Contributions of Beneficial Soil Fungi to Drought Stress Tolerance of Young Citrus[1]

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Abstract

Four arbuscular mycorrhizal (AM) fungal isolates (*Glomus sp.*) from disparate edaphic conditions were screened for effects on whole-plant transpiration of juvenile 'Volkamer' lemon (*Citrus volkameriana* Ten. and Pasq.) plants of similar shoot mass and canopy leaf area. Mycorrhizal and non-mycorrhizal plants were grown in 8-liter containers for 2.5 months under well-watered conditions before subjection to three consecutive soil drying episodes of increased severity (soil moisture tensions of -0.02 [still moist], -0.06 [moderately dry], and -0.08[dry] MPa respectively). Whole plant transpiration measurements were made on the last day of each soil drying episode and measurements were repeated on the first and second days after re-watering, when soil profiles were moist. The percent root length colonized by AM fungi differed among isolates. Three AM fungal isolates, *Glomus* sp. 25A, *Glomus mosseae* (Nicol. & Gerde.) Gerde. & Trappe 114C, and *Glomus intraradices* Schenck & Smith FL 208-3 increased root length and subsequently increased lemon plant water use. Conversely, plants inoculated with *Glomus mosseae* 51C did not enhance lemon plant root length nor improve plant water use compared with nonmycorrhizal control plants. Inoculating citrus with AM fungi that promote root extension may reduce plant water deficit stress under field conditions.

Introduction

Citrus roots normally engage in a symbioses with arbuscular mycorrhizal (AM) fungi (Rayner, 1935), most commonly with fungi of the genus *Glomus* (Nemec, 1978). AM fungi colonize root cortical cells and exchange inorganic nutrients, which are often present in low concentrations in soil, for photosynthate (Menge, 1977). Citrus have a high mycorrhizal dependency (Graham and Syvertsen, 1985) and the potential for AM fungi to increase plant growth has been well documented (Graham, 1986). In addition to facilitating plant nutrient uptake AM fungi can improve *Citrus* water uptake, possibly by stimulating plant root growth (Levy et al., 1983).

The composition and abundance of indigenous AM fungal populations can be altered or suppressed by agricultural practices such as tillage, introduction of exotic weeds and crops, use of agrochemicals (Kleinschmidt

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and Gerdemann, 1972), and irrigation (Graham, 1986). Although citrus orchard soils typically contain several species of AM fungi (Nemec et al., 1981), AM fungal species and even geographic isolates of the same species of AM fungi can differ in their ability to elicit specific host-plant responses. Recent research by Martin and Stutz (1997) showed that an isolate of *Glomus intraradices* Schenck & Smith from the Sonoran desert enhanced sour orange growth compared with an isolate of the same species from Florida when soil was allowed to dry between irrigations. The high mycorrhizal dependency of citrus has stimulated interest in the management of AM fungi to improve plant growth (Camprubi and Calvet, 1996). Such management practices may involve inoculation with superior AM fungal isolates, or even reintroduction of populations of native AM fungal isolates that have been eliminated as a result of contemporary cropping practices.

A first step toward exploiting the potential of AM fungi to improve citrus water relations is to screen mycorrhizal fungal isolates for effectiveness. Objectives of this study were to determine if isolates of AM fungi from disparate edaphic conditions were functionally equivalent with respect to 1) their effect on whole plant transpiration of juvenile 'Volkamer' lemon (*Citrus volkameriana* Ten. and Pasq.) plants of similar shoot biomass and canopy leaf area, under conditions of increased soil water deficit stress and recovery from water stress, and 2) their effect on plant root length.

Materials and Methods

Four AM fungal isolates were used as inoculum in this study: two geographic isolates of *Glomus mosseae* (Nicol. & Gerde.) Gerde. & Trappe, an isolate of *Glomus intraradices*, and an as yet undescribed *Glomus* species. *Glomus mosseae* isolate 51C was started at Arizona State University (ASU) with spores extracted from a trap culture of rhizosphere soil collected from *Prosopis glandulosa* var. *glandulosa* Woot. (honey mesquite) growing in Chihuahuan desert scrub in Padre Canyon, TX, USA. *Glomus mosseae* 114C was started at Arizona State University with spores extracted from a trap culture of rhizosphere soil from *Prosopis velutina* Woot. (velvet mesquite) growing in Sinaloan thorn scrub in Mazacahui, Sonora, MX. The *Glomus intraradices* isolate FL 208-3 originated from a citrus orchard in Orlando, FL, USA (Morton *et al.* 1993) and was obtained from the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, Morgantown, WV, USA). *Glomus sp.* 25A was started at ASU from multiple spores extracted from a trap culture of rhizosphere soil from *Prosopis velutina* growing in Sonoran Desert scrub near Whitman, AZ, USA.

