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Recovery of citrus surface roots following prolonged exposure to dry soil

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Abstract

The effects of prolonged exposure to dry surface soil on the capacity of roots to take up water and phosphorus were examined in mycorrhizal sour orange (*Citrus aurantium* L.) seedlings grown in pots with upper and lower portions separated hydraulically. In the first experiment, upper portions of the pots were either irrigated every 2–3 d, droughted for 14 d, droughted for 43 d, or droughted for 42 d followed by 8 d re-irrigation. Lower portions of the pots were irrigated and fertilized every 2–3 d. Phosphorus uptake capacity was estimated in excised roots using ^{32}P in aerated 50, 750, and 1500 μM P solutions. Exposure to dry soil had no appreciable effect on P uptake capacity. In the second experiment, the ability of intact roots to acquire water and P in the 8 d following rewatering after roots were exposed to localized drought for 14 and 43 d was examined. Roots were observed non-destructively using small transparent tubes (2 cm diameter) and a rigid borescope. Soil water depletion was monitored using time-domain reflectrometry. Phosphorus (^{32}P) was added at various depths in the soil in the upper compartment and uptake was assessed by non-destructively counting beta particle emissions from leaves using a scintillation probe. Similar to the first experiment, localized drought had no effect on P uptake and soil water depletion in citrus roots compared to continuously irrigated plants. Water and P uptake in the first few days apparently occurred from existing roots because of delayed production of new roots in the droughted treatment. Thus, citrus roots exposed to extended periods of dry soil apparently maintain or very quickly recover P and water uptake capacity. This behaviour is consistent with an overall rooting strategy where essentially no surface roots are shed following prolonged exposure to dry soil.

Key words: Drought, split-root, ^{32}P, citrus, uptake kinetics, phosphorus uptake, water uptake, sour orange, *Citrus aurantium*.

Introduction

The soil surface layers, with their accumulation of roots, organic matter and nutrients, commonly undergo large fluctuations in temperature, water content, and nutrient availability. These fluctuations promote pulses of nutrient release by increasing microbial biomass turnover (Reid, 1974; Stark, 1994; Cui and Caldwell, 1997). Moisture and temperature fluctuations may cause opportunities for nutrient uptake by roots in the litter, organic and surface mineral layers to be brief and unpredictable (Grime, 1994). There are at least two basic strategies for exploiting resources in soil surface layers that periodically dry and which may develop high temperatures. One strategy is to grow rapidly relatively thin inexpensive roots that have high absorptive capacity when the soil is wet, and then rapidly shed these roots when the soil becomes dry or otherwise unfavourable. This kind of root strategy is commonly found in hot desert climates with desert succulents (Huang and Nobel, 1992) and in certain desert half-shrubs like *Cryptantha* (personal observation). In addition, many annual crops seem to exhibit this strategy (Eissenstat and Yanai, 1997).

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In citrus, quite a different strategy is exhibited. ^{14}C partitioning in droughted seedling roots of four citrus genotypes was investigated using a vertical split-pot system in which sandy soil was irrigated in bottom pots of all plants and in top pots of control plants (Kosola and Eissenstat, 1994). Sandy soil in droughted top pots was dry within a few days after ceasing irrigation. After 14 d, root length, ^{14}C imported into roots, and percentage of intensely labelled roots were similar in droughted and control treatments. However, after 20 d, C allocation to roots in the top pots of droughted treatments was significantly less than allocation to roots in top pots of irrigated treatments. Very few roots in top pots died as determined by ^{14}C-labelling or direct observation, even after approximately 90 d of exposure to dry soil.

If roots are maintained in dry surface layers, how well do they recover when the soil is rewetted? Little is known on how dry soil affects the physiological capacity of roots to take up water and nutrients. Much of the work has been done on grasses, which typically exhibit substantial reduction in ability to take up water (Wraith and Baker, 1991; Wraith *et al.*, 1995), nitrogen (BassiriRad and Caldwell, 1992*a*; Brady *et al.*, 1995) and phosphorus (Shone and Flood, 1983; Jupp and Newman, 1987) for the first 2–3 d or more after rewetting. Subsequent uptake is often accomplished by new root growth. In the desert shrub, *Artemisia tridentata*, however, roots can maintain nutrient uptake capacity under severe water stress (–5.0 MPa) (Matzner and Richards, 1996). Indeed, P uptake capacity was enhanced in drying soil.

In this study, the extent that exposure of citrus roots to dry soil influences their subsequent ability to take up P and water was examined. Root growth was also carefully monitored during the days immediately following irrigation to determine if new root growth could account for water and nutrient uptake.

Materials and methods

General

Sour orange (*Citrus aurantium* L.) seedlings were grown in vertical split pots where the top soil was hydraulically separated from the bottom soil by a waterproof wax membrane (Nambiar, 1977). Treatments consisted of plants that were continuously watered in the top and bottom halves of the pot and droughted treatments where the top portion was not irrigated for different lengths of time. In the first experiment, specific rates of P (^{32}P) uptake from excised roots was determined in solutions with different P concentrations using the 'tea-bag' technique (Epstein *et al.*, 1963). In the second experiment, recovery of intact roots to rewatering was examined in terms of root growth and water and P uptake.

