



## Radial Hydraulic Conductivity of Individual Root Tissues of *Opuntia ficus-indica* (L.) Miller as Soil Moisture Varies

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Received: 31 January 1995 Accepted: 3 September 1995

The constraints on water uptake imposed by individual root tissues were examined for *Opuntia ficus-indica* under wet, drying, and rewetted soil conditions. Root hydraulic conductivity ( $L_p$ ) and axial conductance ( $K_h$ ) were measured for intact root segments from the distal region with an endodermis and from midroot with a periderm;  $L_p$  was then measured for each segment with successive tissues removed by dissection. Radial conductivity ( $L_R$ ) was calculated from  $L_p$  and  $K_h$  for the intact segment and for the individual tissues by considering the tissue conductivities in series. Under wet conditions,  $L_R$  for intact distal root segments was lowest for the cortex; at midroot, where cortical cells are dead,  $L_R$  for the cortex was higher and no single tissue was the predominant limiter of  $L_R$ .  $L_R$  for the endodermis and the periderm were similar under wet conditions. During 30 d of soil drying,  $L_R$  for the distal cortex increased almost three-fold due to the death of cortical cells, whereas  $L_R$  for the midroot cortex was unaffected;  $L_R$  for the endodermis and the periderm decreased by 40 and 90 %, respectively, during drying. For both root regions under wet conditions, the vascular cylinder had the highest  $L_R$ , which decreased by 50–70 % during 30 d of soil drying. After 3 d of rewetting, new lateral roots emerged, increasing  $L_R$  for the tissues outside the vascular cylinder as well as increasing uptake of an apoplastic tracer into the xylem of both the roots and the shoot. The average  $L_R$  for intact root segments was similar under wet and rewetted conditions, but the conductivity of the tissues outside the vascular cylinder increased after rewetting, as did the contribution of the apoplastic pathway to water uptake.

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**Key words:** *Opuntia ficus-indica*, prickly pear, root hydraulic conductivity, endodermis, periderm, apoplast, lateral root emergence.

### INTRODUCTION

Water moves from the surface of a root to the root xylem through a series of tissues, each with a hydraulic conductivity that can change with root development and with the availability of soil moisture. When the soil is wet, water movement into the plant is generally limited by the root hydraulic conductivity, not by that of the soil (Passioura, 1988; Oertli, 1991; Nobel and Cui, 1992). In turn, root hydraulic conductivity for most plants is limited by the radial conductivity of the tissues outside the xylem, although the xylem or axial conductivity can be limiting near the root tip where the conduits are immature (McCully and Canny, 1988; Frensch and Steudle, 1989). Determining the radial conductivity of individual root tissues can therefore help pinpoint the major constraints on plant water uptake, including changes that may occur during root development as well as soil drying and rewetting.

Of the tissues outside the xylem, the endodermis, whose conductivity depends on the development of Casparian bands and suberin lamellae (Peterson, Murrmann and Steudle, 1993), can be the main barrier to radial water flow (Clarkson and Robards, 1975; Kramer, 1983). A suberized exodermis may also restrict radial water movement for roots of many species (Perumalla and Peterson, 1985;

Shishkoff, 1987), particularly under dry or saline conditions (Walker *et al.*, 1984; Shone and Clarkson, 1988). The roots of most dicotyledons, including the species examined in the present study, *Opuntia ficus-indica* (L.) Miller, exhibit secondary growth and produce a suberized periderm outside the vascular cylinder, which can also influence water uptake (Moon *et al.*, 1986). The epidermis and cortex are composed of relatively thin-walled, unspecialized cells that have higher hydraulic conductivity than other root cell types (Jones *et al.*, 1988; Radin and Matthews, 1989), although the conductivity of these tissues may be disproportionately affected by their sensitivity to soil drying (Clarkson, Sanderson and Russell, 1968; Jupp and Newman, 1987). In addition, the hydraulic conductivity of all tissues outside the vascular cylinder of main roots may be influenced by the emergence of lateral roots (Häussling *et al.*, 1988; Peterson and Moon, 1993).

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), changes for roots of *O. ficus-indica* in response to soil drying and rewetting (North and Nobel, 1992). Root axial conductivity,  $K_h$ , can limit  $L_p$  for roots of this species, especially when embolism occurs during drought; however, root radial conductivity,  $L_R$ , is generally the primary limiter of root water uptake (North and Nobel, 1992). In the present study,  $L_p$  for roots of *O. ficus-indica* in wet, drying,

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and rewetted soil was determined first for intact segments and then for segments after successively removing individual tissues outside the xylem. The radial conductivity of each tissue was calculated by considering the tissue conductivities as occurring in series. Two root regions were examined, allowing a test of the hypothesis that the endodermis is less limiting to  $L_R$  for the younger root segments than is the periderm for the older segments. Whether  $L_R$  for both the endodermis and the periderm would decrease during soil drying was also examined. In addition, the hypothesis that apoplastic water movement in the radial direction is greater at sites of lateral root emergence than elsewhere along the root, at least under drying conditions (North, Huang and Nobel, 1993), was assessed using a mobile fluorescent tracer. Measurements of cell size, cell vitality, suberization, and lignification were made concurrently to investigate the structural bases for differences in tissue hydraulic conductivity.

## MATERIALS AND METHODS

### Plant material

Thirty cladodes (flattened stem segments) of *Opuntia ficus-indica* (L.) Miller (Cactaceae) were obtained from a plantation at the University of California Agricultural Research Station, Riverside, CA, USA and were rooted in  $32 \times 26 \times 12$  cm tubs containing a 1:1 mixture of field soil and sand. Plants were grown in a glasshouse at the University of California, Los Angeles, where they received a mean total daily photosynthetic photon flux of  $38 \text{ mol m}^{-2} \text{ d}^{-1}$  on a horizontal surface (80% of the ambient solar radiation), with daily maximum/minimum air temperatures of approximately  $28/16^\circ\text{C}$ . Soil water potential in the root zone ( $\Psi_{\text{soil}}$ , MPa), which was determined gravimetrically based on soil moisture release curves for sandy soil (Young and Nobel, 1986), was maintained above  $-0.3$  MPa by watering twice weekly for 14 d after root initiation. For plants in drying soil, water was withheld for the next 30 d; at the end of this time,  $\Psi_{\text{soil}}$  was  $-11$  to  $-12$  MPa. After rewetting,  $\Psi_{\text{soil}}$  increased to  $-0.1$  MPa within 1 d and was maintained at this value by daily watering.

