Behavioral Effects of Carbofuran and Phenamiphos on Pratylenchus vulnus. II. Attraction to Bean Roots

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Abstract: Pratylenchus vulnus (L_3, L_4, adults) are attracted to the roots of growing bean seedlings. The attraction is inhibited by treating the nematodes with solutions of carbofuran or phenamiphos at concentrations below those necessary to inhibit motility and dispersion. Nematicide treatments of plants without treatment of nematodes are ineffectual because nematodes are attracted equally well to treated and untreated seedlings. *Key Words:* root-lesion nematodes, non-volatile nematicides, mode of action.

Nematodes respond to the chemical stimuli of their environment. The mechanism of detection and perception of the environment, however, is poorly understood. Seeking activity and feeding-site selection by Tylenchid nematodes has been explained to be a result of an elaborate sensory system (1). There is substantial evidence that emanations from plant roots attract nematodes (4,6,9). Several chemical substances that attract nematodes are known to emanate from the roots of plants (4). Certain nematodes (e.g., *Pratylenchus pratensis* (5) and *P. penetrans* (1)) are attracted to specific areas of host roots.

MATERIALS AND METHODS

Attraction studies were carried out with the equipment and system described previously (9). The containers consisted of aluminum channel sections of different lengths, coated with asphaltum and with the edge inscribed at 1-cm intervals. Longer sections of channel were used for growing plants in sand and shorter channels for containing nematodes uniformly dispersed in sand. For the bioassays a dialysis membrane strip (average pore size 24 Å) was placed between the channels containing plants and nematodes to separate the nematodes from the host roots while allowing for diffusion. Seeds of snap bean, *Phaseolus vulgaris* var. Kentucky Wonder, were germinated in vermiculite in a greenhouse until the seedlings were 4 to 6 cm high. Then the seedlings were removed and washed free of vermiculite, and 12 to 16 were selected for uniformity and transplanted into each plant channel. The plants were grown in a growth chamber regulated to maintain the sand temperature at 23 ± 0.5 C, with a photoperiod of 10 h light and 14 h darkness. The plants were watered with 1/2-strength Hoagland's solution until they were about 10 to 13 cm tall. Each plant dish was then leached with 1 liter of distilled water to reduce the solubles resulting from nutrient watering and root growth, or with nemati-

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cide if nematicidal treatment was desired. About 3,000 to 5,000 nematodes (L_3, L_4, and adults) obtained from carrot stock cultures were pretreated for 12 h in nematicide solution or distilled water. The nematodes were resuspended in the treatment solutions and then poured into the channel container. Quartz sand (150 to 250 μm) was immediately sprinkled into the channel as evenly as possible with the aid of screens. The plant channel and nematode channel were juxtaposed (Fig. 1) and placed in a high-humidity chamber inside the growth

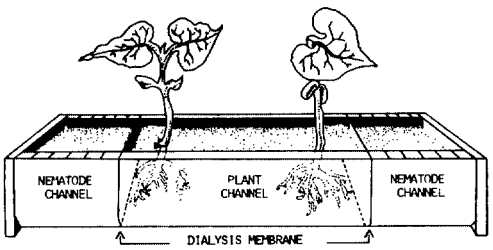

Fig. 1. Schematic diagram of the apparatus used in the attraction experiments.

chamber. After the appropriate incubation period, the assemblies were removed from the humidity chamber. The sand was then removed from the channel in 10-mm sections beginning at the membrane and proceeding outward. Each section was placed in a petri dish and washed three times with 20 to 25 ml of water to extract the nematodes in suspension. The washings were combined, the nematodes allowed to settle overnight, the supernatant decanted, and the nematodes counted. The number of nematodes per 10-mm section of sand was expressed as a percentage of the total number of nematodes recovered from that particular channel container.

