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Hydraulic conductivity of concentric root tissues of *Agave deserti* Engelm. under wet and drying conditions

By GRETCHEN B. NORTH and PARK S. NOBEL

Department of Biology and UCLA–DOE Laboratory, University of California, Los Angeles, California 90024-1786, USA

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SUMMARY

Water moves from the root surface to the root xylem through a series of tissues, each with properties that influence the root radial hydraulic conductivity (L_R). To determine L_R for intact roots, root hydraulic conductivity (L_P) and axial (xylem) conductance (K_h) were measured for root segments of the desert succulent *Agave deserti* Engelm. As layers were successively removed, L_R was calculated for each layer by considering the conductivities in series. For the distal root region, no single tissue limited L_R under wet conditions, although the suberized exodermis and sclerenchyma/endodermis were more limiting per cross-sectional area of the root than were the cortex and stele. At midroot under wet conditions, L_R for the cortex was higher than in the distal region, and cortical cells were dead. After 28 d in drying soil, L_R for the suberized layers decreased by over 70% and thereby became more limiting to L_R of the intact segment, consistent with increases in extracted lipids in these layers. During drying, intercellular lacunae formed in the cortex due to cellular collapse, and cortical L_R decreased. After 7 d of re-wetting following drying, L_R increased for all tissues, but not to its value under wet conditions, reflecting persisting losses in conductivity, especially in the suberized layers. Thus, new apical growth and the development of new laterals are necessary for the complete restoration of root system conductivity. Within 20 mm of the root tip, substantial uptake of the apoplastic tracer 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) occurred, although the exodermis restricted PTS uptake further than 20 mm back from the root tip under wet conditions and all along the root after drying. When the exodermis was removed, PTS moved freely through the cortex in both distal and midroot regions, further suggesting that the cortical apoplast is a significant pathway for radial water flow.

Key words: Water uptake, apoplast, root cortex, exodermis, suberin.

INTRODUCTION

Water movement through roots can be quantified based on the driving force, such as a difference in water potential, and the hydraulic conductivities of the tissues from the root surface to the base of the plant. The root xylem determines the axial (longitudinal) hydraulic conductivity of the root, whereas several tissues contribute to the radial hydraulic conductivity from the root surface to the root xylem. For most roots, the radial hydraulic conductivity is the major limiter of water movement, except in the distal region where the larger vascular conduits are immature and axial conductivity limits water flow (St Aubin, Canny & McCully, 1986; McCully & Canny, 1988; Frensch & Steudle, 1989). Determining the hydraulic conductivities of individual tissues in the radial pathway will therefore help in understanding the major constraints on water movement through roots. Such constraints can limit overall water movement from the soil to the plant, at least under wet conditions when the root hydraulic conductivity is lower than that of the soil or the root-soil interface (Passioura, 1988; Nobel & Cui, 1992).

Root hydraulic conductivity, L_P, a coefficient relating the volume of water flow per unit time to a driving force such as the difference in water potential (Passioura, 1988; Nobel, 1991), has been measured for several species, including the monocotyledonous leaf succulent *Agave deserti* Engelm., the species examined in the present study. Root axial hydraulic conductivity, K_h, has been calculated from conduit diameters in root cross-sections or by measuring flow through root segments (Frensch & Steudle, 1989; North & Nobel, 1991; Melchior & Steudle, 1993). Root radial conductivity, L_R, has been determined less frequently, using pressure probe measurements on individual root cells and osmotically induced backflow (Jones *et al.*, 1983, 1988), root pressure relaxations for excised roots (Steudle & Jeschke, 1983; Steudle, Oren & Schulze, 1987), and measured values of L_P and K_h in models

based on cable theory (Landsberg & Fowkes, 1978; North & Nobel, 1991). For separate root tissues, L_R has been calculated from measurements on individual cells and measured for root 'sleeves' (hollow cylinders of epidermal plus hypodermal tissues; Shone & Clarkson, 1988; Schambil & Woermann, 1989). The contribution of the endodermis to radial conductivity has been assessed by puncturing it and other tissues (Peterson, Murrmann & Steudle, 1993), and the radial conductivity of xylem vessels has been measured using steam-killed roots (Peterson & Steudle, 1993).

The endodermis has long been considered a primary limiter of radial water movement (Clarkson & Robards, 1975; Kramer, 1983; Passioura, 1988). However, the developmental state of the endodermis is critical; after the deposition of Casparian bands but before the development of suberin lamellae, the endodermis may retard the passage of solutes but not water (Peterson *et al.*, 1993). Roots of most angiosperms also have a suberized exodermis (Perumalla & Peterson, 1985; Shishkoff, 1987) that can impose a substantial barrier to water movement, particularly under drying conditions (Shone & Clarkson, 1988). Although the relatively thin-walled, unsuberized cells of the epidermis and cortex can have higher hydraulic conductivities than do other root cell types (Jones *et al.*, 1988), the conductivities of such tissues might change during root development and soil drying due to cell death or the development of intercellular lacunae (Jupp & Newman, 1987; North & Nobel, 1991).

L_P, K_h, and L_R have been determined for *Agave deserti* under a range of soil moisture conditions (North & Nobel, 1991; Huang & Nobel, 1992). In the present investigation, the hydraulic conductivities for roots of two ages in wet, drying, and re-wetted soil were determined first for intact root segments and then for segments with separate tissue layers successively removed by dissection. The radial hydraulic conductivity was calculated for each layer by considering the tissue conductivities as occurring in series. In addition, an apoplastic tracer (Peterson, Emanuel & Humphreys, 1981; Skinner & Radin, 1994) was used to help understand the contributions of the apoplast to radial water transport. Concurrently, properties such as cell size, cell vitality, and the extent of suberization were investigated to help interpret the structural basis of the hydraulic conductivity of the root tissues.

MATERIALS AND METHODS

Plant material

Sixteen plants of *Agave deserti*, previously collected from Agave Hill at the University of California Philip L. Boyd Deep Canyon Desert Research Center (near Palm Desert, CA), were grown in a glasshouse at the University of California, Los Angeles. Plants received a mean total daily photosynthetic photon flux density of 38 mol m^{-2} d^{-1} (80 % of ambient solar radiation), which corresponded to a mean instantaneous value of 800 μmol m^{-2} s^{-1}; daily maximum/minimum air temperatures were *c.* 28 °C/16 °C; and soil water potential (ψ_{soil}) was maintained above −0·3 MPa by watering twice weekly. Plants were 0·26–0·30 m tall with 8–12 unfolded leaves and 20–35 main roots arising from nodes at the base of the stem just below the leaf bases.

