

SUSCEPTIBILITY OF THE LESSER MEALWORM,
ALPHITOBIOUS DIAPERINUS (COLEOPTERA: TENEBRIONIDAE)
TO THE ENTOMOGENOUS NEMATODES
STEINERNEMA FELTIAE, *S. GLASERI* (STEINERNEMATIDAE)
AND *HETERORHABDITIS HELIOTHIDIS* (HETERORHABDITIDAE)

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ABSTRACT

The infectivity of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis heliothidis* for early-stage larvae, late-stage larvae, pupae and adults of the lesser mealworm were evaluated under different habitat conditions. When confined in petri dishes with nematode-treated filter paper, all beetle stages were highly susceptible to parasitism by *S. feltiae*, with LD₅₀ values ranging from 9 to 56 nematodes per host. Early-stage larvae (LD₅₀ = 26) and pupae (LD₅₀ = 36) were more susceptible than late-stage larvae (LD₅₀ = 1,791) and adults (LD₅₀ = 724) to *H. heliothidis*. Only adult beetles (LD₅₀ = 714) were susceptible to *S. glaseri*. Late-stage beetle larvae were more susceptible to *S. feltiae* in rearing medium (LD₅₀ = 24), broiler litter (LD₅₀ = 258) and poultry manure (LD₅₀ = 212) than to *H. heliothidis*, which caused less than 50% mortality at all dose rates in these substrates. Adults were less susceptible than larvae in these substrates, and mortality only exceeded 50% in litter treated with *S. feltiae* (LD₅₀ = 971). Late-stage larvae were highly susceptible to both *S. feltiae* and *H. heliothidis* in sandy loam and clay soils, with LD₅₀'s ranging from 1 to 14 nematodes per larva. Pupal mortality was higher in sandy loam (*S. feltiae* LD₅₀ = 46, *H. heliothidis* LD₅₀ = 444) than in clay soil (*S. feltiae* LD₅₀ = 95, *H. heliothidis* LD₅₀ = 5,796).

Key Words: Lesser mealworm, darkling beetle, *Steinernema*, *Alphitobius diaperinus*, *Heterorhabditis*, *Neoplectana*, DD-136.

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INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a cosmopolitan pest which commonly infests stored products, and is often abundant in poultry litter and manure (Legner and Olton 1970; Pfeiffer and Axtell 1980). In commercial broiler and turkey production facilities, beetle larvae leave the litter and tunnel into building insulation materials, necessitating costly replacement of the insulation (Ichinose et al. 1980; Safrit and Axtell 1984). Tunneling is initiated by larvae when high population densities lead to competition for pupation sites in the soil beneath the litter (Geden and Axtell, unpub. obs.). The beetles are also known to harbor a number of avian pathogens (De las Casas et al. 1972, 1976). Applications of insecticides to litter often fail to provide satisfactory control of this pest, presumably due to the inaccessibility of larvae, pupae and teneral adults within the soil. Evaluations of alternative control strategies are therefore needed.

Entomogenous nematodes of the genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae) parasitize a wide range of insects and have

been the subject of considerable biocontrol research (Gaugler 1981; Poinar 1975, 1979). Although nematode applications to plant foliage have produced disappointing results due to the sensitivity of infective juveniles to dessication (MacVean et al. 1982; Moore 1973; Webster and Bronskill 1968) and ultraviolet light (Gaugler and Boush 1978), *Steinernema* and *Heterorhabditis* species are efficacious for control of some pests which inhabit or pupate in soil (Bedding and Miller 1981; Georgis and Poinar 1984; Kaya et al. 1981; Poinar et al. 1983; Toba et al. 1983).

Our objective was to evaluate the infectivity of three entomogenous nematodes, *Steinernema feltiae* (Filipjev) [= *Neoaplectana carpocapsae* (Wouts et al. 1982)], *S. glaseri* (Steiner) and *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann) for various life stages of *A. diaphanus* under different habitat conditions.

