

Parasitism of *Heterotylenchus autumnalis* Nickle (Nematoda: Sphaerulariidae) to the Face Fly, *Musca autumnalis* De Geer (Diptera: Muscidae)

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Abstract: New information is reported on the parasitism of *Heterotylenchus autumnalis* upon its principal known host, *Musca autumnalis*. Black to brown spots are produced on the cuticle of all infected host larvae where the nematode penetrated. The principal damage to the host is castration of the female. In laboratory tests nematode larvae were not infective and did not leave the hosts before the female fly was 11 days old. Nematode larvae removed from infected male flies infected other hosts, but it is believed that in nature these larvae are unable to leave the host. *Key Words:* Parasitism, *Heterotylenchus autumnalis*, Face Fly, *Musca autumnalis*.

Stoffolano and Nickle (12) found *Heterotylenchus autumnalis* Nickle infecting the face fly in New York State. The nematode is a promising biocontrol agent for this livestock pest since it appears to be host specific, has a life cycle synchronized with that of the fly (11), and is effectively distributed by infected female hosts.

Heterotylenchus is a heteromorphic genus. At present there are six known species (9) and, tentatively, a new one from Australia (6). The life cycle for all species is basically the same, involving alternation of gamogenetic and parthenogenetic generations.

The purpose of this study was to extend knowledge of the life history of *H. autumnalis* in its principal host, *M. autumnalis*.

MATERIALS AND METHODS

REARING: Infected and non-infected face fly cultures were separately maintained in screened cages. Adult flies were exposed to a 2-liter plastic container of fresh cow manure for oviposition. After 24 hr the con-

tainer was removed, covered with a fine screen and incubated 24 hr to stimulate hatch. The manure and fly larvae were then transferred to a shallow enamel pan with a space 5 cm wide at one end filled with sand to allow for pupation. Fly pupae were sifted from the sand and used for experimentation and/or to maintain the adult population. Adults were fed powdered milk, granulated sugar and water. They were maintained at 26.7 C, 50% RH and in continuous light.

INFECTING FLY LARVAE: Twelve days after emergence, infected female hosts were dissected in Ringer's to expose the ovaries containing hundreds of nematodes. Male hosts were not used since they contain fewer nematodes. The infected female was transferred to a petri dish containing fresh cow manure and the nematodes were distributed to various parts of the manure. The uncovered dish was placed in a covered 2-liter, plastic container containing a small amount of water and incubated in a temperature chamber at 26.7 C under constant light. After allowing 24 hr for the nematodes to mate, several larvae of the desired host age were introduced and confined to the dish by a fine net cover. Larvae were removed after 3-5 hr, rinsed in Ringer's to remove clinging nematodes and placed in pans of fresh manure. Later, several larvae were removed,

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dissected and examined for nematodes. Other comparably exposed hosts were allowed to pupate and emerge as adults. These adults were periodically examined for signs of nematode development.

EXAMINATION OF MAGGOTS AND ADULTS FOR INFECTION BY NEMATODES: Under a microscope nematodes can be seen inside the nearly transparent, newly-hatched host larvae while in older less transparent larvae subcutaneous migration can be observed via reflected incident light. In CO₂-anesthetized adult host males the nematodes were observed by transmitted light in the more transparent orange portion of the abdomen. In anesthetized adult females infection was determined by gently drawing out the ovipositor. Using transmitted light nematode larvae can be seen actively moving inside and crawling out of the ovipositor.

INVESTIGATIONS AND RESULTS

LARVAL STAGES THE NEMATODE CAN PENETRATE: Nematodes from the ovaries of two infected flies were placed on the surface of fresh cow manure (2.5 cm deep) in each finger bowl (12.5 cm diameter). These were placed in covered 2-liter, plastic containers. Four age groups (1-2, 2-3, 3-4, and 4-5 days old) each consisting of 20 face fly larvae were placed in each of four nematode-infected finger bowls for 24 hr and then dissected. In order to ensure exposure of 20 larvae one day old or younger, a fifth bowl was set up with 30-40 fly eggs. This entire experiment was repeated one month later. Results (Table 1) indicate the nematode is capable of penetrating all stages of fly larvae.

Nematode larvae were often found crawling on face fly eggs; however, nematodes were never found to have penetrated the egg.

SYNCHRONY OF THE NEMATODE LIFE CYCLE WITH DEVELOPMENT OF THE ADULT FLY: Newly emerged fly larvae were ex-

TABLE 1. Penetration of face fly larvae of various ages by the nematode, *Heterotylenchus autumnalis*.

