Infectivity and Propagation of Entomogenous Nematodes,
Steinernema spp., on the Common Cutworm, *Spodoptera*
litura (Lepidoptera: Noctuidae)^{1,2}

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Inoculation experiments were conducted at 25°C under laboratory conditions to investigate the infectivity and propagation of *Steinernema feltiae* (DD-136), *S. bibionis*, and *S. glaseri* on the last instar larvae and male adults of common cutworm, *Spodoptera litura*. *S. feltiae* was the most infective on *S. litura* larvae, followed by *S. bibionis* and *S. glaseri*. *S. feltiae* was positively attracted to the intact and ligated larvae, efficiently invaded, caused acute death of the hosts, and propagated rapidly and abundantly. *S. bibionis* was weakly attracted to the intact larvae, inefficiently invaded, and slowly developed. These properties led this nematode to low competitive propagation against the other steinernematids which were inoculated at the same time. *S. glaseri* was slowly but steadily attracted, invaded, quickly developed and established a high population in the hosts. The propagation of *S. glaseri*, however, was suppressed by the mixed inoculation with *S. feltiae* and to a lesser extent with *S. bibionis*. All species of steinernematids used propagated more quickly and abundantly in the moths from which the infective juveniles emerged earlier than from the larvae.

The entomogenous nematodes, *Steinernema* spp. and *Heterorhabditis* spp., have been used as biocontrol agents against insect pests (POINAR, 1979). Recently in Japan, attempts to control agricultural and forest pests have been under way (ISHIBASHI et al., 1981; KATAGIRI et al., 1984). The host ranges of steinernematids and heterorhabditids are broad in some species and narrow in others. Although many investigations have been done on the infectivity and host range of these nematodes, rather limited information is available on the factors associated with different infectivity among the nematode species on a selected insect pest. For the efficient control of a target pest, it is important to know the factors involved in the infection process of nematodes as well as to select the most infectious entomogenous nematodes.

The present investigation was conducted to determine the factors which lead the three species of steinernematids used to different infectivity on the larvae and male

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adults of common cutworm, *Spodoptera litura*, which is an important pest on soybean, Japanese yam, and many other crops in Japan. The investigation was also extended to learn the comparative and competitive propagations among these three nematode species in the *S. litura*.

MATERIALS AND METHODS

Nematodes. Three species of entomogenous nematodes were used in this investigation; *Steinernema feltiae* (DD-136), *S. bibionis*, and *S. glaseri*. Since the introduction of these nematodes from the U.S.A. and Australia, they have been cultured at 25°C on chicken liver and/or intestine media (KONDO et al., 1985) or on larvae of the greater wax moth, *Galleria mellonella*.

Insects. The last instar larvae and male adults of common cutworm, *Spodoptera litura* were used. The larvae were reared at 25°C on an artificial diet mainly consisting of crushed kidney beans, wheat germ, dried yeast, and agar. When the feeding larvae were used as hosts, fungi tended to grow and inhibit the nematode propagation in the insect cadavers. Therefore, non-feeding larvae were used within one day after the final molt throughout the present experiment. The male adults of *S. litura* were caught in a pheromone trap (Pherodine II, Takeda Chemical Corp., Osaka) and used within 12 hr after capture.

Effect of moisture on infectivity. A 0.1 to 1.0 ml of 0.1% formalin solution containing ca. 500 each of the infective 3rd stage juvenile (J_{III}s) was inoculated in a plastic petri dish (5.5 cm in inner diam. and 1.3 cm in height) on the bottom of which a sheet of filter paper (6 cm in diam., Toyo #1) was placed. Within 30 min after setting, the insect larvae were individually introduced in the dish. Ten insects were used for each of the various moisture conditions investigated. To maintain a constant moisture, the dishes were encased in a plastic square box, tightly sealed, and incubated at 25°C. The insect mortality was examined 24 and 48 hr after inoculation. Since the infectivity of steinernematids declined under a water saturated condition, all inoculation experiments were conducted under a moisture condition of 0.4 ml per dish.

