Effect of *Pythium ultimum* and Metalaxyl Treatments on Root Length and Mycorrhizal Colonization of Cotton, Onion, and Pepper

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ABSTRACT

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Root length and mycorrhizal colonization of cotton, onion, and pepper inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus intraradices* were generally greater in fumigated soil than in nonfumigated soil. Five weeks after VAM fungus inoculation, 39–42% of roots were colonized in fumigated soil, compared to 21–26% in nonfumigated soil. VAM colonization of roots increased to 64–71% following treatment with the fungicide metalaxyl. Root lengths and VAM colonization of the three crops were reduced significantly in fumigated soil following infestation with *Pythium ultimum* and were similar to those in nonfumigated soil. Metalaxyl did not affect root length or VAM colonization in fumigated soil. *P. ultimum, Fusarium solani,* and *Rhizoctonia solani* were isolated from the roots of cotton, onion, and pepper grown in nonfumigated soil. The most commonly isolated fungus was *P. ultimum.*

Because most agricultural crops are grown without treating the soil with biocides, the potential contribution of vesicular-arbuscular mycorrhizal (VAM) fungi to plant growth can be overlooked. Mycorrhizal fungi increase nutrient uptake and growth in many plants (7,15,17). Crop growth response and percentage colonization of plants inoculated with VAM are often higher in fumigated soil than in natural soil (1,10), even though the application of fumigants reduces or eliminates indigenous VAM fungi (10,13,15). Apparently, the inhibition of mycorrhizal root colonization in natural soil reduces the effectiveness of VAM fungi in enhancing crop growth (9,11).

Root colonization by VAM fungi is usually correlated with the initial timing and rate of colonization. Thus, the first few weeks of plant growth are critical for establishing adequate VAM colonization in annual crops (1,23). *Pythium paroecandrum* Drechs., which was isolated from alfalfa roots and caused preemergence damping-off, might explain the inhibition of VAM colonization the first few weeks after planting (11).

One hypothesis to explain the reduced root colonization by VAM fungi in some natural soils is that pathogens compete with VAM fungi for specific niches, especially during the critical first few weeks of plant growth. Many pathogens, such as *Phytophthora, Fusarium, Rhizoctonia, Pythium,* and *Gaeumannomyces* spp., reduce mycorrhizal root colonization and are extremely common and widespread in agricultural soils (2,8,16,22). Removing these pathogens as competitors may improve VAM colonization in natural soils. Many fungicides improve colonization by VAM fungi (9,14,19), although the mechanism for this improvement is unknown.

This study was undertaken to determine whether VAM colonization of cotton, onion, and pepper is greater in fumigated soil or in nonfumigated soil, to study the effects of *P. ultimum* Trow on VAM colonization and root lengths of these crops, and to examine whether VAM colonization and root length could be increased by treatments with metalaxyl.

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MATERIALS AND METHODS

Plant material. In all experiments, seeds of cotton (*Gossypium hirsutum* L. 'SJ-2'), onion (*Allium cepa* L. 'Burpee Yellow Globe'), and pepper (*Capsicum frutescens* L. 'California Wonder') were surface-sterilized with 20% sodium hypochlorite for 1 min and planted in autoclaved clay pots (volume 500 cm^3) with a sandy loam soil. These crops were chosen because they are annuals and respond positively to VAM.

The sandy loam soil was characterized as follows: saturation percentage 27%, pH 7.7, electroconductivity 4.0 dS/m, Ca 23.5 meq/L, Mg 3.8 meq/L, Na 13.9 meq/L, sodium adsorption ratio 3.8, exchangeable sodium percentage 4.2%, N 632 ppm, P 9.5 ppm, K 142 ppm, Zn 12.8 ppm, Mn 8.6 ppm, Fe 6.5 ppm, Cu 1.7 ppm, organic matter 0.80%, clay 8.3%, silt 28.6%, and sand 63.1%. This soil, which was either not fumigated or was fumigated with methyl bromide (98% methyl bromide + 2% chloropicrin), equivalent to 500 kg/ha, had been used for field trials with vegetable crops the previous 4 yr and was taken from a typical agricultural site in the Citrus Experiment Station, University of California, Riverside.

