Non-Oral Infection of *Steinernema feltiae* (DD-136) to the Common Cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae)¹

Eizo KONDO and Nobuyoshi ISHIBASHI

*Laboratory of Nematology and Entomology, Department of Horticultural Science,
Saga University, Saga 840, Japan*

(Received June 24, 1988)

Histological and scanning electron microscopic observations were made to reveal the infection of *Steinernema feltiae* in the common cutworm, *Spodoptera litura*, via routes other than the alimentary canal. The infective juveniles (JIIIs) invaded the larval, pupal and adult spiracles of *Galleria mellonella*, while they did not invade the *S. litura* spiracles of the last instar larva or pupa aged 3 days after pupation. Many JIIIs congregated in the vesicle under the esophagous of the *S. litura* larva and some JIIIs penetrated the thin cuticular membrane of the vesicle. The nematodes also penetrated wounded tissues on the insect integument. A few JIIIs were assumed to infect the larva through its thin intersegmental membrane, but no direct evidence was obtained. The firm framework of the JIIIs head may be responsible for the mechanical invasion through the cuticular membrane.

INTRODUCTION

The entomogenous nematode, *Steinernema feltiae*, is highly infectious to various species of insects, especially to lepidopterous insects (POINAR, 1979). On the larvae of common cutworm, *Spodoptera litura*, *S. feltiae* is actually more infectious than *S. bibionis* and *S. glaseri* (KONDO and ISHIBASHI, 1986 a).

The parasitism of steinernematid nematodes has been studied in greatest detail on the larvae of the greater wax moth, *Galleria mellonella* (POINAR and HIMSWORTH, 1967); the infective juveniles of steinernematid nematodes invade the insect mainly through the mouth, exsheath in the insect midgut, penetrate the gut wall to the hemocoel, and release their symbiotic bacteria, *Xenorhabdus* spp., causing septicemia (POINAR, 1979). This same pathway has been widely attributed to various nematode-insect associations without careful consideration of the nematode infection process to agriculturally important insect pests which are less susceptible than *G. mellonella* larvae to the nematode-bacterium complex. The mode of nematode invasion to the target seems important for determining the method and quantity of nematodes to be applied, because the nematode infectivity is quite variable depending on the developmental stage and species of insect. The present histopathological and scanning electron microscopic studies were conducted to directly show the possible invasion of *S. feltiae* through routes other than the alimentary canal of *S. litura*.

¹ This work was supported in part by a Grant-in-Aid for Scientific Research (No. 62860006) from the Ministry of Education, Science and Culture, Japan.

MATERIALS AND METHODS

Nematodes. Infective juveniles (J_{III}s) of *Steinernema feltiae* (str. DD-136) were used. They were recovered from chicken offal medium 20–30 days after the commencement of culturing (KONDO et al., 1985).

Insects. The insects used were the common cutworm, *Spodoptera litura*, and the greater wax moth, *Galleria mellonella*. Excepting one study on nematode invasion via spiracles, the last instar larvae of *S. litura* were used.

Invasion via spiracle. The last instar larvae, pupae (3 to 4 days after pupation), and adults of *S. litura* and *G. mellonella* were used. Either side of the insect body was attached to a glass slide using a cyanoacryl binding agent (Alon alpha®). In the case of adult, the scales were brushed off to facilitate adhesion. While being attached, the insects were exposed to 0.1% aqueous solution of sodium hypochlorite for 1 min to clean the body surface, rinsed three times with distilled water, and wiped with filter paper to remove excess water on the body surface. Then, using a fine bamboo spatula, a nematode clump of ca. 10,000 J_{III}s was placed on the side of the insect body near a spiracle. About 6 and 20 hr after the placement, the insects were fixed in buffered 3% glutaraldehyde fixative at 0°C for 48 hr, dehydrated through a graded ethanol series, dried by critical-point drying method, coated with gold with an ion-sputter, and observed under a JSM F-15 scanning electron microscope (SEM) operated at 15 kV. Non-inoculated insects were used to observe spiracle structure. Ten to 15 individuals of each developmental stage were used for both insect species.