Main roots arising from areoles on the part of a cladode below the soil surface were approximately 350–400 mm long and averaged 3.0 mm in diameter after 30 d in wet soil. Little further elongation occurred in wet or drying soil due to the natural death of most main root tips about 40 d after initiation. Two regions of main roots and of first-order lateral roots were examined: distal, from the root tip to 50 mm back; and midroot, from 200 to 250 mm back from the tip.

### Hydraulic conductivity

Roots were excavated using a small spatula and jets of water, excised, and immediately immersed in water. Distal root segments had adhering sheaths of soil particles and mucilage (Huang, North and Nobel, 1993), which preliminary experiments showed did not substantially affect measurements of hydraulic conductivity. Segments 50 mm long from distal and midroot regions were cut under water

with a razor blade. For roots from wet and drying soil, segments were chosen that lacked externally visible lateral roots; after 3 d of rewetting, lateral roots were present on essentially all segments. A 10-mm length of the vascular cylinder exposed by removing outer tissues was inserted into a 10-mm section of Tygon tubing attached to a glass microcapillary (internal diameter 0.4 mm) that was half-filled with water. A silicone and brass compression fitting was then tightened around the tubing to prevent radial leaks (McCown and Wall, 1979; Lopez and Nobel, 1991). Dental impression material (polysiloxane) and two coats of an acrylic co-polymer (Nobel, Schulte and North, 1990) were applied at the junction between the tubing and the vascular cylinder as well as at the distal cut end of midroot segments before immersing the segment in distilled water.

Negative pressure, which was regulated with a needle valve and monitored with a Validyne PS309 digital manometer (Nobel *et al.*, 1990), was applied to the open end of the microcapillary to induce water flow through the segment. After the volumetric flow rate,  $Q_v$  ( $\text{m}^3 \text{ s}^{-1}$ ), became constant at a given pressure ( $P$ , MPa), usually within 10 min,  $L_p$  (root hydraulic conductivity,  $\text{m s}^{-1} \text{ MPa}^{-1}$ ) was calculated as follows (Nobel *et al.*, 1990):

$$L_p = \frac{1}{A} \frac{Q_v}{\Delta P} \quad (1)$$

where  $A$  ( $\text{m}^2$ ) is the root surface area, which was calculated from the root length and radius. For rewetted roots, the surface area of the new lateral roots was less than 10% of the main root surface area and was not included in  $A$ . Radial leaks at the seals were detected by the appearance in the microcapillary of crystal violet dye, which was added at a concentration of 0.005% (w/w) to the immersion water. Axial conductance measured on 20-mm segments cut at the distal end was always much greater than  $L_p$  for intact or sealed segments, indicating that the compression fitting did not unduly constrict the vascular cylinder.

To measure axial (xylem) conductance per unit pressure gradient ( $K_h$ ,  $\text{m}^4 \text{ s}^{-1} \text{ MPa}^{-1}$ ), distal root segments were trimmed by 10 mm at the tip, and distal-end seals were removed from midroot segments. A 1-mm length at the distal cut end was then immersed in 100 mM potassium chloride (Sperry, 1986).  $Q_v$  was measured and used to calculate axial conductance:

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

where the pressure drop  $\Delta P$  was applied across the length  $l$  (m) of the root segment (Gibson, Calkin and Nobel, 1984).

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

$$L_R = \frac{L_p \alpha l}{\tanh(\alpha l)} \quad (3)$$

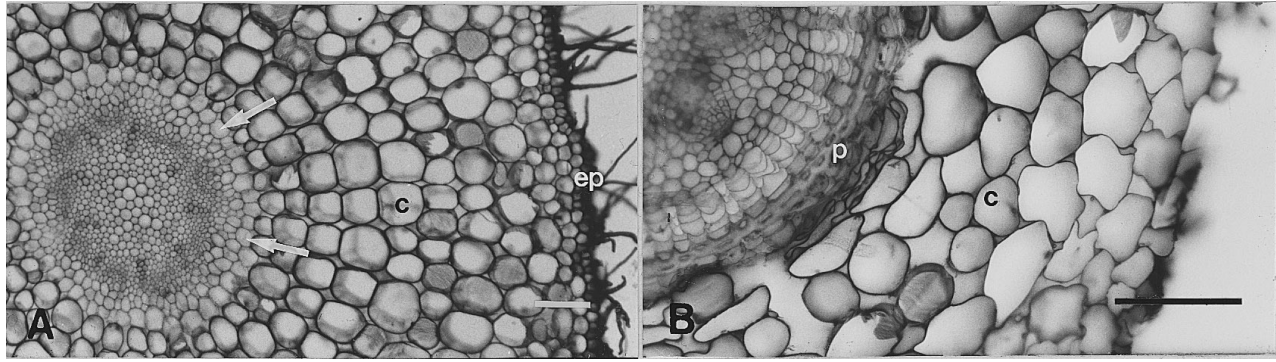


FIG. 1. Cross-sections of root of *Opuntia ficus-indica* (A) from the distal region (25 mm from the tip) under wet conditions and (B) from the midroot region (225 mm from the tip) after 30 d of soil drying; stained with toluidine blue O. ep, Epidermis; c, cortex; arrows indicate endodermis; p, periderm. Bars = 150  $\mu$ m.

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

#### $L_R$ for sequential tissues

After  $L_p$  was measured for an intact root segment, the component tissues were removed in sequence, using fine forceps under a stereomicroscope while the segment was immersed in water. After removal of the outermost tissues, the epidermis and cortex (Fig. 1),  $L_p$  was measured on the stripped segment; then the process was repeated with the next tissue, the endodermis for distal segments (Fig. 1A) and the periderm for midroot segments (Fig. 1B), ending with the vascular cylinder. The epidermis was not considered separately from the cortex because its removal had no measurable effect on  $L_p$ . The occasional distal segments whose endodermis was damaged during removal of the cortex, as determined by subsequent microscopic examination, were not used in calculations of  $L_R$ . For root segments from rewetted plants, tissues were removed without disturbing lateral roots.