Seeds of sour orange were planted in a flat containing sterilized field soil with the top layer of soil (about 1 cm deep) mixed 1 : 1 (v/v) with mycorrhizal inoculum composed of Sudan grass roots infected with *Glomus* sp. FL904 (inoculum potential, 70 propagules mg^{-1} root). This particular mycorrhizal fungal species has been shown to be highly effective at enhancing nutrient uptake of citrus and other species (Sylvia *et al.*, 1993; J Graham, personal communication). The flat was initially watered with distilled water and, beginning about 1 month after planting, seedlings were watered as needed with a solution containing full-strength Hoagland's solution (Hoagland and Arnon, 1939) excluding phosphorus. Seedlings were transplanted into cylindrical polyvinyl chloride (PVC) containers (5 cm i.d., 33 cm in length) about 10 weeks after germination. Perforated PVC caps formed the container bottoms. Containers were hydraulically separated using horizontal cheese cloth barriers containing a mixture of 75% petroleum jelly and 25% paraffin about 5 mm thick (Fig. 1). Upper and lower compartments were irrigated separately with plastic tubing lines entering the sides of the container at the top of each compartment.

In Experiment 1 the soil profile in the containers was reconstructed based on patterns found in the field. Soil (Astatula fine sand, Typic quartzipsamment) was collected from Archbold Biological Station, Lake Placid, FL (Myers and White, 1987). Unsterilized subsurface field soil (15–30 cm depth in the field, 1.3 mg kg^{-1} Mehlich-1 extractable P; Hanlon *et al.*, 1994) was used to fill lower compartments 15 cm deep, followed by the petroleum jelly–paraffin water barrier and then sandy soil from

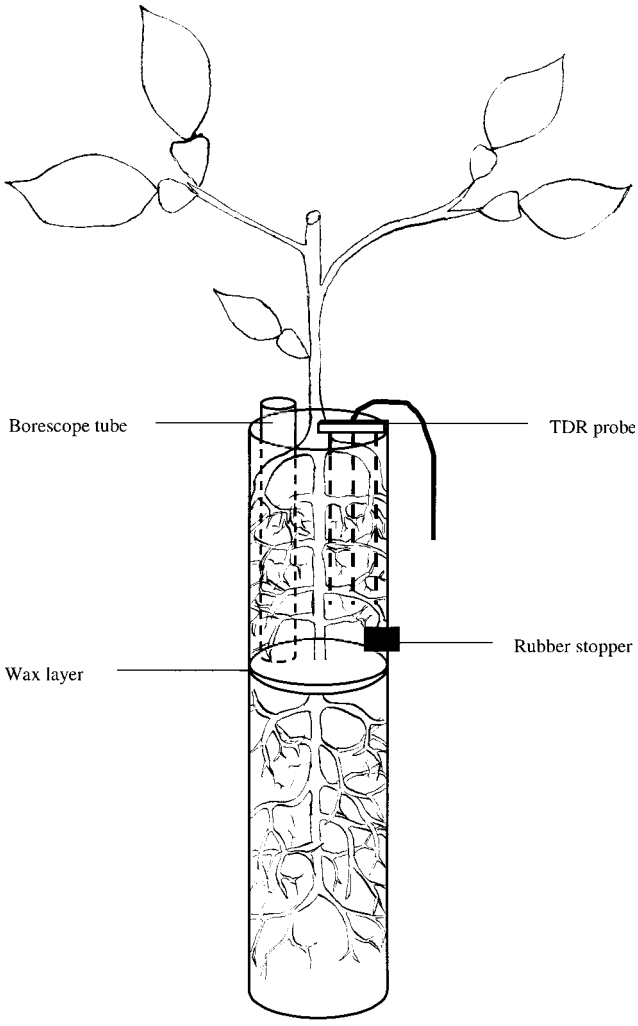

Fig. 1. Schematic of pot design used to separate upper and lower portions of the citrus root system hydraulically. Also shown are the borescope minirhizotron tubes and TDR probes used in Experiment 2.

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the mineral soil surface (0–15 cm depth in the field, 1.6 mg kg^{-1} extractable P) to a 10 cm depth. Duff (incompletely decomposed organic matter including living roots and mycorrhizal inoculum; 2.9 mg P l^{-1} H_2O extract, the recommended extract for organic soils; Hanlon *et al.*, 1994) that originally formed a mat approximately 5 cm thick over the mineral soil in the field was placed around seedling roots to 5 cm depths. The resultant reconstructed soil profile from the top of each cylindrical container to the bottom was 3 cm of free space, 5 cm of duff, 10 cm of surface mineral soil, the petroleum jelly–paraffin barrier, and 15 cm of subsurface mineral soil.

For the second experiment, a very similar fine sand (Candler fine sand, Typic quartzipsamment) was collected to a depth of 10 cm from near the Citrus Research and Education Center in Lake Alfred, Florida; 6–10 extractable P kg^{-1} by Mehlich-1 extraction. There was no duff layer or subsurface soil included in this experiment and the soil was uniform throughout the container. Otherwise, the containers and soil were similar to those in the first experiment (Fig. 1).