Structure of integument and intersegmental membrane. The surface and inner structure of the integument and intersegmental membrane of *S. litura* larva were observed under light microscope (LM) and/or SEM. To observe the surface structure, the larva was pinned down on a paraffin-coated dissecting dish so that the intersegmental membrane was stretched. After being thoroughly rinsed with distilled water, the insect was dissected along the ventral or dorsal line to remove its internal organs. The specimen of integument thus prepared was fixed in 5% glutaraldehyde solution for three days at room temperature and then processed as above for the SEM observation. Observation of the fixed material was also made under a dissecting microscope at a magnification of $\times 60$.

For the SEM observation on the inner structure of the cuticle, the dehydrated specimen was cut with a razor blade just before the critical-point drying. The LM observation was also made on the cuticle which was dehydrated, embedded in SPURR's low viscosity epoxy resin, cut about 10 μ m thick, and stained with a saturated water solution of toluidine blue.

To investigate whether or not the J_{III}s of *S. feltiae* had penetrated the insect cuticle, SEM and LM observations were made on the larva which had been exposed for 24 hr on moist filter paper inoculated with ca. 20,000 J_{III}s. The specimen preparation was the same as described above. A non-inoculated insect served as control.

Invasion via wounded cuticle. The *S. litura* larva was attached to a glass slide, treated with 0.1% sodium hypochlorite solution, rinsed with distilled water, and blotted dry. To observe the healing process by SEM, two punctures were artificially made on the clean insect cuticle with a sterilized needle, and the insects were fixed in cold 5% glutaraldehyde solution 0 to 480 min after the treatment.

To study the nematode invasion via the wound, a nematode clump of ca. 20,000

J_{III}S of *S. feltiae* was placed on the cuticle 5 to 480 min after wounding. The nematodes were allowed to invade for 4 or 20 hr at 25°C and then fixed as described above.

Infection via vesicle. The *S. litura* larva was placed on moist filter paper inoculated with ca. 1,000 or 20,000 J_{III}S of *S. feltiae*. Twelve to 24 hr after exposure, the insects were processed for histological and SEM observations as described in our previous paper (KONDO and ISHIBASHI, 1987). SEM observations were also made both on the larva treated for 1 min in hot water (60°C) to evert out the vesicle, which is located under the posterior portion of the esophagus and the anterior portion of midgut of the insect, and on those treated for 1 hr in 1% aqueous solution of sodium dodecyl sulfate (SDS) to observe the cuticular membrane of the vesicle.

Nematode invasion before exsheathment. To investigate whether or not the inoculated J_{III}S are able to invade the intact last instar larva of *S. litura* before exsheathment, the developmental stage of the nematode in the insect hemocoel was examined after 4 and 6 hr of exposure of the insect on moist filter paper inoculated with ca. 20,000 J_{III}S. Special care was taken when the nematode recovery was made; the body surface of the inoculated insect was rinsed for about 5 min in 0.1% sodium hypochlorite solution to remove any J_{III}S which may have remained there. After being rinsed with distilled water, one of the 1st prolegs was cut; the first drop of hemolymph was discarded and the second one was collected. The developmental stage of the nematodes thus recovered was examined under a light microscope. Fifty of each insect were used for the recovery of nematodes 4 and 6 hr after inoculation.

Stiffness of infective juvenile's head. To investigate the head framework of the J_{III}S, CO₂ was exhausted from the fixed materials within 1 hr after reaching a critical-point temperature of the gas in the course of specimen preparation for SEM observation.

RESULTS

Invasion via spiracle

The structures of spines in the spiracles varied considerably in the developmental stages of *S. litura* and *G. mellonella* (Fig. 1). Generally, the spines of *S. litura* were more dense than those of *G. mellonella*, except for the adults.

The nematode invasion of *G. mellonella* via spiracles was observed throughout all stages: larva, pupa, and adult (Figs. 2D–F). Excepting the adult stage, nematode invasion was not observed through spiracles of *S. litura* (Figs. 2A–C) which were densely armed with spines like a tree branch.

Structure of integument and intersegmental membrane

The structural properties of the notum and intersegmental membrane are shown in Fig. 3. The notum was composed of an epicuticle with regularly arranged elevations, an exocuticle, and an endocuticle with a lamellar structure (Figs. 3B–C). The interspace between the bristle and its socket rim was too narrow to allow the J_{III}S to pass through (Fig. 3A); no detectable changes were found on the base of the bristle socket after nematode inoculation.

