Vesicular-arbuscular mycorrhizal fungus inoculation of sour orange seedlings in Barbados[1,2]

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ABSTRACT

A citrus seedbed was established August 1984 on the west coast of Barbados. Before being planted and inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi, one-half the seedbed was fumigated with methyl bromide; the other half was untreated. Four weeks after fumigation, four treatments, *Glomus intraradices, G. mosseae,* indigenous VAM fungi, and a noninoculated control were established in each half of the test. Early vigorous plant growth in the nonfumigated half of the plot suggested that indigenous VAM may have colonized and stimulated plant growth earlier than the treatments in the fumigated portion. Three months after inoculation, plants in both portions of the plot were growing well. In the fumigated area, application of two *Glomus* species, which were introduced from Florida, resulted in a significant improvement in plant growth over the control. This occurred in spite of the fact that infection levels in control roots were similar to those in inoculated roots. This study suggests that, when possible, alternative pesticides not harmful to VAM fungi should be used in place of methyl bromide fumigation to conserve these fungi in agricultural soils.

INTRODUCTION

Planting young citrus in old citrus soils was shown to result in stunted seedlings nearly 40 years ago. Explanations offered included nutrient depletion, deterioration of soil structure, and unfavorable microbial populations, especially nematodes and pathogenic fungi (14). Chemical fumigation of agricultural soils has restored many such soils to normal crop production, because fumigation temporarily lowers concentrations of soilborne plant pathogens (15, 33). Methyl bromide (MB) fumigation at commercial rates killed over 90% of 10 pathogenic fungi (20). Field studies involving tomatoes (9), beans (3), slash pine (2), and citrus (15) indicated that growth stimulation in MB-treated soil was due to substantial elimination of soilborne pests. However, fumigation also has led to unfavorable effects, such as stunted plant growth. Stunting following fumigation

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was reported with sweetgum (4), poplar (5), peach (12), and citrus (11, 17, 27, 30, 31). Stunted citrus growth following planting on MB-treated citrus nursery soil was linked to the eradication of beneficial vesicular-arbuscular mycorrhizal (VAM) fungi (11).

Menge (16) found MB killed between 90 and 100% of tested VAM fungi at rates lower than are required for pathogen control. Plant growth depression due to fumigation has been overcome both experimentally and commercially by substantially increasing soil phosphorus (P) levels (8, 30), or by inoculation of soil with VAM fungi (11, 21, 23, 30, 31, 32). This growth response has been largely attributed to enhanced P uptake (1, 6, 7, 8, 30) by the fungi. The extended VAM hyphal network also increases water uptake efficiency (8, 13, 25, 26, 29), thereby improving drought tolerance.

Little is known about the behavior of VAM fungi in citrus on the Caribbean islands. Soil P levels in the southeastern Caribbean are generally low (10), and average 25, 3, 27, and 21 p/m (bicarbonate extraction) in citrus soils from Barbados, Grenada, Dominica, and St. Lucia, respectively (18). Fertilizers can be imported, but the cost is prohibitive for marginal agricultural operations. The wide seasonal fluctuations in soil moisture on the islands often result in prolonged drought stress, sometimes leading to tree death. Barbados soils because of their coral origin, are mainly alkaline, a condition known to favor development of *Phytophthora* diseases (28). Significant benefit to plant growth under conditions of these stresses may occur if plants become mycorrhizal.

An experiment was established on Barbados to determine whether soil inoculation with VAM fungi in a citrus nursery site could favorably affect plant growth in MB- and non-MB-treated soil.

MATERIALS AND METHODS

Florida isolates of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *G. intraradices* Schenck and Smith were imported into Barbados from the USDA Horticultural Research Laboratory, Orlando, Florida. These species were grown on roots of sorghum (*Sorghum vulgare*) in steamed Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) in a greenhouse before shipment to Barbados. A third source of VAM fungus inoculum consisted of infected roots obtained from a Barbados orchard showing the highest colonization level during a preliminary survey of VAM fungi in Barbados orchards.

Old sugarcane land located on the west coast of Barbados, which had been fallow for 2 years, was bulldozed, plowed, and rototilled for a 255 square meter nursery seedbed test. The soil, a grey-brown association (mixed smectoid-kandoid), had a pH of 7.6, contained 1.6% organic matter and 3 p/m P. A commercial fumigant with 98% methyl bromide and 2% chloropicrin was introduced at the rate of 680 g/9.29 square m to one-half the experimental site. The other half was not fumigated.

