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Development of the Entomogenous Nematode, *Neoaplectana carpocapsae*[1] (Rhabditida: Steinernematidae), in Insecticide-Killed Beet Armyworm (Lepidoptera: Noctuidae)[2]

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ABSTRACT The entomogenous nematode *Neoaplectana carpocapsae* Weiser invaded and reproduced in larvae of *Spodoptera exigua* (Hübner) killed by the insecticides trichlorfon, mevinphos, methomyl, fenvalerate, and permethrin. When killed *S. exigua* were exposed to infective juveniles of *N. carpocapsae* 48 h after insecticide treatment, 72% to 100% of dead *S. exigua* produced nematode progeny. The nematode progeny were infectious to living *S. exigua*. However, when *S. exigua* was first infected with *N. carpocapsae* and 48 h later topically treated with phenamiphos or methomyl, development and reproduction of *N. carpocapsae* were adversely affected.

The efficacy of *Neoaplectana carpocapsae* Weiser against insects may be impaired if used with chemical pesticides in an integrated pest management program (Kamionek 1979, Hara and Kaya 1982). This is particularly important if *N. carpocapsae* is used as an inoculative biological control agent and its recycling in the insect population is desired. Kamionek (1979) demonstrated that an organophosphate nematicide adversely affected the development and reproduction of *N. carpocapsae* when insects infected first with the nematode were then treated with the chemical. Pye and Burman (1978) showed that larvae of the large pine weevil, *Hylobius abietis* L., killed by chloroform or heat, then invaded by *N. carpocapsae*, could support nematode reproduction. We report here the ability of *N. carpocapsae* to invade and reproduce in insecticide-killed beet armyworm, *Spodoptera exigua* (Hübner), larvae.

Materials and Methods

Spodoptera exigua were reared in the laboratory on artificial diet (Tanada and Chang 1968). The technical grade insecticides used in this study were the organophosphates mevinphos, phenamiphos (nematicide) and trichlorfon, the carbamate methomyl, and the synthetic pyrethroids fenvalerate and permethrin. Stock solutions and dilutions were prepared in acetone. Topical applications of the chemicals were made with a 27-gauge needle and a 0.25 cc tuberculin syringe attached to a microapplicator. For all topical tests, 1.0 μl of the desired concentration was applied to the notum of newly-molted last-instar *S. exigua*. Controls were treated with 1.0 μl of acetone in each replication. Treated caterpillars were placed individually into petri dishes containing ca. 1 g of artificial diet. Each test consisted of treating at least 30 caterpillars per concentration in replicates of 10. Mortality was assessed 48 h after treatment. Moribund caterpillars were considered.

Dead and living caterpillars were weighed and exposed to *N. carpocapsae* (All strain) at 200 infective juveniles per caterpillar as described by Hara and Kaya (1982). After 48 h of exposure to the nematodes, each caterpillar was rinsed in 0.5% sodium hypochlorite for 1 min and placed on another moist filter paper in a dish. Five days after exposure to the nematodes, each caterpillar was placed in a nematode trap. The trap consisted of a 60 × 15-mm petri dish in which a 30 mm diam filter paper disc (Whatman No. 1) was placed on a plastic portion cup-cover (35 ml, Dixie Cup, Easton, PA) cut to 30 mm diam. A caterpillar was placed on the filter paper and the petri dish filled with 5 ml of distilled water. Twelve days after exposure to the nematodes, each caterpillar was dissected and observed for the presence of nematodes. In caterpillars with nematode reproduction, juveniles in the trap and dissected caterpillars were counted using a 1 ml Peters counting slide (Hawsley and Sons, Ltd., Sussex, England).

In another series of experiments, *S. exigua* larvae were first exposed to *N. carpocapsae* as previously described and after 48 h, the dead caterpillars (i.e. killed by the nematode) were treated topically with 1.0 μl of methomyl or phenamiphos at various concentrations. Each treated caterpillar was placed in another dish and five days later transferred to a nematode trap. The trap and caterpillar were examined for nematodes 12 days after exposure to the nematodes. When appropriate, the data were analyzed by ANOVA or chi-square. Percentages were transformed to arcsins before ANOVA.