Fifty uniform, non-mycorrhizal, seedlings (0.15 m mean height) were transplanted into 8-liter polyethylene containers (painted white on the outside surface to increase surface albedo) filled with coarse silica sand (particle size < 4.25 mm) blended with pasteurized soil (3:1, v:v) which had passed through a 2 mm sieve. The soil was a Gilman clay loam collected at a depth of 0.1 to 0.3 m in the A horizon from the ASU Horticultural Resource Center, Tempe, AZ, USA. Soil analysis was as follows: pH=7.3, EC=0.25 dS/m; 11.26 5 g sodium bicarbonate extractable P/g soil; 13.2 g organic matter/kg soil. At potting, seedlings were inoculated with one of four AM fungal isolates or non-inoculated as a control. For AM plants, the appropriate inoculum was placed subjacent to the transplants.

Approximately 33,000 AM fungal propagules of isolates 51C, 114C, and FL-208 3 were added to each pot; inoculum of *Glomus sp.* 25A was limited, so plants assigned this isolate received approximately 7,350 AMF propagules per pot. Control plants were planted without inoculum, but received a nonmycorrhizal inoculum drench in order to establish similar soil microbiota as the other AM fungal treatments.

After transplanting, all plants were fertilized with 11.75 g of isobutylidene diurea slow release fertilizer (20N-0P-16.6K-2Fe-1.4Mn) and 5 g of MgSO4. Additionally, plants inoculated with AM fungi received 21.65 g concentrated superphosphate (0N-19.6P-0K) while plants not inoculated with AM fungi (controls) were given 91.75 g superphosphate (based on unpublished data of Martin and Stutz) in an effort to equalize plant size and P nutrition.

Plants were watered in excess of container capacity with a drip irrigation system that delivered approximately 400 ml deionized H_20/min/pot. For 2.5 months, plants were grown in a glasshouse (26□C day/15□C night , 30% photosynthetically active radiation exclusion) under well-watered conditions. All plants were then subjected to three successive soil drying episodes by withholding irrigations mean soil water tension reached -0.02 MPa, -0.05 MPa, and -0.08 MPa, at which time plants appeared to be under mild, moderate, or severe water deficit stress, respectively.

Whole-plant transpiration was determined on the last day of well-watered conditions (no stress), the last day of

each soil drying episode, and the second day after re-watering when soil profiles remained moist. To determine whole plant transpiration, during the predawn pots were placed in white polyethylene bags sealed around the base of the trunk with wire ties and the containers were weighed. After sunset, the pots were re-weighed and the bags were removed; whole plant transpiration was calculated as the difference in weight. Containers were never continuously bagged for more than 15 hours.

At the end of the study all plants were harvested. Shoots were separated from roots and root systems were carefully extracted from soil by floatation in a large water bath. Root lengths were measured with a digital camera interfaced with a computer (AgVision Digital Imaging System, Pullman, WA). A sample of 1-cm length root pieces (approximately one-gram fresh weight) was collected from each root system and fixed in formalin-acetic acid-alcohol (FAA). Root samples were then cleared, by heating roots at 121□C for 3 minutes in 10% potassium hydroxide, bleached for 30 minutes in alkaline H_2O_2, acidified overnight in 1% hydrochloric acid and stained using 0.05% trypan blue in acidic glycerol (Koske & Gemma 1989). The magnified intersections method (McGonigle et al. 1990) was used to quantify AM fungal colonization of 'Volkamer' lemon root samples. The proportion of root length containing fungal arbuscules, vesicles, and hyphae was determined and used to calculate the percent root length colonized by AM fungi. Roots were oven dried (60□C for 48 hr) and weighed. Phosphorus concentrations of leaves was analyzed using ascorbic methods (Watanabe and Olson, 1965) and all plants were found to have P concentrations sufficient for good growth (16-25 mg/Kg; Davies and Albrigo, 1994).