MATERIALS AND METHODS

Nematode, Beetle and Substrate Sources

The DD-136 strain of *S. feltiae* was used in tests with this nematode species, whereas cultures of *S. glaseri* and *H. heliothidis* were established from more recent North Carolina isolates from Japanese beetle larvae and soil, respectively (Khan et al. 1976; Regniere and Brooks 1978). Nematodes were reared by passage through late-stage larvae of the greater wax moth, *Galleria mellonella* (L.), using methods slightly modified from those of Dutky et al. (1964). After harvest of infective juveniles from host cadavers, nematodes were stored in tissue culture flasks in incubators maintained at 16°C.

Beetle adults and larvae were collected from litter in untreated broiler houses in Burke Co., NC. Larvae were separated into "early" and "late" stage larvae by sifting through a U.S. Standard No. 16 sieve, which segregated larvae into groups of individuals less than (early) and greater than (late) 1 cm in total body length. Pupae were obtained by removal from soil one week after introduction of late-stage larvae.

Tests on filter paper were conducted in plastic petri dishes lined with a single sheet of medium-porosity filter paper (Diam. = 9 cm). Rearing medium tests were conducted with a mixture of dry fly rearing medium (Ralston Purina, St. Louis, MO), yeast and water ("CSMA medium") adjusted to ca. 60% moisture by weight. "Used" poultry litter (consisting of pine shavings, manure and spilled chicken feed) of ca. 30% moisture content from a broiler house and poultry manure (ca. 60% moisture) from beneath caged laying hens were collected at the North Carolina State University poultry research farm in Wake Co., NC. The litter and manure were known to be untreated with insecticides. Two types of soil representative of the floors of North Carolina poultry houses were used in soil tests: soil 1 was sandy loam typical of the coastal plain region of the state and was collected from beneath the litter of a turkey house in Duplin Co., NC; soil 2 was clay characteristic of the piedmont region and was collected from a broiler house in Wake Co., NC.

Litter, manure and soil were frozen and thawed prior to use in tests to kill extraneous arthropods. Tests with these substrates and rearing medium were conducted in screen-topped plastic cups (Cap. = 340 ml). Petri dishes and cups were placed in a rearing room maintained at ca. 26°C after insects and nematodes were introduced.

Susceptibility of Beetles on Filter Paper

Steinernema feltiae and *H. heliothidis* were tested against early and late-stage larvae, pupae and adults of *A. diaperinus* by placing 10 insects in a petri dish lined with filter paper and adding 1 ml of a nematode suspension in water at dosage levels of 0, 1, 10, 100, 1000 and 5000 nematodes per insect (10 dishes/dose/host stage/nematode species). Mortality was recorded 72 hr after exposure to nematodes in tests with larval and adult beetles. Pupal mortality was assessed 7 days after exposure. Adjustments had to be made in tests with *S. glaseri* because of the large size of infective juveniles of this nematode; early-stage larval tests were omitted, and the maximum dose tested against the other host stages was 1000 nematodes per host. Cadavers from each test group were held on moist filter paper and inspected for nematode reproduction and exodus.

LD₅₀ and LD₉₀ values were computed by using the log-probit procedure of the Statistical Analysis System (Ray 1982) after correcting for control mortality (Abbott 1925).

Susceptibility in Rearing Medium, Litter, and Manure

Because *S. glaseri* had relatively low infectivity in the filter paper tests, only *S. feltiae* and *H. heliothidis* were used in subsequent evaluations. In tests with rearing medium, litter, and manure, late-stage larvae and adults were placed in 150 cm³ of substrate (depth = ca. 5 cm) in screen-topped plastic cups and treated with 2 ml of nematode suspensions at the same dosage rates which were used in tests on filter paper. Five cups containing 20 larvae or adults were used for each dosage, substrate and nematode species. Three days after insect and nematode introductions, larvae and adults were removed from the litter by hand and counted. Missing hosts were scored as dead.

Susceptibility of Larvae and Pupae in Soil

In one group of soil tests, 20 late-stage larvae were introduced into cups containing 150 cm³ of soil which had been treated one hr earlier with nematodes at the same dosage levels as in previous tests (5 cups/dose/nematode species). In a second group, larvae were introduced into untreated soil, allowed to pupate (7 days) and were then treated with nematodes as before. Both groups of tests were conducted with both sandy loam and clay soils. Mortality was determined three weeks after the introduction of beetle larvae by counting the number of live adults in each container.

