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Water uptake and structural plasticity along roots of a desert succulent during prolonged drought

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ABSTRACT

Desert succulents resume substantial water uptake within 1–2 d of the cessation of drought, but the changes in root structure and hydraulic conductivity underlying such recovery are largely unknown. In the monocotyledonous leaf succulent *Agave deserti* Engelm. substantial root mortality occurred only for lateral roots near the soil surface; nearly all main roots were alive at 180 d of drought. New main roots were initiated and grew up to 320 mm at soil water potentials lower than – 5·0 MPa, utilizing water from the shoot. The hydraulic conductivity of distal root regions decreased 62% by 45 d of drought and 70% thereafter. After 7 d of rewetting, root hydraulic conductivity was restored following 45 d of drought but not after 90 and 180 d. The production of new lateral roots and the renewed apical elongation of main roots occurred 7–11 d after rewetting following 180 d of drought. Hydraulic conductivity was higher in the distal region than at midroot and often increased again near the root base, where many endodermal cells lacked suberin lamellae. Suberization and xylem maturation were influenced by the availability of moisture, suggesting that developmental plasticity along a root allows *A. deserti* to capitalize on intermittent or heterogeneous supplies of water.

Key-words: *Agave deserti*; endodermis; hydraulic conductivity; root development; xylem.

INTRODUCTION

A trade-off can exist between the ability of a root system to tolerate drought and its ability to respond quickly to brief or intermittent rainfall. Certain root properties associated with drought endurance, such as heavily suberized endodermal and exodermal layers or periderm (Stasovski & Peterson 1993; North & Nobel 1995) and the abscission of fine lateral roots (Huang & Nobel 1992), decrease water uptake by a root system. Yet desert succulents, several of which can endure 2–3 years without rainfall (Szarek, Johnson & Ting 1973; Nobel 1988), can resume stomatal opening and carbon fixation within 1–2 days after the cessation of drought (Szarek *et al*. 1973; Nobel 1988; Ehleringer *et al*. 1991). Such a recovery of shoot function depends on renewed water uptake by the roots. In this regard, the roots of long-lived species in generally unproductive habitats, such as desert succulents, tend to exhibit plasticity, which helps maximize the uptake of intermittently available resources such as water (Grime 1994). Such plasticity can be structural, for example the proliferation of lateral roots in response to a pulse of water (Fitter 1994; Dubrovsky, North & Nobel 1997), or physiological, for example an increase in the rate of water or nutrient uptake by existing roots (Jackson, Manwaring & Caldwell 1990).

Agave deserti, a perennial monocotyledonous succulent native to the Mojave and Sonoran deserts, can endure a year without rainfall (Nobel 1976). The hydraulic conductivity of its root system can decrease by a factor of 10^5 during a 6-month drought (Schulte & Nobel 1989), because of decreases in the hydraulic conductivity of individual roots, in the conductivity of gaps that occur between roots and soil, and especially in the conductivity of the soil itself (Nobel & Cui 1992). In addition, decreases in water uptake can result from the death and abscission of lateral roots (Huang & Nobel 1992). Yet *A. deserti* resumes stomatal opening within 12 h of watering after a drought of 5 months in the field (Nobel 1976), and its succulent leaves recover nearly 30% of their predrought thickness in 24 h (Schulte & Nobel 1989). The structural and physiological characteristics of the root system that permit such shoot recovery were investigated for *A. deserti* during an imposed drought of 6 months. The primary goal was to assess the loss of root function during prolonged drought and to determine whether new root growth was essential for renewed water uptake when the soil was rewetted. Root response to drought and rewetting have been examined for *A. deserti* in the laboratory and the field, but these studies were based on shorter and more sudden drying regimens (e.g. Nobel & Sanderson 1984) in which root systems had not undergone long-term structural changes.

Three hypotheses guided the investigation. One, the relatively long-lived main roots (arising from the stem base) of *A. deserti* can survive a drought of 6 months, aided by the import of water from the succulent shoot but at the cost of greatly reduced root hydraulic conductivity. Two, the roots exhibit plasticity in both structure and hydraulic conductivity; specifically, the distal region (including the root

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tip) is less highly suberized and lignified than the proximal region and consequently has higher conductivity. And three, substantial new root growth after rewetting is required to restore the hydraulic conductivity of the root system to its predrought level. With regard to all three hypotheses, the possible water-uptake redundancy in the root system of *A. deserti* must be considered, as the water needed for shoot recovery may be supplied by relatively few roots or by root regions with sufficiently high hydraulic conductivity.

MATERIALS AND METHODS

Plant material

Thirty plants of *Agave deserti* Engelm. (Agavaceae), collected from Agave Hill at the University of California Philip L. Boyd Deep Canyon Research Center (33°38′ N, 116°24′ W, 820 m) 8 km south of Palm Desert, California, were grown in field soil in a glasshouse at the University of California, Los Angeles. Plants received a mean total daily photosynthetic photon flux of 38 mol m^{-2} d^{-1} (80% of ambient solar radiation), with daily maximum/minimum air temperatures of ≈ 28 °C/16 °C. Soil water potential (Ψ_{soil}), as determined gravimetrically using a moisture-release curve for the field soil (Young & Nobel 1986), was maintained above – 0·3 MPa by watering twice weekly with 0·1-strength Hoagland's solution. Plants were 0·20–0·32 m tall with 8–14 unfolded leaves and 20–35 main roots arising from nodes at the base of the stem just below the leaf bases.