Root lengths were measured using the line intercept method (20) 4 or 5 wk after planting.

Inoculation. Plants were inoculated with the mycorrhizal fungus *Glomus intraradices* Schenck and Smith. The inoculum used was infected roots of greenhouse-grown Sudan grass (*Sorghum vulgare* Pers.) and spores and soil associated with the roots. Approximately 10 g of inoculum was placed 5 cm below the seeds in pots. Plants were grown in a greenhouse at 24 ± 2 C. Fluorescent lights were used to supplement natural light (14-hr light period).

Percentage colonization by VAM fungi was measured after roots were stained in lactophenol trypan blue. One hundred root sites were counted in each sample. Percentage colonization was calculated as the number of colonized sites per 100 sites (24).

Experiment 1: Isolation of microorganisms from roots. Cotton, onion, and pepper seeds were planted and inoculated with *G. intraradices* in fumigated and nonfumigated soil in 30 pots for each crop. Ten days after planting, roots were washed carefully with tap water to remove soil and were immersed for 20 sec in 0.5% sodium hypochlorite. Sodium hypochlorite residues were rinsed off in sterilized water. Roots were cut into 1-cm pieces and placed on three types of media in plastic petri plates 9 cm in diameter. Ten plates, each containing five 1-cm root segments, were used for each crop and each medium, for a total of 90 plates. The media used were potato-dextrose agar (PDA), water agar, and pimaricin-vancomycin-pentachloronitrobenzene (25). The plates were incubated in the dark at 25 C for 48 hr. Microorganisms growing from root pieces were then isolated and identified by R. M. Endo and J. A. Menge (University of California, Riverside).

Experiment 2: Infestation with *Pythium ultimum*. The isolate of *P. ultimum* (P-10) was originally obtained from cotton plants grown at the Citrus Experiment Station, University of California, Riverside, in October 1987. Soil was either fumigated or not fumigated and either infested or not infested with *P. ultimum* (four treatments). Soil was infested by adding approximately 20 g of inoculum of *P. ultimum* to each pot 48 hr before planting. Inoculum was prepared by blending a PDA plate containing a 1-wk-old culture of *P. ultimum* in 50 ml of sterilized deionized water. Ten replicate pots of each crop either infected with *G. intraradices* or not infected (nonmycorrhizal) were used for each of the four soil treatments, for a total of 240 pots. Plants were maintained in the greenhouse at 24 ± 2 C. Percentage VAM colonization and root lengths were measured 4 wk after planting.

Experiment 3: Metalaxyl application. Seeds of cotton, onion, and pepper were planted in pots of fumigated and nonfumigated soil. With each type of soil, 10 replicates were used for each of four treatments for each crop, for a total of 240 pots. Treatments were as follows: control (the seeds were planted without VAM fungus inoculum), VAM (the seeds were inoculated as before with *G. intraradices*), control plus metalaxyl, and VAM plus metalaxyl. Application of metalaxyl consisted of adding 100 ml of 100 μl/L liquid metalaxyl (25% active ingredient) per pot 10 min after planting. This amount is equivalent to 8 mg of active ingredient per 1 m^2. Plants were maintained in the greenhouse at a temperature of 24 ± 2 C. Percentage VAM colonization and root lengths were measured 5 wk after planting.

Experiments 2 and 3 had a completely randomized design, and each experiment was repeated three times.

Table 1. The effect of infection by *Pythium ultimum* on colonization by *Glomus intraradices* in roots of cotton, onion, and pepper

	Root colonization by *G. intraradices* (%)[x]		
Treatment	**Cotton**	**Onion**	**Pepper**
Fumigated soil			
G. intraradices	42 a	43 a	38 a
G. intraradices plus *P. ultimum*	28 b	27 b	25 b
Control[y]	0	0	0
Nonfumigated soil[z]			
G. intraradices	—	26 b	—
G. intraradices plus *P. ultimum*	—	24 b	—
P. ultimum	—	3 d	—
Control	—	10 c	—

[x] Each number is an average of 10 replicates. Means within each column followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).
[y] Neither *G. intraradices* nor *P. ultimum* inoculum. Values were excluded from analysis of variance because all replicates were 0.
[z] Percentage colonization of onion roots in nonfumigated soil includes indigenous colonization. A dash indicates that more than 70% of plants died and statistical analysis could not be done.