Plants were grown in the greenhouse in either Lake Alfred, Florida (for Experiment 1) or State College, Pennsylvania (Experiment 2) over the spring and summer. In both experiments, midday photosynthetic photon flux density (PPFD) at plant height ranged from 110–1100 μmol m^{-2} s^{-1} (Li-Cor quantum sensor, Lincoln, Nebraska) and daily air temperature ranged from 25 °C to 39 °C. Soil temperatures in the middle of the white plant containers ranged from 25 °C to 33 °C. Temperatures were determined with thermocouples. Plants were irrigated and fertilized initially every 2–3 d with one-tenth strength Hoagland's solution (minus P), depending on rates of growth and size of plants. In the second experiment, P and N deficiency symptoms (e.g. yellowish leaves) developed so plants were then fertilized with one-third strength Hoagland's solution including P. At the time of drought initiation, all top compartments in both droughted and irrigated treatments were irrigated until water leached from the soil; thereafter, control plants were irrigated in the top and bottom compartments and droughted plants in the bottom compartments only. Plant shoots were about 30 cm tall at the start of the drought treatment.

Experiment 1: Recovery of P uptake capacity

This experiment had two main treatments: irrigation level (irrigated, droughted) and drought duration (14 d of drought, 43 d of drought, and 42 d drought followed by 8 d re-irrigation). Soil layer (duff, sandy soil) was treated as a split-plot variable. There were eight, seven, and six plants droughted for the 14, 43 and 42 + 8 d drought treatments, respectively. Sample sizes varied because of mortality of droughted plants that had insufficient root development in the lower, continuously irrigated compartment. For each group of droughted plants harvested, an equal number of irrigated plants were harvested.

Stems and roots in duff, upper mineral soil, and lower mineral soil were harvested separately. Arbuscular mycorrhizal fungal (AMF) colonization was determined for fibrous roots from duff and upper sandy soil (by the methods of Graham *et al.*, 1991).

Soil water content in top compartments was determined gravimetrically. The relationship of soil water content to soil water potential (Ψ) was determined with a separate group of nine duff and six upper sandy soil samples that contained 7.1–11.1% and 0.5–1.2% soil water (w/w), respectively. Soil water potentials were determined with screen-cage soil psychrometers (Merrill Specialty Equipment Co., Logan, UT; Brown and Bartos, 1982) attached to a CR7 data logger (Campbell Scientific, Inc., Logan, UT) in a water bath.

To measure specific rates of P uptake, roots were washed free of soil with 1 mM $CaSO_4$ solution buffered with 5 mM MES adjusted to pH 5.5 with 1 M KOH (Sentenac and Grignon, 1985). For each plant, white fibrous roots about 2 cm from the tip were excised; six root samples were cut in mineral soil and three samples in duff. After cutting, sufficient roots to equal at least 10 mg (dry weight) were placed into 2.9 cm diameter plastic cassettes having 1.6 mm holes (Fisher Scientific). Cassettes were immersed in vigorously aerated 200 ml $CaSO_4$/MES solutions and held at 19 °C before labelling. During the 10 min labelling period, temperature was maintained at 28 °C using a water bath. Radiophosphorus (^{32}P) to result in labels ranging from 14–1543 Bq per 10 mg root sample and KH_2PO_4 to result in 50 μM, 750 μM and 1500 μM solution concentrations were added to the $CaSO_4$/MES solutions. These P concentrations were chosen to represent what would be the maximum soil solution P concentration roots may encounter under different field conditions. A soil solution of 1500 μM P is very high but might occur for roots in the vicinity of fertilizer bands in orchards. This high concentration was also chosen because in a previous greenhouse study, non-mycorrhizal sour orange seedling growing in Candler fine sand were P deficient when they received 2 mM P solution weekly (Eissenstat *et al.*, 1993). Although microbial populations may influence P uptake for non-sterile, excised roots, at solution concentrations greater than 50 μM microbes should have little effect (Barber and Frankenburg, 1971). Samples were rinsed twice (3 min each) using solutions of 1500 μM P in $CaSO_4$/MES solution (2–10 °C) to remove ^{32}P adhering to roots. Roots were removed from the cassettes and placed in vials, oven-dried, and weighed. A scintillant (20 ml of 5 mM 7-amino-1,3-naphthalene-disulphonic acid monopotassium salt; ANDA) was added to improve ^{32}P beta emissions counting efficiency (Beckman model LS6000SC liquid scintillation counter).

Specific root length (SRL; cm g^{-1} dw) and root length density (L_v; cm cm^{-3} soil), of the fibrous roots were determined for each drought period and soil layer using a separate group of 18 plants harvested at 0, 14 and 37 d after irrigation was withheld from the top pots in the unirrigated plants (six plants for each harvest; no continuously irrigated plants were available for harvest). Root length was determined by the line intercept method (Newman, 1966; Tennant, 1975). The diameters of 10 randomly selected roots of each sample were determined using a stereo microscope (40× magnification). Roots were then dried (100 °C for 4 h followed by continued drying at 70 °C for 32 h) before weighing.

Phosphate uptake rates (fmol cm^{-1} root length s^{-1}) were expressed on a length basis using estimates of SRL and assuming that continuously irrigated plants had similar root morphology as plants harvested just before drought was initiated (Day = 0). Uptake rate, expressed on a root mass and area basis, was also examined using the diameter estimates and assuming cylindrical geometry. Data were analysed by ANOVA (GLM procedure, SAS Institute Inc., Cary, NC, USA) using a split-plot design with irrigated versus droughted treatments (I), days of drought (D, treated as a fixed variable with three levels: 14 d, 43 d, 43 d + 8 d of irrigation), and the interaction of $I \times D$ as whole-plot variables and soil layer (S) and its interactions as split-plot variables.

