

Field Trials of *Steinernema feltiae* (Nematoda: Steinernematidae) for Control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in Commercial Broiler and Turkey Houses

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ABSTRACT Infective juveniles of the All strain of *Steinernema feltiae* Filipjev were applied (100,000 per square meter) to the soil floors of one broiler and two turkey houses with known recent histories of infestation with lesser mealworm, *Alphitobius diaperinus* (Panzer). After addition of fresh litter and new flocks of birds, beetle populations increased more slowly in treated than in untreated houses on all three farms, but at 10-13 weeks posttreatment adult beetle populations were about equal in treated and untreated houses. Soil samples were bioassayed biweekly for presence of nematodes by adding beetle larvae. Nematodes persisted (63-87% beetle mortality) for 7 weeks posttreatment on two of the farms; on the third farm, beetle mortality was <50% at 3 weeks posttreatment. When soil in plastic containers was treated at varying nematode rates and held for 6 months in a poultry house, beetle mortality ranged from 0 (10⁵ nematodes per square meter) to 48.2% (10⁶ nematodes per square meter).

KEY WORDS *Alphitobius diaperinus*, *Steinernema feltiae*, biocontrol

LESSER MEALWORM, *Alphitobius diaperinus* (Panzer), is a common pest in the litter of commercial poultry houses, where the beetles feed on spilled feed, manure, and dead birds (Legner & Olton 1970, Pfeiffer & Axtell 1980). Although these insects are known to harbor numerous pathogens of avian disease (De las Casas et al. 1972, 1976), their pest status stems primarily from the late instars tunneling into and damaging building insulation materials (Ichinose et al. 1980, Safrit & Axtell 1984). The beetles also pose public nuisance problems during the intervals between flocks when adult beetles leave the poultry houses and enter nearby homes.

Entomogenous nematodes of the genera *Steinernema* and *Heterorhabditis* are promising biological control agents for many insect pests (Poinar 1979) and have been particularly effective when applied to insects that either feed or pupate in soil (Bedding & Miller 1981, Kaya et al. 1981, Poinar et al. 1983, Toba et al. 1983, Georgis & Poinar 1984). In laboratory tests, *Steinernema feltiae* Filipjev was found to be superior to *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann) and *Steinernema glaseri* (Steiner) for control of late-instar lesser mealworm when larvae were introduced into nematode-treated soil (Geden et al. 1985). Therefore, we conducted field evaluations of *S. feltiae* against this pest in commercial broiler and turkey houses.

Materials and Methods

Evaluation of Three Commercially Available Nematode Strains. Bioassays were first conducted to evaluate the relative virulence of juveniles of three strains of *S. feltiae* (All, Breton, and Mexican) which were available in large numbers from Biosis (1057 East Meadow Circle, Palo Alto, Calif.). Tests were conducted on filter paper in petri dishes ($n = 10$ larvae per dish, 10 dishes per dose) and in sandy loam soil in cups ($n = 20$ larvae per cup, 5 cups per dose) with late-instar *A. diaperinus* as described by Geden et al. (1985). Rates of 0, 1, 10, 100, 1,000, and 5,000 nematodes per larva were assayed. For soil tests, these rates corresponded to 0, 3,600, 36,000, 360,000, 3,600,000, and 18,000,000 nematodes per square meter of soil. Nematodes were 2-4 weeks old at the start of the experiment.

Nematode Application Procedure. The All strain of *S. feltiae* was used in the field tests because it was most effective in laboratory bioassays and was available in the quantities needed. Houses were treated after the litter (pine shavings and manure) was removed and the soil floors were exposed. The nematodes were maintained at 10°C in the moist sponges in which they had been shipped until immediately before application. The initial step in nematode application was to rinse each sponge (20 million nematodes per sponge) in 1

Table 1. Relative virulence of the All, Breton, and Mexican strains of *S. feltiae* for *A. diaperinus* immatures on filter paper and in sandy loam soil^a

Nematode strain	LD ₅₀ (95% FL)	LD ₉₀ (95% FL)	Slope (SEM)
Filter paper			
All	5.1 (1.9–11.2)	55.4 (22.3–337.8)	1.24 (0.085)
Breton	7.6 (5.4–10.6)	102.8 (66.4–102.6)	1.13 (0.087)
Mexican	7.9 (5.3–11.3)	165.2 (102.5–306.1)	0.97 (0.103)
Soil			
All	0.7 (0.4–1.1)	6.0 (4.0–11.2)	1.39 (0.072)
Breton	0.9 (0.3–1.9)	188.6 (94.3–480)	0.55 (0.181)
Mexican	1.7 (0–10.8)	106.5 (16.1–166,477.5)	0.71 (0.141)

