Effect of arbuscular mycorrhizal (AM) fungal communities on growth of ‘Volkamer’ lemon in continually moist or periodically dry soil

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Abstract

Citrus volkameriana Tan. and Pasq. (‘Volkamer’ lemon) seedlings were inoculated with five different communities of arbuscular mycorrhizal (AM) fungi collected from citrus orchards in Mesa and Yuma, AZ, USA, and undisturbed North American Sonoran Desert and Chihuahuan Desert soils. Plants were then grown in a glasshouse for four months under continually moist or periodically dry conditions achieved by altering watering frequency so that before watering events container soil water tensions were approximately −0.01 MPa (continually moist) or −0.06 MPa (periodically dry) one half way down the container profile. Plants grown in continually moist soil had greater shoot growth than plants grown in periodically dry soil. Plant P status did not limit growth, and there was no interaction between watering frequency and AM fungal inoculum treatments. Plants inoculated with AM fungi from the Yuma orchard soil had significantly less root dry weight and total root length, and lower photosynthetic fluxes than plants treated with inoculum from the other soils. Specific soil respiration and an estimated carbon cost to benefit ratio were also higher for plants inoculated with AM fungi from the Yuma orchard soil than for plants treated with inoculum from the other soils. The Yuma orchard inoculum was distinctive in that > 80% of the total number of AM fungal spores were from a single species, Glomus occultum. These data showed that root growth suppression of plants treated with the Yuma inoculum, compared with plants treated with inoculum from all other sites, was substantial and greater in magnitude than the effect of periodic soil drying. Suppression of root growth might have resulted from increased AM fungal

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activity resulting in higher carbon costs to the plant. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The potential of arbuscular mycorrhizal (AM) fungi to enhance citrus growth (Menge, 1983), whole plant transpiration (Levy et al., 1983), root hydraulic conductivity (Graham and Syvertsen, 1984) and photosynthesis (Johnson, 1984) has been well documented. Citrus are highly mycorrhizal dependent and citrus roots under orchard condition are normally mycorrhizal (Nemec et al., 1981). AM fungal enhancement of citrus growth has been usually attributed to enhanced phosphorus (P) nutrition of AM plants, but only when soil P supply is limiting (Graham, 1986). Eissenstat et al. (1993) reported a threefold increase in the relative growth rate of citrus seedlings inoculated with *Glomus intraradices* FL208 under conditions of suboptimal soil P. In contrast, Peng et al. (1993) reported a 12% reduction in citrus relative growth rates by *G. intraradices* under conditions of high soil P. Because high energy phospho-nucleotides mediate enzymatically driven plant processes in an non-steady state manner (Eissenstat et al., 1993), it is difficult to identify potential AM fungal effects on plant function that are not directly related to mycorrhizal enhancement of plant P nutrition. There is, however, increasing evidence that AM fungi stimulate citrus root growth independent of P nutrition (Eissenstat et al., 1993, Graham and Syvertsen, 1984; Peng et al., 1993). Since citrus seedlings with small root systems are vulnerable to desiccation (Davies and Albrigo, 1994), inoculation of citrus roots with AM fungal populations that enhance growth might improve transplant survival of seedling nursery stock into field sites.

Previous research documenting the effect of AM fungi on citrus growth and physiology have largely been based on differences between plants inoculated with a single common isolate of AM fungi (typically *G. intraradices*) and non-AM plants. However, citrus orchard soils contain communities of AM fungi rather than a single species (Nemec et al., 1981), and several or all of these species might colonize citrus roots at the same time. The relevance of species diversity to the function of AM fungi in the field is poorly understood (Graham et al., 1996) because data comparing different communities of AM fungi on plant growth and physiology are lacking (Graham, 1986).

