Nematode Parasites of Insects

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The last four speakers talked to you about plant parasitic nematodes which are important pests because they reduce crop yields and require the use of resistant varieties, crop rotations, and other management practices. In the evolutionary scheme, it would seem only fair that some nematodes should be beneficial to man. Fortunately, I am happy to report that there are many nematodes that are beneficial to us. At least three groups of nematodes parasitize insects and they either kill, sterilize, or debilitate in some way untold billions of pest insects each year. We should know more about these nematode parasites of insects and try to encourage their activities in our pest management practices.

We know that nematodes have parasitized insects for a long time because we have found them in Baltic amber and from Rhine lignite (Eocene). The older work, up to 1946, was nicely compiled by van Zwaluwenburg (1928) and La Rivers (1949). In the United States, the life history studies of mermithids on grasshoppers by Christie (1929, 1936, 1937) are excellent and the papers on nematodes of biting flies and other insects by Welch (1959, 1960) in Canada are informative. More recently, Nickle (1967, 1970, 1972a, 1972b) reviewed thhe taxonomy, biology, and pathology of the sphaerulariids, aphelenchoids and the mermithids.

Kinds of Nematode Parasites of Insects, Pathology, and Host Relationships

Insect parasitic nematodes fall into three general taxonomic groupings. Each group differs from the others by both morphological and biological characteristics. The parasitic stages found inside the body cavities of insects are also considerably different morphologically from the infective stages. Generally speaking, if there are one or two large worms in the insect, they are probably mermithids. If there are one or a few medium sized nematodes and about 5,000 larval nematodes, they are probably sphaerulariids or entaphelenchids. If one finds many medium sized

