Combining Fosetyl-Al Trunk Injection or Metalaxyl Soil Drenching with Soil Application of Aldicarb for Control of Citrus Decline

H. F. LE ROUX, Senior Research Pathologist, Outspan Citrus Centre, P.O. Box 28, Nelspruit 1200, Republic of South Africa; F. C. WEHNER and J. M. KOTZÉ, Professors, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, Republic of South Africa; and N. M. GRECH, Senior Agricultural Researcher, Citrus and Subtropical Fruit Research Institute, Private Bag X11208, Nelspruit 1200, Republic of South Africa

ABSTRACT

Le Roux, H. F., Wehner, F. C., Kotzé, J. M., and Grech, N. M. 1991. Combining fosetyl-Al trunk injection or metalaxyl soil drenching with soil application of aldicarb for control of citrus decline. Plant Dis. 75:1233-1236.

Four-year-old citrus trees growing in an orchard infested with *Phytophthora nicotianae* var. *parasitica* and *Tylenchulus semipenetrans* were treated every 3 mo for 2 yr with metalaxyl, fosetyl-Al, and aldicarb alone and in various combinations. Ten and 40 mo after commencement of the treatments, the disease rating of trees treated with fungicides or fungicides + aldicarb was significantly lower than that of untreated trees or trees treated with aldicarb only. The dry root mass per kilogram of soil after 40 mo was greatest in trees treated with fosetyl-Al + aldicarb. Increase in trunk diameter and canopy volume was greatest in trees treated with fungicides + aldicarb. Only trees treated with fosetyl-Al + aldicarb produced a significantly higher fruit yield than control trees harvested at 32 and 44 mo after commencement of treatments. Also, these trees yielded a consistently higher income than the control trees. At the 44-mo harvest, the net income derived from all treated trees, except those given aldicarb only, was significantly higher than that derived from control trees. Only those treatments that included metalaxyl resulted in a reduction in the incidence of *P. n. parasitica* in soil. The population of *T. semipenetrans* on roots and in soil was reduced by treatments containing aldicarb but increased again within 3 mo. None of the treatments had any significant effect on the incidence of *Fusarium solani* or *F. oxysporum* in the roots or soil.

Additional keyword: nematicides

Phytophthora nicotianae Breda de Haan var. *parasitica* (Dastur) G.M. Waterhouse and *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian are the most devastating soilborne fungal pathogens involved in citrus decline (14). The citrus nematode (*Tylenchulus semipenetrans* Cobb) often occurs together with *Phytophthora* in citrus roots, contributing to feeder root damage (2). Chemical control strategies have thus far concentrated on reducing populations of *Phytophthora* with systemic fungicides such as metalaxyl and fosetyl-Al (7,18) or reducing populations of *T. semipenetrans* with nematicides such as aldicarb and fenamiphos (8).

It is logical to assume that treating citrus trees infested with both *Phytophthora* and *T. semipenetrans* with a fungicide plus a nematicide would result in a greater improvement in tree condition than would treatment with only one of the pesticides. This report presents evidence of improved control of decline in citrus trees by treatment of affected trees with metalaxyl or fosetyl-Al in combination with aldicarb.

Accepted for publication 10 December 1990.

MATERIALS AND METHODS

The trial was established in the Northern Transvaal in a 4-yr-old navel orange (*Citrus sinensis* (L.) Osbeck) on rough lemon rootstock (*C. jambhiri* Lush.) orchard that had become naturally infested with *P. n. parasitica* and *T. semipenetrans*. The treatments given at 3-mo intervals over a 2-yr period were: 1) 5% metalaxyl (Ridomil) granules as a soil application at 2 g a.i./m^2 of leaf canopy, 2) 10% fosetyl-Al (Aliette) solution as a trunk injection at the rate of 0.4 ml a.i./m^2 of leaf canopy, 3) 15% aldicarb (Temik) granules as a soil application at 4.5 g a.i./m^2 of leaf canopy, 4) the first and third treatments combined, and 5) the second and third treatments combined. Subsequently, metalaxyl and fosetyl-Al were applied every 6 mo and aldicarb, at 1.875 g a.i./m^2 of leaf canopy, was applied once a year. Trunk injection was performed as described previously (6). Ten randomly selected single trees were used for each treatment, and an additional 10 untreated trees served as controls. The experiment was laid out according to a completely randomized design.

