Effect of Fungicide Applications on Populations of *Phytophthora parasitica* and on Feeder Root Densities and Fruit Yields of Citrus Trees

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ABSTRACT

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Fungicide treatments for control of feeder root rot of citrus caused by *Phytophthora parasitica* were made for 3 yr in four Florida orchards. Treatments consisted of foliar applications of fosetyl-Al at high (FOS-H) and low (FOS-L) frequency and soil applications of metalaxyl (MET). Propagule densities were positively correlated with feeder root densities in some orchards, especially in plots treated with fosetyl-Al. Expression of propagule densities on a root weight rather than a soil volume basis permitted more meaningful comparisons of fungicide effects on soil populations of the fungus. Propagule densities of *P. parasitica* were lower in the metalaxyl treatments and in some cases were lower in the fosetyl-Al treatments than in the untreated controls. Feeder root densities in the four orchards over all 3 yr increased by an average of 29.6, 9.4, and 43.5% above the untreated controls in the FOS-H, FOS-L, and MET treatments. Total fruit and juice yield was increased by the FOS-H and MET treatments in one orchard and by the MET treatment in another orchard, and average fruit weight was increased by all fungicide treatments in a third orchard. Tree appearance was significantly improved by the treatments in three of the four orchards. Feeder root loss in citrus due to infection by *P. parasitica* appears to be substantial and is corrected by applications of fosetyl-Al or metalaxyl. However, the lack of consistent tree response to treatment indicates that fungicide applications may not be economically justifiable in many cases.

Phytophthora foot rot, gummosis, and root rot of citrus are major problems in citrus orchards worldwide (14). The foot rot problem is usually controlled by budding the susceptible scion cultivars on resistant rootstocks, by maintaining bud unions at least 15 cm above the soil line, and by keeping the trunk of the tree dry (5,18). Preventive and curative fungicide applications made as soil treatments, trunk paints, and foliar sprays can effectively control aboveground foot rot and gummosis (2,3,7,11,13).

Feeder root rot, caused by *Phytophthora* spp., is a common problem in citrus nurseries (5,15), and about one-half of the field nurseries assayed in Florida were infested with *Phytophthora parasitica* Dast. (20). The commonly used rootstocks such as sour orange, trifoliate orange, Cleopatra mandarin, Troyer and Carrizo citranges, and Swingle citrumelo range from tolerant to nearly immune to Phytophthora foot rot, gummosis, and scaffold root rot (15,19). When they are overwatered in infested nurseries or inoculated as young seedlings in screening tests for resistance, however, most of these species and cultivars suffer serious root rot (1,4,19). Applications of metalaxyl and fosetyl-Al have proved highly effective for control of feeder root rot problems in nurseries (2,3,9).

Very little is known about the incidence and importance of feeder root rot on bearing citrus trees. Even though many rootstocks are resistant to bark infection, they could be susceptible to feeder root infection. Some rootstocks might tolerate root rot by rapid regeneration of new roots, but Carpenter and Furr (1) found little evidence of differences among cultivars and species in root regenerating ability. Before the development of fosetyl-Al and metalaxyl, no chemical means were available to control root rot and thus determine its impact on tree performance and fruit yield. Recent reports from California (8,10) indicated that feeder root loss from infection by *Phytophthora* spp. could be reduced by fungicide applications and

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fruit yields increased on the highly susceptible sweet orange rootstock.

This study was designed to determine the effect of fosetyl-Al and metalaxyl applications on soil populations of *P. parasitica* and on the feeder root densities, tree condition, and fruit yield of citrus trees on different rootstocks.

MATERIALS AND METHODS

Locations. The four orchards selected for tests were at least 15 yr old and all showed signs of mild decline that could have been attributable to feeder root loss, since all had moderate to high populations of *P. parasitica.* The citrus nematode, *Tylenchulus semipenetrans* Cobb, was found in high populations in one orchard, but that orchard was treated with aldicarb to reduce possible decline due to that factor.