To distinguish between existing roots and new root growth, 1 month before experiments entire root systems were immersed for 10 min in an aqueous solution of neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride, 500 g m^{-3}; Schumacher *et al*. 1983) to stain the existing roots. Plants were then placed in 0·50 m long × 0·35 m wide × 0·15 m deep containers of soil from Agave Hill and watered twice weekly for 30 d before water was withheld. Fifteen days after cessation of watering, to reduce temperature gradients the containers were insulated on all sides with sheets of Styrofoam 20 mm thick and the soil was covered with Styrofoam pellets to a depth of 20 mm; the plants received no further water for up to 180 d. At 45 d of drying, Ψ_{soil} differed according to soil depth because of condensation on the sides and the bottom of the containers, and such differences were monitored throughout the drying period. After rewetting, Ψ_{soil} rapidly increased to – 0·1 MPa and was maintained at that value by daily watering.

Hydraulic conductivity

Roots were excavated using a fine spatula and jets of water, excised, and immediately immersed in distilled water. To measure hydraulic conductivity for distal, midroot, and basal regions, segments 50–70 mm long lacking 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 silicone and brass compression fitting (Lopez & Nobel 1991) was tightened around the tubing to prevent leaks from around the stele. To provide a waterproof seal, dental impression material (polysiloxane) and two coats of acrylic copolymer (Nobel, Schulte & North 1990) were applied at the junction between the tubing and the stele as well as at the distal cut end of midroot and basal segments before immersion of the segment in distilled water. Water flow through the root was induced by applying a negative pressure of 20–50 kPa to the open end of the capillary. Pressure was regulated with a needle valve and monitored with a PS309 digital manometer (Validyne, Northridge, CA, USA). 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 determined 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. Crystal violet dye was added at a concentration of 50 mg kg^{-1} to the immersion solution so that leaks could be detected; if the dye appeared in the capillary, the measurement was disregarded. 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, the waterproof end-seals were removed with a razor blade from midroot segments and distal root segments were trimmed by 10 mm at the tip end to expose cut xylem vessels to the immersion solution. The cut end of the segment (about 1 mm) was immersed in 100 mol m^{-3} potassium chloride to reduce blockage (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}). This flux density 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 $(2\pi r_{root} L_R/K_h)^{1/2}$. Equation 3 was solved by iteration, for which L_R was initially set equal to L_P and gradually increased.

Anatomical measurements

To stain anatomical features differentially, root segments were sectioned with a razor blade and placed in 50 mg kg^{-1} toluidine blue O in distilled water. Other sections were stained with 7 g kg^{-1} Sudan III and IV in ethylene glycol to detect suberin or with 500 mg kg^{-1} phloroglucinol in water followed by 220 g kg^{-1} HCl in water to detect lignin (Jensen 1962). Lignin and suberin, particularly in Casparian bands, were also detected by their autofluorescence (Peterson, Emanuel & Humphreys 1981), which was viewed with a BH-2 microscope (Olympus, Lake Success, NY, USA) fitted with DMU ultraviolet (excitation wavelength 370 nm) or DMV violet (420 nm) filter systems. To assess cell vitality based on fluorescing nuclei, segments were immersed in 10 mg kg^{-1} acridine orange in distilled water for 5 min and viewed with epifluorescence using the DMV filter (Henry & Deacon 1981; Wenzel & McCully 1991). The absence of fluorescing nuclei was considered to indicate cell death only if accompanied by the absence or disintegration of cytoplasm (Wenzel & McCully 1991).

Shoot and root water relations

The water potential of leaves (Ψ_{leaf}) or stems (Ψ_{stem}) was measured by removing a cylinder from midleaf or from midstem using a cork borer 8 mm in diameter, briefly blotting the cylinder, and allowing the tissues to equilibrate in the chamber of a TruePsi thermocouple psychrometer (Decagon, Pullman, WA, USA) for 3 h before measurement. The thermocouple psychrometer was also used to measure root water potential (Ψ_{root}); roots were excavated, wrapped in parafilm, and cut into 5 mm segments inside a humidified chamber. Similar plant samples were frozen, thawed and squeezed through a small tissue press. The osmolality of the expressed liquid was measured with a 5500 vapour pressure osmometer (Wescor, Logan, UT, USA) and used in the Van't Hoff relation to calculate the osmotic pressure (Nobel 1991). The water content of plant material was determined by weighing before and after drying for 48 h in a forced-draft oven at 70 °C.

To investigate the possible transfer of water from the shoot to the roots during drought, the apoplastic tracer sulphorhodamine G (SR; Canny 1990; Canny & Huang 1994) was applied to the stem of intact plants in containers. A reservoir was made by inserting a cork borer 8 mm in diameter through the lowest leaf base and into the stem at a 45° angle; 5 cm^3 of 2 mol m^{-3} SR with sorbitol added to match Ψ_{stem} was dripped into the reservoir through a syringe. The dye was taken up within 8 h, and root sections were examined microscopically using epifluorescence with either the DMV filter (causing the SR to fluoresce yellow-green) or a DMG filter (excitation wavelength 545 nm, causing the SR to fluoresce red).

