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Survival and Movement of Insect Parasitic Nematodes in Poultry Manure and Their Infectivity Against *Musca domestica*

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Abstract: Survival, infectivity, and movement of three insect parasitic nematodes (*Steinernema feltiae* All strain, *S. bibionis* SN strain, and *Heterorhabditis heliothidis* NC strain) in poultry manure were tested under laboratory conditions. The majority (70–100%) of the nematodes died within 18 hours after exposure to the manure. Nematodes exposed to manure slurry for 6 hours killed at least 95% of the house fly larvae, *Musca domestica,* but nematodes exposed for 12 hours achieved less than 40% larval mortality. The majority (90–97%) of the three nematode species applied to the manure remained on the surface. Poor survival and limited movement of nematodes in poultry manure appear to make them unlikely candidates for biocontrol of filth flies in this habitat.

Key words: biological control, insect parasitic nematode, house fly, infectivity, movement, survival.

Insect parasitic nematodes of the genera *Steinernema* (= *Neoaplectana*) and *Heterorhabditis* symbiotically associated with *Xenorhabdus* bacteria are pathogenic to a wide range of insect pests (16). The development of low-cost in vitro, mass-rearing methods for some of these nematode species (2) has increased the feasibility for field experimentation on some important insect pests with modest success (11,17).

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a major economic pest for poultry producers and the object of numerous biological control efforts (1,15,19,20). The application of *Steinernema* spp. and *Heterorhabditis* spp. against house fly larvae on filter paper substrate resulted in substantial mortality, but control in laboratory and field trials using poultry manure as a substrate has been inconsistent (5,15,18,19,21). The failure of the nematodes to survive in manure was reported as a possible explanation for the poor control of house fly larvae (5,15,18). Our objective was to determine the survival and movement of insect parasitic nematodes applied to poultry manure and their infectivity against house fly larvae.

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Materials and Methods

Experiments were conducted in the laboratory at 23–25 C. All tests of significance were done with the Duncan's multiple-range test.

Nematode culture: The nematode species *Steinernema feltiae* Filipjev All Strain, *S. bibionis* Steiner SN strain, and *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) NC strain tested in this study were cultured separately in larvae of the greater wax moth, *Galleria mellonella* L. (4). After extraction, infective nematodes were stored in distilled water at 5–9 C for 2 weeks before application.

Nematode survival: Approximately 100,000 infective juveniles of a particular nematode species in 5 ml water were applied to each of 30 petri dishes (90 mm d), containing a 3-mm layer of moist (ca. 65% moisture) poultry manure. Manure had been field-collected and frozen to kill any existing arthropods. At 3-hour intervals, three petri dishes were randomly selected to determine the survival of nematodes. Four manure samples (each ca. 55 g) were removed from each dish and placed in another dish containing 20 ml water and the number of dead and live nematodes was determined. Nematodes applied to a petri dish containing a 3-mm layer of water served as control.

Nematode infectivity: Approximately 200,000 infective juveniles of a particular nematode species in 1 ml water were placed

TABLE 1. Survival of infective juveniles of three insect parasitic nematodes applied to poultry manure.

Hours after treatment	Survival in manure (%)			
	S. feltiae	*S. bibionis*	*H. heliothidis*	Check†
3	68.3 b	73.6 ab	56.7 b	99.2 a
6	54.6 b	58.9 b	62.5 b	99.4 a
9	59.9 b	58.4 b	44.2 bc	97.9 a
12	33.7 c	49.3 b	26.4 c	98.1 a
15	26.4 c	34.1 c	11.1 d	99.6 a
18	9.3 d	30.4 c	0.0	98.4 a
24	0.0	6.6 ed	0.0	97.6 a
36	0.0	2.4 e		98.5 a
48		0.7 f		98.6 a

Mean of three replicates. Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

† All nematode species had similar survival rates; therefore, the percentage average is presented.

TABLE 2. Mortality of house fly larvae in poultry manure slurry infested with three insect parasitic nematodes.