Different species, and even geographic isolates of the same species of AM fungi might vary with respect to their ability to colonize roots and improve plant growth (Camprubi and Calvet, 1996; Graham et al., 1982, 1996). Relatively high water and nutrient soil inputs might, over time, favor proliferation of species or
strains of AM fungi colonizing citrus roots that, while tolerant of cultural management practices, are less likely to enhance plant growth (Kurle and Pfleger, 1994). Increased below-ground carbon allocation to AM fungi colonizing plant roots might result in plant growth depression if not compensated for by an increase in carbon assimilation (Graham et al., 1996; Graham and Eissenstat, 1998). In this study, we compared effects of AM fungal communities from orchard soils and undisturbed desert sites on growth and physiology of ‘Volkamer’ lemon grown under continually moist or periodically dry soil conditions achieved by altering watering frequency.

2. Materials and methods

Communities of AM fungi from two Arizona, USA, citrus orchard soils and from three undisturbed desert soils in the Southwestern United States were used as inoculum in this study. AM fungal populations from citrus orchards originated from rhizosphere soil of either ‘Volkamer’ lemon rootstock grown at the Yuma Mesa Agricultural Experiment Station in Yuma, AZ, USA (32.6°N, 114.6°W), or from rhizosphere soil of sour orange (Citrus aurantium L.) rootstock grown at a commercial orchard in Mesa, AZ, USA (33.2°N, 111.5°W). AM fungal populations from desert locations originated from rhizosphere soil of Prosopis spp. growing in the central Sonoran Desert near Verde River, AZ, USA (33.3°N, 111.4°W), the western Sonoran Desert near Borrego Springs, CA, USA (33.1°N, 116.2°W), or the western Chihuahuan Desert near Padre Canyon, TX, USA (31.4°N, 105.6°W).

Soil from each site was used to establish trap cultures of each AM fungal community (Stutz and Morton, 1996). The AM fungal communities from citrus orchard soils were cultured for one cycle (3.5 months) on sudan grass (Sorghum sudanense [Piper.] Staph.). Desert populations were cultured for three cycles on sudan grass to increase the AM fungal inoculum potential (Stutz and Morton, 1996). Colonized root pieces, hyphae, rooting substrate, and spores from the trap cultures were used as inoculum. The AM fungal species composition of each inoculum was determined by extracting spores from a 100 cm³ subsample via wet sieving, decanting and sucrose gradient centrifugation (Daniels and Skipper, 1982). A dissecting microscope was used to count spore morphotypes. Each morphotype was mounted in PVLG and Meltzer’s/PVLG (Koske and Tessier, 1983) and identified based on subcellular characteristics.

Fifty uniform (0.10 m tall), two-month old, non-mycorrhizal ‘Volkamer’ lemon seedlings were potted into 8 l plastic pots filled with a pasteurized potting mixture of coarse sand (particle size < 4.25 mm) blended with soil (3 : 1 by volume) which had been passed through a 2 mm sieve. The soil was a Gilman clay loam collected at a depth of 0.1–0.3 m in the A horizon from the Arizona State
University Horticultural Resource Unit, Tempe, AZ, USA. Soil analysis was as follows: pH 7.3, EC = 0.25 dS/m, 11.26 mg sodium bicarbonate extractable P/g soil, and 13.2 g organic matter per kilogram soil. During the study all plants were grown in a temperature-controlled glasshouse under natural irradiance (air temp = 30°C day/21°C night, mean maximum PAR = 1200 mmol/m²/s, VPD range = 1 to 2 KPa).

At potting, inoculum from one of the five AM fungal populations was placed subjacent to the transplants. Inoculum potential was previously determined using the most probable number method (Alexander, 1982), and each pot received approximately \(1.5 \times 10^6\) AM fungal propagules. All plants were fertilized with 12 g of 20N–0P–16.6K–2Fe–1.4Mn (slow release, N source as isobutylidene diurea) and 22 g of 0N–19.6P–0K–12S (concentrated superphosphate).