Each half of the test site contained four treatments (two *Glomus* treatments, infected grove roots, and a sterile sand control) with four replications per treatment. Treatments were established in August 1984 in a randomized complete block design. Each half of the plot contained four rows, each row containing each treatment. Treatment rows were 3.2 m long; treatments within each row were separated by 1 m; rows were 1.5 m apart, and 2.0 m separated each half of the plot. A 30-cm-wide ditch was dug between the plots to trap and divert water away from the fumigated plot. Inoculum was applied to a 5-cm-deep trench in each treatment row. Each *G. mosseae* treatment consisted of 3.5 kg of sand containing hyphae, infected root material, and about 180,000 spores. Each *G. intraradices* treatment contained hyphae, infected root fragments, and about 105,000 spores in 3.0 kg of sand. Seventy grams of the local root inoculum were applied per replicate, and when they were processed by the Phillips and Hayman technique (24) and examined microscopically for infection, they contained high concentrations of vesicles, hyphae and arbuscules. Two centimeters of soil was used to cover the inoculum; on top of that, 100 Florida State Department of Agriculture certified sour orange seed were planted per treatment and covered with soil to ground level. Four weeks after fumigation, a trickle irrigation system was installed for regular irrigation. All plots were hand weeded. The plots were fertilized seven times, 6 weeks apart, with NH_4S0_4 used in the first two applications and $KN0_3$ in the remaining treatments. Fertilizer was applied in a 0.5-m band down the row at a rate of 136 g/m^2.

Three and nine months after germination, root pieces were randomly selected within treatment replicates and processed by the Phillips and Hayman method (24) for fungus evaluation. One hundred 1-cm-long root pieces per treatment replicate were rated for number of vesicles and density of hyphae, and percentage of infection determined as described by Nemec and O'Bannon (23). Three and seven months after seed germination, plant height was measured; stem caliper was taken on the seventh month. Root pieces were collected only from the interior 2.2 m of each replicate to minimize contamination from adjacent treatments; 10 plants representative of the growth in each replicate were measured. Roots of grass plants surrounding the test site were collected in October 1984, and also processed for VAM fungus evaluation with the Phillips and Hayman technique (24). They were rated for infection in the same way citrus roots were rated. Periodically during the course of the test, soils were sampled and wet-sieved to collect and identify indigenous VAM-fungus species.

RESULTS

Over 50% of the seed planted germinatd and survived in all treatments, except the fumigated, noninoculated one (table 1). The seedlings in this treatment grminated uniformly, but by three months after germi-

TABLE 1.—*Sour orange seedling survival in the vesicular-arbuscular mycorrhizal (VAM) fungus seedbed test plots in Barbados*

Treatment	Seedling survival (%)[1]	
	Nonfumigated[2]	Fumigated[2]
Glomus intraradices	62.3 a	52.5 a
G. mosseae	57.8 a	62.3 a
Indigenous VAM	63.8 a	61.3 a
Control	70.8 a	44.5 b

[1]Data were collected 3 months after germination.

[2]Numbers followed by different letters within each column are significantly different from one another ($P = < 0.05$) using Tukey's test.

nation, the more severely stunted ones died. There was no difference in seedling survival between the fumigated and nonfumigated soil treatments (table 1).

Following germination, seedlings in the nonfumigated portion of the plot quickly turned green and readily began to grow. Conversely, seedlings in the fumigated portion of the plot for the first 2 months remained stunted and chlorotic. By the third month after germination, most seedlings in the fumigated inoculum-amended portion of the plot began to grow rapidly. For the remainder of the experiment, growth of plants in both portions of the plot was generally similar.

Plant height overall was greater in the nonfumigated area than in the fumigated portion in December 1984 and April 1985; however, no significant differences in height occurred among the treatments in the nonfumigated area (table 2). The height of plants inoculated with *G. intraradices*

TABLE 2.—*Development of sour orange seedlings grown from seed planted August 1984 in soil amended with Glomus mosseae, G. intraradices, and indigenous vesicular-arbuscular mycorrhizal (VAM) fungi in a Barbados seedbed*

Treatments	Plant growth[1]		
	Stem height (mm)[2]		Stem caliper (mm)[2]
	December 1984	April 1985	April 1985
Nonfumigated area			
G. intraradices	218 ab	734 ab	8.3 ab
G. mosseae	242 a	796 a	8.9 a
Indigenous VAM	239 ab	774 ab	8.2 ab
Control	222 ab	722 abc	7.8 ab
Fumigated area			
G. intraradices	188 ab	696 abc	8.7 a
G. mosseae	194 ab	669 bc	7.8 ab
Indigenous VAM	190 ab	628 c	7.4 b
Control	113 b	489 d	6.0 c

[1]Data are means of 10 plants per replicate.