Age of fly larva when exposed (days)	Replicate 1		Replicate 2	
	No. Inf.	No. Not Inf.	No. Inf.	No. Not Inf.
1	1	11	1	19
2	6	14	7	6
3	1	19	4	15
4	4	16	7	12
5	1	19	7	13

posed to nematodes, allowed to pupate and emerge as adults which were then dissected at various times following emergence to determine the minimum time for larval nematodes to become infective and to further investigate the nematode's life cycle in adult hosts.

Adult gamogenetic female nematodes with yellow intestinal pigmentation were found in newly emerged flies. Parthenogenetic females of various sizes were found in 3-day-old adult hosts. As larvae inside the egg shell and during early development, parthenogenetic females were brown but later became white (Fig. 1, A). Five days after adult emergence parthenogenetically produced eggs were present in the host's hemocoel. Parthenogenetic eggs were white while those of gamogenetic females were brown (Fig. 1, A). At 7 days all stages of the nematode were present, but no larvae were in the host's ovipositor. After 9 days many larval nematodes had invaded the host's ovaries and ovipositor. Nematodes removed from 9-day-old females were not infective. Those from 11-day-old females, however, were able to infect new hosts. Larval nematodes were still present in 17-day-old adult hosts but their infectivity was not tested.

THE INFECTIVITY OF NEMATODES FROM MALE FLIES: Nematodes removed from 12-day-old adult male flies were placed on the surface of fresh manure. Twenty-five

fly maggots were then exposed to the nematodes for 4–5 hr and allowed to develop to adults. Adult hosts were dissected when they were 12 days old. None of the surviving five male fly adults dissected were infected; however, two of the six females contained nematodes in all stages of development. One female fly contained many nematode larvae in its ovaries. These were used to test their ability to infect new hosts. Dissections of host larvae 2 days after exposure to these nematodes indicated that infection of new hosts had occurred.

DISPERSAL OF FREE-LIVING NEMATODE LARVAE: Fresh cow manure was placed in eight aluminum, 23-cm diameter and 3.8-cm deep, pie plates to measure dispersal of the nematodes in artificial cow pats. Larval nematodes from one infected female fly were placed in the center of each plate on top of the manure. Each day, starting one day after introducing nematodes, core samples were taken from the manure in one of the plates to determine how far the nematodes had moved laterally. The core sampler was a piece of glass tubing (1.3 cm diameter bore). The pie plates were divided by using two pieces of string intersecting each other at the center of the plate and perpendicular to each other. Core samples (16 per plate) were taken on the four radii (1.2, 3.8, 6.4, and 8.9 cm from the center). The second day, nematodes were found 6.4 cm from the center in one direction, but not at all in samples taken along the perpendicular axis. In samples taken on the third and fourth days, nematodes were not found beyond 3.8 cm. Not until the fifth day were nematodes found 8.9 cm from the center. Nematodes were found on the sixth day in all samples except one and on the seventh day, nematodes were found in all samples taken.

HOST REACTIONS TO THE NEMATODE: Black to brown spots were observed on the

cuticle of several maggots during the experiment to determine which host larval stage the nematodes could penetrate. During the repeat of this experiment, maggots were examined for concurrence of infection and spotting. Out of seven infected 5-day-old fly larvae, five had spots and of 13 maggots not infected, seven had spots (Table 1). Among the 4-day-old fly larvae, three of seven infected and two of 12 non-infected had spots. Three-day-old fly larvae had four out of four infected larvae and nine out of 15 non-infected maggots with spots. At 2 days, five of seven infected and one of six non-infected fly larvae had spots; and finally, among the fly larvae less than 1 day old, one infected maggot had spots while none of the 19 non-infected had spots. Larvae often contained fewer nematode larvae inside than was suggested by the number of spots present on the cuticle. Conversely, non-infected larvae were often found with spots.

EFFECT OF THE NEMATODE ON THE HOST: Dissections of 5-day-old adult face flies showed that ovaries of non-infected females were fully developed (Fig. 1, B) while infected females of the same age had undeveloped ovaries. Infected female hosts contained ovaries full of larval nematodes instead of eggs (Fig. 1, C) about 9 days after eclosion. When the ovary sacs were ruptured the larval nematodes spilled out (Fig. 1, D). Examination of several 7-day-old, infected face fly males revealed no gross abnormalities of the testes. The sperm were all motile and apparently normal. Nematode larvae were found in the head, thorax, abdomen and legs of infected female adults.

