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Effect of Host Age and Nematode Strain on Susceptibility of *Spodoptera frugiperda* to *Steinernema feltiae*[1]

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Abstract: Median lethal concentrations (LC_{50}) were determined for four nematode populations (two strains of *Steinernema feltiae*, a *S. feltiae* hybrid, and *S. bibionis*) against fifth-instar fall armyworm (*Spodoptera frugiperda*) larvae and for the most virulent of these nematodes against different instars and stages of the insect. Based on lack of overlap of 95% fiducial limits, there were significant differences in virulence among the four nematodes. The LC_{50} ranged from 7.6 to 33.3 nematodes/0.7 ml water, and slopes of the log dose-probit regression lines were similar except for the *S. feltiae* All strain. First-instar fall armyworms suffered virtually 100% mortality from the *S. feltiae* Mexican strain at 1.0 nematode/0.7 ml, and LC_{50} were 2.3 and 7.9 nematodes/0.7 ml in third-instar and fifth-instar larvae, respectively. Pupae had 7–20% mortality at doses ranging from 30 to 60 nematodes/0.7 ml.

Key words: entomogenous nematode, median lethal concentration, *Neoaplectana, Steinernema bibionis, Steinernema feltiae,* virulence.

Steinernema feltiae Filipjev and other steinernematid nematodes are potential biological control agents for a major insect pest, *Spodoptera frugiperda* (J. E. Smith), the fall armyworm. Although *S. feltiae* has failed to control insects in many situations, it is thought to have excellent potential for certain pests in cryptic habitats and soil (5). The fall armyworm often occurs in just such habitats; medium-sized and large larvae damage corn and sorghum by feeding in the whorl of the plant or in corn ears, and they pupate in the soil (4,10,12).

Ideally, the most virulent nematode strain against one or more ages of the target insect should be selected before field trials. This is seldom done, although there is evidence that nematode strains differ in virulence to insect hosts (2,11,13) and that various ages and stages of host insects differ in susceptibility (7,8). Fall armyworm is susceptible to *S. feltiae* (9), but how nematode strains and host age affect this susceptibility is unknown.

Our purpose was to test several strains of *S. feltiae* and one isolate of *Steinernema bibionis* (Bovien) for differences in virulence to fall armyworm and to determine whether various ages and stages of the insect differed in their susceptibility to the most virulent nematode.

MATERIALS AND METHODS

Four nematode populations were used: the All, Mexican, and a hybrid (DD-136 × Breton) strains of *S. feltiae,* and *S. bibionis.* The populations were initially recovered from Georgia, Mexico, G. O. Poinar (University of California, Berkeley), and France, respectively. The nematodes were produced in vitro (BIOSIS, unpubl.) and stored in damp sponges at 4 C until they were used in the experiments. The fall armyworms were collected from corn at Hammond, Louisiana, in June 1985. The insects were reared on artificial diet (6) without formalin, and moths were confined in 3.8-liter ice cream cartons with 10% sucrose solution and paper toweling for an oviposition substrate.

The insects were bioassayed against the nematodes in petri dishes (1.5 cm deep × 6 cm d). Appropriate numbers of infective-stage juvenile nematodes in 0.7 ml water were placed in each dish, followed by two layers of no. 1 filter paper and one test

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TABLE 1. Susceptibility of fifth-instar *Spodoptera frugiperda* to four nematode populations.

Nematode	Total sample size (n)†	Median lethal concentration, LC_{50}‡	95% fiducial limits	Slope (± SE)
S. feltiae Mex.	174	7.6	5.5–11.9	1.36 (± 0.28)
S. feltiae All	200	16.0	13.0–19.4	2.33 (± 0.33)
Hybrid§	270	22.8	16.2–41.0	1.23 (± 0.23)
S. bibionis	298	33.3	24.4–57.9	1.53 (± 0.28)

† Not including controls: n for controls ranged from 35 to 60 with no mortality.
‡ Nematodes/0.7 ml water.
§ *S. feltiae* DD-136 × *S. feltiae* Breton.

insect. The 0.7-ml volume of water was sufficient to moisten the filter paper but not enough to drown the small insects. Fall armyworms were tested as larvae—first, third, and fifth instars—and as pupae. The larvae were tested on the day they molted to their respective instars, and the pupae were tested within 3 days of pupation. There were at least five doses plus a control group (water with no nematodes in the dish) for each nematode strain or host age tested. Initially, 30–65 insects were tested per dose except in the pupal experiment (see Tables 1, 2). A problem with first-instar larvae escaping from the petri dishes was alleviated to some degree by taping the crack between the top and bottom of the dish.