Attraction and invasion. At 6, 12, and 24 hr after the introduction of *S. litura* larva in the petri dish, nematodes were recovered from the dish lid, from the insect body surface, and from the host body. The nematodes on the underside of the lid were collected by rinsing them off with 25 ml of formalin solution. Nematodes on the body surface were isolated by dipping the insects for 30 min with occasional agitation in 10 ml of formalin solution in a plastic centrifuge tube (15 ml in capacity). Then the insects were individually transferred to a petri dish containing no nematodes, and further incubated at 25°C. The total incubation time was 48 hr after the inoculation for all insects exposed to the nematodes for different time periods. During this incubation, the insect tissues were largely disintegrated by the symbiotic bacteria (*Xenorhabdus* spp.) and the invading nematodes developed large enough for easy and accurate counting. These infected insects were then dissected longitudinally along the ventral line and placed for 4 hr on the surface of a 1/2 diluted solution of physiologically balanced saline (KONDO and ISHIBASHI, 1984) in a petri dish with the dissected side down. The nematodes appearing in the dish and those remaining in the insect body were counted as invaders. Statistical differences among data were analyzed by DUNCAN's multiple range test.

Fig. 1. Schematic drawings of *S. litura* larvae inoculated with steinernematids. A: Non-ligated intact larva, Mo: mouth part, An: anus. B: ligated larva. An, Mi, and Po indicate the anterior, middle, and posterior parts of the insect. C: isolated abdomen (IA) with the exposed tissues (ET) at both ends.

Fig. 2. Effect of moisture on the infectivity of steinernematids. Open and solid symbols indicate the mortality of the intact last instar larvae of *S. litura* 24 and 48 hr after the inoculation with ca. 500 infective juveniles of *S. feltiae* (circle), *S. bibionis* (triangle), and *S. glaseri* (square) per petri dish (5.5 cm in inner diam., 1.3 cm in height).

Infectivity on intact and ligated larvae. The larvae molting to the last instar (head slippage stage) were individually introduced in a petri dish and kept at 25°C. Within 12 hr after the final molt, the insects were ligated by cotton thread at the intersegments between the 3rd thoracic and 1st abdominal body segments and also between the 5th and 6th abdominal ones. From these insects, the anterior and posterior body parts were either undetached (Fig. 1B; hereafter called as ligated larva) or detached at one body segment apart from the ligation sites (Fig. 1C; hereafter called isolated abdomen). Non-ligated intact larvae were used as control (Fig. 1A). For each treatment, 20 insects were used. These insects were individually transferred to a petri dish containing ca. 500 JIIS of *S. feltiae*, *S. glaseri* or *S. bibionis*, and kept at 25°C. Forty-eight hours after inoculation, nematodes were recovered from the body surface and from the body of the insects. In case of the isolated abdomen, nematodes on the body surface included those in the exposed tissues. After isolating nematodes on the body surface, the insects were frozen at -30°C; once frozen, they were cut with a surgical knife at both sides of each ligation site. The middle parts of the insect body were separately soaked in diluted saline at room temperature. After thawing, the body pieces were dissected longitudinally, transferred to a centrifuge tube containing 10 ml of saline solution, and vigorously bubbled for about 30 sec using a pipette with a notched mouth opening of ca. 5 mm. The nematodes thus released were counted.

Comparable and competitive propagation. A single species inoculation of steinernematids was conducted to compare the propagation of *S. feltiae*, *S. bibionis*, and *S. glaseri* in the intact larvae and male adults of *S. litura*. Two days after the inoculation with ca. 500 JIIS, the inoculated insects were individually placed on a moist filter paper strip (13 × 130 mm) in a test tube (18 mm in diam. and 180 mm in length) and incubated at 25°C. The nematodes emerging from the insect cadavers were recovered at 24 hr intervals for 21 days by washing them out with 15 ml each of the formalin solution.

The mortality and body weight of the inoculated *S. litura* larvae were measured at 24 hr intervals for 5 days. Change in the body weight was expressed as the rate of weight loss against the weight before inoculation. For control, the weight loss of the non-inoculated starving larva was measured.