At 10, 20, and 40 mo after the start of the experiment, four standardized, 300-g soil samples containing feeder roots were collected at a depth of 200 mm on the north, south, east, and west sides of the trees within the drip zone. The four samples from each tree were pooled and mixed thoroughly, and the roots were sieved from the soil. Then, 100 randomly selected feeder root tips were cut into segments 5 mm long, sterilized superficially for 30 sec with 3% sodium hypochlorite, rinsed in sterile distilled water, and aseptically blot-dried. Fifty root segments from each pooled sample were plated on selective medium (19) for the recovery of *P. n. parasitica*, and 50 segments were plated on potato-dextrose agar supplemented with 250 mg ml^{-1} of chloramphenicol (Chloromycetin) for the recovery of *Fusarium solani* (Mart.) Sacc. and *F. oxysporum* Schlechtend.:Fr., two fungi commonly associated with roots of declining citrus trees (16). Plates were incubated at 27 C, and the number of segments yielding the respective fungi was recorded after 5 days. The incidence of *P. n. parasitica* in the pooled soil samples from each tree was estimated according to the citrus leaf disk method (10) using five subsamples from each pooled sample.

At the 40-mo sampling, 10 g of each soil subsample was serially diluted and plated on the selective medium of Kannwischer and Mitchell (12) containing 50 mg ml^{-1} of hymexazol. The number of colonies of *P. n. parasitica* that developed after 5 days of incubation at 27 C was recorded. The technique described by Van der Vegte (20) was used to obtain *T. semipenetrans* female counts on root samples collected after 20 and 40 mo, and the method of Whitehead and Hemming (22) was used to determine the incidence of *T. semipenetrans* stage 2 juveniles in soil after 40 mo.

The trunk diameter of each tree was measured 300 mm above soil level at the start of the experiment and again after 10, 20, and 40 mo. The severity of canopy decline symptoms was rated on a scale of 0 (healthy) to 4 (dead) after 10 and 40 mo, and canopy volumes were calculated at the end of 40 mo using the method described by Burger et al (5). Dry feeder root mass per kilogram of soil was determined after 40 mo by collecting four standardized 1-kg subsamples within the drip zone of each tree at a depth of 200 mm. The feeder roots were collected by sieving, dried for 48 hr at 70 C, and weighed. Fruit yield and size were determined at the end of the second, third, and fourth seasons (i.e., 20, 32, and 44 mo after the start of the experiment); all the fruit from each tree was picked, weighed, and sized. Income per tree was calculated on the basis of the prevailing export market prices, and the total cost (excluding application costs) of each treatment was subtracted from the gross income to determine the net profit, excluding normal production costs.

The experiment was laid out using a randomized block design. An analysis of variance was performed on the data, and the significance of the treatment differences was determined using the Student-Newman-Keuls multiple range test.

RESULTS

P. n. parasitica was recovered infrequently from citrus roots after 10 mo (fewer than two positive isolations out of 500), irrespective of treatment (Table 1). The recovery rate after 20 mo was similarly low, with roots from trees treated with fosetyl-Al, fosetyl-Al + aldicarb, and metalaxyl + aldicarb yielding few positive isolations of the pathogen. *P. n. parasitica* could not be detected after 10 mo by the leaf disk method in any of the soils receiving a metalaxyl application. After 20 and 40 mo, however, the fungus was detected in these soils, although numbers were significantly lower with the metalaxyl treatment (either alone or in combination with aldicarb) than in the control treatment (Table 1). When soil was assayed by the disk method (10), the incidence of *P. n. parasitica* in the metalaxyl treatment did not differ significantly from the control treatment after 40 mo, but metalaxyl-treated soil yielded significantly fewer colony-forming units of *P. n. parasitica* using selective media than did comparable control soil samples (Table 1). None of the treatments had any significant effect on the incidence of *F. solani* or *F. oxysporum* in the roots.