Experiment 2: Root growth and P and water uptake following rewetting

This experiment examined the effects of three levels of drought duration (0, 14 and 43 d) on the recovery of intact roots to drought. One group of plants was rewatered after 14 d of drought and the initiation of new root growth was monitored

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non-destructively with small minirhizotrons (root observation tubes). A second group of plants ($n=7$ for control; $n=4$ for droughted) were rewatered after 43 d of drought using ^{32}P-labelled water (1.036 MBq $H_2(^{32}P)O_4^-$ in 50 ml of water per pot). The radiophosphorus was added with a syringe to vertical holes about 1 mm in diameter at about 8 cm deep at numerous locations in the upper compartment to improve spatial uniformity of the isotope. For this portion of the experiment, any leachate was collected and re-added to the plants. Plants were harvested 8 d later. Root growth, soil water depletion and ^{32}P uptake were monitored over the 8 d following rewatering.

Water content in the pots was determined non-destructively twice daily using time domain reflectrometry (TDR; Topp, 1993). TDR probes (unbalanced design; each probe containing three stainless-steel rods 11 cm long and 1.6 mm in diameter) were buried vertically in the upper compartment at the time of transplanting (Fig. 1). Root growth in the top compartments was monitored using small-diameter minirhizotrons (clear tubes of butyrate plastic, 2 cm in diameter and 15 cm in length) and a rigid borescope (Olympus model G080–055–110–55) connected to a video camera (Bartz Technology, Santa Barbara, CA) and a SONY Hi-8 tape recorder. Minirhizotrons were placed vertically in the top compartments at the time of transplanting (Fig. 1). Each tube was scribed with two transects of 5×5 mm windows and sealed at the bottom with a rubber stopper and acrylic cement. Light penetration was prevented by wrapping the portion of the tube above the soil surface with black electrical tape. When not in use, a removable stopper was used to seal the top of the tube. Root images were recorded 1 d prior to rewatering and every other day thereafter in both droughted (14 d and 43 d) and continuously watered treatments. Videotapes were played directly onto a Macintosh 7500 computer using Apple Video software. Number of new roots in each 5×5 mm window in control and rewatered pots was determined. When a new root was first detected, it had generally already extended across the entire window (i.e. >5 mm in length). Thus, the number of new roots should be highly correlated with new root length visible on the tube.

Phosphorus uptake was non-destructively estimated by monitoring radioactivity of leaves with a solid scintillant probe (Nuclear Enterprises Beta Probe BP4/4) that was attached to a scalar ratemeter (Ludlum Model 2200, Sweetwater, Texas). The target leaf was pressed against the window of the probe with a custom-made cuvette (Jupp and Newman, 1987) and counted for 24 s, depending on activity. The circular probe window was 5 cm in diameter and each leaf covered 50% or more of the window when counted. The same marked (with transparent tape) section of two leaves of each plant was monitored daily and harvested at the end of the experiment to allow expression of radioactivity on a mass basis.

Plants were harvested 8 d following rewatering and divided into leaves, stem, taproot, fine roots in the top compartment, and roots in the bottom compartment. Root length was determined using a desktop scanner and root length software (Delta-T scan, Delta-T Devices LTD, Cambridge, England). Dry mass of each fraction was determined after drying tissue for 48 h at 70 °C. To determine radiophosphorus content, subsamples were counted using a liquid scintillation counter (Packard Tri-Carb 1500) after ashing in a muffle furnace for 7 h at 480 °C and suspending in 0.1 N HCl solution.

Results

Recovery of P uptake capacity (Experiment 1)

Root length distribution and morphology differed between the soil compartments (Table 1). Because there was no effect of drought duration on root length or morphology, these data were combined across irrigation treatments. Root length density was about 2–3-fold higher in upper sandy soil than in either the duff or lower mineral soil. Fine roots in duff had lower SRL and larger average diameters than roots in upper sandy soil ($P<0.01$ for each). No continuously irrigated plants were harvested for root morphology in Experiment 1. Duff retained more water than upper mineral soil during the drought period (as determined by soil water content), but water in the duff was bound much more tightly to the solid matrix (as estimated by soil water potential; Table 2). Because SRL in duff and upper sandy soil did not change due to drought duration ($P=0.19$, data not shown), differences in SRL between duff and sandy soil were evidently not a response to soil dryness. Percentage incidence of AMF infection of duff and upper sandy soil roots was 86.9% and 84.7%, respectively.

Despite the very dry soil and the length of drought, rates of P uptake by excised roots in solution within 3–4 h of harvest were very similar for roots exposed to different lengths of drought ($P\geq0.14$, Table 3; Fig. 2). There was, however, a significant interaction of soil layer with irrigation regime ($P<0.05$, Table 3; Fig. 3). Roots that had been in dry sand took up P at significantly faster rates than did roots that had been in dry duff regardless of solution P concentration (Fig. 3). Because roots in dry duff had more mass (lower SRL) and more surface area (larger diameter) per unit length than roots in dry sandy soil in the upper compartment (Table 1), differences in P uptake capacity between dry duff and dry sand shown in Fig. 3 would be accentuated if P uptake rates were expressed on a mass or surface area basis.