Hours after applying nematodes	Mortality (%)			
	S. feltiae	*S. bibionis*	*H. heliothidis*	Check†
3	100.0 a	100.0 a	100.0 a	100.0 a
6	100.0 a	97.9 a	100.0 a	100.0 a
9	86.3 b	82.6 a	56.2 b	100.0 a
12	36.9 c	17.4 c	22.7 c	100.0 a
15	11.9 c	13.6 c	2.4 d	100.0 a

Mean of five replicates, five larvae/dish, 1,000 nematodes/larva. Means followed by the same letter in each column are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

† All the nematode species gave 100% control.

in each of 20 petri dishes (90 mm d) containing a 3-mm layer of a concentrated manure slurry. The slurry was made by mixing 100 g poultry manure (ca. 60% moisture) with 10 ml water and sieving the mixture through a 74-μm-pore sieve. Infectivity of nematodes was determined as follows: Approximately 5,000 infective juveniles in 1 ml slurry were removed from the petri dish every 3 hours and placed into a petri dish (60 mm d) containing two #2 Whatman filter papers and five second-instar house fly larvae reared on a commercial fly medium. Mortality of the larvae was recorded 72 hours after treatment. The control treatment was conducted as follows: A suspension of a particular nematode species was prepared, and every 3 hours 5,000 nematodes in 1 ml water were placed into a petri dish (60 mm d) containing two filter papers and five second-instar house fly larvae. Each treatment was replicated five times.

Nematode movement: Vertical columns, 6 cm long and consisting of 2-cm sections of plastic tubing (5 cm inner d) were joined together with adhesive tape and filled with moist (ca. 60% moisture) poultry manure. Each section held approximately 150 g moistened manure. One milliliter water containing 100,000 infective juveniles of a particular nematode species was added in small drops to the surface of the manure at the top of the vertical column. The tubes were capped with aluminum foil and maintained at 23–25 C. The manure columns were not compacted more than would occur from the natural weight of the manure. After 10 hours the manure surface was sprayed with 10 ml water and the nematodes recovered were counted. The plastic tubes were separated and the nematodes recovered by the Cobb sieving and gravity method (3) were counted. Three different columns were evaluated for each nematode species.

RESULTS AND DISCUSSION

The poultry manure had lethal effects on all nematode species (Table 1). Ammonia, salts, or other materials associated with poultry manure were probably toxic and inactivated the nematodes rather quickly (Poinar, unpubl.). Nematode mortality exceeded 65% 15 hours after treatment (Table 1). Previous tests showed that nematodes need at least 24 hours exposure to house fly larvae for successful control (5,15,18). A few infective juveniles of *S. bibionis* were more tolerant to the manure and were recovered alive after 48 hours in the manure. The ability of this nematode to become quiescent in certain environments (12) could have affected its survival in the manure. Some species of *Steinernema*

TABLE 3. Vertical distribution of three insect parasitic nematodes applied to poultry manure 10 hours after placement of nematodes at the manure surface.

Depth (cm)	Nematodes recovered (%)		
	S. feltiae	*S. bibionis*	*H. heliothidis*
0	97.3 a	90.1 a	92.2 a
0–2	2.7 c	8.7 b	7.1 b
2–4	0.0	1.2 cd	0.7 d
4–6	0.0	0.0	0.0
Mean total no. of live nematodes recovered/tube	9,020	12,605	8,670

Mean of three replicates. Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

are known to be capable of adapting to environmental changes and may survive 2–9 months in the soil (6,13).

The manure slurry appears to have sublethal effects on the nematode species (Table 2), as did organophosphate and carbamate insecticides (10). In our study, the three nematode species caused 95% mortality of house fly larvae 6 hours after adding the nematodes to the slurry (Table 2). Mortality declined below 40% 12 hours later possibly because the manure slurry has adverse effects on nematode motility, dispersion, and attraction to the insect host.

Most individuals of each nematode species remained on the surface of the manure (Table 3). Similar results were reported when these nematodes were applied to the soil surface (7–9,14). None of the *S. feltiae* infective juveniles were recovered below the 0–2-cm layer. A few infective juveniles of *S. bibionis* and *H. heliothidis* were recovered 2–4 cm from the surface. Both species appear to have a greater tendency to move downward than does *S. feltiae*, as reported in different soil types (7,9). Nematode movement is important because the depth at which fly larvae are found varies with manure type, temperature, and moisture (20).

The poor survival and limited movement of nematodes in poultry manure appear to make them unlikely candidates for biological control of filth flies in this habitat.

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