After a one-week acclimatization period, half of the plants were randomly assigned to either a continually moist or periodically dry watering treatment. Over a four-month period, plants subjected to the continually moist treatment were watered in excess of container capacity when soil matric potential (water tension) decreased to −0.01 MPa (approximately a 3-day cycle). Plants subjected to the periodically dry treatment were watered in excess of container capacity when potting soil water tensions fell to −0.06 MPa (approximately a 12-day cycle). Soil water tension was monitored with tensiometers (Soilmoisture Equipments, Santa Barbara, CA, USA) inserted into the soil so that the ceramic tips were located approximately one-half the distance between the surface and the container bottom and 4 cm from the container wall.

Approximately three months after the initiation of watering frequency treatments, leaf gas exchange and water potential measurements and soil respiration measurements were made on the second day after an irrigation event when soil water tension and plant water status were nearly uniform in all treatments. Leaf gas exchange measurements were made at mid-day on recently expanded leaves of similar appearance (approximately the seventh leaf from the stem apex) on each plant using an infra red gas analyzer (IRGA, LI-COR 6200; LI-COR, Lincoln, NE, USA) and 0.25 l chamber attachment. A pressure bomb (Soil Moisture Equipments, Santa Barbara, CA, USA) was used to measure predawn and mid-day leaf water potential. Each water potential measurement was based on a single leaf per plant using recently fully expanded leaves of similar appearance (approximately the eighth leaf from the apex). For soil respiration measurements, polyvinyl chloride cylindrical collars (7.0 cm height, 10.1 cm inside diameter) were inserted into the potting soil to a depth of about 2 cm one day before the measurements were made. A soil respiration chamber (LI-COR 6000-09) attached to the IRGA was fitted to the collar such that the chamber air supply manifold was 1–2 cm above the potting soil surface. A foam gasket was placed between the collar and the chamber to prevent air leaks. Soil respiration per pot was derived from the average of four consecutive CO₂ flux measurements.
At the end of the study, total stem length was measured and all plants were harvested. Leaves and stems were separated from roots. Roots were washed free of soil and fresh root weights were recorded. Canopy leaf area and root lengths were measured using a digital camera interfaced with a computer (AgVision Digital Imaging System, Pullman, WA, USA). All roots were inspected optically (with the aid of a stereomicroscope) for pathogens and symptoms of disease and appeared to be healthy. A sample of 1 cm length root pieces (approximately 1 g fresh weight) was collected from each tree and fixed in a formalin–acetic acid–alcohol solution. Roots were stained using 0.05% trypan blue in acidic glycerol (Koske and Gemma, 1989). The magnified intersections method (McGonigle et al., 1990) was used to quantify AM fungal colonization of roots. Roots and shoots were oven dried at 65°C for 48 h and dry weights were measured. An estimation of the carbon cost to benefit ratio \( R_T/A_T \) of plants in response to treatments was calculated as

\[
R_T/A_T = (R_{SP} \times R_{DW} \times SA)/(A \times LA),
\]

where \( R_{SP} \) is the specific soil respiration \((\mu mol/m^2/s/g\) root dry weight), \( R_{DW} \) the total root dry weight (g/plant), \( SA \) the container soil surface area \((0.042 m^2)\), \( A \) the carbon assimilation flux \((\mu mol/m^2/s)\), and \( LA \) is the canopy leaf area \((m^2/plant)\).

Phosphorus concentrations in dry, pulverized leaves were determined by the ascorbic method (Watanabe and Olson, 1965). Dry, pulverized tissue was boiled in ethanol to remove sugars. Starch was extracted from the remaining tissue pellet by digestion in a solution of a-amylase and amyloglucosidase. Carbohydrate concentrations in the resulting solutions were determined using a modified anthrone method with phenol substituted for anthrone (Scott and Melvin, 1953).

