RESEARCH PAPER

Permeability of *Iris germanica*’s multiseriate exodermis to water, NaCl, and ethanol

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Abstract

The exodermis of *Iris germanica* roots is multiseriate. Its outermost layer matures first with typical Casparian bands and suberin lamellae. But as subsequent layers mature, the Casparian band extends into the tangential and anticlinal walls of their cells. Compared with roots in which the endodermis represents the major transport barrier, the multiseriate exodermis (MEX) was expected to reduce markedly radial water and solute transport. To test this idea, precocious maturation of the exodermis was induced with a humid air gap inside a hydroponic chamber. Hydraulic conductivity (*L*<sub>ppc</sub>) was measured on completely submerged roots (with an immature exodermis) and on air-gap-exposed root regions (with two mature exodermal layers) using a pressure chamber. Compared with regions of roots with no mature exodermal layers, the mature MEX reduced *L*<sub>ppc</sub> from 8.5×10<sup>−8</sup> to 3.9×10<sup>−8</sup> m s<sup>−1</sup> MPa<sup>−1</sup>. Puncturing the MEX increased *L*<sub>ppc</sub> to 19×10<sup>−8</sup> m s<sup>−1</sup> MPa<sup>−1</sup>, indicating that this layer constituted a substantial hydraulic resistance within the root (75% of the total). Alternatively, a root pressure probe was used to produce pressure transients from which hydraulic conductivity was determined, but this device measured mainly flow through the endodermis in these wide-diameter roots. The permeability of roots to NaCl and ethanol was also reduced in the presence of two mature MEX layers. The data are discussed in terms of the validity of current root models and in terms of a potential role for *I. germanica* MEX during conditions of drought and salt stress.

Key words: Casparian bands, hydraulic conductivity, *Iris germanica*, multiseriate exodermis, NaCl permeability, pressure chamber, radial transport pathways, root pressure probe, suberin lamellae.

Introduction

Roots of *Iris germanica* have a number of unusual anatomical features that make them instructive for permeability and modelling studies (Kroemer, 1903; Peterson and Perumalla, 1990; Zeier and Schreiber, 1998; Meyer et al., 2009). (i) The root diameter is wide (up to 2.5 mm), as is the central cortex. (ii) The endodermis is composed of palisade-like cells that have Casparian bands and suberin lamellae as close as 30 mm from the root tip in optimal growing conditions. (iii) The multiseriate exodermis (MEX), composed of up to four centripetally maturing layers, has Casparian bands in the tangential walls of adjoining layers in addition to the anticlinal walls of the cells (Fig. 1). Meyer *et al.* (2009) termed this structure a continuous circumferential Casparian band. (iv) Immature areas (windows) in the exodermis through which lateral roots emerge are absent. Such windows are known to occur in roots of other species such as *Phragmites australis* and *Oryza sativa* (Soukup *et al.*, 2002; Armstrong and Armstrong, 2005). (v) Passage cells are absent from mature exodermal layers; i.e. all cells have suberin lamellae (Fig. 1). These substantial wall modifications of the MEX can be expected to increase the resistance to the radial movement of water and solutes through the transcellular and apoplastic pathways.

Roots have been referred to as composite structures since they have several types of cell layers that all contribute to the radial transport properties of these organs (Steudle and Peterson, 1998). In spite of this, roots have been largely treated as osmometers with a membrane-equivalent barrier
comparable to the membrane found in a cell; the 'root membrane' was assumed to be the endodermis (Steudle and Jeschke, 1983; Dainty, 1985; Steudle and Brinckmann, 1989). This may be a reasonable approach for young, thin, non-exodermal roots with a diameter of a millimetre or less, or for the fine roots of most trees that contribute most of the surface area responsible for the uptake of water and nutrients (Steudle et al., 1987; Rüdinger et al., 1994; Steudle and Meshcheryakov, 1996). In thin roots, the storage of water in the central cortex may be negligible, allowing root hydraulics to be measured from transients in the flow of small volumes of water. Such transients are produced with a root pressure probe or by a high-pressure flow meter (HPFM) (Tyree et al., 1994, 1995; Knipfer and Steudle, 2008; Joshi et al., 2009). The existence of an exodermis may require an extension of root models because its hydraulic resistance (in series with that of the endodermis) would add to the overall resistance of the root. When roots are thin, this may reduce the water flow accordingly as derived from transient water flow across young roots of Zea mays (Zimmermann and Steudle, 1998). The situation may be different in thicker roots in which there is substantial water storage in the central cortex. A significant hydraulic capacity of the tissue between the endodermis and exodermis (i.e. a large central cortex) would tend to complicate the interpretation of transients in water flow as produced by the root pressure probe and HPFM techniques (Joshi et al., 2009). It may well be impossible to use these techniques to measure root hydraulic conductivity from pressure relaxations or initial water flow. Instead, instruments that apply steady-state flows such as the pressure chamber may be more suitable in this case.

Ion movement into the root is profoundly affected by the endodermis and exodermis. Passage of ions through the apoplast is essentially prevented by Casprian bands (de Ruz de Lavison, 1910; Baker, 1971; Peterson, 1987; Enstone et al., 2003; Ranathunge et al., 2005). In addition, ions are virtually blocked from contacting the plasma membranes by suberin lamellae (Evert et al., 1985). Thus, for ion uptake into the symplast to occur in regions of *I. germanica* roots with a mature exodermis, some epidermal cells must be alive. Subsequent inward symplastic transport of ions across the exodermis requires at least some of its cells to be alive and connected by plasmodesmata (Fig. 1). This anatomical feature is known to occur in *O. sativa* and *Z. mays* (Clark and Harris, 1981; Wang et al., 1995). Ions unable to traverse cell membranes should be efficiently blocked by the cell-wall-modifying structures of the multiseriate exodermis (Fig. 1).

In the present study, the suitabilities of the root pressure probe and the pressure chamber were assessed for measuring the hydraulic conductivity of *I. germanica* roots. To ascertain the effect of the exodermis on overall root hydraulic conductivity, a comparison was made between roots with an immature exodermis and root segments in which two exodermal layers had matured. Similarly, the effect of the exodermis on NaCl (both ions are virtually membrane impermeant) and ethanol (a membrane-permeant molecule) permeability were made. The extent to which a symplastic pathway across the exodermis might be present was determined by testing epidermal cell viability.

### Materials and methods

#### Plant material and growth conditions

Soil-grown *I. germanica* L. plants were carefully removed from outdoor plots at the University of Bayreuth, Germany, in early May 2006. Rhizomes and their subtending adventitious roots were rinsed clean of adhering soil and then transferred to a 10-l hydroponic tank that was completely filled with nutrient solution [macronutrients (mM): 0.09 (NH₄)₂SO₄, 0.07 MgSO₄, 0.06 Ca(NO₃)₂, 0.05 KH₂PO₄, 0.05 KNO₃, 0.05 Fe(III)-EDTA, 0.03 K₂SO₄; micronutrients (μM): 4.6 H₃BO₃, 1.8 MnSO₄, 0.3 ZnSO₄, 0.3 CuSO₄; pH 5.5–6.0]. The tank was placed inside a growth cabinet [25/23 °C (day/night); 16 h photoperiod, 300 μmol m⁻² s⁻¹ PAR], and the solution was continuously aerated and exchanged with fresh solution weekly (Fig. 2A). During culturing, new adventitious roots emerged from each rhizome and grew into the nutrient solution.

It was known from the previous work of Meyer et al. (2009) that the maturation of *I. germanica*’s second exodermal layer was delayed in fully submerged roots (up to 170 mm in length), but accelerated in regions exposed to an air gap (110–170 mm from tip). In the current work, once several new adventitious roots (>60 mm in length) had formed, some of the hydroponic tanks were only partially filled so that a 60-mm air gap was present.
between the solution surface and the base of the rhizome (Fig. 2F). The relative humidity in the air gap measured with a digital hygrometer/thermometer was 92% (Control Company, Friendswood, TX, USA). To prevent the rhizomes from dehydrating in this area, they were wrapped in paper towel saturated with nutrient solution. To ensure that at least two exodermal layers had matured, roots were exposed to the air-gap condition for at least 14 d.

Anatomical analyses and preparation for physiological tests
Casparian bands and suberin lamellae in the exodermis and endodermis were detected by staining freehand cross-sections of the roots with berberine hemisulphate followed by aniline blue for Casparian bands, or with Sudan red 7B for lipids (Brundrett et al., 1988, 1991). Cell vitality was assessed by placing intact roots in uranin (disodium fluorescein) or Evan’s blue (Stadelmann and Kinzel, 1972; Taylor and West, 1980; Barrowclough and Peterson, 1994). For the former, specimens were viewed with ultraviolet light (UV filter set: excitation filter BP 365, dichroitic mirror FT 395, barrier filter LP 397) and for the latter, white light using an epifluorescence microscope (Carl Zeiss, Oberkochen, Germany). Photographs were taken with a digital camera (Cool Snap; Visitron Systems, Puchheim, Germany).