Dead maggots were often found on the surface of the manure. Sometimes they were brown compared to their normal white or yellow color. The brown maggots were flaccid and contained large numbers of nematode larvae.

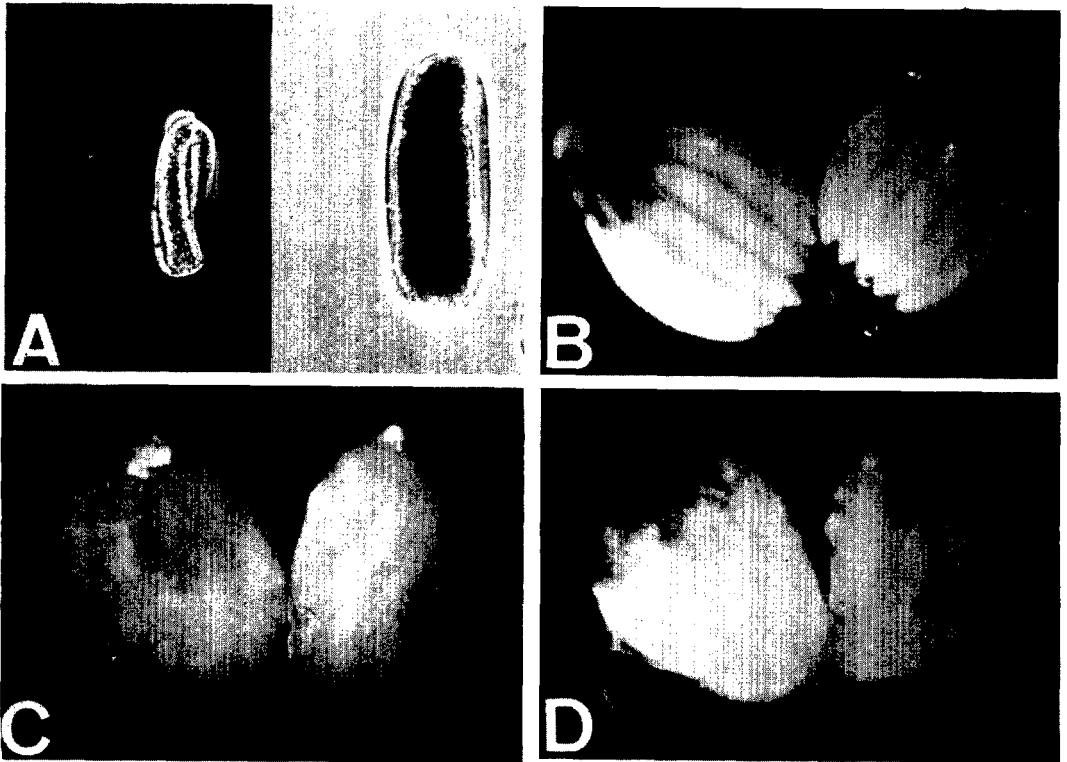


FIG. 1. *Heterotylenchus autumnalis* egg types and pathogenesis in host ovaries. A. Egg laid by parthenogenetic female containing gamogenetic larva ($274.3\times$) and egg laid by gamogenetic female containing the brown, parthenogenetic female larva ($236.3\times$); B. Ovaries of non-infected face fly containing several individual eggs each terminated by a dark respiratory mast ($11.6\times$); C. Ovaries of an infected face fly containing thousands of male and female gamogenetic larvae instead of eggs ($11.6\times$); D. Same ovaries as in C. but with the ovary sac ruptured releasing the nematode larvae ($11.6\times$).

EFFECT OF THE HOST ON THE NEMATODE: Larval nematodes in male face flies were smaller in size and fewer in number than those in females of comparable age.

DISCUSSION

The basic heterogonic life cycle of *H. autumnalis* is the same for all species of *Heterotylenchus* presently described while major differences between these species, other than taxonomic and host, include type of reproduction of the two types of females, stage of the host infected and mode of exit from the host.

PENETRATION OF THE HOST: Infective

larvae of *H. autumnalis* can penetrate all larval stages of face fly in the laboratory (Table 1), but whether they do so in nature is not known.

Only two members of this genus are known to penetrate the larval cuticle of their host, but in neither case is it known which larval stage they penetrate. These are *H. aberrans* Bovien (2) and *H. pawlowskyi* Kurochkin (8), parasites of the onion maggot and a flea, respectively. The remaining members of the genus are reported to penetrate host pupae (14). No entomophilic nematode penetrates the host egg and I know of only one report of possible penetration

into the adult. Wachek (14) stated that he did not observe infection but suggested that *Sphaerularia bombi* Dufour penetrate the hibernating adult bumblebee queen; however, Nickle (10) stated it penetrated the host larvae.