To identify existing roots for subsequent estimations of new root age, 1 month prior to experiments entire root systems were immersed for 10 min in an aqueous solution of neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride, 0·5 g l^{-1}; Schumacher *et al.*, 1983); thus new roots were unstained. Plants were then placed in 32 cm long × 26 cm wide × 12 cm deep tubs containing soil from Agave Hill and were watered twice weekly for 30 d before water was withheld. Based on soil moisture release curves for the field soil (Young & Nobel, 1986), ψ_{soil} in the root zone was determined gravimetrically: ψ_{soil} was −0·1, −7·1, and −11 MPa at 0, 14, and 28 d of drying, respectively. After rewetting, ψ_{soil} increased to −0·1 MPa within 1 d and was maintained at this value by daily watering. Two types of root segments from main roots averaging 600 mm in length and 3·4 mm diam. were investigated: distal (from the root tip to 100 mm back) and midroot (from 250 to 350 mm proximal to the tip). Under both wet and drying soil conditions, the distal region of the root was less than 20 d old, and the midroot region was between 40 and 60 d old.

Hydraulic conductivity

Roots were excavated using a fine spatula and jets of water, excised, and immediately immersed in distilled water. The 100 mm segments from distal and midroot regions lacking visible lateral roots were cut under water with a razor blade. Tissues external to the stele were removed from a 10 mm length of the proximal end of a root segment, and the exposed stele was inserted into a 10 mm section of Tygon tubing attached to a glass capillary (internal diameter 0·8 mm) that was half-filled with water. A silicon and brass compression fitting was tightened around the tubing to prevent leaks around the stele (McCown & Wall, 1979; Lopez & Nobel, 1991). Dental impression material (polysiloxane) and two coats of acrylic co-polymer (Nobel, Schulte & North, 1990) were applied at the junction between the tubing and the stele as well as at the distal cut end of segments from midroot before immersion of the segment in distilled water. Water flow through the root was induced by applying negative pressure to the open end of the capillary. Pressure was regulated with a needle valve and monitored with a Validyne PS309

digital manometer (Nobel *et al.*, 1990). When the volumetric flow rate, Q_v ($m^3 s^{-1}$), became constant at a given pressure (P, MPa), usually within 10 min, L_P ($m s^{-1} MPa^{-1}$) was calculated as follows (Nobel *et al.*, 1990):

$$L_P = (\Delta Q_v/\Delta P)(1/A), \quad (1)$$

where A (m^2) is the root surface area, calculated from root length and radius. Leaks were detected by the appearance in the capillary of crystal violet dye, which was added at a concentration of 0·005 % (w/w) to the immersion solution. Axial conductance measured on 20 mm segments open to solution at the distal end was always much greater than L_P for intact or sealed segments, suggesting that the stele was not crimped by the compression fitting, consistent with microscopic inspection.

To measure axial (xylem) conductance, distal root segments were trimmed by 10 mm at the tip end and distal-end seals were removed from midroot segments. About 1 mm at the cut end of the segment was immersed in 100 mol m^{-3} potassium chloride (to reduce blockage of pit membrane pores; Sperry, 1986). Q_v was measured as for L_P and was used to calculate the root axial conductance per unit pressure gradient (K_h, $m^4 s^{-1} MPa^{-1}$):

$$K_h = Q_v/(\Delta P/l) \quad (2)$$

where the pressure drop ΔP was applied along the length l (m) of the root segment (Gibson, Calkin & Nobel, 1984).

The volumetric flux density ($m^3 m^{-2} s^{-1}$) of water at the root surface divided by the difference in water potential (MPa) from the root surface to the root xylem equals the root radial conductivity, L_R ($m s^{-1} MPa^{-1}$). It was calculated from measured values of L_P and K_h together with the length l and the radius (r_{root}, m) of the root segment (Landsberg & Fowkes, 1978):

$$L_R = L_P\, \alpha l/\tanh(\alpha l), \quad (3)$$

where α equals $(2\pi r_{root} L_R/K_h)^{1/2}$; L_R was initially set equal to L_P and gradually increased to solve eqn 3 by iteration.

L_R *for concentric tissues*

After L_P was measured for a root segment with its radial tissues intact (Fig. 1*a*), the epidermis plus exodermis were stripped from the underlying cortex (Fig. 1*b*) using fine forceps under a stereomicroscope while the segment was immersed in water; regions < 2 mm from proximal and distal seals were not stripped. L_P was then measured on the stripped segment, and the process was repeated after removing the cortex (Fig. 1*c*) and then the endodermis and adjacent sclerenchyma (Fig. 1*d*).

L_R for an intact root segment equals the reciprocal of the sum of the reciprocals of L_R for the epidermis/exodermis, the cortex, the sclerenchyma/endodermis, and the stele. After L_P was measured for the innermost tissue (the exposed stele), K_h was measured and L_R was calculated using eqn 3, based on the surface area of the intact root segment. L_R was then calculated for the next outer layer (the endodermis and sclerenchyma) and for the successive outer layers by considering the radial conductivities in series:

$$L_{R,n} = 1/(1/L_{R,i+j+\ldots+n} - 1/L_{R,i+j+\ldots n-1}). \quad (4)$$

Neither the epidermis nor the sclerenchyma could be removed without damaging the underlying layers (the exodermis and the endodermis, respectively), so these two layers were not treated separately. To investigate the conductivity of the epidermis/exodermis at the root tip, L_P for the entire 100 mm segment was measured before and after this layer was removed from a 10 mm long zone at the tip.

Anatomical measurements

To investigate anatomical features, root segments were sectioned with a razor blade and stained with 0·05 % (w/w) toluidine blue O in distilled water. Other sections were stained with 0·7 % (w/w) Sudan III plus Sudan IV in ethylene glycol to detect suberin or with 0·5 % (w/w) phloroglucinol in water followed by 20 % HCl to detect lignin (Jensen, 1962). Lignin and suberin were also detected by their autofluorescence (Peterson *et al.*, 1981), which was viewed with an Olympus BH2 microscope fitted with DMU ultraviolet or DMV violet filter systems (excitation wavelengths 370–420 nm). To assess cell vitality based on fluorescing nuclei, segments were immersed in 0·001 % (w/w) acridine orange in distilled water for 5 min and viewed with epifluorescence using the DMV filter (Henry & Deacon, 1981; Wenzel & McCully, 1991). For cells not cut by sectioning, the absence of fluorescing nuclei was used together with other evidence (such as the absence of cytoplasm) to indicate cell death.

