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Citrus root responses to localized drying soil: A new approach to studying mycorrhizal effects on the roots of mature trees

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Abstract

Because fine roots tend to be concentrated at the soil surface, exposure to dry surface soil can have a large influence on patterns of root growth, death and respiration. We studied the effects of arbuscular mycorrhizas (AM) formation on specific root length (SRL), respiration and mortality of fine roots of bearing red grapefruit (*Citrus paradisi* Macf.) trees on Volkamer lemon (*C. volkameriana* Tan. & Pasq.) rootstock exposed to drying soil. For each tree, the fine roots were removed from two woody lateral roots, the roots were surface sterilized and then each woody root was placed in a separate pair of vertically divided and independently irrigated soil compartments. The two split-pot systems were filled with sterilized soil and one was inoculated with arbuscular mycorrhizal fungi (*Glomus etunicatum/G. intraradices*). New fine lateral roots that emerged from the woody laterals were permitted to grow inside the pots over a 10-month period. Irrigation was then removed from the top compartment for a 15-week period. At the end of the study, roots inoculated with AM fungi exhibited about 20% incidence of AM formation, whereas the uninoculated roots were completely void of AM fungi. Arbuscular mycorrhizal roots exhibited lower SRL, lower root/soil respiration and about 10% lower fine root mortality than nonmycorrhizal roots after 15 weeks of exposure to dry surface soil. This study demonstrates the feasibility of examining mycorrhizal effects on the fine roots of adult trees in the field using simple inexpensive methods.

Abbreviations: AM – arbuscular mycorrhizal; NM – nonmycorrhizal; SRL – specific root length

Introduction

Comprehensive analysis of trends in published research in the last four decades shows that ecological studies on mycorrhizase have been very biased towards laboratory and greenhouse studies as opposed to field studies (Klironomos and Kendrick, 1993). Similarly, studies of root responses of woody species using seedlings have prevailed over studies using adult trees (e.g., Eissenstat et al., 1993; Peng et al., 1993; Rygiewicz and Andersen, 1994). The present experiment demonstrates the feasibility of producing a field system for comparing phenological and physiological responses of arbuscular mycorrhizal and nonmycorrhizal roots in the same adult tree. Particularly, this system was utilized for exploring the direct effects of arbuscular mycorrhizal (AM) formation on fibrous root mortality under localized water stress.

Root death is a very important but poorly understood component of plant carbon budgets. Carbon investments for shedding or maintaining roots may be considerable, especially under stress conditions. In forest ecosystem studies, the most frequently suggested cause of root death is water deficit (Deans, 1979; Persson 1979). Responses of root systems to

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drought vary from strategies of rapidly shedding roots exposed to dry soil to producing long-lived roots that survive in dry soil (Eissenstat, 1997). For instance, the desert succulent, *Agave deserti*, grows new roots rapidly after rain and then sheds them when the soil dries (Huang and Nobel, 1992), whereas citrus roots may tolerate dry surface soil for more than 90 days (Kosola and Eissenstat, 1994). Dry conditions near the soil surface are extremely common in the field while deeper in the soil profile water may be sufficient for maintaining plant water status. Periodic dry soil conditions can impede water and nutrient uptake; nonetheless, a plant must continue translocating assimilates to roots in order to keep them alive. We simulated the situation where surface roots are in dry soil for varying lengths of time while deeper roots are in wet soil. Under this localized drought, we characterized root respiratory responses of adult citrus trees as affected by AM fungi, in order to examine potential linkages between root respiratory rates and root mortality.

Costs of growing and maintaining AM roots in citrus are a significant component of daily respiratory expense (Peng et al., 1993). Under stress situations colonization by AM fungi may influence root survival by affecting total carbon expenditure. In order to demonstrate this type of relationship it is important to study first the role of colonization by AM fungi on root respiratory responses under stress. Data of root respiration can be incorporated into a cost-benefit analysis to help understand root-shedding strategies. This approach assumes that root mortality is a result of plant allocation strategies that optimize carbon-use efficiency. Under conditions that impede water and nutrient absorption such as dry surface soil, carbon expended in root maintenance would provide little or no immediate benefit. Shedding surface roots in this instance eliminates the cost of root maintenance, but forces the plant to assume the future cost of regrowing roots when soil conditions are more favorable. If plants act to optimize resource capture per resource expended for each root, there should be a balance between the integrated lifetime costs of maintenance and construction of individual roots and the benefits gained during the lifespan of the roots (Eissenstat and Yanai, 1997).