As only one nematode strain or host age could be tested at a time, the first combination tested (the *S. feltiae* Mexican strain vs. fifth-instar larvae) was included as a positive control in each subsequent test; each of 20–30 insects was exposed to eight nematodes in 0.7 ml water (ca. the median lethal concentration—[see Table 1]). Mortality of these positive controls ranged from 45 to 53%, indicating that the bioassay was functioning similarly throughout the experiments. The insects in the first combination tested were checked for mortality every 2 hours for 56 hours; in the remainder of the tests, the insects were checked only at 16 and 52 hours after exposure to the nematodes.

A line was estimated by probit regression for each nematode strain or host age tested. Median lethal concentrations (LC_{50}) and associated statistics were estimated (MicroProbit 3.0 for the IBM PC, Sparks and Sparks, unpubl. program) by the method of probit analysis (3), with control mortality corrected by Abbott's formula (1).

RESULTS

The nematodes differed in their virulence toward *S. frugiperda* based on lack of overlap of 95% fiducial limits of the LC_{50} (Table 1). The *S. feltiae* Mexican strain had the lowest LC_{50} and was thus the most virulent of the four populations. Slopes of all regression lines were similar except for the *S. feltiae* All strain which had a steeper slope than the rest. The highest LC_{50} (*S. bibionis*) was 4.4× greater than the lowest LC_{50} (*S. feltiae* All). Of the 72 fifth-instar fall armyworms killed by the *S. feltiae* Mexican strain, > 60% died between 38 and 50 hours after exposure to the nematode and 100% were dead after 52 hours (Fig. 1). The median lethal time was 42.5 hours (95% fiducial limits = 41.1–42.6 hours).

Pupae as well as larvae were susceptible to the *S. feltiae* Mexican strain; the larvae became slightly less susceptible with age (Table 2). LC_{50} and associated statistics could not be estimated for first-instar larvae or pupae, because the former had 100% mortality at even one nematode/0.7 ml and the latter did not have 50% mortality at the highest nematode dose (60 nematodes/0.7 ml). The LC_{50}, 95% fiducial limits, and slope (± SE) were 2.3 nematodes/0.7 ml, 0.9–3.6, and 0.82 (± 0.24), respectively, in third-instar larvae, and 7.9, 5.8–11.7, and 1.64 (± 0.34), respectively, in fifth-instar larvae.