For physiological tests, adventitious, primary roots that had grown either fully submerged (control; Fig. 2A) or with their basal regions in an air gap (Fig. 2F) were excised from the rhizome under water with a razor blade. Submerged roots were then mounted directly into either a root pressure chamber or onto a pressure probe. To test the permeability of just the part of the root that had been exposed to the air gap, this portion was cut away, and its distal end sealed using a combination of polyacrylamide glue (UHU, Bühl, Germany) and beeswax:colophony (1:3, w/w; melting point 40–50 °C, which has been shown not to affect the viability of cells; Ranathunge et al., 2003). To apply the seal, the cut end of the root was first dipped in a pool of glue, the glue was allowed to dry, and then this process was repeated, followed by dipping the same end into molten beeswax:colophony once. During the sealing process, the remainder of the root segment was kept hydrated by gently wrapping it with watersaturated tissue paper. Once the wax mixture had cooled and hardened, the segment was mounted into either the pressure chamber or the pressure probe. In the latter case, the effectiveness of the glue–wax seal was confirmed when a positive internal root pressure was attained.

Root pressure probe experiments
Measurements using the root pressure probe were conducted as described previously (Steudle et al., 1987; Steudle and Frenc, 1989). The base of each excised root was tightly mounted into the probe with a custom-made silicone seal. To minimize effects due to unstirred layers along the root surface, roots were bathed in a...
turbulent nutrient solution (the same solution used during growth in the hydroponic tank). For roots of *I. germanica*, stable root pressures were rather low (*P*ₚₒ = 0.05–0.08 MPa) and 7–10 h were required before this pressure developed (see Supplementary Figs S1, S2 at *JXB* online). Once the pressure was stable, four to six hydrostatic pressure relaxations were conducted by increasing or decreasing the pressure with the rod of the probe (∆*P*ₚ = 0.04–0.08 MPa; see Supplementary Fig. S3 at *JXB* online.). The transient change in volume induced with the pressure probe ranged from 6.41 × 10⁻¹² to 1.28 × 10⁻¹¹ m³, which was four to five orders of magnitude smaller than the average cylindrical volume of submerged (2.83 × 10⁻³ m³) and air-gap-exposed (2.29 × 10⁻³ m³) roots. Responses in pressure by the root were recorded; in accordance with previous studies, the fast phase was used, which occupied ~75% of the total change in pressure (Steudle and Frehse, 1989; Knipfer et al., 2009; Yoshikawa et al., 2009). The root constants (*k*ᵣₒ), or the half-times (*T*ᵣᵢ/2) of the pressure relaxations, were determined and used to calculate the root’s hydraulic water conductivity (*L*ᵣ in m s⁻¹) and *A*ᵣ (in m²), which referred to the surface area of the root, *A*ᵣ, calculated from the length and diameter of the root, assuming a cylindrical shape:

\[
A_r = \frac{\pi d^2}{4},
\]

\[
L_r = \frac{k_{hr}}{T_{r/2}},
\]

Here, (∆*P*ᵣ/∆*V*ᵣ) is the elastic modulus of the system (in MPa m⁻³). The elastic modulus was measured by inducing a rapid change in volume of the measuring system (Δ*V*ᵣ) with the rod of the probe and measuring the corresponding change in root pressure (Δ*P*ᵣ). The validity of Eq (1) assumes a constant cylindrical geometry for each tested root.

Osmotic hydraulic conductivity (*L*ᵣₒ) and solute permeability (*P*ᵣₒ in m s⁻¹) were measured using the root pressure probe, but the force was applied by changing the osmolarity of the external medium with nutrient solution (∼3 mMol/kg, equivalent to 3 mM of osmotic concentration) amended with either ∼40 mMol/kg NaCl or ∼200 mMol/kg ethanol (EtOH). For each experiment, the osmolality of each solution was measured cryoscopically with a cryometer (Osmotak 030; Gonotec, Berlin, Germany). These solutes and concentrations have been used in the past, and are not toxic (Steudle and Frehse, 1989; Ranathunge et al., 2003). External application of the test solutions created an osmotic pressure gradient between the outside and inside of the root. The reduced external water potential resulted in a net flow of water out of the root as measured by a decrease in root pressure. The half-life of this recorded pressure change was used to calculate *L*ᵣₒ in m s⁻¹ as in Eq (1). In some cases, a net flow of the solute into the root occurred in response to a concentration gradient, causing transients in root pressure. From the second phase of these transients, the permeability coefficient (*P*ᵣ) of the solute (NaCl or ethanol) was worked out assuming a membrane-equivalent barrier in the root (Steudle and Jeschke, 1983; Dainty, 1985). Analogous to Eq (1), the root constant of solute permeability (*k*ᵣ) was related to *P*ᵣ by:

\[
k_r = \frac{\ln(2)}{T_{r/2}} = A_r \cdot \frac{P_r}{V_r},
\]

where *V*ᵣ is the volume of the vessel lumens (0.13–0.4% of total root volume, which was estimated from cross-sections). The biphasic reaction described above was reversible, i.e., when the external solute solution was changed back to the original, there was a net flow of water into the root and of solute out of it (exosmotic). Again, *L*ᵣₒ and *P*ᵣ were calculated from the recorded changes in pressure. During osmotic pressure relaxation, the shrinking or swelling of root cells was relatively small due to the relatively high elastic modulus of the cells (Steudle et al., 1987). Hence, the root surface area (*A*ᵣ) remained virtually constant. The same was true for the volume of the rigid xylem vessels (*V*ᵣ). The reflection coefficient (σᵣᵢₒ) of each solute was calculated from the pressure/time curve using the following equation (Steudle et al., 1987):

\[
J_r = \frac{Q_r}{A_r},
\]

where *P*ᵣₒ and *P*ᵣ are the original and minimum root pressures of pressure/time curves, respectively, (∆*P*ᵣ = RT × ACᵣ is the change in external osmotic pressure caused by the osmoticum (NaCl or ethanol), and *t*ᵣ the time required to reach *P*ᵣᵢₒ following a step change in the external concentration at *t* = 0.

For experiments involving roots from the air-gap growth condition, the complete hydrostatic and osmotic methods described above were conducted twice per root. The first series of measurements was done when the root was intact. The second series was taken after the multiseriate endodermis had been punctured so that its role in permeability could be estimated (Steudle et al., 1993). Each root was punctured eight times using a glass microcapillary that had a uniform diameter of 100 μm along its length and a slightly tapered end <100 μm long. Since air-gap-exposed root segments were relatively thick (average diameter of 2.5 mm), puncturing was easily accomplished with the endodermis remaining unscathed in >80% of cases. Puncturing caused the root pressure to drop slightly and stabilize (see Supplementary Fig. S4) (Supplementary data are available at *JXB* online). Wounds were observed following the experiments by staining with Evan’s blue to detect dead cells (Taylor and West, 1980), and making cross-sections to view the puncture depth. Additional cross-sections were stained with berberine–hemisulphate and aniline blue to detect the presence of the continuous circumferential Casparian band (Brundrett et al., 1988). These sections were viewed and photographed with the microscope and camera described above.

For the submerged and air-gap growth conditions, five or six roots were tested. Submerged roots used for permeability testing had an average length of 100 mm, whereas air-gap-exposed root segments used for permeability testing were, on average, 220–260 mm from the root tip. The endodermis of the latter segments was punctured using the method described above. In cases where the endodermis was accidentally wounded, root pressure dropped to zero, and no further permeability tests could be conducted. The effect of puncturing the biseriate exodermis on hydraulic and osmotic water flow was established by taking the ratio between the *L*ᵣ of intact compared with punctured, air-gap-exposed root segments for each case.

