Influence of Metalaxyl on Three Nematodes of Citrus[1]

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Abstract: Metalaxyl significantly reduced populations of *Pratylenchus coffeae, Radopholus similis,* and *Tylenchulus semipenetrans* in roots of *Citrus limon* (rough lemon) under greenhouse conditions. Postinoculation treatment of roots of rough lemon seedlings was not as effective in reducing nematode populations as was treatment before inoculation. Fewer nematodes infected metalaxyl-treated roots than nontreated roots. However, incubation of nematodes in metalaxyl did not inhibit nematode motility or their ability to locate and infect roots. Cellular responses to nematode injection differed between treated and nontreated tissues. Metalaxyl appeared to confer nematode control by modifying citrus roots such that a normally susceptible rootstock became tolerant. *Key words: Pratylenchus coffeae, Radopholus similis, Tylenchulus semipenetrans,* chemical control.

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Metalaxyl, *N*-(2,6-Dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester, which is effective in controlling *Phytophthora* foot and root rot (1,2,3,5), was registered for use in Florida on citrus for the control of *Phytophthora parasitica* in 1980. Interest in studying the influence of metalaxyl on nematodes of citrus was generated when preliminary studies suggested that this acyl alanine compound might control *Heterodera glycines* (Dropkin, personal communication). The purpose of this study was to determine if metalaxyl adversely influenced the development of *Pratylenchus coffeae, Radopholus similis,* and/or *Tylenchulus semipenetrans* in citrus and the manner in which metalaxyl reduced nematode populations.

MATERIALS AND METHODS

The influence of metalaxyl on nematode population development in *Citrus limon* L. (rough lemon) roots was studied in glasshouse experiments. Six-month-old rough lemon seedlings were transplanted into 20-cm pots containing either steam-sterilized Astatula fine sand (AST) (hyperthermic, uncoated; typic quartzipsamments) or a steam-sterilized soil mix (MIX) (AST:vermiculite:peat, 2:1:1). Seedling height was recorded at time of transplanting. AST was infested with 500 *P. coffeae* or 250 *R. similis;* MIX was infested with 4,000 *T. semipenetrans.* Adults and juveniles of *R. similis* and *P. coffeae* and juveniles and males of *T. semipenetrans* were added as aqueous suspensions to a furrow in the center of each pot. The experiment was maintained under *Phytophthora parasitica*-free conditions.

Treatments were (i) seedling root systems incubated in an aqueous solution of 112 ppm metalaxyl for 2 hours at 26 C, rinsed free of excess test material, and transplanted into infested soil; (ii) infested soil drenched with 250 ml of 112 ppm metalaxyl 10 days before transplanting; (iii) infested soil drenched with 250 ml of 112 ppm metalaxyl 31 days after transplanting; and (iv) no metalaxyl applied to trees growing in infested soil. A second set of seedlings grown in noninfested soil also received these treatments. Upon completion of all treatments, the pots (six replicates/treatment) were randomized and plants were grown for an additional 6 months at 26 C ± 4.

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At harvest, increases in plant growth were determined by measuring the area (cm^2) of leaves produced during the experiment with a leaf surface area meter (Li-Cor, Model LI-3000). Root systems were weighed and the general root condition was noted. Fibrous roots were removed from seedlings and rinsed free of excess soil, and nematodes extracted for 7 days at 26 C (6). Nematodes were recovered in 50 ml of water and counted. The roots were dried for 24 hours at 76 C and weighed, and data expressed as nematodes per gram root dry weight. Following a preliminary experiment, the experiment was repeated twice. Data presented are from one experiment. The commercial carrier of metalaxyl (35 ppm) was also evaluated to determine its influence on nematode population development in a similar but separate experiment.