The intersegmental membrane consisted of two portions: a smooth nearly semi-spherical portion and that covered with cuticular projections like those on the notum (Figs. 3D–F). The smooth portion was thinner than the other portion. Throughout the experiment, no nematodes were observed to penetrate the membrane; dark pig-

Fig. 1. Structure of spiracle spines of larva, pupa, and adult of *Spodoptera litura* and *Galleria mellonella*. Length of bars in all pictures indicates 30 μ m.

Fig. 2. Invasion of infective juvenile of *Steinernema feltiae* (N) through larval, pupal, and adult spiracles of *S. litura* and *G. mellonella*. Insects were fixed 4 hr after nematode inoculation, excepting **F** which was fixed 20 hr after inoculation. Length of bars in all pictures indicates 100 μ m.

mented spots were often observed on the thin and smooth portion of membranes of the inoculated insects (Fig. 3G), however, no signs were detected on the corresponding portion of the non-inoculated ones. The spots were more frequently observed on the deeply invaginated membrane on the notum than on other parts of the body. Hemolymph components were also sometimes observed on this portion (Fig. 3H).

Fig. 3. Notum and intersegmental membrane of *S. litura* larva. **A**: surface view of notum with a bristle (Br). **B** and **C**: cross sectional view of notum under SEM (**B**) and light microscope (**C**). Ep, epicuticle. Ex, exocuticle. En, endocuticle. **D**: surface view of intersegmental membrane. Sm, smooth portion. Rm, rough portion. **E**: Cross section of smooth portion. **F**: cross section of rough portion. **G**: black pigmented spots on smooth portion of intersegmental membrane of inoculated larva. **H**: hemolymph components on pigmented intersegmental membrane of inoculated larva. Length of bars indicates $10\ \mu\text{m}$ for **A–F** and **H**, and $100\ \mu\text{m}$ for **G**.

Fig. 4. Surface of wound site showing healing process of a wound artificially made by a needle on cuticle of *S. litura* larva. Numerals in each picture indicate time after wounding. Length of bars in all pictures indicates $30\ \mu\text{m}$.

Invasion via wounded cuticle

Figure 4 shows the healing process of the wounded cuticle of an *S. litura* larva. Several types of intact hemocytes were exuded from holes on the cuticle (Fig. 4A).

Fig. 5. Scanning electron (A–G) and light micrographs (H) of *S. feltiae* invasion through wounded cuticle of *S. litura* larva. **A**: invading infective juvenile (N) inoculated 15 min after artificial wounding. **B**: tail portion of nematode in insect tissue 15 min after wounding. Black triangle indicates exsheathed cuticle of infective juvenile. **C**: enlarged view of **B** showing many bacteria on the wound surface indicated by a white circle in Fig. 5B. **D**: infective juveniles and exsheathed cuticle on insect tissue 30 min after wounding. **E**: exsheathed nematode cuticle remaining on insect tissue 90 min after wounding. **F**: nematode penetrating wounded tissue. **G**: nematode penetrating insect tissue 480 min after wounding. Note nematode embedding its anterior body portion in insect tissue. **H**: many nematodes penetrating insect tissue 480 hr after wounding. Wounded tissue turned black due to melanization. Fixation was made 20 hr after nematode inoculation for **B** and **C** and 4 hr for the others. Length of bars indicates 10 μm for **C** and 100 μm for the others.

When wounding was made in the cold fixative (0 hr after wounding), the fixed wound remained whitish because the melanization was inhibited or suppressed. Soon after wounding, the wound became black due to a melanization accompanied by the hemocyte breakdown to form a sheet covering the hole on the cuticle (Figs. 4B–C). Within 60 min after it was inflicted, the wound was healed; it was completely covered with a smooth sheet originating from the breakdown products of hemocytes (Fig. 4D).

SEM observations clearly demonstrated the nematode invasion via the wounded cuticle (Fig. 5). When the nematodes were inoculated 15 min after wounding, little covering with the smooth sheet was seen because many nematodes moved into the hemocoel via the wound and soon killed the insect (Fig. 5A); a dense colony of bacteria often formed around the wound (Figs. 5B–C) when the fixation was made 20 hr after nematode inoculation. The nematodes penetrated the partially healed cuticle when the inoculation was made 30 min later (Fig. 5D). Shed coverings of J_{III}s were frequently observed on the wound (Fig. 5E); some J_{III}s seemed to have penetrated the wounded cuticle after molting. The ensheathed nematode embedded its anterior body portion in the healed cuticle 150 to 480 min after inoculation (Figs. 5F–G). LM observation showed a mass of nematodes on the melanized tissue on the cuticle 480 min after inoculation (Fig. 5H).