Table 2. The effect of *Pythium ultimum* infection on root length of cotton, onion, and pepper plants inoculated or not inoculated with *Glomus intraradices*

	Root length (cm)[x]		
Treatment	**Cotton**	**Onion**	**Pepper**
Fumigated soil			
G. intraradices	49 a	25 a	45 a
G. intraradices plus *P. ultimum*	27 b	15 de	18 b
P. ultimum	14 b	14 ef	9 c
Control[y]	34 ab	20 bc	52 a
Nonfumigated soil[z]			
G. intraradices	—	18 cd	—
G. intraradices plus *P. ultimum*	—	11 fg	—
P. ultimum	—	9 g	—
Control[y]	—	10 g	—

[x] Each number is an average of 10 replicates. Means within each column followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).
[y] Neither *G. intraradices* nor *P. ultimum* inoculum.
[z] A dash indicates that more than 70% of plants died and statistical analysis could not be done.

Table 3. Colonization by *Glomus intraradices* in roots of cotton, onion, and pepper plants treated or not treated with metalaxyl in fumigated or nonfumigated field soil.

	Mycorrhizal colonization (%)[w]		
Treatment	**Cotton**	**Onion**	**Pepper**
Fumigated soil			
G. intraradices	40 b	42 b	39 b
G. intraradices plus metalaxyl	51 b	40 b	37 b
Control[x,y]	0	0	0
Control plus metalaxyl[y]	0	0	0
Nonfumigated soil[z]			
G. intraradices	21 c	24 c	26 c
G. intraradices plus metalaxyl	71 a	71 a	64 a
Control[x]	6 d	4 d	3 e
Control plus metalaxyl	9 d	7 d	11 d

[w] Each number is an average of 10 replicates. Means within each column followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).
[x] No *G. intraradices* inoculum.
[y] Values were excluded from analysis of variance because all replicates were 0.
[z] Percentage colonization of roots in nonfumigated soil includes indigenous colonization.

RESULTS

Isolation of microorganisms from roots. Three species of fungi—*P. ultimum, F. solani,* and *R. solani*—were isolated from the roots of cotton, onion, and pepper grown in nonfumigated soil. *P. ultimum* was most prevalent, appearing in 85 of 90 plates; *F. solani* appeared in 13 plates and *R. solani* in 4. Other microorganisms, especially bacteria, were isolated from both fumigated and nonfumigated soil. However, these three fungi were the only microorganisms present on roots in nonfumigated soil but not in fumigated soil.

Effect of *P. ultimum* on VAM colonization and root length. In fumigated soil, mycorrhizal colonization of cotton, onion, and pepper 4 wk after inoculation was 1.5-fold greater than in fumigated soil infested with *P. ultimum*. However, the percentage of VAM colonization in onion in nonfumigated soil was similar to that in fumigated soil infested with *P. ultimum* (Table 1). Many of the cotton and pepper plants in the nonfumigated soil died before mycorrhizal colonization could be determined (Tables 1 and 2), apparently as a result of *P. ultimum*, which was present at 38.3 propagules per gram of soil.

The length of onion, cotton, and pepper roots was more than 30% lower in fumigated soil infested with *P. ultimum* (Table 2). *P. ultimum* was present in these soils at 33.5 propagules per gram of soil. In nonfumigated soil, root lengths of onion not inoculated with VAM fungi were similar whether or not the soil was infested with *P. ultimum* (Table 2).

Effect of metalaxyl on mycorrhizal colonization and root length. The percentage VAM colonization of cotton, onion, and pepper in nonfumigated soil infected with *G. intraradices* and treated with metalaxyl was 2.4–3.4 times greater than that in plants not treated with the fungicide (Table 3). About 40% of the roots of these crops were colonized in fumigated soil, whether treated with metalaxyl or not (Table 3). Root lengths were similar in metalaxyl-treated and untreated fumigated soil and in metalaxyl-treated nonfumigated soil but were up to 50% lower in nonfumigated soil not treated with metalaxyl (Table 4).

