Comparative Efficacy of Preplant Fumigation and Postplant Chemical Treatment of Replant Citrus Trees in an Orchard Infested with *Tylenchulus semipenetrans*

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ABSTRACT

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Preplant soil fumigation with methyl bromide at 49 and 98 g/m² was compared with various postplant nematicide and fungicide programs in a replant citrus orchard infested with the citrus nematode *Tylenchulus semipenetrans* and the fungal pathogens *Phytophthora nicotianae*, *Fusarium solani*, and *F. oxysporum*. Postplant treatments comprised multiple applications of fenamiphos at 4.0 g a.i./m² soil, aldicarb at 4.5 g a.i./m² soil, metalaxyl at 4 g a.i./m² soil, fosetyl-Al stem painting at 400 g a.i./liter, and combinations of fenamiphos + fosetyl-Al and aldicarb + fosetyl-Al at the same rates as for single treatments. *P. nicotianae* could not be detected in the entire experimental site after replanting, but populations of *F. solani* and *F. oxysporum* showed only a temporary decline following site preparation. *T. semipenetrans* did not re-establish in any of the treatments within the first 2 years. Numbers of juveniles remained low in most treatments during the third year, but thereafter both juveniles and females increased significantly in all except the fumigated plots. Female populations on roots of citrus trees planted in fumigated soil remained suppressed for 8 years and the trees developed more vigorously and produced higher yields and larger fruit than those in non-fumigated soil. Compared with the control, net income for the period 4 to 8 years after planting increased by 101 and 46% in plots fumigated with 49 and 98 g/m², respectively. With the exception of aldicarb, all other treatments showed net losses.

In South Africa (6,17-19), as in many other parts of the world such as Arizona, Australia, California, Florida, Texas, and Israel, citrus trees replanted to soils which have been under citrus for an extended period often show retarded growth (29,40). This has been attributed to a number of factors, with the accumulation of root-invasive organisms in such soils being regarded as the most likely cause (37). Organisms commonly implicated are the citrus nematode *Tylenchulus semipenetrans* Cobb (1,2,4,5) and the fungal pathogens *Phytophthora nicotianae* Breda de Haan, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (13,35), and *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyder & Hans. (10,14,23,24,26,32). *Radopholus citrophilus* Huettel, Dickson & Kaplan, which is a severe problem on citrus in Florida's central ridge, is not present in South Africa; however, *Pratylenchus coffeae* (Zimmermann) Filipjev, Schuurmans & Stekhoven is of minor importance (21).

Zebediela Estate, situated near Potgietersrus in the Northern Province of South Africa, produces citrus on an area of approximately 2,000 ha (9). Most of the older trees are grafted on rough lemon (*Citrus jambhiri* Lush) rootstock, which is susceptible to both the citrus nematode and *Phytophthora* spp. (37), and are consequently in a state of progressive deterioration. Decline is further aggravated by irrigation with water containing moderately high levels of chloride (200 to 350 mg/liter; 9), which is conducive to *T. semipenetrans* (20). Rootstocks tolerant to the nematode can lose this attribute under these conditions (9).

Replant orchards at Zebediela Estate are often established in fallowed soil which had been under citrus for up to 50 years. Root pathogens consequently have to be managed by chemical treatment of replanted trees or by soil fumigation with methyl bromide (MBR) prior to replanting. The efficacy of MBR for eradicating *T. semipenetrans* has been reported on various occasions (8,22,28,34), but the compound has yet to be compared with alternative methods of nematode control over an extended period of time. Our investigation, which spanned a period of 8 years, compared the effectiveness of preplant MBR fumigation and postplant chemical treatment for control of the citrus nematode in a replant situation.

MATERIALS AND METHODS

Land preparation and treatments. An orchard at Zebediela Estate with severe decline and a history of infestation by *T. semipenetrans*, *P. nicotianae*, *F. solani*, and *F. oxysporum* Schlecht. emend. Snyder & Hans. was fallowed and ripped to a depth of approximately 40 cm in November 1984. The soil was a loamy sand with 10 to 15% clay, pH (water) 8.3, and resistance 630 ohm, containing 30 ppm P, 195 ppm K, 969 ppm Ca, 312 ppm Mg, 40 ppm Na, and 48 ppm Cl. After the soil was levelled with a disc, nine treatments (Table 1), each comprising eight replicate plots (68 by 4 m), were established in randomized complete blocks. In January 1985, plots destined for preplant treatment were strip-fumigated under clear polyethylene sheeting (4 m wide, 30 µm thick) with a mixture of hot (60°C) MBR/chloropicrin gas (ratio 49:1) at rates of 49 and 98 g/m² MBR. The plastic covers were removed after 2 days.