**Pressure chamber experiments**

A small, custom-made pressure chamber was used as an alternative instrument for measuring the hydraulic conductivity of *I. germanica*’s adventitious roots. This chamber had a volume of 6 × 10⁻³ m³ or 60 ml, and was filled with nutrient solution (as above). It was equipped with a screw cap that had a small hole in the centre (3.2 mm diameter) where a single root could be mounted using a silicone seal. At the same time, the excised end of the root was mounted by means of a silicone seal into a small tube into which a narrow, graduated capillary had been sealed. Initially, water was injected into the small tube until the liquid entered the capillary. Roots from both the submerged and air-gap treatments were mounted in this way. Pneumatic pressure was applied to the chamber and water flow (in m³ s⁻¹) was monitored. Pressure was increased gradually in steps of 0.05 MPa up to a maximum of 0.30 MPa. Measurements were taken at 0.00, 0.10, 0.20, and 0.30 MPa. After reaching each of these pressures, the system equilibrated for 60 min during which readings were taken every 10 or 15 min. At each pressure, rates of water flow were plotted against time, and the slopes from the linear parts of the line (*Q*ᵣ in m³ s⁻¹) were used to calculate the rate of water flow (*J*ᵣ in m² s⁻¹):

\[
J_r = \frac{Q_r}{A_r},
\]
slope and hydraulic conductivity. Once the solutes in the xylem were sufficiently diluted and osmotic gradients negligibly small, \( J/J_0 \) became linear (Fiscus, 1975; Zimmermann and Steudle, 1998).

Hydraulic conductivity (\( L_{\text{pc}} \) in m s\(^{-1}\) MPa\(^{-1}\)) was determined from the slope of the linear part of the \( J/J_0 \) curve. In the pressure chamber experiments, effects of unstirred layers due to a concentration polarization of nutrient ions at the exodermis or endodermis could be neglected because of the low concentration of these solutes in the nutrient medium.

Experiments involving puncturing of the biseriate exodermis of air-gap-exposed roots were also conducted. Two series of increasing pneumatic pressures were conducted per root—first before puncturing the biseriate exodermis and then after puncturing. Following the first set of measurements, the pressure in the chamber was released through a valve. Then the chamber was separated from the screw cap and graduated capillary, which held the mounted root, by fixing the cap and capillary in a stationary position and unscrewing the chamber from the cap. The exodermis of the exposed root was then punctured using the technique described above. Then the chamber was refilled with nutrient solution and screwed back on to the cap so that water flow measurements could be repeated. Five or six roots were used for the submerged and air-gap growth conditions. From the punctured air-gap roots, a complete data set was obtained for repetitions that did not have a damaged endodermis. The effect of puncturing the biseriate exodermis on hydraulic water flow was established by taking the ratio between the \( L_p \) of intact compared with punctured, air-gap-exposed root segments for each case.

**Resistance of the exodermis to water and solute flows**

Hydraulic resistances \( (R_a) \) were calculated from \( L_{\text{pc}}, L_{\text{pco}}, L_{\text{pc}} \), and the respective surface area \( (A) \). Likewise, resistances to solute flow \( (R_s) \) were obtained from \( P_{\text{st}} \):

\[
R_w = \frac{1}{L_p \times A} \quad \text{and} \quad R_s = \frac{1}{P_{\text{st}} \times A} \quad (5)
\]

\( L_p \) values were typically calculated with the root surface area. However, in some cases the \( L_p \) was calculated with the endodermal surface area [Eqs (1) and (4); see Results]. Resistance values were used to determine the fold change in the resistance of the root to hydraulic or solute flow between completely submerged roots with an immature exodermis and air-gap-exposed roots with a biseriate exodermis. Similarly, the fold change in resistance was determined between the intact biseriate exodermis and its punctured counterpart.

**Statistical analyses**

To test whether or not root water and solute permeabilities were significantly different with the maturation of the biseriate exodermis, two-tailed, unpaired \( t \)-tests (\( \alpha=0.05 \)) were employed. Similar tests were conducted to test whether the permeabilities were significantly different between roots with an immature exodermis and root segments with a punctured biseriate exodermis. On the other hand, one-tailed, paired \( t \)-tests (\( \alpha=0.05 \)) were used to determine whether or not the water and solute permeabilities of the intact root segment with a biseriate exodermis increased significantly after it was punctured. The \( t \)-tests described above were also used to determine differences in hydraulic and solute resistances.

**Results**

**Root anatomy**

Development of the exodermis in submerged and air-gap-exposed roots was followed by staining for Casparian bands and suberin lamellae. Differences were evident when comparing roots of similar length that had been grown in these conditions. In completely submerged roots with lengths of \( \leq 200 \) mm, the first exodermal layer usually matured 80 mm from the root tip. This layer had typical Casparian bands occupying its anticlinal walls (Figs 2A, E, 3A). Submerged roots 100 mm in length were used for both pressure chamber and pressure probe experiments. In the case of the pressure chamber, the proximal (basal) 30 mm of the root were sealed into the instrument so that the exposed part on which measurements were made had a uniformly immature exodermis (0EX). In the case of the pressure probe, \( \sim 15 \) mm were sealed into the instrument so that 94% of the exposed root had an immature exodermis.

In contrast, the basal part of roots exposed to a humid air gap (average relative humidity 92%) for 14 d had a uniformly developed biseriate exodermis with its characteristic continuous circumferential Casparian band (2EX) (Figs 2C, F, 3B). (It was this region that was used to test radial water and solute permeability.) On these same roots, the part that remained submerged (i.e. below the air gap) exhibited gradual exodermal maturation, similar to the completely submerged roots (see Fig. 2E, F). Exodermal cells that had Caspian bands also had suberin lamellae as the two structures were deposited concurrently (Fig. 3C).

Epidermal cell viability was examined in submerged and air-gap-exposed roots using uranin. In the submerged epidermis, uranin accumulated in all cells; hence, they were all alive (Fig. 2D). On the other hand, in the air-gap-exposed epidermis, uranin accumulated in \( \sim 50\% \) of the cells indicating that half remained alive (Fig. 2B).

Endodermal development (specifically deposition of suberin lamellae and tertiary walls) was not noticeably affected by the growth conditions (Fig. 3A, B). By 100 mm from the tip, the majority of its cells had reached full maturity, and some passage cells remained irrespective of growth conditions. Caspian bands in the endodermis were initially small and offset towards the pericycle. Later, in many endodermal cells the Caspian bands extended through the anticlinal walls, suberin lamellae were deposited, and U-shaped tertiary wall thickenings were formed (Fig. 3D). However, in the few passage cells of the endodermis where suberin lamellae were not deposited, Caspian bands were not elongated.

To visualize the location and depth of the wounds after puncturing, air-gap-exposed roots that had their two exodermal layers punctured (2EX-P) were stained with Evan’s blue (Fig. 3E). The 40-mm-long root segments had a surface area that ranged from \( 2.1 \times 10^{-4} \) to \( 3.2 \times 10^{-4} \) m\(^2\). The surface area of the punctured tissue was \( 6.3 \times 10^{-6} \) m\(^2\), equalling \( 2-3 \times 10^{-2}\% \) of the total root surface area. From the cross-sections of punctured tissue, it could be observed that the wounds penetrated only about half way through the central cortex, leaving the endodermis intact (Fig. 3F).

There were no apparent differences in the size of intercellular air spaces of the central cortex between submerged and air-gap-exposed roots.
Measurements of hydraulic conductivity

Water flow, as measured with the pressure chamber ($L_{p_{pc}}$), was established for three cases of interest: (i) roots with no mature exodermal layers (0EX), (ii) root segments with two mature exodermal layers (2EX), and (iii) roots with two mature exodermal layers that had been punctured (2EX-P). In all cases, at each step in chamber pressure, the cumulative volume of solution that was transported across the root increased linearly with time (Fig. 4). At 0 MPa, water was exuded over time by a 0EX root due to root pressure (Fig. 4A). However, in the 2EX and 2EX-P roots, there was no water exudation indicating fairly low if any root pressure (Fig. 4B, C). As a consequence, water flow (Fig. 5) was $>0$ at 0 MPa for 0EX roots, whereas it was 0 for 2EX and 2EX-P roots. To demonstrate steady-state water flow, the slopes from the cumulative water flow graphs were plotted against the changes in pressure (Fig. 5). Generally, as pressure increased, water flow through the roots also increased. A typical trend was observed for the 0EX roots: the flow rate was initially slow but accelerated at greater pressures, resulting in a curvilinear response previously explained by Fiscus (1975) as a dilution effect. This effect refers to a decrease in the concentration of solutes in the xylem sap as the inflow of water increases, hence the osmotic contribution to the driving force becomes negligible (Sands et al., 1982; Zimmermann and Steudle, 1998). However, for 2EX the rate of water flow increased only linearly with increasing pressure. Such a linear increase may be due to the fact that both xylem sap concentration and water inflow were already low, tending to nullify the effect.

