in previous work conducted by F.E. Larson. Refinements are still needed in application protocol. Unfortunately, cost and government approval are still major issues to resolve.

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Effects of Soil Amendments and Tillage on Vesicular-Arbuscular Mycorrhizal Fungus Colonization of Citrus

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ADDITIONAL INDEX WORDS. symbiosis, *Glomus*, fertilizers, roots

SUMMARY. Three sites (A-C) were prepared for citrus groves in Florida from 1985 to 1986. Nine soil amendments applied at either one or two rates were deep-tilled 3.9 to 4.9 ft (1.2 to 1.5 m) deep and 4.9 ft (1.5 m) wide in the row before planting. Maximum treatment rates were limestone 50,000 lb/acre (56 000 kg·ha⁻¹), phosphoclay at 80,000 lb/acre (89 600 kg·ha⁻¹), humate at 77,612 lb/acre (86 912 kg·ha⁻¹), shrimp waste at 73,052 lb/acre (81 805 kg·ha⁻¹), peat at 250,000 lb/acre (280 000 kg·ha⁻¹), bentonite clay at 73,052 lb/acre (81 805 kg·ha⁻¹), mined gypsum at 2000 lb/acre (2240 kg·ha⁻¹), calcium humate at 2000 lb/acre (2240 kg·ha⁻¹), and phosphogypsum at 10,000 lb/acre (11 200 kg·ha⁻¹). Deep-tilled controls and a no-till control were established at sites A and B and a deep-tilled control at site C. A fourth grove (site D) was planted in 1970 and included the treatments surface tillage (ST), deep tillage (DT), and DT plus lime (DTL) at 45,407 lb/acre (50,848 kg·ha⁻¹). Mycorrhizal fungus infection was found in roots in 1987 at sites A and B and in 1989 in site C. Fungus infection ranged from 6% (no-till control) to 64% at site A, 64% to 81%

at site B, and 15% to 47% at site C. At all sites, amendments did not increase percentage infection and vesicle and hyphae ratings significantly over the deep-tilled control. At site A, percentage infection in the limestone treatment was the highest (64%) and was significantly higher than infection in phosphogypsum, peat, and the no-till control treatments. No treatment had a suppressive effect on infection. Mycorrhizal infection in roots was high (94% to 95%) at site D but did not differ significantly between treatments.

Organic and inorganic natural products as amendments to agricultural soils have affected soil chemistry and structure, plant disease development and plant growth, production, and development (Berry, 1982; Lumsden et al., 1983; Ozores-Hampton and Bryan, 1993; Roe et al., 1993; Sekiguchi, 1977). In view of the increasing interest in the use of organic products to replace fertilizers and pesticides to provide a safer environment, it is essential to learn more about their impact on beneficial soil microflora, which includes mycorrhizal fungi.

Most literature dealing with effects of composts, organic fertilizers, and amendments on vesicular-arbuscular mycorrhizal (VAM) fungi has reported studies done in controlled environments, such as pots and tubs (Guttay, 1982; Maronek et al., 1980; Menge et al., 1982; Nemec, 1992). Such studies are appropriate for commercial production in soils and soilless media in containers, but results are not necessarily applicable to field-plot production of crops. Although limited, studies of field soils treated with manures and composts indicate that organic manures stimulated a more normal development of VAM fungi in roots than inorganic forms of nitrogen and no fertilizer treatments (Azcon et al., 1978; Reed and Frémont, 1935; Sabet, 1945) and increased VAM fungus spore numbers in soil (Kruckelmann, 1975).

It is well established that high levels of mineral fertilizers can often result in a low level of mycorrhizal fungal infection (Hayman, 1982) in roots of plants in pots and the field. However, it has not been established what effect a wide range of soil amendments have on indigenous VAM fungi in crop plant roots in the field. A large selection of soil amendments has been added to soils

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planted with citrus to study their effects on plant growth, production, tree health, and soil microflora. The purpose of this research is to report on the development of VAM fungi in roots of citrus trees grown in these plantings and to examine the effects of some of these amendments on the host in pot studies conducted in a greenhouse.