Valencia orange (*Citrus sinensis* (L.) Osb.) scions on Volkamer lemon (*Citrus volkameriana* Ten. & Pasq.) rootstock (18-month-old trees, 17 trees per plot, spaced 4 m within rows and 6 m between rows) were planted 1 month after fumigation. The following granular nematicides were applied 3 weeks later to a surface of 1 m² around each tree: fenamiphos (Nemacur 10G) at 4.0 g a.i./m², aldicarb (Temik 15G) at 4.5 g a.i./m², and metalaxyl (Ridomil 5G) at 4.0 g a.i./m². The fungicide fosetyl-Al (Aliette; 400 g a.i./liter), was painted on stems of trees from the soil surface to the first branch. Combined nematicide/fungicide treatments (fenamiphos + fosetyl-Al and aldicarb + fosetyl-Al) were applied at the same rates as above. Soil was irrigated immediately after application of the chemicals with the equivalent of 25 mm precipitated water. Control plots were not fumigated or treated with nematicides and fungicides. The experimental site was managed according to standard practices of the estate. Further postplant treatments were scheduled (Table 1), increasing the surface area receiving nematicides proportionally to the increase in root zone.

Mycological and nematological observations. One week after fumigation, 10 soil samples were arbitrarily collected to a depth of 30 cm in each of the control and fumigated plots. Samples were serially diluted and plated on potato-dextrose agar supplemented with 250 mg/liter chloromyc-

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cetin, and on the selective medium of Kannwischer and Mitchell (12) containing 50 mg/liter hymexazol, for enumerating *F. solani*, *F. oxysporum*, and *P. nicotianae*. *T. semipenetrans* juveniles were determined in the same soil samples according to the method of Whitehead and Hemming (41). Further counts were done annually until 1989 on eight soil and root samples taken to a depth of 20 cm midway between the stem and dripline of each of three trees in each replicate. The same sampling procedure was followed for determining nematode female populations in roots in 1990, 1991, and 1992 using the following procedure for each sample (38). Roots were retrieved from soil and shaken to dislodge loosely-adhering soil, after which the tertiary roots were cut into segments 2 cm in length. Root segments (3 g) were gently rinsed with water on a coarse sieve to remove the remaining soil particles, then blended for 20 s at 12,600 rpm in 200 ml water. The resultant suspension was decanted through a series of three sieves with aperture sizes of 1 mm (top), 150 µm (middle), and 38 µm (bottom). Residues remaining on the top sieves were washed through with a jet of water. Material retained on the bottom sieve was rinsed back onto a 25-µm-aperture sieve with 2% acetic acid, after which the latter sieve was transferred to a staining bath containing a solution of 188 ml liquid phenol, 165 ml lactic acid, 318 ml glycerol, 10 g 1% acid fuchsin, 10 g 1% orcein, and 200 ml distilled water, prepared 24 h before use. After 1 h, the stain was drained and the root segments rinsed until the residue was nearly colorless. Segments were washed into a 250-ml beaker and the volume made up to 100 ml with water. A few drops of 5% acetic acid were added and the root segments were rinsed as air was bubbled through the sus-

pension. Sub-samples (1 ml) were transferred to counting dishes and the stained females counted at 25 to 60× magnification under a dissecting microscope.

Tree response. Stem diameter of the trees was measured 10 cm above the bud union in 1985, 1988, 1989, and 1991. Tree canopy volumes were estimated according to the formula of Serfontein and Catling (31) in 1990 and 1992, and fruit yield and size for each tree were determined from the fourth to the eighth season (1988 through 1992). Leaves from untreated trees, aldicarb-treated trees, and trees planted to soil fumigated with 98 g/m² MBr were analyzed for inorganic elements in 1992.

Economic impact. Cost-efficacy of the various treatments was calculated from the following values, standardized to 1992 levels. All fruit smaller than 63 mm in diameter were regarded as destined for the processing plant, generating \$47/ton. Of the fruit larger than 63 mm, 60% was considered to have been sold on the local market at an average price of \$1.34 per 10-kg pocket, whereas the remaining 40% was regarded as exportable, fetching a free-on-board price of between \$3.71 and 4.89 per 15-kg carton. Gross income, based on a density of 417 trees/ha, was calculated for each treatment. Expenses (excluding labor cost), also standardized to 1992 levels, were subtracted from the gross income of each treatment to determine the net profit.