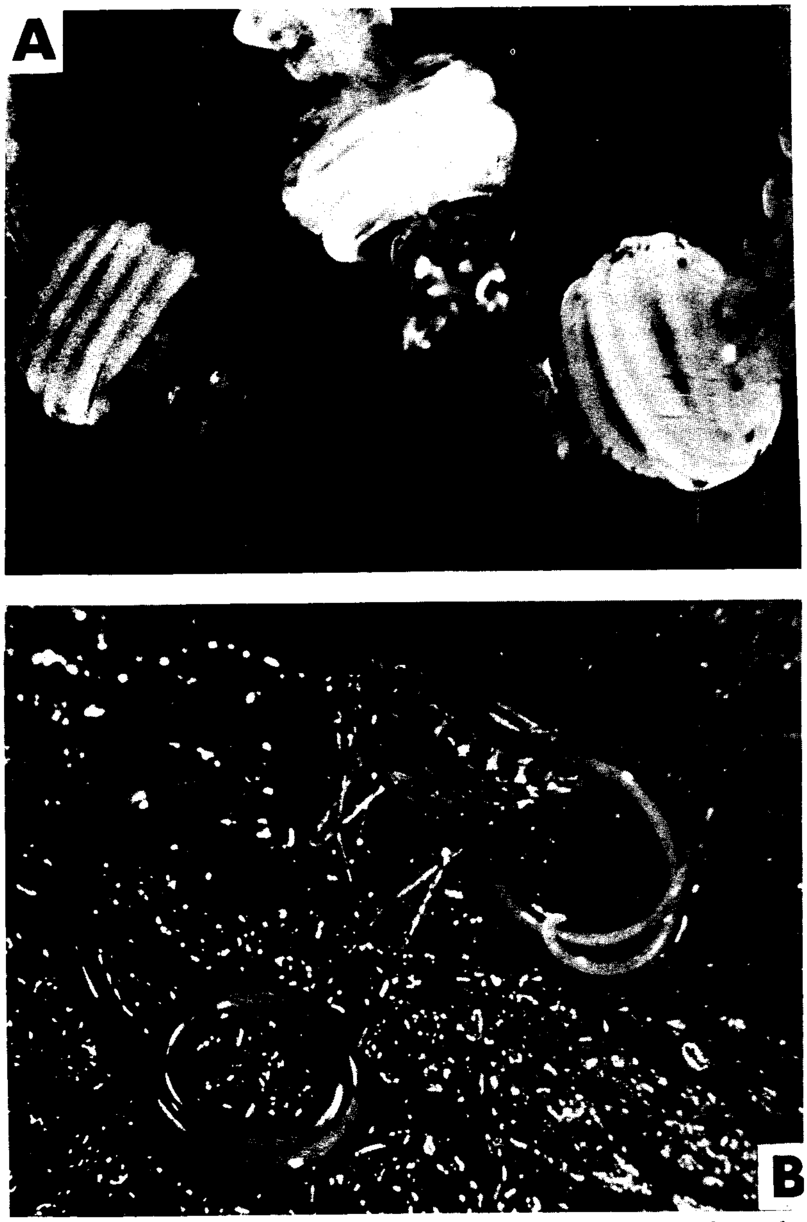

Fig. 1. Mosquitoes parasitized by mermithid nematodes. A. Culicine larvae parasitized by *Reesimermis nielseni*. B. *Aedes sollicitans* adult parasitized by *Perutilimermis culicis*. (Courtesy of Petersen and Chapman).

Fig. 2. Grasshopper parasitized by a mermithid. (Courtesy of USDA photo file).

males and females in an almost dead or dead insect, along with about 100,000-200,000 small nematode larvae and bacteria, it is probably a species of *Neoaplectana*. Further identification should be left to experts who have access to adequate libraries and collections. The pathology caused by the three groups of nematodes is also different. *Neoaplectana dutkyi* (DD-136) causes a rapid death of the insect, often within 24 hours. Mermithids cause the death of the insect when they emerge from the host because they make a large hole in the insect's body wall causing the loss of essential body fluids. Sphaerulariids and entaphelenchids usually cause a debilitation, reduced egg production, or sterility of the insect.

Mermithids

Percent parasitism by mermithids is a good indicator of percent mortality. Some mermithids kill larval insects before pupation *Reesimermis nielseni* (Fig. 1A) and others are found in adult insects *Perutilimermis culicis* (Fig. 1B). The former type of mermithid is, therefore, not well distributed, whereas the latter is well distributed. Some mermithids have