It is widely accepted that mycorrhizas improve P nutrition of host plants. The effects of AM fungi on drought resistance are still controversial. Several reports have considered that drought resistance may be improved as a function of enhanced P nutrition in the host plant, including studies in citrus (Graham et al., 1987; Nelsen and Safir, 1982). In contrast, colonization by AM fungi has been reported as a direct cause of improved drought resistance of host plants through mechanisms as diverse as enhanced water uptake, osmotic adjustment, altered elasticity of the cell walls or altered symplastic water content (Augé and Stodola, 1990), although carefully controlled studies in wheat and safflower have been unable to support such claims (Bryla and Duniway 1997a, 1997b, 1997c). Other direct effects of AM fungal colonization on roots have also been reported to be independent of P nutrition, such as increased resistance to pathogens and herbivores (Gange et al. 1994; Newsham et al. 1995), and alteration of root morphology and architecture (Hooker et al., 1992; Berta et al., 1993; Berta et al., 1995; Hooker et al., 1992). Nevertheless, the evidence to support direct effects of AM formation in plant-herbivore interactions is not solid enough; because most studies did not test AM plants and NM plants at the same P status and/or lacked P deficient controls (e.g. Gange et al. 1994 and Newsham et al. 1995). To avoid inclusion of responses of improved P-nutrition instead of direct effects of mycorrhizas on root lifespan, we tested our hypothesis under an optimum P-supply.

In this study we tested a new field technique designed to compare roots of adult trees with differential colonization by AM fungi. By using this system we examined the effects of AM formation on roots exposed to dry surface soil, in particular, effects on specific root length (SRL), root respiration, and root mortality.

Materials and Methods

The experiment was conducted in an orchard at the Citrus Research and Education Center (CREC) of the University of Florida, Lake Alfred, Florida. In August, 1993, roots of adult trees of Volkamer lemon (*Citrus volkameriana* Tan. & Pasq.) were grown in adjacent pots inside a field root chamber (Figure 1) to test the effect of AM fungal colonization on fine root death and root/soil respiration under surface drought. One chamber was placed adjacent to each tree and buried about 1 m from the bole of the tree. Volkamer lemon was chosen as a rootstock because of its rapid root growth and high mycorrhizal dependency (Graham et al., 1991). Each wooden chamber was covered with an insulated lid to exclude rainwater and sunlight.

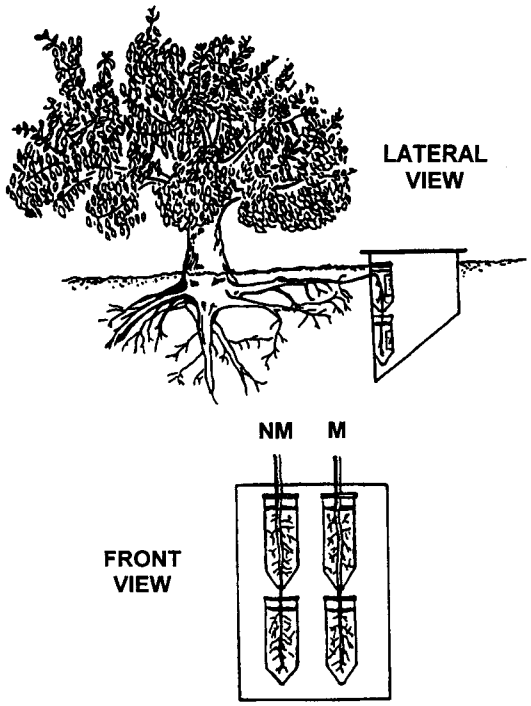

Figure 1. Diagram of the field root chambers used in the study. Each split-pot system consisted of two vertically arranged plastic pots with a transparent plastic window that was used for root mapping. One split-pot contained arbuscular mycorrhizal roots from an adult tree (M) and the other nonmycorrhizal roots from the same tree (NM).

Two sets of split-pots were placed vertically inside the wooden chamber (40 × 45 × 50 cm). Each split-pot system consisted of two vertically arranged 500-mL plastic pots (Deepots, Stuewe and Sons, Corvallis, Oregon, USA) modified with a transparent plastic window (5 × 5 cm) that was used for fine root mapping. One pair of vertical pots was used for arbuscular mycorrhizal roots (M) of adult trees; the second for nommycorrhizal roots (NM) of the same tree.