To investigate possible apoplastic pathways for water movement in intact and stripped root segments, root segments were immersed in a 0·001 % (w/w) solution of 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS), a mobile apoplastic dye that does not bind to cell walls (Peterson *et al.*, 1981; Skinner & Radin, 1994), and a negative pressure of 30 kPa was applied for 1 h to induce flow. To investigate the diffusive movement of PTS, other root segments were sealed at their cut ends and left in the dye for 1 h without the application of pressure. After the root segments were washed for 1 h, sections were cut with a razor blade, mounted in immersion oil, and viewed immediately with epifluorescence, using the DMV filter.

Lipids were extracted from tissues of roots that were initially 30 d old and kept for 28 d more in

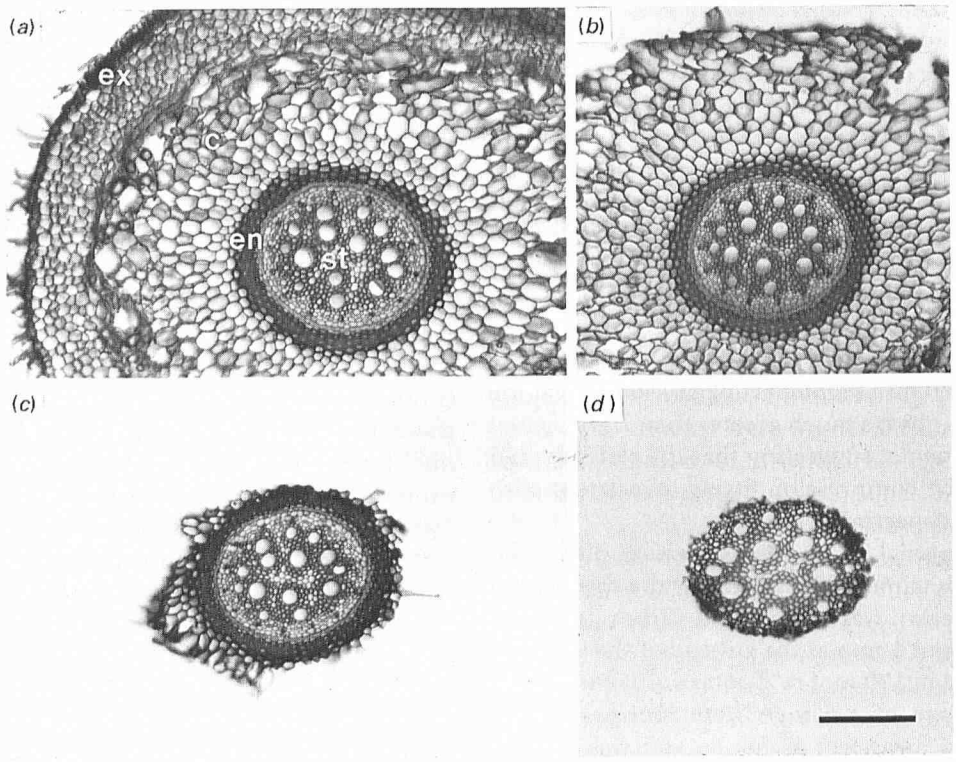

Figure 1. Transverse sections from the midroot region (250 mm proximal to the tip) of a root of *Agave deserti* after 28 d in drying soil: (*a*) intact, showing epidermis/exodermis (ex), cortex (c), sclerenchyma/endodermis (en), and stele (st); (*b*) with epidermis/exodermis removed by dissection; (*c*) with the cortex also removed; and (*d*) with all tissues external to the stele removed. Fresh sections were stained with toluidine blue O; scale bar, 250 μm.

wet soil ($\psi_{soil} > -0{\cdot}3$ MPa) or in drying soil ($\psi_{soil} = -12$ MPa at 28 d). The epidermis/exodermis was removed from a 10 mm region at the root tip, and this tissue plus the sclerenchyma/endodermis were removed from a 10 mm region at the proximal end of the part of the root initiated 58 d earlier; the tissues were immersed in liquid N_2 for 1 h prior to freeze-drying under vacuum for 24 h. Dried tissues were placed in pre-weighed aluminium dishes, weighed, and covered with 2:1 chloroform:methanol (Vogt, Schönherr & Schmidt, 1983) at 4 °C for 1 h. The solvent was then removed by pipette and fresh solvent was added, a process that was repeated four times. Tissues were dried under vacuum for 24 h before obtaining final dry weight. The fraction of extracted lipids in the tissue was calculated as (initial dry weight − final dry weight)/(initial dry weight).

Most data were statistically analysed by ANOVA, followed by pairwise comparisons using the Student–Newman–Keuls method; data based on numerical counts were analysed using the Mann–Whitney rank sum test.

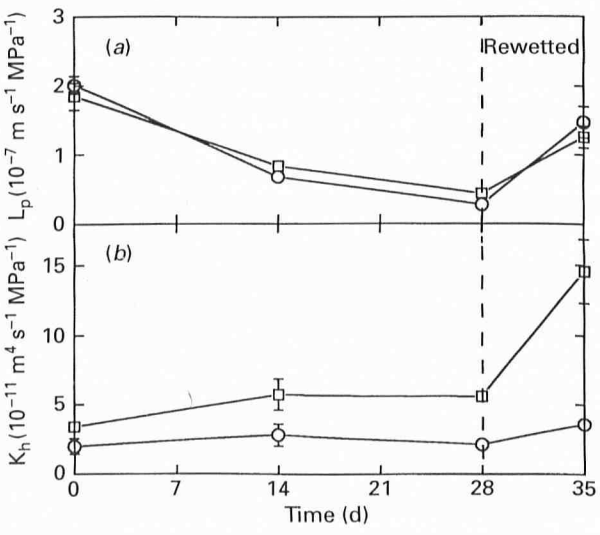

Figure 2. (*a*) Root hydraulic conductivity (L_p) and (*b*) axial (xylem) hydraulic conductance K_h for distal (○) and midroot (□) regions of *Agave deserti* during soil drying and re-wetting. At 0, 14, and 28 d of drying, ψ_{soil} was −0·2, −7·1 and −11 MPa, respectively. Data are means ± SE (unless smaller than the symbol) for three roots from each of n = three plants.