The sites used were: 1) a Pineapple sweet orange (*Citrus sinensis* (L.) Osb.) orchard on Cleopatra mandarin (*C. reshni* Hort. ex Tan.) rootstock planted on a spacing of 7.6 × 4.6 m on an Oldsmar fine sand near Immokalee; 2) a Ruby Red grapefruit (*C. paradisi* Macf.) orchard on sweet orange rootstock planted on a spacing of 9.1 × 6.7 m on a Winder sand near Fort Pierce (Fort Pierce I); 3) a Hamlin sweet orange orchard on sour orange (*C. aurantium* L.) rootstock planted on a spacing of 8.2 × 6.1 m on Pineda sand near Fort Pierce (Fort Pierce II); and 4) a Hamlin sweet orange orchard on sweet orange rootstock planted on a spacing of 8.2 × 8.2 m on a Tavares fine sand near Lakeland.

Treatments. The four treatments were: 1) foliar applications of fosetyl-Al (Aliette 80WP) at high frequency (FOS-H), 2) foliar applications of fosetyl-Al at low frequency (FOS-L), 3) soil applications of metalaxyl (Ridomil 2E) (MET), and 4) no fungicides (control).

At the Immokalee and the two Fort Pierce locations, fosetyl-Al applications were made with concentrate speed sprayers that applied 1.1–2.5 hl/ha of spray mix, depending on the type of sprayer. At the Lakeland site, fosetyl-Al was applied only as a high-frequency treatment with a handgun sprayer, using about 11.3 hl/ha spray mix. At all four locations, fosetyl-Al was applied at 5.61 kg/ha of Aliette 80WP (4.55 kg a.i./ha). At the Immokalee and the two Fort Pierce sites, metalaxyl applications were made as soil surface sprays using 4.61 L/ha of Ridomil 2E (1.1 kg a.i./ha of treated surface). Applications were made using 1.4–2.7 hl of spray material per hectare of treated surface area, depending on the tree spacing; approximately 50% of the orchard soil surface area was treated. At the Lakeland location, metalaxyl was applied as a soil drench in basins formed around the drip line of each tree using 24.5 L/ha of Ridomil 2E (5.9 kg a.i./ha) applied in about 71 hl/ha of water.

Fungicide application dates were in spring (March-April), early summer (May-June), late summer (July-August), and fall (September-October). The FOS-H applications were made on all dates in 1985 and 1986 and in spring, early summer, and late summer in 1987. The FOS-L applications were made in early and late summer in 1985 and 1986 and in spring, early summer, and late summer in 1987. The MET applications were made in spring, late summer, and fall in 1985; early summer, late summer, and fall in 1986; and early and late summer in 1987.

Each fungicide treatment at the Immokalee and the two Fort Pierce locations was replicated five times on four-tree plots arranged in a randomized complete block design. Each plot consisted of four trees in a row, with a single buffer tree between the plots. An untreated buffer row was left between the treated rows. At the Lakeland site, affected trees were scattered throughout the orchard, and 30 trees in mild decline and 30 in moderate decline were selected for the test. Blocks of 12 trees, each containing six trees in mild decline and six trees in moderate decline, were chosen, and the FOS-H, MET, and control treatments were randomly assigned to two trees in mild decline and two trees in moderate decline. Five replicate blocks were used.

Data collection. For determination of propagule densities of *P. parasitica* and root densities, sample collection and handling methods developed previously were used (16). Soil cores were collected with a standard auger at the drip line of the trees and passed through a screen with 3-mm openings to separate roots from soils. Feeder roots, defined as those less than 2 mm in diameter, were dried to a constant weight at 60 C, and root density was expressed as milligrams of dry weight per cubic centimeter of soil. Soil was moistened and incubated at room temperature (21–23 C) for 1–5 days. A 10-cm^3 subsample was suspended in 0.25% water agar, and 1 ml was plated on each of five plates of PAR medium (6,16). After incubation at 28 C for 3 days, the plates were washed and the colonies counted.

