Infectivity of *Steinernema feltiae* in Fenamiphos-treated Sand

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The entomopathogenic nematode *Steinernema feltiae* is compatible with many chemical pesticides including insecticides, miticides, fungicides, and herbicides (1,3,4,7,9,10). Certain organophosphate and carbamate pesticides, however, adversely affect *S. feltiae* (5,6). Removal or dilution of the pesticides with water usually results in nematode recovery, infection of the insect host, and normal development (5,6). Studies were conducted by placing the infective stage of the nematode in a pesticide solution or suspension. The purpose of our study was to determine the infectivity of *S. feltiae* in sand treated with a granular formulation of the organophosphate pesticide, fenamiphos.

The All strain of *S. feltiae*, cultured on *Galleria mellonella* as described by Dutky et al. (2), was stored at 10 C and used within 6 weeks after harvest. Fenamiphos (ethyl-3-methyl-4-[methylthiol]-phenyl[1-methylethyl] phosphoramidate) (Mobay Chemical Corp., Kansas City, MO) was obtained as a 15% granular formulation. Sand (particle size from 0.21 to 0.85 mm) was mixed with granules containing fenamiphos (mixture hereafter referred to as fenamiphos) at the rates of 30, 60, and 120 mg/kg dry sand in a twin shell blender for 10 minutes. These fenamiphos rates equaled 4.5, 9.0, and 18.0 mg a.i./kg dry sand, respectively. These rates approximate low, medium, and high field rates of fenamiphos incorporat-

ed into soil to a depth of 10 cm. Two hundred twenty-five grams of dry sand-fenamiphos mixture was placed in a 177-ml styrofoam cup. Forty milliliters of distilled water (0.18 g water/g sand) was added to the cup; 1 hour later, 1,600 *S. feltiae* in 0.5 ml water were placed on the sand surface. The cup, covered with the bottom of a petri dish (100 × 15 mm), was maintained at room temperature (25 ± 2 C) until five last-instar *Galleria mellonella* larvae were added to each cup to assay for nematode activity. Seven days later, the *Galleria* larvae were examined for nematode infection. In trial 1 the *Galleria* larvae were added immediately after nematode placement, and in trials 2 and 3 the larvae were added 7 days after the nematodes were placed on the sand. Controls for the experiments were untreated sand (no chemicals or nematodes in trials 1, 3) and sand with nematodes only (trials 1–3). There were four replicates for each treatment with individual cups serving as a replicate.

To determine the infectivity of extracted *S. feltiae* after exposure to fenamiphos-treated sand, the same procedure as described in the preceding paragraph was followed except that 3,000 infective nematodes were added to each cup. After 4 days, the nematodes were extracted from the sand with a 38-μm-pore sieve and transferred with other debris to a Baermann funnel for final extraction for 24 hours. Extraction efficacy was 35% for untreated nematodes. The nematodes extracted were counted, the numbers alive and dead were determined, and the live nematodes were bioassayed against *Galleria*. For the bioassay, four or five *Galleria* larvae were exposed to 200–250 *S. feltiae* (30 per larva) in 1 ml water in a petri dish lined with two

TABLE 1. Mortality of *Galleria mellonella* larvae placed on the surface of sand 7 days after the sand was treated with fenamiphos and *Steinernema feltiae*.[†]

Fenamiphos concentration (mg/kg dry sand)	Total no. insects	$\bar{x}$ % dead ± SD	
		With nema	Without nema
0	40	100 ± 0	0 ± 0
4.5	39	22.5 ± 24.9	22.5 ± 18.3
9.0	40	11.9 ± 26.4	5.6 ± 11.3
18.0	39	0 ± 0	34.4 ± 38.1

† *Galleria* larvae in sand with no nematodes had no mortality.

TABLE 2. Mean number of *Steinernema feltiae* extracted, and percentage alive and infective to *Galleria mellonella* larvae after 4 days exposure to fenamiphos-treated sand.

Fenamiphos concentration (mg/kg dry sand)	$\bar{x}$ no. ± SD S. feltiae extracted	$\bar{x}$ % ± SD S. feltiae alive	$\bar{x}$ % ± SD Galleria infected
0	1,145 ± 413	90.1 ± 4.1	86.7 ± 12.6
4.5	787 ± 662	95.7 ± 5.5	92.2 ± 7.5
9.0	714 ± 448	90.3 ± 9.2	75.0 ± 27.8
18.0	847 ± 619	93.7 ± 4.7	76.7 ± 22.5

filter papers (Whatman #1, 9 cm d). The *Galleria* were examined for nematode infection 7 days later. There were three trials with four replicates in each trial.

Additional cups containing the treatments but without *S. feltiae* were planted with tomato (*Lycopersicon esculentum* UC 82B) seedlings to determine if the fenamiphos rates adversely affected the development of the root-knot nematode, *Meloidogyne incognita*. After 40 ml water was added to the sand, 1,000 *M. incognita* J2 in 1 ml water were placed in each cup as described previously. Seven days later a tomato seedling was planted in each cup, and 60 days after planting the roots were examined for presence or absence of galls.

Statistical analyses were done by linear regression and, wherever applicable, arcsine or log transformation of the data was done before an analysis.

