness at 19 °C.

Roots were 15–17 mm long after 9–10 d of culture when they were taken for experiments. They were gently excavated from the vermiculite to avoid injury. Roots touching the bottoms of the pots were not used.

Anatomical characterization of roots

Functional xylem was detected by the method of Peterson and Steudle (Peterson and Steudle, 1993). Roots were excised under already collected was not used. bubbles from an aquarium pump and absorbent paper to wick water up the sides served to increase the humidity. After 40 min the mini-potometers were filled with degassed, distilled water. Then an onion was gently removed from the vermiculite, any remaining growth medium rinsed off with tap water, and its root system placed in the chamber as shown in Fig. 1. The root to be tested was pretreated with (i) deionized water for 20 min to serve as a control, or (ii) 50 μM HgCl₂ for 20 min, or (iii) 5 mM 2-mercaptoethanol for 10 min, or (iv) 50 μM HgCl₂ for 20 min followed by 5 mM 2-mercaptoethanol for 10 min. After rinsing briefly with deionized water, the root was threaded through all three mini-potometers. The three tubes were positioned at the desired locations along the root and the openings sealed with a mixture (modified from Cruz et al., 1992) of 90% anhydrous lanolin and 10% paraffin wax (melting point 51 °C). After 30 min, by which time even low flow rates were achieved, measurements of water uptake were made at intervals of 5 or 10 min for a period of 6 h. Occasionally, one of the seals would fail during the experiment and bubbles would enter the tube around the root.

Immediately after the rate measurements, the root was cut from the bulb and carefully slid out of the mini-potometers. The root water potential was measured by a PMS Model 600 pressure bomb. (Note that measurement of water potential by a pressure bomb gives the average water potential of the root. This varies from the root surface to the xylem, being lowest in the latter. Thus, values obtained are overestimates of the xylem water potential, but probably not by a factor of more than two.
Roots were excised from the bulb and their cut ends sealed with sticky wax (Kerr Brand, Emeryville, CA, USA). Each sealed root was immersed in a solution of 50 μM HgCl$_2$ for 20 min and then hung by a thread in a separatory funnel so that the root was suspended in air. H$_2$S gas was generated by dripping 5 M HCl onto FeS granules and was vented into the funnel for 10 min. Black deposits of mercuric sulphide were evident upon microscopic examination with white light.

Viability tests
The viability of epidermal cells was tested in whole mounts of roots with disodium fluorescein as described previously (Barrowclough and Peterson, 1994), and with Evan’s Blue (Fisher et al., 1985). In total, 20 roots were examined at 10 mm intervals from tip.

The toxicity of 50 μM mercuric chloride applied for 20 min to onion roots was investigated by staining intact roots with Evan’s blue (Fisher et al., 1985) and by staining freehand, paradermal sections with disodium fluorescein (Fisher et al., 1985). Two indicators of vitality were automatically sensed during the experiments. In pressure probe trials, maintenance or loss of turgor pressure after treatment with 25, 50 and 100 μM HgCl$_2$ was evident. In mini-potometer experiments, only 50 μM HgCl$_2$ was used, but any unexplained, drastic increases or decreases in hydraulic conductivity would signal non-specific toxic effects. Reversal of the mercury effect by 2-mercaptoethanol was established in both cell pressure probe and mini-potometer experiments.

Statistics
All data were analysed using Systat version 7 for Windows 95. Data examined in all tests were tested for normality by means of a normal-quantile plot. The homogeneity-of-variance assumption was tested in each case by plotting of the residuals against the estimates. Cell pressure probe data were analysed by ANOVA followed by a Tukey’s test (Sokal and Rohlf, 1995). Mini-potometer data were measured by repeated measures ANOVA followed by a protected LSD (Steel and Torrie, 1980). In all cases $\alpha=0.05$.

Photography
Photographs of the sections were taken using 35 mm Kodak Colour Slide Ektachrome Elite 200 ISO film. Developed slides were scanned into a computer using Polaroid Sprintscan 35. Plates were created using Adobe Photoshop 3.0.5.