RESULTS

Results of all tests are presented in Table 1.

Susceptibility of Beetles on Filter Paper

Larvae, pupae and adults of *A. diaperinus* were susceptible to *S. feltiae* and *H. heliothidis* when hosts were confined in petri dishes with nematode-treated filter paper. Late-stage larvae were less susceptible to *H. heliothidis* (LD₅₀ = 1,791, LD₉₀ = 125,250) than were early-stage larvae (LD₅₀ = 26, LD₉₀ = 1,836). *Steinernema feltiae* was more infective than *H. heliothidis* in every test. Late-stage larvae were the most susceptible beetle stages to *S. feltiae*, with an LD₅₀ of 9 and LD₉₀ of 61 nematodes per host larva. Only adult beetles were susceptible to *S. glaseri* (LD₅₀ = 714, LD₉₀ = 2,370). Nematodes successfully reproduced and produced

Table 1. Infectivity of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis heliothidis* against *A. diaperinus* on nematode-treated filter paper, and of *S. feltiae* and *H. heliothidis* in treated rearing medium, broiler litter, poultry manure and soil.

Nematode species and beetle stage	Mean % mortality* at nematode dose					LD ₅₀ (No. nematodes/host)	LD ₉₀ (No. nematodes/host)	Slope
	(No./host)							
	1	10	100	1,000	5,000			
<i>Filter paper</i>								
<i>S. feltiae</i>								
larvae, early-stage	3.3	23.4	58.7	90.2	97.8	56	997	1.0
larvae, late-stage	11.1	43.4	98.0	100.0	100.0	9	61	1.5
pupae	1.0	17.3	92.8	100.0	100.0	24	91	2.2
adults	4.0	24.2	62.6	94.9	100.0	42	616	1.2
<i>H. heliothidis</i>								
larvae, early-stage	16.3	37.8	66.3	87.8	93.9	26	1,836	0.7
larvae, late-stage	3.0	1.0	24.2	38.4	64.6	1,791	125,250	0.7
pupae	4.1	5.1	82.6	100.0	100.0	36	195	1.7
adults	0.0	7.1	11.1	43.4	92.9	724	10,516	1.1
<i>S. glaseri</i>								
larvae, late-stage	1.0	1.0	7.0	11.0	—	—	—	—
pupae	1.0	0.0	3.0	7.0	—	—	—	—
adults	0.0	0.0	3.0	62.6	—	714	2,730	2.2
<i>Rearing medium</i>								
<i>S. feltiae</i>								
larvae, late-stage	11.2	21.3	91.0	100.0	100.0	24	93	2.1
adults	0.0	3.0	6.1	18.2	44.4	—	—	—
<i>H. heliothidis</i>								
larvae, late-stage	0.0	0.0	3.4	16.8	30.3	—	—	—
adults	0.0	0.0	0.0	2.0	11.1	—	—	—

<i>Broiler litter</i>								
<i>S. feltiae</i>								
larvae, late-stage	1.0	4.2	35.8	65.3	97.9	258	3,483	1.1
adults	0.0	5.1	10.2	38.8	87.8	971	14,417	1.1
<i>H. heliothidis</i>								
larvae, late-stage	2.1	5.2	7.4	9.5	13.7	—	—	—
adults	1.0	4.2	2.0	2.0	17.3	—	—	—
<i>Poultry manure</i>								
<i>S. feltiae</i>								
larvae, late-stage	2.3	7.0	52.3	68.6	84.9	212	7,372	0.8
adults	0.0	0.0	0.0	3.0	17.2	—	—	—
<i>H. heliothidis</i>								
larvae, late-stage	3.5	5.8	5.8	22.1	25.6	—	—	—
adults	0.0	0.0	2.0	1.0	0.0	—	—	—
<i>Soil: sandy loam</i>								
<i>S. feltiae</i>								
larvae, late-stage	37.2	98.9	100.0	100.0	100.0	1	4	2.6
pupae	3.2	29.8	72.3	79.8	95.7	46	1,510	0.9
<i>H. heliothidis</i>								
larvae, late-stage	12.8	47.9	87.2	100.0	100.0	10	118	1.2
pupae	4.2	13.8	22.3	56.4	85.1	444	23,703	0.7
<i>Soil: clay</i>								
<i>S. feltiae</i>								
larvae, late-stage	16.3	68.5	100.0	100.0	100.0	4	18	1.6
pupae	5.4	16.1	50.5	77.4	98.9	95	2,254	0.7
<i>H. heliothidis</i>								
larvae, late-stage	7.4	41.1	88.4	98.9	100.0	14	130	1.3
pupae	5.4	5.4	6.4	8.6	72.0	5,796	682,309	0.6

* Corrected for control mortality by Abbott's formula.

new generations of infective juveniles in every test and at every dosage in which infected cadavers were obtained.