Each tree had both M and NM roots. In the NM treatment, one woody root (diameter about 5 mm) was excavated from each of ten, 6-year-old Volkamer lemon rootstocks (*C. volkameriana* Tan. & Pasq.) supporting shoots of red grapefruit trees (*Citrus paradisi* Macf.). Total tree height was around 3 m. Fine laterals of the woody root were removed, the surface of the woody root sterilized (NaOCl 5%, for 2 min) and then the root was passed through an opening in the wooden chamber wall under the soil and then through the split-pots. The hole in the chamber wall was larger than the diameter of the root to allow for radial growth. The gap was filled with a flexible, waterproof sealant (Terostat). Pots were filled with an autoclaved sandy soil (Astatula fine sand, *Typic quartzipsamment*, Avon Park, Florida, USA). Mycorrhizal roots were treated in the same way but the soil was first inoculated with ca. 100 g of air-dried fragments of mycorrhizal Sudangrass (*Sorghum bicolor* L. Moench cv. 'Sudanense') colonized by *Glomus etunicatum* (FL 312 and FL 329) and *G. intraradices* (FL 208). A plastic screen in the bottom of the pots that allowed excess water to drain freely retained the soil inside the pots. The section of the root between the top and bottom pots was covered with petroleum jelly to avoid desiccation.

The overall process of excavation, sterilization and placement of two roots of a tree (M and NM treatments) in the split-pot system within the chambers took about one day. The excavation required between 2–4 h, and it was the key factor associated with successful regrowth of the roots after transplanting. Care was taken to avoid mechanical damage to the roots during excavation. The process was generally scheduled in early hours of the morning or late afternoon to avoid desiccation of roots due to exposure to the hot and dry conditions of midday.

Growing conditions for roots

Roots were transplanted during the same week in August, 1993, in order to minimize the potential effect of differential root age on respiratory rates, and, subsequently, on root mortality (Palta and Nobel, 1989). Roots were irrigated and fertilized to induce rapid growth of new laterals inside the pots. The M and NM roots in the pots required approximately 10 months to proliferate on the plastic windows. Nutrients and water were added to bottom and top pots three times per week with a 1/500 dilution of a commercial formula [Premier 10-10-10 (N, P_2O_5, K_2O equivalent) with micronutrients, Growers Fertilizer Corp., Lake Alfred, FL]. A total of 300 mL of diluted solution was applied to each pot to provide approximately 180 mg of N, 80 mg of P per week. The system was flushed biweekly with water to prevent salt accumulation. The soil in the pots was drenched with 50 ppm of the fungicide, metalaxyl (Ridomil), every 40 days to suppress infestations of *Phytophthora nicotianae*. Previous studies in onion (*Allium cepa*) (Sukarno et al., 1993, 1996) indicate that this fungicide can reduce plant growth in the absence of AM fungi as well as reduce colonization by AM fungi and reduce P inflow per meter of living external hyphae. Metalaxyl does not appear to affect P transfer across the fungal-plant

interface. Nonetheless, because of our experience with *Phytophthora* infestations causing severe root mortality in citrus, we chose to use metalaxyl despite its potential to reduce plant growth and colonization by AM fungi. Outside the split-pot system, each adult tree was watered adequately and fertilized weekly with a 1/1000 dilution of a liquid fertilizer formula (6-2-8 N, P_2O_5, K_2O equivalent; 3.75 L per tree), that provided approximately a total of 675 g of N and 300 g of P per year to each tree.

Application of localized surface drought and harvesting of the roots

When sufficient number of lateral fine roots (20–30 roots, equivalent to 50–100 cm of total root length) were visible on the transparent windows of the top and bottom pots in each split-pot system, the application of water and nutrients was withheld in the top pots. Bottom pots continued to be fertilized and watered normally throughout the experiment. Drought in the top pots began simultaneously (10 June 1994) for the M and NM roots inside five root chambers, and continued for 15 weeks until the final harvesting date (23 September 1994). Another two chambers were selected for a localized drought of 8 weeks (water was withheld beginning on 14 July 1994) and two additional chambers were selected later for 5 weeks of drought (water was withheld beginning on 3 August. 1994). The harvesting date was the same for all the three drought duration regimes.