No *Pythium* spp. were isolated from roots grown in fumigated soil. Similar results were found when experiments were repeated.

DISCUSSION

When pathogens were not controlled using metalaxyl, root length and percentage colonization of cotton, onion, and pepper were greater in fumigated soil than in nonfumigated soil (Tables 1 and 3). In our field soil, this appears to be a result of the presence of *P. ultimum,* a common pathogenic fungus that was isolated from roots of all three crops. *Pythium* spp. are ubiquitous and are well-known as damping-off organisms associated with root tips during the first few weeks of plant growth, when colonization by mycorrhizae is most important. *Pythium* spp. may interfere with VAM colonization by reducing root growth, damaging roots, and altering root exudation (21) or by competing with VAM fungi during the first few weeks of plant growth when initial mycorrhizal colonization occurs.

Hwang (11) reported that the poor growth of alfalfa seedlings with "alfalfa sickness" is caused by *P. paroecandrum* and *P. sylvaticum* W. A. Campbell & J. W. Hendrix. The addition of VAM fungi partially alleviated the problem, and a combination of metalaxyl and VAM fungus inoculation resulted in the production of healthy alfalfa seedlings in "alfalfa sickness" soil (11). The research reported here further suggests that metalaxyl, a systemic fungicide, can be used both to control root rot caused by oomycetes and to increase VAM colonization and root length of cotton, onion, and pepper in nonfumigated soil (Tables 3 and 4). Similarly, Jabaji-Hare and Kendrick (12) reported that the systemic fungicide fosetyl-Al increased VAM colonization of leek roots, and Groth and Martinson (9) reported that soil incorporation of metalaxyl increased VAM colonization of maize and soybean roots. These results may explain why fungicides increase VAM colonization in some soils (14,19).

The percentage of VAM colonization of onion in nonfumigated soil was similar to that in fumigated soil infested with *P. ultimum,* probably because the populations of *P. ultimum* in the soils were similar (38.3 and 33.5 propagules per gram of soil, respectively). Furthermore, VAM colonization in nonfumigated soil was not influenced greatly by infestation with *P. ultimum* (Table 1). This result is to be expected because *P. ultimum* is already prevalent in this nonfumigated soil, and the additional inoculum did not affect VAM colonization any more than the endemic populations.

The results of this study show that metalaxyl enhances VAM colonization of cotton, onion, and pepper significantly more in nonfumigated soil than in fumigated soil (Table 3). Soil fumigation kills most living soil organisms, including some beneficial microorganisms such as bacteria and actinomycetes, which may improve VAM colonization (3–6,18) as well as pathogenic microorganisms such as *P. ultimum,* which may inhibit VAM colonization. Because metalaxyl acts against *P. ultimum* and not against beneficial microorganisms, VAM colonization is greater in soils treated with the fungicide. Furthermore, the native mycorrhizal inoculum in nonfumigated soil combined with the added VAM inoculum results in a higher mycorrhizal inoculum potential and thus more VAM colonization when the competition from *Pythium* spp. is reduced.

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Table 4. Root length of cotton, onion, and pepper plants treated or not treated with metalaxyl and inoculated or not inoculated with *Glomus intraradices*

	Root length (cm)[y]		
Treatment	**Cotton**	**Onion**	**Pepper**
Fumigated soil			
G. intraradices	177 a	49 a	47 a
G. intraradices plus metalaxyl	197 a	47 a	48 a
Control[z]	179 a	48 a	47 a
Control plus metalaxyl	179 a	46 a	49 a
Nonfumigated soil			
G. intraradices	82 b	28 b	24 b
G. intraradices plus metalaxyl	185 a	45 a	47 a
Control[z]	83 b	27 b	23 b
Control plus metalaxyl	168 a	46 a	48 a

[y] Each number is an average of 10 replicates. Means within each column followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).
[z] No *G. intraradices* inoculum.

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