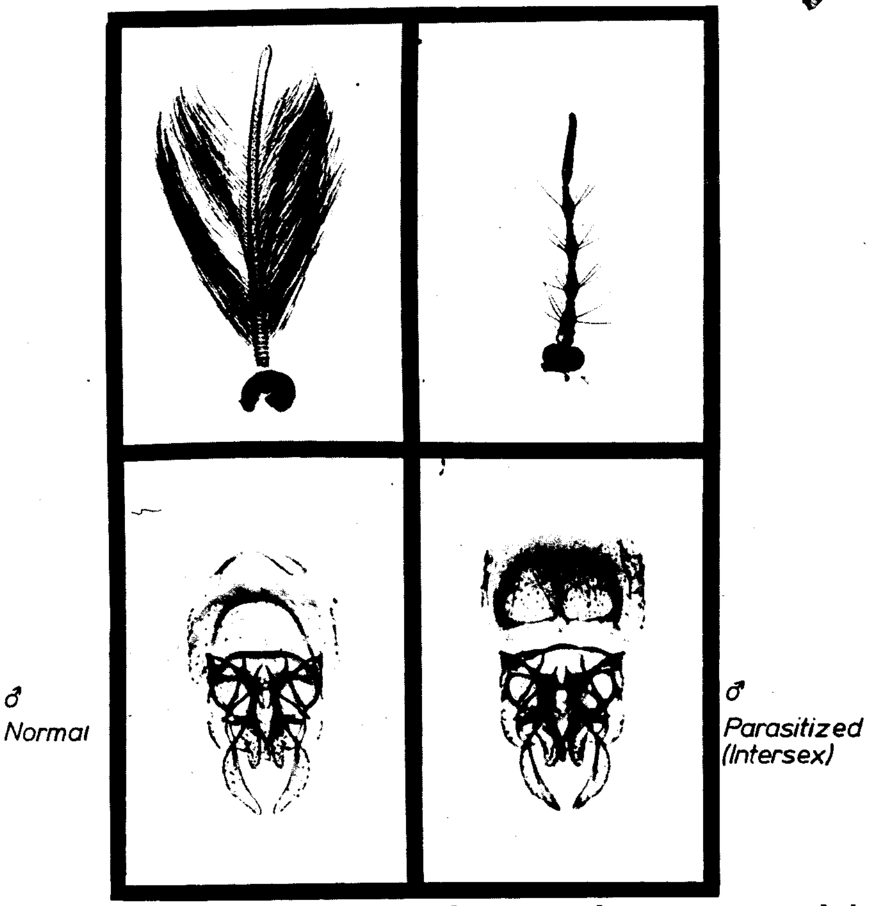

Fig. 3. Photographs of normal male, *Chironomus anthracinus* antennae and abdomen and those of a male, *C. anthracinus* parasitized by *Hydromermis rosea.* (Courtesy of Wülker.)

wide host ranges such as *Hexamermis albicans* on terrestrial insects and *Reesimermis nielseni* on 30 species of mosquitoes (Petersen, Chapman, and Willis 1969). Other mermithids seem to be host specific such as, *Perutilimermis culicis* (Petersen and Willis 1969). Mermithid nematodes have been known to cause epizootics in populations of several kinds of insects including; grasshoppers, chironomids, black flies, mosquitoes, walking-sticks, and some lepidopterans.

Christie and Thorne (personal correspondence) have contended that in Eastern North America, grasshopper populations remain at a low level because of mermithid parasitism. The critical habitat requirement for the parasite is about 40 inches of rainfall. In the west rainfall is not as abundant and therefore not conducive to high levels of mermithid parasitism. Though *Mermis nigrescens* is known throughout the world including the United States, *Agamermis decaudata* (Fig. 2) is only known from the Eastern United States.

Morphological changes in insects, caused by mermithid parasitism, are noted on adult chironomids and ants. Chironomids seem to have many mermithid parasites (Fig. 5A) and no doubt their population levels are influenced by mermithid parasites. Figures 3 and 4A show the morphological changes found by Wülker (1964) on the insect caused by the mermithid parasite. The male takes on some of the morphology of the female and the female has a few male characteristics. Ants (Fig. 4B and Fig. 5B) are also modified morphologically by mermithid parasitism. These intercaste modifications found by Wheeler (1928) are so striking that they have been given discrete names, such as, mermithogynes, mermithergates and mermithostratiotes. Mermithogynes are forms that are intermediary between the workers and fecundated females, whereas mermithostratiotes are female forms that have the head shape of soldiers. Mermithogynes and mermithergates possess swollen abdomens, whereas their heads and thoracies remain smaller. It is not known whether the fire ant has a mermithid parasite, but if it does, it should be introduced into the southeastern United States.

The tent caterpillar (Fig. 6B) and other lepidopterans, including the gypsy moth in Europe, are often parasitized by mermithids, sometimes as high as 65 percent. These parasitic associations have not received much attention in the United States. The Japanese beetle grub has recently been found to have a mermithid parasite (Fig. 6A) and is currently under study.

Black fly populations have been known to be reduced by 95-99 percent by mermithid parasitism. There is currently much activity in regards to the feasibility of utilizing mermithids in control of black flies. One project concerns the use of mermithids both native and exotic to improve control of the black fly vector of Onchocerciasis in West Africa.

Certain mermithids also parasitize spiders (Fig. 7) crustaceans, leeches, nematodes, and other invertebrates.

Neoaplectanids

These nematodes usually have a bacterium associate which they release into the haemolymph of the host insect causing a septicemia.