The experiment was arranged in a split plot (continually moist and periodically dry) completely randomized block design with five AM fungal inoculum treatments and five replications for a total of 50 plants. All data was subjected to the general linear model (GLM) procedure (SAS Institute, Cary, NC, USA) for analysis of variance. Percent AM root colonization data were arcsin transformed to approximate a normal distribution for analysis of treatment effects. Actual percentage means were reported. Comparisons among treatments were made with Duncan’s Multiple Range Test.

3. Results

**AM species composition of inoculum and mycorrhizal colonization responses.** A range of 4–7 AM fungal species were detected in the inoculum mixtures (Table 1). The lowest number of species was detected in the Yuma orchard inoculum while the highest number was in the Borrego Springs desert inoculum.
Glomus microaggregatum was the only species detected in all of the inoculum treatments. The Yuma orchard inoculum was distinctive in that > 80% of the total number of AM fungal spores were from a single species, Glomus occultum. There was no interaction effect of inoculum and watering frequency on AM fungal colonization of plant roots. Plants exposed to periodic soil drying had less total colonization, arbuscules, and vesicles than well-watered plants (Table 2). There were no differences in total colonization between inoculum treatments; however, arbuscular colonization was greatest in plants treated with AM fungi from the Yuma orchard. Plants treated with the Mesa orchard or Verde River desert inoculum had a similar number of vesicles and more than plants grown with other inocula. No septate hyphae were observed in the roots, nor was any necrosis of cortical cells observed.

Plant growth responses and tissue P levels. Plant growth was not affected by the interaction of watering frequency and inoculum treatments, thus, we present plant growth responses to watering and mycorrhizal treatments separately. At harvest, plants grown in periodically dry soil had significantly less total stem length and shoot dry weight, lower shoot to root dry weight ratio (S/R), less canopy leaf area, and a lower canopy leaf area to root length ratio (LA/RL) than plants grown in continually moist soil (Table 3). Root dry weight, total root

<table>
<thead>
<tr>
<th>AM fungal species and authority</th>
<th>Relative inoculum abundance (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
</tr>
<tr>
<td>Acaulospora trapezi Ames &amp; Linderman</td>
<td>21</td>
</tr>
<tr>
<td>Glomus eburneum Kennedy, Stutz &amp; Morton</td>
<td>0</td>
</tr>
<tr>
<td>Glomus etunicatum Becker &amp; Gerdemann</td>
<td>2</td>
</tr>
<tr>
<td>Glomus intraradices Schenck &amp; Smith</td>
<td>11</td>
</tr>
<tr>
<td>Glomus macrocarpum Tulasne &amp; Tulasne</td>
<td>0</td>
</tr>
<tr>
<td>Glomus microaggregatum Koske, Gemma &amp; Olexia</td>
<td>30</td>
</tr>
<tr>
<td>Glomus mosseae (Nicol. &amp; Gerd.) Gerdemann &amp; Trappe</td>
<td>1</td>
</tr>
<tr>
<td>Glomus occultum Walker</td>
<td>0</td>
</tr>
<tr>
<td>Glomus spurcum (Pfeiffer, Walker &amp; Bloss) Kennedy, Stutz &amp; Morton</td>
<td>33</td>
</tr>
<tr>
<td>Glomus versiforme (Karsten) Berch</td>
<td>2</td>
</tr>
<tr>
<td>Glomus spp. AZ112b</td>
<td>0</td>
</tr>
</tbody>
</table>

a Number of AM fungal spores for each species as a percentage of the total number of spores in a 100 cm³ sample.

b Undescribed species number refers to accession number at International Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi, West Virginia University, Morgantown, WV, USA.
length, and leaf P concentrations were not significantly affected by watering frequency treatments.

AM fungal population treatments had similar effects on plant growth with a few exceptions (Table 3). Plants treated with the Padre Canyon or Verde River inoculum had greater shoot dry weight than plants treated with the Yuma orchard inoculum. Plants treated with the Yuma orchard inoculum had lower total root length.