Results

S. exigua larvae killed by trichlorfon, mevinphos, methomyl, fenvalerate, and permethrin were invaded by *N. carpocapsae* at levels of 82.3% to 100%. If the caterpillars were alive after insecticide or control treatments, 95.3% to 100% were infected and contained nematodes. Chi-square analysis showed that the percentages of living and insecticide-killed hosts invaded by the nematode were not significantly different ($P > 0.01$). Analysis of variance showed no significant difference in percentages of insecticide-killed *S. exigua* invaded among insecticides or concentrations. However, the percentages of *S. exigua* larvae with nematode progeny were significantly different ($P < 0.01$) between liv-

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ing hosts and hosts killed by trichlorfon, mevinphos, and methomyl (Table 1). Nematode progeny occurred in 72.2% to 83.3% of the trichlorfon-, mevinphos-, and methomyl-killed hosts, and in 93.3% to 100% of larvae alive after these insecticide treatments. In an infected caterpillar without nematode progeny, few living or dead juveniles or adults were present. With fenvalerate and permethrin, percentages of *S. exigua* larvae with nematode progeny in living or insecticide-killed hosts were not significantly different ($P > 0.01$).

Nematode progeny consisted of infective juveniles occurring both in the trap and the caterpillars. The infectivity of these juveniles to *S. exigua* larvae was 95% to 100% for insecticide-killed and living hosts. Significantly more infective juveniles were produced in living hosts, including controls ($\bar{x}$ = 132,943) as compared to dead hosts ($\bar{x}$ = 22,984) (Table 2). However, the number of infective juveniles per mg of caterpillar were not significantly different ($P > 0.01$) in comparisons between living and insecticide-killed hosts. Living and dead hosts had means between 586 and 871 juveniles per mg of larva for all treatments (Table 2).

Methomyl or phenamiphos adversely affected the development and reproduction of *N. carpocapsae* when *S. exigua* larvae were first infected with the nematode and then treated with the chemicals (Table 3). At 10.0 mg/ml of methomyl, 20% of the treated *S. exigua* larvae had living nematode adults only, and 13% had dead nematode adults only. As the concentration of methomyl increased, there were increases in the percentages of dead nematode adults and progeny. At 10.0 to 100.0 mg/ml of phenamiphos, >80% of the treated *S. exigua* larvae contained dead nematode progeny.

Discussion

Insecticide-killed hosts can provide a resource for development and reproduction of *N. carpocapsae*. This finding is in agreement with the report of Pye and Burman (1978), who found that the infection process of *N. carpocapsae* was not passive since dead insects could be colonized. Possibly, *N. carpocapsae* located and invaded dead insects by using chemical stimuli such as excretory products contaminating the integument of the host insect (Schmidt and All 1978, 1979). The total nematode progeny per host produced by insecticide-killed and living *S. exigua* larvae was significantly different, but the number of nematodes per mg of host did not differ significantly. This was due to a 5- to 7-fold weight loss of the killed *S. exigua* larvae, which resulted in reduced nematode progeny.

With insecticide-killed hosts, the limited toxicity of trichlorfon, mevinphos, and methomyl to *N. carpocapsae* may be explained by metabolism of the insecticide before the death of the insect. Therefore, the concentration of the chemical in the insect was not high enough to adversely affect the development and reproduction of the nematode. However, phenamiphos or methomyl adversely affected the development and reproduction of *N. carpocapsae* when the insects were first infected with the nematode and then treated with the chemicals. Nematode progeny were killed in phenamiphos-treated hosts, while nematode development before reproduction was

Table 1. Development of *N. carpocapsae* in *S. exigua* that were alive or dead after insecticide treatments

Chemical	Conc (mg/ml)	Living *S. exigua*		Dead *S. exigua*	
		No. live[a]	% with nematode progeny[b]	No. dead[a]	% with nematode progeny[c]
Trichlorfon	0.0	30	100	0	—
	50.0	15	100	15	80.0
	100.0	3	100	27	82.3
Mevinphos	0.0	30	100	0	—
	5.0	19	95.3	11	83.3
	10.0	10	93.3	20	76.1
	50.0	2	100	28	72.2
Methomyl	0.0	30	96.7	0	—
	1.0	17	100	13	75.6
	5.0	6	100	24	83.3
	10.0	3	100	27	77.8
	50.0	0	—	30	83.3
Fenvalerate	0.0	30	100	0	—
	1.0	18	100	12	91.7
	5.0	11	100	19	100
	10.0	0	—	30	96.7
Permethrin	0.0	40	95.0	0	—
	0.1	13	100	27	96.5
	0.5	6	100	34	93.8
	1.0	0	—	40	92.5

[a] Combined data of at least three replicates with 10 insects per replicate.