Recovery of P and water uptake, root growth (Experiment 2)

Compared to continuously watered plants, citrus seedlings exposed to 14 d or 43 d of dry soil in the upper compartment did not exhibit a burst of new root growth immediately after rewatering (Fig. 4). After 14 d of drought, new root growth in the irrigated and droughted plants was nearly identical. After 43 d of drought, there was some evidence that root growth was inhibited in the droughted plants for about 4–5 d before there was a burst of new root growth.

After rewatering, soil water depletion of plants that did not receive water in the upper compartment for 43 d was very similar to that of continuously watered plants (Fig. 5a). Although non-significant, there was some evidence that irrigated plants took up slightly more water in the first 24 h, but these differences disappeared by the end of the first day. Similarly, after rewetting, phosphorus uptake as indicated by ^{32}P activity in leaves was very similar in the continuously irrigated and partially droughted plants (Fig. 5b). Three days after rewatering,

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Table 1. *Characteristics of greenhouse-grown plant roots (fine roots only) in Experiment 1*

Values are pooled averages from samples taken at 0, 14 and 37 d of drought (SE in parentheses; $n=18$ for duff and upper soil, $n=17$ for lower sandy soil because of one pot with no roots in the bottom compartment on day 0) in the droughted treatment. Because there was no effect of drought duration on root length or morphology ($P \geq 0.19$), these data were pooled. Row values designated by the same letter are not significantly different (Duncan's Multiple Range Test, $P=0.05$; data were log transformed before analysis).

	Duff	Upper sandy soil	Lower sandy soil
Specific root length (cm g^{-1} dw)	1073 (71) b	1306 (52) a	1408 (90) a
Root diameter (mm)	0.75 (0.03) a	0.66 (0.02) b	0.81 (0.02) a
Root length density, L_v (cm cm^{-3} soil)	2.65 (0.34) b	6.21 (0.53) a	2.94 (0.63) b

Table 2. *Soil water content (w/w) and estimated total water potential (Ψ) (SE in parentheses) at the end of Experiment 1*

Days of drought	Treatment	*n*	Duff		Upper sandy soil	
			% Water	Ψ (MPa)[a]	% Water	Ψ (MPa)
14	Droughted	8	9.0 (0.1)	−6.0	0.8 (0.1)	−0.3
	Irrigated	8	39 (0.4)		18 (0.2)	
43	Droughted	7	9.5 (0.1)	−4.3	0.9 (0.0)	−0.2
	Irrigated	7	41 (0.2)		17 (0.2)	
42 (+8 d irrigated)	Droughted	6	38 (0.7)		17 (0.5)	
	Irrigated	6	43 (0.6)		16 (0.3)	

[a]Based on a water release curve of a separate set of soil samples. See text for more detail.

Table 3. *Probabilities of significance (P) for the effects of drought, drought duration, and soil layer on P uptake rate (fmol cm^{-1} s^{-1}) of sour orange seedlings using a split-plot statistical model for each solution phosphate concentration*

Source	df	Phosphate concentration (μM)		
		50	750	1500
Labelling containers (blocks)	2	0.13	0.17	0.03
Irrigated versus droughted (*I*)	1	0.27	0.99	0.70
Days of drought (*D*)	2	0.14	0.92	0.45
$I \times D$	2	0.96	0.20	0.27
Plant ($I \times D$)[a]	35	<0.01	<0.01	0.04
Soil layer (*S*)	1	<0.01	<0.01	<0.01
$S \times I$	1	0.02	0.03	0.01
$S \times D$	2	0.96	0.34	0.74
$S \times I \times D$	2	0.27	0.87	0.40
Experimental error	75–76			

[a]The type III mean square for Plant ($I \times D$) was used as the whole-plot error term.

approximately 20% of the P (relative to the total taken up during the 8 d observation period) and 60% of the water (an additional 20% was lost to evaporation) was acquired by the partially droughted and continuously irrigated plants (Fig. 5), even though the partially droughted plants had essentially no new root growth during this time period.

Plant biomass was slightly larger in the plants exposed to drought, primarily because the smaller droughted plants died when irrigation was halted in the upper compartment because of insufficient roots in the lower compartment (Table 4). As expected, partially droughted plants tended to have a higher proportion of roots in the lower compartment than continuously watered plants; otherwise, biomass allocation to leaves, stems and roots were generally similar. For roots in the upper compartment, total root length, SRL and mycorrhizal colonization (>70%) were also very similar between droughted and continuously irrigated plants (data not shown, $P \geq 0.36$).

Consistent with non-destructive observations of radioactivity in leaves (Fig. 5), total radiophosphorus acquisition was very similar in partially droughted and continuously watered plants (Table 4). Essentially no radioactivity was detected in the roots or soil in the lower compartment.