At the end of the drought period, soil samples were taken from the top pots of roots exposed to different drought duration regimes in order to determine soil water content gravimetrically. Following removal of irrigation, this sandy soil typically dries quickly for the first week and then changes little in water content (Kosola and Eissenstat, 1994). In both M and NM treatments, soil water content was 5.5% after 5 weeks of drought and, from 8 to 15 weeks of drought, soil water content never exceeded 2% (< -0.1 MPa).

This study did not make comparisons with well-watered controls as done previously (Kosola and Eissenstat, 1994) because the focus was to compare maintenance (basal) respiration with root mortality, which can not be done easily in wet soil. Under wet conditions, most root respiration is associated with root growth and ion-uptake (Kosola and Eissenstat, 1994; Veen, 1981); consequently, there should not be any direct relationship in wet soil between root/soil respiration and root lifespan. For general interest, we did retain one root box that had no interruption in water and nutrient supply over the course of the study. Data of the well-watered control are not presented in detail. As expected, when we exposed roots to localized drought for less than 5 weeks, root respiration for M and NM treatments was much higher than when experiencing droughts of longer duration. Similarly, fine root mortality was very low (<2%) for both M and NM roots over the experimental period when drought lasted less than 5 weeks.

Measurements of root mortality and root growth

Fine root mortality (% cumulative dead to total length) was determined in top pots of M and NM roots by tracking the fate of individual fine roots that were visible from the plastic windows (within a 25-cm^2 square). Except for brief periods when roots were traced, the plastic windows were covered with a flexible opaque plastic covering (Loretex T-4000, Research Plastics Inc.). New and preexistent roots were mapped with different colored pens so that root growth could be tracked. The cohort of roots produced between transplanting into the pots and the start of the drought was followed for 15 weeks for both M and NM roots. Fine roots were marked as dead when they disappeared from the window entirely or showed symptoms typical of decay (e.g., brown and translucent appearance). Fine roots were mapped at 0, 2, 4, 6, 7, 10, 14 and 15 weeks after the onset of the drought. The plastic windows with the root maps were collected at the end of the experiment and the images were digitized using a flat-top office scanner. Root lengths were measured from the digitized images using the program 'Rootlaw'(Pan and Bolton, 1991). Root growth was expressed as visible root length (cm) increase with time.

Respiratory Measurements

Root/soil respiration was estimated as the flux of CO_2 from the top pots of M and NM roots throughout the drought period, during the mornings of the same dates when fine roots were mapped. Fluxes of CO_2 were measured using a dynamic ('instantaneous') method (Norman et al., 1992) and a closed gas exchange system at the soil surface with a metal chamber that was inserted into the head space of the top pots of each split-pot system. The 0.43-L cylindrical chamber (5.7-cm diameter) was connected to a LI-6200 gas exchange system (LICOR, Inc. Nebraska, USA). The chamber was built according to the basic design of

a LI-6000-09 soil respiration chamber (LICOR, Inc., Nebraska, USA), except that a split lid was used to fit around the main root at the top of pots, which was sealed with foam gaskets and flexible sealant (Terostat). Because the measurement period was short (1–3 min. depending on the magnitude of the flux), no attempt was made to stabilize the humidity of the air circulating through the system; therefore, the air was not desiccated or humidified before entering the root chamber. The sensor head of a soil respiration chamber (LICOR part 9960-035) was attached to the main console of the LI-6200 to provide sensors for measurements of air and soil temperature. Except during measurements, an insulated lid covered the root box and split-pot system. Temperatures inside the root box generally tracked soil temperatures at a 10-cm soil depth. Soil temperature was monitored with permanently installed chromel-constantan thermocouples that were placed into the soil at the center of the top pots (ca. 5 cm inside the pot, and 10 cm depth). We did not measure temperature consistently at the surface of the plastic windows (where fine roots where mapped), nonetheless, we did not expect the temperature at the surface of the plastic pots to be much different because soil temperatures inside the pots were similar to air temperatures inside the covered chambers, probably due to the relatively small volume of the pots. Differences in soil temperature never exceeded 3 °C in top pots of M and NM roots (Figure 2C). Root/soil respiration and soil temperature were measured simultaneously for the top pots during the drought. Root/soil respiration rates were expressed as the flux of CO_2 per unit of soil surface.

Root/soil respiration included fungal respiration. Thus, these respiratory measurements of undisturbed roots, soil and AM mycelium allowed continuous tracking of the fate of NM and M roots on the same tree.