The influence of metalaxyl on nematode infection of citrus roots was studied in the laboratory. Root systems of 6-month-old seedlings were incubated in either 112 ppm metalaxyl or water (0 ppm) for 2 hours. Root systems were then rinsed free of excess test solution and equal numbers of intact fibrous roots were distributed among plastic cups (30-ml capacity), which were then filled with either AST or MIX. Ten cups (replicates) of each treatment were inoculated with 250 *P. coffeae,* 120 *R. similis,* or 1,000 *T. semipenetrans.* After 7 days at 26 C, the roots from each cup were removed, lightly rinsed free of excess soil, and weighed. Roots were stained in cold acid fuchsin in lactophenol for 72 hours, rinsed free of excess stain with water, and destained for 24 hours in clear lactophenol. Roots were then diced and ground in 40 ml H_2O for 1.5 min with a tissue homogenizer. The slurry was passed through a 250-μm and a 38-μm sieve. Nematodes were collected from the latter and centrifuged for 5 min at 4,850 × *g;* the supernatant was discarded, the pellet resuspended in 10 ml of water, and nematodes counted. Data are expressed as nematodes per gram root fresh weight. The experiment was repeated twice, and data from one experiment are presented.

The influence of metalaxyl on nematode infection was examined in a second set of experiments. Nematodes were incubated in metalaxyl (112 ppm), solvent (2.2% acetone in tap water, or 0.6% Ciba-Geigy commercial carrier), or water for 16 hours at 26 C on a rotary shaker (175 rpm). Intact rough lemon root systems were divided equally among six plastic cups per nematode species. Nematodes were rinsed free of test solution with tap water and added to the cups. Root systems were removed 7 days later and sample handling was similar to that described in the previous experiment, except that roots were not soaked in test materials. The experiment was repeated twice, and data from one experiment are presented.

The influence of metalaxyl on nematode motility (4) was studied by incubating *P. coffeae, R. similis,* and *T. semipenetrans* in tap water, 0.6% Ciba-Geigy commercial carrier, 2.2% acetone, and in 50, 100, 500, 1,000, and 2,000 ppm metalaxyl for 24 hours at 26 C. Treatments (1,000 nematodes/vial) were replicated 10 times, and the experiment was repeated three times. Data were expressed as reduced nematode motility in relation to control treatments (nematodes in tap water).

To determine if metalaxyl influences the plant-nematode interaction at the cellular level, intact root systems of rough lemon seedlings were soaked in 112 ppm metalaxyl, 35 ppm carrier, or water for 2 hours, rinsed free of excess test solution, planted in AST or MIX, and inoculated with *P. coffeae, R. similis,* or *T. semipenetrans.* Roots were harvested 2 weeks following inoculation, fixed in 10% alcoholic formalin, dehydrated through a TBA series, and embedded in paraffin. Serial sections (12 μm) were stained with safranin/fast green, and examined with a light microscope (160 ×–1,000 ×).

RESULTS

Metalaxyl (112 ppm) significantly reduced populations of *P. coffeae, R. similis,* and *T. semipenetrans* in citrus roots (Table 1). Incubation of seedling root systems for 2 hours before transplanting suppressed population development of all three nematode species and was the most effective method of metalaxyl application tested. Preplant and postplant drenching of nematode-infested soil reduced *R. similis*

Table 1. Influence of metalaxyl (112 ppm) on *Pratylenchus coffeae* (PC), *Radopholus similis* (RS), and *Tylenchulus semipenetrans* (TS).

Treatment*	Nematodes per gram root dry weight		
	PC	RS	TS
None	2319.7 a†	2228.0 a	2575.2 a
Post	1755.5 ab	984.0 b	1280.7 b
Pre	550.0 ab	431.8 c	727.3 bc
Dip	298.8 b	247.7 c	126.4 c

*None = no metalaxyl; post = drench of soil 31 days after planting; pre = drench of soil 10 days before planting dip = 2 hour incubation of root system in metalaxyl before planting. Experiment harvested 6 months after treatments completed.

†Values followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$). No nematodes were detected in noninoculated controls.

and *T. semipenetrans* but not *P. coffeae* populations. Nematodes were not detected in root systems of plants grown in non-infested soil. The commercial carrier of metalaxyl did not affect nematode population development (Kaplan, unpublished data).

Root growth of noninfected citrus seedlings was stimulated by metalaxyl when seedlings were either incubated in an aqueous solution (112 ppm) for 2 hours before planting or when potting media were drenched 10 days before planting (Table 2). Metalaxyl application 31 days after planting did not appear to influence root growth.