RESULTS

Mycological and nematological observations. Fumigation with MBr drastically reduced numbers of *P. nicotianae*, *F. solani*, and *F. oxysporum* in soil (Table 2), but population densities of the latter two species soon recovered to their original levels. *P. nicotianae*, on the other hand, disappeared from all plots within 6 months (*data not shown*). *T. semipenetrans*, which occurred at a density exceeding 20,000 juveniles per 250 ml soil prior to site preparation, could not be detected during the first 2 years after replanting (Table 3). By the third year (1987), juveniles were recorded sporadically in non-fumigated soil but populations remained suppressed in fumigated plots. Reduced numbers were also evident in the fenamiphos, metalaxyl, and aldicarb + fosetyl-Al treatments in 1988, and in the fenamiphos + fosetyl-Al treatment in 1988 and 1989. A constraining effect of MBr on *T. semipenetrans* female populations was apparent in root samples collected 8 years after fumigation, notably in those from plots which had received 98 g/m² MBr. Metalaxyl drenching was associated with an increase in female numbers in 1991.

Tree response. Treatment-related differences in tree response became evident in 1988, with trees in plots which had been fumigated with 98 g/m² MBr showing enhanced stem distension compared with

Table 2. Fungal pathogen populations (propagules/g soil) in replant citrus soil at Zebediela Estate 1 week after fumigation with methyl bromide (MBr)

Treatment	<i>Phytophthora nicotianae</i>	<i>Fusarium solani</i>	<i>Fusarium oxysporum</i>
Control	354 a ^z	290 a	90 a
MBr (49 g/m ²)	6 b	0 b	0 b
MBr (98 g/m ²)	0 b	0 b	0 b

^z Mean of 10 soil samples from each of 8 replicate plots. Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (*P* = 0.05).

Table 1. Summary of treatments and parameters monitored in a replant site at Zebediela Estate^s

1985 ^{u,v}	1986 ^{u,v}	1987 ^{u,v}	1988 ^{v,w}	1989–1993 ^{v,w,x}
Untreated	Untreated	Untreated	Untreated ⁱ	Untreated ^y
Methyl bromide (49 g/m ²)	Untreated	Untreated	Untreated ⁱ	Untreated ^z
Methyl bromide (98 g/m ²)	Untreated	Untreated	Untreated	Untreated ^{y,z}
Fenamiphos (4 × 2 g)	Fenamiphos (2 × 2 g)	Fenamiphos (2 × 2 g)	Fenamiphos (2 × 2 g) ⁱ	Fenamiphos (2 × 2 g) ^z
Aldicarb (4 × 4.5 g)	Aldicarb (4 × 4.5 g)	Aldicarb (4 × 4.5 g)	Aldicarb (1 × 1.875 g) ⁱ	Aldicarb (1 × 1.875 g) ^{y,z}
Metalaxyl (2 × 4 g)	Metalaxyl (2 × 4 g)	Metalaxyl (2 × 4 g)	Metalaxyl (2 × 4 g) ⁱ	Metalaxyl (2 × 4 g) ^z
Fosetyl-Al (2 × 400 g/liter) stem paint	Fosetyl-Al (1 × 400 g/liter) stem paint	Fosetyl-Al (1 × 400 g/liter) stem paint	Fosetyl-Al (1 × 400 g/liter) stem paint ⁱ	Fosetyl-Al (1 × 400 g/liter) stem paint ^z
Fenamiphos (4 × 2 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Fenamiphos (2 × 2 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Fenamiphos (2 × 2 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Fenamiphos (2 × 2 g) + fosetyl-Al (2 × 400 g/liter) stem paint ⁱ	Fenamiphos (2 × 2 g) + fosetyl-Al (2 × 400 g/liter) stem paint ^z
Aldicarb (4 × 4.5 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Aldicarb (4 × 4.5 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Aldicarb (4 × 4.5 g) + fosetyl-Al (2 × 400 g/liter) stem paint	Aldicarb (1 × 1.875 g) + fosetyl-Al (2 × 400 g/liter) stem paint ⁱ	Aldicarb (1 × 1.875 g) + fosetyl-Al (2 × 400 g/liter) stem paint ^z

^s Soil was prepared in 1984 to 1985 and replanted in 1985.

ⁱ *Tylenchulus semipenetrans* juvenile populations/250 ml soil or /10 g roots.

^u Fungal analysis.

^v Tree stem diameter.

^w Tree yield.

^x *T. semipenetrans* adult female populations/10 g roots.

^y Leaf analysis.

