INFLUENCE OF SOIL PESTICIDES ON VESICULAR-ARBUSCULAR MYCORRHIZAE IN A CITRUS SOIL [INFLUENCIA DE PESTICIDAS DEL SUELO SOBRE LAS MICORRIZAS VESICULO-ARBUSCULARES EN TERRENOS DE CITRICOS].

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ABSTRACT

Four fumigants were applied to soils amended with either *Glomus mosseae* or *G. fasciculatus* to determine their effect on these vesicular-arbuscular mycorrhizae (VAM) and subsequent development of citrus seedlings. Fumigation with methyl bromide or chloropicrin effectively controlled VAM at rates of $\geq$ 242 kg/ha, or $>$ 163 liters/ha, respectively. Seedling growth was reduced in fumigated soils because of lack of VAM compared to nonfumigated VAM-infected seedlings. In soils treated with a low rate of either 1,3-dichloropropene (168 liters/ha) or ethylene dibromide (141 and 235 liters/ha), VAM were not eliminated and seedling growth was stimulated.

Key Words: EDB, DD, Telone, fumigants, nontarget effects.

INTRODUCTION

Several vesicular-arbuscular mycorrhizal (VAM) fungi have a beneficial effect on citrus through their role in phosphate assimilation (3,4). Some citrus varieties are highly mycorrhizae-dependent (4) and grow poorly or not at all under nonmycorrhizal conditions (4, 5).

There are several reports on the deleterious effects of some pesticides on VAM in citrus soil (4, 7, 12) and other soils (2, 9, 10, 11). In Florida, the practice of fumigating citrus seedbeds with methyl bromide, in some instances, has resulted in poor seedling growth (13). The purpose of the two tests reported here was to evaluate several commonly used pesticides at three rates on two VAM found in Florida citrus soils, and to relate this to subsequent effects on a major mycorrhizae-dependent citrus rootstock used in Florida.

MATERIALS AND METHODS

In greenhouse studies, the soil used was an Astatula fine sand subsoil (94-97% sand, 0.25% organic matter). In the first test, soil was steam-pasteurized and 14.2 liters of soil was amended with soil containing *Glomus mosseae* and blended in a soil mixer. The *G. mosseae* inoculum source was from citrus seedlings grown in steam-pasteurized soil. Mycorrhizae-amended soil containing approximately 56,000 viable chlamydospores/14.2 liters was placed in 30 liter sealable containers and treated by

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pipetting chemicals into the soil and sealing the containers (fumigants and rates in Table 1). Because of its vapor pressure, methyl bromide was chilled and prepared by a method reported by Abdalla and Lear (1). A natural mycorrhizal and a non-mycorrhizal soil served as nontreated controls. After 4 days, container lids were removed and the soil was aerated by stirring at intervals for 4 days. After 8 days, soils in each container were put in 8-15 cm clay pots. Each pot, except the nonmycorrhizal control, contained about 4,300 chlamydospores. Four sour orange *(Citrus aurantium* L.) seedlings were grown in each pot and the treatments were randomized on a greenhouse bench and grown for 8 months at ambient greenhouse temperatures. All seedlings were fertilized at monthly intervals with 200 cm^3 of a liquid 12-0-6 solution containing urea nitrogen. At harvest, plants were removed and assayed for shoot and root oven-dry weights. To determine mycorrhizal occurrence, 25 g of soil/replication was processed by wet sieving (8) to remove chlamydospores.

The same chemicals and rates were used in the second test, and *G. fasciculatus* inoculum from unpasteurized naturally infested citrus soil was used. Each pot, except the nonmycorrhizal control, contained approximately 800 chlamydospores. The test was terminated after 7 months.

In the second test, the low numbers of chlamydospores revealed by soil examination at harvest were not considered a reliable indicator of mycorrhizal activity. Therefore, roots were stained with acid fuchsin in lactophenol and cleared in lactophenol (6); 25 one-cm-long sections/replication were examined with a microscope for the presence of hyphae and vesicles.

Because the inoculum source in the second test came from naturally infested field soil, plant roots were examined for *Phytophthora parasitica* commonly associated with citrus soils.

Table 1. Fumigants and rates used in the citrus mycorrhizae-pesticide studies.

Chemical	Designation	% active ingredient	Rate/ha[1] Lo	Med	Hi	Unit
Ethylene dibromide	EDB	83	141	235	468	liters
1,3-dichloropropene	1,3-D	92	168	336	675	liters
Chloropicrin	CP	99	163	326	655	liters
Methyl bromide	MB	98	242	487	976	kg

[1]Rates used 1/2, 1, and 2X's the rate for a nursery fumigation.