Materials and methods

Four citrus grove sites were evaluated in these studies. Three groves treated with amendments and planted in 1985 and 1986 (sites A–C) were primarily established to determine the effects of amendments on two major citrus diseases—foot and root rot (causal agent = *Phytophthora parasitica* Dast) and blight. The fourth grove (site D) was established in 1970 as part of the USDA and University of Florida soil–water–atmosphere–plant (SWAP) experiment to study tillage, lime, and water management of citrus (Knippling and Hammond, 1971). The soils at sites A and D are classified as spodosols of the Oldsmar and Wabasso series. The soil at site B was an Astatula fine sand and the soils at site C were Myakka and Immokalee fine sands. Soil amendments were strip-applied 5.2 ft wide to the soil surface of sites A, B, and C and

deep-tilled 3.9 ft deep down the center of rows before planting. Each amendment replicate consisted of four, six, or nine trees, depending on site, spaced 24.3 ft apart in the row. Soil amendments were arranged in a randomized complete-block design. At site D the experimental design consisted of nine main plots each 300 × 360 ft arranged in three blocks of three plots each. Within each block, three soil modification treatments were assigned at random to the main plots. The soil modification treatments at site D were surface tillage (ST) to a depth of 5.9 inches, deep tillage (DT) to a depth of 42 inches, and DT to a depth of 42 inches plus lime (DTL) of 45,407 lb/acre mixed throughout the tilled soil profile. ‘Valencia’ sweet orange [*Citrus sinensis* (L.) Osb.] budded on rough lemon [*Citrus limon* (L.) Burm. f.] was planted in sites A, B, and C and ‘Pineapple’ sweet orange on rough lemon planted at site D. Additional plot history was provided by Knippling and Hammond (1971).

The soil amendments and soil modification treatments of all sites and location, dates amendments were applied and trees planted, rates of treatments used, trees per replicate, number of replicates (plots) used, and dates

VAM fungi were analyzed in citrus roots are reported in Table 1. The amendments used were a reed sedge peat from a central Florida site near Mountverde, shrimp hull waste from a Mississippi Gulf Coast shrimp processing facility, humate marketed as Leonardite Plus (Agra Chem Sales, Avon Park, Fla.), bentonite clay, mined gypsum, calcium humate marketed as Agraset (Agra Chem Sales), phosphogypsum, and phosphoclay from a central Florida phosphate mine and lime for sites A, B, and C from commercially available limestone. Those amendments subject to leaching (phosphogypsum, humate, gypsum, and calcium humate) were reapplied annually to the soil surface at sites A, B, and C. A deep-tilled mix control and a no-mix control were used at sites A and B; only a deep-till mix control was used at site C. The lime source at site D was dolomite and it was applied once only before planting. Groves at all sites were managed (fertilization, pest control, cultivation, and irrigation) according to current citriculture methods. Fertilizers applied followed the guidelines recommended by the Univ. of Florida for citrus (Koo, 1984).

Fibrous roots were dug from three trees per replicate of four replicates at

Table 1. Florida citrus groves treated with soil amendments and dates roots were examined for vesicular-arbuscular mycorrhizal (VAM) fungi.

Grove and location ^a	Date amendments applied	Date trees planted	Soil amendment or treatment applied	Rate (lb/acre)	VAM fungi analysis	Trees/replicate/no. replicates (plots)
Site A	Nov. 1985	Feb. 1986	Lime	50,000	Jan. 1987	9/4
			Phosphogypsum	10,000	Jan. 1987	
			Phosphoclay	80,000	Jan. 1987	
			Reed sedge peat	250,000	Jan. 1987	
Site B	Jan. 1986	Feb. 1986	Phosphogypsum	10,000	Mar. 1987	6/6
			Humate	77,612	Mar. 1987	
			Phosphoclay	80,000	Mar. 1987	
			Peat	250,000	Mar. 1987	
Site C	Aug. 1984	Feb. 1985	Gypsum	1,000	Nov. 1989	4/6
			Gypsum	2,000	Mar. 1986, Nov. 1989	
			Shrimp shell waste	18,263	Nov. 1989	
			Shrimp shell waste	73,052	Mar. 1986, Nov. 1989	
			Humate	14,610	Nov. 1989	
			Humate	73,052	Mar. 1986, Nov. 1989	
			Bentonite clay	73,052	Mar. 1986	
			Peat	233,765	Mar. 1986	
			Calcium humate	2,000	Mar. 1986	
Site D	Mar. 1970	Nov. 1970	Surface tillage	---	Sept. 1979	6/6
			Deep tillage	---	Sept. 1979	
			Deep tillage + lime	45,407	Sept. 1979	

^aSite A = Univ. of Florida Agricultural Experiment Station, Ft. Pierce; Site B = Ft. Meade, commercial grove; St. Cloud, commercial grove; Ft. Pierce, Univ. of Florida soil–water–atmosphere–plant (SWAP) experiment.