Propagule densities were calculated per cubic centimeter of soil volume and per milligram of root. Propagule and root densities were determined for a composite sample of four to six cores taken within each replicate plot of each treatment on each sample date. Simple correlation coefficients between root and propagule densities were calculated within each fungicide treatment.

Canopy condition was rated on a scale of 0 = healthy to 3 = severe decline in the fall of the year at each site, considering primarily the foliage density and leaf size.

Trees were harvested and the fruit yield of each plot was determined annually after maturity had been reached for each cultivar. Hamlin oranges were harvested in December-January, Pineapple oranges in February-March, and grapefruit in April-May. At least 40 fruit were collected at random within each plot, and the average fruit weight was determined. The juice was extracted and the percent juice, total soluble solids, percent acid, and other juice characteristics were determined by a computerized citrus juice analyzer (Toledo Scales, Toledo, OH). The total soluble solids produced per hectare was calculated from the fruit yield, juice percentage, and the percent soluble solids in the juice.

RESULTS

The propagule densities of *P. parasitica* per unit volume of soil varied greatly with sample date in all orchards but generally were moderate to high in 1985, low in 1986, and high again in 1987 (Fig. 1). Root densities were less variable and showed no particular trend with time in any orchard throughout the experiment. Root densities were generally higher on fungicide-treated trees, but the pattern over time was similar in treated and untreated plots (Fig. 1).

There was a significant positive correlation between root density and propagule density in the FOS-H-treated plots in three of the four orchards in the test (Fig. 1). In contrast, root and propagule densities in the control and in the MET-treated plots were significantly correlated in only one of the four orchards tested.

Over the 3-yr test period, the number of propagules per cubic centimeter of soil in the MET treatment was significantly lower than in the controls in three of the four tests conducted (Table 1). The number of propagules per cubic centimeter of soil in the FOS-H and the FOS-L treatments was significantly lower than in the controls at the Fort Pierce II and Immokalee sites. Feeder root densities in the FOS-H and MET treatments were significantly higher than in the controls at all four sites tested. Feeder root densities in the FOS-L were significantly greater than in the controls only at the Immokalee location. However, when propagule densities were expressed on the basis of root weight rather than soil volume, the propagule densities in the fungicide-treated plots were significantly lower than in the controls in almost every case (Table 1).

Canopy condition ratings were consistently lower in the MET and FOS-H treatments than in the control in the Immokalee and both Fort Pierce locations (Table 2). In addition, canopy condition rating in the FOS-L treatment was lower than in the control at the Fort Pierce I site. There was no significant difference in canopy rating between fungicide-treated and control trees at the Lakeland site.

The average fruit yield per tree was greater in the FOS-H and the MET treatments than in the controls at the Immokalee site, and yield was greater in the MET treatments than in controls at the Fort Pierce II site. At the other locations, the yield of the treated trees was occasionally significantly higher than the untreated controls in an individual year but was not significantly greater over all years collectively (Table 2). When total soluble solids were taken into consideration, results were similar,

Table 1. Mean propagule densities of *Phytophthora parasitica* and feeder root densities in four Florida citrus orchards treated with fosetyl-Al and metalaxyl