^a $n = 100$ larvae per dose; doses were 0, 10, 100, 1,000, and 5,000 nematodes per larvae.

liter of water to prepare a concentrated suspension of 20,000 nematodes per milliliter. A 45-ml aliquot of this suspension was then mixed with 8 liters of water in a conventional watering can, and the contents of the can (900,000 nematodes) were sprinkled evenly over a 8.4-m² area of soil delineated by movable plastic frames. This procedure was repeated until the entire soil floor of each house received ca. 100,000 nematodes per square meter.

Description of Study Sites. Nematodes were applied on three farms, none of which received any insecticide treatments during the experiment. Farm 1 (Rutherford County, N.C.) consisted of two broiler houses (9.1 by 91.4 m) with clay floors typical of the piedmont area of the state. Each housed ca. 15,000 female birds. Both were deep-litter houses, and at the time of cleanout had pine-shaving litter that had been used for two years by previous flocks. Pretreatment counts of beetles were made by placing 10 tube traps (Safrit & Axtell 1984) in each house, 5 along both of the long walls in each house. Traps were collected 1 week later (5 July) and counts were made of late instars (>1.5 cm in total body length) and adults. Birds were removed on 9 July, litter was cleaned out on 10 and 11 July, nematodes were applied to one of the houses on 12 July, new pine shavings were introduced on 13 July, and new birds were placed in the houses on 22 July. These chicks were brooded in half of the house for the first 19 days, after which the brooders were removed and the birds had access to the entire house. Traps were placed in both houses (treated and control) on 22 July as before, and counts were made at weekly intervals until 2 November. The sampling period included two flocks; the first flock was removed on 5 September, fresh shavings were placed on top of the litter (not removed), and the second flock was placed in each house on 17 September.

Farm 2 (Wayne County, N.C.) consisted of three turkey houses. The floors of the houses had a sandy loam base typical of the coastal plain area of the state and were topped with a 15-cm pad of clay for increased elevation. Litter was replaced with fresh pine shavings after every flock, and the soil

was routinely disinfected with cresylic acid between flocks. Birds were moved from the brooder houses after 11–12 weeks. Pretreatment counts were made in all three houses during the last week of one flock cycle as before (9 July), after which two houses (treated and control) were selected for the experiment. Birds were removed on 9 July, litter was removed on 16 and 17 July, nematodes were applied on 18 July, new shavings were introduced on 18 July, and new birds were placed in the the houses on 25 July. Traps were placed in the houses (10 per house as before) on 25 July, and weekly counts were made until the birds were removed on 1 October.

Farm 3 (Duplin County, N.C.) consisted of two turkey brooder houses (9.1 by 121.9 m) and six grow-out houses (where birds are raised to market weight) with pine shaving litter on top of indigenous sandy loam soil. The two brooder houses were used for this study (treated and control) because the litter was replaced after each flock. Pretreatment counts were made 2 weeks after the litter was removed from the previous flock (9 July) by placing traps in soil depressions beneath the feeders that contained residual litter and beetles (10 traps per house). Nematodes were applied on 16 July, new shavings were introduced on 17 July, and new birds were placed in the houses on 25 July (treated house) and 29 July (control house). Traps were placed in the houses on 25 July as before and weekly counts were made until the birds were moved out and into the grow-out houses on 8 September (treated house) and 11 September (control house).

Nematode Persistence on Treated Farms. Nematode persistence was monitored by collecting soil samples (250 cm³) from the floor of each house (10 samples per house) on a biweekly basis beginning 1 week after nematode treatment. Each sample was placed in a screen-topped plastic cup (soil depth, 7 cm), returned to the lab, and 20 late-instar *A. diaperinus* larvae were added. After 3–4 weeks at 25°C, the number of adult beetles was counted. In pretreatment bioassays, there were no significant differences ($P > 0.05$, one-way ANOVA) in beetle survival in soil from control and treated houses on any of the farms before nematode application.

