BIOLOGY OF *SEINURA PARATENUICAUDATA* GERAERT

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Summary. The life cycle and feeding of *Seinura paratenuicaudata* was studied on 1% water agar *in vitro* using *Aphelenchus avenae* as prey. *Seinura paratenuicaudata* reproduces uniparentally. Eggs are laid only after it has fed upon its prey. The first moult occurs inside the egg and the second stage juvenile hatches out. Oesophageal gland secretions and mechanical pressure, exerted by both ends but not the stylet of the developing J_2, are involved in rupturing the eggshell. The secretion of dorsal oesophageal glands is also involved in exsheathment during further moulting. The life cycle is completed in 4-5 days. For feeding, the predator inserts its stylet into the prey body and this is followed by injection of the contents of the dorsal oesophageal glands, which paralyse the prey. The body contents of *A. avenae* are subsequently sucked out by the predator. Studies on host preference of *S. paratenuicaudata* revealed that it feeds and reproduces on *Aphelenchoides bicaudatus, A. composticola, Aphelenchus avenae, A. radicicolus, Ditylenchus myceliophagus* and second stage juveniles of *Heterodera cajani, Meloidogyne incognita* and *M. javanica,* but not on larval stages or adults of *Helicotylenchus dihystera, Hoplolaimus indicus, Longidorus pisi*, second stage juveniles of *Heterodera avenae, H. sorghi* or *H. zeae,* or males of *M. incognita* or *M. javanica*. It feeds and reproduces on J_2 of *Anguina tritici* and J_3 of *Subanguina chrysopogoni* but the majority of the eggs produced are unfertile. Temperature also influences the feeding and reproduction of this predator. The optimum temperature for reproduction is between 20 and 35 ^{0}C. The nematode fails to feed or multiply at 11 and 15 ^{0}C but remains active.

Seinura paratenuicaudata Geraert was frequently encountered, along with myceliophagous nematodes, in samples collected from mushroom houses in Haryana State of India. The populations of this nematode were high, especially during the months of February-March, when the mushroom season was towards its end. In order to explore the possibility of using this nematode for the biological control of nematode pests of mushroom, its biology and predator-prey relationship were studied and are discussed hereunder.

MATERIALS AND METHODS

Populations of *Seinura paratenuicaudata* and *Aphelenchus avenae* Bastian were extracted from compost samples collected from mushroom beds. The identification of *S. paratenuicaudata* was based on measurements and morphological characters, which were similar to those of the original description (Geraert, 1962). *Aphelenchus avenae* was cultured on mycelia of white button mushroom, *Agaricus bisporus* (Lange) Singer, grown on Potato Dextrose Agar in 150 ml conical flasks. For culture of *S. paratenuicaudata* (the predator), around 1,000 *A. avenae* were transferred to 5-cm Petri plates containing 1% water agar and 35-40 females of the predator were subsequently introduced. The plates were kept at room temperature (28-31 ^{0}C) for observation. Embryogenesis was studied by the hanging drop method. For study of post-embryonic development, newly hatched juveniles were transferred to 5-cm Petri plates containing *A. avenae* on 1% agar and their further development was studied by making observations on the Petri plates at regular intervals and/or making water mounts for study under a compound microscope.

To study the host range of *S. paratenuicaudata,* ca 200 adult females of *Aphelenchoides bicaudatus* (Imamura) Filipjev *et* Sch. Stek., *A. composticola* Franklin, *Aphelenchus avenae, A. radicicolus* (Cobb) Steiner, *Ditylenchus myceliophagus* Goodey, *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus indicus* Sher, *Longidorus pisi* Edward, Misra *et* Singh, and *Mylonchulus* species, second stage juveniles of *Anguina tritici* (Steinbuch) Filipjev, *Heterodera avenae* Wollenweber, *H. cajani* Koshy, *H. sorghi* Jain, Sethi, Swarup *et* Srivastava, *H. zeae* Koshy, Swarup *et* Sethi, *Meloidogyne graminicola* Golden *et* Birchfield, *M. incognita* (Kofoid *et* White) Chitw., *M. javanica* (Treub) Chitw. and third stage juveniles of *Subanguina chrysopogoni* Bajaj, Dabur, Paruthi *et* Bhatti, and males of *M. incognita* and *M. javanica* were inoculated, along with five females of *S. paratenuicaudata,* separately in Petri plates containing 1% water agar. These plates were kept at room temperature and observations were recorded daily on feeding, egg laying and development of *S. paratenuicaudata.*