After  $L_p$  was measured for the vascular cylinder,  $K_h$  was measured and  $L_R$  was calculated using eqn (3), based on the surface area of the intact root segment. Because the radial conductivities are in series, the reciprocal of  $L_R$  for an intact root segment equals the sum of the reciprocals of  $L_R$  for the epidermis/cortex, the endodermis/periderm, and the vascular cylinder. Thus,  $L_R$  for the  $n$ th tissue ( $L_{R,n}$ ) was calculated for the endodermis/periderm and for the epidermis/cortex from:

$$L_{R,n} = \frac{1}{\frac{1}{L_{R,i+j+\dots+n}} - \frac{1}{L_{R,i+j+\dots,n-1}}} \quad (4)$$

#### Anatomical measurements

To investigate general anatomical features, roots 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 50% ethanol 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, Emanuel and Humphreys, 1981) when viewed with an Olympus BH2 microscope fitted with DMU ultraviolet and DMV violet filter systems (peak excitation wavelengths of 370 and 405 nm, respectively). Cell vitality was assessed by immersing segments in 0.001% (w/w) acridine orange in distilled water for 5 min and viewing with epifluorescence using the DMV filter (Henry and Deacon, 1981; Wenzel and McCully, 1991). The absence of fluorescent nuclei in cells undamaged by sectioning was used only with other evidence (such as the absence of cytoplasm) to indicate cell death.

To investigate possible apoplastic pathways for water movement in excised as well as undisturbed roots, 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS), a mobile fluorescent tracer that is presumably confined to the apoplast and does not bind to cell walls (Peterson *et al.*, 1981; Skinner and Radin, 1994), was used. Some root segments were prepared as for measurements of  $L_p$ , immersed in a 0.01% (w/w) solution of PTS, and a negative pressure of 30 kPa was applied for 1 h to induce flow. To examine apoplastic uptake in undisturbed roots, six plants, two each from wet, drying, and rewetted soil, were watered with 2 l of 0.02% PTS after the drainage holes in the tubs had been plugged with silicone sealant, and the plants were left to transpire in the glasshouse overnight. Two additional plants in wet and rewetted soil were watered with PTS immediately after the cladodes were severed from the root systems, thereby eliminating transpiration, and left in the glasshouse overnight. Root systems were carefully excavated, and root segments were washed in running water for 10 min. Sections were cut with a razor blade, mounted in immersion oil, and immediately viewed with epifluorescence, using the DMV filter.

The amount of soluble lipids in the periderm was determined for midroot segments of roots after 30 d in wet or drying soil. The periderm was removed under a stereomicroscope from eight 50-mm segments per soil treatment and dried under partial vacuum for 24 h. Tissues from two roots from the same soil treatment were combined and weighed, then immersed for 24 h each in methanol,

chloroform, and methanol (Vogt, Schönherr and Schmidt, 1983). The last solvent was removed by pipette, and the tissues were dried under partial vacuum for 24 h before the final dry weight was obtained. The fraction of extracted lipids in the periderm was calculated as (initial dry weight – final dry weight)/(initial dry weight).

Data were statistically analysed by Student's *t*-test or by ANOVA followed by pairwise comparisons using the Student-Newman-Keuls method. Unless indicated otherwise, data are presented as mean  $\pm$  s.e. ( $n$  = number of independent measurements); not significant indicates  $P > 0.05$ .

## RESULTS

### Hydraulic conductivity

Under wet conditions, the hydraulic conductivity ( $L_p$ ) was 30 % higher ( $P < 0.05$ ) at midroot than for distal root segments (Fig. 2A). Neither 30 d of drying nor 3 d of rewetting significantly changed  $L_p$  for distal segments, whereas at midroot  $L_p$  decreased to 17 % of its initial value during 30 d of drying and increased to only 62 % of its initial value during subsequent rewetting ( $P < 0.05$ ). After rewetting,  $L_p$  was not significantly different for distal and midroot segments (Fig. 2A).

Axial conductance ( $K_h$ ) under wet conditions was more than two-fold higher at midroot than for distal root segments ( $P < 0.01$ ; Fig. 2B). As for  $L_p$ ,  $K_h$  for distal segments was not significantly affected by drying or rewetting. However, during 30 d of drying,  $K_h$  at midroot decreased to 40 % of its value under wet conditions ( $P < 0.05$ ) and increased to 60 % of its initial value during 3 d of rewetting (Fig. 2B).

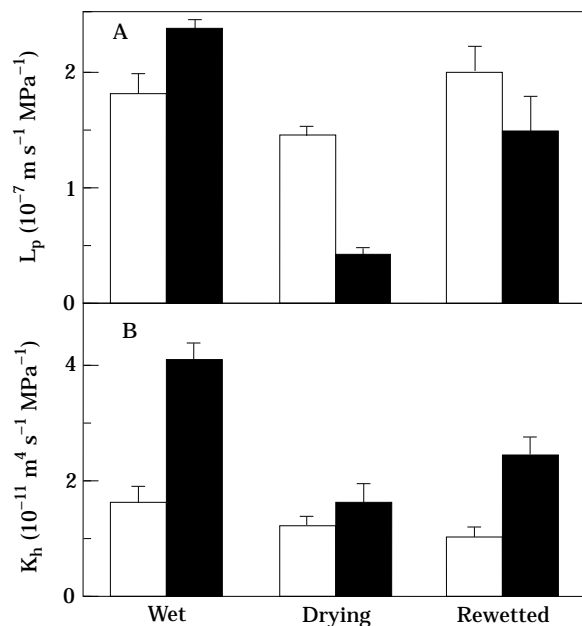


FIG. 2. Root hydraulic conductivity,  $L_p$ , (A) and axial (xylem) hydraulic conductance ( $K_h$ ) (B) for distal (□) and midroot (■) segments of *O. ficus-indica* in wet soil ( $\Psi_{\text{soil}} \geq -0.3$  MPa), after 30 d in drying soil ( $\Psi_{\text{soil}} = -12$  MPa), and after 3 d of rewetting ( $\Psi_{\text{soil}} = -0.1$  MPa). Data are means  $\pm$  s.e. for  $n = 5$  plants.