To determine the competitive propagation among nematodes, the three species of steinernematids were inoculated separately or in combinations on the *S. litura* larvae. The number of J_{III}s inoculated per insect was ca. 500 for a single species inoculation, and 250 each for the mixed inoculations with two or three species. For all inocula, 0.4 ml of 0.1% formalin solution was used for suspending nematodes. About 30 min after inoculation, *S. litura* larvae were individually placed in a petri dish. After two days incubation at 25°C, the insects were rinsed in the formalin solution to wash off the nematodes on the insect body surface and then individually kept in a test tube with a moist filter paper strip.

The nematodes emerged from the insect cadavers were recovered and counted at 24 hr intervals for 21 days. In case of the mixed inoculation, the species determination was made on the J_{III}s by their body size.

When *S. feltiae* and *S. glaseri* were simultaneously inoculated, some insects had one or the other of them and others both. To investigate the relations between the infection time and the infectivity and propagation, *S. feltiae* was inoculated 0, 1, 2 and 3 days after the inoculation with *S. glaseri*. The nematodes were recovered and counted at 24 hr intervals for 14 days. The species determination was also made on the recovered J_{III}s. The yields of nematodes were determined 21 days after the inoculation with *S. glaseri*.

RESULTS

Effect of moisture on infectivity

Infectivity of *S. feltiae*, *S. bibionis*, and *S. glaseri* on the last instar larvae of *S. litura* was higher in this order. However, their infectivity was markedly affected by the moisture conditions (Fig. 2). Within the range of moisture examined, the infectivity of these steinernematids tended to decrease with increasing moisture. The infectivity was obviously lowered under water saturated conditions: 0.8 ml or more per dish. The optimum moisture range for infectivity was similar for all nematode species used: around 0.4 ml per dish, which corresponds to about 60% saturation.

Attraction and invasion to intact larvae

There were differences in the number of steinernematids which moved to the petri dish lid (Fig. 3). When a larva was not introduced in the dish, the J_{III}s of *S. feltiae*, *S. glaseri*, and *S. bibionis* were recovered from the lid in this order. Of ca. 500 J_{III}s inoculated, the number recovered from the lid 24 hr after inoculation was ca. 210 for *S. feltiae*, ca. 80 for *S. glaseri*, and ca. 50 for *S. bibionis*.

The mobility of J_{III}s to the lid was altered by the introduction of a *S. litura* larva in the dish. The number of *S. feltiae* recovered from the lid significantly decreased by the insect introduction. Reversed tendency was observed for *S. glaseri*. An almost equal number of *S. bibionis* was isolated from the lid of a petri dish with or without a *S. litura* larva.

Attraction and invasion to the intact *S. litura* larvae were also variable among

nematode species (Fig. 4). *S. feltiae* was quickly attracted and invaded the host. About 70% of the inoculated nematodes were recovered from the insects 48 hr after inoculation. The total number of *S. bibionis* recovered from the body surface and the body of *S. litura* larva reached maximum 12 hr after inoculation, ca. 45 per insect, and then decreased because some JIII's on the body surface left the host without invading it.

Compared to the above two species, *S. glaseri* was less attracted to *S. litura* larvae. However, the number of nematodes invading the host increased slowly but steadily. About 20 nematodes were recovered per insect 48 hr after inoculation.

Fig. 3. Mobility of the infective juveniles of *S. feltiae* (circle), *S. bibionis* (triangle), and *S. glaseri* (square) to the lid of a petri dish with or without *S. litura* larva. Symbols with different small letters indicate a significant difference at 5% level by Duncan's multiple range test.

Fig. 4. Attraction and invasion of the three species of steinernematids to the intact *S. litura* larva. Open, solid, and shaded symbols respectively indicate the number of steinernematids recovered per insect from the body surface, body, and both.

Table 1. Number (A) and percentage (B) of adult nematodes recovered from intact *S. litura* larvae exposed for different times to moist filter paper containing ca. 500 infective juveniles of *Steinernema* spp.

