Nematode Response to Carbofuran

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Abstract: Higher populations of *Meloidogyne incognita* larvae and *Pratylenchus penetrans* were recovered from soil treated with carbofuran 10 and 15 days after treatment, respectively, than were recovered from untreated control soil. The number of *P. penetrans*, however, was lower 50 days after treatment, and symptoms developed only occasionally on the root systems of host plants. Populations of *Tylenchorhynchus claytoni* inoculated at different distances from the base of corn seedlings growing in carbofuran-treated soil did not move toward the plant, whereas they were attracted in untreated soil from a distance of 12 cm. *P. penetrans* moved at random in treated agar medium when inoculations occurred 4 cm away from the root tips of tomato seedlings under aseptic conditions. Those nematodes that reached the roots were never observed feeding during a 20-day observation period. Specimens of *P. penetrans* placed on the developing roots moved at random and never penetrated. In contrast, numerous *P. penetrans* penetrated roots of seedlings growing in untreated medium. *Key words:* control, mode of action, behavior.

Little is known about the mode of action of nematicides. It is common practice to evaluate a compound by determination of population changes at certain times after treatment. In some cases, plant response is taken into account and yield increase measures the efficacy of the chemical tested. I believe that knowledge of the mode of action of a pesticide is of primary importance in a determination of the best method of evaluating efficacy. The problem is not great when nematodes are killed because the performance of such a nematicide can be determined easily. An active compound, however, may not necessarily kill nematodes but may act in other ways. Sensory organs of nematodes may be affected and, when a compound is systemic, the plant itself may not produce the stimuli required by nematodes to initiate the feeding process. If the chemical expresses its activity in one of these ways, nematode counts, at least in the soil, are useless. Of course, populations in soil will eventually decrease, as nematodes die from starvation when not able to feed.

Preliminary studies (3) showed that higher numbers of root-knot nematodes were recovered from soil treated with carbofuran than from untreated soil. Tomato seedlings grown in this soil, however, were free from the usual swellings.

The present study was undertaken to investigate the factor or factors responsible for the absence of symptoms on the root systems of plants grown in nematode-infested soil treated with carbofuran.

MATERIALS AND METHODS

Extraction of nematodes: Greenhouse-grown tomato roots containing egg masses of *Meloidogyne incognita* (Kofoid and White) Chitwood were cut into pieces and comminuted for 30 sec in a small amount of water in a food blender. The mixture was poured through a sieve of 6.35-mm openings to remove larger debris. The resulting mixture was poured onto a 2-cm layer of sand (steam-sterilized) contained in a wooden flat. A second layer of sand covered the mixture. Three days later, when about 50% of the eggs had hatched at greenhouse temperatures of 23-25 C, the content of the flat was mixed, and enough infested sand was mixed with steam-sterilized sandy-loam soil so that a concentration of about 800 root-knot nematode larvae and eggs/500 cc soil contained in a 10-cm plastic pot was obtained. *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa and *Tylenchorhynchus claytoni* (Steiner) were extracted from callus culture (5) by means of a modification of the Baermann funnel technique (2). Nematodes in 5 ml of water were put around the base of seedlings by removing some soil before inoculation and then replacing it. Nematodes were recovered from infested soil by wet screening using a sieve of 44 μ openings and by processing the collected residue with the sugar flotation method (1). Nematodes were recovered from root systems with the modified Baermann funnel technique over a period of 12 days. Tests with *T. claytoni* and *M. incognita* were kept in a greenhouse at 23-25 C, whereas tests with *P. penetrans* were kept in a growth

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chamber of 21 C and a light intensity of 2,000 ft-c/12 hr day.

Soil treatment: Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), as Furadan® 5% dust (a registered trademark of FMC Corporation) was incorporated into root-knot-infested soil by means of the Twin Shell® blender (The Patterson-Kelley Co., Inc. East Stroudsburg, Penn.) at 10 ppm. A 3-week-old tomato seedling, *Lycopersicon esculentum* Mill. 'Heinz 1350', was transplanted into each of six pots of treated soil. Six untreated plants were held as controls.

Ten days after treatment, the root systems of tomato plants were examined for swellings, and 250 cc of soil from each replicate was processed for nematode recovery. The second-stage larvae of *M. incognita* recovered were counted under a stereoscopic microscope. Larvae collected were put around the base of 2-week-old tomato seedlings growing in 5-cm pots to check infectivity of the larvae. Root galling of these plants was determined 2 weeks later.

