Nematicidal Control of Heterodera tabacum

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ABSTRACT

Bay 68138 [ethyl-4-(methylthio)-*n*-tolyl isopropylphosphoramidate] reduced invasion of roots by larvae of the tobacco cyst nematode, *Heterodera tabacum* (TCN), but did not kill eggs in cysts. Vorlex (80% 1,3-dichloropropene and 20% methyl isothiocyanate) and Telone (1,3-dichloropropene and related C_3 chlorinated hydrocarbons) killed eggs within cysts; little infection was obtained when cysts were moved from soil treated with Telone or Vorlex into untreated soil. *Heterodera tabacum* was controlled in the field for only 1 season by a single application of either 60 gal/acre (500 liter/ha) of Telone or 25 gal/acre (209 liter/ha) of Vorlex or by split applications of either 30 gal/acre (250 liter/ha) of Telone or 20 gal/acre (167 liter/ha) of Vorlex. The populations were high again by harvest time. Populations were still low at harvest time after use of 40 lb./acre (40 kg/ha) of 68138. In early summer, Vorlex, Telone, and 68138 controlled TCN larvae 45 cm deep in the soil where populations in untreated soil were already lower than at the soil surface. In greenhouse trials, methomyl (Lannate) [*s*-methyl *N*(methylcarbamoyl) oxy] (thioacetimidate) gave good control 3 weeks after addition to soil, but not 8 weeks after addition. Thiabendazole gave excellent control in these greenhouse tests. Phytopathology 60:411-414.

Use of nematicides against cyst nematodes is limited because most hosts have too low a value per acre to economically justify their use. However, the high return resulting from control of the tobacco cyst nematode (TCN) *Heterodera tabacum* Lownsbery & Lownsbery warrants use of nematicides in fields of shade-grown cigar-wrapper tobacco. The use of a few nematicides and organic fertilizers to control TCN has been reported (5, 6). This report compares control of *H. tabacum* by several organic fertilizers and nematicides, and compares killing of encysted eggs by two fumigant nematicides and a nonfumigant nematicide.

MATERIALS AND METHODS.—*Effects of nematicides on larval invasion of roots.*—Effects of nematicides and fertilizers on *H. tabacum* were determined in containers holding 50 lb. (22.7 kg) of heavily infested soil that was placed out of doors from late May to late August 1967. Materials used and rates of application are given in Table 1. Vorlex and Telone were injected into the middle of the can of soil moistened to 65% field capacity, and the upper 2.5 cm of soil was kept moist for 48 hr to reduce loss of the fumigant. One treatment was 30 gal/acre (250 liter/ha) of Telone applied in two applications. Applications were 10 days apart. Methomyl, Bay 5461, and Bay 68138 were watered onto the soil, using a 3.8 liter of water/can of soil. Thiabendazole, TH 285-N, and the three fertilizers were mixed with the soil. Each treatment was applied to three cans of soil.

Effectiveness of treatments was assessed 3 weeks after soil was treated. Two aliquots of 100 g of soil were taken from each can of soil and placed in small cups which were seeded with eggplants. Four days after emergence, roots were removed and stained with acid fuchsin-lactophenol (5). Larvae of *H. tabacum* were counted in 6 cm of roots from each cup, or six counts/treatment. Effectiveness of the treatments was assayed again on tobacco 8 weeks after treatment. For this assay, enough soil to fill two pots 15 cm in diam was taken from each can. Each pot received one tobacco plant 7.5 cm tall. After 2 months, the roots were washed and examined for browned areas. The amount of browning was rated on the basis of 1 = no browning; 2 = slight; 3 = moderate; and 4 = severe browning. The fresh wt of the tops of the plant was determined. Also, 100 g of soil from each pot was planted to eggplants to determine possible changes in populations of *H. tabacum* during growth of tobacco.

Telone, Vorlex, 68138, and TH 285-N were applied in the fall in a field of fine sandy loam heavily infested with *H. tabacum*. Telone and Vorlex were applied 20 cm deep at 25-cm intervals with a chisel applicator on four plots 5 by 20 m. Telone was used at two rates, 30 gal/acre (250 liter/ha) applied in two applications and 60 gal/acre (500 liter/ha) applied in one application. Vorlex was also used at two rates, 20 gal/acre (167 liter/ha) applied in two applications, and 25 gal/acre (209 liter/ha) applied in one application. The single applications and the first of the double applications were made on 27 September 1967. The second application was made 10 October, after the field was replowed and disked on 9 October.