Root biomass and measurements of root colonization by AM fungi

Inoculated (M) and uninocculated (NM) roots were harvested after the drought period to measure root biomass, fine root length/mass ratio or specific root length (SRL) and AM colonization. The dry weight of coarse (dia. > 2 mm) and fine roots (dia. < 2 mm) was measured for bottom and top pots to determine allocation patterns among the different treatments. Root length of fine roots was determined for fine roots of bottom and top pots using the line-intersect method (Newman, 1966; Tennant, 1975). Incidence of infection by AM fungi was determined by collecting 20 randomly selected samples of fine root segments of 1-cm length from the last harvest date from each pot, clearing the roots with KOH followed by acidified NaOCl and then staining the roots with 0.05% trypan blue-lactophenol (Graham et al., 1991). The incidence of colonization by AM fungi was expressed as the percentage of 20 1-cm root segments cointaining both hyphae and arbuscules. No vesicles were observed in any of the colonized root segments.

Statistical Analysis

Data of biomass of fine roots and coarse roots, SRL and incidence of AM fungal colonization were analyzed in a two-way ANOVA as a stripped-split-plot design for the effects of mycorrhizal treatment (M and NM) and pot position (top and bottom) and the interaction of these factors. Means were compared by a Duncan's test between M and NM treatments and top and bottom pots. Data of AM fungal colonization expressed in percentages of root length were analyzed after square root transformation by the formula: $\sqrt{(x + 0.5)}$. Data of fine root mortality and root/soil respiration were analyzed by paired t-test to compare the results of M versus NM roots after the drought period (top pots only). Data of fine root mortality expressed in percentages were analyzed after arcsine transformation. All statistical analysis was performed using the SAS statistical program (version 6, SAS Institute, NC).

Results

The incidence of AM colonization at the end of the experiment was significantly higher in inoculated roots (M) than uninoculated roots (NM) after 8 to 15 weeks of exposure to dry surface soil (Table 1). Although root colonization at the end of the experiment averaged only 21%, the roots that were not inoculated were completely void of colonization after 10 months of establishment and the periods of 5, 8 or 15 weeks of localized drought. The percentages of colonization were very similar in fine roots of the top (dry) and bottom (wet) pots of inoculated (M) and uninoculated (NM) roots.

Fine and coarse root biomass were not significantly different ($P < 0.05$) between M and NM roots of adult trees after 8 or 15 weeks of localized drought (Table 1). Similar results were found for the roots in the

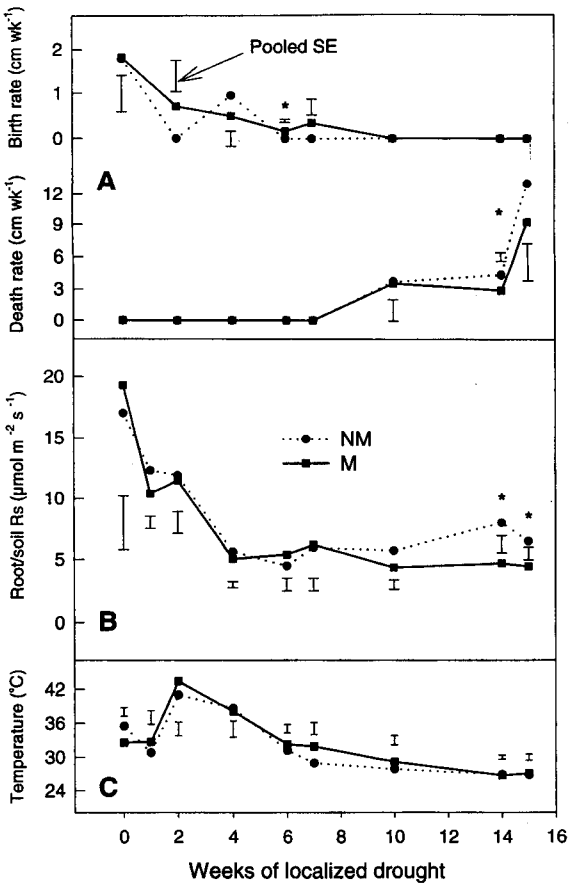

Figure 2. (A) Root/soil respiratory rates (μmol CO_2 m^{-2} s^{-1}) of arbuscular mycorrhizal fine roots (M) and nonmycorrhizal fine roots (NM) from adult trees exposed to dry surface soil for 15 weeks. (B) Root growth and death rates (cm week $^{-1}$) of the cohort of roots traced on the transparent windows of the top pots of M and NM roots of adult trees during 15 weeks of localized drought. (C) Soil temperature (°C) at 10-cm depth in the top pots of M and NM roots. Data were analyzed by paired *t*-test (n = 5, differences are significant at: * $0.01 < P < 0.05$; ** $P < 0.01$).

