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Utillization of Vesicular-Arbuscular Mycorrhiza in Crop Production

J.H. Haas, J. Krikun, J.A. Menge

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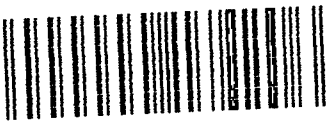

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Utilization of vesicular-arbuscular mycorrhiza in crop production

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UTILIZATION OF VESICULAR-ARBUSCULAR MYCORRHIZA IN CROP PRODUCTION

BARD Grant No. I-0916-85RC -- FINAL REPORT -- 20 August 1990

Jerry H. Haas, John A. Menge and James Krikun

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C. ABSTRACT

Soil treatments and VAMF inoculation were studied in crops of cotton, onion and pepper. Metalaxyl treatments suppressed Pythium-incited disease and encouraged VAMF colonization of roots, especially in nonfumigated soil. Soil solarization also encouraged VAMF colonization and yield. A combination of solarization followed by VAMF inoculation has great potential for use on nonfumigated soil. The importance of effective VAMF species, availability of young susceptible roots for VAMF infection, and the proper placement of VAMF inoculum in relation to the seed was confirmed; the placement studies were done in fumigated and nonfumigated soil in field plots with cotton and onion.

The response of celery, melon, onion and pepper to VAMF and P fertilization was investigated in field plots in a high P-sorbing loessial soil. Where VAMF were absent, even the highest P level was not sufficient for maximum yields. Melon did not evidence potential for benefits by VAMF augmentation as a horticultural practice. Transplanted pepper growing in a Typic Torriorthents soil had greater total crop yield and a higher percent of that yield with large-size fruits when VAMF inoculation and P fertilization were practiced; fertilized P alone was not sufficient for maximum yields.

A study was completed of the VAMF species present in avocado orchards in Israel and California. There were significant differences in the species present and in the soil chemical and physical characteristics, despite the similarities in climate in the two growing areas. The data obtained will be important for studies of VAMF supplementation in avocado nurseries.

D. PROPOSAL OBJECTIVES

We proposed to continue research initiated in BARD grant no. I-0169-80 which was on the same general subject. In the present project the emphasis was to be on field trials to determine whether mycorrhizal augmentation or encouragement has a commercially-viable potential.

This objective was to be achieved by:

1. Conducting trials on vesicular-arbuscular mycorrhizal fungus (VAMF) supplementation in plots with and without soil treatment which reduce viable VAMF populations in soil.

2. Investigate crops other than citrus and pepper which were the principle crops studied, respectively, by the California (CA) and Israel (IL) groups in the previous BARD-supported project.

3. Testing interaction effects of VAMF with other horticultural practices.

E. REPORT

Almost all of the research which was conducted during the period of the grant has been submitted, accepted, or is in press or published. The manuscripts or reprints are an integral part of this section of the report.

In CA, the effects of soil treatments and VAMF inoculation were studied in crops of cotton, onion and pepper. Metalaxyl treatments suppressed Pythium-incited disease and encouraged VAMF colonization of roots, especially in nonfumigated soil (Afek et al., 1990). Soil solarization also encouraged VAMF colonization and yield. A combination of solarization followed by VAMF

inoculation has great potential for use on nonfumigated soil (Afek et al., 1991a). The importance of effective VAMF species, availability of young susceptible roots for VAMF infection, and the proper placement of VAMF inoculum in relation to the seed was confirmed with these crops and for three VAMF species (Afek et al., 1991b); the placement studies were done in fumigated and nonfumigated soil in field plots with cotton and onion.

In IL, the response of celery, melon, onion and pepper to VAMF and P fertilization was investigated in field plots in a high P-sorbing loessial soil (Krikun et al., 1990). Where VAMF were absent, even the highest P level (25 mg bicarbonate-extractable P kg^{-1} soil) was not sufficient for maximum yields with celery, onion and pepper. Melon did not evidence potential for benefits by VAMF augmentation as a horticultural practice. Haas et al. (1991) showed that transplanted pepper growing in a Typic Torriorthents soil type, had greater total crop yield and a higher percent of that yield with large-size fruits when VAMF inoculation and P fertilization were practiced; fertilized P alone was not sufficient for maximum yields.

Haas and Menge (1990) completed a study of the VAMF species present in avocado orchards in IL and CA. There were significant differences in the species present and in the soil chemical and physical characteristics, despite the similarities in climate in the two growing areas. The data obtained will be important for continued studies of VAMF supplementation in avocado nurseries.

During a six-mo study visit with D. M. Sylvia, Univ. Florida, Haas participated in the third year of a 3-yr field experiment on the interaction of VAMF inoculation and plant water stress in corn. He collected crop physiology data. The results are being prepared for publication and an abstract of some of the data has been published (Sylvia et al., 1990).

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

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ABSTRACT

Afek, U., Menge, J. A., and Johnson, E. L. V. 1990. The effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion, and pepper. Plant Dis. 74:117-120.

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

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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

Treatment	Root colonization by *G. intraradices* (%)[x]		
	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*

Treatment	Root length (cm)[x]		
	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.

Treatment	Mycorrhizal colonization (%)[x]		
	Cotton	Onion	Pepper
Fumigated soil			
G. intraradices	40 b	42 b	39 b
G. intraradices plus metalaxyl	51 b	40 b	37 b
Control[y,z]	0	0	0
Control plus metalaxyl[z]	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[y]	6 d	4 d	3 e
Control plus metalaxyl	9 d	7 d	11 d

[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] No *G. intraradices* inoculum.
[z] 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.

ACKNOWLEDGMENTS

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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*

Treatment	Root length (cm)[1]		
	Cotton	Onion	Pepper
Fumigated soil			
G. intraradices	177 a	49 a	47 a
G. intraradices plus metalaxyl	197 a	47 a	48 a
Control[2]	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[2]	83 b	27 b	23 b
Control plus metalaxyl	168 a	46 a	48 a

[1] 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 ($P = 0.05$).
[2] No *G. intraradices* inoculum.

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Simulation of the Date of Maturity of *Plasmopara viticola* Oospores to Predict the Severity of Primary Infections in Grapevine

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ABSTRACT

Tran Manh Sung, C., Strizyk, S., and Clerjeau, M. 1990. Simulation of the date of maturity of *Plasmopara viticola* oospores to predict the severity of primary infections in grapevine. Plant Dis. 74:120-124.

A study of the dynamics of *Plasmopara viticola* oospore maturation during 3 yr in the Bordeaux area of France showed significant differences among years. The date of oospore maturity and subsequent disease severity in spring appeared to be associated with the amount of rainfall after oospore formation. For example, heavy rainfall from September to February was generally associated with early oospore maturity and severe disease in May or June. To predict the date when most oospores are mature (DOM), a climate-based model called "Prediction of Oospore Maturity" (POM) was developed. The POM model, based on daily rainfall beginning in September, permits calculation of DOM as early as the end of January. POM calculations based on climatic data recorded since 1977 confirmed that the earlier the DOM, the more severe disease was in the spring. Four levels of predictable risk are proposed based on the time required for oospore maturation. Although the POM model needs validation, it already shows promise in grape disease management.

Additional keywords: downy mildew, epidemiology, risk modeling

Oospores constitute the primary means of winter survival of *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni and are the initial inoculum for grapevine downy mildew. Previous studies of this organism have focused on the prediction of primary infection dates in spring and have not allowed precise determination of the relationship between the evolution of oospore maturation and the severity of subsequent infections. However, simulations of disease risk made with the "EPI model" developed by Strizyk (11,12) showed that winter climatic data must be incorporated to give accurate predictions (6,8). As early as the end of March, the model provides an indication of primary infection risk by using climatic data starting from October. According to simulations conducted for a 12-yr period (1977-1988), risk is highest when rain is abundant during the oospore maturation period.

Using a technique for assessing the dynamics of oospore maturation and germination (9), we tried to better understand the role of oospores in an epidemic. We studied the maturation of oospores and the initial development of downy mildew in the Bordeaux area of France over 3 yr and developed a model for predicting the dates of oospore maturity. The a posteriori validation of this model over several years demonstrates its ability to predict disease severity in spring (May-June).

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MATERIALS AND METHODS

Dynamics of oospore maturation. Infected leaves containing oospores of *P. viticola* were collected on 24 October 1984, 22 October 1985, and 20 October 1987 from an experimental vineyard belonging to the INRA Research Center in Bordeaux. Under a binocular microscope, leaf disks 6 mm in diameter containing oospores (more than 1,000/cm^2) were punched out with a cork-borer and stored in plaster modeling tubes (40 mm long, inner diameter 12 mm, outer diameter 30 mm) (9). Tubes containing leaf disks were buried under 5 cm of soil and exposed to natural vineyard conditions. Disks were subsampled from the tubes every 15 days beginning in January. Two hundred oospores per sample were dissected from the leaf disks and placed on 1% water agar in petri dishes. The percentage of oospores capable of germinating at 20 C in a lighted incubator (i.e., the percentage of mature oospores) was assessed daily with a microscope (9).

Climatic records. Climatic conditions were recorded by the Regional Service of the National Meteorological Institute. They consisted of daily rainfall in millimeters (*Rd*) from 21 September 1977 to 31 March 1988 and monthly average rainfall (*RM*) calculated from 1946 to 1987.

Assessment of the intensity of primary infections. Disease severity of downy mildew was rated annually from 1977 to 1988 on a scale of 1-4 from the observations published by Plant Protection Services in forecasting bulletins (8). Disease ratings were based on the severity of early downy mildew on leaves and bunches (gray rot symptoms) until end of bloom. Ratings were made in the Bordeaux production area (100,000 ha) on susceptible cultivars. The disease ratings, followed by the years given each rating from 1977 to 1988, are as follows: 1 = disease absent or minimal, with no economic incidence before véraison (1984, 1986, 1987); 2 = disease present in a few vineyards, inducing moderate but not severe damage (1978, 1979, 1981); 3 = disease present in most vineyards in the area, sometimes very severe and leading to significant economic losses (1980, 1982, 1983); and 4 = disease present in almost all vineyards, inducing very severe damage (100% crop loss in vineyards not properly treated with fungicides) (1977, 1985, 1988).

RESULTS

Biological basis for the model. *Dynamics of oospore maturation in 1985, 1986, and 1988.* Our study of the development of mature oospores in 1985, 1986, and 1988 showed that the maturation period can be subdivided into three phases (Fig. 1). In the first phase,

Interaction among mycorrhizae, soil solarization, metalaxyl, and plants in the field

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ABSTRACT

Afek, U, Menge, J. A., and Johnson, E. L. V. 1990. Interaction of mycorrhizal fungus, soil solarization and metalaxyl, and the effect of it on plants in the field. Plant Disease 74:

In an attempt to increase growth response, yield and VAM colonization of cotton, onion and pepper, in natural soil, field trials were conducted in the summers of 1988 and 1989. In these trials, treatments such as soil solarization, soil solarization plus 2-wk tarp coverage after planting and metalaxyl were applied to VAM-inoculated and noninoculated plants and in fumigated and nonfumigated soil. Greatest cotton fresh wt, 1022, 1230 and 1150 g/plant, as well as, boll number, 66, 80 and 78/plant were achieved in VAM-inoculated nonfumigated soil following soil solarization, soil solarization plus 2-wk tarp covered and metalaxyl treatments, respectively. Greatest fresh wt of onion was achieved in VAM inoculated nonfumigated, solarized soil, with and without 2-wk tarp coverage after planting. In pepper, VAM inoculated plants fresh wt and fruit wt in non-fumigated soil were 1.5 to 2.0 and 1.3 to 2.0-fold greater than in fumigated soil respectively. In both fumigated and nonfumigated soil, best results of pepper fresh wt and fruit wt were achieved after soil solarization and soil solarization plus 2-wk tarp covered treatments. Significant correlations were found among VAM percent colonization at the fifth wk after planting and fresh wt and yield of the three crops. Maximum VAM colonization, 65% of the total roots, occurred in VAM inoculated cotton plants five wk after planting in nonfumigated, solarized soil plus 2-wk tarp coverage. With onion the best colonization occurred in VAM inoculated plants 2 and 3.5 wk after planting in both fumigated and nonfumigated, solarized soil and solarized soil plus 2-wk tarp coverage. However, 5 wk after planting best results were in nonfumigated soil treated with metalaxyl, soil solarization and soil solarization plus 2-wk tarp coverage. Maximum VAM colonization of 59% was achieved at the fifth wk after planting in nonfumigated, solarized soil plus 2 wk tarp coverage. Maximum cotton, onion and pepper root lengths of 149, 51 and 94 cm, 3.5 wk after planting, were achieved following soil solarization plus 2 wk tarp coverage after planting, respectively.

It is well documented that vesicular-arbuscular mycorrhizae (VAM) fungi increase nutrient uptake and growth in many plants (19,34,42,43). Most agriculture crops are grown without treating the soil with biocides which reduce or eliminate indigenous VAM fungi (9,18,28,31). Unfortunately, most experiments successful in increasing VAM colonization and growth response of plants treated with VAM inoculum have been done in sterilized or fumigated soil (2,3,18,32,43).

The reason for poor growth response of plants in natural soil following treatment with artificial VAM inoculum may be that certain microorganisms interfere with mycorrhizal development (3,5,16,33,44). Metalaxyl, a systemic fungicide which controls root rot and damping-off caused by oomycetes, has also been reported to increase VAM colonization of plants (3,17,31,36).

Since 1976 many experiments have been done to evaluate the potential of soil solarization for the reduction of pathogen (and other pests) populations, disease control and yield increase (1,22,25,26,41,46,48). At present, covering (tarping or mulching) soils with transparent polyethylene when appropriate climate conditions prevail, is the means for capturing solar energy to heat soil under field conditions (22,23,27,40).

Long-term effects of solarization on disease control and crop yields extending for a second or even a third crop have been observed with a variety of pathogens and crops (24,41,48). So far, a combination of VAM inoculum application and soil solarization has not been investigated. This study attempted to determine the effect of combination of VAM inoculum with soil solarization and with metalaxyl on VAM colonization and yield of cotton, onion and pepper in the field.

MATERIALS AND METHODS

Field site. An agricultural sandy loam soil site planted with vegetables the previous 5 yr on the Citrus Experiment Station, University of California, Riverside was selected for the field plots. The soil was characterized as follows: saturation percentage (SP) - 27%, pH - 7.7, electroconductivity (EC) - 4.0 dS/m, Ca - 23.5 me/l, Mg - 3.8 me/l, Na - 13.9 me/l, sodium adsorbtion ration (SAR) - 3.8, exchangeable sodium percentage (ESP) - 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 (OM) - 0.80%, clay - 8.3%, silt - 28.6% and sand - 63.1%. One half of the site was fumigated with 98% metalaxyl bromide plus 2% chloropicrin equivalent to 500 kg/ha and the other half was not fumigated. There were six 40-m-rows long X 30 cm wide in each half, four rows for each crop. Treatments consisted of a 1.5-m-row with a 0.5-m-buffer between treatments. A four-m-buffer was left at the beginning and the end of each row. Treatments included: 1) control (without mycorrhizal inoculation) 2) VAM inoculation (with G. intraradices) 3) control plus soil solarization 4) VAM inoculation plus soil solarization 5) control plus soil solarization plus 2-wk tarp coverage after the planting 6) VAM inoculation plus soil solarization plus 2-wk tarp coverage after planting 7) control plus metalaxyl 8) VAM inoculation with metalaxyl. All treatments were done

in both fumigated and nonfumigated soil. The field experimental design was randomized block with 4 blocks for each treatment.

Plant material and VAM inoculum. Seeds of cotton (Gossypium hirsutum L.) cv. SJ-2, onion (Allium cepa L.) cv. Burpee Yellow Globe and pepper (Capsicum annuum L.) cv. California Wonder were surface disinfested with 20% sodium hypochlorite for 1 min before planting. These crops were chosen since they are annuals and respond positively to VAM colonization.