[2]Numbers followed by different letters in each column are significantly different from one another ($P = < 0.01$) using Tukey's test.

in the fumigated area was not significantly different from the height of plants in each of the four treatments in the nonfumigated area on both dates. In the fumigated area, plant height was significantly greater in all fungus treatments than in the control in the April sampling. In the nonfumigated area, plant stem caliper was not significantly different among treatments. In the fumigated area, stem caliper of plants in all three fungus treatments was significantly better than that of the control, and only *G. intraradices* was superior to the indigenous source of inoculum (table 2).

In December 1984, three months after seed germination, VAM fungus infection was as high in all treatments of the fumigated area as it was in the nonfumigated control (table 3). By June 1985, overall vesicle ratings and percentage of infection were higher in the treatments of the fumigated area than they were in December, but overall hyphal ratings had declined. Percentage of infection in both fumigated and nonfumigated areas of the plot was similar in June. Statistical analysis of infection data did not reveal any apparent trend, nor indicate that any treatment was superior to another.

Mean values for infection in grass roots collected in October 1984 at all four corners of the plot were 0.71 for vesicles, 1.62 for hyphae and 100.0 for percentage of infection. Very few chlamydospores were present in soil wet-sieved from this area.

TABLE 3.—*Development of vesicular-arbuscular mycorrhizal (VAM) fungi in sour orange seedling roots in fumigated and nonfumigated plots of the Barbados seedbed*

Treatment	VAM fungus infection ratings[1,2]					
	Vesicles		Hyphae		Infection (%)	
	December	June	December	June	December	June
Fumigated area						
Glomus intraradices	0.32	0.36	1.45	1.07	73	89
G. mosseae	0.51	0.64	1.86	1.29	89	93
Indigenous VAM	0.13	0.45	2.14	1.10	76	98
Control	0.09	0.88	1.63	1.45	84	83
LSD 0.05	ns	0.38	0.54	ns	ns	12.1
Nonfumigated area						
G. intraradices	–	0.58	–	0.56	–	92
G. mosseae	–	0.86	–	0.58	–	93
Indigenous VAM	–	0.37	–	1.02	–	91
Control	0.10	0.67	1.83	1.19	82	97
LSD 0.05		0.26		0.20		ns

[1]Ratings: 0 = no vesicles nor hyphae; 1 = 1-50 vesicles or light hyphal colonization; 2 = 51-100 vesicles or moderate hyphal development; and 3 = more than 100 vesicles or heavy hyphal colonization. Percentage of infection based on number of root pieces containing either hyphae or vesicles or both.

[2]Data are means of 100, 1-cm-long root pieces per treatment replicate.

DISCUSSION

Successful fumigation of a low-P Barbados soil resulted in temporary stunting of citrus seedlings in control plots, a phenomenon commonly associated with fumigated soils because native VAM fungi are partially killed (11). Similarly, response of seedlings to added inoculum was delayed by 2 to 3 months, but this delay did not appear to be induced by bromine toxicity. Usually, 2 to 14 days' aeration is sufficient to remove toxic bromine levels from fumigated soil sown with citrus seed (19), and aeration of our plot exceeded this interval. Also, there was no evidence that root disease was responsible for the plant growth delay in the treatments receiving inoculum. Roots appeared healthy at each sampling for mycorrhiza infection and when the plants were dug for transplanting in December 1985.

The delay in plant growth in the fumigated portion of the plot may be due to the placement and type of inoculum used. The form of introduced inoculum applied to the soil in the plot essentially consisted of chlamydospores, whereas, the resident inocula were primarily infected roots as determined by wet-sieving soil and evaluating root infection. Although the level of introduced inocula was in excess (21, 22) of that needed for root infection, the resident inocula were more evenly distributed and may have survived *in situ* better than the introduced inocula before seed germination. The high percentage of VAM fungus infection in citrus roots in the nonfumigated control treatment was equal or superior to infection in the inoculated treatments in the fugimated area, and thus is indicative of the survival and efficiency of inocula in native grass roots.

Seedling survival in the fumigated control was significantly poorer than in the other treatments, an implication that they were nonmycorrhizal long enough to sustain a higher mortality than in the other treatments.