Pea seedlings, *Pisum sativum* L. 'Hundredfold', 3 cm tall, were transplanted into soil treated as previously described with carbofuran at 10 and 25 ppm and inoculated with about 4000 *P. penetrans*. Each treatment was replicated four times. Two weeks later, nematodes were recovered from the soil and from the root systems of pea plants. This test was repeated in a similar manner, except that carbofuran was used at 10 and 20 ppm, nematodes were extracted 50 days after treatment and two replicates were used.

In order to investigate whether nematodes were attracted to the roots, the following experiment was carried out. Five trays (40 × 5 × 5 cm) were filled with soil treated with carbofuran at 10 ppm, and five other trays were filled with untreated soil and held as controls. A corn seedling, *Zea mays* L. 'Seneca Chief', 4 cm tall, was transplanted at the center of each of the 10 trays. About 5000 specimens of *Tylenchorhynchus claytoni* (Steiner), extracted from callus culture, were deposited at distances of 2.5, 5, 8, 10 and 12 cm from the base of the corn seedlings. One tray was used for each distance. Two inoculations were made for each tray at equal distances from the plant. This test was not replicated.

Ten days after inoculation, soil sections were removed from each tray every 2 cm from both sides of the plant. Soil samples taken at equal distances for each tray were combined and processed for nematode recovery by the sugar flotation method.

Agar medium treatment: A 0.75% water agar was prepared and put into two flasks and autoclaved at 120 C for 15 min. When the temperature of the medium dropped to 45 C, a quantity of Furadan 5% dust equivalent to 10 ppm was added to one flask, and the same amount of Attaclay® (hydrated aluminum magnesium silicate, Attapulgus Clay Co., used to prepare the Furadan formulation) was added to the other flask as a control. Both flasks were hand shaken for 30 sec to disperse the dusts, and clear plastic petri dishes were filled to about two-thirds from each medium. Heinz 1350 tomato seeds were surface-sterilized in 3% sodium hypochlorite for 20 min and washed three times in sterile water. The seeds then were transferred to petri dishes containing 2% water agar. Three days later, germinating seeds were put into the previously prepared test petri dishes. Three seedlings were deposited aseptically at one edge of the dish 1 cm away from the wall and 1 cm from each other. The following day about 150 *P. penetrans* were aseptically extracted from callus culture and deposited by micro-pipette in 0.2 ml water on the agar medium 4 cm from the root tips of the seedlings. During the 20-day test period, 2 ml of a modified Hoagland solution (7) was added to each dish three times. The solution had been sterilized previously by passing it through a 0.20-μ opening biological filter. The experiment was repeated in a similar manner, except that nematodes were deposited on the roots of the growing seedlings. Each test consisted of four replicates. Observations on nematode behavior and for symptom development were made daily during the test period. Two weeks after inoculation, roots inoculated directly were stained with acid fuchsin in lactophenol (6) to observe nematodes within the tissues.

RESULTS

Soil treatment: Only an occasional swelling was found on the root systems of tomato plants grown in soil infested with *M. incognita* and treated with carbofuran. In contrast, the root systems of the control plants were heavily galled. The population of *M. incognita* larvae recovered from treated soil averaged 267/pot; and from untreated soil, 38/pot. When larvae from treated as well as untreated soil were put

TABLE 1. Average number of *Pratylenchus penetrans* recovered from the soil and root systems of pea plants 14 days and 50 days after treatment with carbofuran.

Treatment and concentration (ppm)	No. of nematodes/ pot from soil days after treatment		No. of nematodes/ root system days after treatment	
	14	50	14	50
Carbofuran 10	608	12	137	30
Carbofuran 20		12		26
Carbofuran 25	1138		7	
Control	230	4	1116	1098

around tomato seedlings growing in untreated soil, galls developed on the root systems in all cases. The severity of galling was proportional to the number of nematodes introduced.

Greater numbers of *P. penetrans* were recovered from carbofuran-treated soil than from the untreated controls, and more were recovered after 2 weeks at 25 than at 10 ppm (Table 1). After 50 days, nematode populations were low in both treated and untreated soil.