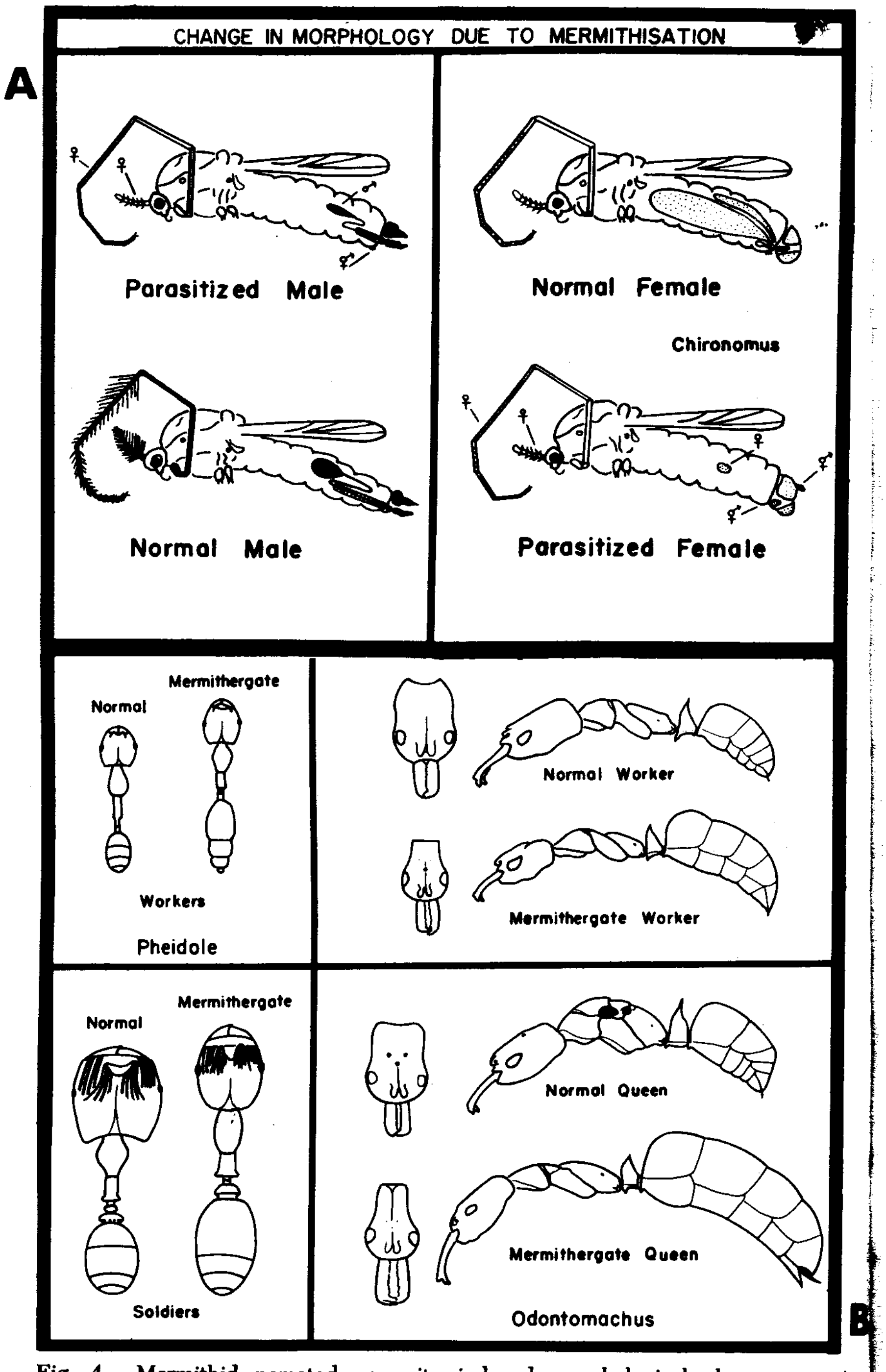

Fig. 4. Mermithid nematode parasite induced morphological changes on A. Chironomids (Courtesy of Wülker) and B. Ants (After Wheeler).