Shoot water loss to the roots was also investigated by comparing the water relations during drought of plants with roots to those with roots removed. Ten plants were maintained for 30 d without watering in a Conviron E-15 environmental chamber (Controlled Environments, Asheville, NC, USA) with 25 °C/15 °C day/night air temperatures and a 12 h photoperiod, with a total daily photosynthetic photon flux of 26 mol m^{-2} d^{-1}. At 0 d and 30 d, leaf cores were taken from mid-leaf on the youngest fully expanded leaf to determine water potential and water content, and stomatal conductance was measured with a LI-COR 1600 porometer (LI-COR, Lincoln, NE, USA) at 0·5 h after the lights went off. All plants were then excavated, and the roots were removed at the base of five plants. Cut root bases were covered with polysiloxane to reduce evaporation, and all plants were returned to their containers, where the soil was replaced and firmly tamped at the base of the shoot. Plants were returned to the chamber for an additional 45 d without water, and stomatal conductance was monitored weekly. At 45 d, Ψ_{leaf} and leaf water content were measured and the shoot water content of each plant was determined after drying in the oven for 7 d.

Data were statistically analysed by *t*-test or by ANOVA followed by pairwise comparisons using the Student–Newman–Keuls method.

RESULTS

Soil water potential

During the first 45 d of soil drying, the soil water potential (Ψ_{soil}) in the upper 10 cm of the containers decreased rapidly and was two to three times more negative than Ψ_{soil} in the bottom 5 cm (Fig. 1). At 90 d of drying, Ψ_{soil} was – 21 MPa in the top 5 cm, which averaged 1·5 and 3·0 times more negative than Ψ_{soil} in the middle 5 cm and the bottom 5 cm of the containers, respectively ($P < 0{\cdot}01$). At 180 d, Ψ_{soil} was similar in the top and middle regions of the containers and about 1·5 times more negative than at the bottom ($P < 0{\cdot}01$). Condensation occurred on the sides and bottom of the containers up to 145 d of soil drying, leading to moisture gradients analogous to heterogeneity of soil moisture in the field.

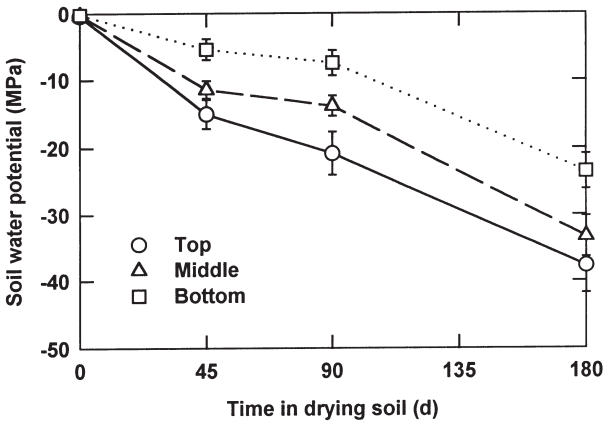

Figure 1. Soil water potential (Ψ_{soil}) for the top (○), middle (△), and bottom (□) 5 cm of soil in containers *of Agave deserti* during 180 d of soil drying. Data are means ± SE for four plants.

Root survival and growth

The main roots of *Agave deserti* experienced less than 5% mortality during 180 d of soil drying (Table 1). In contrast, nearly all fine lateral roots in the top 5 cm of soil died by 180 d (Table 1). In the bottom 5 cm of soil, 81% of fine lateral roots were alive at 180 d of drying, and lateral roots and main roots in this region were flattened against the bottom of the container. A film of moisture occurred directly under such roots, even at 180 d of soil drying.

Under moist conditions (0 d of soil drying), the main roots of *A. deserti* elongated 8·7 mm d^{-1} (Table 2). At 45 d of soil drying, the main roots elongated at 30% of their initial rate, decreasing to 5% at 180 d. New main roots continued to be initiated during 90 d of soil drying, but not thereafter. New apical growth for existing main roots was evident about 2 d after rewetting, but the time required for resumption of the initial elongation rate nearly tripled between 45 and 180 d of drought (Table 2). The duration of drought had no effect on the number of new primary lateral roots produced by main roots in response to rewetting, although the time required for such new roots to appear more than doubled between 45 and 180 d of soil drying ($P < 0{\cdot}05$; Table 2).

Root hydraulic conductivity

The root hydraulic conductivity (L_P) for distal root segments (including the root tip) decreased to about 38% of its initial value at 45 d of soil drying and to about 30% at 180 d (Fig. 2). During 7 d of soil rewetting following 45 d of soil drying, L_P increased to 62% of its value under moist conditions, although rewetting did not similarly restore L_P after 90 or 180 d of drying (Fig. 2). Rapid water uptake after prolonged drought was therefore not attributable to increases in L_P of existing roots. Axial conductance (K_h) for these distal segments increased 20-fold during 180 d of soil drying because of the maturation of late metaxylem vessel elements and was significantly increased by rewetting only after 45 d of drying ($P < 0{\cdot}05$; Fig. 2).