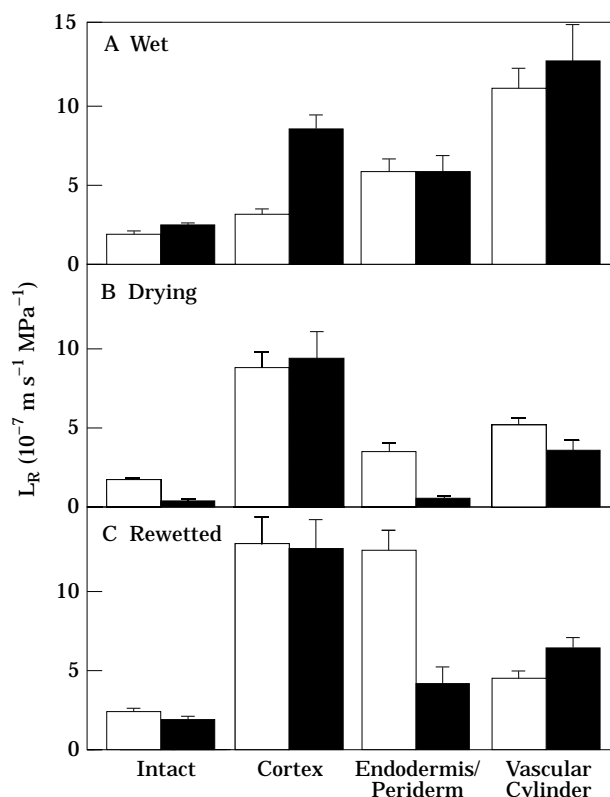


FIG. 3. Radial conductivity ( $L_r$ ) for distal (□) and midroot (■) segments of *O. ficus-indica* under wet conditions ( $\Psi_{\text{soil}} \geq -0.3$  MPa) (A), after 30 d in drying soil ( $\Psi_{\text{soil}} = -12$  MPa) (B), and after 3 d of rewetting ( $\Psi_{\text{soil}} = -0.1$  MPa) (C).  $L_r$  for intact root segments was calculated using  $L_p$  and  $K_h$  (Fig. 2) in eqn (3) and for individual root tissues using eqn (4). Data are means  $\pm$  s.e. for  $n = 5$  plants.

### Hydraulic conductivity of sequential tissues

For both distal and midroot segments under wet conditions,  $L_r$  was greater for the vascular cylinder than for the other tissues (Fig. 3A).  $L_r$  for the endodermis of distal root segments was similar to  $L_r$  for the periderm at midroot under wet conditions. For the cortex, on the other hand,  $L_r$  was nearly three times higher at midroot than for distal root segments ( $P < 0.001$ ; Fig. 3A).

During 30 d of soil drying,  $L_r$  for intact distal root segments decreased slightly but not significantly, whereas  $L_r$  for the distal cortex increased nearly threefold ( $P < 0.001$ ; Fig. 3B). For the endodermis and vascular cylinder of distal segments,  $L_r$  during drying decreased by 39 and 53 % ( $P < 0.05$  for both), respectively. At midroot,  $L_r$  for intact segments decreased by 83 % during drying ( $P < 0.001$ ; Fig. 3B).  $L_r$  for the midroot cortex was unchanged during drying, whereas  $L_r$  for the periderm and vascular cylinder decreased by 90 % ( $P < 0.001$ ) and 72 % ( $P < 0.01$ ), respectively.

During 3 d of rewetting,  $L_r$  for intact distal root segments increased by 31 % over its initial value ( $P < 0.05$ ), reflecting an overall four-fold increase in  $L_r$  for the cortex ( $P < 0.001$ ) and a doubling in  $L_r$  for the endodermis ( $P < 0.01$ ; Fig. 3C). After rewetting,  $L_r$  for the vascular cylinder of distal segments remained about 40 % lower than its initial value.

TABLE 1. Cortex properties for roots of *Opuntia ficus-indica* in wet soil ( $\Psi_{\text{soil}} \geq -0.3$  MPa) and after 30 d in drying soil ( $\Psi_{\text{soil}} = -12$  MPa). Data are means  $\pm$  s.e. for measurements in the distal region (25 mm from the tip) or midroot region (225 mm from the tip) of roots from  $n = 5$  plants (40 cells for diameters)

Region	Soil	Cell diameter ( $\mu\text{m}$ )	Number of cell layers	Cell condition
Distal	Wet	113 $\pm$ 5	10.1 $\pm$ 1.4	Living
	Dry	95 $\pm$ 7	9.3 $\pm$ 1.5	50% dead
Midroot	Wet	97 $\pm$ 8	8.2 $\pm$ 1.3	Dead
	Dry	73 $\pm$ 4	8.1 $\pm$ 1.6	Dead

At midroot after rewetting,  $L_r$  for intact segments was 82% of its initial value,  $L_r$  for both cortex and periderm were similar to initial values ( $P > 0.05$  in both cases), and  $L_r$  for the vascular cylinder was about half of its initial value ( $P < 0.05$ ; Fig. 3C).

#### Changes in the epidermis and the cortex

The epidermis was the outermost layer for distal and midroot segments, although epidermal cells were dead at midroot for both wet and drying soil. For distal root segments under wet conditions, epidermal cells were alive at 50 mm from the root tip; they had a mean radial length of  $15.3 \pm 0.9$   $\mu\text{m}$  and a tangential length of  $16.4 \pm 0.5$   $\mu\text{m}$  (eight cells measured in each of  $n = 5$  roots). Despite the presence of a soil sheath that adhered to the roots more tightly in drying soil, epidermal cells after 30 d of soil drying were dead at 25–35 mm from the root tip. At midroot under all soil conditions, epidermal cells were dead and collapsed. The epidermis represented 1.0% or less of the root cross-sectional area for all root segments.