The numbers of *T. claytoni* recovered from soil at each distance from the corn seedlings for each inoculated area are reported in Table 2. In all cases, nematodes were recovered from the soil adjacent to the corn seedlings grown in untreated soil and predominated in the direction toward the plant. When the soil was treated, nematodes were recovered from the soil sections next to the plant only when inoculations occurred at 2.5 cm from the seedling. In all other cases, nematodes remained near the point of inoculation but predominated in the direction away from the plant. *T. claytoni* was recovered also from areas near the edges of the trays. By the end of the experiment, root systems had grown into these zones.

Agar medium treatment: Twenty-four hours after inoculation with *P. penetrans* 4 cm away from a seedling, a number of nematodes had reached the roots and were moving around the root tips in the untreated dishes. No nematodes were observed near the roots in the treated plates, but specimens were motile near the area of inoculation. Forty-eight hours after inoculation, lesions developed on the roots of tomato seedlings in untreated medium, whereas no nematodes were present near the roots in the treated medium, but were still near the point of inoculation. Three days after inoculation, in one treated replicate several

TABLE 2. Number of *Tylenchorhynchus claytoni* recovered 10 days after inoculation from soil treated with carbofuran at 10 ppm and with nematodes placed at different distances from corn seedlings.

Inoculation distance from the plant (cm)	Sampling distance from the plant (cm)	No. of nematodes	
		Control	Carbofuran
2.5	2	1700	193
	4	450	422
	6	31	8
	8	7	3
	10	2	5
	12	2	0
	14	0	0
	16	0	0
	18	0	0
	20	0	0
5.0	2	42	0
	4	66	37
	6	316	873
	8	36	10
	10	17	5
	12	7	3
	14	2	0
	16	0	0
	18	0	0
	20	0	0
8.0	2	14	0
	4	10	2
	6	200	1
	8	150	321
	10	80	260
	12	12	15
	14	3	1
	16	0	0
	18	0	0
	20	0	0
10	2	5	0
	4	9	0
	6	9	0
	8	18	0
	10	140	331
	12	107	160
	14	22	12
	16	4	2
	18	2	0
	20	0	0
12	2	10	0
	4	6	0
	6	2	0
	8	8	0
	10	32	0
	12	350	887
	14	51	116
	16	24	0
	18	11	0
	20	5	0

nematodes were near the roots, but none was observed attempting to feed. These nematodes moved away by the 4th day and no lesions appeared on the roots, whereas the number of lesions in the controls increased.

Most of the nematodes in treated agar were showing random movement; those that reached the roots were not near the zone of elongation, and, in some cases, they were in a different plane with respect to the roots, and no lesions developed indicating no feeding. Occasional nematodes were seen also with their bodies oriented perpendicularly to the surface of the medium, whereas in the controls the movement was always parallel to the plane of the medium. Nematodes in the controls were seen to penetrate roots. Ten days after inoculation, there were no moving nematodes in the untreated agar – they had either penetrated or died; whereas they were present in treated medium. By this time a number of nematodes had died in both treated and untreated agar.

Twenty-four hours after *P. penetrans* was inoculated on the developing seedlings, lesions developed on the roots of the control plants, and all nematodes were moving near the elongating roots. In carbofuran-treated medium, no lesions were seen and nematodes were not touching the roots. Forty-eight hours after inoculation, all *P. penetrans* were near the roots in the controls, whereas in carbofuran treatments, many were away from the roots, and, since no lesions appeared, those near the roots apparently did not feed. Four days after inoculation, all living nematodes had penetrated the roots and the number of lesions increased, whereas no symptoms appeared on seedlings in carbofuran-treated medium, and most nematodes were in the agar. In one case several nematodes were seen near the roots, but no symptoms developed. These nematodes later moved away from the roots. As previously observed, higher numbers of *P. penetrans* were moving in agar and no lesions developed on roots growing in treated medium. A few specimens were still near the roots. At this stage of the experiment in untreated agar, a number of *P. penetrans* were coming out of the lesions and collecting near the newly formed root tips. This became more evident 2 days later, when lesions were also found away from the point of inoculation (Fig. 1A). At the same time, no lesions were present on roots in carbofuran treatments (Fig. 1B).