Fig. 3. Photomicrographs from *I. germanica* roots grown in submerged or air-gap hydroponic conditions showing cross-sections of the roots unless otherwise stated. Values in mm refer to distances from the root tips. (A) 100 mm. Stained with berberine–aniline blue. This specimen typifies the basal region of submerged roots, exhibiting a typical Casparian band in the endodermis (arrow) and in the outermost cells of the exodermis (arrow). (B) 100 mm. Stained with berberine–aniline blue. This specimen typifies the air-gap-exposed root region, exhibiting a continuous circumferential Casparian band in the multiseriate exodermis (yellow arrows) and a typical Casparian band in the endodermis (white arrow). (C) 120 mm. Stained with Sudan red 7B. The outer part of a root with two mature exodermal layers that contain suberin lamellae (arrowheads). (D) 80 mm. Stained with Sudan red 7B. Endodermis with suberin lamellae (black arrowheads) in all cells except the passage cells (*). Suberin was not deposited in the U-shaped wall thickenings (white arrowhead). (E) 255–247 mm. Treated with Evan’s blue. Whole mount of an air-gap-exposed root with a punctured exodermis (arrowheads). Scale bar 1 mm. (F) 220 mm. Cross-section treated with Evan’s blue. This specimen demonstrates the depth of a puncture within the root (arrowheads). The wound penetrated the epidermis, exodermis, and half of the central cortex; the endodermis was unscathed. Scale bars (except for (E)) 100 μm. Abbreviations: ep, epidermis; ex, exodermis; iex, immature exodermis; en, endodermis; pe, pericycle; xy, xylem pole. *Passage cells.
of dilution of osmotic pressure in the xylem. Even more unusual was the water flow for 2EX-P, which was initially very rapid, but then began to plateau at 0.3 MPa. Such a trend suggested that water flow was reaching a maximum in the plateauing region, similar to the plateau in water flow measured for Picea mariana roots (see Colombo and Asselstine, 1989). The slopes of the linear parts of each curve yielded the hydraulic conductivity (\(L_{ppc}\)). There was a considerable variability in the \(L_{ppc}\) of different roots, which may depend on differences in individual growth of those roots. These differences were much larger than those caused by non-linearities in the increases in flow rates of individual roots in response to pressure changes. Therefore, to arrive at mean values of \(L_{ppc}\) for the different treatments, values of \(J_v\) of all roots were not pooled. Rather, \(L_{ppc}\) values of different roots were averaged (see Table 1).

When \(L_{ppc}\) values of roots with a biseriate exodermis were compared with those of roots with an immature exodermis, \(L_{ppc}\) was shown to be significantly reduced. The average \(L_{ppc}\) of 0EX roots was \(8.5\times10^{-8}\) m s\(^{-1}\) MPa\(^{-1}\), whereas the \(L_{ppc}\) of 2EX was \(3.9\times10^{-8}\) m s\(^{-1}\) MPa\(^{-1}\) (Table 1). Thus, the hydraulic resistance of roots with a biseriate exodermis was 3.1-fold greater than that of 0EX roots (Tables 2, 3). This decrease in \(L_{ppc}\) (or increase in resistance) could have been a consequence of the maturation of the exodermis and/or differences in endodermal permeability of I. germanica’s multiseriate exodermis | 1917

![Fig. 4. Cumulative water flow through roots during pressure chamber experiments. Each graph represents typical results. (A) 0EX roots: submerged with an immature exodermis. (B) 2EX roots: air-gap exposed with two mature exodermal layers. (C) 2EX-P roots: air-gap exposed with two mature exodermal layers that were punctured. Legends inset in graphs.](image)

![Fig. 5. Steady-state water flow per unit root surface area with an increasing driving force (i.e. induced hydrostatic pressure changes). This graph displays typical results. Dashed lines indicate the slope used to calculate \(L_{ppc}\) for 0EX and 2EX-P. Legend inset in graph.](image)

**Table 1.** Root hydraulic conductivity values calculated from pressure chamber and pressure probe experiments

Conductivity data are means±standard deviations, and the numbers of replicates are in parentheses.

| Instrument | \(L_p\) (10\(^{-8}\) m s\(^{-1}\) MPa\(^{-1}\)) |
|------------|-----------------|-----------------|-----------------|
|            | 0EX             | 2EX             | 2EX-P           |
| Pressure chamber | 8.5±0.9\(^a\) (5) | 3.9±2.1\(^a\) (5) | 19±16\(^b\) (5)\(^a\)93±91\(^b\) (5) |
| Pressure probe\(^a\) | 39±13\(^a\) (6) | 23±9\(^a\) (6) | 41±24\(^a\) (6) |
| Pressure probe\(^b\) | 240±100\(^b\) (6) | 130±56\(^b\) (6) | 220±140\(^b\) (6) |

\(^a\) or \(^b\) indicates a significant difference within each row \((P<0.05)\). 0EX roots with no mature exodermal layers; 2EX, root segments with two mature exodermal layers; 2EX-P, root segments with two mature exodermal layers that were punctured.

\(^a\) \(L_p\) values calculated using root surface area.

\(^b\) \(L_p\) values calculated using endodermal surface area.

\(L_{ppc}\) 2EX and \(L_{ppc}\) 2EX-P are statistically similar (result not shown in table).
Table 2. Root hydraulic (above) and solute (below) resistances

Values of hydraulic resistance were calculated from \( L_{\text{ppc}} \) values obtained from pressure chamber and pressure probe experiments [Table 1; resistance = \( 1/L_{\text{ppc}} \) / \( \text{surface area} \)]. Values of solute resistance were calculated from \( P_{sr} \) values obtained from pressure probe experiments [Table 5; resistance = \( 1/P_{sr} \) / \( \text{surface area} \)]. Data are means \( \pm \) standard deviations.

| Type of measurement | 0EX | 2EX | 2EX-P |
|---------------------|-----|-----|-------|
| Hydraulic resistance \( (10^{10} \text{ MPa s m}^{-3}) \) |       |     |       |
| PC                  | 2.2 \( \pm \) 0.27* | 6.9 \( \pm \) 3.5** | 1.7 \( \pm \) 0.76* |
| RPP                 | 0.61 \( \pm \) 0.30* | 1.7 \( \pm \) 0.9** | 1.2 \( \pm \) 0.57** |
| RPP (NaCl)          | 29 \( \pm \) 5*     | 220 \( \pm \) 98**  | 19 \( \pm \) 10*    |
| RPP (EtOH)          | 47 \( \pm \) 12*    | 310 \( \pm \) 110** | 118 \( \pm \) 99*   |
| Solute resistance \( (10^{10} \text{ s m}^{-3}) \) |       |     |       |
| RPP, \( P_{sr} \) (NaCl) | 1050 \( \pm \) 230* | n.m.** | 60 \( \pm \) 51*** |
| RPP, \( P_{sr} \) (EtOH) | 140 \( \pm \) 38*   | 1070 \( \pm \) 430** | 160 \( \pm \) 140*  |

* *, **, or *** indicate a significant difference within each row (\( P < 0.05 \)).

Table 3. Fold change in resistance of the root to water and solute flows after maturation of the biseriate exodermis, or after puncturing the exodermis

These fold change values were calculated from the resistance values in Table 2.

| Type of measurement | Fold change in resistance |
|--------------------|--------------------------|
| 0EX vs 2EX         | 2EX vs 2EX-P             |
| PC                 |                          |
| Hydraulic resistance | +3.1                      | -4.1 |
| RPP                | +2.8                      | -1.4 |
| RPP (NaCl)         | +7.4                      | -11  |
| RPP (EtOH)         | +6.7                      | -2.6 |
| RPP, \( P_{sr} \) (NaCl) | n.m.**                   | n.m. |
| RPP, \( P_{sr} \) (EtOH) | +7.6                      | -6.8 |

When the root pressure probe was used, it was necessary to measure its elastic modulus to calculate hydraulic conductivity \( (L_{p}) \). Values of elastic moduli (mean \( \pm \) standard deviation) were 3.3 \( \times \) \( 10^{-9} \) \( \pm \) 0.90 \( \times \) \( 10^{-9} \) and 5.4 \( \times \) \( 10^{-9} \) \( \pm \) 1.8 \( \times \) \( 10^{-9} \) MPa \( \text{m}^{-3} \) for 0EX and 2EX or 2EX-P, respectively. Maturation of the biseriate exodermis reduced \( L_{p} \) significantly (Table 1); this was equivalent to a 2.8-fold increase in resistance (Tables 2, 3), which may have also been due to differences in the maturation state of the endodermis. Puncturing the biseriate exodermis resulted in a ratio (2EX:2EX-P) ranging from only 1:1 to 1:2.5 (average 1:1.7), indicating a rather small increase in \( L_{p} \), or a 1.4-fold loss of resistance (Tables 3, 4). Interestingly, the absolute values of \( L_{p} \) were significantly greater than those of \( L_{ppc} \). This indicated that, because of the large storage capacity of the root’s central cortex, the pressure probe measured the hydraulic resistance of mainly the endodermis rather than that of the entire root. For a proper comparison in this situation, \( L_{p} \) was recalculated on the basis of the surface area of the endodermis of the outer part of the root. Consequently, the \( L_{p} \) values increased tremendously (Table 1), but there was essentially no change in the ratio of 2EX:2EX-P (Table 4) and no change in the fold change in resistance (Tables 2, 3).