Fig. 6. Aggregation of *S. feltiae* infective juveniles (N) in ventral eversible vesicle (Ev) of *S. litura* larva. **A**: transverse section of vesicle in non-inoculated larva. Sg, subesophageal ganglion. Tg, thoracic ganglion. Cm, cuticular membrane of vesicle. Vc, vesicular cell. **B**: cuticular membrane of vesicle remaining intact after SDS-treatment. **C**: side view of everted vesicle after hot water treatment at 60 °C for 1 min. H, insect head. T1, 1st thoracic leg. **D**: posterior portion of nematodes in orifice of vesicle (Ov). **E**: ventral view of a larva with nematodes in partly everted vesicle. **F**: posterior portion of nematodes in nearly completely everted vesicle. **G** and **H**: transverse section of a larva showing nematode aggregation (N) in vesicle. Note many nematodes in vesicle near orifice and a few in deeper portion of vesicle. B, brain. Es, esophagus. M, midgut. **I**: transverse section of vesicle containing nematodes. Vn, ventral nerve cord. **J**: nematode aggregation in vesicle. Length of bars indicates 100 μ m for **A**, **D**, **F**, **G**, **I** and **J**, and 500 μ m for **B**, **C**, **E** and **H**.

Infection via vesicle

The vesicle was located between the alimentary canal and the sternum. It opened under the subesophageal ganglion and extended to an anterior portion of the midgut.

Fig. 7. Nematode invasion through everseable vesicle of *S. litura* larva. **A**: necrotic reaction (NV) in vesicle 24 hr after nematode inculation. Nematodes are seen in vesicle and in insect hemocoel. **B**: granulated vesicle (12 hr). Nematodes were already in vesicle and in hemocoel. **C**: black pigmented spots (Bs) on vesicle of inoculated insect (12 hr). **D**: nematode penetration through vesicle wall (12 hr). **E**: whole vesicle with a large hole (18 hr). **F** and **G**: enlarged view of hole in vesicle (18 hr). Note bacteria (B) on trachea near hole in Fig. 7G. Length of bars indicates 100 μ m for **A**, **B**, **E** and **F** and 30 μ m for **C**, **D** and **G**.

Fig. 8. Infective (**A**, **C**) and parasitic (**B**, **D**) juveniles of *S. feltiae* recovered from hemolymph of intact *S. litura* larva 4 (**A**, **B**) and 6 hr (**C**, **D**) after inoculation with ca. 20,000 infective juveniles. Cu, cuticle of second stage juvenile covering body of infective juvenile. Lo, lumen of esophagus. Length of bars indicates 20 μ m for all pictures.

Its length was about 1.3 mm in the 5th instar larva and about 2.1 mm in the last instar on average. The vesicle was composed of a single layer of cells and the cuticular membrane (Fig. 6A) which remained intact after the treatment with SDS (Fig. 6B). Hot water treatment caused the eversion of the vesicle in the 5th instar (Fig. 6C) but not in the last instar larvae of *S. litura*.

The J_{III} s entered the vesicle (Figs. 6D–F) and densely aggregated there (Figs. 6G–J); some of them moving into a deeper portion (Fig. 6G).

Fig. 9. Head structure of *S. feltiae* infective juvenile which was prepared by quickly exhausting CO_2 gas after reaching its critical point temperature. **A**: anterior portion of nematode body constricted at about $12\ \mu\text{m}$ behind head tip. An, annulation (An). **B**: whole body of treated nematode. Note body constriction at similar portion of all nematodes.

Some vesicle cells of the inoculated insects showed necrosis (Fig. 7A) and granulation (Fig. 7B) 12 and 24 hr after nematode inoculation. These changes were not observed in the vesicle of a non-inoculated insect. On the cuticular membrane of the vesicle, nematode inoculation caused pigmented spots (Fig. 7C), the diameter of which was generally smaller than the body diameter of the J_{III} s. When the inoculation was made using as many as 20,000 J_{III} s per insect, some nematodes penetrated the vesicle wall (Fig. 7D) and made rather large holes through which they were observed inside the vesicle (Figs. 7E–G). The holes were limited to that part of the vesicle having no cuticular projections. The bacteria were often observed around these holes 18 hr or more after inoculation (Fig. 7G).