Results

Structural features of the roots
Protoxylem, early metaxylem, and late metaxylem could be distinguished from one another by vessel diameter, which was evident even in squashed preparations. The only vessels which were functional (i.e. conducting dye and consequently stained by it) in any part of the 15 mm long roots were those of the early metaxylem. Of the 20 roots examined, the early metaxylem had matured within 25 mm of the root apex (Fig. 2); the average distance of maturation was 19 mm.

Casparian bands and suberin lamellae were present in both the endodermis and exodermis at varying distances.
Fig. 2. Diagram of an onion root (not to scale) showing positions of mature early metaxylem (central, scalariform structure), Casparian bands (dashed lines), Casparian bands plus suberin lamellae (solid lines) in the endodermis (edge of stele) and exodermis (near root surface). Segment A, immature endodermis and exodermis; segment B, endodermis with Casparian bands, immature exodermis; segment C, developing exodermis with Casparian bands and sometimes also suberin lamellae; segment D, endodermis with Casparian bands, exodermis with Casparian bands and suberin lamellae; segment E, endodermis and exodermis with both Casparian bands and suberin lamellae. The locations of zones 1, 2 and 3, which were used for hydraulic conductivity measurements, are indicated on the right.

from the tip so that five developmental stages could be identified (segments A–E in Fig. 2). Based on this information, three zones, all with mature early metaxylem, were selected for hydraulic conductivity measurements. Zone 1, the most apical (30–40 mm from the root tip), had Casparian bands in the endodermis. Zone 2, the next developmental stage (55–65 mm from the root apex) had, in addition, Casparian bands and suberin lamellae in the exodermis. Zone 3 (100–110 mm from the tip) had, in addition, suberin lamella in most of endodermal cells (Fig. 2).

Most of the epidermal cells remained alive over the length of the root. The majority of the cells were stained with fluorescein and had excluded Evan’s blue (data not shown).

Hydraulic conductivity (Lp) of zones in intact roots

Water movement from the mini-potometer tubes into the root began slowly but reached a steady state within 30 min. The rate then usually remained constant for the duration of the experiment. Occasionally, water flow in one of the tubes halted for a time, and then resumed its original rate. This may have been an artefact of the meniscus temporarily sticking in the tube. At no time did the flow occur. Lp, with Tukey’s test, α = 0.05, n = 10 per zone per treatment). (A) Hydraulic conductivities of zones of intact roots measured with a mini-potometer. The first letter above each column indicates statistical differences between treatments within each zone (ANOVA with protected LSD, α = 0.05, n = 10 roots per treatment). (B) Hydraulic conductivities of individual cortical cells measured with a cell pressure probe. The letter above each column indicates statistical differences between treatments within each zone (ANOVA with Tukey’s test, α = 0.05, n = 20 per zone per treatment).

Fig. 3. Mean hydraulic conductivities of three anatomical zones of onion roots (± SE). See Fig. 2 for an explanation of the zones. Prior to measurement, roots were pretreated with one of the following: distilled water (●), 25 μM HgCl2 (□), 50 μM HgCl2 (■), 50 μM HgCl2 followed by 5 mM 2-mercaptoethanol (○), 5 mM mercaptoethanol (■), 5 mM 2-mercaptoethanol (□).
of suberin lamellae in the endodermis between zones 2 and 3 did not have a significant effect on the measured \( Lp \) (Fig. 3A).

Treatment of the roots with 50 \( \mu \)M HgCl\(_2\) prior to the experiment significantly reduced \( Lp \) in the two oldest regions, but not in the youngest (Fig. 3A; Table 1). All mercury treatment effects were reversed by 2-mercaptoethanol which, by itself, did not significantly change the \( Lp \) of any root zone (Fig. 3A).