		Propagules/cm^3[x]				Root density (mg/cm^3)				Propagules/mg root			
Orchard	Fungicide	1985-86	1986-87	1987-88	$\bar{x}$	1985-86	1986-87	1987-88	$\bar{x}$	1985-86	1986-87	1987-88	$\bar{x}$
Immokalee	Fosetyl-Al(H)[y]	19.0*[z]	7.3	22.8*	16.4*	0.62	0.49*	0.57*	0.56*	33.9*	16.2*	37.4*	29.2*
	Fosetyl-Al(L)[y]	24.2	7.6	27.6*	19.8*	0.65*	0.46*	0.56*	0.56*	41.9*	17.1*	46.9*	35.3*
	Metalaxyl	23.8	3.4*	13.4*	13.6*	0.60	0.56*	0.80*	0.66*	41.7*	6.7*	15.4*	21.3*
	Control	27.8	8.4	33.0	23.0	0.59	0.40	0.45	0.48	57.1	30.2	78.5	55.3
	LSD$_{0.05}$	4.5	1.8	4.1	2.1	0.04	0.03	0.04	0.02	10.0	9.4	7.9	5.2
Fort Pierce I	Fosetyl-Al(H)	5.6*	0.5*	33.0	13.0	0.73	0.71*	1.04*	0.83*	9.2*	0.7*	34.9*	15.0*
	Fosetyl-Al(L)	11.0	0.2*	29.3	13.5	0.64	0.54	0.71	0.63	18.1	0.2*	76.4	31.6
	Metalaxyl	6.5	0.4*	27.6	11.5	0.74	0.67*	0.95*	0.79*	15.1	0.6*	31.1*	15.6*
	Control	8.3	0.8	26.0	11.8	0.65	0.57	0.63	0.61	19.2	2.5	73.6	31.8
	LSD$_{0.05}$	2.2	0.2	NS	NS	NS	0.05	0.10	0.05	6.4	0.7	24.0	8.2
Fort Pierce II	Fosetyl-Al(H)	17.8*	8.2*	19.0*	15.0*	0.60	0.48*	0.56*	0.55*	33.7*	17.9*	36.0*	29.2*
	Fosetyl-Al(L)	20.0	7.0*	23.2*	16.8*	0.61*	0.41	0.55*	0.52*	35.8*	15.9*	42.8*	31.5*
	Metalaxyl	18.0*	3.8*	13.4*	11.8*	0.67*	0.64*	0.82*	0.71*	27.8*	6.5*	15.3*	16.6*
	Control	22.4	15.5	37.0	24.9	0.56	0.43	0.45	0.48	42.7	39.0	84.6	55.4
	LSD$_{0.05}$	3.1	1.9	4.0	1.8	0.04	0.03	0.03	0.02	5.9	4.7	9.0	3.8
Lakeland	Fosetyl-Al(H)	35.8	19.6	11.4*	22.3	0.84*	0.66*	0.61*	0.70*	45.0*	36.4*	21.7*	34.3*
	Metalaxyl	27.4*	10.6*	9.2*	15.8*	0.78*	0.88*	0.69*	0.77*	45.4*	14.3*	20.3*	26.7*
	Control	34.8*	17.4	20.4	24.2	0.60	0.44	0.42	0.49	72.2	47.3	50.1	56.5
	LSD$_{0.05}$	6.1	2.3	3.2	2.4	0.08	0.07	0.10	0.05	18.2	7.2	8.9	7.0

[x] Means for each year are the average of five sampling times in five replications of four trees each.

[y] H = high frequency, usually four applications per year; L = low frequency, usually two applications per year.

[z] * = Significantly different from the untreated control at $P \leq 0.05$; NS = not significant.