The numbers of *T. semipenetrans* females on roots and of stage 2 juveniles in the soil were reduced 10–56% by aldicarb treatments, but these reductions were not statistically significant (Table 2).

The disease ratings of control and aldicarb-treated trees were significantly higher than those of the other treatments after 10 and 40 mo. After 10 mo, the trunk diameters of trees treated with metalaxyl + aldicarb had increased significantly more than those of trees receiving other treatments (Table 3). After 20 mo, the increase in trunk diameter was significantly greater in trees treated with metalaxyl + aldicarb or fosetyl-Al + aldicarb than with the other treatments. After 40 mo, however, only the treatment with fosetyl-Al + aldicarb showed a significantly greater increase in trunk diameter over that of the control trees. The canopy volume of trees receiving metalaxyl + aldicarb or fosetyl-Al + aldicarb was significantly greater than that of control trees after 40 mo. The root mass per kilogram of soil was greatest in the fosetyl-Al + aldicarb treatment, although the metalaxyl + aldicarb treatment yielded more roots per unit mass of soil than the control or fosetyl-Al treatments.

No statistically significant differences in mean fruit yield per tree or fruit sizes were observed between the various treatments at the 20-mo harvest, although the yield and size factor resulted in a significantly higher income being derived from trees treated with fosetyl-Al + aldicarb than from untreated trees (Tables 4 and 5). Only trees receiving the fosetyl-Al + aldicarb treatment produced a significantly larger crop at the 32- and 44-mo harvests, partly because of the higher percentage of larger fruit borne by these trees. The net income derived from trees treated with fosetyl-Al + aldicarb at the 32-mo harvest also was significantly higher than with all the other treatments. At the 44-mo harvesting, however, gross and net income derived from all treated trees, except those treated only with aldicarb, was significantly higher than that derived from control trees.

DISCUSSION

This investigation illustrates the benefit of treating simultaneously for *Phytophthora* and *T. semipenetrans* when both organisms are present in a citrus planting. Combining an aldicarb soil drench with a fosetyl-Al trunk injection not only proved to be superior in increasing tree vigor and fruit yield but was also cost-effective throughout the course of the experiment. *Phytophthora* and *T. semipenetrans* apparently exist together in many citrus areas. In

Table 1. Effect of trunk injection of fosetyl-Al or soil drenching with metalaxyl, alone or combined with soil application of aldicarb, on incidence of *Phytophthora nicotianae* var. *parasitica* in the rhizosphere of rough lemon rootstocks grafted with navel orange

Treatment	Isolation of *P. n. parasitica* from roots[w] 10 mo	20 mo	Incidence of *P. n. parasitica* in soil[x] 10 mo	20 mo	40 mo	Number of *P. n. parasitica* propagules at 40 mo[y]
Fosetyl-Al trunk injection	+	−	15 b[z]	41 b	29 b	35 b
Metalaxyl soil application	+	+	0 a	31 ab	16 ab	7 a
Aldicarb soil application	+	+	15 b	42 b	38 b	32 b
Fosetyl-Al trunk injection + aldicarb soil application	+	−	13 b	37 ab	34 b	35 b
Metalaxyl soil application + aldicarb soil application	+	−	0 a	17 a	7 a	3 a
Control	+	+	22 b	44 b	31 b	44 b

[w] *P. n. parasitica* was never recovered from more than 0.4% of 500 root segments from each treatment plated on selective medium.
[x] Each value is the mean percentage of citrus leaf disks (10) colonized by *P. n. parasitica* out of 200 disks representing 40 soil samples from 10 trees.
[y] Each value is the mean number of colony-forming units of *P. n. parasitica* in 40 soil samples from 10 trees.
[z] Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