The statistical comparison between the values of \( L_{p} \) for 2EX and of \( L_{ppc} \) for 2EX-P was based on the rationale that only the endodermal hydraulic conductivity was measured with the pressure probe and that the same was true when punctured root segments were tested with the pressure chamber. The compared \( L_{p} \) values were statistically similar (Table 1), further proving that only the endodermis was tested in these specific cases.

The third method of measuring hydraulic conductivity was using osmotic gradients as the driving force (\( L_{ppc} \)). In this case, values of \( L_{ppc} \) were lower by one to two orders of magnitude than those of \( L_{p} \) and \( L_{ppc} \) (compare Tables 1 and 5). This indicated that, in the presence of osmotic gradients, water flow was from cell-to-cell rather than apoplastic, which was in line with earlier results of root hydraulics (see Discussion). As expected, with the maturation of 2EX, the average \( L_{ppc} \) for both NaCl- and EtOH-treated roots decreased, compared with 0EX (Table 5). Accordingly, when 2EX was punctured, the NaCl \( L_{ppc} \) method.) These ratios indicated a 4.7-fold increase in \( L_{ppc} \) and a similar loss of resistance, when the exodermis was punctured (Tables 3, 4). Assuming that puncturing the exodermis caused its resistance to be negligible, the endodermis would then become the dominant resistance. [Support for this assumption comes from comparing the \( L_{ppc} \) at 0EX with the \( L_{ppc} \) 2EX-P, which were statistically the same (Table 1).] For this case, \( L_{ppc} \) 2EX-P data were recalculated using the endodermal surface area instead of the surface area for the outer part of the root. The recalculations increased the \( L_{ppc} \) of 2EX-P (Table 1) by 23-fold (on average) compared with that of 2EX (Table 4). Nonetheless, the loss of exodermal resistance was still 4.1-fold (the same as measured before \( L_{ppc} \) 2EX-P calculation) because when calculating resistance, \( L_{p} \) values are multiplied by the corresponding surface area [see Eq (5); Tables 2, 3].

The importance of the exodermis to water flow was ascer-
increased by an average of 11-fold (or a 11-fold loss of resistance), while the EtOH \( Lp_{\text{pro}} \) increased by an average of 4.1-fold (or a 2.6-fold loss of resistance) (Tables 2, 3, 6). When the \( Lp_{\text{pro}} \) 2EX-P data was recalculated with the endodermal surface area, the values increased as expected (Table 6). Now, in punctured root segments, NaCl-\( Lp_{\text{pro}} \) was an average of 58-fold greater and EtOH-\( Lp_{\text{pro}} \) an average of 20-fold greater than their intact counterparts.

**Measurements of root permeability to solutes**

The root pressure probe was employed to measure the permeability (\( P_{sr} \)) and reflection coefficient (\( \sigma_{sr} \)) for NaCl and ethanol (Table 5) using the membrane-equivalent root model (see Introduction). NaCl permeated extremely slowly through 0EX roots (\( 8 \times 10^{-11} \text{ m s}^{-1} \)) and for 2EX roots, NaCl permeation was reduced to an undetectable level (Fig. 6A). In contrast, for 2EX-P roots, NaCl permeated rapidly and variably (1.3±0.6\( \times 10^{-8} \text{ m s}^{-1} \); Fig. 6B). These same trends were observed for the \( \sigma_{sr} \) values of NaCl (Table 5).

In contrast to NaCl, EtOH permeated across roots of all treatments. For 0EX roots, EtOH \( P_{sr} \) was \( 0.14 \times 10^{-8} \text{ m s}^{-1} \). This was reduced by a factor of five to \( 0.027 \times 10^{-8} \text{ m s}^{-1} \) in 2EX roots (Table 5), bringing about a 7.6-fold increase in resistance (Tables 2, 3; Fig. 6C). Puncturing the exodermis increased EtOH \( P_{sr} \) from \( 0.027 \times 10^{-8} \) to \( 0.64 \times 10^{-8} \text{ m s}^{-1} \), lowering the resistance by 6.8-fold (Tables 2, 3, 5). The trends observed for the \( \sigma_{sr} \) values of EtOH were similar to those observed for \( P_{sr} \) (Table 5).

**Discussion**

**Root anatomy and cell viability**

Adventitious roots of *I. germanica* were chosen for testing radial water and solute permeabilities because the multiseriate exodermis, with its continuous circumferential Casparian band and suberin lamellae, would form a complete structure restrictive to both apoplastic and transcellular flows (see Introduction; Fig. 1). As more exodermal cell layers became encrusted with Casparian bands and suberin lamellae, the apoplastic and transcellular paths would become more restricted. However, the symplastic path would still have been open if plasmodesmata linked the
Table 6. The effect of puncturing the biseriate exodermis on the osmotic hydraulic conductivity of the roots

Values not within parentheses were calculated using root surface area. Values within parentheses were calculated using endodermal surface area. Paired experiments where * or ** within each row indicates a significant difference between 2EX and 2EX-P (P<0.05).

| Solute | Rep | \( Lp_o (10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}) \) | Ratio comparison |
|--------|-----|--------------------------------|-----------------|
|        | 2EX | 2EX-P | 2EX:2EX-P | 2EX:2EX-P |
| NaCl   | 1   | 0.11  | 1.2 (6.5) | 1:11 (1:57) |
|        | 2   | 0.15  | 1.9 (8.6) | 1:13 (1:57) |
|        | 3   | 0.19  | 2.4 (12)  | 1:12 (1:64) |
|        | 4   | 0.27  | 2.9 (15)  | 1:11 (1:57) |
|        | 5   | 0.30  | 3.1 (16)  | 1:11 (1:55) |
|        | Avg | 0.21±0.08* | 2.3±0.8** (12±4.3)** | 1:11 (1:58) |
| EtOH   | 1   | 0.069 | 0.11 (0.58) | 1:1.5 (1:8.4) |
|        | 2   | 0.080 | 0.18 (0.93) | 1:2.2 (1:12) |
|        | 3   | 0.11  | 0.38 (1.9)  | 1:3.6 (1:18) |
|        | 4   | 0.14  | 0.83 (3.7)  | 1:6.0 (1:27) |
|        | 5   | 0.20  | 1.4 (7.1)   | 1:7.2 (1:36) |
|        | Avg | 0.12±0.05* | 0.58±0.54** (2.8±2.7)** | 1:4.1 (1:20) |

2EX \( Lp_o \)=conductivity before puncturing; 2EX-P \( Lp_o \)=conductivity after puncturing the same root segment.

epidermal, exodermal, and central cortical cells. There were two indicators that these plasmodesmata did, in fact, remain intact. First, in submerged roots virtually all the epidermal cells were alive, and even after a 14-d exposure to an air gap, about half remained alive. [The root epidermis was surprisingly resistant to stress since those of *O. sativa* and *Z. mays* quickly died under similar conditions (Barrowclough and Peterson, 1994; Enstone and Peterson, 1998).] This means that the plasmodesmata were connected to living exodermal cells. Interestingly, even under stress, ions could still be transported across the plasma membrane of a living epidermal cell, entering the symplast to traverse the exodermis, and continue flowing through the plasmodesmata of other living cortical cells. Second, exodermal cells also remained alive as they could eventually develop to State III, in which they had deposited lignified tertiary cellulosic walls (Meyer et al., 2009). Thus, in *I. germanica* roots the symplast would be available as a path for radial transport across the multiseriate exodermis (Fig. 1).

Hydroponically grown roots of *I. germanica* had anatomies similar to those previously described in detail by Meyer et al. (2009). Roots completely submerged in hydroponic solution had delayed exodermal maturation so that 94% of the tested root length lacked mature exodermal layers while the remaining 6% had one mature exodermal layer. Root regions exposed to a humid air gap had two mature exodermal layers while the remaining 6% had one mature exodermal layer. According to the anatomical data of Meyer et al. (2009) and those of the present paper, endodermal maturation was not visibly affected by these growth conditions. (Specifically, suberin lamellae and tertiary wall deposition in the endodermis was not affected by the differing growth conditions. Endodermal Casparian bands and suberin lamellae had already been deposited prior to air-gap exposure.) But, in the current work, 0EX root segments had the full range of endodermal maturation states, while 2EX roots had a uniformly mature endodermis. Presumably, the younger regions of 0EX roots with an endodermis in the process of maturing would be more permeable to water than older regions where most of the endodermal cells contain suberin lamellae. Hence, it was only in experiments with 2EX roots where the exodermis was punctured (without affecting the endodermis) that the properties of this structure to reduce both water and solute permeability were demonstrated unequivocally.