In contrast to the uniformly low hydraulic conductivity of older existing roots, roots newly initiated during drought showed greater plasticity in both conductivity and structure. For a 300 mm long main root initiated during 90 d of soil drying, radial hydraulic conductivity (L_R) in the distal region was $5{\cdot}2 \times 10^{-8}$ m s^{-1} MPa^{-1}, 24% lower in the midroot region, and twice as high in the basal region ($P < 0{\cdot}05$: Fig. 3). The axial conductance (K_h), in contrast, was low in the distal and basal root regions and high in the midroot region (Fig. 3). For similar roots from four plants at 90 d of soil drying, L_R for the basal region was $1{\cdot}2 \pm 0{\cdot}3 \times 10^{-8}$ m s^{-1} MPa^{-1}, and K_h was $6{\cdot}0 \pm 1{\cdot}4 \times 10^{-11}$ m^4 s^{-1} MPa^{-1}. In all cases, L_P was only slightly lower than L_R, indicating that L_R was the principal determinant of root hydraulic conductivity.

Main roots that were initiated during 90 d of soil drying, including the root whose hydraulic conductivity is shown in Fig. 3, differed structurally from older roots that existed before soil drying began. Such differences paralleled the differences in L_P and K_h. In the distal, midroot, and basal regions, younger roots had more living cells in the cortex than did older roots ($P < 0{\cdot}05$; Table 3), in which cortical cells typically lacked cytoplasm. In the distal and basal regions, where L_R was relatively high, younger roots had fewer endodermal cells with suberin lamellae than did older roots (Table 3); further, in a relatively short section (10–30 mm long) of the basal region of younger roots, very few cells had such lamellae in comparison with more distal

Table 1. Survival of roots of *Agave deserti* during 180 d in drying soil in containers 0.15 m deep; main roots were ≥ 3·0 mm in diameter and originated at the base of the stem, whereas fine lateral roots were ≤ 2·0 mm in diameter and branched from the main roots. Data are mean percentages for a particular type of root on a plant ± SE; $n = 4$ plants

	Percentage of roots surviving		
Time in drying soil (d)	Main roots	Fine lateral roots in top 5 cm	Fine lateral roots in bottom 5 cm
45	99 ± 1	66 ± 7	98 ± 4
90	98 ± 9	30 ± 6	92 ± 10
180	96 ± 9	7 ± 1	81 ± 8

Table 2. Growth of main roots and initiation of lateral roots of *Agave deserti* during soil drying and after rewetting; the distal regions of main roots were located in the bottom 5 cm of the soil. Apical elongation was determined by measuring the length of main root regions that were not stained with neutral red. Data are ± SE; $n = 4$ plants

Time in drying soil (d)	Apical elongation of main roots (mm d^{-1})	Time after rewetting to resumption of initial elongation rate (d)	Time after rewetting to appearance of new lateral roots (d)	New lateral roots per main root after 7 d of rewetting (no.)
0	8·7 ± 0·2	–	–	–
45	2·6 ± 0·3	3·9 ± 0·7	2·7 ± 1·3	2·7 ± 1·3
90	0·7 ± 0·2	8·8 ± 0·7	4·6 ± 2·6	3·0 ± 1·4
180	0·4 ± 0·1	11·0 ± 1·3	6·5 ± 1·6	3·2 ± 1·8

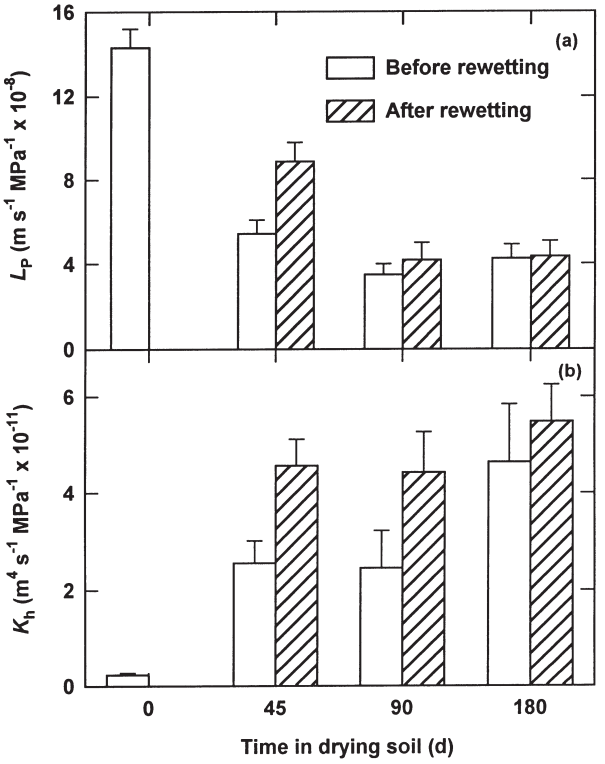

Figure 2. (a) Root hydraulic conductivity (L_P) and (b) axial hydraulic conductivity (K_h) for roots of *Agave deserti* before (open bars) and after (shaded bars) 7 d of rewetting during 180 d of soil drying; distal root regions were located in the bottom 5 cm of the soil. Data are means ± SE for four plants.

regions (Fig. 4a,b). Also in the basal region of younger roots, the late metaxylem was less lignified than at midroot and 34% of the vessels elements had cross walls; in contrast, no cross walls were observed in the midroot region of younger roots or in the midroot or basal regions of older roots, consistent with their higher K_h (Table 3). Similarly, for three roots from two plants in which a root touched the container wall at one location (where a film of moisture was available) and then entered drier soil, vessel elements in the late metaxylem were less lignified in the proximal region (against the container wall) than in the distal (younger) root regions that were in drier soil (Fig. 4c,d).