Nematode Persistence in Soil at Different Application Rates. The persistence of *S. feltiae* in soil at varying dosage rates was further evaluated by applying nematodes to sandy loam soil (6 cm deep) in plastic pails (height, 17 cm; diameter, 20.5 cm) at rates of 0, 10³, 10⁵, and 10⁶ nematodes per square meter (10 pails per dose). The soil was collected from the untreated house on farm 2. After treatment, soil was covered with used poultry litter, screen covers were added, and the containers were held in a poultry house for 6 months (August–January). The house was closed and temperature was regulated to some extent (ca. 23–33°C) by exhaust fans. Soil bioassays for nematodes

Table 2. Trap counts of late-instar and adult of *A. diaperinus* on farm 1 (broiler houses with clay soil) before and after soil treatment with *S. feltiae* at 100,000 nematodes per square meter

Date	Wk post-treatment	\bar{x} no. larvae per trap			\bar{x} no. adults per trap		
		Control	Treated	F	Control	Treated	F
5 July	-1	223.7	221.5	0.002	137.6	279.4	7.693 ^a
9-13 July	0	Birds removed, litter removed, nematodes applied, fresh shavings placed					
22 July		Birds placed					
30 July	+2	0.1	0.0	1.000	4.5	3.3	0.346
6 Aug.	3	0.3	0.4	0.086	10.3	17.8	0.523
13 Aug.	4	27.0	26.5	0.001	24.0	27.2	0.042
20 Aug.	5	240.6	187.7	0.203	60.3	19.4	10.942 ^a
27 Aug.	6	431.5	308.1	1.950	82.8	35.6	8.874 ^a
3 Sept.	7	406.8	260.9	9.647 ^a	123.8	35.0	31.062 ^a
5 Sept.		Birds removed, litter tilled and top-dressed with fresh shavings					
10 Sept.	+8	62.9	55.3	0.754	130.1	12.6	25.208 ^a
17 Sept.		Birds placed					
24 Sept.	10	0.5	0.3	0.545	7.7	2.3	12.979 ^a
3 Oct.	12	25.8	4.7	14.711 ^a	47.8	6.1	7.598 ^a
10 Oct.	13	46.5	5.5	7.244 ^a	48.0	46.6	0.005
17 Oct.	14	127.9	92.3	0.265	76.0	61.2	0.183
24 Oct.	15	240.4	148.2	1.416	231.4	137.5	2.742
31 Oct.	16	267.9	226.0	0.391	262.3	234.9	0.344
2 Nov.		Birds removed					
7 Nov.	17	105.8	38.0	3.545	174.6	157.6	0.121

^a $P < 0.05$, one-way ANOVA for a given date and life stage; $n = 10$ traps per house per week; $df = 1,18$.

were then conducted as before with late-instar *A. diaperinus* larvae.

Statistical Analyses. LD₅₀'s and LD₉₀'s for the three nematode strains were estimated by the PROBIT Procedure of the Statistical Analysis System (SAS Institute 1982). Within-farm differences in adult and larval beetle population sizes between control and treated houses were evaluated by subjecting weekly trap collection data (number of individuals per trap, 10 traps per house) to one-way analysis of variance using the ANOVA procedure of SAS. In soil assays for nematode persistence, larval mortality in the treated soil was corrected for control mortality (soil from the untreated house) by Abbott's formula (Abbott 1925).

Results

Comparison of Nematode Strains. The All, Breton, and Mexican strains of *S. feltiae* were of similar virulence in assays where beetle larvae were exposed to nematode-treated filter paper for 3 days (Table 1). In sandy loam soil, however, the All strain caused higher mortality than either the Breton or the Mexican strains.

Effects of Nematode Treatment on Beetle Populations. On all three farms, adult beetle populations increased more slowly in the treated than in the control houses following a period of low population levels in all houses during the first 4-6 weeks after litter replacement.

On farm 1, adult beetles in both houses were present in low numbers (<5 per trap) after litter placement, compared with pretreatment counts of 138 and 279 beetles per trap in the control and treated houses, respectively (Table 2). Adult beetle populations began increasing again at ca. 3 weeks

posttreatment (control house = 10; treated house = 18), suggesting the emergence of new generations of adults. During weeks 4-8 posttreatment, adult populations in the control house increased rapidly, peaking at 130 beetles per trap (8 weeks posttreatment). In contrast, beetles in the treated house remained at low, stable levels (19-36 per trap) during this time period. Differences in larval numbers were small and were significantly only in week 7 posttreatment of this flock.

Although significantly fewer adult beetles were collected in the treated house on weeks 10 and 12 than in the control house, beetle populations increased rapidly in both houses in subsequent weeks and no further significant between-house adult differences were observed. Significantly fewer larvae were collected in the treated house on weeks 12 and 13.