Table 2
Effect of watering frequency and inoculum treatments on percent root length colonized by AM fungi

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Arbuscules</th>
<th>Vesicles</th>
<th>Hyphae only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watering</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continually moist</td>
<td>31 a b</td>
<td>15 a</td>
<td>19 a</td>
<td>64 a</td>
</tr>
<tr>
<td>Periodically dry</td>
<td>23 b</td>
<td>3 b</td>
<td>16 a</td>
<td>43 b</td>
</tr>
<tr>
<td><strong>Inoculum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borrego Springs</td>
<td>29 ab c</td>
<td>6 b</td>
<td>16 a</td>
<td>49 a</td>
</tr>
<tr>
<td>Padre Canyon</td>
<td>21 b</td>
<td>4 b</td>
<td>17 a</td>
<td>44 a</td>
</tr>
<tr>
<td>Verde River</td>
<td>21 b</td>
<td>12 ab</td>
<td>23 a</td>
<td>58 a</td>
</tr>
<tr>
<td>Mesa</td>
<td>25 b</td>
<td>17 a</td>
<td>20 a</td>
<td>59 a</td>
</tr>
<tr>
<td>Yuma</td>
<td>39 a</td>
<td>5 b</td>
<td>13 a</td>
<td>57 a</td>
</tr>
</tbody>
</table>

*a Values are treatment means, n = 25 for watering frequency treatments and n = 5 for mycorrhizal inoculum treatments.

*b Means followed by a different letter are significantly different (a = 0.05) according to LSD, or

*c Duncan’s Multiple Range Test.

Table 3
Effect of irrigation frequency and inoculum treatments on total stem length, shoot and root dry weights, shoot to root dry weight ratio (S/R), canopy leaf area, total root length, canopy leaf area to total root length ratio (LA/RL) and leaf phosphorus concentration (P) of Citrus volkameriana

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem length (g/plant)</th>
<th>Shoot dry weight (g/plant)</th>
<th>Root dry weight (g/plant)</th>
<th>S/R</th>
<th>Canopy leaf area (m²)</th>
<th>Root length (m/plant)</th>
<th>LA/RL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watering</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continually moist</td>
<td>0.70 a b</td>
<td>5.9 a</td>
<td>6.1 a</td>
<td>0.96 a</td>
<td>1.02 a</td>
<td>2.88 a</td>
<td>0.39 a</td>
<td>2.1 a</td>
</tr>
<tr>
<td>Periodically dry</td>
<td>0.60 b</td>
<td>4.1 b</td>
<td>5.4 a</td>
<td>0.77 b</td>
<td>0.73 b</td>
<td>2.74 a</td>
<td>0.28 b</td>
<td>2.1 a</td>
</tr>
<tr>
<td><strong>Inoculum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borrego Springs</td>
<td>0.59 ab</td>
<td>5.6 a</td>
<td>6.0 a</td>
<td>0.93 a</td>
<td>0.86 ab</td>
<td>2.8 a</td>
<td>0.30 ab</td>
<td>2.1 b</td>
</tr>
<tr>
<td>Padre Canyon</td>
<td>0.66 a</td>
<td>5.3 ab</td>
<td>6.3 a</td>
<td>0.84 a</td>
<td>0.87 ab</td>
<td>3.3 a</td>
<td>0.26 b</td>
<td>1.8 b</td>
</tr>
<tr>
<td>Verde River</td>
<td>0.71 a</td>
<td>5.4 ab</td>
<td>6.4 a</td>
<td>0.84 a</td>
<td>0.91 ab</td>
<td>3.1 a</td>
<td>0.29 ab</td>
<td>1.8 b</td>
</tr>
<tr>
<td>Mesa</td>
<td>0.77 a</td>
<td>5.7 a</td>
<td>6.4 a</td>
<td>0.84 a</td>
<td>1.12 a</td>
<td>3.0 a</td>
<td>0.37 a</td>
<td>2.3 ab</td>
</tr>
<tr>
<td>Yuma</td>
<td>0.51 b</td>
<td>3.3 b</td>
<td>3.6 b</td>
<td>0.91 a</td>
<td>0.63 b</td>
<td>1.7 b</td>
<td>0.37 a</td>
<td>2.7 a</td>
</tr>
</tbody>
</table>

*a Values are treatment means, n = 5.