68138 and TH 285-N were spread on the soil surface of four plots 2.4 by 20 m on 12 October 1967, and rotovated into the soil to a depth of 15 cm. An 86% emulsifiable concentrate of 68138 was used in applying 40 lb/acre (40 kg/ha) of the active ingredient. A 15% granular formulation of TH-285-N was used in applying 100 lb/acre (100 kg/ha) active ingredient.

Soil samples for assays of the TCN populations in field plots were taken to a depth of 15 cm on 2 November 1967, 18 June 1968, and 7 September 1968. Two cups of soil from each plot were seeded to eggplants, and root invasion was determined by counting TCN larvae in 6 cm of roots from each sample.

On 10 July 1968, samples were taken at different depths down to 45 cm to determine the depth of control given by different treatments. The sample from the top 22.5 cm included all soil which had been plowed or disturbed after application of the nematicides. These

samples were seeded to eggplants, and root invasion by TCN larvae determined as before.

Roots and tops of tobacco were examined. Root sections 1 mm in diam were washed and stained in acid fuchsin-lactophenol, and *H. tabacum* larvae were counted in 6 cm of roots from each plot. The possibility that treatments might influence entrance of hatched *H. tabacum* larvae into tobacco roots was studied by extracting those larvae still in the soil by the flotation centrifugation method (4) from 100 g of soil from each plot.

Effects of the nematicides on eggs inside cysts.—Attempts were made to determine whether the nonfumigant nematicide 68138 kills encysted eggs, emerged larvae, or both. Eight months after treatment with 40 lb./acre (40 kg/ha) of 68138, the soil was divided into three lots. One aliquot of this soil was seeded directly to eggplant seed. Cysts harvested from a second aliquot of this soil were mixed with sand in volume equal to the soil sample. This mixture was then seeded to eggplants. Residual effects of 68138 were determined by mixing cysts from untreated soil with the third lot of soil previously treated with 68138, then planting eggplant seeds. Untreated but infested soil was also seeded directly to eggplants.

In further work on the effects of nematicides on encysted eggs, samples were taken from field plots on 29 July and divided into two parts. Eggplants were seeded directly into one part. Cysts were harvested from the other part and mixed with an equal volume of sand; eggplants were then seeded into this sand-cyst mixture. TCN larvae were counted in 6 cm of roots from each sample.

RESULTS.—Three weeks after treatment of soil in cans, the 10 gal/acre (83 liter/ha) rate of Vorlex was as effective as the 50 gal/acre (416 liter/ha) rate of Telone in reducing damage by *H. tabacum* to eggplants (Table 1). Thiabendazole, TH 285-N, methomyl, and 68138 gave excellent control. Telone applied at a rate of 30 gal/acre (250 liter/ha) gave poor control whether applied in one or two applications. The three fertilizers gave some control. Bay 5461 had no effect. Eight weeks after treatment, tobacco planted in the soil methomyl did not control tobacco cyst nematodes, and the three organic fertilizers gave no control. Thiabendazole caused a slight reduction in growth of tobacco.

Vorlex, Telone, 68138, and TH 285-N applied in the field in the fall of 1967 reduced populations of *H. tabacum* in the plowed depth of soil to varying degrees (Table 2). Six weeks after application, 68138 was the

TABLE 1. Effects of soil treatment with various nematicides and fertilizers on invasion of eggplant and tobacco roots 3 to 8 weeks later by *Heterodera tabacum* larvae

Treatment	Amount of active ingredient /Acre	Amount of active ingredient /Hectare	No. larvae in 6 cm eggplant roots: Before tobacco[a]	No. larvae in 6 cm eggplant roots: After tobacco[b]	Tobacco root injury, 4 = severe[c]	Wt of tobacco plant, g
Untreated			200+[d]	200+	3.2	21
Telone[e]	15 Gal	125 Liter	125	148	2.0	26
Telone	30 Gal	250 Liter	39	28	1.8	33
Telone	30 Gal	250 Liter	51	38	1.5	53
In 2 applications						
Telone	50 Gal	416 Liter	2	35	1.5	26
Vorlex	10 Gal	83 Liter	2	39	1.8	29
Vorlex	15 Gal	125 Liter	1	17	2.0	47
Vorlex	20 Gal	167 Liter	0	4	1.5	56
Vorlex	25 Gal	209 Liter	0	6	1.3	60
Thiabendazole	50 Lb.	50 Kg	2	1	2.4	17
Thompson-Hayward 285-N	25 Lb.	25 Kg	35	31	2.0	45
Thompson-Hayward 285-N	50 Lb.	50 Kg	3	28	1.1	45
Thompson-Hayward 285-N	100 Lb.	100 Kg	0	1	1.4	36
Bay 5641	40 Lb.	40 Kg	182	200+	3.3	23
Bay 68138	40 Lb.	40 Kg	1	0	1.3	65
Methomyl	40 Lb.	40 Kg	1	182	3.1	33
6-3-6 fertilizer[f]	2000 Lb.	2000 Kg	88	200+	2.2	29
10-6-4 fertilizer[g]	2000 Lb.	2000 Kg	51	150	3.5	20
Castor pomace	2000 Lb.	2000 Kg	48	200+	2.0	36