Hydraulic conductivity (\( Lp \)) of individual cortical cells

The \( Lp \) values of individual cells in the outer cortex of the three zones, measured from water flows generated by a cell pressure probe, did not differ significantly from each other (Fig. 3B). However, treatment with 50 \( \mu \)M mercuric chloride significantly inhibited the \( Lp \) of cells only in zone 1 (Figs 3B, 4). As in the case of \( Lp \) measurements, the effect was reversed by 2-mercaptoethanol which, by itself, was benign (Figs 3B, 4). The lower concentration of HgCl\(_2\) (25 \( \mu \)M) tested was ineffective (Figs 3B, 4), while the higher (100 \( \mu \)M) was toxic, causing the cells to lose turgor rapidly during the experiment (Fig. 4).

Permeability of roots to mercuric chloride

To test whether or not HgCl\(_2\) penetrated all three zones of the root uniformly, \( Hg^{2+} \) was precipitated as mercuric sulphide (HgS) with \( H_2S \) gas after treatment. In zone 1, black precipitates of HgS were evident in the cells of the outer cortex (Fig. 5A), but in zones 2 and 3, such precipitates were seen only in the epidermis (Fig. 5B, C, respectively). Black deposits were absent from control roots not treated with either HgCl\(_2\) or \( H_2S \) gas (Fig. 5D), as well as from roots treated with only HgCl\(_2\) or \( H_2S \) (Barrowclough, 1998). Evidently, mercuric chloride did not pass through the mature exodermis in amounts large enough to cause visible precipitates with \( H_2S \). The sensitivity of the technique was not tested, but should have been rather high due to the extremely low solubility of mercuric sulphide.

By assuming that all cells of the root have the same \( Lp \), it is possible to calculate the resistances to water flow that would hypothetically occur if all water were flowing through the cell-to-cell path in its passage from the soil solution to the lumina of the xylem (Table 1). These values for all three root zones were at least 4-fold higher than the resistances calculated from \( Lp \) values obtained experimentally with mini-potometers (Table 1). The discrepancy between the sum of the resistances of the cells \( (\Sigma R_c) \) and the root resistance \( (R_r) \) increased with root age, so that in the oldest zone tested, the \( \Sigma R_c \) was 12-fold higher than \( Lp \) (Table 1). The effect of mercuric chloride was greater on the \( \Sigma R_c \) then on the \( R_r \) in the youngest zone of the root (Table 1). This comparison could not be made for the older two zones because the mercuric chloride did not reach the cells of the central cortex.

Discussion

Onion roots proved an ideal test system to investigate the effects of root anatomy on radial hydraulic conductivity. The roots were easy to handle, and had consistent anatomical changes in the endodermis and exodermis along their lengths. In addition to measurements of water flow through three individual zones along the root length, parallel experiments were conducted to measure the hydraulic conductivity of individual cells in the outer cortex of each zone. A novel aspect of this study was the inclusion of a HgCl\(_2\) pretreatment in both types of experiments to assess the importance of water channels in controlling water flow.

The values obtained for onion root hydraulic conductivity \( (Lp) \) in the three tested zones were comparable to those obtained earlier (Melchior and Steudle, 1993). In the present study, the average \( Lp \), of zone 2 (55–65 mm from the root tip) was 2.4 \( \times \) \( 10^{-7} \) MPa s m\(^{-1}\) and

Table 1. Comparison of cell and root resistances in onion (MPa s m\(^{-1}\) \( \times \) \( 10^{-6} \))

For an explanation of the root zones, see Fig 2. Root resistances \( (R_r) \) were reduced from the original values by 20\% to remove the resistance of the vessel walls which are in series with the other resistances and should not be included for comparative purposes. The actual number of living cells from the epidermis to the pericycle, inclusive, usually varied between 12 and 14. An intermediate value of 13 was chosen to calculate the sum of the resistances of the cells \( (\Sigma R_c) \). It was assumed that the hydraulic conductivity of every cell was equal to the average of cortical cells measured in all three zones of untreated roots. Treatment with 50 \( \mu \)M mercuric chloride is indicated by (Hg).