On farm 2, pretreatment adult counts were low (control = 12; treated = 44), and populations remained low during the first 5 weeks posttreatment (Table 3). Significantly fewer adults were trapped in the treated house in weeks 6-9. No significant differences were observed in adult populations on the final sampling dates of weeks 10 and 11. Differences in larval numbers were significant only in weeks 5 and 7.

On farm 3, where pretreatment counts were made after litter removal, adult numbers were significantly higher in the treated (79 per trap) than in the control house (23 per trap); larval numbers were negligible in both houses (Table 4). Numbers of larvae and adults were low following flock introduction 1 week posttreatment and remained low (<13 adults per trap) in both houses until week 7. On this date and on the final sampling date of week 8, significantly fewer adults were trapped in

Table 3. Mean trap counts of late-instar and adult of *A. diaperinus* on farm 2 (turkey houses with clay soil over sandy loam) before and after soil treatment with *S. feltiae* at 100,000 nematodes per square meter

Date	Wk post-treatment	\bar{x} no. larvae per trap			\bar{x} no. adults per trap		
		Control	Treated	F	Control	Treated	F
9 July	-1	46.2	56.2	0.146	11.7	43.8	4.124
10 July							
16-18 July							
25 July							
1 Aug.	2	0.6	1.2	0.453	17.8	15.2	0.107
8 Aug.	3	15.0	2.1	1.411	18.6	4.5	18.354 ^a
15 Aug.	4	29.9	42.5	0.825	11.6	7.1	2.416
22 Aug.	5	190.5	93.2	13.654 ^a	4.7	7.1	0.964
29 Aug.	6	209.7	143.3	2.089	27.2	7.3	7.233 ^a
5 Sept.	7	155.7	66.0	16.126 ^a	78.2	18.5	12.366 ^a
12 Sept.	8	109.1	93.1	0.267	184.3	56.6	19.492 ^a
19 Sept.	9	142.3	124.1	0.153	197.4	110.3	11.235 ^a
26 Sept.	10	143.7	123.7	0.269	202.0	145.5	1.377
1 Oct.							
3 Oct.	11	135.4	78.2	1.463	144.5	70.9	2.277

^a $P < 0.05$, one-way ANOVA for a given date and life stage; $n = 10$ traps per house per week; $df = 1,18$.

the treated house than in the control house. No significant larval population differences were observed on this farm.

Nematode Persistence on Treated Farms. Nematodes persisted in the soil for varying amounts of time on the three farms (Table 5). Persistence on farm 1 (indigenous piedmont clay soil) remained high (>73% beetle mortality) and changed relatively little during the first 7 weeks posttreatment. A drop to 15.5% was observed in week 9, followed by an increase to 36.0% in week 11. Beetle mortality then decreased to <20% on weeks 13 and 15. Persistence on farm 2 (indigenous sandy loam topped with imported piedmont clay) was also high (73-81%) in the first few weeks posttreatment, then declined gradually. In contrast, the soil of farm 3 (indigenous sandy loam) caused beetle mortality ranging from 63.0 to 78.5% between weeks 1 and 7, after which mortality dropped to 0% on the final sampling dates of weeks 9 and 11.

Nematode Persistence in Soil at Different Application Rates. No mortality was observed among

beetle larvae that were exposed to soil treated 6 months previously with *S. feltiae* at 10^4 nematodes per square meter (not presented in a table). Mortality of 11.8% was observed in soil treated with 10^5 nematodes per square meter, which was the rate used in the field trials. At the higher rates of 10^6 and 10^7 nematodes per square meter, beetle mortality was 34.1 and 48.2%, respectively. Mean control mortality was 15%.

Discussion

Soil treatments with entomogenous nematodes of the genera *Steinernema* and *Heterorhabditis* have provided moderate to high degrees of control against a number of coleopteran pests, including elm leaf beetle, *Pyrrhalta luteola* (Muller) (Kaya et al. 1981), Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Welch & Briand 1961), sugarcane wireworm, *Limoniopsis californicus* (Mannerheim) (Toba et al. 1983), black vine weevil, *Otiorhynchus sulcatus* (F.) (Bedding & Miller

Table 4. Mean trap counts of late-instar and adult *A. diaperinus* on farm 3 (turkey houses with sandy loam soil) before and after soil treatment with *S. feltiae* at 100,000 nematodes per square meter