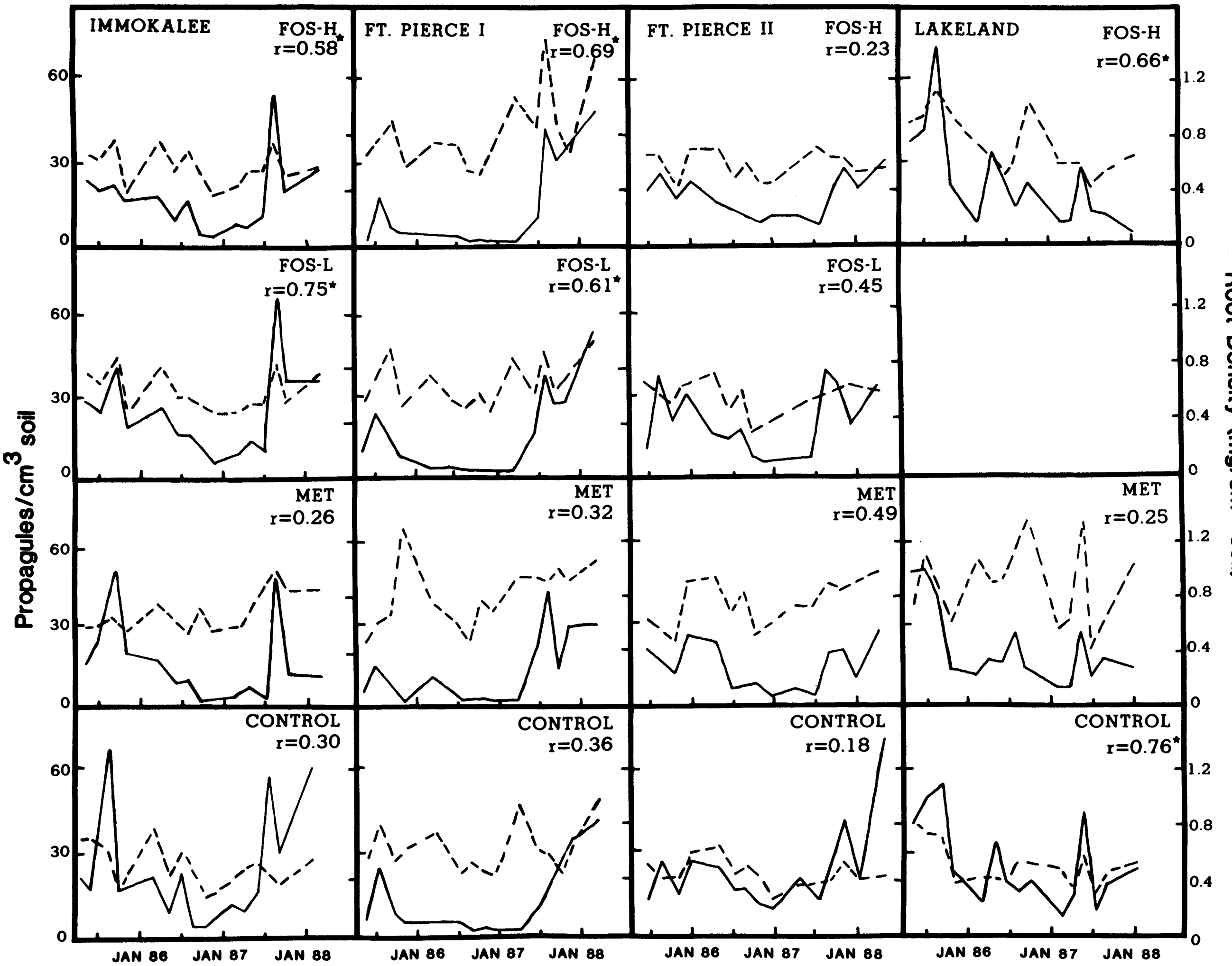

Fig. 1. Propagule densities of *Phytophthora parasitica* (solid lines) and root densities of citrus trees (dashed lines) treated by high-frequency foliar applications of fosetyl-Al (FOS-H), low-frequency applications of fosetyl-Al (FOS-L), or soil applications of metalaxyl (MET) or untreated control trees at four orchard sites; the FOS-L treatment was not applied at the Lakeland site. The simple correlation coefficient (*r*) for propagule vs. root density was calculated for each treatment at each site. * = Significant at $P \leq 0.05$, ** = significant at $P \leq 0.01$.

with the MET and FOS-H treatments having higher yields than the controls at the Immokalee site and the MET treatments having higher yields at the Fort Pierce II site. All three fungicide treatments had significantly greater average fruit weight of grapefruit than the controls at Fort Pierce I, but the weight of oranges at the other three locations did not differ between treatments.