Modification of the attraction response of *P. vulnus* to bean root emanations by carbofuran and phenamiphos was established by the following treatments: a) both channels—nematode and plant—were drenched with the same nematicide concentration; b) only nematode channels or plant channels were drenched; c) treatment solutions included 0.17*M* acetone, 0.001, 0.0001, 0.00005, and 0.00001 m*M* phenamiphos, and 0.005, 0.001, and 0.0005 m*M* carbofuran. In addition, bean foliage treatments were carried out by immersing the whole stem foliage area 5 to 6 cm above sand level in carbofuran or phenamiphos solution for various periods (Table 1). A 4.06-m*M* stock solution of each compound was made up in 0.6*M* acetone to aid dissolution, plus 0.1% detergent to assist wetting of the foliage. The stock solution of both chemicals was then diluted threefold to 0.15 m*M*. Thus, four concentration levels were tested: 4.06, 1.35, 0.45, and 0.15 m*M*. To prevent chemical contamination of the sand, the plant channels were held inverted until the foliage dried. In addition, strips of filter paper were placed around bean stems to preclude drainage of water of guttation, or condensation from the foliage or petioles to the sand. The experiments were replicated six to eight times, and the experimental designs were randomized blocks. Where indicated, data were subjected to analysis of variance and Duncan's multiple-range test.

RESULTS

Influence of bioassay time on attraction to emanations: The control curve shows that in the absence of plants it was possible to prepare a channel container in which nematodes were distributed evenly, and to maintain that distribution indefinitely. The control curve was not different ($P = 0.01$) from that obtained with a 24-h exposure to bean root emanations. However, both of these curves were different from others obtained with the longer bioassay exposure periods (Fig. 2).

Table 1. Attraction response of *P. vulnus* (L_3, L_4, adults) to bean roots after 48 h. Bean foliage immersed in different concentrations of carbofuran (C) or phenamiphos (P) for various immersion periods.
+ = normal attraction, x = phytotoxic, xx = very phytotoxic

Concentration (m*M*)	Nematicide	Immersion period		
		5 sec	50 sec	300 sec
4.06	C	+	+	+ (x)
	P	+	+ (x)	+ (xx)
1.35	C	+	+	+
	P	+	+	+ (x)
0.45	C	+	+	+
	P	+	+	+
0.15	C	+	+	+
	P	+	+	+
Control		+	+	+

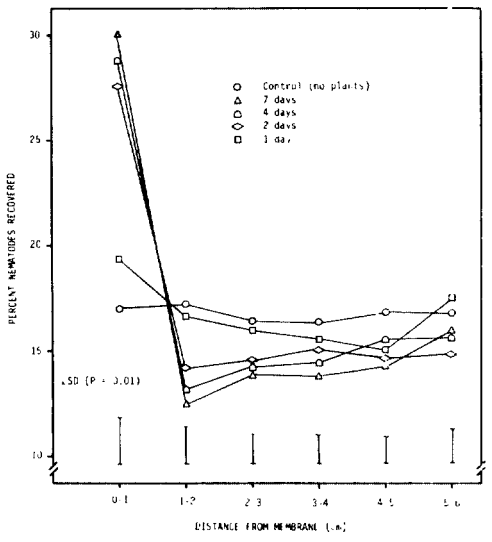

Fig. 2. Percent of *P. vulnus* (L_3, L_4, adults) per 1-cm section of channel relative to the distance from the membrane for different attraction times. Percentages based upon total number of nematodes recovered from each channel. Each point represents the mean of eight replicates.

Influence of nematicides on attraction: The effect of nematicides applied at various concentrations as a sand drench on nematode redistribution in both plant and nematode channels is illustrated in Fig. 3 (carbofuran) and Fig. 4 (phenamiphos). In 0.005 m*M* carbofuran drenches, *P. vulnus* did not redistribute under plant influence (Fig. 3); the nematode distribution was not significantly different from that of untreated nematodes under no plant influence. In a 0.0005 m*M* drench of carbofuran, *P. vulnus* redistributed under plant influence in the same fashion as untreated controls under plant influence. At a concentration of 0.001 m*M* carbofuran, *P. vulnus* redistributed, but the redistribution was significantly different from that with both higher and lower doses.