**Measurements of radial water permeability**

The radial water permeability of *I. germanica* roots was measured using various approaches: pressure probe to measure hydraulic conductivity and osmotic hydraulic conductivity, and a pressure chamber to measure hydraulic conductivity. Water permeability values varied among each of these approaches (see below).

The fact that the osmotic hydraulic conductivity (\( Lp_o \)), as measured with the pressure probe, was 14- to 33-fold lower than that measured with the pressure chamber in the presence of a hydraulic gradient, was not unusual. Osmotic permeabilities for water have typically been reported as being lower than hydraulic values due to the nature of the forces driving the flows (Steudle and Fresch, 1989; Cruz et al., 1992; Steudle et al., 1993; Rüdinger et al., 1994; Steudle and Meshcheryakov, 1996; Steudle and Peterson, 1998). Osmotic water flow has to occur from cell layer to cell layer across all root tissue layers, including the exodermis, in order for a net equilibrium in water potential to be attained. When *I. germanica* roots were bathed in ethanol or NaCl, water traversed both 0EX and 2EX roots slowly. Water flow across the exodermis may have occurred primarily through the symplastic pathway, bypassing the continuous circumferential Casparian band and suberin lamellae (see Fig. 1). When this layer was punctured, its selective properties were diminished and \( Lp_o \) increased dramatically as one would expect when the pore formed after removal of the capillary remains partially open (see Discussion in Steudle et al.,
In this case, the endodermis was the primary resistance to $L_{pr}$. In transpiring plants, a hydrostatic gradient across the apoplast is the dominant driving force for radial water flow, but water can still flow through the parallel symplast and through aquaporins along the transcellular path (Maurel, 1997; Steudle and Peterson, 1998; Tyerman et al., 1999, 2002; Steudle, 2001; Javot and Maurel, 2002). When transpiration rates are reduced or even stopped, osmotic gradients across the symplastic and transcellular pathways become more important for radial water flow (Steudle and Peterson, 1998). However, when EtOH or NaCl were applied externally to $I. \text{germanica}$ roots, a hyposmotic shock from the sharp increase in external solute concentration may have caused some of the aquaporins to close (Ye et al., 2004; Boursiac et al., 2005). Such a closure of aquaporins may have contributed to the lower osmotic water conductivity measurements compared with the hydraulic conductivity measurements.

Pressure probe experiments yielded hydraulic conductivity values that were markedly greater than data obtained with the pressure chamber. Measurements of $L_p$ depended on transient flows of volumes of water that were small compared with the root’s storage capacity for water (Knipfer and Steudle, 2008; Joshi et al., 2009). When water was injected into the root xylem as a pulse in pressure probe experiments, it should not pass through the entire root, but should be stored in $I. \text{germanica}$’s large central cortex with 12–18 cell layers. The storage capacity should also be increased by the existence of air-filled intercellular spaces in the central cortex. Hence, in cases like the roots of $I. \text{germanica}$, it is concluded that the pressure probe largely measured the endodermal $L_{pr}$. This conclusion should also hold for the situation when water flows across thick roots are largely transcellular (due to a high activity of aquaporins). In this case, steady-state techniques (i.e. the pressure chamber) should be used to evaluate the overall root $L_p$, rather than techniques by which initial water flows are measured (i.e. the HPFM or root pressure probe). On the other hand, the combined use of initial and steady-state techniques may provide a way to quantify the contribution of different series resistances within the root cylinder. This was accomplished here for $I. \text{germanica}$, where the single equivalent membrane model of the root breaks down and the exodermis rather than the endodermis dominates resistance to water uptake. To date, the composite transport model of the root usually used for arrays of transport arranged in parallel (cell-to-cell compared with apoplastic) would have to be extended by a series element (exodermis).

When the pressure chamber was employed to measure the hydraulic conductivity of $I. \text{germanica}$ roots, large volumes of water were induced to flow through the roots. Under these conditions, water storage areas in the stele and cortex should have been filled and a steady state accomplished. Problems with unstirred layers may have been relatively small because water flow was directed from outside the root to the xylem, tending to dilute the xylem sap (Zimmermann and Steudle, 1998; Knipfer et al., 2007). Hence, the pressure chamber technique measured the $L_{ppc}$ across both the endodermis and exodermis.

Which device is then best for measuring the hydraulic conductivity of $I. \text{germanica}$ roots? From the present results, there are several indications that the pressure chamber
measured flow across the whole root (including the exodermis), while the pressure probe measured flow across primarily the endodermis. (i) The volume flow of water is large and steady with the pressure chamber, but low and transient with the pressure probe. (ii) Puncturing *I. germanica*’s exodermis caused a statistically significant reduction in hydraulic resistance (4.1-fold loss) as measured with the pressure probe, whereas with the pressure probe, puncturing resulted in a statistically insignificant reduction in resistance (1.4-fold loss). See Tables 2 and 3. (iii) Pressure chamber *L*\_pc values for punctured 2EX segments were statistically equivalent to pressure probe *L*\_p values for intact 2EX root segments. (iv) When pressure chamber *L*\_pc values were recalculated using endodermal surface area and then compared with pressure probe *L*\_p values calculated on the basis of whole-root surface area, the values were the same statistically between 0EX, between 2EX and between 2EX-P (see Table 7 and footnote). Clearly, the use of the pressure chamber is necessary when measurement of total root hydraulic conductivity in thick roots such as those of *I. germanica* is required. The pressure probe is suited for measuring hydraulic water flow across thinner roots. It should be stated that an HPFM could not be used as an alternative to the pressure chamber since the HPFM also measures transient changes rather than steady flow (see Discussion in Joshi et al., 2009). The use of the HPFM should be affected by internal unstirred layers, which are negligible when using the pressure chamber or the pressure probe (Knipfer et al., 2007).

According to the key hydraulic conductivity values measured with the pressure chamber (*L*\_pc), the maturation of two exodermal layers significantly reduced the permeability of roots to water. Deposition of a continuous circumferential Casparian band and suberin lamellae resulted in a substantial increase in the overall hydraulic resistance, corresponding to a reduction in *L*\_pc (see Tables 1 and 3). Although the multiseriate exodermis reduced radial water flow, it was not completely blocked. Some water would have moved through the symplast and possibly also through the continuous circumferential Casparian band or suberin lamellae if pores existed in these structures (Ranathunge et al., 2005; Waduwara et al., 2008). Puncturing the biseriate exodermis, but not the endodermis, separated the direct contribution of both the exodermis and endodermis to hydraulic resistance. According to the results of these experiments, the exodermis was the most hydraulically resistant structure in the root, accounting for an average of 75% of the root’s resistance to water flow. In other words, the hydraulic resistance of the remaining undamaged tissues contributed 25% to the overall resistance. Assuming that the majority of this 25% refers to the endodermis, with an average diameter of 0.5 mm (whereas the diameter of the exodermis was on average 2.5 mm), then the endodermal *L*\_pc was ~23-fold greater than that of the exodermis (see Table 4). These results highlight the importance of *I. germanica*’s exodermis as a very hydraulically resistant structure. In future, this work could be extended by similarly puncturing 0EX roots.

It is understood that water flow through plant roots is, to a large extent, regulated by aquaporins. Unfortunately, for technical reasons it was not possible to assess the activity of the aquaporins in the large central cortex of *I. germanica* roots due to their size and the mechanical strength of the exodermis. The only case in which hydraulic conductivity has been measured at both the cell and root level was in young *Z. mays* roots (Zimmermann et al., 2000). In roots grown in mist culture, the exodermis had matured whereas in hydroponics it remained immature. The *L*\_p of mist-cultured roots was smaller than that of hydroponically cultured roots by a factor of four. However, the *L*\_p of the cortical cells was not affected by growth condition. It was concluded that the mature, uniseriate, uniform exodermis was responsible for the decline in hydraulic conductivity. Although previous evidence is limited, it does not seem unreasonable to conclude that the observed reduction in hydraulic conductivity in *I. germanica* roots was due to the maturation of two exodermal layers in which passage cells were lacking.