Root and shoot water potentials

The water potentials (Ψ_{root}) for the distal, midroot, and basal root regions were similar at 0 d of soil drying, decreasing by about 70% during 180 d of drought (Fig. 5a). At the same time, the osmotic pressure for the three root regions increased about two-fold (Fig. 5c), implying a reduction in root turgor pressure. In this regard, the turgor pressure of the basal region exceeded that at midroot at 180 d of drought ($P < 0{\cdot}05$). The water potentials of the stem (Ψ_{stem}) and leaf (Ψ_{leaf}), which were relatively high and similar throughout 180 d of soil drying, decreased by about 50%, while their osmotic pressure increased by about 60% (Fig. 5b,d). At 180 d of soil drying, Ψ_{leaf} and Ψ_{stem} were higher than Ψ_{root} for the midroot and basal root regions ($P < 0{\cdot}05$).

Water transfer from the shoot to the roots

Eight h after the tracer dye sulphorhodamine G was applied to the stems of plants of *A. deserti* at 180 d of soil drying, sections of main (Fig. 6a) and lateral roots (Fig. 6b) exhibited fluorescence, indicating water transfer from the shoot to the roots. In contrast, when the tracer was applied to the stems of well-watered plants, fluorescence was observed in the leaves but not in the roots, indicating that the tracer moved toward regions of lower water potential. The transfer of water from the shoot to the roots was also indicated by a greater decrease in leaf water content for plants with intact roots than for plants with roots removed before drought. Specifically, the amount of water lost by the shoot during 45 d of soil drying, including the loss by transpiration, was 13·2 ± 1·1 g for plants with intact roots as opposed to 8·7 ± 0·7 g for plants with roots removed ($P < 0{\cdot}05$). Stomatal conductance was similar for plants with and without roots 21 d after drying was begun, as was the leaf water potential (Ψ_{leaf}) at 45 d of drying, indicating that shoot water transfer to the roots and not water loss resulting from transpiration led to the differences in leaf water content.

DISCUSSION

The main roots of *Agave deserti* that existed before the onset of soil drying survived 6 months without additional water, but with a 70% reduction in root hydraulic conductivity, while the survival of lateral roots depended on their location in the soil. The condensation of water led to differences in water potential of as much as 10 MPa between the

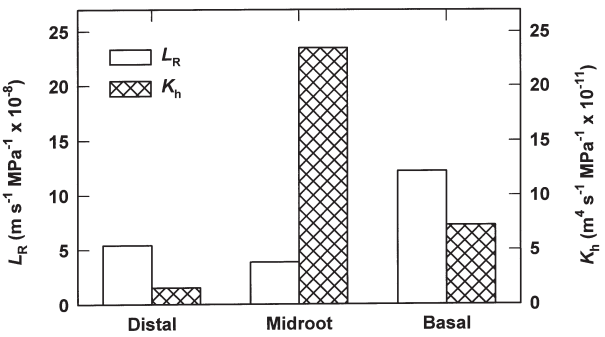

Figure 3. Radial hydraulic conductivity (L_R; open bars) and axial hydraulic conductivity (K_h, shaded bars) for a representative young root of *Agave deserti* at 90 d of soil drying. Data are for 50 mm segments from the distal (includes the root tip), midroot (200–250 mm proximal to the tip), and basal (10–60 mm from the shoot base) regions; the distal root region was located in the bottom 5 cm of the soil.

Root age and region	Living cortical cells (% of total)	Endodermal cells with suberin lamellae (% of total)	Metaxylem vessel elements with cross walls (% of total)
Younger roots			
Distal	98 ± 6	20 ± 2	57 ± 7
Midroot	17 ± 2	96 ± 9	0 ± 0
Basal	66 ± 8	15 ± 3	34 ± 7
Older roots			
Distal	32 ± 5	76 ± 8	64 ± 7
Midroot	0 ± 0	94 ± 7	0 ± 0
Basal	6 ± 1	92 ± 10	0 ± 0

Table 3. Cellular characteristics for main roots of *Agave deserti* at 90 d of soil drying. Younger roots were initiated after soil drying was begun and were ≈ 300 mm long; older roots were established before soil drying and were 500–600 mm long; distal regions of all main roots were located in the bottom 5 cm of the soil. Cells were examined at 10 mm from the root tip in the distal region, 150 mm from the tip in the midroot region, and 30 mm from the shoot base in the basal region. Data are means ± SE; n = 4 plants

surface soil and that near the bottom of the container, this heterogeneity in soil moisture being analogous to that occurring in the field as a result of differences in soil depth and to topographic features such as rocks. In the case of *A. deserti* at a site in the Sonoran Desert, for example, moisture accumulates under rocks, where lateral roots preferentially occur (Nobel, Miller & Graham 1992). Roots of *A. deserti* that survived 6 months of drought showed the morphological adaptation of flattening against the walls of the container, thereby trapping a film of moisture that could be absorbed. Similarly, the roots of the woody chaparral species *Arctostaphyllos viscida* and *Arbutus menziesii* flatten when they grow in rock crevices, forming cortical extensions to maximize water absorption (Zwieniecki & Newton 1995).