Discussion

This study differs from most studies in that the soil water stress imposed was localized, as might occur when a plant

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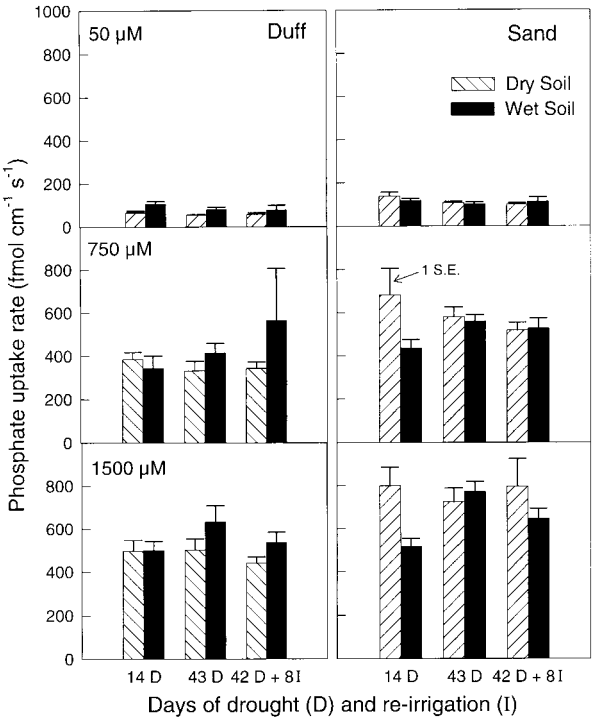

Fig. 2. Phosphate uptake rate from 50, 750 and 1500 µM P solutions by excised sour orange roots exposed for various lengths of time to dry or continuously moist duff or sandy soil. There were no significant effects of drought duration on phosphate uptake rate ($P \geq 0.14$; Table 3).

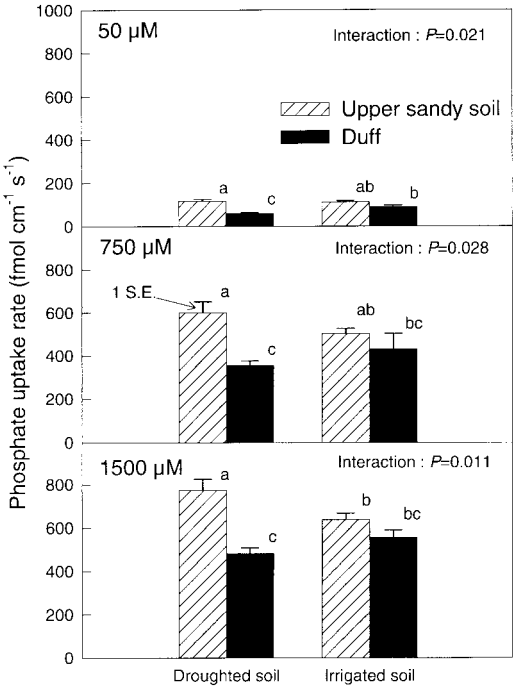

Fig. 3. The interaction of soil layer and irrigation treatment for phosphate uptake rate from 50, 750 and 1500 µM P solutions by excised sour orange roots. For each P solution concentration, bars designated by a different letter are significantly different (Duncan's Multiple Range Test, $P < 0.05$).

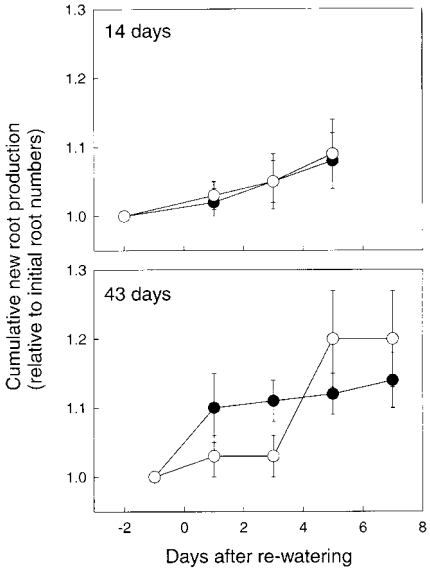

Fig. 4. Root growth following rewetting for citrus roots exposed to dry soil for 14 d or 43 d. Root growth is expressed as new root production (relative to number of existing roots, ±SE) for citrus seedlings continuously irrigated (●—●) and those whose surface roots were not irrigated (○—○) for 14 d (Top) and 43 d (Bottom).

has access to deep water, but where the soil near the surface is dry. When soils dry from the surface to depths of just 10–20 cm, often greater than 50% of the total roots of a plant may be exposed to desiccating conditions (Eissenstat and Van Rees, 1994). Access to deep sources of moisture will often allow plants to maintain their water balance even though a majority of their shallow roots are unable to acquire appreciable water and nutrients. It was anticipated that prolonged exposure to dry surface soil should have greatly diminished the ability of shallow roots to take up water and P when soil was rewetted, at least initially. But even within 24–48 h after rewetting, roots exposed to dry soil for over 40 d had very similar rates of water and P uptake to those never exposed to dry soil (Figs 2, 5). The rapid uptake of water and nutrients likely occurred mainly from existing roots because new root growth was generally slow (<3% until after 3 d in the 43 d droughted plants) and generally similar to that in controls (Fig. 4), and excised roots generally exhibited no decrease in P uptake capacity (Fig. 2). Thus, these data overall demonstrate that citrus seedlings with access to deep soil moisture have the ability to maintain or recover very quickly P and water uptake capacity of their shallow roots even if these shallow roots are exposed to very dry soil (< −4 MPa) for an extended length of time (>40 d).