This study consisted of five treatments (four fungal treatments and one non-inoculated control) and 5 single tree replications arranged in a randomized complete block design. Data were analyzed using Duncan's multiple range test (SAS version 6.03, SAS Institute Inc., Cary, NC).

Results and Discussions

AM fungi did not colonize control plants, while mycorrhizal plants were colonized to varying extents depending on the AM fungal isolate (Table 1). *Glomus sp.* 25A colonized the greatest percent root length despite using fewer propagules for inoculation than the other AM fungi. *Glomus mosseae* 51C colonized the least percent root length. *Glomus mosseae* isolate 114C, and *Glomus intraradices* colonized the lemon plant roots to a similar extent.

Canopy leaf area and shoot mass were not affected by mycorrhizal treatments ($p = 0.133$ and $p = 0.898$ respectively, data not shown), evidence that shoots and whole-plant leaf transpirational surface area of mycorrhizal and control plants were similar. Although P concentrations varied between mycorrhizal treatments, all plants had leaf P concentrations that were sufficient for citrus (Davies and Albrigo, 1994). *Glomus sp.* 25A significantly increased leaf P concentration of lemon plants compared with all other treatments (Table 2). *Glomus mosseae* 114C induced significantly greater leaf P concentrations compared with non-mycorrhizal control plants, but did not significantly increase P nutrition compared with *Glomus mosseae* 51C and *Glomus intraradices*, which had similar leaf P concentrations with non-mycorrhizal control plants.

Three isolates of AM fungi, *Glomus sp.* 25A, *Glomus mosseae* 114C, and *Glomus intraradices*, significantly increased plant root length (by approximately 20%) compared with non-mycorrhizal control plants (Table 2). Our results agree with observations of Levy et al. (1983) that rough lemon (*Citrus jambhiri* Lush) seedlings colonized by *Glomus intraradices* tended to have longer root lengths compared with non-mycorrhizal plants. *Glomus mosseae* isolate 51C did not increase root length relative to non-mycorrhizal control plants. Camprubi and Calvet (1996) also found an isolate of *Glomus mosseae* was less infective (colonized less percent root length of citrus) and less effective (in terms of stimulating shoot growth) than either *Glomus intraradices* or mixed species inoculum. Neither root length nor root dry mass was significantly correlated with leaf P concentrations ($p = 0.42$ and 0.89, respectively).

Whole-plant transpiration generally agreed with mycorrhizal status and plant root length. Under well-watered conditions, plants colonized with *Glomus sp.* 25A and *Glomus intraradices* transpired significantly more water than control plants (Table 3), possibly because mycorrhizal induced root extension improved plant water uptake. Levy and Krikun (1980) and Levy et al. (1983) also observed increased whole plant transpiration of mycorrhizal rough lemon seedlings when soil was moist. *Glomus mosseae* 51C did not affect whole plant transpiration.

Soil drying reduced whole plant transpiration for all plants. Under mild water stress (mean soil water tension = -0.02 MPa) conditions, all mycorrhizal plants tended to transpire more water than control plants, with

Glomus intraradices significantly increasing whole plant transpiration compared to non-mycorrhizal plants. Whole-plant transpiration was similar for all plants on the two recovery days from mild soil water deficit stress, though *Glomus mosseae* 114C, *Glomus sp.* 25A, and *Glomus intraradices* all tended to increase whole plant transpiration compared to non-mycorrhizal control plants and plants colonized with *Glomus mosseae* 51C.

Under moderate soil water stress conditions (mean soil water tension = -0.05 MPa), whole-plant transpiration was less than 50% that of the values achieved under no stress conditions. Although transpiration data did not significantly differ among fungal treatments, there was a trend toward *Glomus mosseae* 114C, *Glomus sp.* 25A, and *Glomus intraradices* having decreased whole plant transpiration compared to non-mycorrhizal plants. Levy et al. (1983) also found mycorrhizal citrus in containers had decreased transpiration under water stress, because mycorrhizal plants had depleted container soil water sooner than non-mycorrhizal plants.

AM fungi altered whole-plant transpiration during recovery, with plants inoculated with *Glomus mosseae* 51C transpiring more water than plants colonized with *Glomus intraradices* FL 208-3. Mycorrhizal plants recovered from moderate water deficit stress more quickly than non-mycorrhizal plants as evidenced by increased transpiration of mycorrhizal plants compared with non-mycorrhizal plants (Table 3).