Table 1. Total mass, specific root length (SRL) and incidence of arbuscular mycorrhizal colonization (%) of fine roots and total mass of coarse roots in the pots at final harvest (8- and 15-week drought data combined). Data were analyzed by a two-way ANOVA (n = 7), and means were compared by a Duncan test between arbuscular mycorrhizal roots (M) and nonmycorrhizal roots (NM) and top and bottom pots (different letters are significant at: * $0.01 < P < 0.05$). Pooled s.e. is shown in parentheses

		Fine roots			
		Incidence of VAM colonization (%)	Biomass (g)	SRL (cm g^{-1})	Coarse roots (g)
Top	NM	0.00 b	3.82 b	2490 a	3.95 a
(dry)	M	20.7 a	4.74 b	1790 b	3.97 a
		(10.4)	(0.87)	(470)	(0.54)
Bottom	NM	0.00 b	6.26 a	2220 a	2.04 ab
(wet)	M	21.2 a	7.07 a	1860 b	2.18 ab
		(8.00)	(1.83)	(180)	(0.79)

bottom (wet) pots. Fine root biomass was larger in the bottom than top (dry) pots ($P < 0.05$), even though bottom roots were confined to the bottom pots within the chambers and did not grow out of the pots into untreated areas of soil. Differences between bottom and top pots can be associated with differential moisture, nutrients or relative location of the drought along the root axis.

Fine roots inoculated with AM fungi (M roots) exhibited significantly lower specific root length (SRL) than uninoculated fine roots (NM roots) after 8 and 15 weeks of drought ($P < 0.05$; Table 1). Similarly, higher SRL in NM than M roots was found in the bottom pots. There was no difference in SRL between the bottom (wet) and top (dry) pots ($P < 0.05$).

Fine root mortality was very low (2%) in M and NM roots after 5 weeks of drought, but increased to 26% and 28% after 8 weeks in M and NM roots, respectively, and continued to increase thereafter with a rapid peak near the end of the experiment (from 14 to 15 weeks) (Table 2, Figure 2A). While fine root mortality was very similar between NM and M roots when exposed to less than 8 weeks of localized drought, at 15 weeks NM roots exhibited higher fine root mortality (42%) than M roots (33%). Total root length present in the observation surface of top pots before the application of the drought averaged 100.0 cm in NM roots and 95.2 cm in M roots, and total root death after the 15-week drought averaged 42.1 cm and 32.4 cm, respectively. Weekly estimates of fine root mortality of the plants exposed to 15 weeks of drought (Figure 2A) exhibited a similar time course to those exposed only to 5 and 8 weeks of drought at a later starting date (Table 2).

Drought depressed fine root growth equally in NM and M root systems. Root growth was about 2 cm $week^{-1}$ for the two-week period prior to drought for both M and NM roots (top pot only; Figure 2A). In the following weeks, root growth decreased steadily. During the last 7 weeks of drought there was no growth recorded for either M or NM roots. The drought treatments did not affect overall tree water relations, because only a minimal portion of the root system was exposed to drought. Shoots of trees grew normally throughout the study and never exhibited symptoms of water or nutrient stress.

Before drought started, root/soil respiration was not significantly different between M and NM roots for the group of roots exposed to dry surface soil for 15 weeks (Figure 2B). Root/soil respiration rates diminished similarly in both M and NM roots during the first seven weeks of drought. After 8 weeks, NM roots tended to exhibit higher root/soil respiration than M roots (Figure 2B). These differences were significant by 14 weeks of drought ($P < 0.05$).

Total root mass data at the end of each drought period (8 and 15 weeks of drought) were used to estimate the rates of root respiration per unit mass of roots. Similar patterns of divergence in root/soil respiration were observed when respiration was expressed per unit of root mass (Table 2). Nonmycorrhizal roots exhibited significantly higher root/soil respiration per unit root mass than M roots ($P < 0.05$) at 15 weeks of exposure to surface dry soil (Table 2). Estimates of root/soil respiration per unit root mass before the drought are not presented because data of root mass were only available at final harvest (i.e., at the end of the drought).