^z Economic analysis.

the fosetyl-AI treatment or combinations of fosetyl-AI and nematicides (Table 4). Stem diameter of the former trees also exceeded that of fenamiphos-treated trees in 1989 and, in 1991, that of all treatments except fumigation with 49 g/m² MBr and aldicarb drenching, with the latter two nonetheless performing better than the remaining treatments. Increase in canopy volume followed a similar pattern. Trees in fumigated plots produced more extensive foliage than the control from 1990 onward, whereas those receiving aldicarb had larger canopies than the control in 1992 only (Fig. 1).

Leaf analysis indicated differences in magnesium, iron, and manganese content between treatments, but none of these were considered sufficient to have an effect on tree health. Nitrogen and especially chloride levels were generally high, whereas concentrations of phosphorus, potassium, zinc, boron, sulphur, and sodium were mostly lower than those recommended. The low potassium and high nitrogen content resulted in the nitrogen:potassium ratio exceeding the optimal level of 1:3. Leaves from the MBr treatment nevertheless had a ratio nearest to the optimum.

Trees in fumigated plots gave the highest cumulative fruit yield over the trial period, but those receiving aldicarb and aldicarb + fosetyl-AI also produced more fruit than the control (Fig. 2). Preplant fumigation with 98 g/m² MBr improved yield aggregate by 53, 25, and 20% over that of the control, aldicarb, and aldicarb + fosetyl-AI treatments, respectively, with the lower MBr rate performing equally well. Application of fenamiphos, metalaxyl, fosetyl-AI, and fenamiphos + fosetyl-AI had no significant effect on yield.

Economic impact. Besides enhancing total fruit yield, fumigation with MBr at both rates (49 and 98 g/m²) improved fruit size, resulting in increases of 119 and 110% in gross and 78 and 46% in net income over the control, respectively (Fig.

3). Fumigation with 49 g/m² MBr provided the greatest net income due to the lower input cost. All other treatments, with the exception of aldicarb, gave net losses.

DISCUSSION

Before it was replanted, the orchard used for this experiment supported populations of *T. semipenetans*, *P. nicotianae*, *F. so-*

Table 4. Stem diameter increase of citrus trees in a replant orchard at Zebediela Estates after preplant methyl bromide (MBr) fumigation and postplant nematicide and fungicide treatments^z

Treatment	Increase in stem diameter (mm) since 1985		
	1988	1989	1991
Control	59.7 ab	63.7 ab	98.0 a
MBr (49 g/m ²)	61.9 ab	65.9 ab	116.1 cd
MBr (98 g/m ²)	66.6 a	70.0 a	119.0 d
Fenamiphos	56.5 ab	60.5 b	102.4 ab
Aldicarb	59.8 ab	63.8 ab	113.0 cd
Metalaxyl	60.2 ab	64.2 ab	97.8 a
Fosetyl-AI	55.5 b	59.5 b	101.0 ab
Fenamiphos + fosetyl-AI	56.4 b	60.4 b	100.8 ab
Aldicarb + fosetyl-AI	57.4 b	61.4 b	102.6 ab

^z Mean of 8 replicates each consisting of 17 trees. Values in columns followed by the same letter do not differ significantly according to Student-Newman-Keul's multiple range test ($P > 0.05$).

Fig. 1. Effect of preplant methyl bromide (MBr) fumigation and postplant nematicide and fungicide treatments on canopy development of citrus trees in a replant orchard at Zebediela Estate (see Table 1 for treatment details). Bars with the same letter do not differ significantly according to Fisher's least significant difference ($P > 0.05$).

Table 3. *Tylenchulus semipenetans* populations in citrus soil and roots in a replant orchard at Zebediela Estates after preplant methyl bromide (MBr) fumigation and postplant nematicide and fungicide treatments

Treatment	<i>T. semipenetans</i> juveniles/250 ml soil					<i>T. semipenetans</i> females/10 g roots		
	1985 ^w	1986 ^x	1987	1988	1989	1990 ^y	1991	1992
Control	0	0	*z	19,700 cd	17,000 c	18,586 bc (100)	31,125 bc (100)	27,400 bcd (100)
MBr (49 g/m ²)	0	0	0	284 a**	528 a**	4,477 a (50)	3,950 a (12.5)	2,425 a (25)
MBr (98 g/m ²)	0	0	0	367 a**	657 a**	365 a (25)	300 a (12.5)	350 a (12.5)
Fenamiphos	0	0	*	2,550 a	5,929 abc	13,950 ab (100)	21,325 abc (75)	22,800 bcd (87.5)
Aldicarb	0	0	*	10,433 abc	10,729 abcd	20,412 bc (87.5)	43,200 cd (100)	32,100 cde (100)
Metalaxyl	0	0	*	1,000 a	3,814 abc	14,455 ab (100)	57,900 d (100)	31,100 bcde (100)
Fosetyl-AI	0	0	*	13,067 abc	11,429 abcd	20,601 c (87.5)	33,850 bc (100)	18,000 abcd (100)
Fenamiphos + fosetyl-AI	0	0	*	1,150 a	2,357 ab	12,564 ab (100)	13,300 ab (100)	34,175 cde (100)
Aldicarb + fosetyl-AI	0	0	*	4,483 ab	9,585 abc	13,986 ab (100)	32,425 bc (100)	35,950 de (100)

^w Mean of 10 soil samples from each of eight replicate plots (1985 only).