Ten days after inoculation, a group of nematodes in carbofuran treatments were about 2 mm away from the root tips. By the next day, these nematodes had moved in mass following the root in its growth. A day later they had stopped, whereas the root continued to grow. Three days later their movement slowed down, and by the 4th day they were dead. As many as thirty dead nematodes were seen near the growing region of the roots. Successive observations revealed that in all replicates, several nematodes had died near the roots. Nematodes were considered dead after they were seen in a relaxed position for at least 2 days. Dead specimens were present throughout the medium, but no lesions appeared on the roots. As observed in the previous test, some nematodes were perpendicular to the agar surface, and they died in this position. Lesions on the roots of the controls had enlarged by the end of the experiment, and they were no longer individually distinguishable. Table 3 presents the average number of *P. penetrans* and lesions produced on roots of tomato seedlings.

DISCUSSION

Higher numbers of *M. incognita* and *P. penetrans* were recovered from carbofuran-treated than from untreated soil 10

TABLE 3. Average number of *Pratylenchus penetrans* and lesions on roots of tomato seedlings growing in agar medium treated with 10 ppm carbofuran.

Treatment	Placement of nematodes	Nematodes in the agar, days after treatment		Nematodes on the roots, days after treatment		Number lesions, days after treatment		
		5	6	5	6	5	6	20
Carbofuran	On the roots		51		3	0	0	0
	4 cm from roots	93		1		0		
Control	On the roots		0		1		25	54
	4 cm from roots	2		11		22		

