Growth of *Citrus volkameriana* inoculated with AM fungi in moist or periodically dry soils.

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Abstract

'Volkamer' lemon (Citrus volkameriana Tan. and Pasq.) seedlings were inoculated with either of five communities of arbuscular mycorrhizal (AM) fungi collected from either citrus orchards in Mesa and Yuma, Arizona or from undisturbed Sonoran or Chihuahuan desert soils. Plants were then grown for four months under low or high irrigation frequency treatments such that soil water tension reached about -0.01 MPa (moist) or -0.06 MPa (periodically dry), respectively. Plants grown in moist substrate had greater shoot mass than plants grown in periodically dry substrate. Plants inoculated with AM fungi from the Yuma orchard soil had significantly less shoot and root mass, higher specific soil respiration rates, and lower photosynthesis rates than plants treated with inoculum from other soils. Plant phosphorus nutrition did not limit growth. These data show that growth of 'Volkamer' lemon seedlings can be substantially affected by arbuscular mycorrhizal fungal communities in moist or periodically dry soils.

Introduction

The potential of arbuscular mycorrhizal (AM) fungi to enhance citrus growth has been well documented. AM fungi can also host plant uptake of phosphorus (Graham, 1986). There is increasing evidence that AM fungi effect citrus root growth independent of phosphorus nutrition (Peng et al., 1993.) AM fungal stimulation of citrus root growth may be beneficial for nursery or outplanting stock since their limited root system makes seedlings vulnerable to desiccation (Davies and Albrigo, 1994.) Alternately, increased below ground carbon allocation of AM plants can also result in plant growth depression if not compensated for by increased carbon acquisition.

Much of the research documenting the effect of AM fungi on citrus growth and physiology is based on differences between plants inoculated with a single isolate of AM fungi (usually *Glomus intraradices* Schenck & Smith) and non-AM plants. However, citrus orchard soils contain communities of arbuscular mycorrhizal (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 AM fungal diversity to the functioning of mycorrhizae in the field is not yet known. Data from comparisons of AM plants, the normal condition of field grown citrus, inoculated with different communities of AM fungi on plant growth and physiology are lacking (Graham, 1986.) It was anticipated that the growth of 'Volkamer' lemon (*Citrus volkameriana* Tan. and Pasq.) seedlings would be altered by AM fungal communities from orchard soils compared to AM fungal communities of desert origin. Thus, the objective of this study was to test the hypothesis that AM fungal communities differentially affect citrus growth under moist or periodically dry conditions.

Different AM fungal species, and even geographic isolates of the same species, can vary with respect to their ability to colonize roots and improve plant growth (Camprubi and Calvet, 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 management

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practices, are less likely to enhance plant growth (Kurle and Pfleger, 1994).

Materials and Methods

Communities of AM fungi from two Arizona citrus orchard soils and from three undisturbed desert soils in the Southwestern United States were used as inoculum in this study. Citrus orchard AM fungal populations originated from rhizosphere soil of either 'Volkamer' lemon rootstock growing at the Yuma Mesa Agricultural Experiment Station in Yuma, Arizona (32.4°N, 114.4°W), or from rhizosphere soil of sour orange (*Citrus aurantium* L.) rootstock from a commercial orchard in Mesa, Arizona (33.2°N, 111.5°W). The non-agricultural, undisturbed desert locations included central Sonoran desert near Verde River, Arizona (33.7°N, 111.6°W), western Sonoran desert near Borrego Springs, California (33.2°N, 116.3°W), and western Chihuahuan desert near Padre Canyon, Texas (31.7°N, 106.0°W).