RESULTS

Hydraulic conductivity

Under both wet and drying conditions, root hydraulic conductivity (L_p) of *Agave deserti* was similar for the distal and midroot regions (Fig. 2*a*). During 28 d of soil drying, L_p decreased 87 % for the distal region and 77 % at midroot. After 7 d of re-wetting, L_p for both root regions was restored to an average of

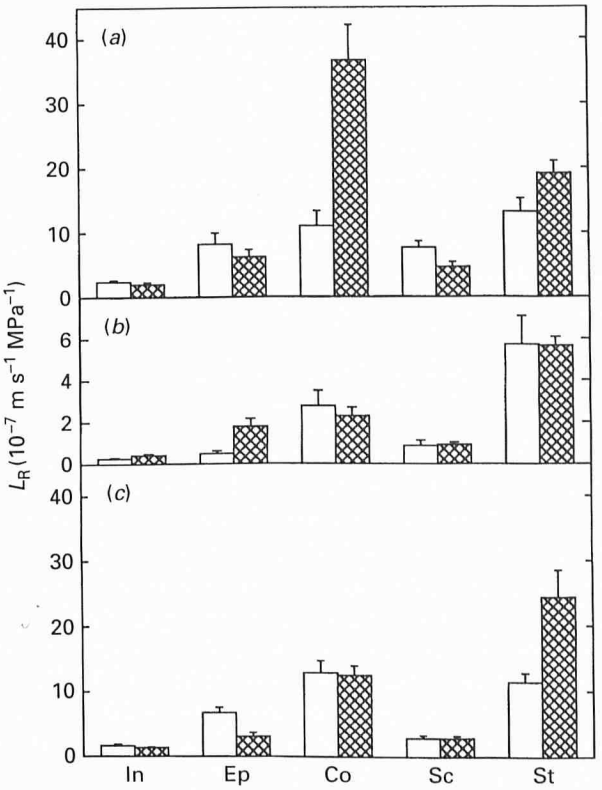

Figure 3. Radial conductivity (L_R) for the distal root region (open bars) and the midroot region (crosshatched bars) under (*a*) wet conditions ($\psi_{soil} = -0{\cdot}1$ MPa), (*b*) after 28 d in drying soil ($\psi_{soil} = -11$ MPa; note change in scale on ordinate), and (*c*) after 7 d of re-wetting ($\psi_{soil} = -0{\cdot}1$ MPa). L_R for intact root segments was calculated using L_p and K_h (Fig. 2) in eqn 3 and for individual root layers using eqn 4. Data are means ± SE for three roots from each of n = three plants. In, Intact; Ep, epidermis/exodermis; Co, cortex; Sc, sclerenchyma/endodermis; St, stele.

70 % of its initial value (Fig. 2*a*); roots then had 23 ± 4 mm (mean ± SE; $n = 8$) of new growth at their tips.

Under wet conditions, axial hydraulic conductance along the root xylem (K_h) was 70 % higher at midroot than for the distal region (Fig. 2*b*). During 28 d of soil drying, K_h changed only slightly for the distal region but increased 70 % at midroot. During 7 d of re-wetting following drying, K_h for the distal region nearly doubled and for the midroot region approximately tripled (Fig. 2*b*).

Under wet conditions, root radial hydraulic conductivity for an intact root segment was similar for distal and midroot regions, although cortical L_R was about three-fold higher at midroot than for the distal region (Fig. 3*a*). For individual tissues of the distal region, L_R's were also relatively similar, being about two-fold higher for the stele than for the least conductive layer, the sclerenchyma/endodermis (Fig. 3*a*). For the midroot region, however, L_R was eight-fold higher for the cortex than for the sclerenchyma/endodermis (Fig. 3*a*).

During 28 d in drying soil, L_R for intact root segments decreased 88 % at the distal region and 78 % at midroot (Fig. 3*b*). For the distal region, L_R decreased most for the epidermis/exodermis and sclerenchyma/endodermis during drying; at midroot, the greatest decrease in L_R occurred for the cortex. L_R for the stele remained substantially higher during drying than for the other root tissues, despite decreases of 57 and 70 % for the distal and midroot regions, respectively (Fig. 3*b*).

At 7 d of re-wetting following 28 d of drying, L_R for intact root segments was ~ 60 % of its value under wet conditions for both distal and midroot regions (Fig. 3*c*). L_R for the epidermis/exodermis at the distal region increased during re-wetting to 83 % of its value under wet conditions and to 49 % for the midroot region. L_R was similar for the cortex of the two root regions after re-wetting, as was also the case for the sclerenchyma/endodermis. L_R for the stele increased to about its value under wet conditions for the distal region and was slightly higher than initially at midroot after 7 d of re-wetting (Fig. 3*c*).

Table 1. *Tissue properties for roots of* Agave deserti *in wet soil ($\psi_{soil} = -0{\cdot}1$ MPa) or after 28 d in drying soil ($\psi_{soil} = -11$ MPa)*

Region/ soil	Number of cell layers		
	Exodermis	Cortex	Sclerenchyma
Distal			
Wet	2·3 ± 0·3	16·8 ± 1·2	2·1 ± 0·5
Dry	4·2 ± 0·4*	14·3 ± 1·6	4·8 ± 0·4*
Midroot			
Wet	4·0 ± 0·2	15·3 ± 0·9	4·2 ± 0·5
Dry	4·9 ± 0·3	13·8 ± 0·7	4·9 ± 0·4
	Cell diameter (μm)		
Distal			
Wet	31·9 ± 2·9	56·4 ± 5·4	28·1 ± 1·5
Dry	29·7 ± 2·2	46·6 ± 2·2	30·2 ± 0·9
Midroot			
Wet	30·6 ± 2·2	61·8 ± 5·2	31·1 ± 1·0
Dry	25·1 ± 3·2	41·1 ± 3·5**	33·8 ± 2·5

*, ** Significant differences ($P < 0{\cdot}05$ or $< 0{\cdot}01$, respectively) between tissues under wet and drying conditions.

Data are means ± SE for measurements made at 50 mm (distal region) or 275 mm (midroot region) from the tip of two roots from each of n = four plants (64 cells for diameters).