[a] Eggplant seeded 3 weeks after treatment of soil.

[b] After tobacco was grown for 2 months, 100 g of soil were removed from each pot and planted to eggplants.

[c] Root injury on tobacco planted 8 weeks after soil treatment was found as follows: 1 = no browning; 2 = slight; 3 = moderate; and 4 = severe browning.

[d] *H. tabacum* counts: rates were more than 200, too abundant to count accurately.

[e] Telone, 1,3-dichloropropene and related C_3 hydrocarbons; Vorlex, 80% Telone and 20% methyl isothiocyanate; thiabendazole, 2-(4'thiazolyl)-benzimidazole; Thompson-Hayward 285-N, 4-chloropyridine-*N*-oxide; Bay 5461, *s,s*-diethyl chloromethylphosphonodithioate; Bay 68138, ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate; and methomyl, *s*-methyl *N* [methylcarbamyl]oxy-thioacetimidate.

[f] An organic fertilizer composed primarily of cottonseed meal and containing 6% *N'*, 3% available P_2O_5 and 6% K_2O.

[g] An organic fertilizer obtained from animal residues and containing 10% N, 6% available P_2O_5, and 6% K_2O.

TABLE 2. Control of *Heterodera tabacum* in the field with fall application of nematicides

Treatment	Rate/acre	No. larvae in 6 cm of eggplant roots			No. larvae in 150 g of soil around tobacco roots	Larvae in 6 cm of tobacco roots
		1967	1968			
		2 November	8 July	7 September	13 June 1968	13 June 1968
Untreated		190	200+	200+	124	61
Vorlex	25 Gal[a]	30	14	117	14	19
Vorlex	10 Gal + 10 gal[b]	2	8	106	9	6
Telone	15 Gal + 15 gal[b]	1	11	98	2	14
Telone	60 Gal	2	6	64	2	3
Thompson-Hayward 285-N	100 Lb.	43	113	137	103	93
Bay 68138	40 Lb.	0.1	12	26	18	1

[a] Applied in one application on 27 September 1967.
[b] Applied in two applications on 27 September and 10 October 1967.

most effective treatment. Vorlex using a total of 20 gal/acre (167/liter ha) in two applications, Telone using a total of 30 gal/acre (250 liter/ha) in two applications, and Telone using 60 gal/acre (500 liter/ha) in a single application were very effective. TH 285-N gave poor control. In June, after plowing and planting of tobacco, treatments were evaluated by means of eggplant assays or by counts of larvae in tobacco roots and in soil around tobacco roots. Data indicated that Vorlex, Telone, and 68138 were giving good control. TH 285-N was ineffective. By September, populations of *H. tabacum* were high again in all plots except those treated with 68138.

During the summer, Vorlex and 68138 controlled *H. tabacum* effectively down to 45 cm (Table 3). In two cases (68138 and 25 gal/acre [209 liter/ha] of Vorlex), control was slightly better below a depth of 22.5 cm than above this depth. In the untreated plots, populations of *H. tabacum* decreased with depth of soil at 30 cm and 37.5 cm.

Growth of the tobacco was greatly improved by all treatments except TH 285-N. Roots from plots treated with 68138, Vorlex, and Telone were white with little browning. Those in plots treated with TH 285-N and control plots showed many browned areas.

Abundant invasion of roots by *H. tabacum* larvae was obtained in sand containing cysts from soil treated with 68138, but little invasion was obtained from cysts remaining in the treated soil (Table 4). Larvae from cysts transferred from untreated soil to treated soil invaded roots abundantly. Evidently, the residues of 68138 remaining in the soil were insufficient to prevent infection.