DISCUSSION

The positive relationship between propagule densities of *P. parasitica* and citrus feeder root densities was especially apparent on trees treated with fosetyl-Al. In previous work (16), we found no relationship between root and propagule densities when samples were collected in areas where feeder roots were present. In that study (16), the comparisons were made on a sample-to-sample basis, whereas in this study, correlations were made on a time course basis. Since correlations were always positive and often significant (Fig. 1), it appears that high populations may result from the presence of abundant feeder roots that provide substrate for multiplication of *P. parasitica.* It appears conceivable that under some circumstances, fungal populations may be high where roots are still growing vigorously and low where root rot has substantially reduced feeder root density at some time in the past. Propagule densities also vary from one year to the next in the same orchard and with sampling time during the year but are never consistently high in any one season (17). Thus, establishing a threshold for treatment may be more difficult than previously anticipated (8,12,14). Propagule densities may be a better indicator of favorable conditions for feeder root rot in the recent past than a predictor of future damage.

Fungicide treatment greatly affected the relationship between root and propagule densities. When applied as a foliar spray, fosetyl-Al is translocated downward to the roots and prevents infection (2), but it probably has no direct effect on propagules in the soil. This product presumably increased feeder root densities by reducing root rot. Where fosetyl-Al treatments prevented increases in soil populations of *P. parasitica* in these tests, they probably did so by limiting the multiplication of the fungus on treated roots. Thus, differences in propagule densities on fosetyl-Al-treated trees seemed more substantial when densities were expressed on the basis of root weight rather than soil volume.

In contrast to fosetyl-Al, metalaxyl directly kills the fungus in the soil (2,3) and thus reduces the number of propagules per unit volume of soil. By reducing infection, it also increases feeder root densities. When propagule densities were expressed on a root weight basis, the effect of metalaxyl also appeared more substantial. Because fungicide treatments increase feeder root densities and thus the amount of substrate available for multiplication of the fungus, expression of densities on a soil volume basis minimizes the apparent disease control. In a previous study (14), we saw no advantage to expression of propagule densities on a root weight basis, since variance of propagule densities was similar with both means of expression. However, expression of densities on a root weight basis seems more appropriate when comparing fungicide treatments.

The FOS-H, FOS-L, and MET treatments increased feeder root density over that of the untreated controls an average of 29.6, 9.4, and 43.5%, respectively, when considered across all orchard sites and across all years. However, the increase in feeder roots was not accompanied by a corresponding increase in total fruit yield in all cases. Where citrus is produced primarily for juice, the yield in total soluble solids per hectare is of primary importance. Fungicide treatment had small significant effects on various juice quality characteristics in some years, but there was no consistent pattern (*data not shown*). When fruit quality as well as quantity was taken into consideration, yield was affected by fungicide treatments again only at the Immokalee and Fort Pierce II sites. Fruit size is an important characteristic for producers of fresh market fruit. In these tests, all fungicide treatments produced small, but consistent, increases in average weight of grapefruit at the Fort Pierce I site but did not affect orange sizes at the other three sites.

The lack of an increase in productivity of citrus trees at some sites in response to the increased feeder root densities brought about by fungicide treatment is difficult to explain. At the Immokalee site, where yields were increased, the improvement in feeder root densities was no greater than at the other sites (Table 1). The orchard in which a yield response was observed was on Cleopatra mandarin rootstock, which is considered more tolerant to *Phytophthora*-induced diseases than sweet orange rootstock (15), the rootstock used at the Fort Pierce I and Lakeland sites, where no yield response was found.