Tissue dimensions

For the two root regions, epidermal cell size was not affected by soil moisture, averaging 10·2 ± 0·7 μm (mean ± SE for $n = 16$ roots) in the radial direction. The number of cell layers in the exodermis in the distal region under wet conditions doubled during soil drying ($T = 10$; $P < 0{\cdot}05$; Table 1). At midroot, the exodermis had twice as many layers as in the distal region, and this number increased somewhat

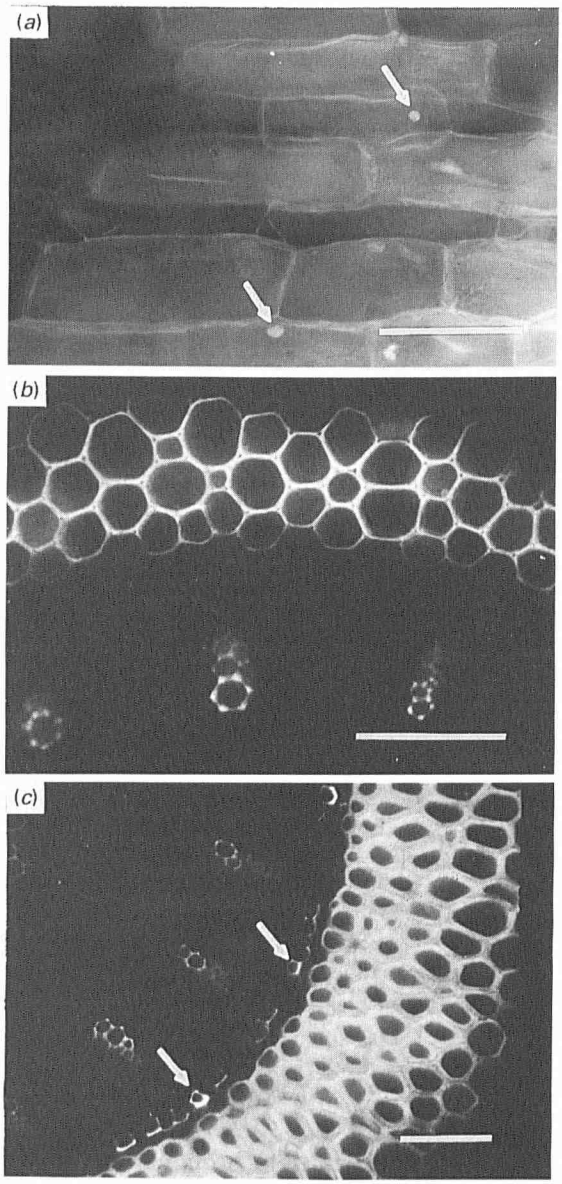

Figure 4. Light micrographs using epifluorescence of roots of *Agave deserti*: (*a*) longitudinal section through exodermis at 250 mm from the root tip after 28 d in drying soil, stained with acridine orange; arrows indicate fluorescing nuclei; (*b*) cross-section at 80 mm from a root tip under wet conditions; lignified cell walls in sclerenchyma outside the endodermis (with no suberin lamellae) and in protoxylem and early metaxylem vessels in the stele show white autofluorescence, and (*c*) cross-section at 5 mm from the tip of a root after 28 d in drying soil; autofluorescent suberin lamellae in endodermal cells indicated by arrows. Scale bars, 100 μm.

during drying. Exodermal cell diameter did not change significantly during drying for either the distal or the midroot region. The number of cell layers in the cortex was not affected by soil drying or root region, although cortical cell diameter decreased by 33 % for the midroot region during drying ($F_{1,6} = 10{\cdot}9$; $P < 0{\cdot}01$; Table 1). As for the exodermis, the number of cell layers in the sclerenchyma outside the endodermis in the distal root region approximately doubled during drying ($T = 10$; $P < 0{\cdot}05$); the number at midroot was also twice as high as in the distal region (Table 1). Endodermal cell size, which was unaffected by drying, was $18{\cdot}6 \pm 0{\cdot}7$ μm in the radial direction and $11{\cdot}4 \pm 1{\cdot}2$ μm tangentially. Under both wet and drying conditions, stele diameter averaged 733 ± 88 μm ($n = 8$ roots) at 50 mm from the root tip (distal region) and 794 ± 87 μm at 275 mm from the tip (midroot region).

Changes in epidermis/exodermis and sclerenchyma/endodermis

Under both wet and drying conditions, the cell walls of the exodermis stained with Sudan dyes and exhibited bright white fluorescence within 10 mm of the root tip, indicating the presence of suberin. In the distal region more than 10 mm back from the root tip, approximately 30 % of the uncut exodermal cells showed fluorescent nuclei after being stained with acridine orange and were judged to be living (Fig. 4*a*). In the midroot region, ~ 10–20 % of the cells showed fluorescent nuclei, even after 28 d of soil drying.

In the distal region under wet conditions, the cell layers immediately outside the endodermis were lignified but not suberized. After soil drying for the distal region and for the midroot region under both wet and drying soil conditions, suberin was present in the sclerenchyma. In the endodermis, Casparian bands were present within 5 mm of the root tip under both soil conditions. Endodermal cells lacked suberin lamellae in the distal region under wet conditions (Fig. 4*b*). In contrast, after 28 d of soil drying, some endodermal cells with suberin lamellae were present within 5 mm of the root tip (Fig. 4*c*), and over 90 % of the endodermal cells in the midroot region had suberin lamellae. Both the exodermis and the sclerenchyma/endodermis became more suberized during 28 d in drying soil, resulting in a greater amount of extracted lipids than under wet conditions ($F_{1,6} = 12{\cdot}8$; $P < 0{\cdot}05$; Table 2). Specifically, the exodermis (with epidermis adhering) stripped from a 10 mm long zone at the tip of roots in drying soil had twice as much extracted lipids as that from roots in wet soil. Under drying conditions, the epidermis/exodermis and sclerenchyma/endodermis stripped from the proximal end of roots initiated 58 d earlier had 20 and 34 % more extracted lipids, respectively, than under wet conditions ($F_{1,6} = 6{\cdot}6$; $P < 0{\cdot}05$; Table 2).