'Volkamer' lemon seeds were germinated in polyethylene trays containing a steam-sterilized Supersoil and Perlite (1:1 by volume) propagation substrate, then grown for two months in a laboratory (temp. = 22°C; fluorescent lighting, PAR = 55 mmol m-2 sec-1). Seedlings were moved to a glasshouse (temp. = 30°C, PAR @ 1200 mmol m-2 s-1, VPD range = 1 to 2 MPa) where fifty uniform plants (0.10m tall) were potted into 8-L plastic pots filled with a pasteurized and screened (2 mm sieve) rooting substrate mixture of coarse sand and soil (3:1 by volume). The soil was a Gilman clay loam collected at a depth of 0.1 to 0.3 m in the A horizon from the Arizona State University Horticultural Resource Unit, Tempe, AZ. 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/kg soil.

At potting, the appropriate inoculum 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 x 106 AM fungal propagules. All plants were fertilized with 12 g of isobutylidene diurea, (IBDU) slow release fertilizer (20% N - 0% P -16.6% K - 2% Fe -1.4% Mn) and 22 g of concentrated superphosphate (0% N - 19.6% P -0% K).

After a one-week acclimatization period, half of the plants were randomly assigned to either a moist or periodically dry irrigation treatment. Over a four-month period, seedlings subjected to the moist treatment were irrigated when rooting substrate matric water potential (water tension) decreased to -0.01 MPa (approximately a three-day cycle). Seedlings under the periodically dry treatment were irrigated when rooting substrate water tension fell between -0.05 and -0.07 MPa (approximately a 12-day cycle).

When irrigated, all plants received 400 ml deionized H_20 through an electronically controlled drip irrigation system. Rooting substrate water tension was monitored with tensiometers (Soilmoisture Equipment Inc., Santa Barbara, CA, USA) inserted into the rooting substrate such that the ceramic tips were approximately located one-half the distance from the rooting substrate surface and the container bottom and 4 cm from the container wall.

Approximately three months after the start of irrigation treatments, gas exchange measurements were made on recently expanded leaves of similar appearance (approximately the seventh leaf from the stem apex) on each plant using a portable photosynthesis system (LI-COR 6200; LI-COR, Lincoln, NE, USA). Leaf photosynthesis measurements were made on the first day after watering and the last day before watering (soil water tension = -0.01 or 0.05 MPa) on all plants. On the following irrigation cycles, soil respiration measurements were made on the first day after watering and the last day before watering (soil water tension = -0.01 or -0.05 MPa) for each pot. For measuring soil respiration, polyvinyl chloride collars (7.0-cm height, 10.1 cm inside diameter) were inserted into the rooting substrate to a depth of about 2 cm one day before the measurements were made. A soil respiration chamber (LI-COR 6000-09) attached to a portable photosynthesis system was fitted to the collar such that the chamber air supply manifold was 1-2 cm above the rooting substrate surface. At the time leaf and soil gas exchange measurements were taken, shoot size, and consequently soil water tension, varied among treatments at the end of the dry cycle. Thus for treatment comparison purposes, data was reported for plants on the first day of an irrigation cycle only, when soil water tension and plant water status was uniform among treatments.

At the end of the study all trees were harvested. Leaves and stems were separated from roots. Roots were washed free of substrate and fresh root weights were recorded. All roots were inspected optically (with the aid of a stereo and a compound

microscope) for pathogens and symptoms of disease and appeared to- be healthy. -A sample of 1 -cm length root pieces (approximately one g fresh weight) was collected from each tree and fixed in formalin-acetic acid-alcohol. Roots were then cleared, by heating roots at 121°C for 3 minutes in 10% potassium hydroxide, bleached for 30 minutes in alkaline H_20_2, acidified for 12 hours in 1% hydrochloric acid and stained using 0.05% trypan blue in acidic glycerol (Koske & Gemma 1989). The proportion of root length containing fungal arbuscules, vesicles, and hyphae was determined to calculate the percentage of root length colonized by AM fungi. Roots and shoots were oven dried at 65°C for 48 hours and dry weights were measured. Phosphorus concentration in dry, pulverized leaves were determined by ascorbic methods (Watanabe and Olson, 1965).