Plants were inoculated with the mycorrhizal fungis Glomus intraradices Schenck and Smith (isolate 185 collected from Citrus sp., Ventura, California, 1975). Inoculum consisted of mixed roots and soil from sudan grass (Sorghum volgaris Pers.) which had been infected with the mycorrhizal fungus for 9 mo. Inoculum potential was calculated by the most probable number (MPN) equation.(4,12) and adjusted for identical inoculum potential during both years. Inoculum was applied by spreading by hand 170 g per 1 m row for a total of 144-m-rows, 3 cm below seeds at planting. Root lengths were measured using the line intercept method (37). Percent colonization by VAM was calculated after staining with lactophenol trypan blue as a number of colonized sites per total sites X 100 (39).

Soil solarization and tarping. Soil solarization was used on half of the site. It was applied to both fumigated and nonfumigated soil, moistened by irrigation for the purpose of improving heat conduction (29). A sprinkler system was placed between rows and the plots were irrigated for 24 hr and then removed. The soil was covered with a transparent polyethylene plastic sheet (0.1 mm) for 1 mo (May 22 - June 22, 1989). Seeds were planted 24 hr after removing the tarp. Two plots in each block, after soil solarization, were recovered with a tarp as in the soil solarization treatment in order to heat the soil, and holes were opened for the growing plants. Soil temperature was measured and recorded using all metal stem thermometers (FISHERbrand).

Soil temperatures before the planting were measured every day at 4:30 p.m. during May 22-June 22, 1989 (maximum soil temp occurred at 4:30 - 5:00 p.m.). Soil temperatures were measured with and without a tarp 0, 5, 10, 20 and 30 cm deep. During the first 20 days of tarp covering the sky was cloudy and the surface temp. was 28-33 C. Maximum temp 0, 5, 10, 20 and 30-cm deep under the tarp was: 41, 41, 40, 26 and 26 C compared to maximum temp without a tarp of 33, 32, 30, 23 and 22 C respectively. During the last 11 days the surface temp (without a tarp) was much higher (45-50 C) and maximum temp in these depths under the tarp was 54, 53, 50, 37 and 33 C in comparison to 50, 43, 37, 27 and 25 C without a tarp respectively.

Soil temp with and without a tarp after planting (holes were open in the tarp for the growing plants) were also recorded during the first 2 wk (the tarp was removed 2 wk after planting). Temp 0, 5, 10, 20 and 30-cm-deep with a tarp were 4-5, 4-5, 3-4, 2-4 and 2-3 C higher than without a tarp respectively.

Metalaxyl application. Application with metalaxyl consisted of drenching 1 l of 700 Cml/l liquid metalaxyl (25% active ingredient) per treatment (1.5 m X 0.35 m) 1 hr after the planting. This amount is equivalent to 333 mg active material per 1 mC2.

Harvesting. Seeds of cotton, onion and pepper were planted on June 23, 1989. Percent colonization by VAM was measured 2, 3.5 and 5 wk after the planting. Root length was measured 3.5 wk after planting. The final harvest was done four mo after the planting. Fresh wt was measured in all of the plants. In addition, cotton boll number was counted and pepper fruit wt was weighed.

Data were analyzed by an Anova Statistical Program. Correlation coefficients were computed by a Lotus statistics package. Experiments were repeated twice in the summers of 1988 and 1989 and similar results were obtained.

RESULTS

VAM percent colonization. Maximum VAM colonization of VAM-inoculated cotton plants, was over 65% at the fifth wk after planting in nonfumigated soil in the solarized plus 2-wk tarp coverage (Fig. 1). The percent colonization by VAM in inoculated cotton plants 3.5 and 5 wk after planting in fumigated soil was 39 and 41% compared to 23 and 30% in nonfumigated soil respectively. However, the reverse occurred when the soil was treated with solarization, solarization plus 2-wk tarp coverage and metalaxyl (Fig. 1). In these treatments the percent colonization of cotton plants in nonfumigated soil 3.5 and 5 wk after planting was significantly higher than in fumigated soil.

In onion colonization best results of the inoculated plants, 2 and 3.5 wk after planting were achieved both in fumigated and nonfumigated soil solarized soil and solarized soil plus 2-wk tarp coverage. However, 5 wk after planting all three best results were in nonfumigated soil treated with metalaxyl, soil solarization and soil solarization plus 2-wk tarp coverage. Maximum VAM colonization of onion of 59% was achieved at the fifth wk after planting in nonfumigated, solarized soil plus 2-wk tarp coverage (Fig. 2).

In pepper 2 wk after planting in nonfumigated solarized soil plus 2 wk covered with a tarp, VAM percent colonization of the inoculated plant was reached at 31% significantly higher than the other

treatments at that time. At the fifth wk after planting the best results were achieved in nonfumigated, solarized soil with or without 2-wk tarp coverage. Maximum VAM colonization of inoculated pepper plants increased up to 63%, 3.5 wk after planting in nonfumigated solarized soil plus 2-wk tarp coverage (Fig. 3).

VAM percent colonization of plant control (without mycorrhizal inoculation) of all treatments for all times was 1-3% in fumigated soil and 3-16% in nonfumigated soil (Fig. 1-3).

Root length. Root length of cotton, onion and pepper was measured 3.5 wk after planting. Maximum lengths were achieved, both in fumigated or nonfumigated soil, in all three crops, following solarization plus 2 wk tarp coverage after planting treatment. In fumigated soil, cotton root length, not inoculated and inoculated with VAM, increased significantly by 40% and 57% after soil solarization plus 2 wk tarp coverage, respectively.

In nonfumigated soil, root length of cotton not inoculated and inoculated with VAM, after soil solarization plus 2 wk tarp coverage, soil solarization and metalaxyl treatments increased significantly by 126%, 204%, 63%, 77%, 65% and 120% respectively (Table 1). Onion root length, not inoculated and inoculated with VAM, after soil solarization plus 2 wk tarp coverage, in fumigated soil, increased significantly by 27% and 31%, respectively. In nonfumigated soil, onion root length, not inoculated and inoculated with VAM, after soil solarization plus 2 wk tarp coverage, soil solarization and metalaxyl treatments incrased significantly by 175%, 155%, 125%, 45%, 93% and 70%, respectively (Table 1).

Pepper root length, not inoculated and inoculated with VAM, after soil solarization plus 2 wk tarp coverage, in fumigated soil, increased significantly by 50% and 91% respectively. In nonfumigated soil, pepper root length, not inoculated and inoculated with VAM, after soil solarization plus 2 wk tarp coverage, soil solarization and metalaxyl treatments increased significantly by 104%, 185%, 52%, 90%, 64% and 128%, respectively (Table 1).

Final yield. Cotton, onion and pepper were harvested 4 mo after planting and inoculation. VAM inoculation significantly increased the fresh wt of cotton plants by 58% in nonfumigated soil and by 345% in fumigated soil. In nonfumigated soil, VAM plus solarization increased the fresh wt of cotton 81% over the noninoculated control and by 87% over a solarization tratement without VAM inoculation. In nonfumigated soil, the VAM plus solarization plus tarp treatment increased the fresh wt of cotton by 118% over the untreated control and by 165% over the solarization plus tarp treatment without VAM inoculation. In nonfumigated soil the VAM plus metalaxyl treatment increased the fresh wt of cotton by 103% over the untreated control and by 58% over the metalaxyl treatment without VAM inoculation. In fumigated soil the VAM plus metalaxyl treatment increased the fresh wt of cotton by 520% over the untreated control and by 1100% over the metalaxyl treatment without VAM inoculation.

Cotton fresh wt, in fumigated soil, with VAM inoculum treated with solarization and solarization plus 2 wk tarp coverage were higher by 859% and 837% than the same treatments without VAM inoculum, respectively. Fresh wt of cotton plants inoculated with VAM in nonfumigated soil were more than double that of inoculated plants in fumigated soil. In the control without VAM fresh wt of plants in nonfumigated soil was about ten times greater than the control in fumigated soil. Best yields in fumigated and nonfumigated soil were achieved with VAM and solarization, solarization plus 2-wk tarp coverage and metalaxyl treatments (Fig. 4). Cotton boll number was clearly related to fresh wt of plants. Boll number of VAM inoculated cotton plants in nonfumigated soil was 1.8 - 2.8-fold higher than of those in fumigated soil. Highest boll yields per plant, 78, 80 and 66 were reached VAM inoculation after metalaxyl, solarization plus 2-wk tarp coverage and solarization treatments.

Fresh wt of VAM-inoculated onion plants (treatment no. 2) were significantly higher than of the same treatment in nonfumigated soil. However, in nonfumigated soil, fresh wt of onion plants after soil solarization, either with or without 2-wk tarp coverage was greater than all of the other results. There was no significant difference among treatments of VAM-inoculated onion fresh wt in fumigated soil. All of the control (without VAM) onion fresh wt in nonfumigated soil was 2.3-5.4-fold greater than the control onion plants in fumigated soil (Fig. 5). VAM inoculation significantly increased the fresh wt of onion plants by 57% in nonfumigated soil and by 417% in fumigated soil. In nonfumigated soil VAM plus solarization increased the fresh wt of cotton 177% over the noninoculated control and by 163% over a solarization treatment without VAM inoculation. In nonfumigated soil, the VAM plus solarization plus tarp treatment increased the fresh wt of cotton by 200% over the untreated control and by 68% over the solarization plus tarp treatment without VAM inoculation. VAM plus metalaxyl treatment, in nonfumigated soil, increased the fresh wt of onion by 140% over the untreated control and by 126% over the metalaxyl treatment without VAM inoculation. In fumigated soil the VAM plus metalaxyl treatment increased the fresh wt of onion by 390% over the untreated control and by 550% over the metalaxyl treatment without VAM inoculation. Onion fresh wt, in fumigated soil, with VAM inoculum treated with solarization and solarization plus 2 wk tarp coverage were higher by 716% and 626% than the same treatments without VAM inoculum, respectively (Fig. 5).

Pepper fresh wt inoculated with VAM, signifi cantly increased by 84% in nonfumigated soil and by 269% in fumigated soil. In nonfumigated soil VAM plus solarization increased the fresh wt of pepper 100% over the noninoculated control and by 76% over a solarization treatment without VAM inoculation. In

nonfumigated soil, the VAM plus solarization plus tarp treatment increased the fresh wt of pepper by 134% over the untreated control and by 81% over the solarization plus tarp treatment without VAM inoculation. VAM plus metalaxyl in nonfumigated soil, increased the fresh wt of pepper 96% over the untreated control and 52% over the metalaxyl treatment without VAM inoculation. In fumigated soil the VAM plus metalaxyl treatment increased the fresh wt of pepper by 380% over the untreated control and by 236% over the metalaxyl treatment without VAM inoculation. Pepper fresh wt, in fumigated soil, with VAM inoculum treated with solarization and solarization plus 2 wk tarp coverage were higher by 258% and 400% than the same treatments without VAM inoculum, respectively (Fig. 6). Pepper fruit wt was clearly related to fresh wt of plants. VAM inoculated plant fresh wt and fruit wt in nonfumigated soil were 1.5-2.0 and 1.3-2.0-fold greater than in fumigated soil, respectively. In both fumigated and nonfumigated soil best results of plant wt and fruit wt were achieved following soil solarization and soil solarization plus 2-wk tarp covered treatments. Pepper fresh wt and fruit wt of the controls (without VAM) in nonfumigated soil was 3-5 and 2-2.8-fold greater than of pepper fresh wt and fruit wt in fumigated soil respectively (Fig. 6).

Correlation coefficients. Correlation coefficients were computed for the relationship between VAM percent colonization of plants at the fifth wk after planting (and inoculating) and fresh wt or yield. All weights and yield of the plants were found positively significant correlated with VAM percent colonization of the plants as follows: In fumigated soil cotton plant fresh wt rC2=0.898, P0.01 cotton plant boll number rC2=0.822, P0.05 onion plant fresh wt rC2=0.928, P0.01 pepper plant fresh wt rC2=0.950, P0.001 pepper plant fruit wt rC2=0.834, P0.05. In nonfumigated soil cotton plant fresh wt rC2=0.918, P0.01 cotton plant boll number rC2=0.846, P0.01 onion plant fresh wt rC2=0.900, P0.01 pepper plant fresh wt rC2=0.872, P0.01 pepper plant fruit wt rC2=0.941, P0.001.

DISCUSSION

To date, experiments which have been most successful in increasing percent VAM colonization, wt and yield of crop plants have been performed in fumigated/sterilized soil (18,32,43). In this study we have attempted to find new approaches such as soil solarization which can replace, or at least, reduce fumigants in the soil and allows high VAM colonization of plants.

Many experiments have been carried out and reported to evaluate the potential of soil solarization in pathogen (and other pest) population reduction, disease control, weed control and yield increase (1,20,22,23,27). Although, non of them discussed what is the effect of soil solarization on VAM colonization of plants and what is the effect on growth response and yield of plants following a combination of VAM application and soil solarization together. Our results show that soil solarization does not damage native VAM whereas fumigation with methyl bromide controls the most of the population of native VAM (Fig. 1-3). Other studies (9,13,28,30), as well as results of this study, show that indigenous VAM is very important for growth response and yield of plant (Fig. 4-6). Several researchers reported that failure of VAM colonization of plants in natural soil are caused by microganisms which compete with mycorrhizal fungi on root sites and interfere with mycorrhizal development (5,33,44). Similarly, this research suest that control or elimination of microorganisms which can compete or interfere with mycorrhizal development improve VAM colonization, growth response and yield of crops. Our results show VAM colonization of inoculated plants 3.5 and 5 wk after planting is higher in fumigated than in nonfumigated soil (treatment 2 Figs. 1-3). However, this situation has been reversed and VAM colonization of plants in nonfumigated soil but solarized is higher than in fumigated soil probably because of control of this microorganisms (treatments 4,6 Figs. 1-3).

In a previous study Afek et al. (3) reported that in the greenhouse VAM percent colonization of plants in nonfumigated soil treated with metalaxyl increased apparently following controls of Pythium ultimum. Likewise this study, which had been conducted in the field, shows similar results (Figs. 1-3).

Other researchers reported that metalaxyl improves VAM colonization (17,31,36) whereas Pythium spp. caused poor VAM colonization of plants (3,21). Cook et al. (10) reported that soil solarization controls 80-90% of the Pythium spp. population in the soil and increases growth response of wheat. It might be that reduction of the Pythium population in the solarized soil also increases VAM colonization of the plants. Furthermore, results of this research, show that soil solarization and metalaxyl improve VAM colonization of cotton, onion and pepper more in nonfumigated soil than it does in fumigated soil (Figs. 1-3). That means that not only control of microorganisms involved in increasing VAM colonization of plants.

This investigation suests that soil fumigation kills most living organisms including some beneficial microorganisms such as bacteria and actinomycetes which may improve VAM colonization (6,7,8,11,35), as well as microorganisms which may inhibit VAM colonization (3,16,44). Since metalaxyl acts against P. ultimum and not against beneficial microorganisms, VAM colonization of the root crops is higher in nonfumigated soil. Katan (23) suests that soil solarization, in addition to control of patho?-gens in the soil, may increase the number of beneficial microorganisms in the soil. Solarized soils undergo significant changes in their temperature and moisture regions, the inorganic and organic composition of their solid, liquid, and gaseous phases and their physical structure, all of which in turn effect the biotic and abiotic components (23,38,47). Such changes may explain why in fumigated soil where

most microorganisms were controlled anyway, VAM percent colonization of cotton and onion increase following soil solarization (Figs. 1,2). Another factor which can enhance VAM colonization is high temperature. Heating the soil, especially during the first 2 wk after planting, which is critical for establishing adequte VAM colonization in annual crops (2,45) increased VAM colonization in this research (Figs. 1-3). It might be that heating the soil after planting, even by 3-4 C at the upper 20 cm, as in our study, increase VAM colonization by enhanced root growth response (Table 1), root exudation and VAM spore germination which can improve VAM colonization of plants (14,15). However, our results show that higher VAM percent colonization of the crops 5 wk after planting lead to greater wt and yield of the crops 4 mo after planting (Figs. 4-6) and these factors were significantly correlated. Finally, this study leads to the conclusion that a combination of vesicular-arbuscular mycorrhizae with soil solarization can be one of the best approach to replace, or at least, to reduce the use of chemicals in agriculture. We believe that the future for mycorrhizae as a biofertilizer, lies not in fumigated but in natural soil, especially now when agriculture growers approaching a crisis because of pesticide and toxic chemicals found in food, water and the environment.

