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SHORT COMMUNICATION

Infectivity of *Steinernema* spp. (Nematoda: Steinernematidae) to Adult Litter Beetles, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in the Laboratory

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Three species of Steinernema, S. carpocapsae, S. feltiae, *and* S. scapterisci *consisting of 12 different strains, were tested for their infectivity towards adults of the litter beetle* Alphitobius diaperinus*. Of the five most promising nematode strains, LC_{50} values ranged from 1.5 to 77.0 nematodes per host in the filter paper assays. Assays in poultry litter material revealed LC_{50} values to be 5.8 and 14.6 nematodes per host for the Mexican* S. carpocapsae *strain and Pye* S. feltiae *strain.*

Keywords: Alphitobius diaperinus*,* Steinernema*, entomopathogenic, biological control*

The litter beetle, *Alphitobius diaperinus* (Panzer) is one of the most important insect pests of commercially raised poultry, both as a nuisance and as a vector of disease. The litter beetle is omnivorous, feeding on dead birds as well as chicken feed, manure and other organic material found in broiler houses (Pfeiffer and Axtell, 1980). This insect is also known to harbor numerous avian pathogens (De la Casa *et al*., 1972; De la Casa *et al*., 1976). Late instar litter beetles also cause damage by tunneling into building insulation materials (Safrit and Axtell, 1984), and the beetles pose public nuisance problems due to the beetles attraction to light (Gall, 1980).

One of the primary reasons why entomopathogenic nematodes failed in the past for biological control of insect pests is because of the wrong choice of nematode species or strain (Georgis and Gaugler, 1991) and results can vary greatly among strains of the same species (Taylor *et al*., 1998; Shapiro and McCoy, 2000). According to Stock *et al*. (2001), there are 25 recognized species in the genus *Steinernema*, and Hominick *et al*. (1997) recognizes eight species in *Heterorhabditis*. Furthermore, many of the species contain numerous strains (Poinar, 1990). Three species of entomopathogenic nematodes have been tested for infectivity

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against immature litter beetles: *S. carpocapsae* (Weiser) (All, Breton, and Mexican strains), *S. glaseri* (Steiner), and *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) (Geden *et al*., 1985, 1987; note: *S. carpocapsae* was listed as *S. feltiae* in Geden *et al*., 1985, 1987). The only *Steinernema* species which have been evaluated against adult litter beetles in both filter paper and broiler litter assays is *S. carpocapsae* All strain. This nematode strain had LC_{50} values of 42 and 971 nematodes per adult beetle for filter paper and litter assays, respectively. These nematodes represent only a small portion of the variety of nematodes that are known to infect insects. It is conceivable that nematode species or strains exist that are more virulent than the *S. carpocapsae* All strain. Our objective was to expand comparative testing of entompathogenic nematodes for infectivity towards litter beetles in the lab in order to find promising strains for future field applications.

Species, strains, and origins of nematodes used are presented in Table 1. Classification of nematode strains to species follows that of Poinar (1990) and Hominick *et al*., (1997), except for *S. feltiae* SD-1, SD-7, and SD-34W. These nematodes were classified to species based on PCR-RFLP using the restriction enzymes *Alu* I and *Hinf* I following Taylor and Szalanski (1999), and using DNA sequence information from Szalanski *et al*., (2000). Nematodes were reared using late-instar greater wax moths, *Galleria mellonella* (L.), obtained from Grubco (Hamilton, OH) using methods similar to Dutky *et al*., (1964). Nematodes were harvested with White traps (White, 1927), quantified by counting the number of live nematodes in five, 5 µl samples and using the average to dilute with tap water to suspensions for the required concentration. Nematodes were stored in tissue culture flasks at 6°C for less than 1 month for the beetle assays. Beetle adults were collected from litter in broiler houses untreated with insecticides in Washington Co., AR, and maintained in colonies at the Department of Entomology, University of Arkansas. Adults were collected from pipe traps (Safrit and Axtell, 1984) placed in the litter material overnight.