The outermost one or two layers of the cortex were slightly lignified but did not exhibit suberin deposits in the radial walls or suberin lamellae after staining with Sudan dyes or viewing with epifluorescence. For both distal and midroot segments, the cortical cells decreased in diameter during 30 d of soil drying ( $P < 0.05$ ; Table 1). Intercellular spaces and fissures were enlarged or created by such cell shrinkage, particularly in the outer layers of the cortex at midroot. Under wet conditions, cortical cells in the distal root region were alive, with fluorescent nuclei apparent after staining with acridine orange (Fig. 4). After 30 d of soil drying, about 50% of the cortical cells in the distal region (25 mm from the root tip) lacked fluorescent nuclei. As for epidermal cells, cortical cells at midroot were dead for wet and drying soil; such cells lacked nuclei and also were frequently filled with gas bubbles (Fig. 5). Under wet and drying conditions, the cortex represented about 71% of the root cross-sectional area for distal root segments. At midroot under wet conditions, the cortex represented  $48 \pm 4\%$  of the cross-sectional area, declining to  $40 \pm 3\%$  ( $P < 0.05$ ) after 30 d in drying soil.

#### Changes in the endodermis and the periderm

Under wet conditions, an endodermis was present just inside the cortex of the distal root region (Fig. 1A), with Casparian bands evident at 10 mm from the root tip and a few cells with suberin lamellae in radial and tangential cell walls present at 25 mm. At 50 mm from the root tip, about 50% of the endodermal cells had suberin lamellae. After 30 d in drying soil, Casparian bands were present within 5 mm of the root tip, and over 50% of the cells had suberin lamellae at 25 mm from the tip after 30 d in drying soil. The dimensions of the endodermis were not affected by soil drying (Table 2), this layer representing about 2% of the root cross-sectional area under wet and drying conditions.

Under wet conditions, the periderm began to develop at about 70–90 mm from the root tip. After 30 d in drying soil, one to two layers of periderm were present at 50 mm from the tip. At midroot under wet conditions, eight layers of periderm (strictly, phellem; the unsuberized phelloderm cells were not considered) were present (Table 2). Of these, one to two layers had thickened cell walls that stained more strongly for lignin than for suberin (cf. Figs 6 and 7). During drying, the number of periderm layers increased by 34% ( $P < 0.01$ ; Table 2), and the number of layers with thickened cell walls approximately doubled. Under wet conditions, the periderm represented  $16.1 \pm 0.3\%$  of the root cross-sectional area, increasing to  $22.5 \pm 1.8\%$  after 30 d in drying soil ( $P < 0.05$ ). Extracted lipids represented  $3.9 \pm 1.4\%$  of the periderm dry weight under wet conditions and  $3.2 \pm 0.5\%$  after soil drying ( $n = 8$  plants).

#### Changes in the root tips, vascular cylinder, and lateral roots

In wet soil, root tips were white and turgid. After 30 d of soil drying, root tips were darker and less turgid; also, the distal 15 mm of the vascular cylinder was then discoloured and easily removed from the proximal portion. In the proximal 35 mm of distal root segments and at midroot, parenchyma cells in the vascular cylinder were turgid and alive during soil drying and rewetting. For distal root segments, no secondary xylem was present under wet conditions; after soil drying, secondary vessels and xylem fibres were present at 25 mm from the tip. Fibres were abundant in the vascular cylinder at midroot, particularly after soil drying. Under wet and drying conditions, the vascular cylinder represented about 25% of the cross-sectional area of distal root segments and about 37% at midroot.

For distal root segments under wet conditions, no lateral root primordia were observed. During drying,  $4.3 \pm 0.3$  primordia developed per 10 mm of the distal segment ( $n = 5$  plants), appearing as hemispherical swellings on the vascular cylinder. At midroot for wet and drying soil,  $3.2 \pm 0.4$  and  $3.6 \pm 0.6$  lateral root primordia per 10 mm of main root, respectively, were present internal to the periderm of the main root. After 3 d of rewetting, new lateral roots had an average maximum length of about 8 mm (measured from the surface of the main root).