Nematode invasion before exsheathment

In addition to the parasitic juveniles, the ensheathed J_{III} s were recovered from the *S. litura* hemolymph obtained 4 and 6 hr after inoculation (Fig. 8). Among all nematodes recovered, the infective juveniles accounted for 48% (31 out of 65) and 43% (102 out of 236) in the insects 4 and 6 hr after inoculation, respectively.

Stiffness of infective juvenile's head

The head of ensheathed J_{III} s was hemi-spherical and had no prominent projections. When the J_{III} s were exposed to physical stress during the specimen preparation as described above, their anterior body portion remained nearly intact (Fig. 9), however, the rest of the body shrank considerably and/or twisted. The constriction of the nematode body occurred at almost the same location in all specimens examined, about $12\ \mu\text{m}$ behind the head tip.

DISCUSSION

Infective juveniles of steinernematid nematodes invade the insects mainly through the alimentary canal (POINAR and HIMSWORTH, 1967; POINAR, 1979; KONDO and ISHIBASHI, 1987); however, POINAR (1979) also reported other routes for the invasion. It is critically important to learn the mode of invasion to determine the method and quantity of nematode application.

TRIGGIANI and POINAR (1976) showed that *S. feltiae* infective juveniles broke through the tracheae and entered the hemocoel of an adult California oakworm, *Pryganidia californica*, in which the mouth and anus were sealed with wax. BEDDING and MOLYNEUX (1982) showed that *Heterorhabditis bacteriophora* and *H. heliothidis* invaded *G. mellonella* larva via spiracles. The present SEM observation clearly demonstrated the *S. feltiae* invasion via spiracles of adult *S. litura* and *G. mellonella*. Considering that the pupae were killed by this nematode invasion through spiracles, the nematodes in the tracheae might have penetrated the tracheal wall into the hemocoel thus causing septicemia in the insect.

The entry of steinernematids via spiracle is considered to be a main infection route in insect pupae which have neither mouth nor anus (POINAR, 1979; HARA and KAYA, 1983; KAYA and GRIEVE, 1982). Nematode infectivity of pupae considerably differs depending on insect species; the soil- or litter-pupating insects are generally less susceptible than those which pupate above ground (KAYA and HARA, 1981). For instance, they showed that *S. feltiae* was less infectious to pupae of *Pseudaletia unipuncta* and *Spodoptera exigua* than to those of *G. mellonella* of which the cuticle was less sclerotized than that of other insect species examined. Our observation also showed that the sclerotization of *S. litura* pupa greatly affected the infectivity of *S. feltiae*; *S. feltiae* infected pupae within two days after pupation but not those aged 3 days or older (KONDO and ISHIBASHI, 1984, 1986 b). Structure of the spiracle also seems related to the susceptibility of insect larvae to the nematode; *S. feltiae* invaded the spiracle of *G. mellonella* larva but not of *S. litura* larva in which the spiracle was densely armed with spines which may prevent the nematode from entering the tracheal system. These results indicate that the structure and hardness of the trachea may be responsible for insect susceptibility to nematode infection.

A few investigators consider that a wound on the cuticle is a means of nematode entry into the insect hemocoel (KAYA and HARA, 1980). The present SEM observation demonstrated that infective juveniles of *S. feltiae* had the ability to penetrate wounded tissue on the *S. litura* integument. Also, *S. feltiae* infective juveniles densely accumulated in the vesicle of *S. litura* larva and some ensheathed nematodes penetrated the cuticular membrane of the vesicle, the function of which is not yet fully understood.

FINNEY and WALKER (1977) suggested that in bark beetle larvae, the soft cuticle of the intersegmental folds facilitated the nematode entry to host insects. Considering the following facts, *S. feltiae* seems to have a potential for invading *S. litura* larvae via the deeply invaginated cuticular intersegmental membrane: the infective juveniles propagating in the insect cadavers frequently came out through the intersegmental membranes of the host; a few nematodes infected ligated larvae; and black pigmented spots were often observed on the smooth portion of the thin intersegmental membrane of inoculated larvae.