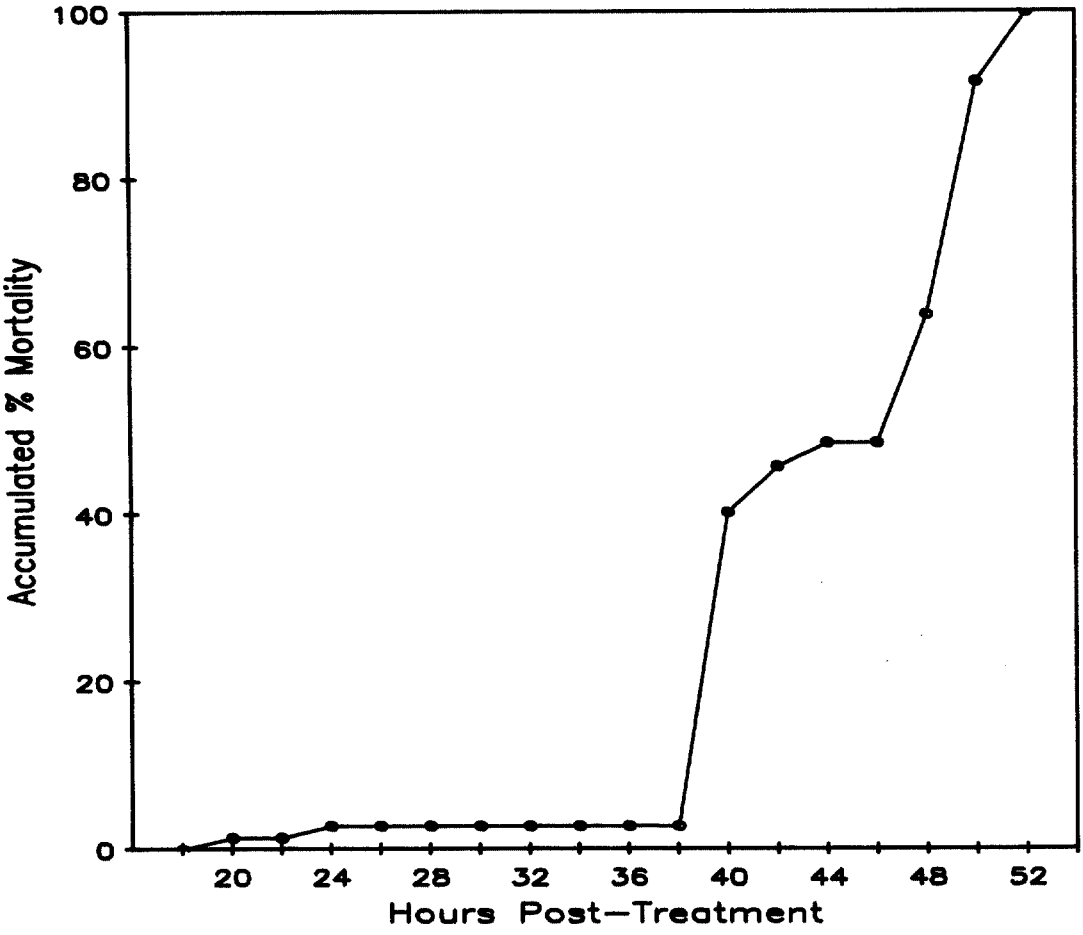

FIG. 1. Time : mortality response of fifth-instar *S. frugiperda* larvae exposed to the *S. feltiae* Mexican strain. The figure is based on the 72 insects killed by this nematode in the "strains" experiment (Table 1).

DISCUSSION

Our results demonstrated differences in virulence among three strains (including a hybrid) and two species of nematodes to fall armyworm. We observed three levels of virulence among the four nematode populations based on lack of overlap of 95% fiducial limits; the *S. feltiae* Mexican strain was the most virulent, the *S. feltiae* All strain and the *S. feltiae* hybrid were intermediate, and *S. bibionis* was the least virulent. The 4.4× difference among nematode populations in our experiment is probably sufficient to affect short-term biological control of fall armyworm. Other researchers have demonstrated variations in virulence among strains of *S. bibionis* in *Lucilia cuprina* (Weidemann) (11) and *Heliothis punctiger* Wallengren (2), *S. feltiae* in *Leptinotarsa decemlineata* (Say) (13), and *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann) in *Calliphora vicina* Robineau-Desvoidy (2).

Age-related differences have been observed in susceptibility of Lepidoptera to *S. feltiae*, though not previously in *S. frugiperda*. Kaya (7) observed significantly greater mortality caused by *S. feltiae* (= *Neoaplectana carpocapsae*) in 3–8-day-old larvae of *Spodoptera exigua* (Hübner) than in 0–1-day-old larvae, whereas our experiment demonstrated a decrease in mortality as the insects aged. This difference may have been related to the bioassay method; infection in the Kaya experiments may have depended to some degree on the insect in-

TABLE 2. Susceptibility of different larval instars and pupae of *Spodoptera frugiperda* to *Steinernema feltiae* Mexican strain.

Dose (nematodes per 0.7 ml)	% mortality (n)			
	1st instar	3rd instar	5th instar	Pupae
0	32.4 (34)	12.0 (50)	0 (30)	0 (30)
1	100 (26)	38.0 (50)	10.0 (30)	
3	100 (36)	50.0 (50)	20.0 (30)	
5	100 (29)	67.3 (49)		0 (10)
7	97.1 (34)	66.0 (50)		
8			50.0 (30)	
10	97.1 (34)	66.0 (50)	60.0 (30)	0 (10)
13			63.3 (30)	
20				0 (30)
30				6.7 (30)
40				20.0 (30)
50				10.0 (20)
60				15.0 (20)

gesting nematodes along with artificial diet. Kaya and Hara (8) demonstrated that lepidopterous pupae are susceptible to *S. feltiae*. Seven insect species that pupate in soil had 0–47% mortality when exposed to 200 infective juveniles, which is similar to the 7–20% mortality we observed with fall armyworm pupae.

Our results are encouraging for possible use of *S. feltiae* for biological control of fall armyworm in corn. The *S. feltiae* Mexican strain is virulent, which would reduce the number of nematodes required to be delivered to the insect in the field. The nematode kills most insects within 2 days, which would limit the amount of damage to the plant. Certain other potential microbial control agents for fall armyworm, such as the nuclear polyhedrosis virus (NPV) or microsporidia, require a week or more to kill the insect (Fuxa, unpubl.). Additionally, fifth-instar larvae were only slightly less susceptible to *S. feltiae* than first or third instars; therefore, timing of application would not be as critical with *S. feltiae* as with other biological agents. In contrast, > 200 times and > 1 million times as much NPV is required to kill fifth-instar fall armyworms as is required for third or first instars, respectively (Mitchell and Fuxa, unpubl.). Finally, the searching ability of the juvenile nematodes might allow them to search and destroy fall armyworms that have burrowed into host plant parts where chemical insecticides are ineffective.

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