New main roots of *A. deserti* were initiated during up to 3 months of drought, when the soil water potential (Ψ_{soil}) near the surface and in the middle of the containers became

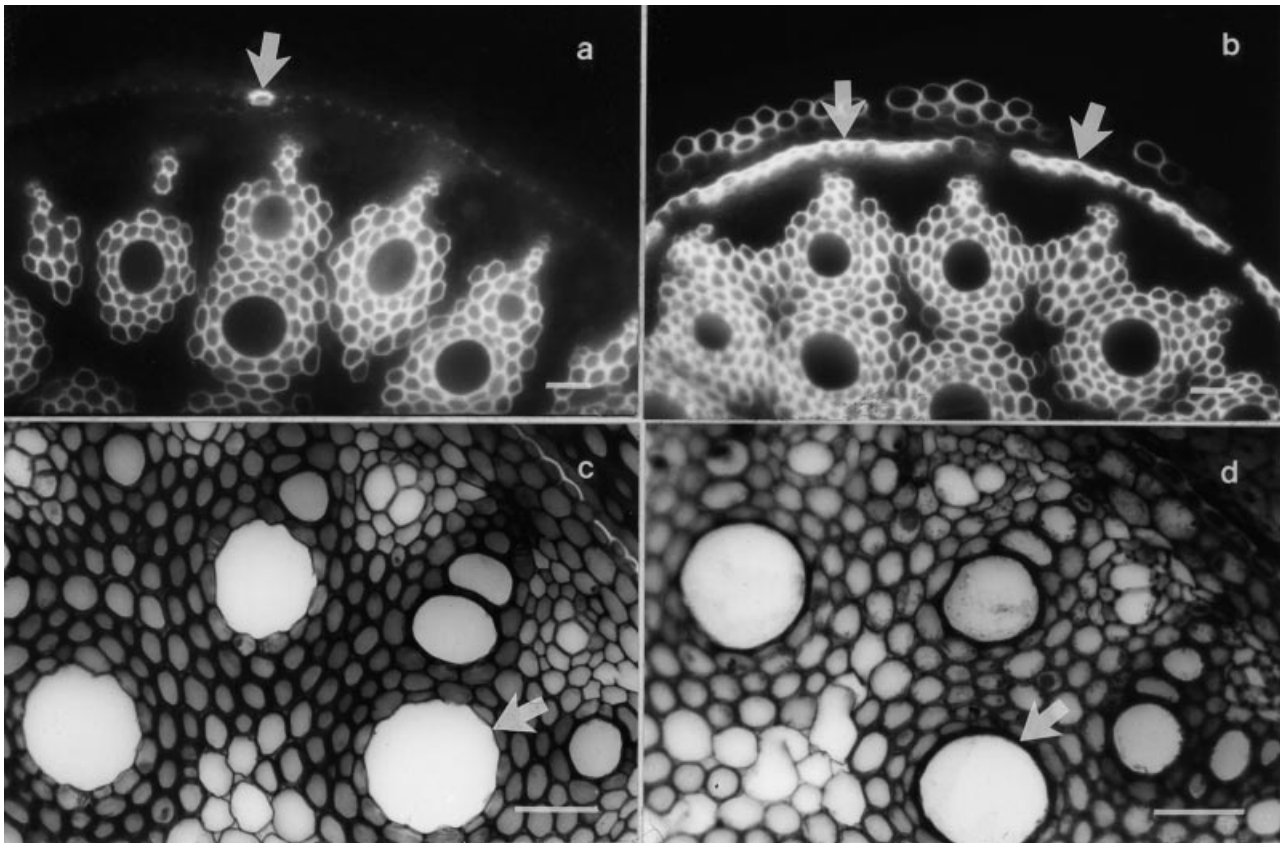

Figure 4. Micrographs of cross-sections of a young root of *Agave deserti* at 90 d of soil drying, made at (a) 20 mm and (b) 40 mm from the shoot base; and at (c) 100 mm and (d) 130 mm proximal to the root tip. For (a) and (b), tissues that are lignified or suberized or both appear white because of autofluorescence under UV light, and arrows indicate endodermal cells with suberin lamellae. For (c) and (d), sections were stained with toluidine blue O, and lignified walls appear dark; arrows indicate vessel elements in the late metaxylem, which are more lignified at 100 mm than in the more proximal section. Bars represent 50 μm.