When *S. feltiae* and *Galleria* were placed on the sand surface on the same day as the fenamiphos granules, nematode activity was totally suppressed. No *Galleria* larvae (n = 58) became infected in the pesticide treatment, but if the pesticide was not present, *Galleria* larvae (85%, n= 20) were infected. Placement of the *Galleria* on the sand surface 7 days after nematode and fenamiphos treatment resulted in nematodes killing significantly fewer *Galleria* as fenamiphos concentrations increased ($P < 0.01$, $r^2 = 0.56$, $F = 3.67$, df = 31) (Table 1). At fenamiphos concentrations of 0, 4.5, and 9.0 mg/kg sand, nematode progeny were observed, respectively, in 92.5% (37 of 40),

77.8% (7 of 9), and 75% (3 of 4) *Galleria*. *Galleria* mortality attributed to causes other than nematode was high in the fenamiphos-treated sand (Table 1). These insects were black because of a secondary bacterial invasion and probably died from exposure to the pesticide.

There was wide variation in number of nematodes extracted from sand (Table 2). Although the untreated sand had the highest mean number of nematodes extracted, the variance was high and no significant difference among the treatments was observed ($P > 0.05$). Survival of the extracted nematodes showed no significant differences and was > 90% in all treatments. These nematodes were infectious to *Galleria* larvae and caused mortality of >75% (Table 2). No significant differences in *Galleria* mortality were observed among the treatments.

The levels of fenamiphos used in our studies prevented the root-knot nematode from infecting tomato roots. In a qualitative assessment, a high degree of galling was observed in the control plants but no galling was observed in the fenamiphos-treated sand.

The presence of fenamiphos in sand adversely affects the ability of *S. feltiae* to infect an insect host. Fenamiphos was nematostatic in sand over a 4–7-day exposure period, but the nematodes were infectious when they were extracted from sand. Although fenamiphos and *S. feltiae* are not compatible, they can possibly be used to-

gether if they are separated spatially or temporally. Hara and Kaya (6) suggest that *S. feltiae* can be used before the pesticide or the pesticide and nematode applications can be spaced apart to allow for pesticide degradation. Recent studies by Petersen et al. (8) showed that fenamiphos was found at detectable levels in turfgrass clippings and thatch 2 months after application and in soil 6 months after application. The biological significance of the residual fenamiphos on *S. feltiae* is unknown. In essence, the use pattern of *S. feltiae* will depend on the insect's distribution on and in the soil and the residual amount of fenamiphos. For example, when fenamiphos is used for plant-parasitic nematode control and an insect pest (such as a cutworm) occurs subsequently in the upper soil layer, *S. feltiae* can be effective provided there is no adverse effect of the pesticide on the nematode. If *S. feltiae* and fenamiphos are applied simultaneously, the nematodes may still be effective against an insect pest after the pesticide is diluted or degraded over time. Prolonged exposure to this pesticide, however, may reduce the efficiency of the nematodes to infect the insect host or may cause nematode mortality. At this time, the complex nature and the many unanswered questions of integrating a chemical pesticide nematostatic or toxic to entomopathogenic nematodes preclude the use of this approach for practical insect control. Studies to determine the effect of this pesticide on entomopathogenic nematodes in the field are needed.

LITERATURE CITED

1. Dutky, S. R. 1974. Nematode parasites. Pp. 576–590 *in* F. G. Maxwell and F. A. Harris, eds. Proceedings of the summer institute in biological control of plant insects and diseases. Jackson: University Press of Mississippi.

2. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. Journal of Insect Pathology 6:417–422.

3. Fedorko, A., M. Kamionek, J. Kozlowska, and E. Mianowska. 1977. The effects of some carbamide herbicides on nematodes from different ecological groups. Polish Ecological Studies 3:23–28.

4. Fedorko, A., M. Kamionek, J. Kozlowska, and E. Mianowska. 1977. The effects of some insecticides on nematodes of different ecological groups. Polish Ecological Studies 3:79–88.

5. Fedorko, A., M. Kamionek, J. Kozlowska, and E. Mianowska. 1977. The effect of vydate–oxamyl on nematodes of different ecological groups. Polish Ecological Studies 3:89–93.

6. Hara, A. H., and H. K. Kaya. 1983. Toxicity of selected organophosphate and carbamate pesticides to infective juveniles of the entomogenous nematode *Neoaplectana carpocapsae* (Rhabditida: Steinernematidae). Environmental Entomology 12:496–501.

7. Kovacs, A. 1982. Sensibilita' del nematode entomopatogeno *Neoaplectana carpocapsae* Weiser ad antiparassitari. Atti Giornate Fitopatologiche 3:11–19.

8. Petersen, D., W. Winterlin, and L. R. Costello. 1986. Nemacur residues in turfgrass. California Agriculture 40 (3 and 4):26–27.

9. Prakasa Rao, P. S., P. K. Das, and G. Padhi. 1975. Note on compatibility of DD-136 (*Neoplectana dutkyi*), an insect parasitic nematode with some insecticides and fertilisers. Indian Journal of Agricultural Science 45:275–277.

10. Welch, H. E. 1971. Various target species: Attempts with DD-136. Biological Control Programmes Against Insects and Weeds in Canada 1959–1968, Technical Communication Number 4:62–66.