Feeding of *S. paratenuicaudata* was studied by placing a small drop of water on a glass slide and adding an equal volume of melted 2% water agar to it. A single female of *S. paratenuicaudata* and seven females of *A. avenae* were added to the drop, which was then covered

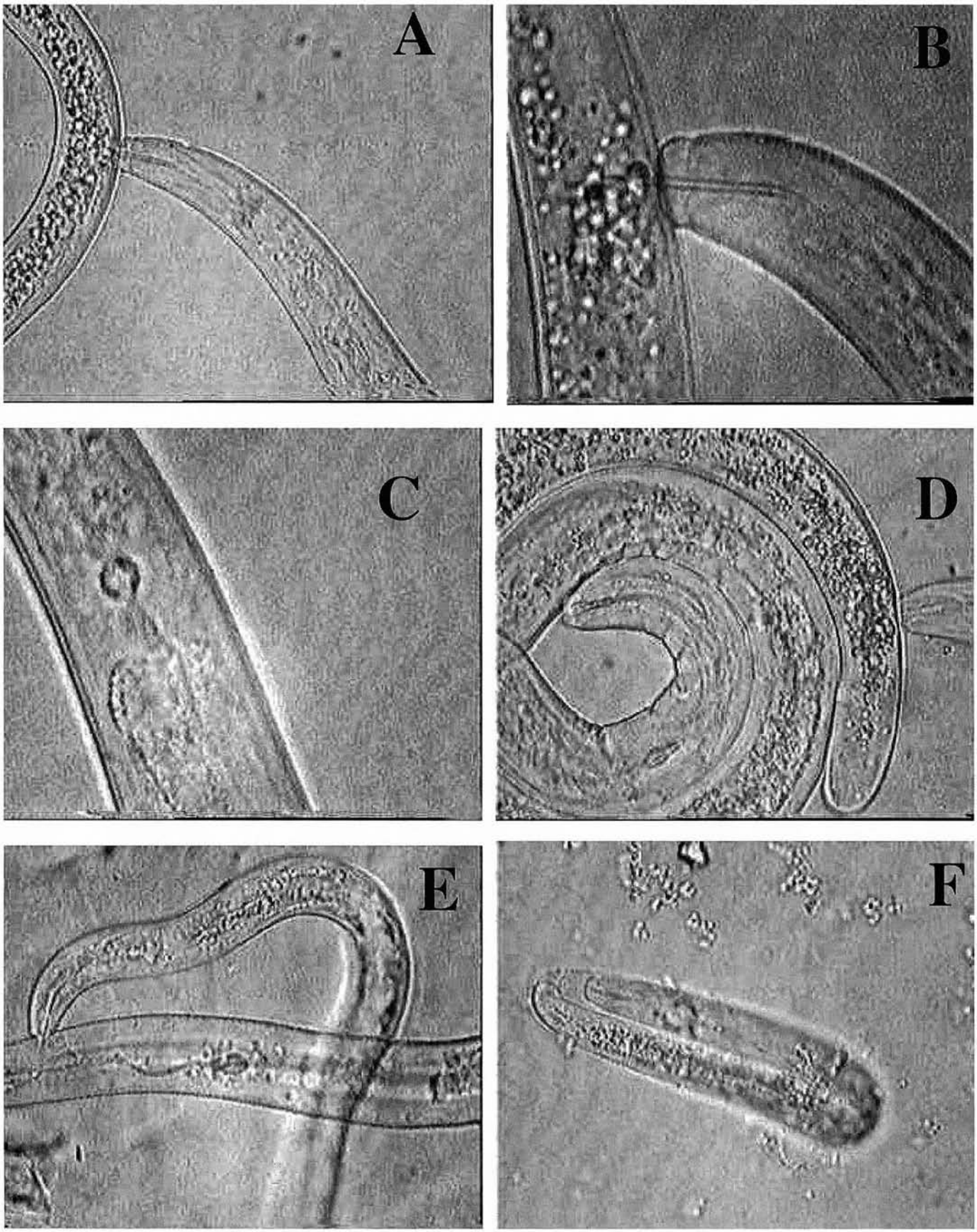

Fig. 1. A-E, Feeding of *Seinura paratenuicaudata* on *Aphelenchus avenae*: A, female feeding near intestinal region; B, magnified view of anterior region showing stylet inside prey body; C, accumulation of dorsal oesophageal gland secretions in the ampulla; D, female feeding on paralysed coiled female; E, J_2 feeding in the oesophageal region. F, embryonic development of *S. paratenuicaudata* J_2.

with a 22-mm square cover glass. The slides were kept in the dark in Petri plates lined with moist filter paper when not under observation. The various stages of the feeding process were observed under a compound microscope and also recorded on videocassette through a CCTV attached to the microscope for critical analysis.