Susceptibility of Larvae and Adults in Rearing Medium, Litter, and Manure.

High rates of infection of beetle larvae were found with *S. feltiae* in rearing medium ($LD_{50} = 24$, $LD_{90} = 93$); adults were much less susceptible, with maximal mortality of 44.4% at the highest nematode dosage of 5,000 juveniles per beetle. Low larval and adult mortality was obtained in tests with *H. heliothidis* in this substrate, with maximal mortalities of 30.3 and 11.1%, respectively, at 5,000 nematodes per host.

In broiler litter, beetle larvae ($LD_{50} = 258$, $LD_{90} = 3,483$) and adults ($LD_{50} = 971$, $LD_{90} = 14,417$) were susceptible only to *S. feltiae*. Litter treated with *H. heliothidis* resulted in low rates of mortality among larvae (13.7%) and adults (17.3%) at the highest nematode dosage.

Beetle larvae in treated manure were also susceptible to *S. feltiae* ($LD_{50} = 212$, $LD_{90} = 7,372$), but low mortality (17.2%) was observed among adults exposed to the highest nematode dosage. No mortality was found among adults exposed to *H. heliothidis* in manure, and larval mortality was low (25.6%) at the highest nematode dosage.

Susceptibility of Larvae and Pupae in Soil

Larvae were highly susceptible to infection by *S. feltiae* and *H. heliothidis* in both sandy loam and clay soils. This was especially true in tests with *S. feltiae*, where an LD_{50} of 1 and LD_{90} of 4 in sandy loam and an LD_{50} of 4 and LD_{90} of 18 in clay was observed. *Heterorhabditis heliothidis* was somewhat less infective than *S. feltiae* in both sandy loam ($LD_{50} = 10$, $LD_{90} = 118$) and clay ($LD_{50} = 14$, $LD_{90} = 130$). Pupae were less susceptible to *H. heliothidis* ($LD_{50} = 444$, $LD_{90} = 23,703$) than to *S. feltiae* ($LD_{50} = 46$, $LD_{90} = 1,510$) in sandy loam. Pupae in clay were less susceptible to both nematode species than they were in sandy loam, and were much more susceptible to *S. feltiae* ($LD_{50} = 95$, $LD_{90} = 2,254$) than to *H. heliothidis* ($LD_{50} = 5,796$, $LD_{90} = 682,309$).

DISCUSSION

Larvae and pupae of *A. diaperinus* were refractory to parasitism by juveniles of *S. glaseri* when insects were confined in petri dishes with nematode-treated filter paper, although adults were susceptible at the highest dose rate of 1,000 nematodes per beetle. Since *S. glaseri* is restricted in its mode of infection to entry through natural host openings, and infective juveniles of this species are quite large, the lower infection rates among larvae was probably due to the smaller size of these openings (mouth, anus, spiracles) in larvae compared to those of the adults. Most reports of infections with this nematode have been with large beetle larvae, especially members of the Scarabaeidae (Poinar 1979). Our low pupal infection rates also suggest that the adult infection rate was enhanced by the "feeding" activities of the adults on the treated filter paper. Similar results were observed in a study with house flies, where adult flies were susceptible to *S. glaseri* at high dose rates, whereas mature fly larvae were refractory to infection (Geden et al., in prep.).