Plant growth data in table 2 and fungus infection data in table 3 indicate that in spite of the fact that control roots became heavily infected the third month after seed germination in both fumigated and nonfumigated plots, plants in the *G. intraradices* and *G. mosseae* treatments in fumigated soil outgrew their respective control plants and produced healthier, more salable plants. These data suggest that the two introduced species were more effective than the indigenous species that survived in the fumigated control plot. Although VAM species cannot be identified in roots, *G. intraradices* was detected in roots of its treatment by the chlamydospores it produced. Fresh chlamydospores of both species were found in soils around roots near the end of the test. This finding suggested that the introduced species had infected plants in the plot. We do not know at this time whether these two species are resident

in Barbados soils, nor do we know the identity of the indigenous species. Wet-sieving of soils from this plot in Barbados did not extract enough viable spores to adequately identify the few *Glomus* spores found to species. Moreover, U.S. Federal Plant Quarantine did not permit export of soils to the United States for spore identification.

This study indicates that when it is necessary to fumigate soil to control root disease problems, a serious consideration should be given to the survival of indigenous VAM fungus species. When it is not possible to reintroduce VAM fungi into fumigated soil via inocula, suitable plant growth may be obtained by using preinfected or preinoculated plant material. Alternatively, disease control may be achieved by using pesticides that are not harmful to VAM fungi, or by appropriate agronomic practices. Conservation of VAM fungi in this way will ensure that their biological activity is preserved for optimum plant growth response.

The relatively high infection levels present in roots in this test site were not indicative of VAM-fungus infection levels in grove trees on Barbados. In an earlier study (18) of infection in fruiting citrus trees in 24 Barbados plantings, mean vesicle, hyphal, and percentage of infection levels were 0.27, 0.48, and 40.0, respectively. The overall higher infection parameters in this study probably occurred because of the optimal cultural care given to the plantings; groves on the island receive minimal care and are not irrigated.

RESUMEN

Inoculación de arbolitos de naranjas agrias en Barbados con un hongo micorrizógeno vesicular

Un semillero de cítricos se sembró en agosto de 1984 en la costa occidental de Barbados. Antes de sembrarlo e inocularlo con hongos micorrizógenos vesiculares (HMV) la mitad del semillero se fumigó con bromuro metílico. Cuatro semanas después de la fumigación, las dos mitades se sometieron a cuatro tratamientos: *Glomus intraradices, G. mosseae*, hongos HMV indígenos y un testigo sin inocular. El crecimiento vigoroso inicial de los arbolitos en la mitad sin fumigar sugiere que los HMV indígenos habían colonizado y estimulado el crecimiento antes que en los tratamientos en la mitad fumigada. Tres meses después de la inoculación las plantas en las dos secciones crecían bien. En la sección fumigada la aplicación de las especies de *Glomus*, introducidas de Florida, mejoraron significativamente el crecimiento de los arbolitos. Esto ocurrió a pesar de que la infección en las raíces testigo era muy parecida a la de las raíces sin inocular. El estudio sugiere que, cuando sea posible, se alternen las aplicaciones de fungicidas que no sean perjudiciales a los hongos HMV en vez de aplicar bromuro metílico para que estos hongos perduren en suelos agrícolas.

LITERATURE CITED

1. Baylis, G. T. S., 1959. Effect of vesicular-arbuscular mycorrhiza on the growth of *Griselinia littoralis* (Cornaceae). *New Phytol.* 58:274-80.