To study the effect of temperature on feeding and reproduction of *S. paratenuicaudata,* 200 *A. avenae* and five females of the predator were transferred to Petri plates containing 1% water agar. The plates were kept at 10, 15, 20, 25, 30 or 35 ^{0}C in Biological Oxygen Demand incubators. There were three replications for each temperature. Observations on feeding and multiplication of *S. paratenuicaudata* were recorded after five and ten days of incubation.

RESULTS AND DISCUSSION

Seinura paratenuicaudata laid eggs only after feeding for 4-5 h. The elongate-conoid eggs with blunt ends were laid singly at a single-cell stage and measured an average of 76 x 23 µm. They reached gastrula and tadpole stages after 8 and 9 h, respectively, from egg laying. The anterior end of the developing embryo started movement inside the eggshell 14 h after initiation of cleavage. The fully formed first stage juvenile underwent the first moult 22 h after egg laying (Fig. 1F). The second stage juvenile (J_2) started moving within the eggshell, with intervening periods of rest, after 23-24 h. It frequently pressed its head against the narrow end of the eggshell. Subsequently, the median oesophageal bulb started pulsating at regular intervals, each pulsation lasting for 15-20 seconds. Within 2-3 h of initiation of movement by the J_2, the eggshell became very elastic and changed its shape as the enclosed juvenile moved. The pulsation of the median bulb later stopped but the movement of the J_2 became brisk, but with rests in between periods of activity. Some 27 h after egg laying, the J_2 broke the eggshell near the narrow end by exerting pressure through the head and tail regions and wriggled out of the eggshell. At no stage was the involvement of the stylet in breaking the eggshell noticed.

The second stage juvenile started feeding soon after hatching. After feeding for 6-7 h it acquired an open 'C' shape and remained motionless for about a day. A new stylet, cuticle lining of the oesophageal lumen, valvular apparatus of the median oesophageal bulb, etc. were formed. Within 2 h of formation of the new stylet and median bulb, the J_3 became very active inside the old cuticle. During the process of ecdysis, pulsation of median bulb occurred at short intervals, each pulsation lasting for 4-12 seconds and accompanied by secretion from the dorsal oesophageal gland. Intermittent pulsation continued for about an hour. Finally, the J_3 moved its anterior region rapidly inside the old cuticle and emerged. The J_3 underwent two more moults to become adult. The adult female was formed within four days of egg laying when food was readily available. However, if no food was provided, the J_2 remained active for several days and failed to develop further. If food was available, the whole life cycle was completed in 4-5 days.

Seinura paratenuicaudata moved randomly before recognising its prey. It showed no ability to follow an escaping prey. After finding a suitable prey, it oriented its head toward the prey body and tightly pressed the lip region against the body wall of the prey (Fig. 1, A-E). The nematode could feed on any part of the prey body but preferred the anterior oesophageal region. After pressing its lip region onto the prey, *S. paratenuicaudata* inserted its stylet into the prey body. Within a few seconds of stylet penetration, the prey was paralysed, probably due to injection of secretions from the dorsal oesophageal glands. The extent of paralysis depended upon the feeding site. When the nematode fed near the oesophageal region, almost the whole prey body became paralysed. However, the stylet and oesophagus of the prey usually continued to twitch if *S. paratenuicaudata* fed posterior to this region. Secretions were seen flowing from the dorsal oesophageal gland through the lumen of the oesophagus and stylet of the predator and into the prey body. During feeding, the median bulb pulsated intermittently at a rate of four pulsations per second and each bout of pulsation continued for 7-17 seconds. During pulsation of the median bulb the contents of the dorsal oesophageal gland that were stored in the ampulla just above the valvular apparatus were injected into the prey body. The ingestion of food took place by drawing out the contents of the prey in globules, which assumed an elongated form during passage through the stylet but rounded up again as they escaped from the stylet tip. Thus, during feeding, the processes of salivation and ingestion of food took place alternately. Single J_2 could feed on a prey for 1.5 to 2 h continuously and kill one to three nematodes before moulting. Adult females, however, fed on and killed 4-6 *A. avenae* in a day.