Table 2. Vesicular-arbuscular mycorrhizal (VAM) fungus infection in roots of 'Valencia' sweet orange on rough lemon from soil amended with four amendments in a Ft. Pierce, Fla., field plot (Site A), January 1987.

Treatment	Rate (lb/acre)	Vesicles ^a (0-3)	Hyphae ^a (0-3)	Infection (%)
Limestone	50,000	0.61 a	0.69 ab	64 a
Phosphogypsum	10,000	0.23 ab	0.18 bc	25 bc
Phosphoclay	80,000	0.29 ab	0.36 abc	33 abc
Peat	250,000	0.27 ab	0.32 abc	26 bc
Control	No till	0.03 b	0.06 c	6 c
Control	Deep-tilled	0.65 a	0.80 a	55 ab

^aRatings: 0 = no vesicles or hyphae, 1 = 1-50 vesicles and trace hyphae development, 2 = 51-100 vesicles and moderate hyphae, and 3 = >100 vesicles and abundant hyphae development. Treatments sharing the same small letter are not significantly different from one another at $P = 0.05$ by Duncan's multiple range test. Data are means of four treatment replicates, three trees per replicate, 100, 0.39-inch-long root pieces per replicate.

Table 3. Vesicular-arbuscular mycorrhizal (VAM) fungus infection in roots of 'Valencia' sweet orange on rough lemon from soil amended with four amendments in a Ft. Meade, Fla. field plot (Site B), March 1987.

Treatment	Rate (lb/acre)	Vesicles ^a (0-3)	Hyphae ^a (0-3)	Infection (%)
Phosphogypsum	10,000	0.75 a	0.95 a	71 a
Humate	77,612	1.06 a	1.38 a	81 a
Peat	250,000	0.81 a	1.09 a	76 a
Phosphoclay	80,000	0.82 a	1.07 a	73 a
Control	No till	0.77 a	1.02 a	64 a
Control	Deep-tilled	0.66 a	0.86 a	66 a

^aRatings: 0 = no vesicles or hyphae, 1 = 1-50 vesicles and trace hyphae development, 2 = 51-100 vesicles and moderate hyphae, and 3 = >100 vesicles and abundant hyphae development. Treatments sharing the same small letter are not significantly different from one another at $P = 0.05$ by Duncan's multiple range test. Data are means of four treatment replicates, three trees per replicate, 100, 0.39-inch-long root pieces per replicate.

Table 4. Vesicular-arbuscular mycorrhizal (VAM) fungus infection in citrus roots of high amendment rates at a St. Cloud, Fla., field plot (Site C), March 1986.

Treatment	Rate (lb/acre)	Vesicles ^a (0-3)	Hyphae ^a (0-3)	Infection (%)
Gypsum	2,000	0.46 ab	0.66 a	66.2 a
Humate	73,052	0.50 ab	0.61 a	61.3 a
Bentonite clay	73,052	0.40 ab	0.61 a	61.0 ab
Peat	233,765	0.48 ab	0.58 a	57.8 ab
Calcium humate	2,000	0.64 a	0.55 a	53.5 ab
Shrimp shells	73,052	0.31 ab	0.48 a	47.5 ab
Control	Deep-tilled	0.26 b	0.19 b	29.7 b

^aRatings = 0-3. 0 = no hyphae and no vesicles; 1 = trace hyphae infection and 1-50 vesicles; 2 = moderate hyphae development and 51-100 vesicles; 3 = abundant hyphae development and ≥101 vesicles. Treatments not sharing the same small letter are significantly different from one another using Duncan's multiple range test ($P = 0.05$). Data are means of three treatment replicates, three trees per replicate, 50, 0.39-inch-long root pieces per replicate.

site A, three trees per replicate of six replicates at site B, four trees of three replicates at site C, and two trees per replicate of three replicates from site D to a depth of 18 inches. They were dug from areas within the row that had received soil amendment treatments. They were pooled and a subsample taken and cut into ≈0.39-inch-long pieces and processed for VAM fungus infection by a modification of the Phil-

lips and Hayman (1970) technique that eliminated the use of phenol. Either 50 or 100 root pieces were examined per treatment replicate, depending on the site selected. All sites were sampled once, except site C, which was sampled twice. Vesicles and hyphae were rated on a scale of 0 to 3: 0 = none, 1 = 1-50 vesicles and trace hyphae development, 2 = 51-100 vesicles and moderate hyphae, and 3 =

>100 vesicles and abundant hyphae development. All tests were set up in a completely randomized block design. Data from all field tests were analyzed by analysis of variance and mean separations determined by Duncan's multiple range test at $P = 0.05$.