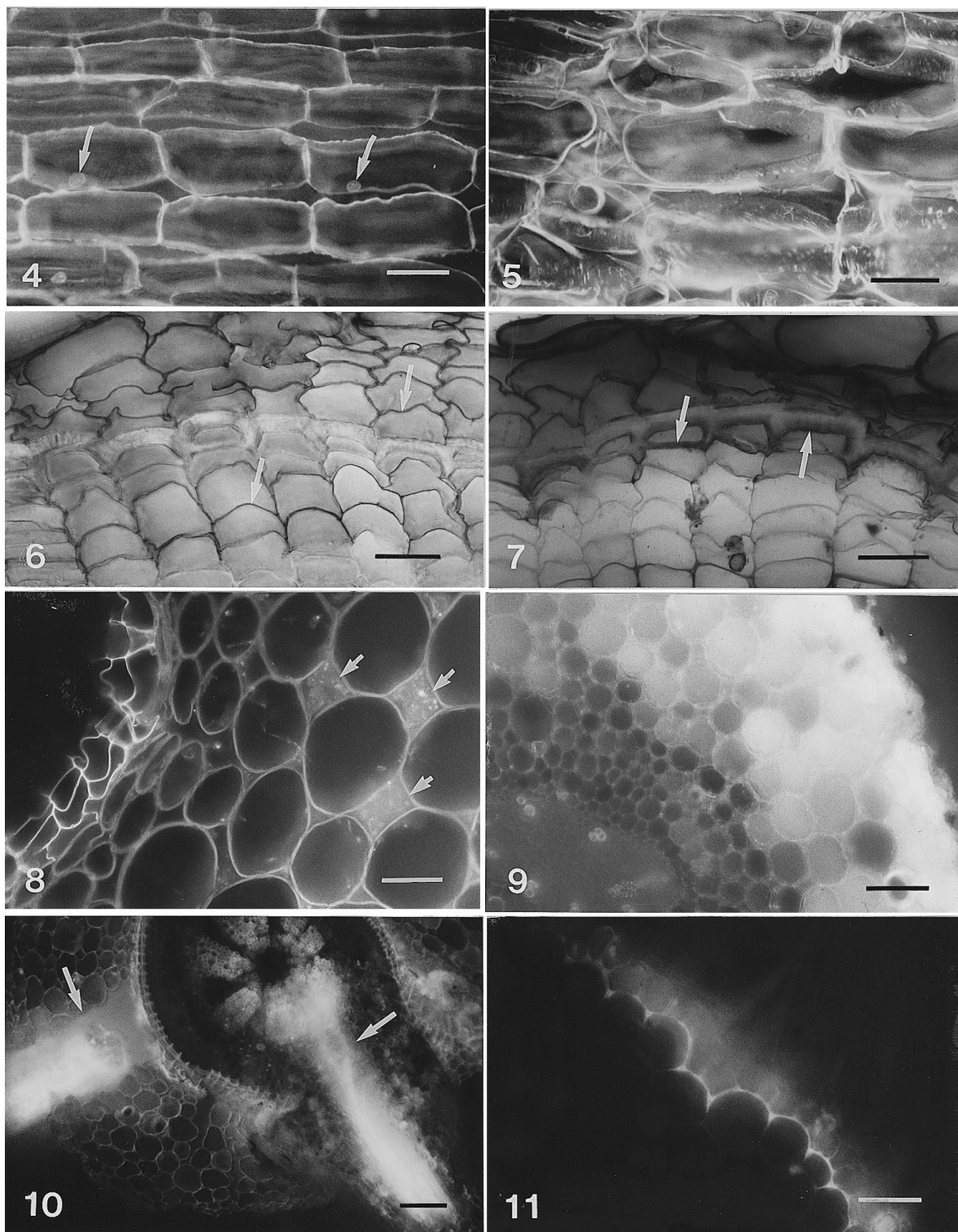


FIG. 4. Light micrograph using epifluorescence of longitudinal section through the cortex at 25 mm from the root tip (distal region) of *O. ficus-indica* under wet conditions, stained with acridine orange; arrows indicate fluorescing nuclei. Bar = 100  $\mu$ m.

FIG. 5. Longitudinal section through the cortex at 225 mm from the root tip (midroot region) after 30 d of soil drying, stained with acridine orange. Bar = 100  $\mu$ m.

FIG. 6. Cross-section through the periderm at 225 mm from the root tip (midroot region) under wet conditions, stained with Sudan dyes; suberized cell walls (indicated by arrows) are dark. Bar = 25  $\mu$ m.

TABLE 2. Properties of the endodermis or periderm for roots of *O. ficus-indica* in wet soil ( $\Psi_{\text{soil}} \geq -0.3$  MPa) and after 30 d in drying soil ( $\Psi_{\text{soil}} = -12$  MPa). Data are means  $\pm$  s.e. for measurements made at 25 mm (distal region) or 225 mm (midroot region) from the root tip for  $n = 5$  plants.

Region	Tissue	Soil	Radial cell length ( $\mu\text{m}$ )	Tangential cell length ( $\mu\text{m}$ )	Number of cell layers
Distal	Endodermis	Wet	$56 \pm 3$	$46 \pm 4$	1
		Dry	$48 \pm 3$	$41 \pm 4$	1
Midroot	Periderm	Wet	$14 \pm 1$	$37 \pm 3$	$8.0 \pm 0.5$
		Dry	$13 \pm 1$	$37 \pm 3$	$10.7 \pm 0.6$

TABLE 3. The uptake of the apoplastic tracer 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) by roots of *O. ficus-indica* in wet soil ( $\Psi_{\text{soil}} \geq -0.3$  MPa) and after 30 d in drying soil ( $\Psi_{\text{soil}} = -11$  MPa) followed by 8 or 80 h of rewetting ( $\Psi_{\text{soil}} = -0.1$  MPa) for  $n = 5$  roots

Treatment	Root region	Tissue	Number of roots showing PTS uptake		
			Wet	Rewetted	
				8 h	80 h
Excised, vacuum applied	Distal	Cortex	3	5	5
		Xylem	0	0	2
	Midroot	Cortex	3	5	5
		Xylem	2	2	3
Attached, transpiring	Distal	Cortex	4	5	5
		Xylem	0	0	1
	Midroot	Cortex	3	5	5
		Xylem	2	1	4
Attached, no transpiration	Distal	Cortex	1	—	3
		Xylem	0	—	0
	Midroot	Cortex	2	—	2
		Xylem	0	—	0

### PTS uptake

No qualitative differences in PTS uptake were apparent between excised roots immersed in PTS with a partial vacuum applied to the vascular cylinder *vs.* root segments from plants that were watered in soil with PTS and then allowed to transpire overnight (Table 3). For these two categories, PTS was observed in the cortex of both distal and midroot segments for wet soil and after rewetting for 8 and 80 h. In the region from 5 to 15 mm from the root tip, PTS-induced fluorescence was apparent throughout the cortex, although less intense in the innermost layers. Proximal to 15 mm, fluorescence in the cortex was much less intense. Although the precise cellular location of the PTS

was difficult to determine near the tip, at 50–100 mm from the tip under wet conditions the tracer was chiefly concentrated in the intercellular spaces (Fig. 8). After 30 d of soil drying followed by 8 h of rewetting, PTS-induced fluorescence in the cortex, where most cells were then dead, was more intense than under wet conditions (Fig. 9). For roots subjected to either vacuum or transpirational pull, PTS appeared in the xylem of distal segments only after 80 h of rewetting, whereas at midroot some PTS was observed in the xylem under all three soil conditions (Table 3). Uptake was particularly evident at the sites of lateral root branching after 80 h of rewetting (Fig. 10), although PTS was observed in the xylem of only two out of eight lateral roots examined.

When transpiration was eliminated by removing the

FIG. 7. Cross-section through the periderm in the midroot region of the same root as in Fig. 6, but stained with toluidine blue O; lignified regions in cell walls (indicated by arrows) stained darker than suberized regions. Bar = 25  $\mu\text{m}$ .

FIG. 8. Cross-section at 75 mm from the tip of a root under wet conditions exposed to the apoplastic tracer PTS in soil for 8 h; arrows indicate intercellular locations of PTS. Bar = 50  $\mu\text{m}$ .