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Table 1. Root lengths of cotton, onion and pepper 3.5 wk after planting in the field, inoculated or not inoculated with Glomus intraradices

	Root length (cm)[z] Treatments			
Cotton	Onion	Pepper	Fumigated soil	
Control (without mycorrhizal inoculation)	94	33	55	
VAM (G. intraradices)	80	29	49	
Control + soil solarization	98	27	47	
VAM + soil solarization	107	30	40	
Control + soil solarization + 2 wk tarp coverage after planting	131	42	83	
VAM + soil solarization + 2 wk tarp coverage after planting	126	38	94	
Control + metalaxyl	93	34	50	
VAM + metalaxyl	86	29	43	Nonfumigated soil
Control	60	16	25	
VAM	49	20	21	
Control + solarization	98	36	38	
VAM + soil solarization	87	29	40	
Control + soil solarization + 2 wk tarp coverage after planting	136	44	81	
VAM + soil solarization + 2 wk tarp coverage after planting	149	51	60	
Control + metalaxyl	99	31	41	
VAM + metalaxyl	108	34	48	
LSD (P=0.05)	21	8	12	

[z]Each number is an average of 4 replicates.

FIGURES LENGED

Fig. 1. Mycorrhizal colonization (%) of cotton plants 2 (A), 3.5 (B) and 5 (C) wk after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CO) (not inoculated) 2. VAM inoculation (VA) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solarization (CO, SO) 4. VAM inoculation plus soil solarization (VA, SO) 5. control plus soil solarization plus 2-wk tarp coverage (CO, TR) 6. VAM inoculation plus soil solarization plus 2-wk tarp coverage (VA, TR) 7. control plus metalaxyl (CO, ME): and 8. VAM inoculation plus metalaxyl (VA, ME).

Fig. 2. Mycorrhizal colonization (%) of onion plants 2 (A), 3.5 (B) and 5 (C) wk after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CO) (not inoculated) 2. VAM inoculation (VA) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solarization (CO, SO) 4. VAM inoculation plus soil solarization (VA, SO) 5. control plus soil solarization plus 2-wk tarp coverage (CO, TR) 6. VAM inoculation plus soil solarization plus 2-wk tarp coverage (VA, TR) 7. control plus metalaxyl (CO, ME) and 8. VAM inoculation plus meta laxyl (VA, ME).

Fig. 3. Mycorrhizal colonization (%) of pepper plants 2 (A), 3.5 (B) and 5 (C) wk after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CO) (not inoculated) 2. VAM ino culation (VA) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solarization (CO, SO), 4 VAM inoculation plus soil solarization (VA, SO) 5. control plus soil solarization plus 2-wk tarp coverage (CO, TR) 6. VAM inoculation plus soil solarization plus 2-wk tarp coverage (VA, TR) 7. control plus metalaxyl (CO, ME) and 8. VAM ino culation plus metalaxyl (VA, ME).

Fig. 4. Cotton fresh wt per plant (A) and boll number per plant (B) harvested 4 mo after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CON) (not inoculated) 2. VAM inoculation (VAM) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solariza tion (CON, SOL) 4. VAM inoculation plus soil solarization (VAM, SOL) 5. control plus soil solarization plus 2-wk tarp coverage (CON, TAR) 6. VAM inocu lation plus soil solarization plus 2-wk tarp coverage (VAM, TAR) 7. control plus metalaxyl (CON, MET) and 8. VAM inoculation plus metalaxyl (VAM, MET).

Fig. 5. Onion fresh wt per plant harvested 4 mo after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CON) (not inoculated) 2. VAM inoculation (VAM) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solarization (CON, SOL) 4. VAM ino culation plus soil solarization (VAM, SOL) 5. control plus soil solarization plus 2-wk tarp coverage (CON, TAR) 6. VAM inoculation plus soil solarization plus 2-wk tarp coverage (VAM, TAR) 7. control plus metalaxyl (CON, MET) and 8. VAM inoculation plus metalaxyl (VAM, MET).

Fig. 6. Pepper fresh wt per plant (A) and fruit wt per plant (B) harvested 4 mo after planting and inoculating in fumigated and nonfumigated soil treated as follows: 1. control (CON) (not inoculated) 2. VAM inoculation (VAM) (inoculated with the mycorrhizal fungus Glomus intraradices) 3. control plus soil solarization (CON, SOL) 4. VAM inoculation plus soil solarization (VAM, SOL) 5. control plus soil solarization plus 2-wk tarp coverage (CON, TAR) 6. VAM inoculation plus soil solarization plus 2-wk tarp coverage (VAM, TAR) 7. control plus metalaxyl (CON, MET) and 8. VAM inocula tion plus metalaxyl (VAM, MET). tion plus metalaxyl (VAM, MET).

Fig 1.

% COLONIZATION OF COTTON (2, 3.5, 6 WK)

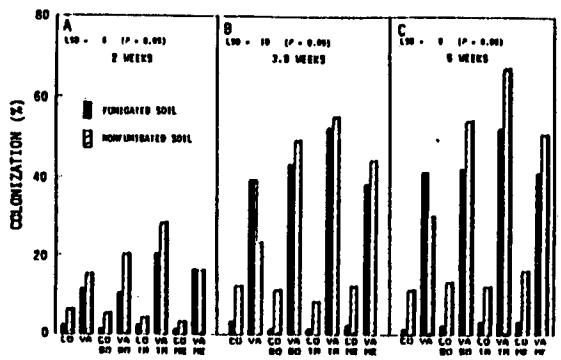


Fig.2

% COLONIZATION OF ONION (2, 3.5, 6 WK)

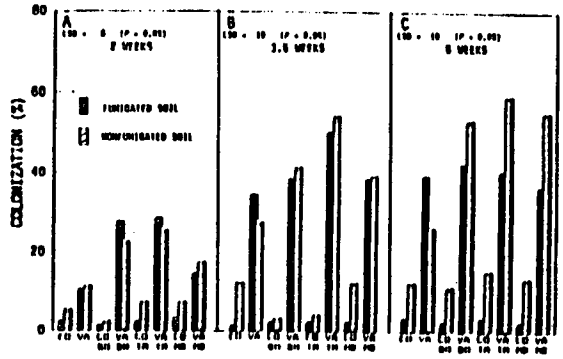


Fig 3

% COLONIZATION OF PEPPER (2, 3.5, 6 WK)

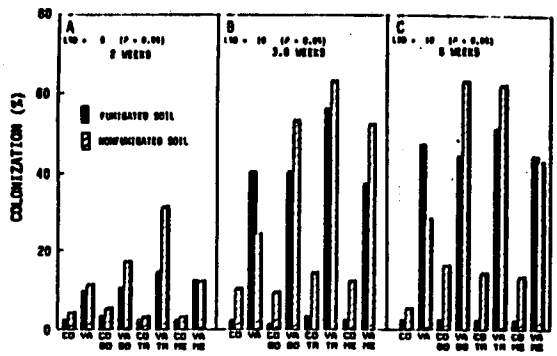


Fig 4

COTTON FINAL HARVEST FRESH WT.(6 MONTHS)

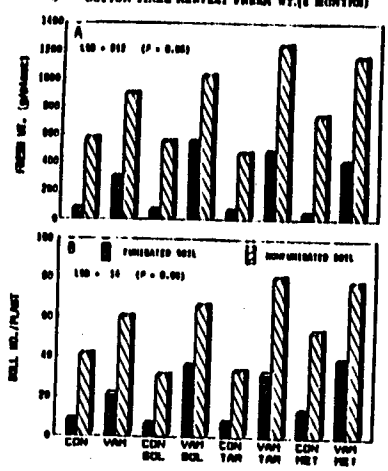


Fig. 5

ONION FINAL HARVEST FRESH WT (6 MONTHS)

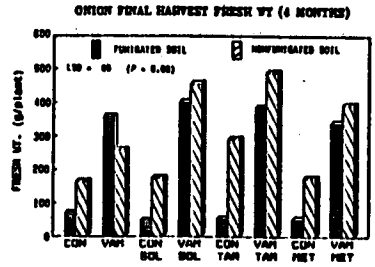
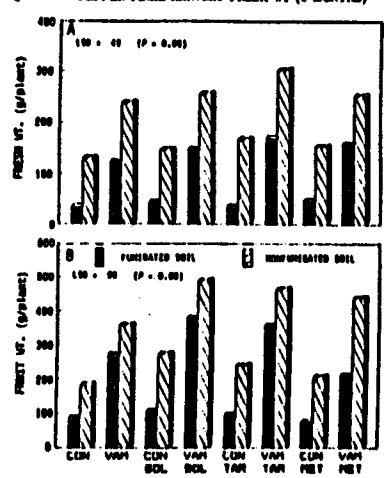


Fig. 6

PEPPER FINAL HARVEST FRESH WT (6 MONTHS)



The Effect of Mycorrhizal Species, Root Age and Position of Mycorrhizal Inoculum on Colonization of Cotton, Onion and Pepper In Greenhouse and Field Experiments

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Additional index words. Mycorrhizae, cotton, onion, pepper, VAM.

Abstract. The length of time required for vesiculararbuscular mycorrhiza (VAM) colonization, the effect of root age, and the position of VAM inoculum with respect to the root system were tested on cotton, onion and pepper. Colonization of onion by Glomus deserticola began 3 days after inoculation and reached 50% of the total root length after 21 days. Colonization by G. mosseae and G. intraradices began after 12 days and attained 15% and 37% after 21 days, respectively. In cotton, colonization with G. deserticola and G. intraradices began 12 days following inoculation and increased to 20% and 18% after 21 days, respectively. Colonization of cotton by G. mosseae was poor. In pepper, colonization with G. deserticola, G. mosseae and G. intraradices began 3, 6 and 6 days after inoculation and after 21 days reached 60%, 13% and 10%, respectively. In a second experiment, rapid colonization by G. deserticola took place in 3dayold onion seedlings and increased to 51% 3 days after inoculation. Tendayold and 17dayold seedlings were far less responsive to VAM colonization but became highly infected at 30 days when new roots were produced. In a third experiment, inoculum placement 3 cm below seeds at planting in the field was the most effective for promoting colonization of cotton and onion by VAM. In fumigated field soil, mycorrhizae increased cotton growth an average of 28% when inoculum was applied below seeds compared to one or twosided band applications. Even in nonfumigated field soil, inoculum placed 3 cm below the seed and inoculum placed in a band at one side 2 wk after planting significantly increased cotton growth. In onion, mycorrhizal inoculation improved growth in fumigated soil when it was placed below the seed, but did not stimulate growth in nonfumigated soil.

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Mycorrhizal fungi increase nutrient uptake and growth of many plants (Dodd et al., 1983 Harley and Smith, 1983 Haas et al., 1987 Menge, 1983 Mosse, 1973). It is now feasible to introduce mycorrhizal fungi in the field as a modern agriculture practice, especially for crops planted in poor soil with few or no indigenous mycorrhizal fungi (Jeffries, 1987). While success in achieving effective mycorrhizal associations with crop plants growing in sterilized soil has been achieved, the ultimate success for agricultural use of VAM fungi will occur when they can be used dependably to improve performance of crops grown in nonfumigated soil.

The effectiveness of VAM inoculum depends on the colonization potential of the inoculum (Alexander, 1965 Daniel et al., 1981), proper placement (Afek et al., 1989 Menge and Timmer, 1982), proper timing (Smith et al., 1979), age of roots (Hepper, 1985) and the susceptibility of crops to VAM colonization (Hepper, 1985 Sanders et al., 1977). Total root colonization by VAM fungi sometimes negatively correlated with the length of time between seed germination and the initial mycorrhizal infection (Afek et al., 1988 Smith et al., 1979). The purpose of this study was to determine the length of time required for colonization of onion, cotton and pepper roots by VAM fungi under greenhouse conditions and to examine the efficiency of VAM colonization of cotton and onion in the field with respect to placement of the inoculum in the soil. The effect of root age on VAM colonization of onion root also was studied. All of these experiments were conducted in the continuing effort to improve growth responses of onion, pepper and onion to mycorrhizal inoculation in nonfumigated soil.

Materials and Methods

Plant material and VAM inoculum. Seeds of 'SJ2' cotton, (Gossypium hirsutum L.) 'Burpee Yellow Glove' onion (Allium cepa L.) and 'California Wonder' pepper (Capsicum annuum L.) were surface disinfested with 20% sodium hypochlorite for 1 min and rinsed twice in distilled sterilized water for 1 min each time before planting. These crops were chosen since they are annuals and respond positively to VAM colonization in previous studies (Afek et al., 1990). Sandy loam soil which was used for both greenhouse and field experiments was characterized as follows: P 9.5 ppm by Olsen analysis (Chapman and Pratt, 1961), which is considered a low level (Graham and Leonard, 1982 Waterer and Coltman, 1989b), N 632 ppm (Schuman and Stanley, 1973), Zn 12.8 ppm, Mn 8.6 ppm, Fe 6.5 ppm, Cu 1.7 ppm (Page, 1982), Ca 23.5 me/l, Mg 3.8 me/l, Na 13.9 me/l, K 142 ppm, saturation percentage (SP) 27%, pH 7.7, electroconductivity (EC) 4.0 dS/m, sodium adsorption ratio (SAR) 3.8, exchangeable sodium percentage (ESP) 4.2%, organic matter (OM) 0.80%, clay 8.3%, silt 28.6% and sand 63.1% (Quick and Rible, 1960). This soil, which was either fumigated or not fumigated with methyl bromide (98% MB + 2% chloropicrin, equivalent to 500 kg/ha), was used for field trials with vegetable crops the previous 3 years of this study and was taken from an agricultural site at the Citrus Experiment Station,

University of California, Riverside. No fertilizers were used in the experiments.

Plants were inoculated with the mycorrhizal fungi Glomus intraradices Schenck and Smith (isolate 185 collected from Citrus sp., Ventura, California, 1975), G. deserticola Trappe, Blosse and Menge (isolate 01 collected from Citrus sp., Thermal, California, 1975), or G. mosseae (Nicol. and Gerd.) Gerdemann and Trappe (isolate S50 collected from Artemisia sp., Fresno, California, 1981). The inoculum for each mycorrhizal species consisted of mixed roots and soil from sudan grass (Sorghum vulgare Pers) nurse cultures which had been infected with the mycorrhizal fungus for 9 months. Inoculum potential of the mixture was calculated by the most probable number (MPN) procedures (Alexander, 1965 Daniels et al., 1981) and inoculum quantity adjusted so that inoculum potential was identical using Sudan grass as the host.

Percent colonization by VAM was calculated after staining in lactophenol trypan blue, as the number of colonized sites examined randomly, per total sites X 100 (Phillips and Hayman, 1970).

Experiment 1. Determination of the length of time for VAM colonization of cotton, onion and pepper in the greenhouse. Cotton, onion and pepper were inoculated separately with the three VAM fungi, G. intraradices, G. deserticola and G. mosseae, in soil autoclaved twice for 1 hr with a 24 hr interval between autoclavings. Mycorrhizal inoculation was achieved by placing approximately 10 g of inoculum 5cm deep in 500cm53 autoclaved clay pots containing the autoclaved soil.