Under severe soil water stress conditions (mean soil water tension = -0.08 MPa), whole-plant transpiration of all plants was similar and was approximately 10% of values under no stress conditions. Although plants had statistically similar whole-plant transpiration values on the second day of recovery from high soil water deficit stress, transpiration as a function of AM fungal treatment followed trends similar to those observed under no stress conditions.

Plants colonized by *Glomus sp.* 25A, *Glomus mosseae* 114C, and *Glomus intraradices* FL 208-3 fungi are better able to amass soil water than non-mycorrhizal control plants as shown by transpiration data under well-watered conditions and recovery form soil water deficits The AM fungal isolates that stimulated plant root length and increased whole plant transpiration also colonized the greatest root length, so it is possible that AM fungi could have directly enhanced plant water uptake via external hyphae. However, water transport through hyphal entry points has been estimated by Graham and Syvertsen (1984) and is believed to be insignificant under well-watered conditions. Thus, we conclude that improved water uptake by 'Volkamer' lemon was likely the result of AM stimulation of root extension (and consequent increase of root surface area).

How AM fungi increased plant root growth in this study is uncertain. Peng et al. (1993) showed that 'Volkamer' lemon roots colonized by *G. intraradices* were a greater carbon sink than non-mycorrhizal roots which might have limited shoot growth of mycorrhizal plants. Graham et al. (1987) assert that the affect of AM fungi on citrus water uptake is the result of improved plant nutrition, especially P. Although we have reported some apparent differential stimulation of P uptake by AM fungi, these differences did not result in increased shoot size. In addition, citrus root growth was not correlated with leaf P concentrations. However, we did not measure root P concentrations and it is possible that AM fungi enhanced P nutrition in roots. Moreover, Druge and Schonbeck (1992) concluded that increased transpiration and growth of mycorrhizal flax (Linum usitatissimum L.) was related to enhanced root cytokinin levels.

Inoculating citrus rootstock seedlings with AM fungi that stimulate root growth at or before transplanting into field conditions may be utilized as a crop management strategy, particularly under conditions of low soil P availability. Beneficial AM fungal isolates like G. mosseae 114C, Glomus sp. 25A, and G. intraradices FL208 might improve performance of young citrus rootstock by enhancement of root length resulting in greater exploration of soil volume for water. Finally, not all AM fungal isolates cause the same host plant response. The use of G. mosseae 51C, an isolate from a Chihuahuan desert scrub community which was a poor colonizer under well-watered conditions and did not enhance lemon plant root length, may not be recommended as an inoculum for 'Volkamer' lemon.

Literature Cited

Camprubi, A. and C. Calvet. 1996. Isolation and screening of mycorrhizal fungi from citrus nurseries and orchards and inoculation studies. HortScience 31(3):366-369.

Davies, F. S., and L. G. Albrigo. 1994. Citrus. CAB International. Wallingford, UK.

Druge, U. and F. Schonbeck. 1992. Effect of vesicular-arbuscular mycorrhizal infection on transpiration, photosynthesis, and growth of flax (Linum usitassimum L.) in relation to cytokinin levels. Journal of Plant

Physiology 141:40-48.

Graham, J. H. 1986. Citrus mycorrhizae: potential benefits and interactions with pathogens. HortScience 21(6):1302-1306.

Graham, J. H. and J. P. Syvertsen. 1984. Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytologist 97:277-284.

Graham, J. H. and J. P. Syvertsen. 1985. Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytologist 101:667-676.

Graham, J. H., J. P. Syvertsen, and M. L. Smith. 1987. Water relations of mycorrhizal and phosphorus-fertilized non-mycorrhizal *Citrus* under drought stress. New Phytologist 105:411-419.

Kleinschmidt, G. D. and J. W. Gerdeman. 1972. Stunting of citrus seedlings in fumigated nursery soils related to absence of endomycorrhizae. Phytopathology 62:1447-1453.

Koske, R. E. and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92:486-489.

Levy, Y. and J. Krikun. 1980. Effect of vesicular-arbuscular mycorrhiza in *Citrus jambhiri* water relations. New Phytologist 85:25-31.

Levy, Y., J. P. Syvertsen and S. Nemec. 1983. Effect of drought stress and vesicular-arbuscular mycorrhiza on citrus transpiration and hydraulic conductivity of roots. New Phytologist 93:61-66.