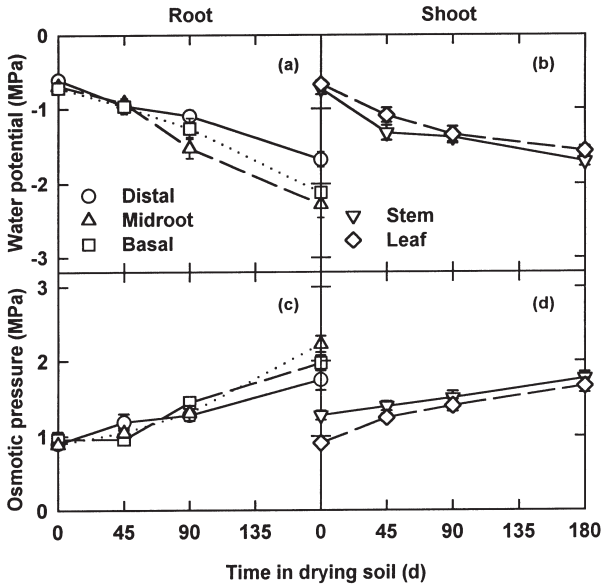

Figure 5. The water potential (a, b) and osmotic pressure (c, d) of the distal (○), midroot (△), and basal (□) root regions and of the leaf (◇) and stem (▽) of *Agave deserti* during 180 d in drying soil; data are means ± SE for four plants.

– 21 MPa and – 14 MPa, respectively. Root apical growth continued, albeit at a much reduced rate, throughout the 6 months of drought; the water required for such root construction and growth came from the succulent shoot, as indicated by the movement of the tracer dye sulphorhodamine G. Root growth for *Glycine max* is similarly supported by water from the shoot, although at a higher substrate water potential (Matyssek, Tang & Boyer 1991). Water moved from the shoot to the roots of *A. deserti* as a result of differences in water potential, with the water potential (Ψ_{root}) at midroot being lower than that of the shoot at 3 and 6 months of drought. The tracer dye accumulated in the protoxylem near the root tip, although Ψ_{root} was higher there than elsewhere in the root; perhaps the living cortical cells near the tip and its relative hydraulic isolation because of the immaturity of the metaxylem contributed to a local water potential gradient causing water movement toward the apical meristem. In any case, water from the shoot moved acropetally through the xylem and helped maintain apical viablity and growth, as occurs for roots of *Lycopersicon esculentum* and *Zea mays* at a Ψ_{soil} of about – 10 MPa (Hunter & Kelley 1946; Portas & Taylor 1976). Rewetting accelerated root apical growth within 2 d, but the time required for the resumption of the pre-drought growth rate doubled between 1·5 and 3 months of drought. At 6 months of drought, 11 d of rewetting were required before the apical growth rate was fully restored.

Lateral root production by main roots of *A. deserti* was not stimulated by soil drying, unlike the case for the cacti *Opuntia ficus-indica* (Dubrovsky *et al*. 1997), *Epiphyllum phyllanthus*, and *Rhipsalis baccifera* (North & Nobel 1994). In addition, the longer the duration of drought, the greater the delay in lateral root emergence in response to rewetting. Considering both main and lateral roots, substantial new growth required more than 7 d of rewetting after a 6-month drought. In the field, therefore, the rapid recovery of shoot function for *A. deserti* after the cessation of prolonged drought (Schulte & Nobel 1989) occurs before renewed root growth. This is analogous to the increased nutrient uptake exhibited by root systems exposed to a pulse of elevated nutrient concentrations, well in advance of root proliferation (Caldwell 1994). Such an increase in nutrient uptake without corresponding root growth can be the result of physiological adjustments (plasticity), allowing existing roots to capitalize on the sudden availability of a resource (Jackson, Manwaring & Caldwell 1990; Grime 1994).

Changes in root hydraulic conductivity (L_p) for *A. deserti* during drought were not consistent with physiological plasticity or structural plasticity, if the latter refers only to changes in root branching and deployment (Fitter 1994). During the first 1·5 months of drought, L_p decreased by about 60% and was restored to about 60% of its pre-drought value by rewetting. During the remainder of the 6-month drought, L_p decreased only slightly and did not respond to rewetting within 7 d. However, younger roots

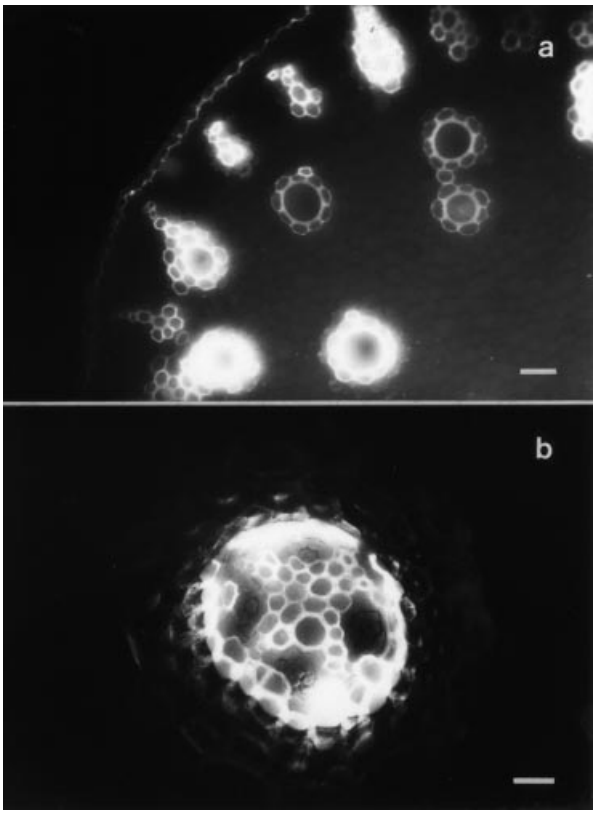

Figure 6. Micrographs of cross-sections of (a) a main root and (b) a lateral root of *Agave deserti* at 180 d of soil drying 8 h after the tracer dye sulphorhodamine G was applied to the stem; white fluorescence indicates dye accumulation (not the autofluorescence of lignified or suberized cell walls). Bars represent 50 μm.