Effects of stripping the epidermis/exodermis at the root tip

To determine the effect of the exodermis at the root tip on hydraulic conductivity, L_p was measured before and after the epidermis/exodermis was re-

Table 2. *Amount of lipids extracted with chloroform/methanol from tissues stripped from 10 mm regions of 58-d-old roots of* Agave deserti *after 28 d in wet or drying soil*

Region	Tissue	Lipids (percentage of dry weight)	
		Wet	Dry
Tip	Epidermis/exodermis	17·0 ± 2·3	34·1 ± 4·2*
Proximal	Epidermis/exodermis	37·1 ± 4·1	44·7 ± 4·1
	Sclerenchyma/endodermis	27·1 ± 2·2	36·9 ± 3·1*

* Significant differences ($P < 0{\cdot}05$) between tissues under wet and drying conditions.

Data are means ± SE for n = four plants.

Table 3. *Hydraulic conductivity* (L_p) *for 100 mm long root segments before and after stripping the epidermis/exodermis from a 10 mm long zone at the root tip of* Agave deserti

Soil	L_p (10^{-7} m s^{-1} MPa^{-1})	
	Before stripping	After stripping
Wet	1·68 ± 0·23	1·96 ± 0·26
Dry	0·56 ± 0·06	1·25 ± 0·19*

* Significant difference ($P < 0{\cdot}05$) within a soil condition.

Data are means ± SE for n = four plants in wet soil ($\psi_{soil} = -0{\cdot}1$ MPa) or after 14 d in drying soil ($\psi_{soil} = -7{\cdot}1$ MPa).

moved from a 10 mm long zone at the tip. Under wet conditions, removal of the epidermis/exodermis at the tip did not significantly change L_p for the entire segment (Table 3). In contrast, under drying conditions, stripping this layer more than doubled L_p for the segment ($F_{1,6} = 12{\cdot}0$; $P < 0{\cdot}05$; Table 3).

Changes in the cortex and stele

In the distal region under wet conditions, cortical cells were turgid and contained cytoplasm, with nuclei that fluoresced after taking up acridine orange (Fig. 5*a*). At ~ 150–180 mm back from the root tip, most cortical cells lacked nuclei; in the midroot region, nuclei were rare under both wet and drying soil conditions. After 28 d in drying soil, cortical cells within 10 mm of the tip remained turgid and alive, whereas about 50 % of the cortical cells lacked nuclei at 20–30 mm from the root tip; at 80 mm from the tip, fluorescent nuclei were not observed, and the cells appeared to lack cytoplasm (Fig. 5*b*).

During soil drying, cortical cells apparently lost turgor and became less rounded, and gaps or lacunae were formed. Such lacunae were not observed within 35 mm of the root tip. For both distal and midroot

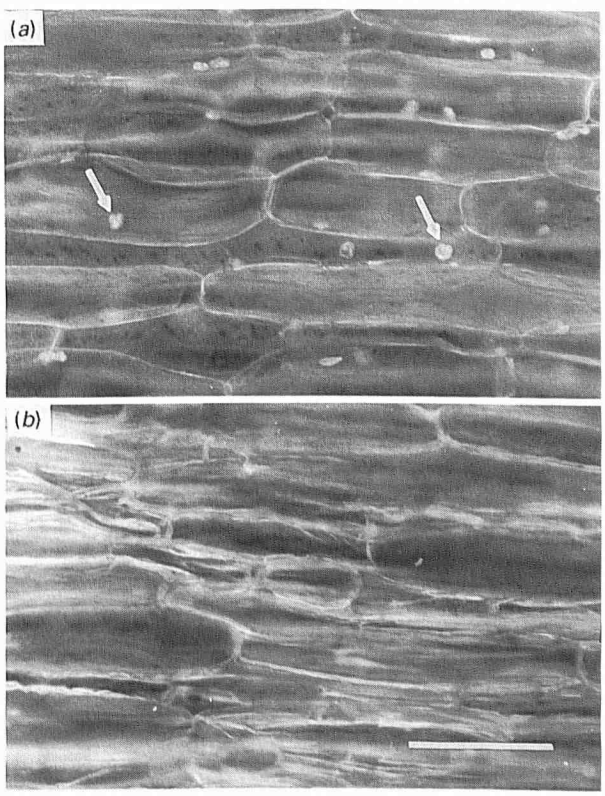

Figure 5. Longitudinal sections of the cortex at 80 mm from a root tip, stained with acridine orange; (*a*) under wet conditions and (*b*) after 28 d in drying soil. Arrows indicate fluorescing nuclei; scale bar, 100 μm.

regions after 28 d of drying, the percentage of cross-sectional area of the cortex comprised by lacunae varied from ~ 7 % (Fig. 1*a*) to almost 50 %, with a mean of 22 ± 6 % ($n = 6$ roots). In addition, gas bubbles emerged from the cortex when newly excised roots were submerged in water.

Under wet conditions, only protoxylem and early metaxylem vessel elements were fully lignified in the distal region (Fig. 4*b*). During drying, a few lignified late metaxylem vessels were observed at 50 mm and further back from the root tip. For the midroot region under wet conditions, approximately 60 % of the late metaxylem vessels were lignified; after