When cysts were taken from field plots and put into sand, only slight invasion was obtained from cysts treated with Telone or Vorlex, whether the cysts remained in or were removed from treated soil; thus, Telone and Vorlex killed the eggs inside the cyst. Abundant invasion was obtained from cysts removed from plots treated with 68138. Thus, 68138 does not kill encysted eggs as Vorlex and Telone do, but apparently acts internally to prevent hatching.

DISCUSSION.—Results contained in this study show that some contact nematicides attack free larvae or prevent emergence, but do not kill encysted eggs. For example, methomyl (Lannate), soon after addition to soil, killed the free larvae but did not kill encysted eggs. In 5 weeks, the eggs hatched and the larvae invaded the roots of tobacco plants as plentifully as they did in plants growing in untreated soil. The toxicant had not penetrated the cysts, and lacked persistence in the soil. Likewise, when cysts were taken from soil treated with 68138, washed, and planted in untreated soil, many eggs hatched and the larvae that developed invaded roots. In contrast, there was little root invasion when cysts were taken from soil fumigated with Telone or Vorlex. Thus, these fumigants killed encysted eggs. Plant injury from nematodes which occurs in the

TABLE 3. Control of *Heterodera tabacum* given by nematicides at different soil depths on 10 July 1968

Nematicide	Rate/acre	Number of *H. tabacum* larvae in 6 cm of eggplant roots grown in soil taken at depths of:			
		0-22.5 cm	22.5-30 cm	30-37.5 cm	37.5-45 cm
Control		200+	200+	154	36
Vorlex—split application	10 + 10 Gal	6	10	2	1
Vorlex	25 Gal	15	5	5	3
Telone—split application	15 + 15 Gal	8	7	2	2
Telone	50 Gal	3	6	6	4
Thompson-Hayward 285-N	100 Lb.	150	193	112	60
Bay 68138	40 Lb.	22	3	0	0

TABLE 4. Root invasion by larvae from cysts of *Heterodera tabacum* in soil treated 8 months previously with Bay 68138, from cysts moved from treated soil to sand and from cysts moved from untreated to treated soil

Source of cysts	Medium for eggplant germination 13 March 1968	No. larvae in 6 cm of roots
Cyst soil treated with 68138[a]	The original cyst soil treated with 68138	1
Cyst soil treated with 68138	Sand[b]	52
Untreated cyst soil	Sand[b]	152
Untreated cyst soil	Soil treated with 68138[b]	133
Untreated cyst soil	Untreated cyst soil	143

[a] Soil mixed with 40 lb./acre of 68138 on 25 May 1967 exposed outside for 3 months, then stored in a dry greenhouse for 5 months.

[b] Soil allowed to dry for 48 hr, then cysts were floated off, caught in a 60 mesh screen, and mixed with an amount of sand equal in volume to the original volume of infested soil.

season following fumigation will depend upon repopulation of the soil by the few nematodes that survived the fumigation. In contrast, with a contact nematicide that kills free larvae but leaves encysted eggs unharmed, nematode damage can occur in mid or late season once the nematicide is gone. After dissipation of the nematicide, these encysted eggs can hatch and provide large numbers of infective nematodes.

Residual 68138 in the cysts or eggs may be effective long after the remainder of the nematicide has dissipated from the soil. However, inhibitory effects of residual 68138 can be eliminated by washing cysts. Presumably, the toxicant is sorbed to the cyst wall.

H. tabacum rapidly repopulated fumigated soil in 1968, just as has been shown for other cyst nematodes (7). Repopulation was reduced by the new contact nematicide, 68138. Good control was given by 68138, Vorlex, and Telone to soil depths of 45 cm. Control by fumigants and 68138 was poorer in the upper 5 cm of soil, apparently due to the rapid loss from this layer by volatilization or leaching. These results suggest that a combination of a fumigant with a surface application of an insoluble nonfumigant nematicide would give optimum control.

The split application of 20 gal/acre (167 liter/ha) of Vorlex gave slightly better nematode control and plant growth than a single application of 25 gal/acre (209 liter/ha) of Vorlex, but few differences in nematode control or plant growth were observed between one and two applications of Telone. Both in this and another cooperative test of split applications, 20 gal/acre (167 liter/ha) of Vorlex was superior to 30 gal/acre (250 liter/ha) of Telone.

This is the first report of the value of thiabendazole as a soil nematicide. It is currently used as anthelmintic (1, 2, 3) and as a fungicide. Further work on nematicidal properties of this compound is in progress.

Further work on suppression of emergence or invasion by 68138 and similar contact nematicides is planned.

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