Exposure time (hr)	<i>S. feltiae</i>		<i>S. bibionis</i>		<i>S. glaseri</i>	
	A	B	A	B	A	B
6	45.5	38.3	4.4	35.6	0.2	8.3
12	176.1	78.6	17.8	56.3	0.5	18.8
24	294.0	92.6	22.2	62.4	2.3	19.8
48	346.0	96.6	18.0	49.5	9.2	36.7

Nematodes were recovered 48 hr after the onset of exposure.
Each value is the mean of 10 replicates.

The development of invading nematodes to adult was different among nematode species (Table 1). For *S. feltiae* and *S. glaseri*, the number and percentage of adults increased with increasing exposure time of *S. litura* larvae to the nematodes. Such a clear tendency was not observed for *S. bibionis*. Of the three steinernematids examined, *S. feltiae* developed to adult more quickly and abundantly than the others. Although the total number of invading *S. glaseri* was fewer, the percentage of adults in the insects steadily increased with time.

Infectivity and propagation on intact larvae

The infectivity of *S. feltiae*, *S. bibionis*, and *S. glaseri* on the intact *S. litura* larva was higher in this order (Fig. 5). Body weight of the uninoculated larva gradually decreased with time by starvation. The mortality of these insects increased after 4 days starving. Of the three steinernematids used, *S. glaseri* showed the weakest infectivity and the mortality of the inoculated insects increased about one day earlier than that of uninoculated control. The body weight of the insects inoculated with *S. glaseri* similarly decreased by 4 days as in control, though the weight became nearly stationary thereafter.

All larvae were killed within 48 hr after the inoculation with *S. feltiae*. Their body weight rapidly decreased during the two days after inoculation and then very slowly thereafter. The mortality and body weight of the insects inoculated with *S. bibionis* were almost intermediate between those of *S. feltiae* and *S. glaseri*. Body weight decreased less extensively by the inoculation with the more infectious steinernematids.

Fig. 5. Changes in the rates of survival and body weight loss of the intact *S. litura* larvae after the inoculation with *S. feltiae* (circle), *S. bibionis* (triangle), and *S. glaseri* (square). Cross indicates the non-inoculated control.

Fig. 6. Invasion of *S. feltiae* (circle), *S. bibionis* (triangle), and *S. glaseri* (square) to the middle body part of the ligated *S. litura* larva. Vertical lines indicate the standard deviations.

Table 2. Propagation of *Steinernema* spp. on intact *S. litura* larvae

Nematodes inoculated	Days after inoculation	Total no. of nematodes recovered per insect	Developmental stages (%)		
			Infective juveniles	Other juveniles	Adults
<i>S. feltiae</i>	4	43,440	0.0	98.6	1.1
	21	59,520	99.8	0.2	0.0
<i>S. bibionis</i>	4	9,837	0.0	99.8	0.2
	21	12,400	98.2	1.8	0.0
<i>S. glaseri</i>	4	927	0.0	98.2	1.8
	21	4,490	98.9	1.1	0.0

Nematodes were recovered 4 and 21 days after the inoculation with ca. 500 infective juveniles per insect.

Each value is the mean of 10 replicates.

Table 3. Growth of *Steinernema* spp. in the isolated abdomen of *S. litura* larva

Nematodes	Number of nematodes recovered per insect	
	Body surface	Body
<i>S. feltiae</i>	111 (39)	371 (36)
<i>S. bibionis</i>	43 (29)	2 (0.1)
<i>S. glaseri</i>	768 (45)	435 (1)

Nematodes were recovered 54 hr after inoculation.

Numerals in parentheses indicate the number of adult nematodes.

Each value is the mean of 15 replicates.

Infectivity on ligated larvae

The J_{III}s of *S. bibionis*, *S. glaseri*, and *S. feltiae* invaded the middle parts of the ligated larvae (Fig. 6). With increasing exposure time of the insects to the nematodes, the number of invaded nematodes increased, though no significant differences were observed among nematode species. The mortality of the ligated insects 72 hr after inoculation was 87% for *S. bibionis*, 73% for *S. feltiae*, 69% for *S. glaseri*, and 13% for non-inoculated control (data omitted).