In 0.001 m*M* phenamiphos solutions, no redistribution of nematodes occurred (Fig. 4); there was no significant difference in nematode distribution between this concentration and controls without plants. At 0.0001 m*M* phenamiphos solution, nematode redistribution adjacent to the membrane was significantly different from that of controls without plants or the 0.001 m*M* phenamiphos solution with plants. At 0.00005 m*M* phenamiphos solution, the threshold concentration, nematode redistribution was significantly less than that of untreated controls under the influence of plants.

When nematode-containing channels

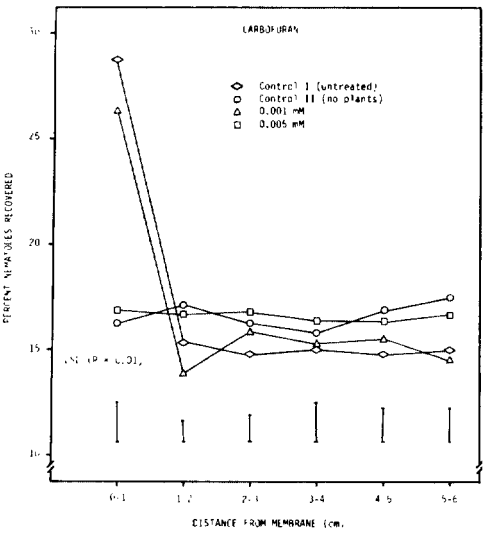

Fig. 3. Percent of *P. vulnus* (L_3, L_4, adults) per 1-cm section of channel relative to the distance from the membrane as affected by different concentrations of carbofuran after 48 h. Percentages based upon total number of nematodes recovered from each channel. Each point represents the mean of eight replicates.

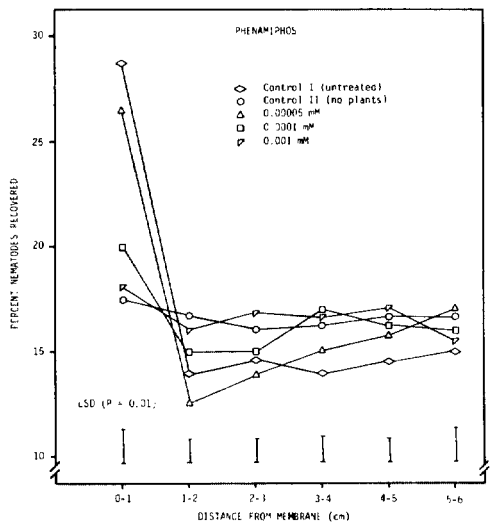

Fig. 4. Percent of *P. vulnus* (L_3, L_4, adults) per 1-cm section of channel relative to the distance from the membrane as affected by different concentrations of phenamiphos solutions after 48 h. Percentages based upon total number of nematodes recovered from each channel. Each point represents the mean of eight replicates.

were drenched and plant channels were not drenched, results were similar to those obtained when both plant channels and nematode channels were treated. When plant channels only were treated, either by drenching or by dipping plant foliage, no inhibition of attraction was obtained. This was true over a broad range of nematicide concentrations, the highest of which caused phytotoxicity (Table 1).

DISCUSSION

P. vulnus responded to a dialyzable stimulating agent from bean roots by accumulating in the 10-mm sand section nearest the membranes separating bean roots from nematodes when bioassay exposure periods exceeded 24 h. Incubation periods exceeding 48 h did not increase the magnitude of the response. The attraction curve suggested that the attraction response was operative within 1 to 2 cm of bean roots. It was difficult to determine whether nematode migration from sections 2 to 6 cm from the membrane was primarily in response to a diffusing chemical stimulant from bean roots or to a population density gradient established by a local area depletion (9) as a consequence of attraction. The indication that *P. vulnus* could be attracted over small distances by diffusing bean root emanations was consistent with observations by Klingler (4) and Steinbach (8) with different nematodes.