Martin, C.A. and J.C. Stutz. 1997. Effect of *Glomus intraradices* isolates under two irrigation frequencies on growth of sour orange. HortScience (in preparation).

Menge, J. A., H. Lembright and E. L. V. Johnson. 1977. Utilization of mycorrhizal fungi in citrus nurseries. Proceedings of the International Society of Citriculture 1:129-132.

McGonigle, T. P., M. H. Miller, D. C. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495-501.

Nemec, S. 1978. Response of six citrus rootstocks to the three species of *Glomus*, a mycorrhizal fungus. Proceedings of the Florida State Horticultural Society 91:10-14.

Nemec, S., J. A. Menge, R. G. Platt, and E. L. V. Johnson. 1981. vesicular-arbuscular mycorrhizal fungi associated with Citrus in Florida and California and notes on their ecology. Mycologia 73:112-125.

Peng, S., D. M. Eissenstat, J. H. Graham, K. Williams and N. C. Hodge. 1993. Growth depression in mycorrhizal citrus at high phosphorus supply. Plant Physiology 101:1063-1071.

Rayner, M. C. 1935. Mycorrhizal habit in the genus *Citrus*. Nature 136:516-517.

Ruiz-Lozano, J. M. and R. Azcon. 1995. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiologia Plantarum 95:472-478.

Watanabe, F. S., and S. R. Olson. 1965. Test of ascorbic method for determining phosphorus in water and sodium bicarbonate extracts from soil. Soil Science Society Proceedings 29:677-678.

Table 1. Arc-sin transformed mean percent root length of young *Citrus volkameriana* plants colonized by different AM fungal isolates after being grown in 8-liter containers for 2.5 months under well-watered conditions followed by three consecutive soil drying episodes. Control plants remained nonmycorrhizal

AM fungal treatments	Total % infection
Glomus sp. 25A	87a[z]
G. mosseae 51C	12c
G. mosseae 114C	47b
G. intraradices 208-3	58b

[z] Mean separation in columns by Duncan's Multiple Range test, 5% level.

Table 2. Mean root length and leaf P concentration of *Citrus volkameriana* plants colonized by different isolates of AM fungi.

AM fungal treatments	Root length (m)	Leaf P concentration (mg/Kg)
Glomus sp. 25A	3.54a[z]	25.50a
G. mosseae 51C	3.06ab	18.00bc
G. mosseae 114C	3.48a	21.25b
G. intraradices 208-3	3.53a	20.00bc
Control	2.90b	16.25c

[z] Mean separation in columns by Duncan's Multiple Range test, 5% level.

Table 3. Root length and whole-plant transpiration of young *Citrus volkameriana* in response to isolates of arbuscular mycorrhizal (AM) fungi 2.5 months after root inoculation and growth under well-watered conditions (no stress), and subsequent exposure (S) and recovery (R) to three consecutive soil drying cycles of low (-0.02 MPa), moderate (-0.05 MPa), and high (-0.07 MPa) water stress, respectively.

AM Fungal Treatments	Whole Plant Transpiration (ml H_2O/plant/14 hours)									
	No Stress	Mild			Moderate			High		
		S	R1	R2	S	R1	R2	S	R1	R2
Glomus AZ112	158.5 a	127.7 ab	146.8 a	82.1 a	47.0 b	108.7 a	108.7 a	7.2 a	72.6 a	72.6a
G. mosseae 51C	117.2 b	109.9 ab	119.9 a	65.0 a	74.4 a	88.5 ab	88.5 ab	10.2 a	62.7 a	62.8a
G. mosseae 114C	135.4 ab	116.7 ab	124.4 a	70.1 a	52.6 ab	94.2 ab	94.2 ab	8.2 a	92.1 a	72.1a
G. intraradices 208-3	154.0 a	133.4 a	143.6 a	78.4 a	46.7 b	99.3 ab	99.3 ab	6.6 a	80.7 a	80.7a
Control	110.8 b	100.9 b	128.9 a	63.0 a	57.8 ab	81.2 b	81.3 b	10.2 a	60.3 a	60.4a

[z] Values are treatment means, n = 5. Mean separation in columns by Duncan's Multiple Range test, 5% level.

[y] Pearson's correlation coefficients showing the correlation of root length to whole-plant transpiration.