2. Bengston, G. W., and G. C. Smart, Jr., 1981. Slash pine growth and response to fertilizer after application of pesticides to the planting site. *For. Sci.* 27 (3):487-502.
3. Bezdicek, D. F., G. T. Vigue and D. B. Burke. 1981. Effect of soil fumigation on field bean. *Agron. J.* 73:1062-065.
4. Bryan, W. C. and P. P. Kormanik. 1977. Mycorrhizae benefit survival and growth of sweetgum seedlings in the nursery. *South. J. For.* 1:21-3.
5. Clark, F. B., 1963. Endotrophic mycorrhizae influence poplar seedling growth. *Science* 140:1220-221.
6. Cooper, K. M., 1984. Physiology of VA mycorrhizal associations. p. 173-174. *In*: Powell, C. L. and Bagyaraj, D. J., Eds. VA mycorrhiza, CRC Press, Inc., Boca Raton, FL.
7. Daft, M. J. and T. H. Nicholson, 1966. Effect of endogone mycorrhiza on plant growth. *New Phytol.* 65:343-350.
8. Hardie, D. and I. Leyton. 1981. The influence of vesicular- arbuscular mycorrhizae on growth and water relations of red clover in phosphate deficient soil. *New Phytol.* 89:599-608.
9. Jaworski, C. A., S. C. Phatak, A. W. Johnson and S. M. McCarter. 1981. Tomato production and soil pest control in relation to width of fumigated and mulched bed and soil fumigation rate. *HortScience* 16 (5):667-69.
10. Jones, W. W. and T. Embleton. 1973. Soils, soil management, and cover crops. pp. 98-121. *In*: Reuther, W., Ed. The Citrus Industry, Univ. Calif., Berkeley, Vol. III.
11. Kleinschmidt, G. D. and J. W. Gerdemann, 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endophytes. *Phytopathology* 62:1447-453.
12. LaRue, J. H., W. D. McClellen and W. L. Peacock. 1975. Mycorrhizal fungi and peach nursery nutrition. *Calif. Agric.* 29:7.
13. 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 Phytol.* 93:61-6.
14. Martin, J. P., 1948. Effect of fumigation, fertilization, and various other soil treatments on growth of orange seedlings in old citrus soils. *Soil Sci.* 66:273-288.
15. Martin, J. P., D. G. Aldrich, W. S. Murphy and G. R. Bradford. 1953. Effect of soil fumigation on growth and chemical composition of citrus plants. *Soil Sci.* 75:137-51.
16. Menge, J. A., D. E. Munnecke, E. L. V. Johnson and D. W. Carnes. 1978. Dosage response of the vesicular-arbuscular mycorrhizal fungi, *Glomus fasciculatus* and *G. constrictus* to methyl bromide. *Phytopathology* 68:1368-372.
17. Menge, J. A., 1982. Effect of soil fumigants and fungicides on vesicular-arbuscular mycorrhizal fungi. *Phytopathology* 72 (8):1125-132.
18. Michelini, S., S. Nemec and L. E. Chinnery, 1986. Vesicular- arbuscular mycorrhizal fungus infection of citrus roots on four islands in the Caribbean. XII Inter. Hort. Cong. Supplement, p. 20. 1986. Abs.
19. Milne, D. L., 1974. Citrus seedbeds: Methyl bromide and mycorrhizae. *Bromides Agric.* 40:3-7.
20. Munnecke, D. E., J. L. Bricker and M. J. Kolbezen, 1978. Comparative toxicity of gaseous methyl bromide to ten soilborne phytopathogenic fungi. *Phytopathology* 68:1210-216.
21. Nemec, S., 1983. Inoculation of citrus in the field with vesicular-arbuscular mycorrhizal fungi in Florida. *Trop. Agric.* 60 (2):97-101.
22. ——, 1984. Application of VA mycorrhizal fungi in fruit crops - field inoculations. p. 35-45. *In*: J. J. Ferguson, Ed. Applications of mycorrhizal fungi in crop production, Univ. Fla., Inst. Food Agric. Sci.
23. —— and J. H. O'Bannon, 1979. Response of *Citrus aurantium* to *Glomus etunicatus* and *G. mosseae* after soil treatment with selected fumigants. *Plant Soil* 53:351-59.

24. Phillips, J. M. and D. S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55:158-61.
25. Safir, G. R., J. S. Boyer, and J. W. Gerdemann, 1971. Mycorrhizal enhancement of water transport in soybean. *Science* 172:581-83.
26. ——, J. S. Boyer and J. W. Gerdemann, 1971. Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiol.* 49:700-03.
27. Schenck, N. C. and D. P. H. Tucker, 1984. Endomycorrhizal fungi and the development of citrus seedlings in Florida fumigated soils. *J. Amer. Soc. Hort. Sci.* 99 (3):284-87.
28. Schmitthenner, A. F. and C. H. Canaday, 1983. Role of chemical factors in development of *Phytophthora* diseases. pp. 189-96. *In*: Phytophthora, its biology, taxonomy, ecology, and pathology, D. C. Erwin, S. Bartricki-Garcia, and P. H. Tsao, Eds. Am. Phytopathol. Soc. St. Paul, MN.
29. St. John, T. V., 1980. Root size, root hairs, and mycorrhizal infection: a re-examination of Baylis' hypothesis with tropical trees. *New Phytol.* 84:483-87.
30. Timmer, L. W. and R. F. Leyden, 1978. Stunting of citrus seedlings in fumigated soils in Texas and its correction by phosphorus fertilization and inoculation with mycorrhizal fungi. *J. Amer. Soc. Hort. Sci.* 103 (4):533-37.
31. —— and ——, 1978. Relationship of seedbed fertilization and fumigation to infection of sour orange seedlings by mycorrhizal fungi and *Phytophthora parasitica*. *J. Amer. Soc. Hort. Sci.* 103 (4):537-41.
32. Tommerup, I. C. and L. K. Abbott, 1981. Prolonged survival and viability of VA mycorrhizal hyphae after root death. *Soil Biol. Biochem.* 13:431-33.
33. Vaartaja, 0., 1964. Chemical treatment of seedbeds for control of nursery diseases. *Bot. Rev.* 30:1-91.