Student's *t*-test comparisons ($P = 0.05$) between infected and noninfected treatments indicated that neither *P. coffeae* nor *T. semipenetrans* significantly reduced root growth. However, lesions were apparent on *P. coffeae*-infected roots which received metalaxyl 31 days postinoculation or to which no metalaxyl was applied. *Radopholus similis* was associated with numerous brown roots and reduced root growth when metalaxyl was applied 31 days postinoculation, or when plants which did not receive metalaxyl. *Radopholus similis*- and *P. coffeae*-infected root systems were comparable in size and general appearance to noninfected root systems when metalaxyl was applied by either of the described preplant methods.

Metalaxyl did not significantly increase foliar growth of noninfected citrus seedlings grown in AST, although increased growth occurred when seedling root systems were incubated in metalaxyl and then planted in MIX (Table 3). *Tylenchulus semipenetrans* infection appeared to prevent increased foliar growth in the heavier soil type. Foliar growth of *P. coffeae*-infected citrus was not significantly different (Student's *t*-test, $P = 0.05$) from that of noninfested plants, with the exception of seedlings which underwent preplant incubation in metalaxyl. Trees which were inoculated with *P. coffeae* and whose root systems incubated in metalaxyl (112 ppm for 2 hours) had more new foliage than trees from any other treatment.

Radopholus similis-infected seedlings had significantly less foliar growth than seedlings of comparable treatments grown in noninfested Astatula fine sand except where metalaxyl was applied before plant-

Table 2. Influence of metalaxyl (112 ppm) on root growth of rough lemon seedlings infested with *Pratylenchus coffeae* (PC), *Radopholus similis* (RS), or *Tylenchulus semipenetrans* (TS).

Treatment*	Root growth (grams fresh weight)				
	PC/AST†	RS/AST	(—)/AST	TS/MIX	(—)/MIX
Dip	15.1 a‡	14.2 a	14.2 a	19.9 a	19.1 a
Pre	12.5 a	11.1 a	14.1 a	15.5 a	18.5 a
Post	7.5 b	3.8 b	8.2 b	13.4 a	11.6 b
None	5.6 b	3.4 b	7.4 b	10.1 a	8.6 b

*Dip = 2 hours incubation of root system in metalaxyl before planting; pre = drench of soil 10 days before planting; post = drench of soil 31 days after planting; none = no metalaxyl. Experiment harvested 6 months after treatments completed.

†AST = Astatula fine sand; MIX = Astatula fine sand:vermiculite:peat (2:1:1). (—) = no nematodes.

‡Values followed by the same letter in columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

Table 3. Influence of metalaxyl (112 ppm) on plant growth (leaf surface area:cm²) of rough lemon seedlings infested with *Pratylenchus coffeae* (PC), *Radopholus similis* (RS), and *Tylenchulus semipenetrans* (TS).

Treatment*	Leaf surface area (cm²)				
	PC/AST†	RS/AST	(—)/AST	TS/MIX	(—)/MIX
Dip	387.9 a‡	48.2 a	312.9 a	305.8 a	349.8 a
Pre	215.2 b	236.5 a	193.1 a	298.7 a	300.6 ab
Post	198.9 b	123.5 b	207.4 a	273.0 a	260.3 b
None	246.5 b	133.4 b	232.1 a	250.2 a	263.2 b

*Dip = 2 hours incubation of root system in metalaxyl before planting; pre = drench of soil 10 days before planting; post = drench of soil 31 days after planting; none = no metalaxyl. Experiment harvested 6 months after treatments completed.

†AST = Astatula fine sand; MIX = Astatula fine sand:vermiculite:peat (2:1:1). (—) = no nematodes.

‡Values followed by the same letter in columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

ing, as a soil drench or as a preplant seedling treatment. In those cases, treatment was associated with reduced nematode populations and larger root systems.

Significant reductions in infection of citrus roots by *P. coffeae* ($P = 0.1$), *R. similis*, and *T. semipenetrans* ($P = 0.01$) (Table 4) occurred when roots were incubated in metalaxyl before inoculation. Conversely, incubation of inoculum in metalaxyl (112 ppm) before inoculation did not reduce the numbers of nematodes infecting roots (Table 5). Nor did metalaxyl (112 ppm) inhibit motility of the three nematode species (Fig. 1a,b). Sensitivity to solvents differed between species (Fig. 2). *Pratylenchus coffeae* and *T. semipenetrans* motility was reduced by 0.6% commercial carrier (Ciba-Geigy) (Fig. 2), while the carrier had no adverse effect on *R. similis*. Conversely, *R. similis* was sensitive to 2.2% acetone (Fig. 1a, 0 ppm metalaxyl), which served as a good solvent for tests involving *P. coffeae*

Table 4. Influence of 2-hour preplant dip of rough lemon seedlings in metalaxyl (112 ppm) on infection of fibrous roots by *Pratylenchus coffeae* (PC), *Radopholus similis* (RS), and *Tylenchulus semipenetrans* (TS).