that were initiated during drought showed a more complex pattern of hydraulic conductivity. For such roots, radial hydraulic conductivity (L_R, the principal determinant of L_P) was about twice as high for the basal region as for the mid-root and distal regions at 3 months of drought, indicating an absorptive region located near the shoot base. Anatomically, this region differed from the others in having fewer endodermal cells with suberin lamellae, which led to its relatively high L_R (North & Nobel 1995). Such structural or developmental plasticity within individual roots thus affected water uptake by the root system of *A. deserti* after drought. Specifically, such regions of the newly developed roots would be ready to take up water immediately upon rewetting, unlike the existing roots with greatly reduced L_P.

The low percentage of endodermal cells with suberin lamellae and the high percentages of both living cortical cells and metaxylem vessel elements with cross walls in the basal region, which would lead to relatively high radial conductivity and low axial conductance, were unexpected in that such features are generally characteristic of younger root regions of *A. deserti*, particularly under drying conditions (North & Nobel 1991, 1995; Huang & Nobel 1992). The proximity of the basal root region to the succulent shoot suggests that the better hydration of the tissues in that region compared with the more distal root regions may have delayed the deposition of suberin and lignin in cell walls. In this regard, the turgor pressure (calculated as the water potential plus the osmotic pressure) tended to be higher in the basal region than at mid-root, indicating a difference in tissue hydration that could have affected developmental processes. Similarly, the development of the metaxylem was apparently linked to the availability of soil moisture, as vessel elements in proximal (older) root regions that developed in relatively moist soil next to the walls of the containers were less lignified than vessel elements in distal (younger) regions of the same roots in drier soil. A similar delay in tissue maturation could occur in root regions developing in moist microsites in the field, such as under rocks (Nobel *et al*. 1992). Root age and tissue maturation are also uncoupled for roots of *Cicer arietinum* in heterogeneously moist soil (Spaeth & Cortes 1995), although soil moisture has no effect on anatomical development for roots of *Zea mays* in partitioned containers with different soil water contents (Watt *et al*. 1996). In the basal root regions of *A. deserti* where metaxylem vessel elements were immature, axial hydraulic conductivity (K_h) was lower than at midroot; however, the relatively high L_R in such regions led to high L_P despite the reduced axial flow.

Absorptive root regions could be crucial for the water balance of *A. deserti*, other desert perennials, and plants in general during prolonged drought. In particular, the location of such regions near the shoot base of *A. deserti* could help intercept rainfall that is directed downward as stemflow (Martinez-Meza & Whitford 1996) by the leaves, which occur in a basal rosette. Furthermore, upper root regions could respond to light rainfall that moistens only the top 20–40 mm of soil. Because root regions with a relatively high L_P tend to lose water to a drier soil as readily as they take it up from a wetter soil (Caldwell & Richards 1989), their location near the shoot base would also be advantageous because of cooler temperatures and higher soil water potentials directly under the massive shoot compared with those in the surrounding soil. In addition, a relatively high root L_P does not necessarily lead to water loss to the soil, as the overall conductivity of the root–soil system during drought is limited by the low conductivities of root–soil air gaps and the dry soil (Nobel & Cui 1992; North & Nobel 1997). The primary contribution of absorptive root regions located under rocks might be to prolong water uptake as the surrounding soil dries out. Absorptive root regions located near the base of the plant and under rocks could contribute disproportionately to the recovery of water uptake by *A. deserti* after drought, despite their limited surface area. On the basis of plant excavations and allometric analysis (Hunt & Nobel 1987) as well as determinations of whole-plant water uptake and loss (Alm & Nobel 1991), the root system of *A. deserti* lacks redundancy; that is, L_P measured on representative roots and multiplied by the total root surface area and the soil–root water potential difference under wet conditions approximately equals an independent measurement of transpirational water loss under wet conditions. In the absence of new root growth following rewetting after a 6-month drought, in which the average L_P decreased 70%, the contribution of absorptive root regions would therefore be necessary to permit recovery of shoot water content, which occurs within 3–5 d (Schulte & Nobel 1989).

With regard to the three hypotheses guiding this investigation, the first was largely supported: main roots of *A. deserti* survived a 6-month drought, utilizing water imported from the shoot, but with a 70% reduction in the hydraulic conductivity of existing roots. The second hypothesis, that roots exhibit plasticity in structure and hydraulic conductivity, was also supported, although the higher conductivity and tissue immaturity of the basal root regions of younger roots were unexpected, suggesting that tissue maturation in roots of *A. deserti* can be linked to moisture availability. The third hypothesis was not supported, in that substantial new root growth did not occur within 7 d of rewetting, nor did older existing roots recover their predrought hydraulic conductivity. This suggests a new hypothesis: root regions with higher-than-average hydraulic conductivity develop where moisture continues to be available during drought, such as from the shoot or from patches in the soil, and are crucial to the quick recovery of *A. deserti* upon the cessation of drought.

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