Discussion

The results of the present study demonstrate the feasibility of manipulating AM fungal colonization on trees in the field using quite simple techniques. In this study we achieved an incidence of AM formation in about 20% of the fine root segments on one woody lateral while excluding AM formation in the fine roots of another woody lateral (Table 1). Moreover, we would expect higher colonization by AM fungi under conditions where soil P was lower and where the fungicide, metalaxyl, was not required to control *Phytopthora* infestations. The main shortcomings to this approach were that the roots were are still grown in pots and not directly in the soil, and that the method was quite labor intensive.

We provide the first field demonstration that colonization by AM fungi can indeed subtly decrease root death under localized drought. Previously, many investigators have argued that mycorrhizas may improve plant water relations under drought but never have demonstrated mycorrhizal effects on localized root survival in dry soil. Very low fine root mortality was observed during the first 5 weeks of exposure to dry soil, but increased to more than 25% by 8 weeks and reached 33% (M plants) compared to 42% (NM plants) by 15 weeks. The time course of root respiration was similar to that of root growth and death. For instance, the initial drop in root/soil respiration (Figure 2B) coincided with decreasing root growth rates for M and NM roots in the first month of exposure to dry surface soil (Figure 2A). This reduction in root

Table 2. Mortality of fine roots (% cumulative dead to total length), root/soil respiration per soil surface and root/soil respiration per unit of root mass of adult tree roots harvested after 5, 8 and 15 weeks of localized drought. Data were analyzed by paired *t*-test (for 5 and 8 weeks of drought, $n = 2$; for 15 weeks of drought, $n = 5$; differences between arbuscular mycorrhizal roots (M) and nonmycorrhizal roots (NM) are significant at: * $0.01 < P < 0.05$, ** $P < 0.01$). Root mortality data were analyzed after arcsine transformation of the percentages. Pooled s.e. is shown in parenthesis. Root/soil respiratory rates (Rs) were estimated by dividing total respiration by the final root mass. No data was available for root/soil respiration per unit root mass for 5 weeks of drought

Weeks of drought		Fine root mortality (%)	Root/Soil Rs (μmol m^{-2} s^{-1})	Root/Soil Rs (nmol g^{-1} s^{-1})
5	NM	2.29	3.80	-
	M	1.75	7.09	-
		(2.30)	(1.98)	-
8	NM	27.9	2.18	0.94
	M	25.7	2.91	1.09
		(3.32)	(0.88)	(0.30)
15	NM	42.0 *	6.49 *	1.99 *
	M	33.3	4.48	1.32
		(3.87)	(1.08)	(0.22)

respiratory activity of M and NM roots could be a direct response to reduction in growth and ion-uptake respiration (Kosola and Eissenstat, 1994; Veen, 1981). Contrary to what was observed in the first month, the increase in respiration after 8 weeks of localized drought was probably related to an increase in root death and subsequent metabolism of root constituents by soil microbes in the NM pots. When comparing M and NM roots, 45% higher respiration in NM than M roots after 15 weeks of drought was associated with about 25% higher mortality in NM than M roots.

Higher rates of root/soil respiration in NM roots could be attributed also to differences in carbon economy of NM and M fine roots under drought. These differences may be important only under prolonged exposure to dry soil, because respiration of M and NM roots were more similar for the first 10 weeks of localized drought than after 15 weeks of drought (Figure 2). Suppression by mycorrhizas of root activity after prolonged exposure to dry soil would prevent excessive carbon expenditure in maintenance respiration. Thus, the ability of AM fungi to reduce root respiration under drought may delay root shedding. In addition, the retention of M roots may also be a way by which the fungus guarantees its survival under hostile environmental conditions.