Sevendayold seedlings of cotton, onion and pepper were planted in the pots with roots in contact with the inoculum. The pots were placed in a completely randomized design on a greenhouse bench at temperatures of 24 C C= 2 C. Fluorescent light was provided to maintain a day length of 14 hr. Photosynthetically active radiation (PAR) within the 400700 nm range was periodically measured in terms of Cm mol m524 S514, average 385 C= 58 (Graham and Leonard, 1982). Five plants were harvested every 3 days for 21 days, their roots stained and examined for percent VAM colonization. The experiment was repeated twice with similar results.

Experiment 2. The effect of onion root age on VAM colonization of onion. Onion seeds were surface sterilized in 20% sodium hypochlorite solution for 1 min and rinsed twice for 1 min each time in distilled sterilized water. The seeds were then placed on a damp filter paper in 9cmdiameter petri dishes in an incubator at 24 C to germinate. After 3 days, when the first root was about 0.5 cm long, 150 germinated seeds were planted into autoclaved soil, in an autoclaved nursery flat, as described previously. Simultaneously, 42 germinated seeds were planted into 21 500cmC3 clay pots (2 plants per pot) containing autoclaved soil, after having placed 10 g inoculum of G. deserticola in contact with the roots system. The 150 plants in the nursery flat were grown for 7, 14 or 21 days. At the end of each time period, 42 plants were transplanted into 21 pots and plants were inoculated with G. deserticola as described previously. All plants were grown in a greenhouse at 24 C C= 2 C. Fluorescent light was provided in addition to natural light (14hrlight period) as described in experiment 1. Plants in three pots were harvested and root colonization estimated 3, 6, 9, 12 15, 18, and 24 d after inoculation for each different preinoculation period. The experiment was repeated twice with simlar results. The experimental design was completely randomized after all pots from all time periods were mixed together.

Experiment 3. Determination of efficiency of VAM colonization of cotton and onion with respect to placement of the inoculum in the field. In May 1987 cotton and onion were planted in double 80mrows in both fumigated with methyl bromide (98% MB + 2% chloropicrin, equivalent to 500 kg/ha) and nonfumigated soil. The field experimental design was a randomized block with 4 blocks. Each treatment contained a 1.5mrow of onions and a 3mrow of cotton. The width of the rows were 30 cm. There were 1316 plants in each plot. One plant from each treatmentblock were randomly harvested 3, 4 and 5 wk after planting and root systems evaluated for VAM colonization. The final harvest for total dry weight was done 16 wk after planting. Treatments included: 1) control without mycorrhizal inoculation 2) inoculation with G. deserticola 3 cm below seeds at planting 3) inoculation with G. deserticola on one side of plant (5 cm deep, 3 cm from plant) 2 wk after planting 4) inoculation with G. deserticola on both sides of plant (5 cm deep, 3 cm from plant) 2 wk after planting 5) inoculation with G. intraradices 3 cm below seeds at planting 6) inoculation with G. intraradices on one side of plant (5 cm deep, 3 cm from plant) 2 wk after planting and, 7) inoculation with G. intraradices on both sides of plant (5 cm deep, 3 cm from plant) 2 wk after planting. Banding was done by dispensing the inoculum by hand using 170 g/m inoculum in each band. No fertilizers were used during the experiment time period.

Experiment 3 was performed one time during the summer of 1987, but similar field trials at 2 sites in 1982 and 2 sites in 1983 produced similar results.

Results

Determination of the length of time for VAM colonization of cotton, onion and pepper under optimum

conditions.

Onion: Colonization with G. deserticola was detected 3 days after inoculation, and increased to 50% after 21 days (Fig. 1). Colonization with G. mosseae began 12 days after inoculation, and increased to 15% after 21 days. Colonization with G. intraradices also occurred by 12 days after inoculation, and increased to 37% after 21 days.

Cotton: Colonization with G. deserticola and G. intraradices was detected 12 days after inoculation, attaining 20% and 18% after 21 days, respectively (Fig. 2). Colonization by G. mosseae was poor.

Pepper: Colonization with G. deserticola was evident at 3 days after inoculation, and G. mosseae and G. intraradices at 6 days (Fig. 3). After 21 days colonization with these fungi,reached 60%, 13% and 10%, respectively.

The effect of onion root age on VAM colonization. Rapid colonization with G. deserticola took place in 3dayold seedlings and reached 51% 3 days after inoculation (seedling age 6 days) (Fig. 4). Tendayold and 17dayold seedlings were more resistant to VAM colonization. Twentyfourdayold seedlings again had rapid colonization. 3

Determination of the efficiency for VAM colonization of cotton and onion with respect to placement of the inoculum in the field. Mycorrhizal colonization of cotton in fumigated soil was best for both G. deserticola and G. intraradices when inoculum was placed below seeds at planting, reaching 29% and 40%, respectively, at 5 weeks after planting, (Table 1). VAM colonization of onion in fumigated soil also was highest when inoculum was placed below the seeds at planting, reaching 32% and 59% for G. deserticola and G. intraradices, respectively, at 5 wk (Table 1). Even in nonfumigated soil at 5 wk after inoculation, with one exception, VAM colonization of onion and cotton reached a maximum when inoculum was placed below seeds at planting (Table 1).

Generally, in fumigated soil, dry weights of VAMinoculated cotton and onion plants were significantly higher than those of noninoculated plants. Further, dry weights of cotton and onion plants from fumigated soil treatments were greatest when VAM inoculum had been placed below seeds at planting (Table 2). In nonfumigated soil, dry weights of cotton plants were increased by inoculation with G. deserticola, and only only when inoculum was placed below seeds at planting or banded on one side 2 wk after planting. There were no significant differences among dry weights of onion in nonfumigated soil (Table 2).

Discussion

Today we have the technology to produce VAM inoculum and apply it as a biofertilizer. However, largescale quanities of VAM for field application cannot be stored for long periods of time free of contamination, as the inoculum is maintained in nonsterile conditions (Menge, 1983 Menge and Timmer, 1982). The efficiency of such use has to be improved so that growth responses are assured with field applications. The effectiveness of VAM inoculum depends on the colonization potential of the inoculum, proper placement, proper timing and the susceptibility of the crops to colonization.

Onion and cotton are known to respond to VAM inoculation (Afek et al., 1988). However, growth responses of these crops in nonfumigated soils where the VAM inoculum is competing with other microorganisms has not been shown to occur effectively (Table 2). One of the keys to eliciting growth responses with VAM fungi in annual crops is thought to be achieving rapid colonization (Haas, et al., 1985 Manjunath et al., 1983). In these experiments, colonization of cotton, onion and pepper, occurred within 615 days under optimal conditions (Fig. 13).

Under field conditions comparable levels of colonization of cotton and onions rarely occurred even after 5 weeks in nonfumigated soils (Table 1). Rapid colonization must be realized in the field if growth responses are to be achieved. Attempts must be made to improve the rates of VAM colonization in the field to approach those achieved under greenhouse conditions. Colonization rates by VAM fungi are more rapid in fumigated soil than in nonfumigated soil (Table 1), indicating that competing microorganisms or native mycorrhizae in nonfumigated soil may effect the infection process (Afek et al., 1990). This may explain why, sometimes, unexpected results occur when inoculum banded on 2 sides is less effective than a single band (Table 2). One of the most important factors that influences VAM colonization of plants is the P level in the soil.

Benefits of VAM fungi are greatest when crops are grown on P deficient or P fixing soils (Graham and Leonard, 1982 Waterer and Coltman, 1989a,b). However, in this study, a Plowlevelsoil (9.5 ppm) (field and greenhouse experiments) was chosen and probably this factor enhances VAM colonization and growth response of the crops following inoculation with VAM.

Some VAM fungal species are more efficient on certain crops than on others (Daniels et al., 1981

Sanders et al., 1977). Differences in inoculum quality of different VAM species in comparative studies may be responsible for some of the reported differences in effectiveness between species or isolates. However, even in this study, where inoculum quantity was adjusted to achieve similar inoculum potentials, G. intraradices and G. deserticola were better colonized, while isolate of G. mosseae was poor for all crops (Fig. 13). Common sense indicates that isolates which naturally produce rapid infection in crops should be selected for field work.

Inoculum placement has been shown repeatedly to be important for field crop growth responses (Afek et al., 1989 Jackson et al., 1972 Menge et al., 1977). A review of the literature (Menge and Timmer, 1982) indicates that layering inoculum beneath seeds so that roots will penetrate the inoculum appears to be more desirable than banding inoculum along the sides or placing inoculum with the seed. Similarly, results of this study show that maximum colonization of plants was achieved when VAM inoculum was placed below seeds at planting (Table 1).

The age and condition of crop roots have a major effect on rapid colonization by VAM inoculum and they are factors over which we have some control. Crop species also differe in the period of time over which their root systems will establish mycorrhizal associations (Hepper, 1985), and in the maximum proportion of the root system that can become mycorrhizal (Buwalda et al., 1982a Buwalda et al., 1982b Jakobsen and Nielsen, 1983 Warner and Mosse, 1982). We found that extensive VAM colonization took place in 3dayold and 30dayold onion seedlings, whereas 10dayold and 17dayold onion seedlings were far less responsive to VAM colonization (Fig. 4). The reason for this appears to be the unique rooting habit of onion whereby new flushes of roots are periodically produced at the base of the bulb and will pass through the VAM inoculum. These new roots are essentially young roots and VAM colonization fluctuates with each flush of young roots as they have a high degree of susceptibility.

In normal root systems, the young potentially colonizable roots are produced as branches from other roots (Hepper, 1985 Mosse, 1975). It then becomes critical that VAM inoculum to be at its optimum potential when the roots pass through because, at early age, they are strongly infected. Other investigators reported that VAM fungus colonization of root plants grown in the field occur, outside the zone of inoculation, at a secondary infection along the roots (Waterer and Coltman, 1989a,b). However, mycorrhizal inoculum should be adjusted for maximum germinationmaximum inoculum potential during the one short "window" of root susceptibility as the roots pass through the inoculum.

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Table 1. The effect of method of mycorrhizal inoculation in the field on mycorrhizal colonization of cotton and onion in fumigated and nonfumigated soil after 5 weeks

Treatment	Cotton		Onion	
	(% colonization)			
	Fumigated	Nonfumigated	Fumigated	Nonfumigated
noninoculated	1[z]	8	1	10
Inoculated with Glomus deserticola below seeds at planting	29	14	32	32
Inoculated with G. deserticola band 1 side after 2 weeks	5	8	13	19
Inoculated with G. deserticola band 2 sides after 2 weeks	4	26	7	19
Inoculated with G. intraradices below seeds at planting	40	20	59	35
Inoculated with G. intraradices band 1 side after 2 weeks	4	12	13	15
Inoculated with G. intraradices band 2 sides after 2 weeks	8	9	19	14
LSD (P=0.05)	9.6	15.1	15.9	14.6

[z]Each number is an average of 4 replicates.

Table 2. Dry weights of cotton and onion plants after 16 weeks of growth as affected by different methods of inoculation of mycorrhizal fungi and by soil fumigation in the field

Treatment	Cotton		Onion	
	(g plant^{-1})			
	Fumigated	Nonfumigated	Fumigated	Nonfumigated
noninoculated	14[z]	69	33	26
Inoculated with Glomus deserticola below seeds at planting	118	157	65	37
Inoculated with G. deserticola band 1 side after 2 weeks	85	148	53	46
Inoculated with G. deserticola band 2 sides after 2 weeks	68	80	51	28
Inoculated with G. intraradices below seeds at planting	106	94	65	37
Inoculated with G. intraradices band 1 side after 2 weeks	96	92	55	30
Inoculated with G. intraradices band 2 sides after 2 weeks	101	98	56	33
LSD (P=0.05)	52.3	47.6	25.1	26.6

[z]Each number is an average of 4 replicates.

Figure Legend

Fig. 1. Colonization of onion 121 days following inoculation with Glomus deserticola, G. mosseae, and G. intraradices in autoclaved soil. The vertical bars are standard errors.

Fig. 2. Colonization of cotton 121 days following inoculation with Glomus deserticola, G. mosseae, and G. intraradices in autoclaved soil. The vertical bars are standard errors.

Fig. 3. Colonization of pepper 121 days following inoculation with Glomus deserticola, G. mosseae, and G. intraradices in autoclaved soil. The vertical bars are standard errors.

Fig. 4. Colonization of 3, 10, 17, and 24dayold onion seedlings 121 days following inoculation with Glomus deserticola in autoclaved soil. The vertical bars are standard errors.

Fig.1

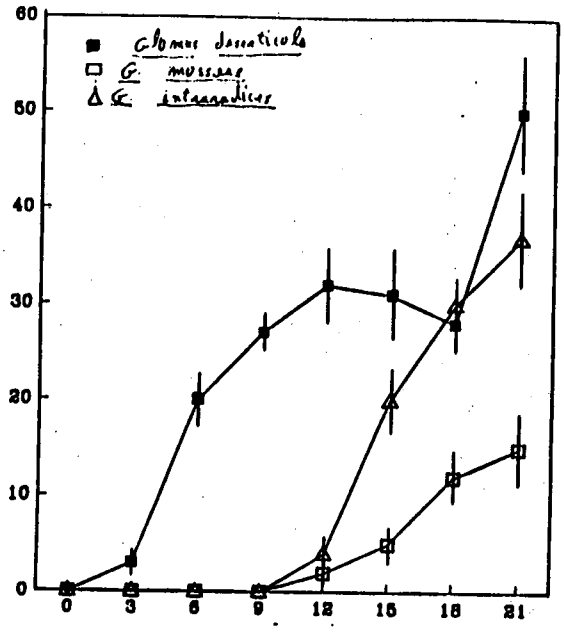


Fig.2

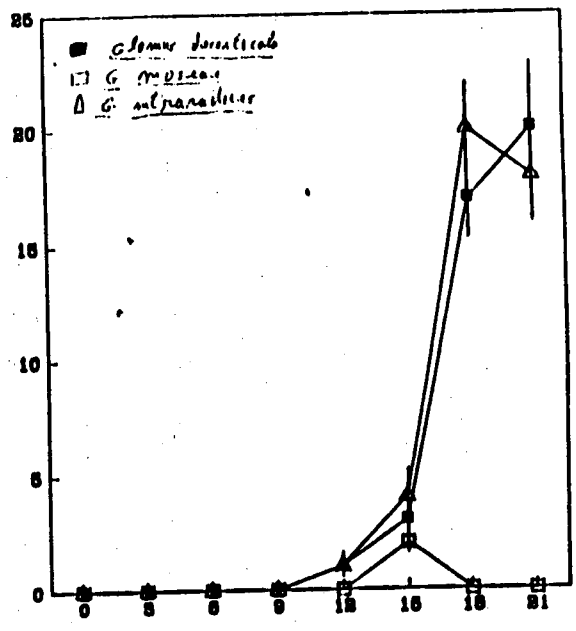


Fig.3

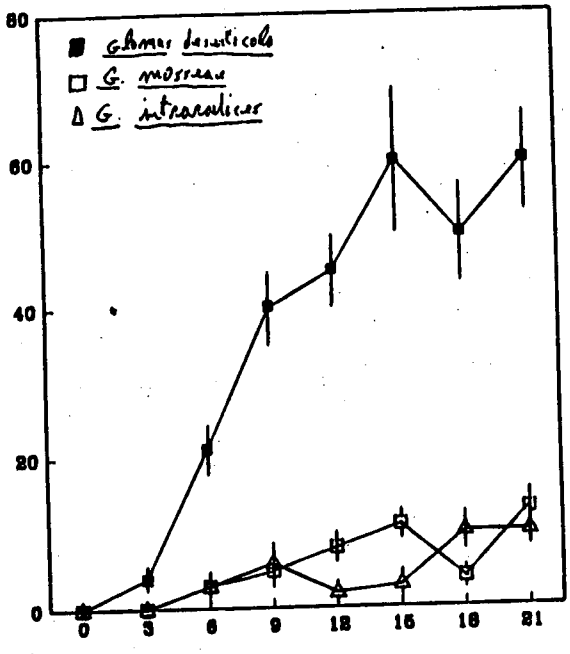
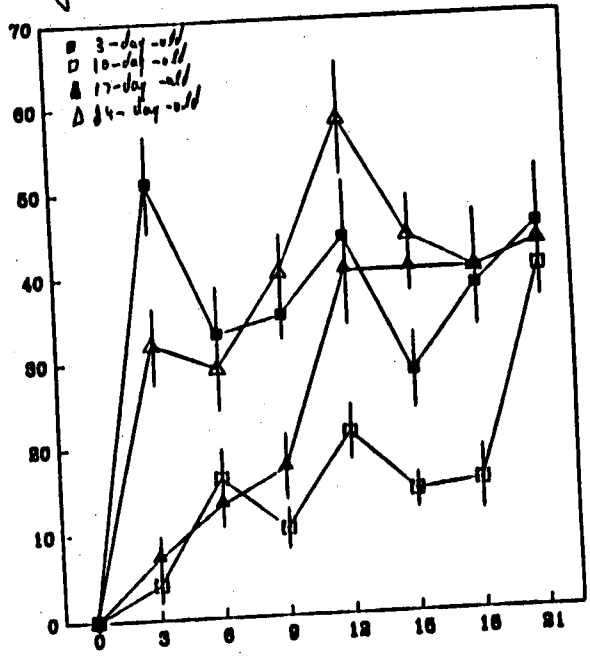