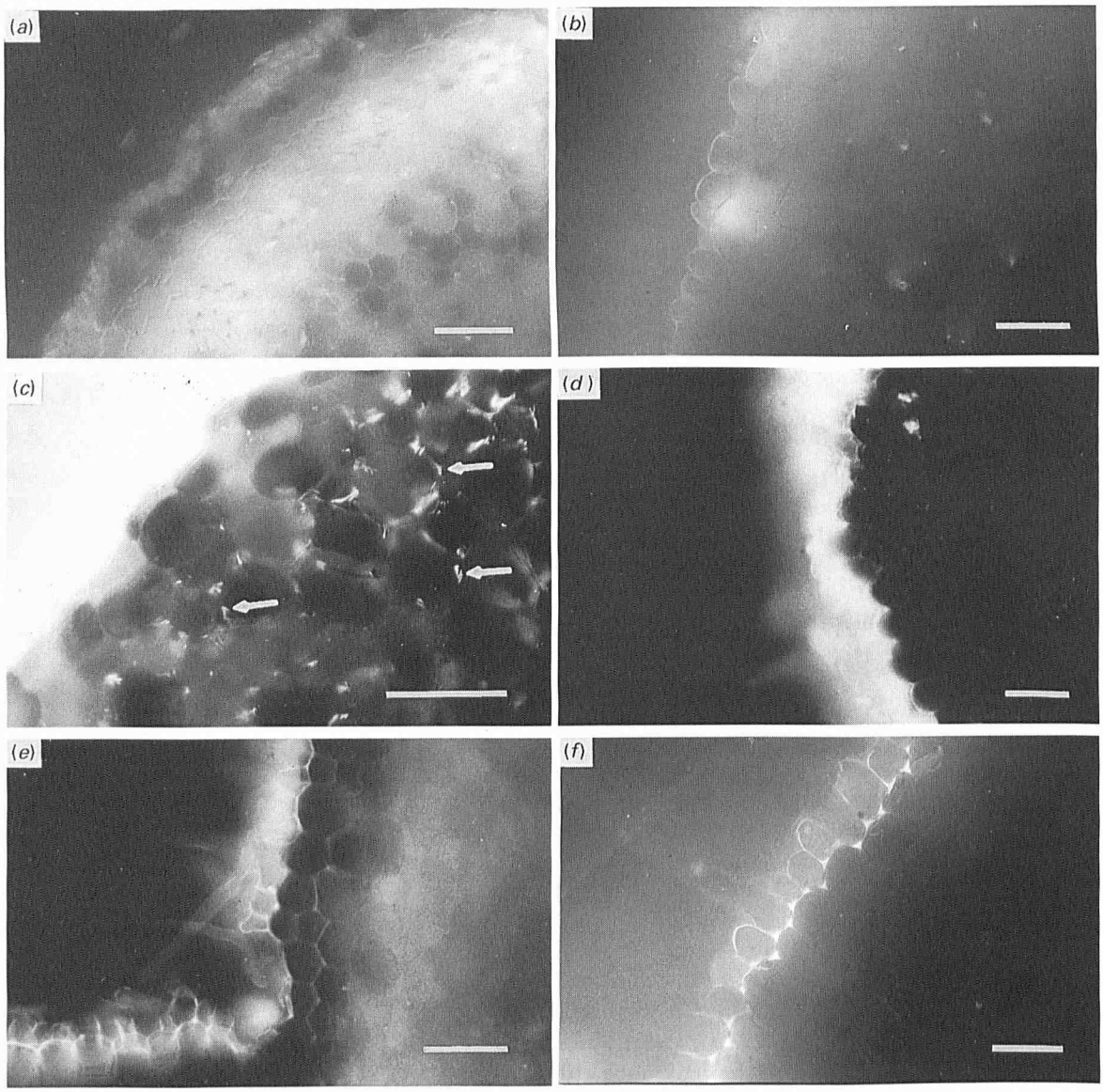

Figure 6. Light micrographs using epifluorescence of cross-sections of roots of *Agave deserti* immersed in PTS for 1 h: (*a*) 8 mm from the tip of a root under wet conditions, with negative pressure applied; (*b*) as for (*a*), but without negative pressure applied; (*c*) as for (*a*), but at 15 mm from the tip; arrows indicate PTS in intercellular spaces; (*d*) as for (*a*), but at 25 mm from the tip; (*e*) as for (*d*), but with a strip of exodermis removed; and (*f*) as for (*a*), but after 28 d in drying soil. Scale bars, 100 μm.

28 d of drying, all vessels appeared fully mature. Parenchyma cells in the stele in both root regions were not visibly affected by soil drying.

Apoplastic pathway

When the apoplastic tracer PTS was added to the immersion solution and negative pressure was applied to a root stele to induce flow, fluorescence was observed in the cortex less than 10 mm from the root tip under wet conditions (Fig. 6*a*). In such roots, PTS appeared to be excluded from most of the exodermis but accumulated in the epidermis and cortex. When negative pressure was not applied, fluorescence induced by PTS was limited to the outer layers of the root (Fig. 6*b*). From 10 to 20 mm back from the tip under wet conditions, PTS-induced fluorescence was more limited, and appeared to be concentrated in the intercellular spaces in the cortex (Fig. 6*c*). Further than 20 mm back from the root tip, apoplastic movement of the tracer was largely restricted at the exodermis (Fig. 6*d*) whether or not negative pressure was applied; partial removal of the exodermis allowed PTS movement throughout the cortex (Fig. 6*e*). Under drying conditions, the uptake of PTS was restricted by the exodermis within 10 mm of the root tip (Fig. 6*f*), although a few exodermal cells fluoresced brightly in all cases. Under both wet and drying conditions, L_p for root segments immersed in PTS averaged $31 \pm 6\%$ of L_p measured for the same segments in water ($n = 6$ roots).

DISCUSSION

Radial conductivity (L_R) for intact root segments of *Agave deserti* was similar to overall hydraulic conductivity (L_P) under wet conditions. During drying, decreases in L_P were due to decreases in L_R, presumably because the maturation of vessels compensated for any drought-induced loss of axial conductance (K_h), such as by embolism (Sperry, 1986; North & Nobel, 1991). After re-wetting, K_h was higher and hence even less limiting than under wet conditions. L_P also increased in response to re-wetting, although not to its value under wet conditions, reflecting a persisting decrease in L_R. Throughout the course of drying and re-wetting, L_R did not differ significantly between the intact distal and midroot regions. In contrast, L_R for individual root tissues differed with root region, reflecting differences in suberization, cell vitality, and tissue morphology.

Under wet conditions, L_R for the individual tissues of the distal region differed by less than a factor of two, indicating that no single tissue predominated in limiting water movement. Hydraulic conductivity is also fairly similar for the individual living tissues of young roots of *Zea mays* (Peterson *et al.*, 1993). However, the contribution of a tissue to the conductivity of an intact root segment of *A. deserti* was not proportional to the tissue's cross-sectional area. Specifically, the epidermis/exodermis and the sclerenchyma/endodermis in the distal region each represented *c.* 5 % of the root cross-sectional area, yet each accounted for *c.* 30 % of the hydraulic resistance. The cortex, in contrast, represented 65 % of the cross-sectional area but only 22 % of the resistance for the distal region, and the stele accounted for 25 % of the area and 20 % of the resistance. For the midroot region under wet conditions, the cortex accounted for 50 % of the area but only 6 % of the resistance, whereas the epidermis/exodermis and the sclerenchyma/endodermis each represented 10 % of the area and 33–43 % of the resistance.