^x Mean of 8 soil samples from each of 3 trees in each of eight replicate plots (1986 to 1989). Values in columns followed by the same letter do not differ significantly according to Fisher's least significant difference ($P > 0.05$).

^y Mean of 8 root samples from each of 3 trees in each of eight replicate plots (1990 to 1992). Values in brackets represent the percentage of trees which tested positive for *T. semipenetans*.

^z * = sporadic recovery of <1,000 juveniles/10g soil; ** = nematodes were only isolated in a few instances, indicating that small areas of soil escaped fumigation.

lani, and *F. oxysporum*. Site preparation reduced *T. semipenetrans* and *P. nicotianae* to undetectable levels. *P. nicotianae* was not detected in further tests. *P. nicotianae* is a poor saprophytic competitor incapable of surviving in the absence of a live host (36). Fallowing and replanting reduce the supply of host roots in soil, depriving such pathogens of a food source and causing a decline in populations. It is not known why propagules of *P. nicotianae* having access to roots of replanted trees perished, but it may have been due to exposure to high temperatures in the sparsely-shaded soil.

In contrast to *P. nicotianae*, *T. semipenetrans* is able to survive in the absence of its host (7,39) and has been detected in citrus soil for up to 9 years after removal of the trees (3,11). The undetectable population of *T. semipenetrans* during the first 2 years of the experiment is possibly due to the lack of tree shade, which created an environment unfavorable

for nematode development. Reynolds and O'Bannon (30) reported a similar phenomenon in Arizona, and O'Bannon et al. (27) showed populations of *T. semipenetrans* to increase maximally at 23°C, with slower development below 20 and above 31°C. Soil temperature in citrus-producing areas of the Northern Province can reach 44°C at a depth of 5 cm during mid- and late summer (January to April; 15), and thermal inactivation of the nematode in the upper soil layers may indeed explain the initial reduction in nematode numbers. Nevertheless, results indicate that tree responses observed were primarily due to effects of treatments on *T. semipenetrans*, or rather on the citrus nematode-*Fusarium* complex, which is known to retard growth of citrus seedlings more pronouncedly than the respective organisms on their own (14,26). Non-involvement of *P. nicotianae* and other pythiaceae fungi was corroborated by the failure of metalaxyl and fosetyl-Al to improve tree performance.

Notwithstanding the proven activity of aldicarb and fenamiphos against *T. semipenetrans* (22,25), this investigation showed that these nematicides provide only transitory suppression. Nematode populations in the aldicarb and fenamiphos treatments started to increase from the third year, indicating that the compounds had lost their efficacy at this stage, probably due to accelerated degradation (33). Augmenting nematicide drenching with fosetyl-Al stem painting did not improve productivity of the trees, thus reconfirming that *P. nicotianae* was not involved in the disease syndrome and emphasizing that a combined nematicide-fungicide program (16) should be considered only where *T. semipenetrans* and *Phytophthora* spp. are both present. Unlike multiple nematicide or fungicide applications, preplant fumigation with MBr not only provided sustained suppression of *T. semipenetrans* but also improved tree performance substantially and, most importantly, surpassed postplant treatments in terms of cost-effectiveness. The lower MBr dosage (49 g/m²), was as effective as the dosage of 98 g/m² in enhancing tree growth, but superior regarding return on input costs. However, the higher rate could be more efficient in the long run as it seemed to provide more persistent suppression of *T. semipenetrans*. As MBr has little residual activity, fumigation must have created an environment unfavorable for the nematode, the nature of which remains unknown.

Fig. 2. Effect of preplant methyl bromide (MBr) fumigation and postplant nematicide and fungicide treatments on the relative fruit yield of citrus trees in a replant orchard at Zebediela Estate (see Table 1 for treatment details). Bars with the same letter do not differ significantly according to Fisher's least significant difference ($P > 0.05$).

Fig. 3. Cost-effectiveness of preplant methyl bromide (MBr) fumigation and postplant nematicide and fungicide treatments in a replant orchard at Zebediela Estate for the first 8 years after replanting (see Table 1 for treatment details).

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