Fig.4



Simultaneous Use of Mycorrhizal Inoculation and Phosphorus Fertigation with Transplanted Pepper[1]

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ABSTRACT

Bell pepper (Capsicum annuum L.) is stunted when grown on a high P-sorbing fossiliferous Typic Torriorthents soil fumigated with methyl bromide to control soilborne pathogens. Our previous experiments with direct seeded pepper showed that both high levels of P fertigation and soil amendments with vesicular-arbuscular mycorrhizal fungus (VAMF, Glomus macrocarpum Tul. & Tul.) were required for maximum crop growth and yield. Two field experiments and one commercial-field trial were conducted to determine if these effects could be repeated with a transplanted cropping system. In one factorial-design experiment there were four levels of initial inoculum (0, 2, 10 and 20% of the growth medium) and three levels of P (0, 0.3 and 1 mM H_3PO_4) applied with N and K at each irrigation in the field. After 31 and 62 d in the field, root colonization by the VAMF increased with the original inoculum levels and decreased with P fertigation levels. At that stage, only P fertilization increased plant biomass. The cumulative total of red-ripe peppers increased from 33 to 42 Mg ha^{-1} with, respectively, 0 and 1 mM P, and with no VAMF. High levels of P fertilization did not obviate the mycorrhizal effect. With 20% VAMF, yield increased from 41 to 48 Mg ha^{-1} at 0 and 1 mM P, respectively. The second field experiment, with 0 and 10% VAMF inoculum and 0.3 and 1 mM P, corroborated the previous results, as did the trial in a commercial crop where plants with and without inoculum were transplanted and fertigated with 1 mM P.

INTRODUCTION

The directed use of vesicular-arbuscular mycorrhizal fungus (VAMF) inoculum in agriculture is limited, in spite of massive evidence of its benefits to plant nutrition, water relations and disease tolerance. Although most of the data are from pot trials, McGonigle (1988) analyzed the published results of 78 field trials reported prior to 1986. In many of them there was no positive yield response. Field plant growth responses are often obtained in soils with low available P, typical of low-input agriculture (non-fertilized) but not of modern cultivation, where yield maximization is desired and fertilization is practiced. Although not thoroughly proven, the currently accepted theory of mycorrhizal function is that the external hyphae of mycorrhizae increase the P-absorbing surface area of the root system (Stribley, 1989). If the available-P is sufficiently high, the non-mycorrhizal root still can absorb sufficient P to maintain maximum growth.

Field responses to inoculation with VAMF have been found even where P fertilization is practiced. They can be expected when at least three conditions are fulfilled: (i) The crop is one which "requires" VAM. The variations in plant species typically have been shown by comparing growth in sterilized vs. non-sterilized soil (Krikun et al., 1990; Plenchette et al., 1983). (ii) The crop is growing on soil with low populations of suitable VAMF, especially as would be expected when soil treatments with fungicides, fumigation or solarization are practiced (Menge, 1982). (iii) The levels of P in the soil solution are too low for near-maximum plant growth even though the available P is considered sufficient. This last condition is common in high P-sorbing soils typical of arid and tropical climates where soil pH usually is high and low, respectively. The importance of the soil P-sorption characteristic in relation to VAM effects has recently been emphasized by Plenchette and Fardeau (1988). In all soils, low levels of soil solution P also may be common during periods of rapid plant growth, when soil water potentials are low, or the desorption of P is too slow to provide, at the root surface, the soil solution ca. 5 uM P required for near-maximum plant growth (Asher and Loneragan, 1967; Fohse et al.,1988).

Haas et al. (1987) identified a commercially used soil/crop combination -- high P-sorbing arid-climate fumigated soil on which bell pepper (Capsicum annuum L.) was grown -- where VAMF inoculation was beneficial. Previous and subsequent work have shown the importance of VAMF in pepper crop production (Waterer and Coltman, 1989). In our field trial there was a synergistic effect of P-fertilization and VAMF supplementation. The crop was direct-seeded in rows and inoculum was incorporated in the row before seeding.

The inoculum used in those tests was a soil culture of the VAMF Glomus macrocarpum Tul. & Tul. (Gerdemann and Trappe, 1974). Only one level of inoculum was tested, the equivalent of 900 L ha^{-1}. For transplanted crops, a more efficient and potentially inoculum-conserving technique would be incorporation of VAMF in seedling growth media. The VAMF would be restricted to the seedling root zone and would be transplanted to the field along with the crop. However, most seedling growth media contain large amounts of organic matter, generally in the form of peat. High organic matter has been reported to reduce the development of endomycorrhiza (VAM) (Brechelt, 1987; Brechelt, 1989;

Schoenbeck, 1984).

The objectives of the work reported here were to determine, with the soil/crop combination previously shown to "require" VAMF for maximum yields, (i) if the beneficial effects of VAMF could be repeated in a transplanted-crop system, (ii) if the synergistic effects of VAMF and P-fertigation could be repeated, and (iii) which VAMF and P-fertilization levels would support maximum yield production and fruit size.

MATERIALS AND METHODS

Many of the techniques for these experiments have been reported previously (Krikun et al., 1987).

Experiment 1

Soil cultures of effective VAMF inoculum containing G. macrocarpum were tested for propagule number by a most probable number (MPN) technique; there were 22 propagules mL^{-1}. The inoculum was mixed at levels of 0, 2, 10 and 20% (volume basis) into a peat:vermiculite seedling growth medium. The mixture was used to fill plastic seedling trays and seeded with bell pepper cv. Maor. Each tray compartment contained 23 mL growth medium.

After 31 d, the seedlings were transplanted to a farm field with Typic Torriorthents, clay soil. The soil had been prepared according to normal commercial practices in the area. These included fertilization with 75 g superphosphate m^{-2} to bring Olsen available-P level to 35 mg kg^{-1}, formation of planting beds 1.65 m wide, and fumigation with 48 g m^{-2} heated methyl bromide.

There were 6.5 seedlings m^{-2}, three rows per bed, and a trickle-emitter irrigation line along each row. The irrigation system was set up so that each field plot, 15 m long and three plant beds wide, could be fertigated (soluble fertilizer added to the water) with one of three solutions containing agricultural grade phosphoric acid at 0, 0.3 or 1 mM P plus N and K. Fertigation was performed every second day with 2:1 NH_4-N/NO_3-N at 3.6 and 7.1 mmol N L^{-1} for 30 and 154 d after transplanting, consecutively. Potassium chloride (2 mmol K L^{-1} was added with every fertigation. Plants from each of the four VAMF-inoculum levels were planted in five replicated, randomized plots of each fertigation-level treatment.

Thirty and 62 d after transplanting, soil and plant samples were collected from the outer beds of each plot. The middle bed was used for fruit yield measurements. Red-ripened pepper fruits were harvested selectively four times, from 116 to 154 d after transplanting. The fruits were graded for size (max. diam.), counted and weighed.

Analysis of variance was performed with the SAS-GLM procedure (SAS Institute, Inc., 1985) using orthogonal linear, square and cubic contrasts of the levels of the mycorrhizal inoculum factor (MYC) and linear and square contrasts of the P-fertigation factor (P).

Experiment 2

Two experiments were conducted to confirm the previous results. One test was of factorial design as in Expt. 1 but with two levels of each factor; inoculum at 0 and 10% VAMF in the seedling growth medium, and P-fertigation in the field at 0.5 and 1 mM P mL-1 irrigation water. The soil type and N and K applications were as in Expt. 1. The initial inoculum contained 17 propagules mL^{-1} according to a MPN test. Thirty-two d after the initial growth-medium infestation, the seedlings, cv. Maor, were transplanted to the field. There were six replicated plots for each treatment. Analysis of variance was done by the SAS-ANOVA procedure (SAS Institute, Inc., 1985).

Experiment 3

A second test was conducted within a commercial field and was not replicated. The seedlings were prepared and transplanted as in Expt. 2. The grower performed all the horticultural treatments and the harvest along with normal crop. The soil type was the same as that used in the other experiments, but fumigation induced stunting had never occurred in this field; we assume that the P-binding capacity of the soil was lower. Plots were 100 m long and 6.4 m wide (four beds of three rows each); one plot had seedlings with 10% VAMF inoculum and the other without. The pepper F1 hybrid 'P-1750' (Sluig & Gruit, Holland) was used and fertigation was with 1 mM P mL^{-1} plus N and K as in Expt. 2.

RESULTS

Root colonization, Experiment 1

At transplanting, the VAMF had not begun to colonize the pepper roots, although some external hyphae were visible on the surface of roots of inoculated plants. In the field, colonization took place and the percent root infection increased with time, i.e., in the upper 30 cm of the soil profile, colonization increased more rapidly than the growth of roots.

There was little interaction between P fertilization and VAMF inoculation treatments (Table 1). The amount of VAMF added to the growth media at seeding significantly affected the amount of colonization at 30 and 62 d after transplanting (Table 1, Figs. 1B and 2B). Mycorrhizal infection increased with increasing inoculum level, except that the highest level (4.4 propagules ml^{-1}) did not increase colonization above the next lowest level (2.2 propagules mL^{-1}). Infection was present even when no inoculum had been added to the original seedling growth medium (Figs. 1B and 2B); apparently the soil fumigation treatment did not eliminate all the indigenous soilborne inoculum.

The effect of P on mycorrhizal formation also was significant (Table 1). Increasing P fertigation depressed mycorrhiza formation (Fig. 1A). This inhibition was present until at least 62 d after transplanting (Fig. 2A), after which root sampling was discontinued.

Root colonization, Experiment 2

The infestation of the seedling growth media again was effective in increasing mycorrhiza development in the field (Tables 2 and 3). There was little P *X* MYC interaction, but between the two P levels used there was no significant effect on colonization (Table 2) during the 47 d that plants were sampled. As in the previous experiment, the fumigation treatment did not completely eliminate VAMF inoculum and there were mycorrhizal roots on non-inoculated plants (Table 3).

Early-season growth after transplanting, Experiment 1

The P-fertigation treatments initiated when the plants were transplanted to the field quickly caused differences in plant height and mass. The P and MYC factors acted independently. After 30 d in the field, plant height and weight had increased linearly with increasing P- fertigation (Table 1). The P effect on plant weight also was present after 62 d (Table 1, Fig 3A). Fertigation with P resulted in increased growth but tissue concentrations of P were not significantly affected, being 0.28 $\pm$0.051%. The treatment means for these data are not shown.

VAMF inoculation did not significantly affect the early season plant height or mass (Table 1, Fig. 3B).

Early-season growth after transplanting, Experiment 2

In this experiment only the moderate and high levels of P fertigation were tested and their effect on growth was not significantly different (Table 2), although the 1 mM P-fertigated plants were smaller, but not significantly different, than the 0.3 mM fertigated ones both 17 and 47 d after transplanting. Apparently, in this season, the high level of P was not required for maximum plant growth, and may even have been an above-optimum concentration which induced Zn deficiency.

There were significant effects between the VAMF-inoculated and non-inoculated plants, but no P X MYC interaction (Table 2). The addition of VAMF to the seedling growth medium decreased plant growth after transplanting (Table 2 and 3). The plants did not appear stunted but, at both 17 and 47 d after transplanting, inoculated plants were significantly smaller than non-inoculated ones.

Effects on yield, Experiment 1

The P and MYC effects acted independently on the number of immature fruit present 62 d after transplanting, total fruit harvested and percent of large-size, marketable fruit in the total yield (Table 1).

Increasing P-fertigation levels caused earlier flowering as evidenced by fruit set (Table 1, Fig. 4A). The VAMF inoculations did not have a significant effect on early fruit setting (Table 1, Fig.4B). The P and MYC response curves for this parameter are similar to that of plant weight at the same growth stage.

The selective harvests for yield were begun when the green fruit had ripened and become red. This was the commercial practice in the locale at which the experiment was conducted. Four harvests were made and the sum of these yields calculated (Table 1, Fig. 4A, 4B and 5). P applications significantly increased yield (Fig. 4A and 4B), as they previously had increased plant dry matter accumulation. VAMF inoculation, which had not significantly affected early season plant growth, resulted in larger yields (Fig. 4B, 5) and a greater proportion of those yields being large fruits (>65 mm) (Table 1, Fig. 4B).

The complete response curve is presented only for total yield (Fig. 5). With no or little VAMF inoculum added to the seedling growth medium, plants P-fertigated with 1 mM phosphoric acid produced 42 Mg ha^{-1}. Yields were further increased by 12-14% by VAMF inoculation. With the highest level of VAMF inoculation, this high yield could be obtained with only 0.3 mM P.

Effects on yield, Experiments 2 and 3

As occurred with early season plant size, the two P-fertigation levels had no significant influence on yield (Table 2). VAMF inoculation increased total yield and large-fruit yield in spite of the

depression in plant growth which the treatment had caused earlier (Tables 2 and 3).

In the non-replicated plot in a commercial grower's field, the VAMF-inoculated plants were seen to be slightly smaller than the non-inoculated ones but no measurements were made. Four selective harvests of red fruit were done over a 7 wk period. At the first harvest, VAMF plants yielded less than non-inoculated ones (11.7 and 14.8 Mg ha^{-1}, respectively) but the percent of larger, marketable fruit (>65 mm) was greater (96% and 91%, respectively)). The total yields and percent marketable fruit after all the harvests, however, were greatest with the VAMF plants.

DISCUSSION

Mycorrhizal fungi are present in all soils where plants have been grown (Mosse, et al., 1981; Harley and Smith, 1983) and their ubiquitousness suggests an ecological importance of the mycorrhizal symbiosis. Lapeyrie and Chilvers (1985) found it crucial for the establishment of Eucalyptus dumosa in a calcareous soil of Australia. In a semi-arid region of Israel, Berliner (1990) studied two adjacent ecosystems, one with trees and dwarf shrubs and the other grassland. Cystus incanus, one of the species which grows only in the former ecosystem, is absent from the latter because of the lack of mycorrhiza and in spite of the higher quantity of available-P in the basalt-derived soil under the grassland.