Under wet conditions, the largest disparity in tissue conductivity between the distal and the midroot region occurred for the cortex. The threefold increase in cortical L_R at midroot was accompanied by the loss of nuclei and cytoplasm, which began at ~ 100 mm back from the tip under wet conditions. A similar increase in hydraulic conductivity occurs for roots that are killed by steaming or other treatments and then subjected to hydrostatic pressure differences (Kramer, 1983; Peterson & Steudle, 1993), perhaps because disrupting the membranes also eliminates osmotic gradients that might oppose inward radial flow (Rygol *et al.*, 1993). For *A. deserti*, the death of cortical cells at midroot under wet conditions was not accompanied by the development of the intercellular lacunae observed after soil drying, thus the cortex retained its structural integrity. Under wet conditions, L_R for the stele was also higher at midroot than in the distal region, perhaps reflecting the shorter distance within the stele between the outermost parenchyma cells and the fully mature, conducting metaxylem vessels.

During drying, the cross-sectional areas of the epidermis/exodermis and the sclerenchyma/endodermis in the distal region each increased to ~ 9 % of the total cross-sectional area due to an approximate doubling in the cell layers of the exodermis and sclerenchyma. Concurrently, L_R for each of these layers decreased by ~ 90 %. At midroot, the exodermis increased by about one cell layer during drying, and L_R for the epidermis/exodermis decreased by 70 %. Increases in the number of suberized cell layers apparently did not account for the decreases in L_R, however. The exodermis for the distal region after drying had the same number of cell layers as the exodermis at midroot under wet conditions, yet L_R was more than ten times lower for the epidermis/exodermis of the distal region. In addition, the number of cell layers in the sclerenchyma at midroot did not change during drying, but L_R for the sclerenchyma/endodermis decreased by 80 %. At both the root tip and at the proximal end of 58-d-old roots, the amount of extracted lipids in the suberized tissues was 20–50 % higher after drying than under wet conditions. Such lipids in suberized cell walls decrease their water permeability (Vogt *et al.*, 1983), particularly after exposure to air or drying conditions (Shone & Clarkson, 1988). For *A. deserti*, the drying of suberized tissues was also important in decreasing conductivity; for example, L_R for the epidermis/exodermis was lower in the distal region after drying than at midroot under wet conditions, although the two regions had similar amounts of extracted lipids.

Stripping the epidermis/exodermis from a 10 mm long zone at the root tip had no effect on root hydraulic conductivity of the distal segment under wet conditions, but more than doubled its L_P during soil drying. The constraint on water movement resulting from the suberization of external tissues at the tip might help keep cortical cells in this meristematic region alive and turgid during drying. The immaturity of late metaxylem vessels near the tip can also help restrict water loss from the cortex to a drier soil (Wang, Canny & McCully, 1991).

Further back from the root tip, cortical cells lost turgidity during drying, and the loss of nuclei and cytoplasm that occurred normally during root development was hastened. Cells collapsed and ruptured, leading to the formation of intercellular lacunae in both distal and midroot regions. Such lacunae have been observed previously in the roots of *A. deserti* (North & Nobel, 1991; Huang & Nobel, 1992) and other species (Robards, Clarkson & Sanderson, 1979; Wullstein & Pratt, 1981) in

response to soil drying and root ageing. The intercellular lacunae could have decreased L_R by forcing water to move in the vapour phase. Lacunae could also have increased the mean path length for water moving in a surface film along cell walls by disrupting the integrity of the cell wall network. In addition, gas that entered the interstitial spaces within and between cell walls during drying would have to be displaced for liquid water to move freely in this part of the pathway.

After re-wetting, L_R for the intact distal root region was somewhat lower than under wet conditions, but L_R for its cortex was higher. This increase in cortical L_R is presumably due to the higher conductivity of the cortical cells that died during drying. At midroot, re-wetting did not fully restore cortical L_R to its initial value, perhaps because of the persistence of lacunae. For both root regions, L_R for the suberized tissues was higher after re-wetting than after drying, but neither the epidermis/exodermis nor the sclerenchyma/endodermis recovered full conductivity. In contrast, L_R for the stele in the distal region increased to its initial value; L_R for the stele at midroot was higher after re-wetting than under wet conditions, reflecting the maturation of the xylem during drying and concomitant decreases in the radial distance to mature late metaxylem vessels. One implication of the persisting reductions in L_R for tissues other than the stele is that apical growth and the production of new lateral roots are necessary to restore hydraulic conductivity of the root system after re-wetting.

New apical growth might also influence the pathway of water movement, because substantial uptake of the apoplastic tracer PTS into the stele occurred only in a 20 mm zone near the root tip under wet conditions. At 10–20 mm from the tip, PTS was concentrated in the intercellular spaces, suggesting flow along rather than within cell walls. Further back from the tip under wet conditions and along the entire root after drying, the movement of PTS was restricted by the exodermis, although PTS apparently moved through a few exodermal cells that behaved as passage cells; these passage cells might correspond to the unsuberized cells in the dimorphic exodermis (Shishkoff, 1987; North & Nobel, 1991). Some exodermal cells remained alive during drying in both distal and midroot regions, similar to the short, unsuberized exodermal cells in *Allium cepa* that survive 200 d of drought (Stasovski & Peterson, 1992). Removal of the exodermis allowed PTS to move freely through the cortex, indicating substantial apoplastic flow through this tissue in both the distal and midroot regions. Moreover, at midroot, where the cortical cells are dead, the only pathway for water flow across the cortex is the apoplast. In the absence of negative pressure applied to the root, little PTS uptake occurred, even near the root tip, suggesting that movement of the tracer in the apoplast occurs preferentially in response to a hydrostatic pressure difference, as also occurs for roots of *Zea mays* (Zhu & Steudle, 1991).

In summary, radial conductivity across roots of *A. deserti* was fairly similar for the component tissues in the distal root region under wet conditions, although the suberized layers were less conductive per cross-sectional area than were the cortex and stele. The limitation imposed on L_R by the suberized layers increased during root development and even more during soil drying. Cortical cells died during root development under wet conditions, leading to a higher L_R for the cortex at midroot than in the distal region. During drying, intercellular lacunae developed in the cortex and reduced cortical L_R. Re-wetting after drying increased L_R for all tissues, but not to its values under wet conditions; thus, new apical growth and the development of new lateral roots are necessary for the complete restoration of conductivity. Uptake of an apoplastic tracer was also substantial only near the root tip, although occasional cells in the exodermis allowed passage of the tracer under all conditions. Removal of the exodermis resulted in free movement of the tracer through the cortex, further suggesting that water moved through the apoplast of this tissue much more readily than through other layers of the root.

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