The tylenchid and mermithid entomogenous nematodes invade the hosts trans-

cuticularly using their stylet or mouth teeth. In the case of *H. bacteriophora* and *H. heliothidis*, the infective juveniles often penetrated the cuticle of *Otiorhynchus sulcatus* and *Lucilia cuprina* larvae and of *Mastotermes darwiniensis* nymphs with the aid of an anterior tooth which was exposed after the loss of the cuticle in the preceding stage (BEDDING and MOLYNEUX, 1982). The anterior tooth may enable those nematodes to enter the body of some insects that are less vulnerable to *Steinernema* species which do not have the tooth. In the present study, non-oral infection of *S. feltiae* in the *S. litura* seems mechanical; the rather firm head framework, the minute diameter of the infective juvenile's head, and the characteristically high internal body pressure seems to be responsible for this mechanical infection. Considering that the ensheathed juveniles of such animal parasitic nematodes as *Necator americanus*, *Strongyloides* sp., and *Ancylostoma caninum* (all of these having no stylet) probably dissolve their way into the host enzymatically (ROGERS, 1962), the possible penetration of the ensheathed juveniles of steinernematid nematodes with the aid of enzymatic degradation of the insect cuticle remains to be further investigated.

REFERENCES

- BEDDING, R. A. and A. S. MOLYNEUX (1982) Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* **28**: 354–359.
- FINNEY, J. R. and C. WALKER (1977) The DD-136 strain of *Neoaplectana* sp. as a potential biological control agent for the European elm bark beetle, *Scolytus scolytus*. *J. Invertebr. Pathol.* **29**: 7–9.
- HARA, A. H. and H. K. KAYA (1983) Susceptibility of *Spodoptera exigua* pupae from different pupation sites to the nematode *Neoaplectana carpocapsae*. *J. Invertebr. Pathol.* **42**: 418–420.
- KAYA, H. K. and A. H. HARA (1980) Differential susceptibility of lepidopterous pupae to infection by the nematode *Neoaplectana carpocapsae*. *J. Invertebr. Pathol.* **36**: 389–393.
- KAYA, H. K. and A. H. HARA (1981) Susceptibility of various species of lepidopterous pupae to the entomogenous nematode *Neoaplectana carpocapsae*. *J. Nematol.* **13**: 291–294.
- KAYA, H. K. and B. J. GRIEVE (1982) The nematode *Neoaplectana carpocapsae* and the beet armyworm *Spodoptera exigua*: Infectivity of prepupae and pupae in soil and of adults during emergence from soil. *J. Invertebr. Pathol.* **39**: 192–197.
- KONDO, E. and N. ISHIBASHI (1984) Infectivity and multiplication of *Steinernema feltiae* (str. Mexican) on the common cutworm, *Spodoptera litura*. *Jap. J. appl. Ent. Zool.* **28**: 229–236 (in Japanese with an English summary).
- KONDO, E. and N. ISHIBASHI (1986 a) Infectivity and propagation of entomogenous nematodes, *Steinernema* spp., on the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Appl. Ent. Zool.* **21**: 95–108.
- KONDO, E. and N. ISHIBASHI (1986 b) Infection efficiency of *Steinernema feltiae* (DD-136) to the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the soil. *Appl. Ent. Zool.* **21**: 561–571.
- KONDO, E. and N. ISHIBASHI (1987) Histological and SEM observations on the invasion and development of entomogenous nematode, *Steinernema feltiae* (str. DD-136), in *Spodoptera litura* (Lepidoptera: Noctuidae) larvae. *Appl. Ent. Zool.* **23**: 88–96.
- KONDO, E., S. HONDA and N. ISHIBASHI (1985) Growth and propagation of entomogenous nematodes, *Steinernema* spp., on several kinds of media prepared by chicken liver and intestine. *Jpn. J. Nematol.* **15**: 1–10 (in Japanese with an English summary).
- POINAR, G. O., Jr. (1979) *Nematodes for Biological Control of Insects*. CRC Press, Boca Raton, Florida, 277 pp.
- POINAR, G. O., Jr. and P. T. HIMSWORTH (1967) *Neoaplectana* parasitism of *Galleria mellonella*. *J. Invertebr. Pathol.* **9**: 241–246.
- ROGERS, W. P. (1962) *The Nature of Parasitism*. Academic Press, N.Y. and London, 287 pp.
- TRIGGIANI, O. and G. O. POINAR, Jr. (1976) Infection of adult Lepidoptera by *Neoaplectana carpocapsae* (Nematoda). *J. Invertebr. Pathol.* **27**: 413–414.