The feeding behaviour and fecundity of *S. paratenuicaudata* varied depending upon its prey species (Table I). It fed and reproduced continuously on females of *Aphelenchoides bicaudatus, A. composticola, Aphelenchus avenae, A. radicicolus* and *Ditylenchus myceliophagus,* J_2 of *Meloidogyne incognita, Heterodera cajani* and *Anguina tritici*, and J_3 of *Subanguina chrysopogoni.* It fed but failed to reproduce on *Mylonchulus* species. Females of *S. paratenuicaudata* feeding upon anguinids (*A. tritici* and *S. chrysopogoni)* became obese and laid numerous eggs. However, about 90% of these eggs were dark black in colour compared to normal light brown eggs and failed to hatch. Variation in the prey preference of *S. paratenuicaudata* was also observed within a prey genus and also within different life cycle stages of a species. It fed and reproduced on *H. cajani* but not on *H. avenae, H. sorghi* or *H. zeae.* Likewise, among the root-knot nematode species tested, it did not feed or reproduce on J_2 of *M. graminicola*, fed but laid few eggs on J_2 of *M. javanica,* and fed and reproduced

Table I. Feeding and reproduction of *Seinura paratenuicaudata* on different nematodes.

Prey		No. of eggs laid/5 ♀♀	Remarks
Species	Stage(s)		
Anguina tritici	J_2	38	95% of eggs turned black, did not hatch
Aphelenchoides bicaudatus	♀, ♂, J_2-J_4	31	
A. composticola	♀, ♂, J_2-J_4	34	
Aphelenchus avenae	♀, ♂, J_2-J_4	40	
A. radicicolus	♀, ♂, J_2-J_4	35	
Ditylenchus myceliophagus	♀, ♂, J_2-J_4	32	
Helicotylenchus dihystera	♀	0	No feeding
Heterodera avenae	J_2	0	No feeding
H. cajani	J_2	22	
H. sorghi	J_2	0	No feeding
H. zeae	J_2	0	No feeding
Hoplolaimus indicus	♀, ♂, J_2-J_4	0	No feeding
Longidorus pisi	♀, J_1-J_4	0	No feeling
Meloidogyne graminicola	J_2	0	No feeling
M. incognita	J_2	24	
M. incognita	♀	0	No feeling
M. javanica	J_2	8	
M. javanica	♀	0	No feeding
Mylonchulus sp.	♀	0	Feeding but no reproduction
Subanguina chrysopogoni	J_2	28	95% of eggs turned black, did not hatch

normally on J_2 of *M. incognita*. Males of *M. incognita* or *M. javanica* were, however, not attacked by this predatory nematode. *Hoplolaimus indicus, Helicotylenchus dihystera* and *L. pisi* were not fed upon by this nematode.

Feeding and multiplication of *S. paratenuicaudata* on *A. avenae* also varied at different temperatures (Table II). The nematodes fed and reproduced at 20, 25, 30 and 35 °C, with maximum reproduction taking place at 30 °C. They did not feed or multiply at 10 and 15 °C, though they remained active. When transferred to 28-31 °C after ten days at 10 or 15 °C, they started to feed and reproduce.

Like other species of *Seinura* e.g., *S. tenuicaudata, S. steineri, S. oxura, S. celeris* and *S. oliveirae*, *S. paratenuicaudata* has a short life cycle of 4-5 days (including 27 h of embryonic development), which is a good attribute for a biocontrol agent. The pattern of embryonic and postembryonic development was similar to other species of the genus, except that the stylet was not involved in rupturing the eggshell. Like *S. tenuicaudata*, the J_2 hatched out of the eggshell, whereas two moults occur inside the eggshell and the J_3 emerges in *S. oxura* (Hechler and Taylor, 1966). Reproduction in *S. paratenuicaudata* is uniparental, closely resembling *S. steineri*. The female is proterandric hermaphrodite as sperms are invariably present in the spermatheca of females when no males are present in the culture plates. Also, young females still enclosed within the cuticle of the J_4 had sperm in their spermatheca. A very few males were occasionally produced in the culture tubes of this species. They are perhaps produced under certain, as yet unknown, environmental conditions, as has been reported for several parasitic nematodes (Poinar and Hansen, 1983). Feeding is essential for reproduction and multiplication of *S. paratenuicaudata*. When the food was available in abundance, egg laying began 4 h after feeding at the rate of 2 eggs/h and continued for several days. However, no reproduction occurred in the absence of prey. Normally, one *S. paratenuicaudata* fed on a single prey but, occasionally, up to six predators could feed simultaneously on a prey. This was especially true when food was scarce, such as near the end of the experiment on host range when the prey population had come down considerably. *Seinura paratenuicaudata* did not feed on eggs of prey nematodes or cannibalistically, as has been reported in *S. demani* and *S. tenuicaudata* (Hechler, 1963; Wood, 1974; Small and Grootaert, 1983).