<table>
<thead>
<tr>
<th>Root zone</th>
<th>( R_c \pm SE )</th>
<th>( R_r ) (Hg)</th>
<th>( R_r ) (Hg)</th>
<th>( R_r ) (Hg)</th>
<th>( \Sigma R_c ) (Hg)</th>
<th>( \Sigma R_c ) (Hg)</th>
<th>( R_r ) (Hg)</th>
<th>( R_r ) (Hg)</th>
<th>( R_r ) (Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 ± 2.8</td>
<td>23 ± 2.4</td>
<td>0.8 ± 0.1e</td>
<td>2.3 ± 0.2c</td>
<td>115 ± 6.7</td>
<td>40 ± 5.5</td>
<td>4.0</td>
<td>5.0</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>4.8 ± 1.2e</td>
<td>25 ± 2.1e</td>
<td>0.8 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>115 ± 6.7</td>
<td>40 ± 5.5</td>
<td>4.0</td>
<td>5.0</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>3.2 ± 0.3f</td>
<td>23 ± 3.5f</td>
<td>0.8 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>115 ± 6.7</td>
<td>40 ± 5.5</td>
<td>4.0</td>
<td>5.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

\*Pooled data.
\#Sum of resistances for 13 cells adjusted for root radius.
\#Significant difference between \( R_c \) and \( R_r \) (ANOVA), \( \alpha = 0.05 \).
\#Significant difference between \( R_c \) and \( R_r \) (repeated measures ANOVA, \( \alpha = 0.05 \), with a protected LSD).
\#Significant difference between \( R_c \) and \( R_r \) (repeated measures ANOVA, \( \alpha = 0.05 \), with a protected LSD).
Fig. 4. Examples of relaxation curves from pressure probe measurements of zone 1 cortical cell membranes of onion. First curves generated by forcing liquid into the cells; second curves generated by withdrawing liquid from the cells. Half-times of the first curves are indicated on each plot. (A) Untreated cell. (B) Root pretreated with 25 μM HgCl₂. (C) Root pretreated with 50 μM HgCl₂. (D) Root pretreated with 50 μM HgCl₂ then 5 mM 2-mercaptoethanol. (E) Root treated with only 5 mM 2-mercaptoethanol. (F) a toxic response after treatment with 100 μM HgCl₂.

that of zone 3 (100–110 mm from the root tip) was $2.8 \times 10^{-7} \text{ m MPa}^{-1} \text{s}^{-1}$ (Fig. 3A). Average values of $L_p$, for onion roots obtained earlier (Melchior and Steudle, 1993; their Fig. 4D) varied between $0.7 \times 10^{-7}$ and $2.8 \times 10^{-7} \text{ m MPa}^{-1} \text{s}^{-1}$ between 45 and 130 mm from the root tip.

The hydraulic conductivities of onion roots changed along their lengths, but did not correlate with increased suberin deposits in the exodermis and endodermis. First, root $L_p$ was actually lowest in the youngest zone which had the least suberin in these layers. The substantial increase in $L_p$ from zone 1 (30–40 mm from the root tip) to zone 2 (55–65 mm from the root tip) could have been due to a reduced axial conductivity in the young zone, as seen previously (Melchior and Steudle, 1993). On the other hand, results of the present study indicated that all metaxylem vessels were functioning 25 mm from the root tip, i.e. distal to zone 1. In general, the axial component of the overall resistance is usually negligible as soon as functional xylem is present (Peterson and Steudle, 1993). Further, balance pressures measured by pressure bomb at zones 1, 2, and 3 were not significantly different from each other (Barrowclough, 1998). Second, there was no significant difference in $L_p$ between the two older zones which differed by the presence of suberin lamellae in the endodermis. The observed change in $L_p$ between zones 1 and 2 cannot be ascribed to differences in cell $L_p$ in the outer cortex, since this was constant in the three tested zones. The vitality of epidermal cells was also rather constant. The greater conductivity of the older root was actually lowest in the youngest zone which had the least suberin in these layers. The substantial zones to water is reminiscent of the results of Hodges and Vaadia, who studied water movement (due to osmotic increase in $L_p$ from zone 1 (30–40 mm from the root tip) to zone 2 (55–65 mm from the root tip) could have forces) in onion roots (Hodges and Vaadia, 1964). Could water channels in the membranes be lowering the resistance of the cell-to-cell path in older zones?