It is instructive to compare hydraulic conductivity values among species, including *I. germanica*. Despite the thickness of these roots, the hydraulic conductivity of 0EX roots was within the same order of magnitude (some 10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}) as those obtained for species with narrower roots such as *Z. mays* and *Sorghum bicolor* (see Cruz et al., 1992; Steudle and Peterson, 1998). To the best of the authors’ knowledge there are, in the literature, only two quantitative comparisons of the overall compared with exodermal hydraulic resistance. (i) In young *Z. mays* roots, a comparison of the hydraulic of roots grown either in hydroponics or mist culture (similar to the conditions in the present study) led Zimmermann and Steudle (1998) to conclude that the development of the exodermis resulted in a decrease in hydraulic conductivity (from 26×10^{-8} to 7.3×10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}). This decrease is equivalent to a 3.6-fold increase in

**Table 7.** Root hydraulic conductivity values calculated from pressure chamber (using endodermal surface area) and pressure probe (using root surface area) experiments

Conductivity data are means±standard deviations, and the numbers of replicates are in parentheses.

| Instrument          | 0EX          | 2EX          | 2EX-P         |
|---------------------|--------------|--------------|---------------|
| Pressure chambera   | 48.7±6.0*     | 18.3±7.7**   | 93±91***      |
| Pressure probeb     | 39±13*       | 23±9*        | 41±24*        |

^a or ^b indicates a significant difference within each row (P<=0.05). 0EX, roots with no mature exodermal layers; 2EX, root segments with two mature exodermal layers; 2EX-P, root segments with two mature exodermal layers that were punctured.

^a *L*\_p values calculated using endodermal surface area.

^b *L*\_p values calculated using root surface area.

*L*\_p, 0EX and *L*\_pc 0EX are statistically similar (result not shown in Table).

*L*\_p, 2EX and *L*\_pc 2EX are statistically similar (result not shown in Table).

*L*\_p, 2EX-P and *L*\_pc 2EX-P are statistically similar (result not shown in Table).
the overall hydraulic resistance, which is within the range of the change in resistance measured for *I. germanica* (from 0EX to 2EX = 3.1-fold increase in resistance, 2EX to 2EX-P = 4.1-fold loss of resistance; see Table 3). In contrast, for *O. sativa* roots, Ranathunge *et al.* (2003) concluded that the endodermis was the major resistance to water flow; although the hydraulic conductivity for the whole root was low (4 × 10⁻⁸ m s⁻¹ MPa⁻¹), the conductivity across only the outer part of root containing a uniseriate exodermis was 30-fold greater (or 120 × 10⁻⁸ m s⁻¹ MPa⁻¹). It was shown previously by Armstrong and Armstrong (2005) that *O. sativa* roots have ‘windows’ in the exodermis, which are regions that lack suberin lamellae and where lateral roots emerge; these ‘windows’ would be less resistant to water flow compared with suberized regions. Also, lateral root production in *O. sativa* is prolific and quite damaging to the exodermis. Lastly, since *O. sativa* roots were hydroponically grown, it is probable that there was reduced production of key suberin lamellae aliphatic monomers (particularly α-OH fatty acids) in the exodermis compared with growth in soil or in humid air (Krishnamurthy *et al.*, 2009; Meyer, 2010).

Like *I. germanica*, *Carex arenaria* has a three- to four-layered multiseriate exodermis and Robards *et al.* (1979) measured its water permeability. The permeability of *C. arenaria*’s isolated exodermis to water (7–15 × 10⁻¹¹ m s⁻¹ MPa⁻¹) was lower by three orders of magnitude than that of *I. germanica*. Hence, while *C. arenaria*’s water conductivity values differ from those of *I. germanica* and *Z. mays*, the exodermis appears to function similarly with regard to the increase in hydraulic resistance. In view of these results, the present findings demonstrate a dominating effect of *I. germanica*’s multiseriate exodermis on hydraulic resistance, which is related to the deposition of a continuous circumferential Casparian band and suberin lamellae in all exodermal cells. In addition, this high hydraulic resistance may also be the result of up-regulation in the production of suberin-associated fatty acids and waxes in the exodermal layers.

### Solute permeability

Solute permeability ($P_{sr}$) of *I. germanica* roots was measured concurrently with measurements of osmotic hydraulic conductivity (see Fig. 6). The permeability of ethanol (a small, uncharged, lipophilic solute) was reduced by nearly an order of magnitude with maturation of the biseriate exodermis, compared with 0EX (see Table 5). Assuming that the symplastic pathway did not change with maturation of the exodermis, the reduction in permeability reflects the importance of the apoplastic and transcellular pathways for permeation of this solute. After some of the exodermal cells were punctured, resistance to ethanol permeation decreased by 6.8-fold, indicating that the exodermis provided a major resistance to ethanol entry into the root.

Reflection coefficients ($\sigma_{sr}$) refer to the passive selectivity of a membrane or tissue to a solute, and are used to express, in a quantitative way, the ability of membranes or cell-wall-modifying structures to resist the flow of solutes. Values of $\sigma_{sr}$ range from 0 to 1, where 0 means that there is no selectivity to solute flow (as compared with water) and 1 means there is a total blockage of solute flow. In the present study, the $\sigma_{sr}$ of ethanol was quite high for *I. germanica*’s intact multiseriate exodermis ($\sigma_{sr}$=0.69; Table 5). In contrast, Miyamoto *et al.* (2001) and Ranathunge *et al.* (2003) measured substantially lower ethanol $\sigma_{sr}$ across the outer part of *O. sativa* roots, a region that included the uniseriate exodermis ($\sigma_{sr}$=0.04–0.13). This is in line with the greater overall permeation resistance of *I. germanica*’s exodermis compared with that of *O. sativa*.

When *I. germanica* roots were exposed to NaCl, a small amount penetrated the 0EX roots but was effectively excluded from the 2EX region (see Table 5). These results explain the earlier findings of Wang (2002) for *Iris hexagona*, a species with a uniseriate exodermis (Meyer *et al.*, 2009). In this species, salt accumulated primarily in the roots but some was also transported to the leaves. Presumably in young root zones where the exodermis was immature, the majority of NaCl flow was blocked by the Casparian band in the endodermis. In older zones, however, based on the current puncturing experiments with the 2EX region, it is predicted that apoplastic NaCl flow is restricted by the Casparian band in the exodermis (see Fig. 1).

The NaCl $\sigma_{sr}$ for *I. germanica* was high regardless of the exodermal maturation stage (0EX=0.92; 2EX=1.0; Table 5). Based on these results, it is unlikely that Na⁺ was transported across the epidermal plasma membranes; hence, the ions were restricted to the apoplast where they came into direct contact with the Casparian bands. For *I. germanica*, the roots behaved as an osmotic cell. This is a special case because the $\sigma_{sr}$ values of NaCl are much greater than those for other species. For example, in young *Z. mays* roots with an immature exodermis, the $\sigma_{sr}$ of NaCl was 0.64 and puncturing the endodermis reduced it to 0.41 (Steudle *et al.*, 1993). Using *O. sativa* roots it was possible to measure the $\sigma_{sr}$ of NaCl for the uniseriate exodermis ($\sigma_{sr}$=0.10) as well as the entire root ($\sigma_{sr}$=0.20–0.30) (Ranathunge *et al.*, 2003). Interestingly, the non-exodermal roots of *Arabidopsis thaliana* had a $\sigma_{s}$ of 0.77 for NaCl (Boursiac *et al.*, 2005). Apparently, in certain species, the endodermis itself can quite effectively restrict the flow of NaCl. The presence of exodermal Casparian bands may function as additional apoplastic solute filters that could be of interest when determining a species’ tolerance to salt.

### Functional significance of the multiseriate exodermis

The multiseriate exodermis in the thick roots of *I. germanica* may be a special adaptation to drought conditions, and would tend to reduce water loss to a relatively dry soil. This is achieved by having the highest resistance to radial water flow in the outer part of the root rather than at the endodermis, thus preserving the central cortex. According to Meyer *et al.* (2009), the majority of species with a multiseriate exodermis (including *I. germanica*) inhabit well-drained soils suggesting that this type of exodermis may play a role in tolerating periodic drought stress. Under favourable growth
conditions with abundant water, there will be zones of exodermal development along the root length beginning with the outermost layer near the root tip and developing centripetally until four layers are mature (Meyer et al., 2009). Therefore, one would expect less resistance to radial water flow closer to the root tip. In the present experiments, the development of a multiseriate exodermis was induced even under rather wet conditions indicating that the responses of roots to environmental changes were fairly sensitive. In the event of a drought, root growth would slow but maturation of the multiseriate exodermis would continue (Perumalla and Peterson, 1985), consequently increasing the number of exodermal layers and the resistance to water loss in younger root regions. This anatomical change could prevent excessive water loss from the root to the dry soil. As extreme examples, C. arenaria and Agave deserti, two species that inhabit dry, sandy substrates and have roots with a multiseriate exodermis, are not very permeable to water (Robards et al., 1979; North and Nobel, 1991, 1995). Hence, the capacity to produce a multiseriate exodermis is one of many important evolutionary specializations that allow some species to tolerate drought-prone habitats.