*b Within treatments, column means followed by a different letter are significantly different according to Duncan’s Multiple Range Test (a = 0.05).
length and root dry weight than plants treated with the other inoculum treatments. Plants treated with the Yuma orchard inoculum also had higher leaf P concentrations than plants grown with the other inoculum treatments. Plants treated with the Mesa or Yuma inoculum differed in canopy leaf area, with the former having significantly greater area than the latter. However, plants treated with the orchard inocula had higher LA/RL than plants treated with the desert inoculum from Padre Canyon. Mycorrhizal inoculum treatments had no effect on S/R.

Physiological responses and carbohydrate analysis. There was a significant interaction between watering frequency and AM fungal inoculum treatments on leaf gas exchange (Table 4). When grown in moist soil, plants treated with the Padre Canyon desert inoculum had the highest leaf carbon assimilation (A) and stomatal conductance (gs). In contrast, plants treated with the Yuma orchard inoculum had the lowest A and gs, while plants treated with the Yuma orchard and Verde River desert inoculum had the lowest water use efficiency (WUE) compared with plants treated with other inocula. Mycorrhizal inoculum treatments had no effect on C1 : CA.

When grown in periodically dry soil, plants treated with Yuma orchard inoculum had the lowest A and WUE, and the highest C1 : CA (Table 4). Plants treated with Borrego Springs or Padre Canyon desert inocula had the highest gs, while plants inoculated with the Verde River or Mesa inocula had the lowest C1 : CA compared with plants treated with the other mycorrhizal inoculum treatments.

Specific soil respiration (RSP) was affected by an interaction of watering frequency and inoculum treatments (Table 4). Plants treated with the Yuma or Mesa orchard inocula had greater RSP than other plants treated with the desert inocula (P = 0.02) when grown in continually moist soil. However, only plants treated with the Yuma orchard inoculum had greater RSP that was between two and three times more than plants treated with the any of the other inoculum treatments when grown in periodically dry soil. Our estimated carbon cost to benefit ratio of plants treated with the Yuma orchard inoculum was about 1.7 (continually moist) or 2.4 times (periodically dry) higher than for plants treated with the other inocula.

Leaf sugar levels were not affected by watering frequency, but leaf starch levels were lower for plants in periodically dry soil compared with plants grown in continually moist soil (Table 5). Plants grown in continually moist soil had higher root sugar and starch concentrations than plants grown in periodically dry soil. Leaf carbohydrate concentrations were not affected by AM fungal population treatment (Table 5). Plants treated with the Borrego Springs desert inoculum had higher levels of root sugar than plants treated with Verde River desert inoculum, and higher levels of starch than plants treated with Verde River desert or Mesa orchard inocula (Table 5). Watering frequency and AM fungal inoculum
treatments did not affect pre-dawn or mid-day leaf water potentials when measurements were taken under similar (>−0.01 MPa) soil water tensions (data not shown).

4. Discussion

These data show that communities of AM fungi can mitigate substantially different effects on growth ‘Volkamer’ lemon plants. The mycorrhizal effects we measured on plant growth may be the result of changes in photosynthate production and/or partitioning (Syvertsen and Graham, 1990). Plants treated with
the Yuma orchard inoculum had root systems that were 43–49% smaller than other plants, and when averaged across both irrigation treatments also had A fluxes that were 18% lower, and $R_{SP}$ and estimated carbon cost to benefit ratio values that were at least 2.0 times higher than plants treated with the other inocula. These data support previous findings that some AM fungi can reduce growth of citrus if the high carbon costs of AM roots are not offset by increased carbon assimilation (Graham et al., 1996).