A decline in root or cell hydraulic conductivity following treatment with HgCl₂ was taken to indicate involvement of water channels. However, as mentioned in the Introduction, such treatments could lead to artifacts due to osmotic and/or toxic effects. In both types of experi-
Fig. 5. Cross-sections of onion roots. (A, B, C) Intact roots were treated with HgCl$_2$ followed by H$_2$S gas prior to sectioning. (A) Zone 1; (B) zone 2; (C) zone 3 (see Fig. 2 for explanations of zones). (D) Untreated control. Bars = 50 μm.

Willaerts, et al., the effect of 50 μM HgCl$_2$ was completely reversed by 2-mercaptoethanol, indicating that a toxic reaction had not occurred. Tests with vital dyes also indicated that a 20 min treatment with 50 μM HgCl$_2$ was not damaging to membranes. This concentration is 10-fold lower than that used in previous studies of tomato root hydraulic conductivity (Maggio and Joly, 1995; Peyrano et al., 1997). It has been claimed that 100 μM HgCl$_2$ is non-toxic (Willmer et al., 1999), but this does not agree with the results of this study. Their observed decline in protoplast volume could have been due to toxicity as measured in the present study. Cell pressure probe experiments were performed quickly, before any appreciable osmotic effect could have occurred. The mini-potometer studies lasted longer, but here the main driving force was the transpiration stream which would override osmotic effects (Steudle and Peterson, 1998). Therefore, the reported data can be taken to indicate a minimum involvement of water channels. Their absolute effect cannot be measured with mercury inhibition, because some channels are known to be insensitive (Daniels et al., 1994). Here, it is being assumed that the proportion of mercury-sensitive to mercury-insensitive channels does not change either along the radial path or along the length of the onion root.

To have any effect at all, it is necessary for the mercurial to reach its target. In the youngest zone (1) used in this study, HgCl$_2$ moved into the central cortex where it measurably reduced the $Lp$ of individual cells. In the other two zones tested (2 and 3), however, a mature exodermis with Casparian bands was present which apparently prevented HgCl$_2$ from diffusing freely into the central cortex. This conclusion was reached from two separate experiments. First, no mercury was precipitated with H$_2$S gas in the cortex of zones 2 and 3 and, second, the $Lp$ of individual cortical cells was not affected by HgCl$_2$ treatment. A reduction of the HgCl$_2$ concentration by as little as 50% was sufficient to reduce its effect to an insignificant level. Therefore, in zones where the exodermis was mature, the majority of living cells in the radial path would have been unaffected by the HgCl$_2$ treatment.

Judging from changes in $Lp$, and $Lp$ brought about by an HgCl$_2$ pretreatment, water channels were present in all tested zones of the roots. Cell $Lp$ was significantly reduced in zone 1, as was root $Lp$, in zones 2 and 3. The effect on $Lp$, in zones 2 and 3 was much greater than in zone 1 (which had no significant inhibition) in spite of the fact that in the older zones, the HgCl$_2$ could have influenced only the membranes of the epidermis and the outer membranes of the exodermal short cells while in the youngest zone, it acted on the central cortical cells. This result indicates that either the cells of the epidermis and/or the short cells of the exodermis have a greater water channel activity than those of the younger zone, or that movement of water in the youngest zone is predominantly apoplastic, while in the older zone, it must pass at least one layer by transcellular flow. Two lines of evidence indicate that substantial water flow in zone 1 occurs in the apoplast. First, as seen in Table 1, the summed resistances of cortical cells (measured from $Lp$ of individual cells and representing the cell-to-cell path)
is 4-fold higher than the resistance of the root (calculated from $L_p$, and representing both the apoplastic and cell-to-cell paths together). Second, HgCl$_2$ treatments, which would reduce water flow in the cell-to-cell path, significantly increased the resistance of individual cells but not of intact roots. The discrepancy between the sum of the cellular resistances and the root resistance is even greater in zones 2 and 3, but this can be explained by forced passage through the membranes in one or more layers when the apoplastic pathway is blocked (i.e., the exodermis and/or the endodermis). The extent of inhibition by HgCl$_2$ in zones 2 and 3 indicates that functioning water channels in the epidermis/exodermis alone, increases the conductivity of the root by up to 720% (excluding the effect of vessel walls, which would remain constant; calculated from Table 1). Thus, the majority of the resistance to water flow in onion roots would probably occur at these layers in the absence of water channels.