There are many unanswered questions concerning the mode of penetration and the behavior of infective larvae of heterotylenchid nematodes: (i) Can the infective larvae identify the preferred host? (ii) Is mating a prerequisite for a gamogenetic female to seek a host and for penetration? (iii) What environmental factors stimulate mating, molting and host penetration? (iv) How do infective nematodes attach to and penetrate the host? (v) What effect does the stage of the host penetrated have on the duration of the nematode's life cycle?

NEMATODE DEVELOPMENT IN ADULT FACE FLIES: The brown pigment found only in unhatched and newly hatched parthenogenetic female *H. autumnalis* (personal observation), *H. aberrans* and *H. boviemi* Wachek (14) has not been investigated. Gamogenetic larvae are always white. The size difference reported by Bovien (2) for the two types of eggs of *H. aberrans* is similar to that reported here for *H. autumnalis*.

The digestive tract pigment of adult gamogenetic females of all *Heterotylenchus* species has not been identified (2, 8, 14 and personal observations) but may be similar to the ommochrome pigments formed as excretory products in insects (4). Wachek referred to the pigment of *H. pawlowskyi* as a metabolic product.

Size variation of parthenogenetic female nematodes has been reported for other members of this genus (2, 8 and 14). Wachek attributed this difference in *H. boviemi* to intra-specific competition, while Kurochkin stated that for *H. pawlowskyi* it was due to differences in their ages.

Results of the present study and those of Jones and Perdue (7) conflict with that of Treece and Miller (13) who reported adult hosts more than eight days old could transmit nematodes to manure. Both this study and that of Jones and Perdue showed that the host must be 11 days or older before it can deposit nematodes into manure.

The effect of retaining gamogenetic nematode larvae in the adult host for extended periods of time should be investigated. Treece and Miller (13) reported nematode larvae in hosts 36 days after adult host emergence; however, observations during this study showed larvae in older hosts were often sluggish and dead.

THE FATE OF NEMATODE LARVAE IN MALE FACE FLIES: Failure of adult male face flies to frequent the breeding media raises several questions concerning the fate of nematode larvae in male hosts. Male hosts contain fewer nematodes than females. The development of nematode larvae in males is slower and is probably due to differences in available nutrients between the two host sexes. Regardless of these differences between host sexes, experimental removal of nematode larvae from male hosts demonstrated they could penetrate and successfully parasitize new hosts. However, absence of nematode larvae from the digestive tract and testes of males suggests a "dead-end" in this sex. Bovien made similar observations with *H. aberrans* (2) and *Scatonema wülkeri* Bovien (1). The exact fate of nematode larvae in male face flies in nature is not known. It is suggested that this apparent "dead-end" in male hosts is compensated for by large numbers of larvae in female hosts, other reproductive adaptations and/or different behavioral patterns of the infected female host during egg laying.

DISPERSAL OF THE NEMATODE: Infected face fly females do not deposit eggs

along with nematodes as do some other dipterous hosts; thus, the parasite is confronted with the problem of all parasites, that of contacting a suitable host. First, however, the nematodes must find a mate. The latter problem is solved because male and female *H. autumnalis* nematode larvae are deposited together in batches. Once mated, many parasites actively search for a host and must disperse from the site of release or emergence from the egg. Results of the present study show that the infective, free-living stages of *H. autumnalis* do not disperse very far before the fly larvae leave the manure to pupate. These results suggest that the nematodes rely on other ways of contacting a new host. Little information is known about the mechanisms used by entomophilic nematodes to contact their host.

EFFECT OF NEMATODE ON HOST: Stoffolano and Nickle (12), Stoffolano (11) and Treece and Miller (13) reported female face flies were castrated by the nematode, but the mechanism remains unknown.

No information, however, has been reported on the effect of the nematode on the male host. Examinations failed to reveal any gross effect on the testes. Squash preparations showed that the sperm were motile and appeared normal. However, Drea (3) reporting on castration of male alfalfa weevils parasitized by a braconid wasp demonstrated that only during mating tests between infected males and non-infected, virgin females could the effect of parasitization on the male weevils be seen. Bovien (2) reported male onion maggots infected by *H. aberrans* were not affected by parasitization. Hopkins and Rothschild (5), however, reported fleas of the genus *Coptosylla* castrated by nematodes (presumably *H. pawlowskyi*) and that the major portion of the male genitalia of the host was destroyed.

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