Date	Wk post-treatment	\bar{x} no. larvae per trap			\bar{x} no. adults per trap		
		Control	Treated	F	Control	Treated	F
10 June							
27 June							
9 July	-1	0.6	1.4	0.917	34.4	78.8	4.572 ^a
16-17 July							
25 July							
1 Aug.	2	0	0	—	1.4	4.2	5.784 ^a
8 Aug.	3	0	0	—	0.8	4.2	7.287 ^a
15 Aug.	4	1.1	2.2	0.612	2.0	12.0	5.435 ^a
22 Aug.	5	30.0	16.2	2.082	6.4	7.3	0.046
29 Aug.	6	25.7	22.3	0.090	6.6	10.3	0.472
5 Sept.	7	46.9	34.3	0.516	37.3	9.1	14.136 ^a
11 Sept.							
12 Sept.	8	7.0	6.4	0.342	110.2	22.6	27.094 ^a

^a $P < 0.05$, one-way ANOVA for a given date and life stage; $n = 10$ traps per house per week; $df = 1,18$.

1981), and corn rootworm, *Diabrotica* spp. (Poinar et al. 1983). In a previous study (Geden et al. 1985), we found that *S. feltiae* (strain DD-136) was more virulent than *S. glaseri* and *H. heliothidis* in assays with *A. diaperinus* larvae. *S. feltiae* was particularly virulent against beetle immatures in sandy loam soil, where mortality rates of 99 and 100% were observed at dose rates corresponding to 36,000 and 360,000 nematodes per square meter, respectively. In the present study, we observed mortality rates of 94, 70, and 56% among immatures exposed to soil treated with the All, Breton, and Mexican strains, respectively, at a rate of 36,000 nematodes per square meter.

At a field application rate of 100,000 nematodes per square meter, a modest but significant degree of control was observed on all three farms. The relatively low level of control that we observed in the field relative to the laboratory assays may be due to several factors. Laboratory assays were conducted by placing larvae in soil that had been treated 1 h earlier with nematodes. In the field, however, numbers of late instars were not present to challenge the treated soil until several weeks posttreatment. Soil bioassays indicated that the nematodes persisted for several weeks; however, a decline was observed by week 5 on farm 2 and week 9 on farm 1. Thus, there was a relatively short time when high populations of late instars coincided with large nematode populations. This short period of overlap may account for the temporary nature of the control that was observed on these farms.

On farm 3 beetle populations remained very low throughout the study, presumably because of the long time interval between litter removal and new flock placement (4 weeks). Beetles that emerged from the soil during this time probably moved out of the empty houses and into the adjacent grow-out houses, leaving a negligible residual population to initiate a new infestation in the brooder houses when new shavings and birds were placed. Despite the low populations on this farm, comparison of Tables 4 and 5 indicates that high nematode persistence between weeks 1 and 5 resulted in a significant reduction in adult emergence in weeks 7 and 8 posttreatment.

The loss of nematode persistence on all three farms was surprising because nematodes emerging from parasitized cadavers were expected to enhance, or at least maintain, parasite populations in the soil. Even in the absence of new hosts, nematode persistence was about as high after 6 months in an environmentally controlled house as in the open-sided treated houses after 9 weeks. High temperatures may have played a role in the decline of nematode populations in the treated houses, because *S. feltiae* suffers high mortality at temperatures >30°C (Gray & Johnson 1983). Although soil temperature within the houses was not monitored, the time interval of greatest loss of nematode persistence (weeks 5–9 posttreatment) includ-

Table 5. Persistence of nematodes in soil of farms treated with *S. feltiae* at 100,000 nematodes per square meter

Wk post-treatment	\bar{x} % mortality of beetle immatures ^a		
	Farm 1	Farm 2	Farm 3
1	86.6	73.0	76.2
3	79.2	81.2	74.8
5	78.2	42.3	78.5
7	73.5	33.5	63.0
9	15.5	16.0	0.0
11	36.0	12.2	0.0
13	15.6	—	—
15	12.3	—	—

^a Corrected for control mortality (3–21%) by Abbott's (1925) formula.

ed a week-long period (3–10 September) during which daily outdoor temperatures exceeded 36°C.

In conclusion, *S. feltiae* may have potential as a biological control agent against lesser mealworm in poultry houses with soil floors. Further work is needed with higher application rates to determine treatment levels and times which will maximize nematode efficacy and persistence.

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