The experiment was arranged in a split plot (moist and periodically dry) completely randomized block design with five AM fungal treatments (two communities of AM fungi from citrus orchards and three communities of AM fungi from non-cultivated desert soils) and five replications for a total of 50 plants. All data was subjected to the general linear model (GLM) procedure (SAS Institute Inc., Cary, NC, USA) for analysis of variance. Actual percentage means were reported. Comparisons among treatments were made with LSD and Duncan's Multiple Range Test.

Results and Discussion

A range of four to seven species of AM fungi were detected in the inoculum mixtures, with the lowest number detected in the Yuma inoculum and the highest in the Borrego Springs inoculum. *Glomus microaggregatum* Koske, Gemma & Olexia was the only species identified in each inoculum. The Yuma inoculum was distinctive in that greater than 80% of the total number of AM fungal spores were from a single species, *Glomus occultum* Walker. AM fungal colonized 64% of total root length for plants that were well watered, but only 43% for plants that were exposed to periodic drying. Plants exposed to periodic soil drying also had less arbuscules and vesicles per root length than plants that were well watered. Plants treated with Yuma inoculum had the most root length occupied by arbuscules. Plants treated with Mesa inoculum had a similar number of vesicles as plants treated with Verde River inoculum but more vesicles than plants treated with other inoculum. All mycorrhizal treatments resulted in similar total AM fungal infection and percent root length occupied by hyphae. There were no interaction affects between inoculum and irrigation frequency in terms of AM colonization. We saw no septate hyphae in the roots, nor was any necrosis of cortical cells observed indicating that roots were not infected with non-treatment fungi.

Plants grown in periodically dry substrate had smaller shoots but similar-sized roots compared to plants grown in moist substrate (Table 1). AM fungal inoculum treatments affected both shoot and root growth. Shoot and root mass were less for plants treated with the Yuma inoculum compared with plants treated with the other inoculum treatments (Table 1). Leaf phosphorus concentrations were not affected by irrigation frequency treatments. Plants treated with the Yuma inoculum had slightly higher phosphorus concentrations than all other plants. Growth was not affected by an interaction of irrigation frequency with AM fungal inoculum treatments.

There was a significant interaction between irrigation and inoculum treatments on leaf photosynthesis. When grown in moist substrate, plants inoculated with the Padre Canyon AM fungi from western Texas had the highest leaf photosynthetic rates (Table 2). In contrast, plants inoculated with the Yuma orchard AM fungi had the lowest photosynthetic rates and water use efficiency (WUE). When grown in periodically dry substrate, plants treated with the Borrego Springs inoculum of eastern California had the highest photosynthetic rates and WUE, while again plants inoculated with the Yuma AM fungi had the lowest photosynthetic rates and WUE. Irrigation frequency treatments did not affect specific soil respiration rates, and there was no interaction between irrigation frequency with AM fungal inoculum treatments. In moist substrate, plants inoculated with the Yuma and Mesa orchard AM fungi had the highest specific soil respiration (Table 2). In periodically dry substrate, specific soil respiration was between two to three times greater for plants inoculated with the Yuma orchard AM fungi compared with plants inoculated with the other AM fungal populations.

These data show that communities of AM fungi can have substantially different affects on plant performance. Mycorrhizal effects on plant growth must be accompanied by changes in photosynthate production and/or partitioning (Syvertsen and Graham, 1990). Plants treated with Yuma inoculum had strikingly smaller root systems than other plants, and had lower photosynthetic and specific soil respiration rates than plants treated with other inoculum. The Yuma inoculum data supports

indicate that AM fungi can reduce growth of citrus if the high carbon costs of AM roots are not offset by enhanced plant carbon acquisition via photosynthesis.