The J_{III}s of these three species of nematodes invaded the isolated abdomen and developed to adult in it (Table 3). The number of adult nematodes recovered from the isolated abdomen 54 hr after inoculation were 36 for *S. feltiae*, 1 for *S. glaseri*, and 0.1 for *S. bibionis*. The adults of *S. feltiae* and *S. glaseri* produced their offspring more rapidly than did *S. bibionis* so that the total number of nematodes recovered was more numerous from the insects inoculated with the former two species: 435 for *S. glaseri*, 371 for *S. feltiae*, and only 2 for *S. bibionis*.

The number of nematodes recovered from the body surface of the isolated abdomen was the greatest for *S. glaseri*, followed by *S. feltiae* and *S. bibionis* (Table 3). The J_{III}s of *S. glaseri* were efficiently attracted to the exposed tissues where they developed to adults and produced many progeny. The J_{III}s of *S. bibionis* were also attracted to the exposed tissue, though the nematode propagation was less abundant. The J_{III}s of

Table 4. Mortality of intact *S. litura* larvae inoculated with *S. feltiae* (Sf), *S. bibionis* (Sb), and *S. glaseri* (Sg) in combinations

Nematodes inoculated			Mortality (%)		
Sf	Sb	Sg	24 hr	48 hr	72 hr
+	—	—	6.7	100.0	100.0
+	+	—	6.7	100.0	100.0
+	—	+	13.3	80.0	100.0
—	+	—	0.0	46.7	80.0
—	+	+	0.0	20.0	40.0
—	—	+	0.0	13.3	60.0
+	+	+	0.0	86.7	100.0
—	—	—	0.0	0.0	6.7

Number of nematodes inoculated (+) was ca. 500 for a single species inoculation and ca. 250 each for a mixed inoculation.

Fifteen insects were used for each combination.

S. feltiae were attracted to the exposed tissues and developed to adults but produced fewer progeny than in the isolated abdomen.

Effect of mixed inoculation on nematode propagation

When one, two, or three species of steinernematids were inoculated in combinations, the mortality of *S. litura* larvae was largely determined by the nematode having the stronger infectivity (Table 4). No synergistic effect on the infectivity was observed by the mixed inoculation of steinernematids.

The propagation of *S. feltiae* was not disturbed by the mixed inoculation with *S. bibionis* and/or *S. glaseri* (Fig. 7). Although no statistical difference was observed, the number of *S. feltiae* in the cadaver increased by the mixed inoculation with *S. bibionis* and decreased with *S. glaseri*.

From the insects inoculated with *S. bibionis* alone, the total number of JIII's emerged from the insect cadavers in 21 days was ca. 12,000 per insect. The propagation of *S. bibionis* was, however, strongly suppressed by mixed inoculation with *S. feltiae* and/or *S. glaseri*.

The propagation of *S. glaseri* was more or less suppressed by the mixed inoculation with *S. feltiae* or *S. bibionis* (Fig. 7). At all inoculation intervals tried, the mortality of *S. litura* larvae was the highest when *S. glaseri* and *S. feltiae* were inoculated at the same time (Fig. 8). Increasing the time intervals between the inoculation with *S. glaseri* first and then with *S. feltiae*, a longer time was required to kill the insects. Generally, the JIII's of *S. glaseri* emerged from the cadavers earlier than those of *S. feltiae*. The total number of JIII's recovered from *S. litura* larvae inoculated with *S. glaseri* and *S. feltiae* decreased with increasing time intervals between the inoculations of the two species (Table 5). In all *S. litura* larvae inoculated with combinations of the three species of steinernematids, JIII's characteristic of the intermediate morphology were not observed.