Metalaxyl	Nematodes per gram root (fresh weight)		
	PC	RS	TS
0 ppm	1071.3	227.4	211.5
112 ppm	734.7*	68.2**	73.8**

*LSD = 0.1.
**LSD = 0.01.

Table 5. Influence of incubation of *Tylenchulus semipenetrans* (TS), *Radopholus similis* (RS) and *Pratylenchus coffeae* (PC) for 16 hours in water, solvent or metalaxyl on nematode infection of rough lemon roots.

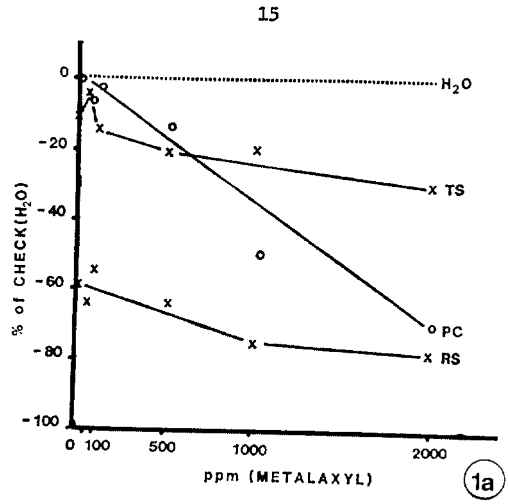
Treatment	Nematode per gram root (dry weight)		
	TS	RS	PC
H_2O*	3966.0 a†	6429.0 a	2856.0 a
Solvent	8961.9 a	5942.5 a	3870.7 a
Metalaxyl	7160.8 a	6009.5 a	2378.0 a

*H_2O = tap water; solvent = 2.2% acetone for *P. coffeae* and *T. semipenetrans*, or 0.6% Ciba-Geigy commercial carrier for *R. similis*; metalaxyl = 112 ppm.

†Values followed by the same letter in columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$). Experiment harvested 7 days after inoculation.

and *T. semipenetrans*. The ED_{50} for the Ciba-Geigy commercial carrier was 12 ppm for *T. semipenetrans* and 17 ppm for *P. coffeae*. However, the carrier (35 ppm) did not affect nematode populations under greenhouse conditions (Kaplan, unpublished data).

Metalaxyl appeared to influence the interaction of the three nematode species within rough lemon roots. *Pratylenchus coffeae* typically forms cavities in the root cortex, where only small changes are observable in cells adjacent to the nematodes. Infection of metalaxyl-treated roots was associated with granular cytoplasm in cells adjacent to the nematode (Fig. 3). *Radopholus similis*, which generally has a strong



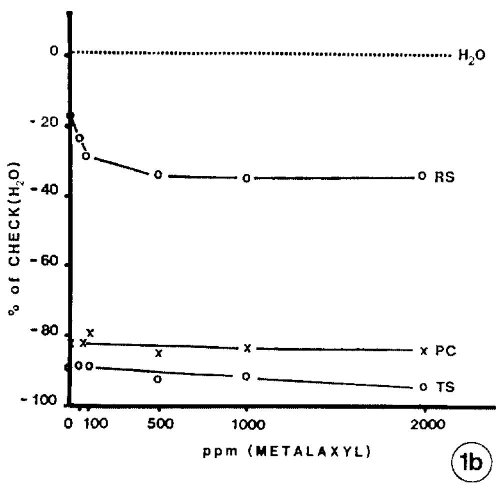


Fig. 1. Influence of 24-hour metalaxyl treatment on *Pratylenchus coffeae* (PC), *Radopholus similis* (RS), and *Tylenchulus semipenetrans* (TS) expressed as percentage of nematode motility in water. 1a) 2.2% acetone as solvent. 1b) 0.6% commercial carrier (Ciba Geigy) as solvent.