Agricultural crops also may not succeed if the mutualistic mycorrhizal symbiosis fails to develop. Thompson (1987) demonstrated the effect of long periods of fallow in reducing inoculum levels. In our agronomic field experiments, and a previously reported field experiment with a direct-seeded pepper crop (Haas et al., 1987), we reduced the normal VAMF levels by methyl bromide fumigation, which is an accepted practice to control soilborne pathogens and weeds. In modern agriculture, where yield maximization is desired, soil treatments to control pathogens generally will reduce VAMF populations (Menge, 1982; Trappe et al., 1984). We have shown that VAMF supplementation can be used to increase P uptake and yields in a P-sorbing soil when the VAMF population in the soil is too low.

Insufficient inoculum levels could be a more frequent phenomenon than realized. Of particular interest is the yield response from VAMF inoculation on the soil where fumigation-induced stunting is not observed (Expt. 3); i.e., in this apparently less strongly P-sorbing soil, 1 mM P fertigation of pepper is recommended for maximum yield and the absence of VAMF inoculum is not recognized as a limiting factor. Marketable yield was increased by 11% when VAMF-inoculated seedlings were used, even though the entire field was fertigated with 1 mM P.

Large scale field experiments with augmented VAMF inoculum have been reported only rarely (McGonigle, 1988; Powell, 1984). We know of no reports where several levels of VAMF inoculum and P fertilization have been tested simultaneously, as in Expt. 1. Response curves developed from this type of experiment are important if the well-developed theories of VAM activity (Smith and Gianinazzi-Pearson, 1988) are to be proven under field conditions (Abbott and Robson, 1984). The curves show the quantitative effects of VAMF inoculum levels, P fertilization levels, and the two together. Also, it gives an opportunity to determine the applicability of VAMF supplementation in agricultural systems where it is feasible to apply both production practices.

Fertigation with P increased P uptake and crop yield. The effect on yield was linear and synergistic with the VAMF effect. Crop yield was maximum when soil P levels were approximately 1.5 μmol P gm^{-1} soil (47 mg kg^{-1}) as determined by $NaHCO_3$ extraction (Haas et al., 1987). This was obtained by fertigation with 1 mM H_3PO_4, a concentration ten times that used by Waterer and Coltman (1989). Their experiments were conducted in a 1:1 mixture of field soil and sand. In more neutral pH soils, dry fertilizer applications can be used to achieve these levels.

It generally is believed that P fertilization can replace the need for VAM. In our experiments this was not true, even when high levels of P were applied with each irrigation. If there had been a large replacement effect there would have been more significant P X VAMF interactions for yield as there were for VAM colonization in Expt. 1 (Table 1). In Expt. 2, where only moderate and high P fertigation levels were compared, there was a significant interaction because VAMF inoculation increased yield while there was no difference between P levels. Inoculation caused a growth depression in this experiment. Still, there was a positive yield response, an effect which would not have been recognized if this had been a pot experiment.

Two commonly used measures of VAMF activity were not useful here. Neither the early season P concentration in plant tissue nor the amount of root colonization by VAMF was correlated with crop yield. If root colonization were well correlated with external hyphal length, the extra P-absorbing surface would increase yield. There is more evidence that, above a very minimum level, VAMF colonization is not correlated with external hyphal length (see Miranda et al., 1989).

Two potential problems with incorporation of VAMF in seedling growth media were not severe enough to negate the positive effects of inoculation. High organic matter in the growth medium did not prevent the development of VAM in the seedlings. Also, root pruning, which normally occurs after transplanting, did not eliminate the continuing colonization of roots developing in the field. In fact, root colonization after transplanting still was correlated with initial VAMF inoculum levels.

The amount of inoculum required in the system described was 150 L ha^{-1} when a 1:9, inoculum:peat-mix ratio was used.

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Fig. 1-4. Response of pepper to P fertigation and vesicular-arbuscular mycorrhizal fungus inoculum (VAMF); Experiment 1. Data are means over four levels of VAMF for P data (1A-4A), and three levels of P for VAMF data (1B-4B); ANOVA probabilities in Table 1. Arrow is SE. Fig. 1 - VAMF colonization of root system in upper 30 cm of soil 31 and 62 d after transplanting. Fig. 2 - Dry weight of above-ground portion 62 d after transplanting. Fig. 3 - Number of fruits on plants 62 d after transplanting. Fig. 4 - Yield from four selective harvests of red-ripe fruits; total is cumulative and marketable is percent fruit > 65 mm (+).

Fig. 5. Total yield of red-ripened pepper fruits in five replicate plots with three levels of P fertigation and four levels of vesicular-arbuscular mycorrhizal fungus (VAMF) inoculum; Experiment 1. Inoculation was in a nursery and plants were transplanted to the field 30 d after seeding. Arrow is SE.

Table 1. Experiment 1, ANOVA; F-value probabilities for effects of phosphorus (P) and mycorrhizal-fungus (MYC) inoculum on root colonization (VAM), plant height (HT) and dry weight (DWT), immature fruit number (NFR) and dry weight (DWFR), per-hectare total fruit yield (YLD) and marketable yield (MYLD), and MYLD as percent of YLD (%MKT). Error df=48.

		Days after transplanting							Harvest no.			
		31			62				3	1+2+3+4		
Source		HT	DWT	VAM	DWT	VAM	NFR	DWFR	YLD	YLD	MYLD	%MKT
P	lin.[+]	.0001	.0001	.01	.0008	.0001	.04	.0001	.0001	.0004	.002	.01
	res.	NS[‡]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MYC	lin.	NS	NS	.001	NS	.0001	NS	.004	.002	.0003	.0003	.001
	sq.	NS	NS	.02	NS	.002	NS	NS	NS	NS	NS	NS
	res.	NS	NS	NS	NS	.02	NS	NS	NS	NS	NS	NS
P*MYC		.7	.7	.2	.5	.6	.8	.0002	.6	.9	.9	.9

[+]Linear (lin.) and square (sq.) orthogonal contrasts and their residuals (res.)
[‡]NS = P>0.05

Table 2. Experiment 2; probabilities of F-values for parameters subjected to factorial ANOVA. Root colonization (VAM), plant dry weight (DWT), average weight per fruit (WT/F), total fruit yield (YLD), marketable yield (MYLD), and MYLD as percent of YLD (%MKT). Error df=20.

	Days after transplanting					Harvest no.						
	17	31	47	17	47	1	2	3		1+2+3		
Source	VAM	VAM	VAM	DWT	DWT	WT/F	WT/F	WT/F	YLD	YLD	MYLD	%MKT
P	NS[+]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MYC	.02	.0001	.0001	.004	.002	.04	.01	.0002	.04	.03	.007	.0003
P*MYC	.2	.9	.7	.2	.5	.9	.6	.6	.5	.2	.8	.6

[+]NS = P>0.05

Table 3. Experiment 2; effect of mycorrhizal inoculum (MYC) on development of VA mycorrhizal fungi (VAMF) and field-grown pepper plant growth and fruit yield.

	Root colonization by VAMF (%)			Shoot dry weight (g)		Fresh weight per fruit (g)			Total harvest (t ha^{-1})		Marketable fruit (t ha^{-1})
	Days after transplanting					Harvest no.					
	17	31	47	17	47	1	2	3	3	1+2+3	1+2+3
-MYC	.003[+]	1.6	2.3	.46	9.3	114	138	102	19.8	40.5	16.1
+MYC	.287	23.7	23.6	.37	6.5	129	164	125	25.3	35.0	21.6
SE	--	--	--	.03	.8	6.8	9.7	5.0	2.8	2.6	1.8

[+] Mean of both P treatments and six replicates.

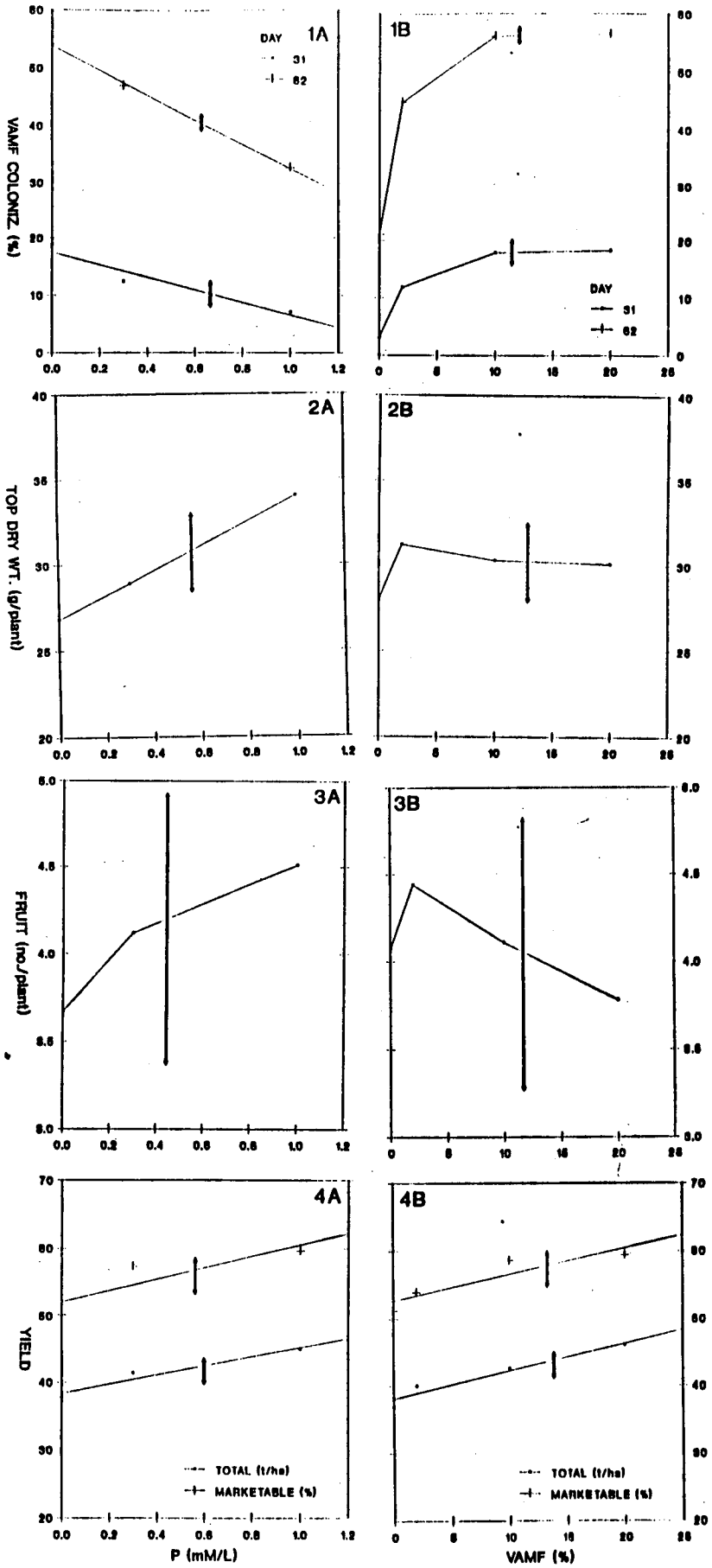
1A
DAY
31
62
VAMF COLONIZ (%)
1B
DAY
31
62
2A
TOP DRY WT. (g/plant)
2B
3A
FRUIT (no./plant)
3B
4A
YIELD
TOTAL (t/ha)
MARKETABLE (%)
P (mM/L)
4B
TOTAL (t/ha)
MARKETABLE (%)
VAMF (%)

Fig. 1-4

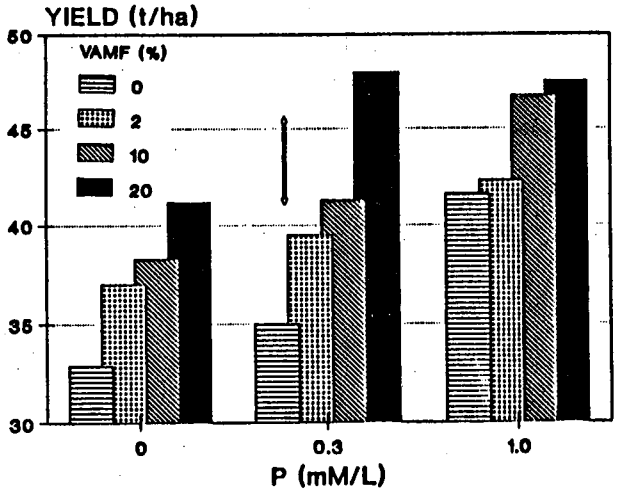

Fig. 5

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VA-mycorrhizal fungi and soil characteristics in avocado (*Persea americana* Mill.) orchard soils

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Abstract

Soils from avocado (*Persea americana* Mill.) orchards in Israel (IS) and California (CA), both sites with a Mediterranean climate, were sampled and analyzed for the species and quantities of vesicular-arbuscular mycorrhizal fungus (VAMF) spores in them, and for soil physical and chemical characteristics.

Numbers of spores were similar in soil from IS and CA but the dominant VAMF species were very different. In IS the most common fungi were *Sclerocystis sinuosa* and *Glomus macrocarpum*. In CA, *Gl. constrictum* was present in every orchard examined and *Gl. fasciculatum* was nearly as widespread. *Acaulospora* spp. and other *Glomus* spp. also were found, including *A. elegans* which has never before been reported from CA.

The differences in VAMF populations and species constituents found on two continents but in areas with similar climates and soil types may be due to host or edaphic factors. Different avocado rootstocks are used in the two countries and lower pH and higher soil fertility levels were present in CA soils.

The total VAMF spore populations in each orchard was about 275 per 100 mL soil. The population level was not correlated with any of the soil physical or chemical characteristics examined nor with avocado cultivar or age. In IS no fungus spores were found in three orchards; available P, Ca, Mg and Cu levels were high in these soils.

Introduction

Vesicular-arbuscular mycorrhizal fungi (VAMF) commonly are associated with the roots of most of the plants growing throughout the world (Mosse *et al.*, 1981). Although the fungi are obligate symbionts they are not highly host specific and one species may be found on various plants in the same locale. Also, one host plant can support mixed populations of VAM-fungus species.

Cultivation in general and monoculture in particular reduce the spectrum of species found in a soil and relatively few species are present after several years of continuous cultivation (Allen and Boosalis, 1983; Daniels and Bloom, 1983). Insufficient information is available to establish whether the climax mycorrhizal species depends upon the crops grown, the edaphic conditions or the climate at a particular location. Nemec *et al.* (1981) compared citrus in California and Florida. In Florida *Gigaspora margarita* Becker and Hall was the most common species found and in California *Glomus fasciculatum* (Thaxter *sensu* Gerdemann) Gerdemann and Trappe was present at 86% of the sites examined. Most citrus isolates of the latter fungus are now known as *Gl. deserticola* Trappe, Bloss and Menge (Trap-

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pe *et al.*, 1984). *Gigaspora margarita* has been reported only once from California (Menge *et al.*, 1983) but *Gl. fasciculatum* is common in Florida (Schenck and Smith, 1981).

This study was initiated to determine the occurrence of VAM fungi from soils of a single host, *Persea americana* Mill. (avocado), in Israel (IS) and California (CA). Avocado is grown extensively in the two localities and as a perennial crop presents a relatively stable environment for the development of a stabilized mycorrhizal population. The climates in the avocado-growing regions of both countries are similar (Mediterranean) and the soil types and growing conditions also are similar. It might be expected that the VAM-fungus species and quantities would be similar also.