When roots are exposed to dry soil, several factors will influence the trade-offs between shedding and maintaining the roots. It was hypothesized that roots are less likely to be shed under conditions where (1) building new roots is relatively expensive; (2) roots recover quickly in ability

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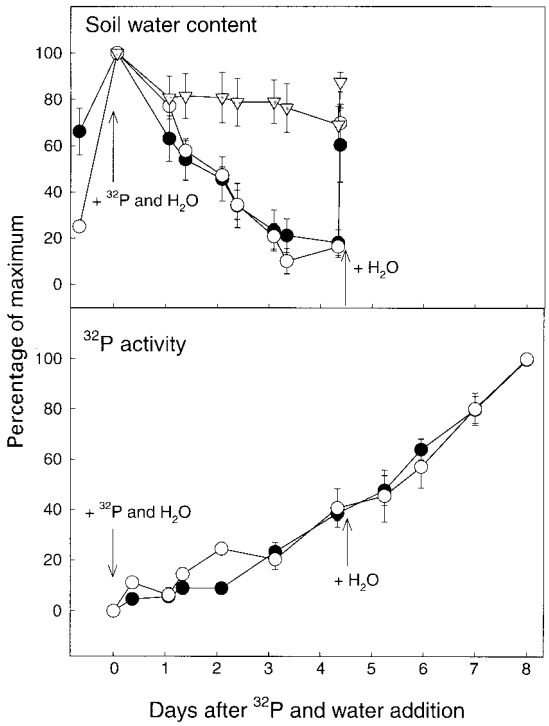

Fig. 5. Water and phosphorus acquisition following rewetting for citrus roots exposed to dry soil. (Top) Soil water depletion (±SE) following irrigation (day 0) of plants continuously irrigated (●—●) and those whose surface roots were not irrigated for 43 d (○—○). Also shown is change in soil water content due to evaporation using pots with dead plants (▽—▽). (Bottom) Radiophosphorus activity (±SE) in target leaves of continuously irrigated citrus plants and those not irrigated for 43 d (symbols same as above) relative to maximum counts per minute determined, after correcting for decay. Absolute radioactivity above background of target leaves for droughted and irrigated plants was 42±20 and 49±15 (SE) Bq (g dw)$^{-1}$, respectively, harvested 8 d after ^{32}P addition and determined by liquid scintillation after correcting for quench, counting efficiency and decay.

to take up nutrients once the soil is rewetted; and (3) carbon costs of maintaining roots during the dry period are relatively low. In this study it has been shown that sour orange, which does not readily shed its roots in dry soil, does exhibit rapid recovery following drought. Other traits associated with the costs and benefits of root shedding also indicate that sour orange is particularly well suited for retaining roots in dry soil.

Citrus roots, especially in seedlings, exhibit virtually no mortality and greatly reduced maintenance respiration in response to localized drought (Kosola and Eissenstat, 1994; Bryla *et al.*, 1997; Espeleta and Eissenstat, 1998). Fibrous roots of citrus rootstocks, in general, and sour orange, in particular, are characterized by slow growth rate (Eissenstat, 1991), coarse diameter (Eissenstat, 1992), high specific gravity (tissue density; Eissenstat, 1991), and an exodermis with heavily lignified secondary walls (Walker *et al.*, 1984; Eissenstat and Achor, 1999). Other plant species, such as certain grasses, have finer roots that are considerably more succulent and exhibit much greater mortality when exposed to localized drought (Huang *et al.*, 1997). Thus, the rapid recovery of existing sour orange roots to localized drought is consistent with its pattern of root construction, maintenance and lack of root mortality. In contrast, species like most festucoid grasses, whose roots lack an exodermis and tend to be succulent with low tissue density would more likely depend on new root growth for resumption of water and nutrient uptake after drought.

The first experiment revealed that there could be subtle effects of soil layer on P uptake rates (Fig. 2). Roots exposed to dry duff had slightly lower P uptake rates than roots in wet duff, whereas roots in dry sand had slightly higher rates of uptake than those roots in wet sand. The very low soil water potentials of dry duff (< −4.0 MPa) compared to dry sand (−0.3 to −0.2 MPa) may have contributed to the slightly different root responses (Table 2). In sandy soils, the exodermis in sour orange likely protects the root from desiccation and deep roots may provide sufficient water to keep the inner cortex well hydrated. There may be soil conditions where this is not the case. If a soil develops very low soil water potentials and maintains close root : soil contact, appre-

Table 4. *Total dry mass, ^{32}P content and percentage allocation (±SE) of citrus seedlings 8 d after watering for plants that had either been continuously watered (Irrigated) or plants whose surface roots were not irrigated for 43 d (Droughted)*

Probability of significance (*P*) is also indicated.[a]

	Dry mass			^{32}P		
	Irrigated	Droughted	*P*	Irrigated	Droughted	*P*
Whole plant content (g or kBq)	13.1±1.18	17.5±1.55	0.048	1.48±0.45	1.71±0.61	0.761
Allocation (%)						
Leaves	36±1	33±2	0.221	10±3	12±7	0.736
Stems	22±1	20±0	0.480	22±11	9±2	0.418
Upper fine roots	13±4	6±3	0.571	18±9	16±7	0.933
Upper tap root	20±1	18±1	0.257	51±10	62±7	0.496
Lower roots	9±4	22±3	0.057	0±0	1±0	0.200

[a] $n=7$ and 4 for Irrigated and Droughted plants, respectively. Data were analysed by *t*-test or Wilcoxon rank test, depending on homogeneity of variance.