Infectivity and propagation on male moth

Compared to the infectivity on *S. litura* larvae, *S. feltiae*, *S. bibionis*, and *S. glaseri*

Fig. 7. Emergence of the infective juveniles (JIII) of steinernematids from *S. litura* larvae inoculated with *S. feltiae*, *S. bibionis*, and *S. glaseri* in combinations. Solid symbols indicate the number of nematodes recovered from the insects inoculated without other species. Open symbols indicate the number of nematodes recovered from the insects inoculated at the same time with the nematodes noted by the abbreviations on the right margin. S.f., S.b., and S.g. indicate *S. feltiae*, *S. bibionis*, and *S. glaseri*, respectively. Results on the nematode emergence from *S. litura* larvae inoculated together with the three species of steinernematids were omitted from the above figures because they generally coincided with the lowest curves.

Fig. 8. Effect of time intervals between the inoculations with *S. glaseri* first and then with *S. feltiae* on the mortality of *S. litura* larvae (solid circle) and on the percent of insects releasing JIII of *S. glaseri* (open square) and *S. feltiae* (open circle). Black and white arrows indicate the inoculation time of *S. glaseri* and *S. feltiae*, respectively.

Table 5. Effect of time intervals between the inoculations with *S. feltiae* (Sf) first and then with *S. glaseri* (Sg) on their propagation in the intact *S. litura* larva

Time difference in days between inoculations	No. of nematodes recovered per insect	Species composition of infective juveniles (%)	
		<i>S. glaseri</i>	<i>S. feltiae</i>
0	48,007	6.0	94.0
1	35,529	6.1	93.9
2	20,120	15.6	84.4
3	19,935	9.8	90.2

Nematodes were recovered 21 days after the inoculation with *S. glaseri*.

Each value is the mean of 20 replicates.

Fig. 9. Mortality of male adults of *S. litura* inoculated with *S. feltiae* (circle), *S. bibionis* (triangle), and *S. glaseri* (square) and the percent moths releasing JIII's of steinernematids. Cross indicates the non-inoculated control.

Table 6. Propagation of *Steinernema* spp. on male adults of *S. litura*

Nematodes recovered	Average number of nematodes recovered per		Infective juveniles (%)
	Insect	Body weight of insect (mg)	
<i>S. feltiae</i>	114,210	892	98.8
<i>S. bibionis</i>	15,720	123	97.9
<i>S. glaseri</i>	11,430	89	96.9

Nematodes were recovered 14 days after the inoculation with ca. 500 infective juveniles per moth.

Average fresh body weight of moths was 128 mg.

showed higher infectivity on male moths (Fig. 9). Fifty percent mortality of the moth was obtained one to two days after inoculation for all nematode species examined and no significant difference was observed among their infectivity on the moths.

From the dead moths, the JIII's emerged more quickly than from the larvae. The time intervals between the 50% mortality of the moth and 50% of the moths from which the JIII's emerged was about 4.5 days for *S. glaseri*, 7 days for *S. feltiae*, and 8 days for *S. bibionis*.

More JIII's were recovered from the moths than from the larvae of *S. litura* (Table 6). The number of JIII's recovered per moth 14 days after inoculation was ca. 113,000 for *S. feltiae*, ca. 15,000 for *S. bibionis*, and ca. 11,000 for *S. glaseri*.

DISCUSSION

The inoculation experiments conducted by many investigators under laboratory conditions have shown steinernematids to have quite different host ranges according to the nematode species: *S. feltiae* has the broadest host range covering over 59 families of insects; *S. glaseri*, originally discovered in the larvae of the Japanese beetle (*Popillia japonica*), prefers to infect beetles (Chrysomelidae, Curulionidae, Elateridae and Scarabidae) as well as various moth larvae (Galleriidae, Noctuidae and Pyralidae); *S. bibionis*, originally found in the bibionid fly larvae, is experimentally infectious to

some insects belonging to Bibionidae, Tipulidae and Galleridae (POINAR, 1979).