Arid soils have high AM fungal diversity (Stutz and Morton, 1996), but intensive field cultivation might select for species or isolates of AM fungi that tolerate horticultural practices, but which are not particularly beneficial to crop plants in terms of increased plant growth (Kurle and Pfleger, 1994). Unlike the other AM fungal populations we evaluated, we found that the AM fungal population from the Yuma orchard site was dominated (based on the proportion of spores) by a single AM fungal species, *G. occultum*. Johnson et al. (1992) found that *G. occultum* proliferated in corn fields, and that spore abundance of *G. occultum* correlated negatively with corn plant dry mass and yield. Graham et al. (1996) also found that aggressive strains of AM fungi enhanced plant P uptake and growth at low soil P, but caused growth depression of citrus at high soil P (Graham et al., 1996). Furthermore, Modjo and Hendrix (1986) linked growth depression of tobacco plants to mycorrhizal colonization under conditions of high soil P.

The Yuma orchard soil was very low in organic matter and available P which might have favored proliferation of aggressive strains of AM fungi that enhance P

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**Table 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Sugar</th>
<th>Leaf Starch</th>
<th>Root Sugar</th>
<th>Root Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watering</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continually moist</td>
<td>4.88 a b</td>
<td>3.10 a</td>
<td>2.33 a</td>
<td>1.41 a</td>
</tr>
<tr>
<td>Periodically dry</td>
<td>4.37 a</td>
<td>2.16 b</td>
<td>1.65 b</td>
<td>0.97 b</td>
</tr>
<tr>
<td><strong>Inoculum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borrego Springs</td>
<td>4.56 a c</td>
<td>2.67 a</td>
<td>2.26 a</td>
<td>1.82 a</td>
</tr>
<tr>
<td>Padre Canyon</td>
<td>4.55 a</td>
<td>2.36 a</td>
<td>2.01 ab</td>
<td>1.29 ab</td>
</tr>
<tr>
<td>Verde River</td>
<td>5.20 a</td>
<td>2.56 a</td>
<td>1.76 b</td>
<td>0.89 b</td>
</tr>
<tr>
<td>Mesa</td>
<td>4.55 a</td>
<td>2.72 a</td>
<td>1.95 ab</td>
<td>0.81 b</td>
</tr>
<tr>
<td>Yuma</td>
<td>4.23 a</td>
<td>2.87 a</td>
<td>1.98 ab</td>
<td>1.13 ab</td>
</tr>
</tbody>
</table>

*a Values are treatment means, $n = 25$ for watering frequency treatments and $n = 5$ for mycorrhizal inoculum treatments.

*b Means followed by a different letter are significantly different according to LSD, or*  
  *c Duncan’s Multiple Range Test ($a = 0.05$).*
uptake. Though possibly beneficial in situ at the Yuma site, *G. occultum*, might have been responsible for the poor growth performance of the P sufficient lemon plants treated with the Yuma orchard inoculum in our study. In support of this hypothesis, we note that plants inoculated with AM fungi from the Yuma orchard soil had higher leaf P concentrations and more arbuscules (sites of carbon and P exchange) per root length than plants treated AM fungi from the other soils. This suggests that increased AM fungal activity might have resulted in higher carbon costs to the plant.

Arbuscular mycorrhizal fungi can induce high rates of root/soil respiration which represent considerable carbon costs to the plant (Peng et al., 1993; Graham et al., 1996). If the carbon sink of AM roots is not compensated for by increased A, then growth depression will result (Graham et al., 1996). High values of $R_{SP}$ might indicate that the carbon cost to benefit ratio of the AM association to the plant is great and/or that fungal activity is high (Eissenstat et al., 1993; Graham et al., 1996). Thus, we conclude that the Yuma orchard inoculum presented lemon plants with the highest carbon cost to benefit ratio, which might explain the reduced root length and lower root dry weights found in those plants.