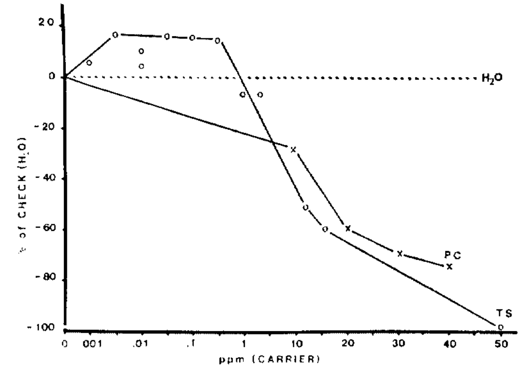


Fig. 2. Influence of the commercial carrier of metalaxyl (Ciba Geigy) on motility of *Pratylenchus coffeae* (PC) and *Tylenchulus semipenetrans* (TS) expressed as percentage of nematode motility in water.

affinity for the phloem parenchyma, was not observed in the stele of treated fibrous roots, and cellular responses to infection of the root cortex were similar to those observed for *P. coffeae* (Fig. 4). *T. semipenetrans* normally infects the root and establishes a feeding site comprised of nurse cells. Metalaxyl reduced the number of nematode feeding sites. Cytoplasm of nurse cells within nematode feeding sites in metalaxyl-treated roots was dense and plasmolysed (Fig. 5).

DISCUSSION

Metalaxyl appears to confer nematode control by modifying citrus roots such that a normally susceptible rootstock (rough lemon) becomes tolerant. Metalaxyl (112 ppm) did not adversely affect nematode motility or chemotropic functions (interaction of nematode with soil and root leading to, and including, infection of plant roots). However, when plant root systems

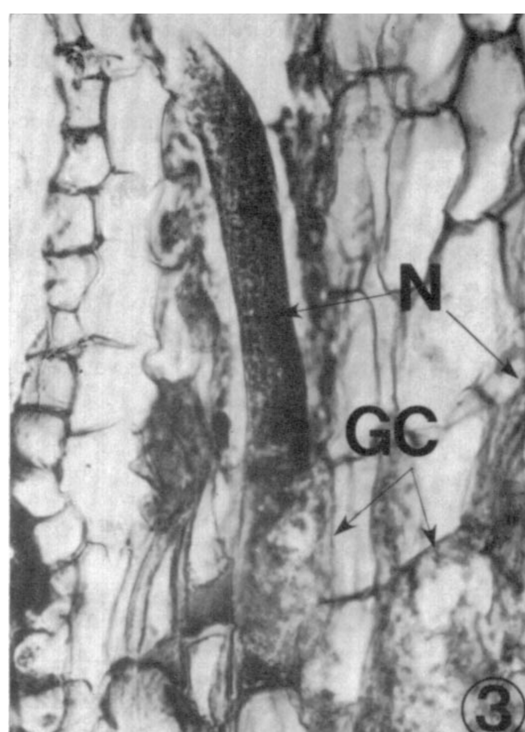


Fig. 3. Infection of metalaxyl-treated rough lemon seedlings by *Pratylenchus coffeae* was associated with granular cytoplasm (GC) in cells adjacent to the nematodes (N). (400 ×)

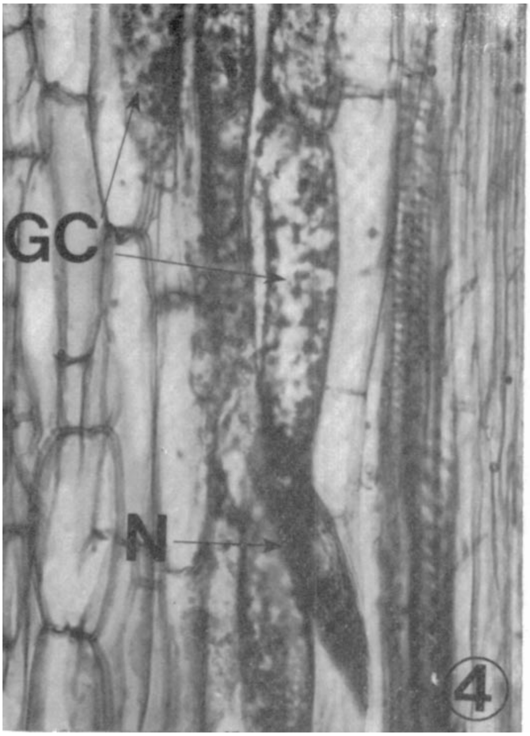


Fig. 4. Infection of metalaxyl-treated rough lemon seedlings by *Radopholus similis* (RS) was associated with granular cytoplasm (GC) in cells adjacent to the nematodes (N). In addition, *R. similis,* which generally has a strong affinity for phloem parenchyma, was not observed in the stele of treated fibrous roots. (400 ×)