Colonization by AM fungi decreased the SRL of roots, as was also noted by Eissenstat et al. (1993) and Peng et al. (1993) for sour orange and Volkamer lemon seedlings, respectively. This response was found only at low-P supply and not at high-P status; therefore, it was concluded that the effect of arbuscular mycorrhizas on SRL was an indirect P effect (Eissenstat et al., 1993). These authors also suggested that the effect of P status on SRL could become less pronounced in large plants. Berta et al. (1995) found that AM formation decreased SRL in micropropagated plantlets of *Prunus cerasifera* L. that were adequately supplied with phosphorus. In our experiment with adult tree roots, arbuscular mycorrhizal formation also reduced SRL for roots that were fertilized frequently with a high-P nutrient solution. These results suggest that root responses to AM fungal colonization such as SRL may depend on whether roots are from adult or from juvenile trees. If SRL indeed decreases in M roots of adult trees, and assuming that higher root diameter is associated with lower SRL (Eissenstat, 1992), then the larger diameter with a lower surface to volume ratio in M than NM roots could influence root tolerance to dry soil. Survival of fibrous roots was even higher in M juvenile trees compared to M adult trees after 15 weeks of drought (93% versus 67% survival, respectively), and that survival was also associated with lower SRL of fibrous roots of M juveniles (SRL of M roots of juveniles: 1200 cm g^{-1} versus 1800 cm g^{-1} of M roots of adult trees; Espeleta and Eissenstat, 1998). Nonmycorrhizal roots may partially compensate for their lack of absorptive surface area compared

to M roots by constructing fine roots with higher SRL (Table 1), but this allocation pattern, which increases the surface-to-volume ratio, may incur the risk of causing roots to be more susceptible to desiccation. From a cost-benefit perspective, survival of thick roots (low SRL) in dry soil has the advantage of avoiding the cost of building new roots of high construction cost per unit of root length (Nobel et al., 1992). These potential linkages between form and function are analogous to parallel tradeoffs found in leaves, where longevity and thickness are often interrelated (Chabot and Hicks, 1982; Chapin et al., 1987; Williams et al., 1989).

Other investigators have demonstrated that colonization by AM fungi can increase root survival by improving resistance to root pathogens. Mechanisms may include increased P nutrition which reduces root exudation, thus, making the roots less attractive to root diseases (Graham, 1988; Stroble and Sinclair, 1992) as well as direct initiation of plant defense compounds (Benhamou et al. 1994).

Mycorrhizas may affect root survivorship in more subtle ways. Compared to NM plants, Hooker et al. (1995) found that root longevity was lower in AM poplar seedlings (*Populus gerosa inter americana*) where cuttings were rooted in previously sterilized soil and grown in a controlled environment. Mycorrhizal colonization had no effect on shoot dry weight or leaf area in this study. The fine roots of seedlings of woody species may differ from those of adults in response to environmental stress. In citrus, for example, we have found substantially higher root survival in seedlings than bearing trees, when both were exposed to dry surface soil for 15 weeks (Espeleta and Eissenstat, 1998).

In general, the comparison of our results and previous studies using citrus seedlings suggests that AM effects on root responses to localized drought may be affected noticeably by tree developmental stage. A parallel study has shown that tree juvenility also affects root morphological attributes such as SRL and enhances fine root survival and respiration in dry soil (Espeleta and Eissenstat, 1998). We show here that arbuscular mycorrhizas can have important effects on root morphology in wet and dry soil (bottom and top pots), and on root respiration and root mortality in dry soil. Nevertheless, differences between top and bottom pots of our experiment should be interpreted carefully, because results may have been influenced also by the relative location of the drought treatment along the axis of the root. Responses of the roots to the localized drought may have been influenced also by their previous exposure to a nutrient-rich patch relative to the majority of roots of the tree. Additionally, we advise caution, however, in the extrapolation of our data on root mortality and root respiration to wet soil conditions. Recent studies of periodic root observation with minirhizotrons have found considerable death of fine roots in adult citrus trees that may be linked to infestations of *Phytophthora nicotianae* in moist soil (Kosola et al., 1995). Similarly, seasonal variation in responses of M and NM roots should also be considered, especially in relation to periods of reproductive allocation (Eissenstat and Duncan, 1992).

In contrast to the fine roots of seedlings, responses of the fine roots of mature trees to AM fungi have been rarely studied. We demonstrated a rather simple approach to obtain M and NM roots in adult trees in the field by removing the fine laterals and letting them regrow under controlled soil conditions. Our technique was successful in producing differential AM fungal colonization even after almost a year in the field. Because small woody (e.g., 3–8 mm) roots rarely have mycorrhizas but still typically are a reservoir of meristems for new lateral roots, our approach should be applicable to many woody species. We believe that our experimental approach could be adapted to many studies in orchards and forests where the effects of mycorrhizas on root behavior are of interest.

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