[b] Nematode progeny consisting of infective juveniles. Mean percent of at least three replicates with 10 insects per replicate. No significant difference ($P > 0.01$) among insecticides or concentrations.

[c] No significant difference ($P > 0.01$) among concentrations of same insecticide. Chi-square analysis shows significant difference ($P < 0.01$) in percentages of *S. exigua* with nematode progeny between living hosts and hosts killed by trichlorfon, mevinphos, and methomyl, but no significant difference with fenvalerate and permethrin.

Table 2. Number of infective juveniles of *N. carpocapsae* occurring in *S. exigua* that were alive or dead after insecticide treatments

Chemical	No. of nematodes[a]			
	Total per *S. exigua*[b]		Per mg of *S. exigua*[c]	
	Living	Dead	Living	Dead
Trichlorfon	105,978 ± 28,436	18,578 ± 6,461	633 ± 178	566 ± 202
Mevinphos	150,334 ± 34,356	24,727 ± 12,362	655 ± 210	810 ± 300
Methomyl	115,425 ± 32,387	21,036 ± 6,775	778 ± 183	714 ± 168
Fenvalerate	154,044 ± 40,497	32,061 ± 4,213	604 ± 136	871 ± 130
Permethrin	127,335 ± 35,780	18,517 ± 8,276	694 ± 248	726 ± 205
Acetone (control)	144,544 ± 31,236	—	847 ± 146	—

[a] Mean number ± SD of infective juveniles occurring in the nematode trap and in the dissected larva ($n = 20$).
[b] Total nematodes per *S. exigua* larva are significantly different ($P < 0.01$) between living and dead hosts.
[c] Larva weighed 48 h post-insecticide treatment. Nematodes per mg of *S. exigua* larva are not significantly different ($P > 0.01$).

Table 3. Toxicity of methomyl and phenamiphos to *N. carpocapsae*-infected *S. exigua*[a]

Chemical	Conc (mg/ml)	Percentages of *S. exigua* with[b]			
		Living nematodes		Dead nematodes	
		Progeny	Adult only	Progeny	Adult only
Methomyl	0.0	100	0	0	0
	1.0	100	0	0	0
	10.0	67	20	0	13
	50.0	47	23	13	17
	100.0	13	20	17	50
Phenamiphos	0.0	100	0	0	0
	1.0	100	0	0	0
	10.0	0	7	93	0
	50.0	0	0	80	20
	100.0	0	0	83	17

[a] *S. exigua* larvae first infected with *N. carpocapsae* and after 48 h treated topically with methomyl and phenamiphos.
[b] Combined data of three replicates with 10 insects per replicate.

affected in methomyl-treated hosts. The toxicity of phenamiphos and methomyl to *N. carpocapsae* agrees with that reported by Kamionek (1979), who found that the organophosphate nematicide thionazin was toxic to *N. carpocapsae* when applied to the greater wax moth, *Galleria mellonella* (L.), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), 48 h after nematode invasion.

Although insecticide-killed insects may serve as hosts for *N. carpocapsae*, the behavior, infectivity, the in vivo or in vitro development and reproduction of the nematode are adversely affected by certain organophosphate and carbamate insecticides and nematicides (Hara and Kaya 1982, Kamionek 1979). If *N. carpocapsae* and pesticides that are toxic to the nematode are used in IPM programs, compatibility may be achieved by altering the use pattern of the pesticide as suggested by Hara and Kaya (1982). With pesticides such as certain synthetic pyrethroids that are not toxic to *N. carpocapsae* (Hara and Kaya 1982), the nematode may be used as an inoculative or long-term biological control agent. Recycling of the nematode in the environment may occur in the nematode-killed and possibly in the insecticide-killed host species. However, in field situations, *N. carpocapsae* is not a generalized biological control agent, but is restricted to insect species or stages highly susceptible to the nematode. These insects must occur in moist habitats that favor the survival and infectivity of the nematode (Gaugler 1981). Field experiments combining *N. carpocapsae* and insecticide treatments will be needed to test the actual potential of this nematode in IPM programs.

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