Arid soils have high AM fungal diversity (Stutz and Morton, 1996), but agricultural practices can select for species or isolates of AM fungi that tolerate agricultural practices but are not particularly beneficial to crop plants. Unlike other inoculum tested, Yuma inoculum 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* was negatively correlated with corn plant dry mass and yield. Likewise, growth depression of tobacco plants in high phosphorus soil has been linked to AM fungi (Modjo and Hendrix, 1986), and aggressive strains of AM fungi which enhance plant P uptake and growth at low soil phosphorus cause growth depression of citrus at high soil phosphorus (Graham et al., 1996).

Yuma orchard soil was very low in organic matter and available phosphorus compared with Mesa orchard soil which might have favored proliferation of aggressive strains of AM fungi which were able to enhance phosphorus uptake. When soil phosphorus is not limiting, as in this study, such fungi would be expected to aggressively colonize citrus roots and, in the absence of phosphorus benefit, suppress plant growth. Thus, aggressive AM fungi, possibly *G. occultum*, might be responsible for the poor performance of plants given Yuma inoculum.

Total internal AM infection was similar for plants of each inoculum treatment but the amount of external mycelium might have differed. In citrus, AM enhancement of plant nutrition is positively related to the amount of extra-radical hyphae produced (Graham et al., 1982). Plants inoculated with Mesa and Yuma inoculum had the greater leaf phosphorus concentrations and more arbuscules (sites of carbon and phosphorus exchange) per root length than plants treated with other inoculum which were evidence of higher AM fungal activity and greater carbon costs. However, leaf phosphorus levels are diluted in fast growing plants so high levels of leaf phosphorus in plants treated with Yuma inoculum might reflect their slower rate of growth in addition to high fungal activity.

Growth suppression of plants treated with the Yuma inoculum, compared with plants treated with other inoculum, was substantial and even greater in magnitude than the effect of decreased irrigation frequency. Previous studies have reported dramatic differences in citrus growth as a function of different mycorrhizal isolates. The cause of differential growth can be phosphorus limitation (Camprubi and Calvet, 1996) or high carbon costs which appears to be the case here. 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 fungus was responsible for poor plant growth. Management of AM fungi that cause growth suppression might involve reducing phosphorus fertilization to enhance the carbon efficiency of the fungi (Graham et al., 1996).

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Table 1. Effect of irrigation frequency and inoculum on shoot and root mass and leaf phosphorus concentration.

Treatment	Shoot mass (g/plant)	Root mass (g/plant)	% leaf P
Irrigation			
Frequent	5.97[z]a[y]	6.13 a	0.21 a
Periodically dry	4.17 b	5.39 a	0.21 a
Inoculum			
Borrego Springs	5.70 a	5.96 a	0.21 b
Padre Canyon	5.60 a	6.34 a	0.18 b
Verde River	5.34 a	6.38 a	0.18 b
Mesa	5.43 a	6.47 a	0.23 ab
Yuma	3.31 b	3.64 b	0.27 a

[z] Values are treatment means, n=5.

[y] Means within treatment followed by a different letter are significantly different according to Duncan's Multiple Range Test (alpha = 0.05).

Table 2. Effect of irrigation and inoculum on carbon assimilation (A-*u*mol/m^2/s), instantaneous water use efficiency (WUE - *u*mol/m^2/s/mmol/m^2/s) and specific soil respiration (SSR - *u*mol/m^2/s/g).

Irrigation (W) Inoculum (M)	A	WUE	SSR
Frequent			
Borrego Springs	9.75[z]	4.12	0.16
Padre Canyon	11.63	4.15	0.12
Verde River	10.30	3.89	0.12
Mesa	10.99	4.18	0.23
Yuma	8.72	3.76	0.23
Infrequent			
Borrego Springs	10.54	4.16	0.14
Padre Canyon	9.97	4.00	0.13
Verde River	10.37	4.49	0.11
Mesa	10.53	4.54	0.16
Yuma	8.72	3.47	0.35
Significance			
W	NS[y]	NS	NS
M	***	***	***
W*M	*	**	NS

[z] Values are treatment means, n=5.

[y] Non-significant, or significant at the 5% (*), 1% (**), or 0.1% level according to ANOVA.