Methods

Sampling

Avocado orchards were sampled in the principal growing regions within each country: in IS the northern half of the country (latitude 31.5–33.5 N) and in CA the San Diego–Santa Barbara area (latitude 32.4–34.5 N). Within each country, the number of orchards sampled in each sub-region was approximately proportional to the percent of avocado acreage in the various sub-regions. Soil samples were collected from 25 orchards in CA and 34 in IS in August; spore populations are near their maximum at that time (Gemma *et al.*, 1989; Saif and Khan, 1975). Two average-looking trees were selected. The leaf litter was removed from an area 1 to 2 m from the tree trunk. Where drip irrigation was being practiced the samples were removed from within 30 cm of an emitter. A 30-cm diameter and 30-cm-deep hole was dug. The soil was mixed thoroughly *in situ* and a 1.5 L sample of the mixture placed in a plastic bag. This treatment was designed to avoid the non-normal distribution of spores found in counts from small core samples (St. John and Koske, 1988).

Spore extraction and identification

The soil samples were refrigerated (4°C) until processing. Within 2 weeks of collection a 200-mL aliquot of each soil was wet sieved (37–650 µm) and layered onto sucrose solution (20, 40, 60%) and centrifuged at 1700 × g (Daniels and Skipper, 1982). Sand and some root fragments settled under the 60% sucrose layer; very light organic matter, including most of the empty fungus spores remained floating on the water layer on top of the gradient. Except for the floating material, the supernatant was removed to a 37-µm sieve, washed, decanted into a grid-marked 5-cm plastic dish, and the VAM-fungi were identified and counted. Spore morphology was ascertained under a compound microscope and identification was made using the keys of Hall and Fish (1979) and Trappe (1982).

Soil analysis

Soil analyses were carried out by the Agricultural Extension Laboratory, Univ. of California. Saturation percentage (grams of water required to saturate 100 g of soil) was determined and electrical conductivity and pH were measured in the water of the saturation paste (Chapman and Pratt, 1961). Available P was extracted from soil by 0.5 *M* sodium bicarbonate (Chapman and Pratt, 1961). Exchangeable soil calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) were measured in lithium chloride and lithium acetate extracts (Yaalon *et al.*, 1962), and soil zinc (Zn), manganese (Mn) and copper (Cu) were extracted using DTPA (Lindsay and Norvell, 1978); all were quantified by atomic absorption spectrophotometry.

Results

Spore numbers and species

The total number of VAMF spores extracted from each of the orchard soils ranged from 0 to 21 mL^{-1} and averaged 3 spores mL^{-1} in both IS and CA (Table 1).

Nine species of VAM fungi were found in IS and six in CA (Table 1); four species were common to both countries. The most ubiquitous species in IS were *Sclerocystis sinuosa* Gerd. and Bakshi, *Gl. macrocarpum* Tul. and Tul., *Gl.*

Table 1. Occurrence of vesicular-arbuscular mycorrhizal fungi in avocado orchards in Israel (IS) and California (CA)

Fungus	Percent orchards containing species		Spore numbers/100 mL soil[a]							
			Israel				California			
	n = 34 IS	n = 25 CA	Min[b]	Mean	Max[b]	SD[b]	Min[b]	Mean	Max[b]	SD[b]
Acaulospora elegans	0	4						12		–
A. laevis	9	0	12	123	300	155				
A. scrobiculata	0	12					4	53	100	48
A. trappei	6	0	12	102	191	127				
Glomus constrictum	3	100	–	195	–	–	2	83	380	89
G. fasciculatum	26	80	4	309	1250	449	40	202	750	187
G. geosporum	26	0	5	136	420	174				
G. macrocarpum	41	24	5	138	648	172	5	56	150	62
G. miscrosporum	6	0	138	1044	1950	1281				
G. mosseae	6	0	10	318	625	435				
Sclerocystis sinuosa	50	20	1[c]	13	42	14	1	1	2	1
Total population/orchard	–	–	0	284	2127	436	45	266	762	167

[a] Counts were made from 200 mL soil samples per orchard.
[b] Minimum, maximum and standard deviation of mean in orchards where the fungus was present.
[c] Number of sporocarps.

geosporum (Nicol. and Gerd.) Walker and *Gl. fasciculatum*. In CA, *Gl. constrictum* Trappe was present in every orchard examined and *S. sinuosa*, *Gl. macrocarpum* and *Gl. fasciculatum* were common also. The number of spores of each of these species found in the orchard soils varied considerably, and was highest for *Gl microcarpum* Tul. and Tul. in IS and for *Gl. fasciculatum* in CA.

Plant and soil characteristics

The avocado orchards in IS tended to be younger and the organic matter in the soils was less than those in CA. The pH of the soils in CA orchards was one unit lower than those in IS. In general, the soils in IS seemed to have been less fertilized and had lower quantities of P and other nutrients (Table 2).

Table 2. Tree age and edaphic characteristics in avocado orchards in Israel (IS, n = 34) and California (CA, n = 25) and the correlation of these values with the number of vesicular-arbuscular mycorrhizal fungus spores in the soils

Variable	Host and soil variables						Correlation coefficient		
	Israel			California			IS	CA	Both
	Min.[a]	Mean	Max.[a]	Min.[a]	Mean	Max.[a]			
Age (years)	2	11	28	3	17	35	.01	.21	.07
Saturation (%)	23	44	58	30	37	56	−.18	−.17	−.16
Organic matter (%)	0.4	1.4	3.2	1.2	3.2	5.3	.15	.02	.05
pH	7.6	7.9	8.4	5.8	6.7	7.5	−.02	−.21	−.02
Conductivity (mS cm^{-1})	.6	1.2	2.9	.6	1.7	4.5	.09	.26	.10
Phosphorus, Olsen ($\mu g\ g^{-1}$)	.1	4.8	14.8	4	22	53	.05	.18	.02
Potassium, acid ($g\ kg^{-1}$)	.1	.3	.9	.4	1.0	1.9	−.26	.35	−.10
Potassium, acetate ($g\ kg^{-1}$)	<.1	.1	.3	<.1	.2	.5	−.30	−.22	.01
Calcium + magnesium ($meq.^{-1}$)	5	15	32	5	12	38	−.04	.21	–
Cooper (ppm)	<.1	.1	.5	.4	4.4	24	−.09	−.21	−.07
Manganese (ppm)	3	14	36	7	61	141	−.06	−.10	−.04
Sodium (ppm)	31	79	340	32	124	481	−.02	.20	.03
Zinc (ppm)	.2	2.7	17	3	27	178	−.19	−.06	−.04

[a] Minimum and maximum values for variables.

Host and soil variables

(000) Haas and Menge IL.SO 8621-14

Table 3. Avocado orchard soil variables associated with the species of vesicular-arbuscular mycorrhizal fungi found in Israel

Fungus	No. of orchards	Soil variables								
		Saturation (%)	Organic matter (%)	pH	Conductivity (mS cm^{-1})	P Olsen (μg g^{-1})	K acid (g kg^{-1})	Ca + Mg (meq L^{-1})	Cu (ppm)	Mn (ppm)
Acaulospora laevis	3	38	1.2	7.9	.8	4	.32	9	.08	21
A. tappei	2	34	.7	7.6	.6	9	.26	7	.07	36
Glomus constrictum	1	57	3.2	8.0	1.1	1	.27	16	.14	31
G. fasciculatum	9	43	1.5	7.9	1.4	4	.34	18	.09	12
G. geosporum	6	45	1.4	7.8	1.2	7	.40	17	.12	18
G. macrocarpum	13	45	1.4	8.0	1.2	4	.30	15	.14	11
G. microcarpum	2	51	1.8	7.9	1.2	2	.11	16	.20	2
G. mosseae	2	29	.6	8.0	.6	8	.29	6	.12	26
Sclerocystis sinuosa	16	46	1.7	7.9	1.1	4	.25	14	.13	18
None	3	45	1.0	7.9	1.7	11	.45	22	.35	13
LSD $P < 0.5$*		19	1.2	0.3	0.9	6	.10	11	.12	15

* LSD based on the harmonic mean (2.8) of the number of orchards.

Correlation of species with soils

The soil characteristics of IS soils containing the various species of VAM-fungi are presented in Table 3. Olsen P levels were highest where no VAMF spores were recovered and lowest where *Gl. constrictum* and *Gl. microcarpum* were present. Also Ca + Mg and Cu levels were high where no VAM fungus spores were found. The characteristics of the CA soils did not differ according to the species of fungi present (Table 4).

Discussion

Mycorrhizal fungi were associated with avocado roots in all of the 25 orchards investigated in CA and in 31 of the 34 investigated in IS. It is likely that VAM fungus spores would have been found in all of the samples if more than one 200-mL aliquot would have been examined. As with most crops, avocado apparently are normally mycorrhizal.

Despite the similarities of host, climate and edaphic factors in the two regions, different fungi were the most prevalent in each country. The most common fungi in IS orchards were *S. sinuosa* and *Gl. macrocarpum*. The latter fungus is very common in IS and was the most prevalent in a survey of onion and pepper crops (Haas and Krikun, 1983). *Glomus constrictum*, which was present in every CA orchard investigated, was present at only a single location in IS. This fungus is often associated with ageing citrus but the ages of avocado orchards in the two countries did not differ significantly.

Table 4. Avocado orchard soil variables associated with the species of vesicular-arbuscular mycorrhizal fungi found in California

Fungus	No. of orchards	Soil variables								
		Saturation (%)	Organic matter (%)	pH	Conductivity (mS cm^{-1})	P Olsen (μg g^{-1})	K acid (g kg^{-1})	Ca + Mg (meq L^{-1})	Cu (ppm)	Mn (ppm)
Acaulospora scrobiculata	3	32	2.0	6.7	2.2	13	.13	17	.13	21
Glomus constrictum	25	37	3.2	6.7	1.7	22	.16	12	.44	61
G. fasciculatum	19	36	3.3	6.6	1.6	22	.14	11	.42	62
G. macrocarpum	5	37	2.8	6.4	2.2	31	.13	17	.78	65
Sclerocystis sinuosa	5	36	3.5	6.3	1.7	26	.13	12	.22	58
LSD $P < .05$*		7	1.4	0.7	1.1	21	.12	9	.76	51

* LSD based on the harmonic mean (5.0) of the number of orchards.

This is the first report of *A. elegans* Trappe and Gerd. in CA. *Acaulospora* spp. appear to be rare in CA, probably because this genus favors acid soil. Most of the agricultural soil in CA is alkaline. However, avocados are one crop that are grown on acid soil (Table 4). *Acaulospora* spp. would be expected to be more prevalent with avocados than other crops only grown on higher pH soil.

The significant differences in species of VAM fungi found in the two localities are probably due to either host or edaphic factors; the climates are very similar. Different rootstocks of avocado are used in the two countries. In IS the common rootstocks are of West Indian and Guatemalan origin, with about 25% originating from Mexican rootstocks; in CA, only Mexican rootstocks were used. There also are several outstanding differences in edaphic characteristics. Soils in IS have a higher pH and a higher clay content (saturation percentage); in CA, soils probably are fertilized more intensively and therefore have higher levels of electrical conductivity, phosphorus, potassium, and minor elements.

Although it is likely that edaphic factors influenced the prevalence of the fungi, none of the fungi was associated strongly with any particular factor (Table 3 and 4). This can be explained by the fact that mycorrhizal spores do not necessarily reflect the abundance of mycorrhizal infection. Some species sporulate far more prolifically than others and to combine spore counts for many species would make a correlation with edaphic conditions difficult, since not all species react similarly to soil conditions. It might be expected that spore numbers of individual species would vary directly with soil factors. In IS, *Gl. constrictum* and *Gl. microsporum* tended to be found where soil phosphorus levels were low, and *A. trappei* Ames and Lindsey, *Gl. mosseae* (Nic. and Gerd.) Gerd. and Trappe and *Gl. geosporum* where the P levels were higher than average. In the three locations where no VAM fungi were found in the soil samples, soil phosphorus levels were even higher. In CA, but not in IS, *Gl. macrocarpum* was present where P levels were elevated. In general, the fungi found in CA were not typically associated with any of the soil or plant characteristics investigated.

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Mycorrhizal dependence of four crops in a P-sorbing soil

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Abstract

The effect of methyl bromide and P fertilization on the growth of four agricultural crops in a P-sorbing soil was studied. Harvestable yields and P tissue content were significantly lower for celery (*Apium graveolens* L.), onion (*Allium cepa* L.) and pepper (*Capsicum annuum* L.) following fumigation. P fertilization up to 330 kg P ha^{-1} as superphosphate could not reverse this effect. The highest P fertilizer treatment had 25.4 $\mu g\ g^{-1}$ bicarbonate-extractable P and 0.26 $\mu g\ g^{-1}$ water extractable P. This P level produced acceptable commercial yields when vesicular arbuscular mycorrhizae (VAM) were present (non-fumigated) and indicate the importance of the VAM association for these crops. In contrast, melon (*Cucumis melo* L.) growth was slightly greater following soil fumigation at all P fertilization rates. These findings indicate that crop VAM dependence and efficacy of VAM biotypes must be considered as essential components for P fertilization recommendations on fumigated P-sorbing soils.

Introduction

The beneficial role of the vesicular arbuscular mycorrhizal (VAM) association in P uptake and growth response under P-limiting conditions has been well established for many agricultural crops (Gerdemann, 1968; Mosse, 1973). Soil disinfection by methyl bromide fumigation or steam is often used to eliminate soil-borne plant pathogens, but such treatments can reduce VAM populations as well (Menge, 1982).

Several studies have indicated that plant stunting following soil fumigation treatments may be due to elimination of VAM (Dodd *et al.*, 1983; Yost and Fox, 1979). Reversal of stunting can be achieved by VAM augmentation or increased P fertilization (Haas *et al.*, 1987). We have found that following methyl bromide fumigation of a P-sorbing soil larger phosphoric acid applications via fertigation were necessary for maximal pepper yields (Haas *et al.*, 1987).

The use of standard P-extraction tests for predicting plant growth response to P additions may be affected by fumigation. Such tests are correlated with plant growth response in non-fumigated soils where VAM are likely to be present. We have found that higher bicarbonate-extractable P levels are required to achieve comparable growth in pepper after soil fumigation (Dodd *et al.*, 1983). Stribley *et al.* (1980) noted that a poor relationship between soil analysis and the growth response to applied P is probably due to differences in mycorrhizal infection.

The objectives of this study were to determine the P requirements of four major crops grown to maturity in a P-sorbing soil following methyl bromide fumigation, and to evaluate the role of two P extraction tests in predicting P addition growth

responses, in the presence and absence of VAM infection.

Materials and methods

Field design

The experiment was carried out at the Gilat Experiment Station. The soil, which is representative of a major area in the northern Negev of Israel, is a deep loess, with a pH of 8.2 (saturated paste); $CaCO_3$, 17%; and clay, 18%. The field selected had been planted for the last 15 years with commercial crops. Prior to this experiment, cotton, potato and wheat were grown in sequence. Extensive soil tests showed that there was little variation in soil-P values. Previous studies showed that the soil contained a highly effective VAM population (Dodd *et al.*, 1983; Haas and Krikun, 1985).

Four soil-P levels were established by adding, 0, 110, 220 and 330 kg single superphosphate P ha^{-1}. The superphosphate was spread manually on pre-established beds and incorporated to a depth of 25 cm with a rotary tiller. The four crops examined were: direct seeded onion (*Allium cepa* L.), melon (*Cucumis melo* L.), celery (*Apium graveolens* L.), and pepper (*Capsicum annuum* L.) transplants from a commercial nursery.

Fertilizer and pest control measures as well as cultivars and dates of planting were as commonly practiced in the region. Supplemental watering was provided as needed via sprinkler irrigation.

Plots consisted of six 1.8-m-wide beds. Three adjacent beds were covered with 0.1-mm-thick, 6-m-wide polyethylene sheeting and fumigated with 60 g m^{-2} of 98% methyl bromide and 2% chloropicrin mixture. This method was found to be sufficient in previous work to eliminate mycorrhizal inoculum (unpublished results). Plot length for every P rate was 8 m. The fumigated plots served as the non-mycorrhizal treatment, and the non-fumigated plots as the mycorrhizal ones (Yost and Fox, 1979). Each treatment was replicated four times.