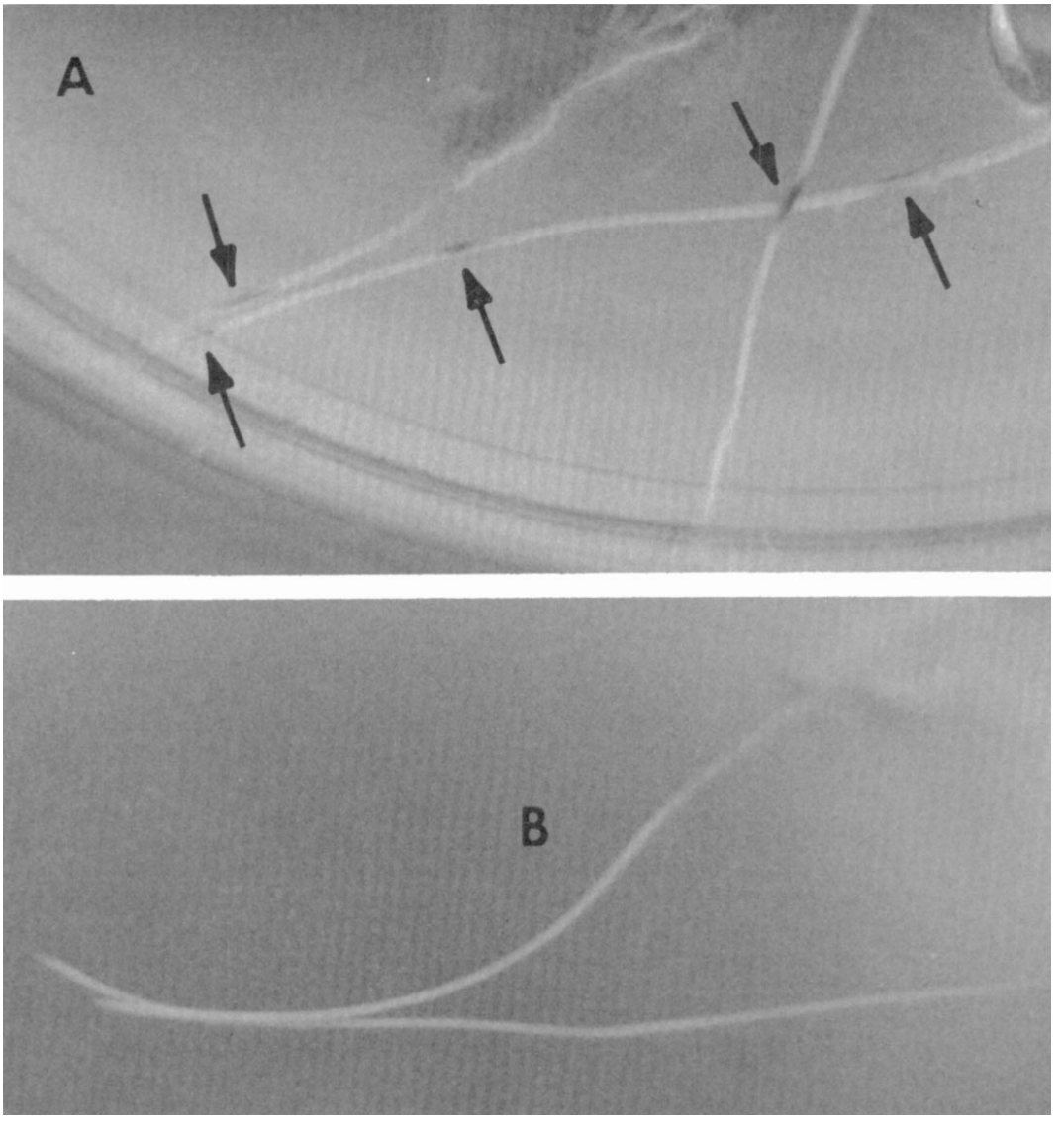

FIG. 1. Root sections of 'Heinz 1350' tomato seedlings eight days after inoculation with *Pratylenchus penetrans*. **A.** Lesions (arrows) developed on roots in untreated agar medium. **B.** No lesions developed on roots in agar medium treated with 10 ppm carbofuran.

and 15 days after treatment, respectively, and in both cases, symptoms on the root systems of host plants were greatly reduced. It would appear that nematodes had not penetrated the roots and, therefore, were recovered from the soil. After 50 days, however, the population of *P. penetrans* in treated and untreated soil was low, and, because they were not recovered from the roots, I assume that they died in the soil without causing infection. The higher numbers in soil treated at 25 ppm than at 10 ppm suggest that higher concentrations of carbofuran affected *P. penetrans* to a greater extent than did lower concentrations resulting in less root penetration.

Since *T. claytoni* remained near the areas of inoculation for 10 days in trays containing treated soil and a corn plant, but moved toward roots in untreated soil, it would seem that nematodes were disoriented in some manner or that the corn roots were not "attractive".

The tests carried out in petri dishes show

that random movement of *P. penetrans* predominated in carbofuran-treated medium when inoculations occurred 4 cm away from the root tips. A number of nematodes reached the root area, then moved away. Probably the root was reached by random movement that continued near the root zone. Random movement was observed also when inoculations occurred near the developing roots. Nematodes in untreated medium moved within a short time to the growing roots and infected them. Nematodes that left roots moved to newly formed rootlets. In contrast, nematodes in carbofuran-treated medium moved about remote from the roots. A number of nematodes later returned to near the root zones, probably as a result of random movement. In most cases, they were not seen at the growing root tips. In one case, a group of nematodes was seen at about 2 mm away from the root tip, and they followed the root growth for 24 hr when they finally stopped. It is not clear whether these specimens were actually following the zone of root elongation under the stimulus of an attractant or were trapped in that area by the root. I did not observe any attempts to feed, however.

Present knowledge about attraction and feeding of plant-parasitic nematodes do not explain the observed phenomenon. Klinger (4) feels that CO_2 produced by roots has to be considered as the long distance attractant which orients the movement of nematodes toward the roots. Other attractants may act in the vicinity of the roots. If we accept this explanation, it would appear that in carbofuran-treated medium *P. penetrans* was unable to detect the CO_2 concentration gradient, and therefore, moved at random. That the orientation was affected is clearly indicated by those specimens which were seen perpendicular to the agar surface. Attractants acting near the roots were also not detected, with one possible exception already mentioned. Previous unpublished experiments showed that *M. incognita* larvae behaved similarly. Thomas (8) observed that feeding by *Criconemoides xenoplax* is initiated after the lip region comes in contact with the roots. Sensory receptors of the nematode are then stimulated and feeding starts. If such stimulation is general, carbofuran may affect these receptors so that feeding cannot start when nematodes reach the root. Since carbofuran is systemic in plants, the possibility also exists that the plant itself may be affected so that attractants are not produced. Somewhat supporting this view was the fact that nematodes, removed from treated soil, infected tomato seedling roots in untreated soil.

The present study suggests that carbofuran controls the plant-parasitic nematodes examined, primarily by affecting the orientation and feeding mechanisms. Reduction of nematode populations in soil may be attributed to starvation, or more likely to a combination of starvation and sublethal toxicity.

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