Table 2. Effect of trunk injection of fosetyl-Al or soil drenching with metalaxyl, alone or combined with soil application of aldicarb, on colonization of rough lemon rootstocks by *Tylenchulus semipenetrans* females and incidence of stage 2 juveniles in the rhizosphere

Treatment	Number of *T. semipenetrans* females per 10 g of roots[x] 20 mo	40 mo	Number of *T. semipenetrans* stage 2 juveniles per 250 g of soil at 40 mo[y]
Fosetyl-Al trunk injection	10,836 a[z]	8,125 a	17,161 a
Metalaxyl soil application	11,080 a	7,344 a	11,541 a
Aldicarb soil application	6,058 a	5,175 a	8,925 a
Fosetyl-Al trunk injection +aldicarb soil application	3,820 a	6,600 a	10,288 a
Metalaxyl soil application +aldicarb soil application	4,112 a	6,265 a	9,122 a
Control	8,733 a	7,367 a	15,587 a

[x] Each value is the mean number in 40 samples representing 10 replicates, determined according to the method of Van der Vegte (20).
[y] Each value is the mean number representing 10 replicates, determined according to the method of Whitehead and Hemming (22).
[z] Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

South Africa, for instance, Martin (15) isolated *Phytophthora* spp. from one-third of old citrus soils and found that *T. semipenetrans* was omnipresent. Similarly, a more recent countrywide survey (M. Rea, *personal communication*) revealed the presence of *P. n. parasitica* and *T. semipenetrans* in 65 and 90%, respectively, of the citrus plantings surveyed. Trials conducted concurrently in other localities (*unpublished*) have produced results corroborating the findings of the present report, which confirms the ubiquity of the *Phytophthora*-nematode association. It should be kept in mind, however, that the rootstock concerned in this study (rough lemon) is susceptible to both *Phytophthora* and the citrus nematode (11,21). With a rootstock tolerant to either one or both of these organisms, the frequency of simultaneous applications of nematicide and fungicide could probably be reduced.

Although study of the nature of any synergy between *P. n. parasitica* and *T. semipenetrans* was not the purpose of this report, certain parameters were investigated to elucidate the effects of the various treatments on tree performance. The results suggest that *P. n. parasitica* contributed most to the decline situation, since treating with only aldicarb resulted in no improvement in tree condition, even though the activity of aldicarb against *T. semipenetrans* is well documented (8,9). Considering that the rate of reduction in nematode numbers achieved by the nematicide in the present investigation was approximately of the same order as that reported previously (3), there is little doubt about the efficacy of the compound. Soil populations of *P. n. parasitica* exceeded the level reported to cause significant feeder root loss in Florida citrus groves, namely, 10–20 propagules per cubic centimeter (17). As a result, application of fosetyl-Al or metalaxyl improved tree vigor to such an extent that treatment became cost-effective.

Populations of *P. n. parasitica* in the soil responded to fosetyl-Al and metalaxyl in keeping with previous reports, namely, a reduction in the number of colony-forming units with metalaxyl (1,7) but not with fosetyl-Al. Difficulties experienced with recovering *P. n. parasitica* from roots preclude conclusions concerning the effect of the various treatments on the degree of root infection by the pathogen. Nevertheless, tree response indicates a reduction in root colonization by treatments that included either fungicide.

Neither fosetyl-Al nor metalaxyl had any significant effect on the colonization of roots by *F. solani, F. oxysporum*, or *T. semipenetrans*. This agrees with the report by Darvas et al (6) that trunk injection with fosetyl-Al does not reduce *F. oxysporum* in avocado roots, but disagrees with reports that metalaxyl can increase the tolerance of citrus roots to nematodes (13) and of potato tubers to *Fusarium* spp. (4).