Fig. 8 A. Adult chironomid (Courtesy of Esser and MacGowan). B. Garden [illegible] (Courtesy of USDA photo file), parasitized by mermithids.

[illegible] work has been done by Dutky and his associates on the species [illegible] known as *Neoaplectana dutkyi* (DD-136). It has a wide host [illegible] killing many species of insects from several orders: Lepidoptera,

Fig. 6. A Japanese beetle grub with a mermithid parasite (Courtesy of Klein) and B. Forest tent caterpillar with mermithid (Courtesy of USDA photo file).

Diptera, Hymenoptera, Coleoptera, Orthoptera, Hemiptera, Homoptera and Isoptera.

The infective stage, which is the ensheathed second stage larva (dauer

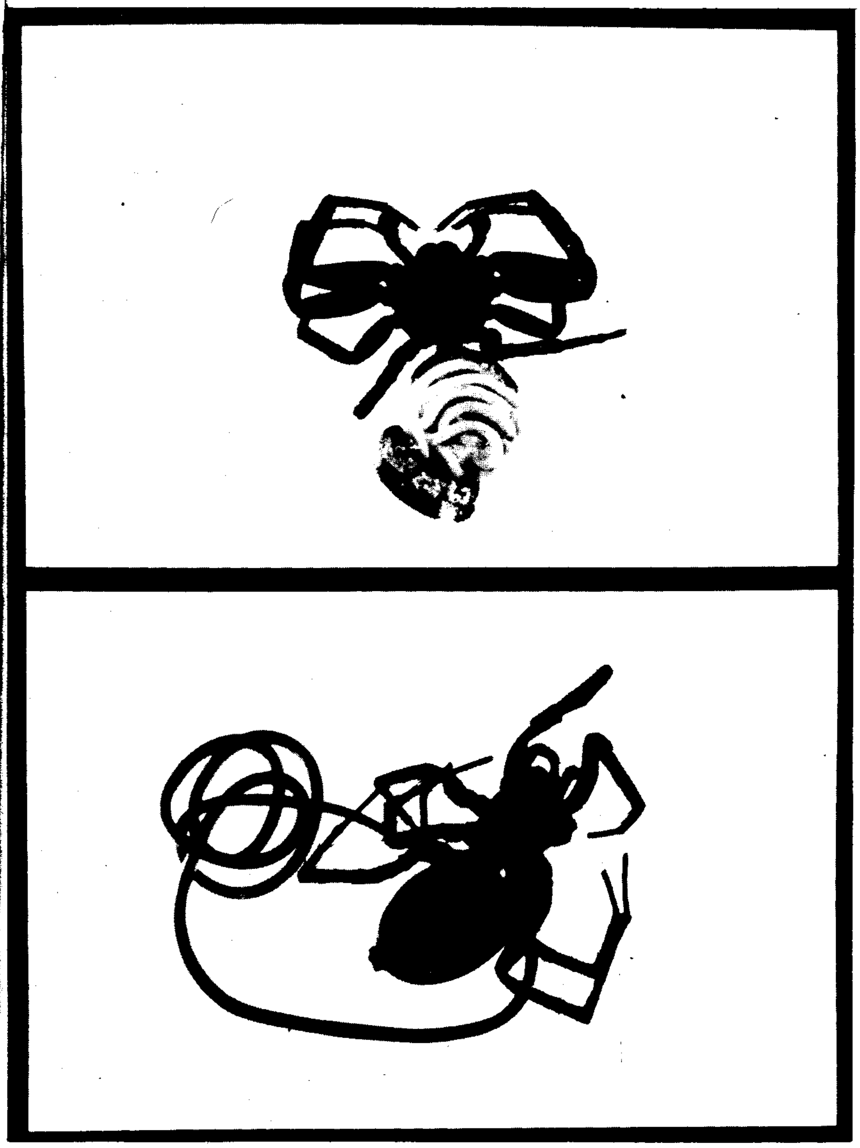

Fig. 7. Spiders parasitized by mermithids (Courtesy of Leech).