At 0.05 m*M* carbofuran completely suppressed dispersion of *P. vulnus* (7), but at 0.005 m*M* over 50% of the nematodes were able to disperse. At this lower concentration the attraction response remained completely inhibited. Even at 0.0001 m*M* of carbofuran, 80% of the nematodes were capable of dispersion but the attraction response was inhibited ($P = 0.01$). Similarly, differences in the distribution patterns between phenamiphos-treated and untreated nematodes indicated an impairment of nematode attraction toward bean roots at concentrations too low to affect motility and dispersion. The inhibition of attraction was proportional to phenamiphos concentration and occurred over a broader range than the carbofuran inhibition. Below the threshold concentration of 0.00005 m*M*, attraction was no different from that of controls with plants ($P = 0.01$). At 0.0001 m*M*, phenamiphos attraction was substantially inhibited, with fewer nematodes redistributing toward the membrane, although dispersion was essentially unhindered (7). At the higher dose of 0.0001 m*M* the nematode distribution pattern was similar to that of the control without plants, but this same concentration rendered nematodes immotile (7).

It is possible that phenamiphos interferes with the attraction response of otherwise active and motile *P. vulnus*, by disrupting sensor or a sensor-control ganglia coordination function that allows the nematodes to orient. These results suggest that *P. vulnus* attraction is inhibited only when the nematodes are exposed directly. When just the plants were treated, no inhibition was observed under the conditions of these experiments. Apparently the attraction response was not impeded by nematicide diffusing from the plant side toward the nematode side, because preliminary experiments had established that both carbofuran and phenamiphos were freely permeable through the dialysis membrane. Furthermore, when a treated nematode channel was placed adjacent to an untreated plant channel, the treated nematodes adjacent to the membrane did not recover as the nematicide concentration adjacent to the membrane decreased as a consequence of diffusion through the membrane into the plant channel.

Nematode attraction to bean roots was nearly completely inhibited at 0.0001 m*M* phenamiphos, whereas a hundredfold increase in concentration was required to fully suppress the motor functions involved in dispersion, and a five-hundredfold increase to suppress those involved in motility. These differences in responses suggested that the chemo-receptor and coordination network by which *P. vulnus* detects and responds to plant attractants was extremely sensitive to phenamiphos, but less so to carbofuran. Whether this effect was the result of a receptor site binding or modification or a block in recognition and integration neuro circuitry therefrom, was not established.

These kinds of behavioral responses are useful to understanding of mode of action of nonfumigant nematicides. As illustrated by phenamiphos concentrations of 0.0001

m*M* in the soil solution, mass action effect would indicate that host root attraction was essentially eliminated as a natural force, reducing the attack pressure of *P. vulnus* on host roots; at a higher concentration of 0.01 m*M*, coordination function involved in dispersion was completely suppressed, while at 0.05 m*M* motility was completely suppressed. Carbofuran behaves similarly, although in a higher concentration range. The current belief is that in field situations most carbamates and organophosphate insecticides/nematicides can only cause nematode immobilization or death in the recommended commercial applications. It is evident that these rates are marginally lethal, so that other effects are responsible in part for the control properties observed in the field. The interference of nematode orientation by some nonfumigant nematicides has been reported recently. Di Sanzo (1) has shown that carbofuran has interfered with the orientation of *Tylenchorhynchus claytoni* to corn roots in sand and *Pratylenchus penetrans* to tomato roots growing on agar.

Aldicarb has been reported to inhibit the orientation mechanism of *Heterodera schachtii* males to the female sex attractant (3). The data and observations offered in this report clearly demonstrate the capability of the carbamate and organophosphate compounds to effect subtle behavioral responses of nematodes under continuous exposure to specific doses that did not affect nematode somatic neuromuscular function. They also show that these behavioral disruptions can remain operative as long as the nematicide concentrations do not fall below threshold levels. Above such levels these compounds may have affected a wide variety of nematode functions impairing their ability to infect.

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