RESULTS AND DISCUSSION

Glomus mosseae sporulated only around plants growing in control mycorrhizal soil or soils treated with EDB (Table 2). MB, CP, and 1,3 D appeared to have significantly reduced VAM development as no chlamydospores were detected in these samples. Growth of sour orange as influenced by VAM alone was significantly greater than seedlings growing in nonmycorrhizal soil (Table 3). EDB at rates used in this study did not inhibit mycorrhizal activity and, in fact, the combination improved plant growth (Table 3, Fig. 1). Growth stimulation of different plants with mycorrhizal-biocide combinations has been reported (3). While no chlamydospores were detected in 1,3-D treated soils, shoot growth was significanlty greater (P : 0.01) than that occurring in

Table 2. Number of viable chlamydospores or percent root segments infected with vesicular arbuscular mycorhizae (VAM).

Fumigant	Rate/ha	*G. mosseae* (chlaymdospores[1])	*G. fasciculatus* (% infection[2])
EDB	141 l/ha	62	76
"	235 "	42	68
"	468 "	48	64
1,3-D	168 "	0	75
"	336 "	0	0
"	675 "	0	0
CP	163 "	0	4
"	326 "	0	0
"	665 "	0	0
MB	242 kg/ha	0	0
"	487 "	0	0
"	976 "	0	0
Control + VAM		42	89
Control - VAM		0	0

[1]No./25 g soil, [2]25 one-cm root segments/replication. Percent infection based on + or - hyphae and/or vesicles.

nonmycorrhizal control plants; however, there was not as great a root effect. Both MB and CP at the low and high rates significantly reduced VAM development, resulting in reduced plant growth comparable to that of non-VAM seedlings (Tables 2 & 3).

High percentages of *G. fasciculatus* root infection occurred in VAM control seedlings, seedlings grown in all rates of EDB and low rates of 1,3-D treated soils (Table 2). Negligible root infection was observed on seedlings from soils receiving the low CP treatment. As might be expected, no root infection was observed with any MB treatment.

Root assay for *Phytophthora* infection indicated its presence on all seedlings in the VAM control, all rates of EDB, the low rate of 1,3-D, and low and middle rates of CP. No infection was observed on seedlings growing in MB-treated soils. This and the previous test suggest the limited biocidal activity of EDB at these rates.

Seedling growth in all treatments was influenced by either *Phytophthora* or mycorrhizae. Seedlings from the pasteurized non-VAM soil were significantly smaller than seedlings from VAM control soil in spite of *Phytophthora* infection in these seedlings. Root weight data showed only those seedlings growing in 1,3-D low rate soil to be significantly greater than all other seedlings (Table 3). While seedlings growing in all MB-treated soils were free of *Phytophthora*, the lack of VAM resulted in less plant development.

Data from these tests have shown that EDB at the rates used did not impede *G. mosseae* or *G. fasciculatus* activity and seedling growth was stimulated; however, higher rates could be biocidal. Similarly, low rates of 1,3-D favored plant growth with VAM, while higher rates appeared to have some adverse effect on VAM. Combining treatment rates (Table 4) demonstrates the effect of VAM as it influences growth and also the inhibitive effect of *Phytophthora* as shown with *G. fasciculatus*. MB and CP effectively inhibited *G. mosseae* and *G. fasciculatus* at all rates, to the detriment of the host.

Table 3. Ovendry *Citrus aurantium* shoot and root weights in *G. mosseae* and *G. fasciculatus* VAM and non-VAM soil following fumigation.

	Mean ovendry weight (g)													
	Low				Med				Hi				Control	
Glomus spp.	EDB	1,3 D	CP	MB	EDB	1,3 D	CP	MB	EDB	1,3 D	CP	MB	VAM	-VAM
G. mosseae														
Shoot	7.7a[2]	4.8b	2.2d	3.7c	8.8a	4.9b	5.2b	4.0b	5.9ab	4.8b	3.6c	1.1d	4.0b	1.9d
Root	4.6a	1.8cd	1.3cd	1.8cd	3.7a	2.1c	2.7b	1.9cd	2.8b	2.3c	2.1c	0.7d	2.5b	1.1d
G. fasciculatus														
Shoot	3.2b	5.7a	2.4cd	2.0cd	2.7c	1.8d	2.6c	2.2cd	2.7c	1.8d	2.4cd	2.5cd	3.1b	1.6d
Root	2.2cd	3.0b	2.2cd	1.9d	2.3c	1.8d	2.5c	2.0cd	2.0cd	2.2cd	2.3c	2.6c	2.4c	1.7d

[1]Rates shown in Table 1.
[2]Numbers followed by the same letters horizontally are not significantly different according to Duncan's multiple range test. Each row analyzed separately.