FIG. 9. Cross-section at 10 mm from the tip of a root after 30 d in drying soil exposed to PTS in soil for 8 h; PTS-induced fluorescence is apparent in all but the innermost layers of the cortex. Bar = 100  $\mu\text{m}$ .

FIG. 10. Cross-section at about 200 mm from the tip of a root after 3 d of rewetting followed by exposure to PTS in soil for 8 h; PTS-induced fluorescence is apparent in the two new lateral roots (arrows), and in the xylem of the main root. Bar = 200  $\mu\text{m}$ .

FIG. 11. Cross-section at 10 mm from the tip of a root under wet conditions, exposed to PTS for 8 h after cladode was removed to prevent transpiration; PTS-induced fluorescence is external to the outer tangential wall of the outermost cortical cells. Bar = 50  $\mu\text{m}$ .



stems (cladodes) just prior to the application of PTS, tracer uptake was restricted (Table 3; Fig. 11). PTS was observed in the cortex of only 30 % of the root segments under wet conditions and 50 % after 80 h of rewetting, and it was not observed in the xylem (Table 3).

## DISCUSSION

Root hydraulic conductivity ( $L_p$ ) and axial conductance ( $K_h$ ) for distal root segments of *Opuntia ficus-indica* were not significantly affected by soil drying or rewetting, reflecting in part the buffering provided by soil sheaths (Huang *et al.*, 1993). For midroot segments, no soil sheath was present, and both  $L_p$  and  $K_h$  decreased more than 50 % during drying and did not recover completely after rewetting. Although  $L_p$  measured in soil would presumably be lower than that measured here due to resistance at the root-soil interface, especially after 30 d of drying (Nobel and Cui, 1992), the relative contribution of root tissues in the radial pathway should be similar. In any case, the observed changes in  $L_p$  of intact root segments represent a composite of changes in the conductivity of individual root tissues, including a shift in the relative contribution of the apoplastic pathway to radial water flow.

Under wet conditions, radial hydraulic conductivity ( $L_R$ ) was similar for intact distal and midroot segments. With respect to individual tissues,  $L_R$  differed most between the distal and midroot cortex, the higher conductivity of the latter reflecting the higher conductivity of dead *vs.* living root tissue (Kramer, 1983; Peterson and Steudle, 1993). For distal root segments, the cortex represented 71 % of the total root cross-sectional area and 55 % of the hydraulic resistance of the intact segment under wet conditions; at midroot, the cortex accounted for 48 % of the area and 32 % of the resistance. On a cellular basis, if  $L_R$  for the cortex were to represent water moving from cell to cell,  $L_R$  for a cortical cell under wet conditions would be  $3.1 \times 10^{-6} \text{ m s}^{-1} \text{ MPa}^{-1}$  (based on a segment with ten living cell layers) for the distal region and  $7.5 \times 10^{-6} \text{ ms}^{-1} \text{ MPa}^{-1}$  at midroot, where the cortical cells were dead. Considering only the distal region, such an  $L_R$  is 10–30 times larger than the cellular  $L_p$  measured with a pressure probe for living cells in roots of *Triticum aestivum* (Jones *et al.*, 1988), *Gossypium hirsutum* (Radin and Matthews, 1989), and *Zea mays* (Zhu and Steudle, 1991). One implication is that  $L_R$  measured for the cortical tissue of *O. ficus-indica*, as for the other species, apparently represents other pathways for water movement in addition to a cell-to-cell pathway across living cortical cells.

Unlike radial conductivity for the cortex,  $L_R$  for the endodermis in the distal root region equalled that for the periderm at midroot under wet conditions. Thus, the Casparian bands and suberin lamellae in the single cell layer of the endodermis imposed the same constraint on water movement as did the eight lignified and suberized layers of the periderm. The endodermis represented 2 % of the root cross-sectional area and 29 % of the resistance, whereas the periderm represented 16 % of the area and 47 % of the resistance. The relatively low resistance (high  $L_R$ ) of the

periderm may reflect the low extent of its suberization, with only 3 % of its dry weight consisting of extracted lipids, as compared with over 50 % for the periderm of stems of *Betula pendula* (Schönherr and Ziegler, 1980) and 30 % for the endodermis and adjacent sclerenchyma of roots of *Agave deserti* (North and Nobel, 1995). In addition, the thickened cell walls present in one to two layers of the periderm were more lignified than suberized. A pathway for water movement through the middle lamellae and the primary, unsuberized cell walls of the periderm is possible (Schönherr and Ziegler, 1980), as is water passage through pit connections or pores in the suberized, lignified walls. In any case, neither the periderm nor the endodermis was the primary limitation on radial water movement for roots of *O. ficus-indica* under wet conditions.

Under wet conditions, the vascular cylinder had the highest  $L_R$  of all the root tissues in both the distal and the midroot regions. For the distal region, the vascular cylinder represented 26 % of the root cross-sectional area and accounted for 16 % of the radial hydraulic resistance of the intact root segment. At midroot, the vascular cylinder represented 38 % of the area, reflecting increased numbers of vessels and fibres, and 21 % of the resistance. Similarly, the vascular tissue of young roots of *Zea mays* accounts for 8–31 % of the total hydraulic resistance (Peterson and Steudle, 1993). For *O. ficus-indica*,  $L_R$  for the vascular cylinder reflected the conductivity of the living parenchyma cells and, at midroot, of the abundant xylem fibres, perhaps explaining why  $L_R$  for the cylinder was three to four times lower than that measured for flow in the radial direction through the cell walls of various hardwoods (Petty and Palin, 1983).

During soil drying, the contributions of the individual tissues to  $L_R$  for the intact root changed substantially. Although  $L_R$  for most tissues decreased,  $L_R$  for the cortex was unchanged at midroot and increased nearly threefold in the distal region during drying. Both the increase in conductivity for the distal cortex and the similarity in  $L_R$  for the cortex of the two regions after drying resulted from the death of over 50 % of the cortical cells, beginning at about 25 mm from the root tip. After drying, the cortex contributed only 19 % of the hydraulic resistance of the intact segment in the distal region and 5 % at midroot. Although the dehydration of the cell walls and the presence of gas bubbles in the dry cortex could have decreased its  $L_R$  at midroot, such a decrease was apparently offset by radial fissures opening between cortical cells, particularly in the outermost layers, through which water could pass easily during the measurement of conductivity.