Table 3. Effect of trunk injection of fosetyl-Al or soil drenching with metalaxyl, alone or combined with soil application of aldicarb, on disease development and growth of navel orange trees on rough lemon rootstocks

Treatment	Disease rating[w]		Increase in rootstock diameter[x] (%)			Dry root weight at 40 mo[y] (g/kg soil)	Canopy volume at 40 mo (m^3)
	10 mo	20 mo	10 mo	20 mo	40 mo		
Fosetyl-Al trunk injection	0.3 b[z]	1.2 b	13 b	40 b	134 ab	3.5 c	16.1 ab
Metalaxyl soil application	0.3 b	1.3 b	14 b	38 b	107 b	5.1 bc	16.9 ab
Aldicarb soil application	0.9 a	1.9 a	15 b	37 b	129 b	5.0 bc	13.0 ab
Fosetyl-Al trunk injection + aldicarb soil application	0.2 b	1.1 b	18 b	49 a	161 a	8.1 a	17.9 a
Metalaxyl soil application + aldicarb soil application	0.4 b	1.1 b	31 a	51 a	147 ab	6.2 b	18.0 a
Control	1.3 a	2.1 a	11 b	38 b	117 b	3.3 c	10.4 b

[w]Each value is the mean of 10 replicate trees rated on a scale of 0 (healthy) to 4 (dead).
[x]Each value is the mean of 10 replicate trees; rootstock diameter was measured 300 mm from soil level.
[y]Each value is the mean of 10 replicate trees.
[z]Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

Table 4. Effect of trunk injection of fosetyl-Al or soil drenching with metalaxyl, alone or combined with soil application of aldicarb, on size and yield of fruit from navel orange trees on rough lemon rootstocks

Treatment	Fruit yield[y] (kg/tree)			Fruit yield at various diameters (mm)[y] (% of total fruit yield/tree) 32 mo			44 mo		
	20 mo	32 mo	44 mo	≤63	64–73	≥74	≤63	64–73	≥74
Fosetyl-Al trunk injection	3.3 a[z]	53 a	102 ab	24 ab	49 a	27 a	20 b	51 a	29 a
Metalaxyl soil application	2.7 a	46 a	100 ab	13 a	52 a	35 a	13 a	48 a	39 ab
Aldicarb soil application	2.6 a	52 a	53 b	29 b	38 a	33 a	24 b	42 a	34 a
Fosetyl-Al trunk injection + aldicarb soil application	3.2 a	73 b	128 a	9 a	40 a	51 b	9 a	40 a	51 b
Metalaxyl soil application + aldicarb soil application	4.0 a	56 a	111 ab	13 a	37 a	50 b	9 a	34 a	57 b
Control	2.6 a	46 a	43 b	14 a	55 a	31 a	14 a	56 a	30 a

[y]Each value is the mean of 10 replicate trees.
[z]Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

Table 5. Effect of trunk injection of fosetyl-Al or soil drenching with metalaxyl, alone or combined with soil application of aldicarb, on income derived from fruit of navel orange trees on rough lemon rootstocks

Treatment	Percentage increase in income over control[y] 20 mo		32 mo		44 mo	
	Gross	Net	Gross	Net	Gross	Net
Fosetyl-Al trunk injection	35 ab[z]	33 ab	21 b	20 b	68 a	66 a
Metalaxyl soil application	65 ab	46 ab	6 b	−4 b	69 a	58 a
Aldicarb soil application	27 ab	7 b	16 b	12 b	3 b	0 b
Fosetyl-Al trunk injection + aldicarb soil application	111 a	89 a	71 a	65 a	86 a	80 a
Metalaxyl soil application + aldicarb soil application	46 ab	34 ab	31 ab	16 b	85 a	70 a
Control	... b	... b	... b	... b	... b	... b

[y]Each value is the mean of 10 replicate trees. Income was calculated according to market values at harvesting. Net income is gross income minus cost of treatment, excluding normal production costs.
[z]Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

ACKNOWLEDGMENTS

We thank R. T. Frean for help in revising this article and Elna Wessels for assistance with statistical analysis of the data.