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ciable water loss from the fibrous roots may occur which is not immediately replenished by water uptake from deep roots. If the fibrous roots develop low water potentials, root capacity for P uptake may diminish. Consequently, sour orange roots with a well-developed exodermis and high tissue density may be very well suited to prolonged periods of drought in Florida's typically sandy soils, but it is not clear that they would recover equally well in heavy textured or organic soils.

It was found that arbuscular mycorrhizal fungi (AMF) heavily colonized the citrus roots and that the drought treatments did not affect percentage colonization. In wet soil, sour orange growth can be greatly enhanced by AMF at concentrations of soil P used in this study (Eissenstat *et al*., 1993). In dry soil, benefits of AMF to plant P acquisition may be enhanced because P is more diffusion limited and extramatrical hyphae can explore small water-filled pores inaccessible to roots (Smith and Read, 1997). Use of excised roots to estimate P uptake capacity severs extramatrical AMF hyphae, removing an important pathway by which roots, especially citrus roots, acquire P. Thus, the examination of P uptake of intact mycorrhizal roots in the second experiment is important in controlling this, and other artefacts associated with excision. The results clearly demonstrate that undisturbed mycorrhizal roots were also unaffected by prolonged exposure to dry soil (Fig. 5).

Surface roots in 'dry' soil might maintain appreciable water and nutrient uptake if water is 'hydraulically lifted' from deep wet soil and leaked from the roots into the dry surface soil at night when transpiration is minimal (Richards and Caldwell, 1987) or if well-hydrated roots exude significant amounts of mucilage (Nambiar, 1976). In this study, mucilage production was not obvious. There is also doubt that hydraulic lift contributed to root P uptake capacity. In a similar experimental system, P uptake in the top compartment of sour orange seedlings exposed to 12 h of light was compared with those exposed to continuous light, which should have reduced nocturnal water leakage (Whaley, 1995). Using ^{32}P and ^{33}P, it was shown that there was very little P (<1% of total) acquired from the top compartment when the top compartment was not irrigated and no evidence that plants when provided a dark period took up more P from the upper compartment than those exposed to continuous light.

Roots of plant species vary in their capacity to recover rapidly after prolonged exposure to dry soil. Similar to sour orange, maintenance of P uptake capacity has also been found using excised roots in seedlings of the desert shrub, *Artemisia tridentata* exposed to slowly drying soil (Matzner and Richards, 1996). Roots exposed to very low soil water potentials not only maintained their uptake capacity for P, but exhibited higher uptake capacity at low (−3.4 to −5.0 MPa) compared to high (0.0 to −1.6 MPa) soil water potentials. In contrast, it has been shown that N (ammonium + nitrate) uptake capacity declined substantially with exposure to relatively little soil water stress (Matzner and Richards, 1996). Most of the change in N uptake capacity occurred between soil water potentials of −0.03 and −0.3 MPa, with little further change between soil water potentials of −0.3 and −5.0 MPa; which is generally consistent with earlier work (BassiriRad and Caldwell, 1992*b*) indicating that nitrate influx of intact roots of *A. tridentata* was diminished for at least 4 d in droughted seedlings compared to well-watered controls.

Some species require more time to recover from localized drought. In the perennial pasture grass, *Lolium perenne*, P influx was reduced for at least 10 d after rewatering (Jupp and Newman, 1987). In the tussock grass, *Pseudoroegneria spicata*, N influx was only 60–70% of controls 4 d after rewatering; these species had not fully recovered their ability to take up N until 14 d after rewatering (BassiriRad and Caldwell, 1992*a*). However, in barley genotypes (Wraith *et al*., 1995) and sorghum (Wraith and Baker, 1991), substantial water uptake occurred only 2–3 d following rewetting when exposed to 2–3 weeks of water deficit. Recovery in many species may involve initiation and elongation of new laterals. In wheat, for example, short seminal roots were unable to take up significant amounts of N until they had broken dormancy and elongated, which occurred 2–4 d after rewetting (Brady *et al*., 1995). Similarly, the rapid recovery of P influx in barley following drought (3 d after rewatering) has been attributed to new root growth (Shone and Flood, 1983). New root growth, however, was not considered a factor in the rapid (first measurement at 4 d) recovery of the cold-desert tussock grass, *Agropyron desertorum* in dry surface soil (BassiriRad and Caldwell, 1992*a*). Although experimental conditions vary, the evidence suggests that species differ in the rate and process by which root systems recover.

Nutrient uptake ability of excised roots may not accurately indicate nutrient uptake by intact roots (Bloom and Caldwell, 1988). In excised corn roots, however, root P-uptake rates of excised roots in solution more closely represented intact root P-uptake rates than did uptake rates of other ions such as nitrate or potassium (Gronewald and Hanson, 1982). There was some corn root P-uptake response to cutting and washing roots in aerated labelling solutions and P-uptake rate increased for 2–3 h after excision. The size of excised corn root segments or their handling did not change P-uptake rate when expressed in $\mu mol\ g^{-1}\ fw\ h^{-1}$. Thus, the use of excised roots is considered to be most useful for comparative investigations, such as the effects of drought on *relative* values of potential uptake.

In conclusion, sour orange typifies a species that builds expensive roots with a prominent exodermis and retains rather than sheds its roots when exposed to dry surface

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soil. For the first few days after the soil is rewetted, sour orange apparently depends mainly on existing roots for rapid acquisition of water and P, rather than rapidly building new roots.

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