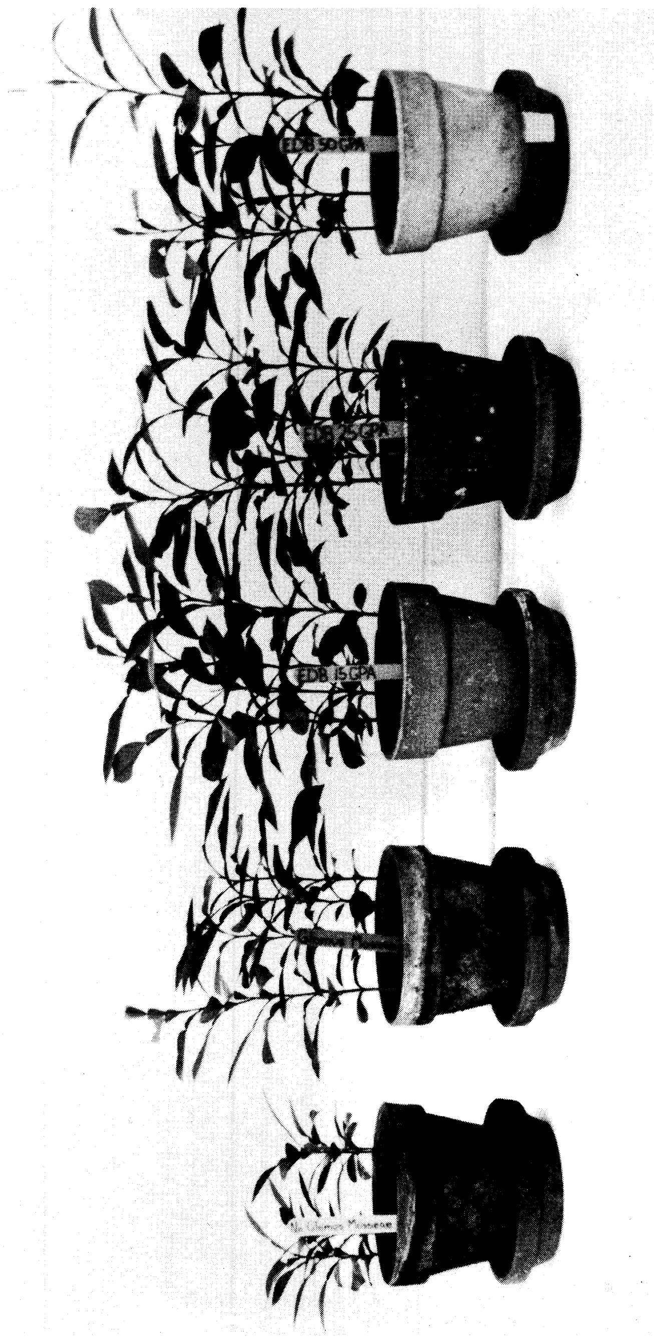

Figure 1. The influence of *Glomus mosseae* and EDB on citrus seedling growth. L to R: Control, no *G. mosseae*; *G. mosseae* only; EDB-141, 235, 468 liters/ha.

Table 4. Growth response of *Citrus aurantium* seedlings in *G. mosseae* and *G. fasciculatus* VAM and non-VAM soil following fumigation.

	Dry weight (g)			
	G. mosseae		*G. fasciculatus*	
Treatment[1]	Shoot	Root	Shoot	Root
EDB	7.46**[2]	3.71**	2.9*	2.1
1,3 D	4.84*	2.13*	3.1*	2.3
CP	3.65	2.06	2.5	2.3
MB	2.94	1.48	2.2	2.1
CK+VAM	4.01*	2.09*	3.0*	1.8
CK - VAM	2.97	1.05	1.6	1.9

[1]Combined treatment means.
[2]*(P : 0.05), **(P : 0.01) using Tukey's Honestly Significant Differences.

RESUMEN

Cuatro pesticidas fumigantes se aplicaron a suelos infestados con *Glomus mosseae* o *G. fasciculatus* para determinar sus efectos sobre micorrizas arbúsculo vesiculares (VAM) y sobre el desarrollo subsiguiente de posturas citricas. La fumigación con bromuro de metilo y cloropicrina controló efectivamente el VAM con las dosis empleadas. El crecimiento de las posturas fue reducido por falta de VAM. En suelos tratados con 1,3-diclororopropeno y bibromuro de etileno, VAM no fue eliminado y hubo estimulación en el crecimiento de las plántulas.

Claves: EDB, DD, Telone, fumigantes, efectos aleatorios.

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