Fungicide treatment significantly reduced populations of *P. parasitica* and increased root densities compared with the untreated controls, but the benefits of treatment were not great in most cases. Thus, a general recommendation for treatment of orchards where feeder root rot occurs cannot be made. We and others (8,12) have suggested that propagule densities may be used as a guide in decisions on whether or not to initiate treatment programs, and that the thresh-

Table 2. The effect of fungicide treatment for control of Phytophthora root rot on tree condition and yield parameters in four citrus orchards in Florida

		Tree decline rating[w]				Yield (kg/tree)				Yield (kg soluble solids/ha)[x]				Average fruit weight (g)			
Orchard	**Fungicide**	**1985–86**	**1986–87**	**1987–88**	$\bar{x}$	**1985–86**	**1986–87**	**1987–88**	$\bar{x}$	**1985–86**	**1986–87**	**1987–88**	$\bar{x}$	**1985–86**	**1986–87**	**1987–88**	$\bar{x}$
Immokalee	Fosetyl-Al(H)[y]	1.2	1.1*[z]	0.5	0.9*	123*	160*	107	130*	3,126*	3,987*	2,521	3,211*	165	168*	165	166
	Fosetyl-Al(L)[y]	1.2	1.3	0.5	1.0	92	147*	103	114	2,204	3,429*	2,354	2,662	166	166*	162	165
	Metalaxyl	1.1	0.8*	0.2*	0.7*	127*	156*	139*	141*	3,273*	3,847*	3,228*	3,449*	165	169*	166	167
	Control	1.3	1.4	0.8	1.2	97	119	88	101	2,327	2,949	2,088	2,454	168	157	162	162
	LSD$_{0.05}$	NS	0.20	0.30	0.25	18	15	22	19	440	342	495	396	NS	7.0	NS	NS
Fort Pierce I	Fosetyl-Al(H)	1.5	1.4	1.3*	1.4*	233	184*	212	210	2,931	2,446*	2,980	2,786	357*	384*	357*	365*
	Fosetyl-Al(L)	1.5	1.4	1.2*	1.4*	210	157	212	193	2,575	1,948	2,923	2,486	368*	376	355*	366*
	Metalaxyl	1.6	1.1*	1.0*	1.2*	216	170	213	200	2,648	2,335*	2,903	2,629	377*	384*	371*	377*
	Control	1.6	1.5	1.6	1.6	238	156	193	196	3,006	2,035	2,601	2,547	335	362	328	342
	LSD$_{0.05}$	NS	0.21	0.17	0.13	NS	22	NS	NS	NS	290	NS	NS	15	18	15	8
Fort Pierce II	Fosetyl-Al(H)	1.6	1.1*	0.5	1.0*	75	101	140	105	1,169*	1,679	2,321	1,723	188	150*	136	158
	Fosetyl-Al(L)	1.5	1.2*	0.6	1.1	51	113	133	99	759	1,833	2,174	1,588	177	150*	136	154
	Metalaxyl	1.3	0.9*	0.2*	0.8*	82*	135*	161*	126*	1,235*	2,212	2,587	2,011*	180	143	142*	155
	Control	1.5	1.5	0.5	1.2	59	123	149	111	863	2,063	2,448	1,791	187	139	134	153
	LSD$_{0.05}$	NS	0.26	0.13	0.13	16	11	11	7	242	NS	NS	108	NS	4	4	NS
Lakeland	Fosetyl-Al(H)	1.5	1.3	1.2	1.3	439	312	218	323	4,454	3,437	2,349	3,413	143	149	141	144
	Metalaxyl	1.6	1.0	1.1	1.2	431	319	267	339	4,493	3,555	2,877	3,642	147	150	149	149
	Control	1.6	1.1	1.3	1.3	463	301	262	342	4,748	3,321	2,788	3,619	144	154	147	148
	LSD$_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[w] Trees rated on a scale from 0 = healthy to 3 = severe decline.

[x] Yield of juice per hectare × percent total soluble solids (sugar).

[y] H = high frequency, usually four applications per year; L = low frequency, usually two applications per year.

[z] * = Significantly different from the untreated control at $P \leq 0.05$; NS = not significant.

old for treatment should be approximately 10–15 propagules per cubic centimeter of soil. Because the relationships between fungal populations, feeder root densities, and yields are not straightforward, establishment of a firm threshold level may not be possible. More research is needed on the dynamics of populations of *P. parasitica*, feeder root turnover, and citrus tree health and productivity before sound recommendations can be made.

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