larva), seeks out the host and penetrates the insect's body through the alimentary canal. After reaching the haemocoel, the sheath is discarded and the nematode releases a bacterium which kills the insect, usually

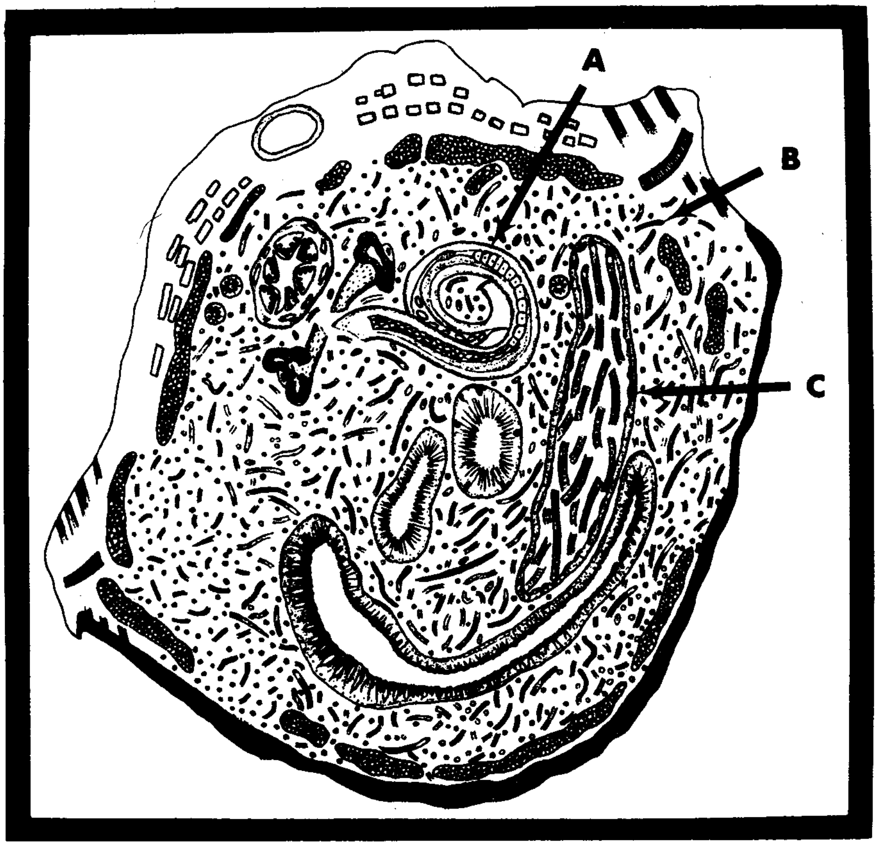

Fig. 8. Cross-section through the abdomen of the bark beetle, *Ips confusus* parasitized by the sphaerulariid nematode, *Contortylenchus elongatus* showing A. Female nematode. B. Her young. C. Another species of nematode in ventriculus.

within 24 hours. The nematodes then feed on the bacteria, become adult, mate, and produce many young. Over 100,000 infective stage larvae can be found in the cadaver of one wax moth larva.

Sphaerulariids and Entaphelenchids

These namatodes parasitize a wide variety of insects, including; bark beetles and other beetles, many flies, fleas, thrips, wood wasps, and other insects. They usually do not bring about a rapid death, as neoaplectanids, or even a later death, as mermithids, unless the infection is heavy.