LITERATURE CITED

1. Allen, R. N., Pegg, K. G., Forsberg, L. I., and Firth, D. J. 1980. Fungicidal control in pineapple and avocado of diseases caused by *Phytophthora cinnamomi*. Aust. J. Exp. Agric. Anim. Husb. 20:119-124.
2. Anonymous. 1984. Integrated pest management for citrus. Division of Agriculture and Natural Resources Publications, University of Califor-

nia, Oakland. 144 pp.
3. Baines, R. C., and Van Gundy, S. D. 1976. Control of the citrus nematode with an insecticide/fungicide. Page 51 in: Summary of Certain Research on Citrus. Citrus Research Advisory Committee, Riverside, CA.
4. Barak, E., Edgington, L. V., and Ripley, B. D. 1984. Bioactivity of the fungicide metalaxyl in potato tubers against some species of *Phytophthora, Fusarium* and *Alternaria*, related to polyphenoloxidase. Can. J. Plant Pathol. 6:304-308.
5. Burger, W. P., Vincent, C. J., Barnard, C., du Plessis, J. A., and Smith, J. H. E. 1970. Metodes waarvolgens die grootte van sitrusbome bepaal kan word. S. Afr. Citrus J. 433:13-15.
6. Darvas, J. M., Toerien, J. C., and Milne, D. L. 1984. Control of avocado root rot by trunk injection with phosethyl-Al. Plant Dis. 68:691-693.
7. Farih, A., Menge, J. A., Tsao, P. H., and Ohr, H. D. 1981. Metalaxyl and efosite aluminum for control of Phytophthora gummosis and root rot on citrus. Plant Dis. 65:654-657.
8. Ferguson, J., and Dunn, R. 1984. Occurrence and control of citrus nematode. Citrus Ind. 65:16-20, 23.
9. Fisher, J. 1986. Research on Temik continues at CREC. Citrus Ind. 67:5, 8, 9, 58.
10. Grimm, G. R., and Alexander, A. F. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. Phytopathology 63:540-541.
11. Hutchison, D. J., and Grimm, G. R. 1973. Citrus clones resistant to *Phytophthora parasitica*: 1973 screening results. Proc. Fla. State Hortic. Soc. 86:88-91.
12. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
13. Kaplan, D. T. 1983. Influence of metalaxyl on three nematodes of citrus. J. Nematol. 15:454-460.
14. Klotz, L. J., De Wolfe, T. A., and Wong, P. 1958. Decay of fibrous roots of citrus. Phytopathology 48:616-622.
15. Martin, J. P. 1960. Fungi and nematodes in South African citrus orchards in relation to the citrus replant problem. Soil. Sci. Soc. Am. Proc. 24:469-472.
16. Nemec, S. 1975. Micro-organisms associated with healthy and sandhill-declined citrus roots. Plant Dis. Rep. 59:210-213.
17. Sandler, H. A., Timmer, L. W., and Graham, J. H. 1987. Effect of systemic fungicides on *Phytophthora* populations, feeder root densities, and yield of citrus. (Abstr.) Phytopathology 77:1691.
18. Timmer, L. W., and Castle, W. S. 1985. Effectiveness of metalaxyl and fosetyl-Al against *Phytophthora parasitica* on sweet orange. Plant Dis. 69:741-743.
19. Tsao, P. H., and Guy, O. D. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. Phytopathology 67:796-801.
20. Van der Vegte, F. A. 1973. A new method of estimating the numbers of citrus nematodes (*Tylenchulus semipenetrans*) in root samples. Nematol. Soc. S. Afr. Newsl. 4:11-12.
21. Van Gundy, S. D., and Kilpatrick, J. D. 1964. Nature of resistance in certain rootstocks to citrus nematodes. Phytopathology 54:419-427.
22. Whitehead, A. G., and Hemming, J. R. 1965. Comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55:25-38.