Conclusions

Hydraulic conductivity measured with the pressure chamber revealed a significant resistance of I. germanica’s intact multiseriate exodermis to radial water flow that was, in fact, dominating when two layers of the exodermis were fully developed. Due to the large water storage capacity of the central cortex, the measurement of transients generated by the root pressure probe resulted in estimates of mainly the endodermal rather than the overall radial hydraulic conductivity. In agreement with the composite transport model of the root, the osmotic permeability of water was much lower than the hydrostatic. Water permeability was greatly reduced in the presence of a multiseriate exodermis, as were the permeabilities of the two test solutes ethanol and NaCl. When the multiseriate exodermis was punctured, its limiting influence on radial water and solute transport was lost. It appears that the multiseriate exodermis is one trait within a suite of specializations that evolved to enable plants to tolerate drought-prone habitats. In thick roots such as those of I. germanica, the existence of series arrays of different hydraulic resistances (exodermis compared with endodermis) extends the composite transport model of the root that has been used so far to explain the role of parallel pathways of different selectivity (cell-to-cell compared with apoplastic).

Supplementary data

Supplementary Fig. S1. Typical root pressure build-up measured with the root pressure probe on an Iris germanica root that was grown completely submerged in hydroponics. Data to the left of the arrow represent the pressure relaxation after tightening the root to the probe. Data to the right of the arrow represent internal root pressure build-up; i.e. a net increase in ion and water influx into the stele.

Supplementary Fig. S2. Typical root pressure build-up measured with the root pressure probe on an Iris germanica root segment that was exposed to a humid air gap in the hydroponic tank. Data to the left of the arrow represent the pressure relaxation after tightening the root to the probe. Data to the right of the arrow represent internal root pressure build-up; i.e. a net increase of ion and water influx into the stele.

Supplementary Fig. S3. Typical root pressure relaxations induced with the root pressure probe; Iris germanica root.

Supplementary Fig. S4. Typical root pressure decrease and stabilization following exodermal puncturing (arrow) on Iris germanica roots; measured with the root pressure probe.

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References

Armstrong J, Armstrong W. 2005. Rice: sulfide-induced barriers to root radial oxygen loss, Fe²⁺ and water uptake, and lateral root emergence. Annals of Botany 96, 625–638.

Baker DA. 1971. Barriers to the radial diffusion of ions in maize roots. Planta 98, 285–293.

Barrowclough DE, Peterson CA. 1994. Effects of growing conditions and development of the underlying exodermis on the vitality of the onion root epidermis. Physiologia Plantarum 92, 343–349.

Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries N, Maurel C. 2005. Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression. Plant Physiology 139, 790–805.

Brundrett MC, Enstone DE, Peterson CA. 1988. A berberine–aniline blue fluorescent staining procedure for suberin, lignin and callose in plant tissue. Protoplasma 146, 133–142.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with Sudan red 7B or Fluorol yellow 088 in polyethylene glycol–glycerol. Biotechnic and Histochemistry 66, 111–116.

Clark LH, Harris WH. 1981. Observations on the root anatomy of rice (Oryza sativa L.). American Journal of Botany 68, 154–161.

Colombo SJ, Asselstine MF. 1989. Root hydraulic conductivity and root growth capacity of black spruce (Picea mariana) seedlings. Tree Physiology 5, 73–81.
North GB, Nobel PS. 1995. Hydraulic conductivity of concentric root tissues of Agave deserti Engelm. under wet and drying conditions. New Phytologist 130, 47–57.

Perumalla CJ, Peterson CA. 1985. Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. Canadian Journal of Botany 64, 1873–1878.

Peterson CA. 1987. The exodermal Casparian band of onion blocks apoplastic movement of sulphate ions. Journal of Experimental Botany 32, 2068–2081.

Peterson CA, Perumalla CJ. 1990. A survey of angiosperm species to detect hypodermal Casparian bands. II. Roots with a multiseries hypodermis or epidermis. Botanical Journal of the Linnean Society 103, 113–125.

Ranathunge K, Steudle E, Lafitte R. 2003. Control of water uptake by rice (Oryza sativa L.): role of the outer part of the root. Planta 217, 193–205.

Ranathunge K, Steudle E, Lafitte R. 2005. A new precipitation technique provides evidence for the permeability of Casparian bands to ions in young roots of corn (Zea mays L.) and rice (Oryza sativa L.). Plant Cell and Environment 28, 1450–1462.

Robards AW, Clarkson DT, Sanderson J. 1979. Structure and permeability of the epidermal/hypodermal layers of the sand sedge (Carex arenaria L.). Protoplasma 101, 331–347.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED. 1994. Hydraulic and osmotic properties of spruce roots. Journal of Experimental Botany 45, 1413–1425.

Sands R, Fiscus EL, Reid CPP. 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. Australian Journal of Plant Physiology 9, 559–569.

Soukup A, Votrubová O, Čičková H. 2002. Development of anatomical structure of roots of Phragmites australis. New Phytologist 153, 277–287.

Stadelmann EJ, Kinzel H. 1972. Vital staining of plant cells. In: Prescott VDM, ed. Methods in cell physiology. New York: Academic Press, 325–372.

Steudle E. 2001. The cohesion-tension mechanism and the acquisition of water by plant roots. Annual Review of Plant Physiology and Plant Molecular Biology 52, 847–875.

Steudle E, Brinckmann E. 1989. The osmometer model of the root: water and solute relations of Phaseolus coccineus. Botanica Acta 102, 85–95.

Steudle E, Frensch J. 1989. Osmotic responses of maize roots. Planta 177, 281–295.

Steudle E, Jeschke WD. 1983. Water transport in barley roots. Planta 158, 237–248.

Steudle E, Meshcheryakov AB. 1996. Hydraulic and osmotic properties of oak roots. Journal of Experimental Botany 47, 387–401.

Steudle E, Murrmann M, Peterson CA. 1993. Transport of water and solutes across maize roots modified by puncturing the endodermis. Further evidence for the composite transport model of the root. Plant Physiology 103, 335–349.
Steudle E, Oren R, Schulze ED. 1987. Water transport in maize roots. Measurement of hydraulic conductivity, solute permeability, and of reflection coefficients of excised roots using the root pressure probe. *Plant Physiology* **84**, 1220–1232.

Steudle E, Peterson CA. 1998. How does water get through roots? *Journal of Experimental Botany* **49**, 775–788.

Taylor JA, West DW. 1980. The use of Evan’s blue stain to test the survival of plant cells after exposure to high salt and high osmotic pressure. *Journal of Experimental Botany* **31**, 571–576.

Tyerman SD, Bohnert HJ, Maurel C, Steudle E, Smith JAC. 1999. Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. *Journal of Experimental Botany* **50**, 1055–1071.

Tyerman SD, Niemietz CM, Bramley H. 2002. Plant aquaporins: multifunctional water and solute channels with expanding roles. *Plant Cell and Environment* **25**, 173–194.

Tyree MT, Patino S, Bennink J, Alexander J. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany* **46**, 83–94.

Tyree MT, Yang S, Cruziat P, Sinclair B. 1994. Novel methods of measuring hydraulic conductivity of tree root systems and interpretation using AMAIZED. *Plant Physiology* **104**, 189–199.

Waduwara CI, Walcott SE, Peterson CA. 2008. Suberin lamellae of the onion root endodermis: their pattern of development and continuity. *Canadian Journal of Botany* **86**, 623–632.

Wang XL, McCully ME, Canny MJ. 1995. Branch roots of Zea. V. Structural features that may influence water and nutrient transport. *Botanica Acta* **108**, 209–219.

Wang Y. 2002. Ecological, physiological and molecular responses of *Iris hexagona* to salinity stress. PhD thesis. Lafayette, University of Louisiana: Louisiana, USA.

Ye Q, Wiera B, Steudle E. 2004. A cohesion/tension mechanism explains the gating of water channels (aquaporins) in Chara internodes by high concentration. *Journal of Experimental Botany* **55**, 449–461.

Zeier J, Schreiber L. 1998. Comparative investigation of primary and tertiary endodermal cell walls isolated from the roots of five monocotyledonous species: chemical composition in relation to fine structure. *Planta* **206**, 349–361.

Zimmermann HM, Hartmann K, Schreiber L, Steudle E. 2000. Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (*Zea mays* L.). *Planta* **210**, 302–311.

Zimmermann HM, Steudle E. 1998. Apoplastic transport across young maize roots: effect of the exodermis. *Planta* **206**, 7–19.