In contrast to that of the cortex,  $L_R$  for the endodermis and periderm decreased during root drying by about 40 and 90 %, respectively; the endodermis then accounted for 48 % of the total hydraulic resistance in the distal region and the periderm accounted for 83 % at midroot. The number of endodermal cells with suberin lamellae in the distal region increased during drying, as did the number of cell layers in the periderm at midroot, although the extracted lipids did not change as a percentage of periderm dry weight. Increasing the number of layers decreases the water permeability of *Betula* periderm (Schönherr and Ziegler,



1980), but the dehydration of the suberin *per se*, or its exposure to the air present in the dead, dry cortex, may have been equally important in reducing the conductivity of the endodermis and periderm for *O. ficus-indica* (Vogt *et al.*, 1983; Clarkson *et al.*, 1987). Similarly, the decrease in  $L_R$  for the vascular cylinder during drying may have been due to an increase in the path length from the outside of the vascular cylinder to the vessels due to an increase in the number of parenchyma cells and fibres within the vascular cylinder.

After 3 d of rewetting, the emergence of new lateral roots coincided with increases in  $L_R$  for the cortex of distal and midroot regions and for the endodermis and the periderm. The effect of lateral root emergence on the hydraulic conductivity of main roots varies with respect to species, root age, and measurement techniques (Peterson and Moon, 1993), but an increase in water uptake by a main root often coincides with the early stages of lateral root outgrowth (Häussling *et al.*, 1988; North *et al.*, 1993; Peterson and Moon, 1993). For main roots of *O. ficus-indica*, 3 d of rewetting caused lateral roots to split the dead cortex, thereby increasing its permeability. The endodermis and periderm were similarly interrupted, although connections between the endodermis of the new lateral roots and the suberized layers of the main root may form subsequently. Rewetting did not fully restore  $L_R$  for the vascular cylinder to its initial value under wet conditions, because of the presence of more cells between the vessels and the outside of the cylinder than before soil drying.

In addition to changes in the radial conductivity of individual root tissues, soil drying and rewetting changed the extent of apoplastic water movement within the tissues. Under wet conditions, the fluorescent tracer PTS was taken up into the cortex within 10 mm of the tip; some of the tracer appeared to enter the cytoplasm or vacuole of the living cells, perhaps analogous to the entry of PTS into 5-d-old protoplasts of *Avena sativa* (Wright and Oparka, 1994). Further back from the tip, PTS uptake was more limited and fluorescence was concentrated in the intercellular spaces. After soil drying, PTS was generally taken up throughout the cortex in the distal and midroot regions, reflecting apoplastic movement through the dead cortical cells.

The periderm was a less effective barrier to apoplastic movement than was the endodermis under wet and rewetted conditions. For roots in wet soil and after only 8 h of rewetting, PTS did not cross the endodermis in the distal root region; in contrast, PTS crossed the periderm at midroot for seven out of 20 roots under these conditions. After 3 d of rewetting, three out of ten distal segments showed PTS in the xylem, whereas seven out of ten midroot segments then exhibited PTS in their xylem. For both distal and midroot regions, PTS entered the vascular cylinder at the sites of lateral root emergence; thus, lateral root outgrowth was responsible for both increased apoplastic uptake and increased  $L_R$  of the tissues external to the vascular cylinder. The effects of lateral root emergence on apoplastic flow and  $L_R$  may be transitory, however, depending on the developmental state of tissues at the junctions between main and lateral roots (Peterson and Moon, 1993). The pattern of PTS uptake was the same for

excised roots to which a partial vacuum was applied and for undisturbed roots that were left to take up PTS during transpiration, suggesting that  $L_p$  measurement did not create artificial pathways for water movement. Roots in soil but with transpiration eliminated by shoot removal did not exhibit PTS uptake in the xylem; thus apoplastic flow due to diffusion or osmotic pressure was less than that caused by hydrostatic pressure, as has been observed for the roots of *Zea mays* (Zhu and Steudle, 1991; Steudle, Murrmann and Peterson, 1993).

Rewetting and the emergence of new lateral roots increased the radial conductivity of the tissues outside the vascular cylinder for both distal and midroot regions of *O. ficus-indica*. Concurrently, apoplastic transport was more important to overall water uptake during the first few days of lateral root emergence than at other stages of root development. One implication is that after drought and rewetting in the field, plants may be more likely to take up certain solutes that travel primarily in the apoplast, such as  $\text{Ca}^{++}$  (Robards, Clarkson and Sanderson, 1979; Häussling *et al.*, 1988). In addition, increased NaCl uptake is correlated with apparent increases in the proportional contribution of apoplastic flow in root systems of *Oryza sativa* (Yeo, Yeo and Flowers, 1987), suggesting that salt-intolerant species such as *O. ficus-indica* (Berry and Nobel, 1985) may be particularly susceptible to salt damage in the first few days after the cessation of drought.

During soil drying, the increase in radial conductivity for the cortex of *O. ficus-indica* was more than offset by decreases in conductivity for the endodermis, periderm, and vascular cylinder. Under field conditions, the decrease in  $L_R$  may limit water movement between the roots and the soil during the early stages of drought, before decreases in the hydraulic conductivities of the root–soil interface and of the soil become greater limiting factors (Nobel and Cui, 1992). After 3 d of rewetting,  $L_R$  for intact root segments, averaged for distal and midroot regions, increased to about that for roots under wet conditions. The changes in conductivity accompanying the emergence of lateral roots should allow for substantial water uptake in the field soon after the cessation of drought, even before new lateral roots constitute much of the total root surface area.

#### ACKNOWLEDGEMENT

Financial support from the US National Science Foundation grants DCB-90-02333 and IBN-94-19844 and the Office of Health and Environmental Research, US Department of Energy, Program for Ecosystem Research, grant DE-FG03-93ER61686 is gratefully acknowledged.

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