In contrast to the many investigations on the host range of a selected nematode, rather limited studies have been done on the comparative infectivity of some nematodes on a selected insect host. In laboratory tests conducted by TOBA et al. (1983), *Steinernema feltiae* caused significantly higher mortality in the larvae of sugar beet wireworm, *Limonijs californicus*, than did *S. glaseri*, but both nematode species were equally effective against the larvae of Colorado potato beetle, *Leptinotarsa decemlineata*. In the present experiments, three species of steinernematids were infective on the larvae and male adults of the common cutworm, *Spodoptera litura*, though their infectivity and propagation on this insect were considerably different.

To kill insects, the infective juveniles (JIIIs) have to be attracted to the insects, invade them and, once inside the hosts, release their symbiotic pathogenic bacteria which cause an acute insect death (POINAR and THOMAS, 1967). Of the three steinernematids examined, attraction to the intact *S. litura* larvae was the most efficient for *S. feltiae*, least efficient for *S. bibionis*, and slow but steady for *S. glaseri*. On the other hand, no differences were observed in the numbers of adult nematodes recovered from the exposed tissue on the isolated abdomen, being indicative of similar attraction to the substances in the insect wound. In laboratory tests, *S. feltiae* has been shown to positively respond to various stimuli: carbon dioxide (GAUGLER et al., 1980), thermal gradient (BYERS and POINAR, 1982), and such excretory products of insects as uric acid, xanthine, allantoin, ammonia and arginine in insect feces (SCHMIDT and ALL, 1979). However, these stimuli seemed to be commonly emitted from various insects and to be responsible for the different attractivity of nematodes to a selected insect. If these stimuli were involved in host-finding of the nematodes, the different attraction to a selected insect might depend on qualitative and/or quantitative differences of these stimuli. In fact, SCHMIDT and ALL (1978) showed that dauerlarvae (JIIIs) of *S. feltiae* were attracted to an aqueous surface wash of *G. mellonella* larvae and suggested that the attraction was to a chemical gradient around the larvae. Considering the application of steinernematids for insect pest control, host attraction of the JIIIs should be examined on the intact insects as well as on their excretion.

Infectivity of *S. feltiae* on the intact *S. litura* larvae was significantly higher than *S. bibionis* and *S. glaseri*. Most JIIIs of steinernematids are known to invade the hosts through the midgut wall via mouth and/or anus (POINAR and HIMSWORTH, 1967). The three species of steinernematids used also invaded the ligated *S. litura* larvae in which the nematodes propagated. *S. feltiae* was as infectious on the ligated insects as on the non-ligated ones. The JIIIs of *S. glaseri* and *S. bibionis* also invaded the ligated insects. Considering that these nematodes were not attracted to nor did they invade the actively moving intact larvae, rather high infectivity on these ligated insects may be, at least partly, due to the lack of mobility because of the ligations. When the isolated abdomen was used, most of the nematodes were so effectively attracted and/or arrested by the exposed tissue that only a few invaded the body of the isolated abdomen. These results indicate that all steinernematids used may transcuticularly invade the *S. litura* larvae, though direct evidence of the penetration through the cuticle is still limited (BEDDING and MOLYNEUX, 1982; KONDO and ISHIBASHI, 1983).

Infection was usually followed by propagation within the hosts unless the insect cadavers were contaminated with microorganisms other than symbiotic bacteria. As the nematode growth and propagation in the hosts primarily depend on the available

symbiotic bacteria (POINAR and THOMAS, 1966), the host defense against the bacteria may determine the rate of nematode growth and propagation, especially in the early stage of infection. Assuming the defense of *S. litura* larvae was very low, the nematodes invading earlier may reach adult earlier, irrespective of their number. The facts obtained from the present experiments did not support such assumption; with a decreasing number of invading nematodes, the rates of development to adulthood decreased. On the contrary, all species of nematodes soon propagated in the exposed tissue on the isolated abdomen, suggesting that the host defense no longer functions there but that enough nutrients are still available for the multiplication of the symbionts. These results may indicate that *S. litura* larvae are defensive to some degree against these three species of steinernematids, while these nematodes and their associated bacteria have different abilities to break that host defense.