Results

Compared to the deep-tilled control, none of the amendments applied to site A affected VAM fungus colonization in roots (Table 2). The lowest VAM fungus development occurred in roots in the no-till control. Percentage infection was highest in the limestone treatment, about twice that present in the other soil amendment treatments. Fungus development was higher in roots of plants at site B (Table 3) than at site A, but no treatment significantly influenced fungus development compared to other treatments.

In the first sampling (March 1986) from the high rates of amendments at site C (Table 4), percentage infection ranged between 29.7% and 66.2%. Infection in the gypsum and humate treatments were significantly higher than infection in the deep-tilled control. Hyphal development was significantly higher in all treatments compared to the control, but only one treatment—calcium humate—contained vesicle numbers significantly higher than the control. In the second sampling of site C (November 1989), from both rates of gypsum, shrimp shells, and humate, infection was overall lower than during the first sampling, ranging from 14.7% in the 73,052-lb/acre rate of shrimp shells to 47.3% in the 18,263-lb/acre rate of that treatment. Infection, vesicle, and hyphal ratings from the second sampling were all nonsignificant. The residual effect from the original application of shrimp shells and the effects from reapplication of gypsum and humate annually had no effect on fungus development 3 years after the beginning of this test.

DT and DTL at site D (Table 5) did not alter fungus development compared to surface tillage 9 years after treatment, but percentage infection was highest in the roots at this site compared to the other sites studied.

Discussion

VAM fungi are found in tropical, temperate, and arctic soils, and are virtually ubiquitous. Some individual spe-

Table 5. Vesicular-arbuscular mycorrhizal (VAM) fungus infection in roots of 'Pineapple' sweet orange on rough lemon from soil managed by deep and surface tillage and deep tillage plus lime in the soil-water-atmosphere-plant (SWAP) grove (Site D) Ft. Pierce, Fla.

Treatment	Vesicles ^a (0-3)	Hyphae ^a (0-3)	Infection (%)
Surface tillage	0.89 b	1.92 ab	94.0 a
Deep tillage	1.22 a	2.24 a	94.0 a
Deep tillage + lime	1.14 ab	1.81 b	95.3 a

^aRatings = 0-3. 0 = no hyphae and no vesicles; 1 = trace hyphae infection and 1-50 vesicles; 2 = moderate hyphae development and 51-100 vesicles; 3 = abundant hyphae development and ≥101 vesicles. Treatments not sharing the same small letter are significantly different from one another using Duncan's multiple range test ($P=0.05$). Data are means of three treatment replicates, three trees per replicate, 50, 0.39-inch-long root pieces per replicate.

cies have a worldwide distribution (Gerdemann, 1975). In cultivated soils, VAM fungi are affected by various agricultural and horticultural practices, particularly fertilizer additions, pesticide applications, and crop rotations (Hayman, 1982).

Although a considerable base of information has been accumulated about effects of pesticides, mineral fertilizers, and crop rotations on VAM balances in soil, very little information has been generated on soils modified with amendments and by tillage. Generally, treatments such as mulches and limestone (Mosse, 1972a), fertilizers, or other additives to soil that improve the crop-growing potential of poor or marginal soils or correct soil pH (Azcon et al., 1978; Crush, 1975; Harinikumar and Bagyaraj, 1988; Mosse, 1972b; Nappi et al., 1980; Sabet, 1945;) enhance the infection of VAM in roots and production of spores in the soil. Similarly, amendments such as sludges and mulches applied for land reclamation confer improvements in pine growth and development of ectomycorrhiza (Marx et al., 1979; Berry and Marx, 1980; Berry, 1982).