Due to the presence of a mature exodermis, it was not possible to gauge the involvement of water channels in the inner cortical cells of the older zones in onion roots. The involvement of water channels on $L_p$, throughout the entire cortex could be tested on roots without an exodermis. Further work with other exodermal species such as maize is also needed because the limited information available indicates that a tonoplast aquaporin is not strongly expressed in the exodermis of this species (Barrieu et al., 1998). As has been pointed out (Yamada et al., 1997; Higuchi et al., 1998), the tissue-specific location of aquaporins often correlates with regions of high water flow, e.g., vascular parenchyma (Barrieu et al., 1998). Aquaporins are also plentiful in root tips and zones of elongation (Yamada et al., 1997; Higuchi et al., 1998; Barrieu et al., 1998), areas which have a low permeability to ions and dyes (Lüttge and Weigl, 1962; Enstone and Peterson, 1992). Similarly, aquaporins are prevalent in the endodermis (Barrieu et al., 1998; Yamada et al., 1995), in which the apoplastic permeability is reduced by the Casparian band. A system similar to the endodermis may also exist in the exodermis of onion. Here, Casparian bands occur in all cells, and long cells have suberin lamellae which cut off plasmodesmata connecting them with all surrounding cells (Ma and Peterson, 1999). Thus, both the transcellular and symplastic components of the cell-to-cell pathway are interrupted in long cells. Radial water flow should occur predominantly through the membranes of the short cells, which occupy only about 20% of the tissue surface (Kamula et al., 1994).

Two models concerning the placement of resistances on the radial path are emerging from this and previous studies. One could be termed the ‘uniform resistance model’, in which the resistance to radial water flow is distributed fairly evenly along the radial path. Roots of hydroponically grown maize in which the exodermis has not matured and the endodermis has not developed suberin lamellae fit this description. Root $L_p$ is constant over the length of the root, cell $L_p$ is not highly variable across the cortex (Frensch et al., 1996), and removal of part of the cortex by dissection brings about only a proportionate increase in $L_p$ (Peterson et al., 1993). The second conception could be termed the ‘non-uniform resistance model’ which applies to zones of onion roots with a mature exodermis. Water channels in some cell layer(s), probably the short cells of the exodermis, are especially important for water flow into the root. When flow through the channels is inhibited or the channels are removed from the membrane, water movement into the root is reduced by a combination of the lipid bilayer, the Casparian bands and the suberin lamellae in the long cells of the exodermis. The latter model would allow a root to react to environmental stresses during which the exodermis covers a large proportion of the root (Perumalla and Peterson, 1986) or is induced (Reinhardt and Rost, 1995), and flow through water channels is inhibited (Yamada et al., 1995; Carvajal et al., 1996; Johansson et al., 1998). Whether or not the non-uniform model can be extended to other exodermal species awaits further experimentation. Other cell layers, such as the endodermis and stelar parenchyma, could play a major role in regulating radial water flow in roots of onion and other species. The non-uniform model could be confirmed by mapping $L_p$ of individual cells in the radial path (from epidermis to pericycle) as the root develops.

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