The infectivity of *Steinernema* spp. towards adult beetles was tested using a filter paper assay. Fifteen adult beetles were placed in a 9-cm Petri dish lined with a filter paper disk (7 cm diameter). One milliliter of a nematode-water suspension ($\approx$ 100 nematodes per beetle) was applied to the filter paper, and replicated 9 times. For all of the assays, water was used for control replicates, temperature was maintained at 27°C, and beetle mortality was recorded at 3 d. Because the *S. carpocapsae* and *S. feltiae* UK, Kapow, Agriotos, Umea, SD1, SD7 strains along with *S. scapterisci* had relatively low infectivity in the filter paper assay, only five strains, *S. carpocapsae* Mexican, Mexican/Breton, and *S. feltiae* Pye, SN and SD34W, were tested for pathogenicity towards adult beetles using a bioassay. Four replicates

TABLE 1. Species and strains of *Steinernema* spp. evaluated for infectivity to litter beetle adults

Species	Strain	Origin	% mortality $\pm$SD at 72 h	Group[a]
S. carpocapsae	UK	United Kingdom	61.8 $\pm$ 21.3	**
S. carpocapsae	Kapow	Kapow, Poland	16.8 $\pm$ 8.7	
S. carpocapsae	Mexican	Mexico	98.3 $\pm$ 3.5	**
S. carpocapsae	Mexican/Breton	Hybridized	75.0 $\pm$ 15.0	**
S. carpocapsae	Agriotos	Russia	36.8 $\pm$ 8.7	**
S. feltiae	Pye	Sweden	100.0 $\pm$ 0.0	**
S. feltiae	Umea	Sweden	0.0 $\pm$ 0.0	
S. feltiae	SN	France	72.3 $\pm$ 21.1	**
S. feltiae	SD1	South Dakota, USA	35.0 $\pm$ 27.7	**
S. feltiae	SD7	South Dakota, USA	25.0 $\pm$ 28.3	**
S. feltiae	SD34W	South Dakota, USA	48.8 $\pm$ 9.4	**
S. scapterisci	—	Uruguay	13.3 $\pm$ 5.3	
Control	—	—	0.6 $\pm$ 1.2	

Data are the mean percentage of adult litter beetles killed in filter paper assays. Each strain was replicated 9 times with 15 litter beetles per replicate, using a concentration of 100 nematodes per beetle.

[a] **indicates significant mortality of adult litter beetles ($P < = 0.001$).

of 15 beetles per strain were inoculated with 1, 5, 10, 50, 75, 100, and 150 nematodes per beetle using the previously described filter paper technique.

The *S. carpocapsae*, Mexican strain and *S. feltiae* Pye strain, were evaluated for their infectivity towards litter beetles in a litter substrate. The litter substrate consisted of a mixture of pine and cedar chips which is commonly used as poultry litter in Arkansas. Thirty five grams of litter was placed in plastic containers (11 cm diameter, 8 cm deep) with screened holes (7.5 cm diameter) in the lid. Fifty millilitres of water was added to each container. Four replicates of 40 adult beetles per strain were inoculated with 1, 5, 10, 50, 75, 100, and 150 nematodes per beetle.

For the susceptibility study of all 12 *Steinernema* strains, nematode strains were ranked by infection frequency and χ^2 goodness of fit was used to compare differences of infection frequency between treatments (SPSS 10.0 for Windows, 1999). Means were compared by a LSD test (SPSS 10.0) to compare treatments with controls. Probit analysis (SPSS 10.0) was used to calculate LC_{50} and LC_{90} values for the filter paper and litter tests using a natural log transformation of the nematode concentrations.

Nine *Steinernema* strains produced significant mortality of adult litter beetles in the 100 nematodes per host filter paper assay (Table 1, $F = 14.421$; $df = 12$; $P < = 0.001$). Based on these results, three strains of *S. feltiae* (Pye, SN, SD34W) and two strains of *S. carpocapsae* (Mexican, Mexican/Breton) were further evaluated for their infectivity towards adult beetles. For the filter paper assays, the LC_{50} value was from 1.5 to 77.0 nematodes per host for the *S. carpocapsae* strains, and 4.2 to 61.8 for the *S. feltiae* strains (Table 2). Rates of infection of adult litter beetles by the *S. carpocapsae* Mexican strain and *S. feltiae* Pye strain was approximately four times less for the litter assay ($LC_{50} = 5.8$ and 14.6) compared to the filter paper assay (Table 2).