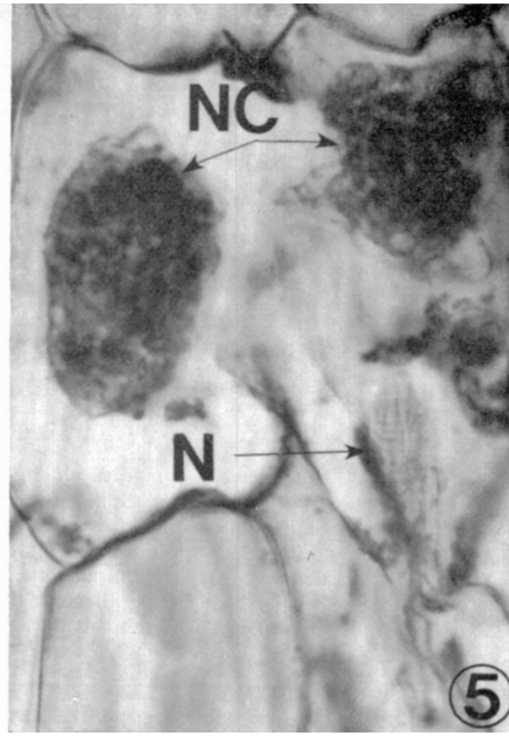


Fig. 5. Infection of metalaxyl-treated rough lemon seedlings by *Tylenchulus semipenetrans* (N) was significantly reduced and cytoplasm of nurse cells (NC) in treated tissue was dense and plasmolysed. (1,000 ×)

were incubated in, or soil-drenched with, metalaxyl before planting, nematode infection and subsequent reproduction, as well as postinfectional cellular responses, in citrus roots differed from those of nontreated plants. The significance of these cellular responses to infection is difficult to assess; however, they suggest that an increased sensitivity to nematode infection exists which may be associated with the limited nematode reproduction observed in metalaxyl-treated plants.

Treatment of nematode-infested soil (preplant drench) with metalaxyl and/or of seedlings before being planted in nematode-infested soil (dip) was more effective than treatment of nematode-infected seedlings (post). Reduced treatment effect has been observed also in *Phytophthora parasitica* control in citrus when metalaxyl was applied to plants after infection had occurred (5).

Although *P. coffeae* and *R. similis* populations were significantly reduced by metalaxyl in this study, nematode frequencies which would normally destroy rough lemon root systems were still detected in symptomless root systems. These nematode species are associated with citrus slump (*P. coffeae*) and spreading decline (*R. similis*), diseases associated with damaged root systems which increase tree susceptibility to environmental stress. The research findings presented herein suggest that these diseases might be controlled by metalaxyl. Field research will be conducted to determine if metalaxyl can reduce disease incidence in nematode-infested citrus groves.

LITERATURE CITED

1. Davis, R. M. 1982. Control of Phytophthora root and foot rot of citrus with systemic fungicides metalaxyl and phosethyl aluminum. Plant Disease 66:218-220.

2. Farih, A., J. A. Menge, P. H. Tsao, and H. D. Ohr. 1981. Metalaxyl and efosite aluminum for control of Phytophthora gummosis and root rot on citrus. Plant Disease 65:654-657.

3. Grimm, G. R. 1982. Metalaxyl for control of

Phytophthora parasitica in Florida citrus nurseries. Plant Disease 66: (In press).

4. Kaplan, D. T., N. T. Keen, and I. J. Thomason. 1980. Association of glyceollin with the incompatible response of soybean roots to Meloidogyne incognita. Physiol. Pl. Pathol. 16:309-318.

5. Timmer, L. W. 1979. Preventative and systemic activity of experimental fungicides against Phytophthora parasitica on citrus. Plant Dis. Rept. 63: 324-327.

6. Young, T. W. 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Dis. Pept. 38:794-795.