Seinura paratenuicaudata fed and reproduced well on all the myceliophagous nematode species tested. However, these processes were variable when plant-parasitic nematodes were offered as food. This ranged from no feeding (e.g., *H. indicus, H. dihystera* and *L. pisi*), though feeding but no reproduction (e.g., *Mylonchulus*

Table II. Effect of temperature on feeding and multiplication of *S. paratenuicaudata* preying on *Aphelenchus avenae*, recorded either 5 or 10 days after inoculation (DAI).

Temperature	Fecundity of *5 S. paratenuicaudata* on 5 *Aphelenchus avenae*				Remarks
	5 DAI		10 DAI		
	Eggs	J_2-J_4, ♀	Eggs	J_2-J_4, ♀	
11 °C	0	0	0	0	Inoculated predators did not feed. When transferred to 25 °C after 10 days, they fed and reproduced.
15 °C	0	0	0	0	
20 °C	8	5	5	11	All food consumed. No further reproduction.
25 °C	30	4	9	30	
30 °C	0	45	-	-	
35 °C	0	32	-	-	

sp.) and feeding but low fecundity (e.g., J_2 of *M. javanica*), to feeding with enhanced fecundity (e.g., J_2 of *A. tritici*). Non-feeding on some species may be due to non-perception of potential attractants secreted by particular nematode species or to the structure of the cuticle. Differences in the fecundity of *S. paratenuicaudata* feeding upon different prey species reflect the role of food quality on reproduction. Increased fecundity of the predator feeding on anguinids was, however, associated with production of black, infertile eggs.

The optimum temperature for feeding and multiplication of *S. paratenuicaudata* is around 30 °C, which explains the maximum recovery of this nematode from mushroom beds at the end of February and during March when the temperature is around 25-30 °C under Haryana conditions. At 11-15 °C, this nematode did not feed or multiply but remained active for more than ten days, becoming inactive (quiescent?) thereafter. At the end of three months, they were still live but inactive and could recover and reproduce when transferred to fresh plates having *A. avenae* at 25-30 °C. This peculiar capability to survive without feeding at low temperature could be exploited for storage of this nematode at low temperature in the off-season ready for subsequent use in the next season of mushroom cultivation.

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LITERATURE CITED

Geraert E., 1962. De nematoden fauna in en om de wortels van *Musa paradiaca normalis*. In Bijdragen tot de kennis der plantenparasitaire en der vrijlevende nematoden van Kongo. Inst. Dierkunde, Rijksuniversiteit, Gent, 7-73.

Hechler H.C., 1963. Description, developmental biology and feeding habits of *Seinura tenuicaudata* (De Man) J.B. Goodey, 1960 (Nematoda: Aphelenchoididae), a nematode predator. *Proceedings of the Helminthological Society of Washington, 30*: 182-195.

Hechler H.C. and Taylor D.P., 1966. The life histories of *Seinura celeris, S. oliveirae, S. oxura* and *S. steineri* (Nematoda: Aphelenchoididae). *Proceedings of the Helminthological Society of Washington, 33*: 71-83.

Poinar G.O. and Hansen E., 1983. Sex and reproductive modifications in nematodes. *Helminthological Abstracts, 52*: 145-163.

Small R.W. and Grootaert P., 1983. Observations on the predation abilities of some soil dwelling predatory nematodes. *Nematologica, 29*: 109-118.

Wood F.H., 1974. Biology of *Seinura demani* (Nematoda: Aphelenchoididae). *Nematologica, 20*: 347-353.