The final yields of *S. feltiae*, *S. glaseri*, and *S. bibionis* in the intact larvae were higher in this order. This result was similar to the propagation on some media (BEDDING, 1981; KONDO et al., 1985). If the factors suppressing nematode growth are negligible, the yields of nematodes may primarily depend on the quality and quantity of nutrients available for the symbionts and, in turn, for the nematodes. Once the infection is established and no post-infectional microbial contamination occurs, the nematodes may propagate in the cadavers until they consume the tissues of *S. litura* larvae.

The infective juveniles (J_{III}s) of *S. feltiae*, *S. bibionis*, and *S. glaseri* are known to have their symbiotic bacteria (AKHURST, 1980) which can be recognized by the particular color appearance of the infected insects, as was observed in the present experiments on *S. litura* larvae. These bacteria are closely associated with the nematode life cycle (POINAR and THOMAS, 1966); the J_{III}s of nematodes carry their symbiotic bacteria into the insect haemocoel, and feed on the propagated bacteria as well as on the insect tissues disintegrated by the bacteria. Mixed inoculation of different species of nematodes onto a single host was expected to provide further information about the role of bacteria on the growth, development, and propagation of the nematodes. In the present experiments, three species of nematodes inoculated in combinations were able to grow and propagate in *S. litura* larvae, though their propagation was considerably different from that of single species infection. The propagation of *S. feltiae* was hardly disturbed by the other nematode species, mainly because of its efficient attraction and invasion to the *S. litura* larvae. *S. bibionis* was a weaker competitor than the other steinernematids; its propagation was considerably suppressed by mixed inoculation with either *S. feltiae* or *S. glaseri*. This result may stem from the low rates of infection and propagation of *S. bibionis* in the early stage of infection, though the pathogenicity of its symbiotic bacteria seemed to be more virulent than or equal to that of *S. glaseri*, judging from the higher mortality of the insects inoculated with *S. bibionis*. Regardless of the low infectivity of *S. glaseri* on *S. litura* larvae, the nematode showed rather high competitive propagation. Both on the culture media and on the exposed insect tissues, *S. glaseri* grew and propagated faster than did *S. feltiae* and *S. bibionis*.

AKHURST and BEDDING (1978) inoculated one each of the steinernematids in *Galleria* larva to facilitate the species determination in the genus *Steinernema*; inoculation with different species resulted in the failure of progeny production. In the present inoculation experiments, we could not observe J_{III}s characteristic of the intermediate morphology.

Clarification of the role of symbiotic bacteria on the induction of J_{III}s is an interesting problem. The J_{III}s have been reported to be induced by overcrowding (POINAR,

1979), food shortage (SULGOSTOWSKA, 1980) and phase change of symbionts from Type 1 to Type 2 (AKHURST, 1980). In the present mixed inoculation experiments, the fates of symbionts in the insect cadavers were not followed, so that the role of these bacteria in JIIs induction remains to be further investigated.

Infectivity of nematodes on insects is different among developmental stages of a host insect. Generally, the soil-inhabiting insects are more resistant than those living above ground (KAYA and HARA, 1981). This was also the case for the common cutworm, *S. litura*. In the course of development from larvae via pupae to adults, a drastic change occurs in the insect physiology which may be associated with change in its cellular and non-cellular defense against nematode parasites. In fact, the defense against nematodes became weaker in adults than in larvae of *S. litura* which resulted in a rapid and considerable propagation of steinernematids. Practically, the male moths can be used as an alternative host of *S. feltiae*, *S. bibionis*, and *S. glaseri* without the expense of rearing the insects, because the moths are easily caught by pheromone trap from June to late-October in western parts of Japan.

Throughout the present experiments conducted under favorable moisture and temperature conditions for the nematode infection, *S. feltiae* (DD-136) showed the highest infectivity on the larvae and adults of the common cutworm, *Spodoptera litura*. However, we observed that the nematode infectivity was considerably affected by the biotic and abiotic conditions of soil in which *S. litura* last instar larvae hid in the daytime, pupated, and emerged as adult moths. To make the nematode an efficient bioinsecticide of *S. litura*, the survival, mobility, infectivity and propagation of *S. feltiae* should be further investigated under field conditions.

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