Plants treated with Yuma orchard inoculum and grown under periodically dry soil conditions had lower $A$ and $g_s$ than other plants, but had $C_l : C_A$ that was similar or higher than other plants. Since $C_l$ did not decrease with $g_s$, $A$ limitation for these plants was at least partly stomatal (Mott, 1988). Leaf P concentrations can also limit $A$ (Syvertsen and Lloyd, 1994), but plants treated with Yuma inoculum had the highest leaf P which is evidence that reduced $A$ was not caused by P limitation. Though leaf P concentrations for all plants were sufficient for normal plant growth, we did not measure levels of other nutrients which might have limited $A$. Morphological differences such as increased specific leaf weight or reduced root hydraulic conductance could reduce $A$ in citrus. However, specific leaf weight (data not shown) was not affected by inoculum and though root conductance was not measured, specific root weight (data not shown), and root : shoot ratio were similar among inoculum treatments, suggesting that root hydraulic conductivity might be similar among treatments.

Although plants treated with the Yuma orchard inoculum had the highest estimated carbon cost to benefit ratio, they did not have significantly different levels of non-structural carbohydrates from plants treated with other inocula. In plants with adequate P nutrition, AM fungi affect root carbohydrates more than leaf carbohydrates (Nemec and Guy, 1982), as observed in this study. Reducing sugars are apparently the most responsive to AM infection, but are the smallest carbohydrate fraction, so differences in sugar and starch levels caused by AM fungi can be subtle (Nemec and Guy, 1982; Graham et al., 1997). Since only total sugars were measured in this study, potential small differences in reducing sugars due to AM treatment were not detectable.
Shoot growth was positively correlated to watering frequency. Plants grown under the periodically dry soil had lower leaf carbohydrate concentrations than plants grown under continually moist conditions. Large citrus seedlings have greater carbohydrate concentrations than small seedlings (Nemec and Guy, 1982), so an analysis of variance with shoot size as a covariate was done and showed that shoot carbohydrates were not different if size was taken into account. Thus, shoot growth was probably not limited by leaf carbohydrate concentrations in the dry treatment, but by some other mechanism.

Shoot growth at low soil water potential can be reduced by changes in the elastic properties of cell walls (Nonami and Boyer, 1990) or by production of abscisic acid in the roots (Saab et al., 1990). None of the inocula showed significant effects in terms of enhancement of leaf water potential or improvement of shoot growth. However, AM fungal inoculum did affect aspects of plant morphology that might enhance plant water status, such as lowered leaf area : root length or root : shoot ratios.

Root growth was not affected by soil drying, but root growth can be less sensitive than shoot growth to soil drying (Hsiao and Jing, 1987). Lower root carbohydrate concentrations in plants grown under periodically dry conditions might have been a result of lower carbon acquisition in these plants since canopy leaf area and LA/RL was significantly reduced in plants exposed to periodic soil drying.

5. Conclusion

Growth suppression of plants treated with AM fungi from the Yuma orchard, compared with plants treated with inocula from desert soils or the Mesa orchard, was substantial and greater in magnitude than the main effects of the watering frequency treatments. Previous studies have reported dramatic differences in citrus growth as a function of different mycorrhizal isolates. The causes of differential growth can be phosphorus limitation (Graham et al., 1982; Camprubi and Calvet, 1996) or high carbon costs (Graham et al., 1997; Johnson et al., 1997; Graham and Eissenstat, 1998). Since P limitation was not a factor in our study, the differences in carbon costs of the populations used probably caused the growth effects observed. Further research is needed to determine which AM fungal isolate or combinations of isolates are responsible for plant growth depression. Single species from the Yuma inoculum could be isolated, and inoculation of citrus with isolates individually and in combination would confirm which fungi were responsible for plant growth suppression. Analysis of the effects of the Yuma population compared with the other inocula on growth of citrus under P limited conditions might provide insight into management strategies to enhance AM fungal benefits in citrus orchards.
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References