All beetle life stages were susceptible to *S. feltiae* and *H. heliothidis* on filter paper, although *S. feltiae* was more virulent in most cases. Under more natural

conditions (rearing medium, litter, manure), *S. feltiae* was much more infective than *H. heliothidis*. This difference was probably due in part to nematode and host behaviors. *S. feltiae* juveniles tended to move to the surface of the substrates while *H. heliothidis* tended to move downwards. *A. diaperinus* adults and larvae generally feed on or just under the substrate surface unless they are disturbed, increasing the opportunities for contact with *S. feltiae* under our experimental conditions. In poultry houses, where bird movement on the litter surface may drive the beetles to a greater depth in the litter, this factor may be of less importance. In a related study, we have also found that *H. heliothidis* is less tolerant than *S. feltiae* of highly organic materials such as the moist manure (ca. 72% moisture) in which house fly breeding typically occurs (Geden et al., in prep.).

In all three semi-natural and natural substrates, adult beetles were less susceptible to infection by *S. feltiae* than were late-stage larvae. These results differed from those of the filter paper trials, where adult and larval susceptibility was comparable. This difference may have been due to fewer nematode-beetle contacts in these treated substrates compared with filter paper, where constant contact was assured. Kaya et al. (1981), in a similar study, showed that both adults and larvae of the elm leaf beetle, *Pyrhalta luteola* (Muller), were highly susceptible to *S. feltiae* on filter paper, but the adults were much less susceptible than larvae on treated elm leaves. Larval scolytid beetles of the genera *Ips* and *Hylobius* have also been found to be more susceptible than adults to infection by *S. feltiae* (Ivanova 1983; Pye and Burman 1978).

Very high infection rates were found among beetle larvae which were allowed to pupate in nematode-treated soil. In contrast with the above tests in organic substrates, larvae were somewhat more susceptible to infection in soil than on filter paper. This was due in part to the longer exposure period in soil, since these tests were allowed to run for 3 weeks compared with 3 days in filter paper tests. In addition, the pre-pupae may have provided a more stationary target for the nematodes to orient towards during pupal cell construction. *Steinernema feltiae* is capable of directed movement towards hosts, a process which is aided by orientation towards carbon dioxide and other host emissions (Gaugler et al. 1980).

Pupae in soil were less susceptible than larvae in soil, and were less susceptible than pupae which were liberated from cells and exposed to nematodes on filter paper. The soil cells which are constructed by larvae prior to pupation therefore appear to provide some protection from infection. Similarly, the lower infection rates in clay compared with sandy loam probably reflected the greater protection conferred by cells made from materials with smaller particle sizes.

Most of the biocontrol research with entomogenous nematodes has shifted in recent years from foliar to soil applications (Gaugler 1981). Soil treatments with *S. feltiae* have given superior control over foliar applications for elm leaf beetles (Kaya et al. 1981). Similarly, higher mortality was observed among Colorado potato beetle pupae in *S. feltiae*-treated soil than among adults and larvae which fed on treated foliage (Welch and Briand 1961b). Toba et al. (1983) observed high mortality among sugarbeet wireworm larvae and moderate Colorado potato beetle pupal mortality in cups containing soil that was treated with infective juveniles of *S. feltiae*. This nematode has also been successfully applied to soil to reduce larval densities of *Hylemya* spp. on tobacco (Cheng and Bucher 1972) and cabbage roots (Verenchuk 1983; Welch and Briand 1961a).

Bedding and Miller (1981) obtained nearly complete control of root-feeding vine weevil larvae on high-value potted plants via soil applications of *H. heliothidis* at levels similar to those which were used in the present study. In a field trial for control of corn rootworm larvae (*Diabrotica* spp.), Poinar et al. (1983) found that applications of *S. feltiae* to soil at planting provided more effective and less costly control of this pest than conventional insecticide treatments.

Several factors indicate that soil treatments with *S. feltiae* between flocks when the litter is replaced may provide effective long-term control of *A. diaperinus* populations. First, the soil in turkey and broiler houses is shaded and remains somewhat moist due to moisture input from manure and waterers within the houses. Second, since *S. feltiae* is infective for all beetle life stages, the insects may become parasitized when they enter the soil as larvae to pupate, while they are pupae, and as they move out of the soil as teneral adults. Finally, since *S. feltiae* successfully reproduces in beetle cadavers, nematode populations may be sustained over time by production of new infective juveniles from host cadavers. Work is currently in progress to evaluate this nematode for beetle control under field conditions.

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