Evidence from this study indicates that soil amendments used in all sites had no detrimental effect on indigenous VAM fungus development in roots and that a few amendments at site C improved infection and fungus development. Increased infection induced by gypsum at site C may have been due to its reduction of *P. parasitica* symptoms the first 3 years of the test (Nemec and Lee, 1992). Four of these soil amendments—phosphogypsum, phosphoclay, humate, and calcium humate—have been used in limited greenhouse pot experiments to determine their effects on infection in citrus. They did not affect infection of *Glomus* spp. any more than they did

in these field plots (unpublished data).

Tillage is used in crop production to prepare soil, control weeds, aerate soils, and incorporate minerals and fertilizers into soil. Results of a few studies addressing this topic and mycorrhizas provided mixed results. Physical soil disturbance disrupted P uptake by young maize (Miller et al., 1994) because mycelia connections from the root to the soil were broken, and tillage reduced the number of chlamydospores more than chemical fallow (Yocum et al., 1985). Tillage in other studies (Douds et al., 1995; Zhai et al., 1990), as in this one, had no significant effect on mycorrhizas.

It is essential that VAMs in agricultural soils be conserved. The beneficial impact they have on plants ranges from improved nutrient uptake to biological control. The observations made in this study support the limited information available on the effects soil amendments have on mycorrhizas. With heightened concern by municipalities about solid waste disposal and the shift toward a more holistic agriculture away from pesticides, more uses for organic wastes in agriculture will be found. The results of this study suggest that organic wastes will conserve and perhaps enhance mycorrhiza activity in soils and plant roots.

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Bloom Thinning of 'Loadel' Cling Peach with a Surfactant: Effects of Concentration, Carrier Volume, and Differential Applications within the Canopy

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ADDITIONAL INDEX WORDS. *Prunus persica*, fruit thinning, fatty amine polymer, hand thinning, fruit set

SUMMARY. In 1994, we established that a surfactant, Armothin (AR), reduced fruit set when applied as 3% and 5% AR at 100 gal/acre with a Stihl mistblower to 'Loadel' clingstone peach [*Prunus persica* (L.) Batsch]. In 1995 we compared 3% AR at volumes of 100 and 200 gal/acre (935 and 1870 L·ha⁻¹, the volumes most commonly used by tree fruit growers in California) applied with commercial airblast sprayer; overthinning resulted with the latter. In 1996, we applied 3% AR at 100 gal/acre and 1% AR at 200 gal/acre. In 1995, differential applications of 3% AR at 100 gal/acre (two-thirds of the material applied to either the upper or lower canopy) reduced fruit

set in the upper canopy in proportion to the amount of chemical applied (twice as much fruit set reduction with twice as much chemical); fruit set in the lower canopy was reduced by an equal amount regardless of amount of chemical used. Salable yields, equivalent to those obtained by hand thinning, and improved fruit size were achieved with all treatments of 3% AR at 100 gal/acre in 1995 with a 76% reduction in hand thinning. Following a low-chill winter (1995-96) with a protracted bloom, flower bud density (return bloom) was significantly greater in 1995 AR-treated trees. In 1996, treatment with AR did not result in fruit set reduction due to the protracted bloom and poor weather conditions before and after bloom. Nonetheless, 1% AR at 200 gal/acre applied in 1996 increased salable yield and increased final fruit mass. Return bloom in 1997 was equal among 1996 treatments.

Thinning blossoms provides early adjustment of the fruit-to-leaf ratio (Havis, 1962), increasing fruit size, which is particularly desirable in early ripening peach cultivars (e.g., 'Loadel') that have a short fruit developmental period and fruit sizing problems (Southwick et al., 1996). Reducing flower buds by surfactants during the latter part of bloom resulted in improved fruit size at harvest in some treatments (Byers and Lyons, 1982, 1985). Under California conditions of low frost and generally good weather at bloom, cling peach fruit set is usually excessive, necessitating heavy thinning. Hand thinning is time-consuming and typically incurs costs in excess of \$283/acre (\$700/ha) in early maturing cling (and freestone) peaches (Southwick and Fritts, 1995). Although bloom thinning may cause overthinning in poor weather conditions at or shortly after bloom, the potential advantages of such a program and lack of suitable chemical thinning agents for small fruit removal warrant development of chemical alternatives to traditional hand thinning at reference date (pit hardening + 10 days). Work by Southwick et al. (1995) has led to the use of gibberellins for flower reduction, which reduces the need for hand thinning. However, growers are unsure of this practice and view it as risky. Initial work (Southwick et al., 1996) suggested that Armothin (AR) [(N,N-bis

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