Plant and soil analyses

Soil-P was extracted by two procedures: with 0.5 *M* $NaHCO_3$ (Olsen and Dean, 1965) or water. In the water extraction, 100 g 65°C-dry soil was moistened with twice the saturation volume, left standing for 18 h, and filtered. The water extractant contained 0.01% $HgCl_2$ to inhibit microbial action.

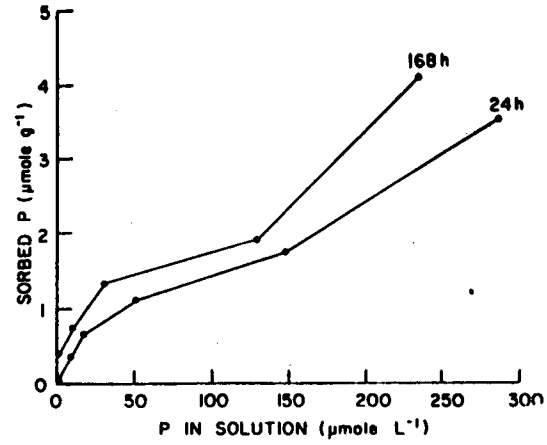

Fig. 1. Sorption isotherms of P in the experimental soil after 24 and 168 h equilibration. Solution (0.01 *M* $CaCl_2$)/soil ratio was 10:1.

Characteristic P sorption isotherms of the non-fumigated soil after 24 and 168 h equilibration in soil/0.01 *M* $CaCl_2$ (1:10) are presented in Fig. 1. The curvilinear portion of the curve is attributed to adsorption reactions, while the slope change indicates formation of a new phase attributed to P precipitation.

Yields were taken from the center bed, and plants for tissue analysis from the inner rows of the border beds. Plant tissues were digested with nitric-perchloric acid. P and several microelements were determined in the digest. Since no major differences were found in microelement content only P results will be presented in this report.

Mycorrhizae

Mycorrhizal infection was evaluated by staining and examining roots (Phillips and Hayman, 1970).

Results and discussion

Effect of soil-P additions on extractable-P in fumigated and non-fumigated plots

The results of 0.5 *M* $NaHCO_3$- and water-

Table 1. Effect of soil P additions on 0.5 *M* $NaHCO_3$ and H_2O-extractable P ($\mu g\ g^{-1}$)

P added	$NaHCO_3$ extracted	H_2O extracted
P0[a]	10.9a[b]	0.10c
P1	15.6b	0.16f
P2	18.4c	0.25g
P3	25.4d	0.26g

[a] P0, no added P; P1, P2, P3 = respectively 110, 220, 330 kg single superphosphate P ha^{-1}, incorporated to 25 cm depth.
[b] Numbers followed by different letters are significantly different at $P < 0.05$ (Duncan's Multiple Range Test).

extractable P are presented in Table 1. Fumigation had no effect on $NaHCO_3$-extractable P levels. Similar results were presented by Yost and Fox (1979). The additions of superphosphate resulted in relatively slight increases in both $NaHCO_3$ and water-extractable P, thus confirming the high-P sorbing capacity of this soil. According to Olsen and Dean (1965) the higher levels of $NaHCO_3$-extractable P (treatments P2, P3) should be sufficient to meet the P requirements of most crops. The wide $NaHCO_3$:H_2O ratio obtained in this soil is in contrast to results obtained in coarser textured soils of the region (Dodd *et al.*, 1983). Sharpley *et al.* (1982) obtained similarly wide ratios in some of the 66 soils tested from various regions in the USA. For some of the soils, ratios ranged from 53 to 73 $NaHCO_3$:H_2O-extractable P. In our case, depending on the treatments, the ratios ranged from 66 to 115.

Effect of fumigation on mycorrhizal infection and growth of test crops

The four crops tested were mycorrhizal in non-fumigated soil at all P levels, whereas those grown in fumigated soils were non-mycorrhizal. At the end of the first month, infection in all crops in the non-fumigated soil was *ca.* 50%. This was also true for melon, which in this experiment as well as in many other examinations, were found to be highly mycorrhizal. With the exception of melon, crop yields at all rates of P were signficantly greater when grown in non-fumigated soil. Table 2 shows further that mycorrhizal plants of pepper, onion and celery growing at the lowest soil-P level, out-yielded non-mycorrhizal plants grown at the highest P level. Our results confirm the findings of others (Plenchette *et al.*, 1983a; Yost and Fox, 1979) that onion and pepper are highly VAM-dependent. In addition, we believe this is the first report of the VAM dependency of celery.

In contrast with Yost and Fox (1979), we could not overcome the VAM deficiency with large applications of fertilizer P. Although the coarseness of phosphate particles may affect the availability of P, we observed a similar response in pot tests when superphosphate was finely ground (Dodd *et al.*, 1983). Non-VAM plants of pepper, onion and celery in general were larger with increasing soil P additions, whereas this general trend was not observed in VAM plants. In contrast to the above three species, melon, as observed by Reuveni *et al.* (1982) and unpublished results did not show deleterious effects due to fumigation.

Table 2. Influence of soil fumigation and P fertilization on yield of four crop species[a]

P rate[c]	Melon		Onion		Pepper		Celery			
							Marketable		Biological	
	M[b]	NM	M	NM	M	NM	M	NM	M	NM
P0	85bc[d]	95bc	90a	27b	620b	18c	620a	0b	1,569a	116c
P1	78c	99ab	85a	36b	670ab	58c	572a	0b	1,563a	533b
P2	84bc	95bc	85a	41b	697ab	59c	636a	0b	1,763a	665b
P3	93bc	114a	91a	49b	748a	75c	599a	0b	1,655a	754b

[a] Melon yield expressed as kg fruit of all three beds; in other crops, yield expressed in g $plant^{-1}$
[b] M = mycorrhizal; NM = not mycorrhizal.
[c] P0, P1, P2, P3 = respectively no P added, and 110, 220 and 330 kg single superphosphate P ha^{-1} added.
[d] Statistical analysis within the crop. Numbers followed by different letters are significantly different at $P = < 0.05$ (Duncan's Multiple Range Test).

Effects of VAM infection and P additions on P content of plant tissues

Representative results of P analysis of the various crops are presented in Table 3. The data clearly indicate the effect of mycorrhizal infection on increasing P levels in the tissues of VAM-dependent crops. In these three crops, plant-P levels in the mycorrhizal-without-P-fertilizer additions were significantly higher than P-fertilized treatments without mycorrhizae. The interactions between mycorrhizae and P fertilization indicate that both increases and decreases in tissue-P can occur. Jarrel and Beverly (1981) noted that such variable effects are a result of the relative difference between the rate of nutrient uptake to the rate of dry matter accumulation. In the case of melon, the large increase in tissue P corresponded with a decrease in yield. The decreasing P tissue concentrations with increasing soil P in mycorrhizal celery may be due to inhibition of mycorrhizal infection (Menge *et al.*, 1978). The importance of differing plant-P levels as a result of fertilizer-VAM interactions is secondary when compared with plant-VAM interactions. Apparently, the P level of VAM-dependent crops was above a critical threshold when the latter were infected with mycorrhizae. When the VAM-dependent crops were grown without mycorrhizae, P levels were clearly below the critical threshold level for normal growth, whereas VAM plants produced excellent yields.

Table 3. Influence of soil fumigation and P fertilization on P content (%) of tissues

	P additions[a]			
	P0	P1	P2	P3
Melon				
M[b]	0.12c[c]	0.10c	0.16ab	0.22a
NM	0.11c	0.10c	0.14bc	0.14bc
Onion				
M	0.13bc	0.21a	0.17ab	0.16ab
NM	0.06d	0.06d	0.08cd	0.09cd
Pepper				
M	0.21b	0.23b	0.23b	0.27a
NM	0.09c	0.11c	0.12c	0.12c
Celery				
M	0.18bc	0.20b	0.29a	0.20b
NM	0.07d	0.13cd	0.13d	0.12d

[a] P0, P1, P2, P3 = respectively no P added, 110, 220 and 330 kg superphosphate P ha^{-1} added.
[b] M = mycorrhizal; NM = non-mycorrhizal.
[c] Statistical analysis within the crop. Numbers followed by different letters are significantly different at $P < 0.05$ (Duncan's Multiple Range Test).

Conclusions

The crops used in our experiment were chosen on the basis of observations made following soil fumigation, a common practice with many of our growers (Krikun *et al.*, 1982). One of our aims was to add enough P to overcome the stunting observed in three of the crops. However, although comparatively large amounts of P were added, it is obvious that P sorption is such as to reduce available P to a level where normal plant growth can not be attained in three of the four crops tested in fumigated soil. Fox and Kamprath (1970) pointed out that some plants supplied with 0.03 ppm P in nutrient solutions will sustain maximum growth. However, a value of 0.2 ppm in the soil solution has been suggested as one at which most plants attain near maximum growth. Based on our water-extraction procedure, this concentration was attained at the two high rates of added P, but non-mycorrhizal plants of the three dependent crops apparently could not take up enough P to attain maximal growth.

Phosphorus extraction from moderate to high pH soils by $NaHCO_3$ is a standard procedure in many studies where the effects of added P are analyzed. According to this test, especially at the high rates, enough P should have been available for normal plant development. However, this was so only when mycorrhizal infection took place. Stribley *et al.* (1980) concluded that "$NaHCO_3$-extractable P is an excellent predictor of phosphate availability as long as the V-A mycorrhizal infection is uniform." While concurring, we believe that for at least some soils the water-extractable P as well as $NaHCO_3$-extractable P and their ratios should also be considered, as Sharpley *et al.* (1982) have shown that a poor correlation exists between $NaHCO_3$- and H_2O-extractable P. Thus, in situations where mycorrhizal inoculum may be absent, low or inefficient, the above information as well as the minimal P requirements for each crop must be known before meaningful predictions as to P res-

ponse can be made (Dodd *et al.*, 1983). That this may be a critical factor in many soils, as exemplified by ours, should not be underestimated. In many regions of the world, arid calcareous soils are being brought under cultivation by the introduction of irrigation. It has been shown that in soils of pH 7.0 and above, a significant correlation exists between P sorption and exchangeable Ca ($r = 0.895$) (Sharpley *et al.*, 1982). Due to the sparse vegetation in these arid regions, V-A mycorrhizal inoculum would be minimal. Thus, if the above is not taken into account, "normal" applications of P, both in quantity and methods of application, may be insufficient for an ever-expanding number of crops (Plenchette *et al.*, 1983a).

Our results emphasize another aspect of the mycorrhizal association. In a previous paper (Dodd *et al.*, 1983) we pointed out, that based on pot studies, the native mycorrhizal population in the soil reported here was much more efficient in P uptake and growth-promoting effects than the populations of soils with lower P-sorbing capability. We postulated that evolutionary pressure selected species and/or biotypes more capable of P uptake in the high P-sorbing soil. Our field results tend to support our previous conclusion, when we consider that at 10 $\mu g\ P\ g^{-1}$ $NaHCO_3$-extractable P, mycorrhizal plants produced higher yields than non-mycorrhizal ones exposed to 25 $\mu g\ P\ g^{-1}$ $NaHCO_3$-extractable P. It is becoming more obvious, as comparative trials are carried out, especially in high P-sorbing soils, that the efficiency of the mycorrhizal population, and the mycorrhizal dependency of the particular crops species examined, must be taken into account in understanding and analyzing P-uptake results.

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Field Response of Corn to Water Management and Inoculation with *Glomus etunicatum*

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Plant water stress is a major factor limiting crop yield. Several authors have suggested that improved crop nutrition may increase drought resistance and yield of crops exposed to water stress. Mycorrhizae improve plant nutrition and are known to affect the water relations of plants grown in chambers and greenhouses; however, there are few data on the response of mycorrhizal plants to water stress in the field. In this paper we summarize the results of a field trial that was conducted over three years to test the effect of mycorrhizae on water-stressed corn.

A field plot of Millhoppper fine sand (loamy, siliceous, hyperthermic Grossarenic Paleudult) was fumigated with methyl bromide and fertilized before each growing season. Two inoculation treatments (inoculated or not with *Glomus etunicatum)* were applied to subplots of three water management treatments each year. Treatments were replicated four times. Inoculum was placed in a furrow 7- to 10-cm deep at an approximate rate of 1500 propagules m^{-1} of row. Corn (*Zea mays* L. "Pioneer Brand 3165") was planted with intra- and inter-row spacings of 20 and 60 cm, respectively.

To monitor root colonization by mycorrhizal fungi, five soil cores were collected from plots to a depth of 30 cm; roots were washed free, blotted dry, weighed, and a 0.5 g subsample was assessed for mycorrhizal colonization. Six to seven weeks after planting, colonization ranged from 0 to 3% on noninoculated plants and from 17 to 54% on inoculated plants. Twelve to thirteen weeks after planting, colonization ranged from 2 to 17% on noninoculated plants and 24 to 45% on inoculated plants. Water management had little effect on root colonization.

There was a linear increase in both total crop biomass and grain yield with increasing water application. Biomass increased from 11,000 to 19,000 kg ha^{-1} when total water input increased from 34 to 52 cm while grain yield increased from 4,500 to 10,200 kg ha^{-1}. Inoculated plants had greater biomass (ca. 1,000 kg ha^{-1}) and grain yield (ca. 800 kg ha^{-1}) than noninoculated plants across all water treatments. There was no interaction between water management and inoculation treatments. Due to the smaller size of water-stressed plants, but a consistent growth response to inoculation across water treatment, the proportional response of corn to inoculation with *G. etunicatum* increased with increasing water stress.

F. COOPERATION

Frequent correspondence and periodic meetings between the principal investigators allowed cooperation in refining plans of the originally-proposed research, in exchanging results and discussions of the grant research and that of other scientists, and in revising particular goals during the period of the grant.

In addition, an Israeli postdoctoral scientist, Dr. Uzi Afek, who had spent some time in the laboratories of Haas and Krikun, was hired by Menge. He also was very active in ensuring close cooperation and communication between the CA and IL groups.

G. ACHIEVEMENTS

The original proposal suggested that "the program will result in

"1) selection of effective strains of VAM-fungi and establish the correlation of colonization rapidity and external mycelium production with strain efficacy;

"2) comparison of the consistent positive growth benefits achieved with inoculation of pepper in fumigated P-sorbing soils, with pepper grown in other arid-land soils;

"3) determination of fertilization practices and inoculum levels required to produce horticulturally acceptable mycorrhizal seedlings of celery, onion, cotton and citrus, for transplanting or as direct seeded plants;

"4) evaluation of the growth and yield effects attributable to VAM-fungus inoculation under various P-fertilization levels under modern agricultural production practices in fumigated and non-sterile soils; and

"5) evaluate the potential for producing VAM-fungus inoculum in the field rather than containers in greenhouse or other controlled environments."

No attempt was made to achieve results (3) and (5) because the original grant was less than half of the proposed budget.

We have demonstrated that the benefits of VAMF inoculation, which usually are shown in greenhouse trials, can be translated to the field. In the greenhouse it usually is impossible to test marketable-yield responses and the root system of test plants are severely restricted and not comparable to that of field-grown crops.

We have produced immediately useful information on (i) inoculum placement in relation to seed, (ii) utility of mixing inoculum with growth media in the nursery for subsequent transfer to the field along with the seedling transplants, (iii) benefits of soil solarization along with VAMF-population augmentation in nonfumigated soil, (iv) importance of controlling plant pathogens in order to achieve maximum VAMF colonization of crop roots, and (v) the inadequacy of P fertilization in replacing VAMF inoculation in high P-sorbing soil, and the ability of the plants to benefit from simultaneous VAMF-inoculation and P fertigation, in spite of the depression of VAMF colonization by P fertilizers.

H. LIST OF PUBLICATIONS

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