Control mortalities were 3% for the filter paper assay and 5% for the litter assay. *Steinernema carpocapsae* and *S. feltiae* were the most promising nematode species, however, strains of these two species varied greatly in their infectivity. Of the 5 strains of *S. carpocapsae* and 6 strains of *S. feltiae* evaluated, levels of mortality ranged from 16.8 to 98.3%, and 0.0 to 100%, respectively. Variability in infectivity among nematode strains has been reported for numerous other insects such as house fly, *Musca domestica* L. (Taylor *et al*., 1998), and the diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Shapiro and McCoy, 2000), and illustrates the importance of evaluating not only different species, but different strains of these entomopathogenic nematodes.

Geden *et al*. (1985) evaluated the infectivity of *S. feltiae* (= *carpocapsae*) All strain, *S. glaseri*, and *Heterorhabditis heliothidis* towards larval and adult litter beetles using both filter paper and litter assays. In this study, the All strain was the most infective nematode strain. In the filter paper assay, both beetle larvae and adults were susceptible to the All strain, with LC_{50} values of 9 and 42 nematodes per host, respectively. In the broiler litter assays, beetle larvae and adults were much less susceptible to nematodes, with LC_{50} values of 258 and 971 nematodes per host, respectively. In contrast, we found that adult beetles were

TABLE 2. Pathogenicity of select *Steinernema feltiae* and *S. carpocapsae* strains towards adult litter beetles

Substrate	Strain	LC_{50}^a	95% FL	LC_{90}^a	Slope ± SE	χ^2
Filter paper	SN	29.3	23.3–36.5	141.8	0.65 ± 0.06	26.3
	SD34W	61.8	45.9–87.2	596.3	0.45 ± 0.05	31.7
	Pye	4.2	2.9–5.8	18.3	0.71 ± 0.06	35.7
	Mexican	1.5	0.7–2.3	7.1	0.65 ± 0.08	47.7
	Mexican/Breton	77.1	63.1–93.8	170.4	1.05 ± 0.11	49.4
Litter	Mexican	5.8	3.8–8.3	46.8	0.61 ± 0.05	12.3
	Pye	14.6	9.2–23.3	93.1	0.69 ± 0.06	22.5

[a] Number of nematodes per host.

highly susceptible to *S. carpocapsae* Mexican and *S. feltiae* Pye strains in our litter environment. These strains have never been evaluated for their infectivity towards adult beetles. Previous persistence studies (Geden *et al*., 1987) have revealed that the nematodes can survive in the poultry environment for at least 7 wk. This infectivity of the nematodes towards the adult stage is important since adults have a lifespan from 77 to over 703 d (Preiss and Davidson, 1971) with females laying an average of 3.5 eggs per day. Presumably, fresh hosts will be available for the nematodes to cycle continuously, thus not only extending the persistence of the nematodes but possibly increasing the population with time.

Although laboratory screening of entomopathogenic nematodes for infectivity can be an important component of developing a biological control program for a particular pest (Ricci *et al*., 1996), relative infectivity among nematodes in the laboratory may not be consistent with what is observed in the field (Grewal and Georgis, 1998). Once the biological candidates are defined based on characters tested in the laboratory, the ultimate test of efficacy must be conducted under field conditions. Within a 1,386 m^2 poultry broiler house, the 1,268 m^2 area of open litter is estimated to have an average weekly population of 2.2×10^6 adult litter beetles during a growout (Strother, 1998). We estimate (unpublished data) that $\approx$5,000 infective juvenile nematodes are produced per adult beetle. Hence, successful nematode reproduction in 16 adult beetles per square-meter of a broiler house may maintain a nematode population adequate for controlling 50% of the adult litter beetles.

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