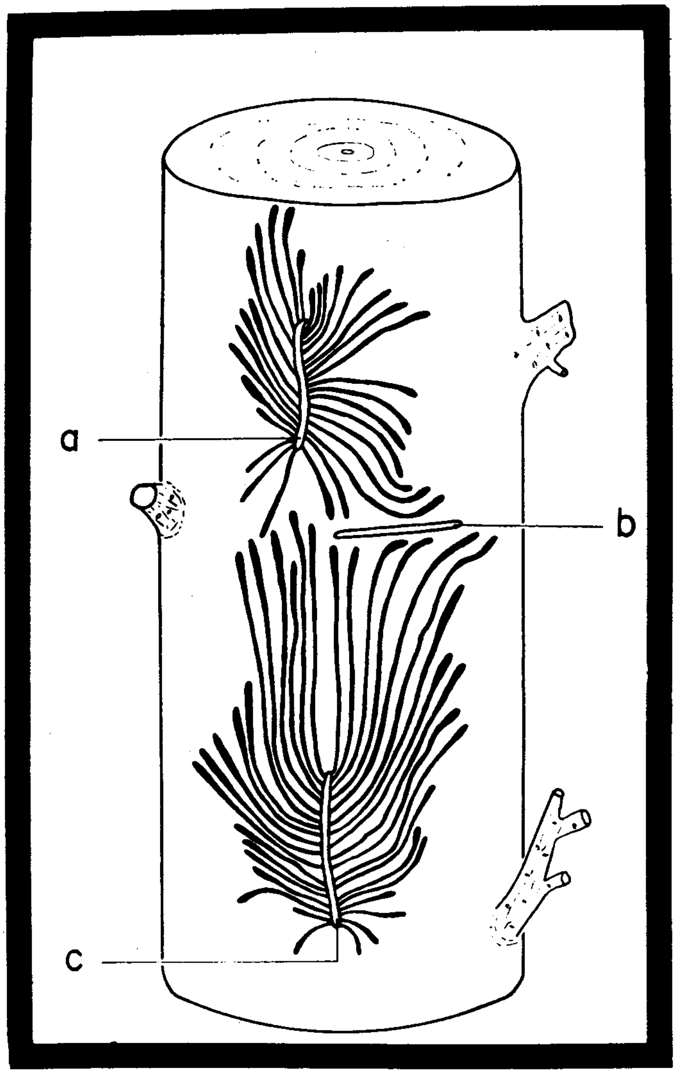

Fig. 9. Gallery patterns of the bark beetle, *Scolytus rugulosus*. a. and c. are normal patterns formed by the female beetle and her young. b. shows an abnormal gallery formed when the female beetle is parasitized by the sphaerulariid nematode, *Neoparasitylenchus rugulosi*.

Normally these namatodes cause debilitation, reduced egg production, late emergence, or complete sterility.

Bark beetles, parasitized by the sphaerulariids, have probably received

Fig. 10. Ovaries of the face fly, *Musca autumnalis*. A. Normal ovaries. B. Ovaries of a fly parasitized by the sphaerulariid nematode, *Heterotylenchus autumnalis*.

more attention (Rühm 1956; Massey 1957; Nickle 1963) than other sphaerulariids. Figure 8 shows a cross section through the abdomen of bark beetle, *Ips confusus*, parasitized by *Contortylenchus elongatus*. This association is fairly typical with one or sometimes more large female nematodes and about 5,000 small larval nematodes free in the haemolymph of the adult beetle. The most interesting nematode parasite induced change in oviposition behavior is shown in Figure 9. Here we see that the normal gallery pattern of the fruit tree shot hole borer is parallel with the stem. When parasitized by the sphaerulariid nematode, *Neoparasitylenchus rugulosi*, the female bark beetle makes a gallery perpendicular to the stem and does not lay eggs (Schvester 1957; Nickle 1971).

The face fly nematode, *Heterotylenchus autumnalis*, was found by Stoffolano and Nickle (1966) and described by Nickle (1967). It has a fairly complicated life cycle with three kinds of female worms and one male worm found at the same time in the fly's body cavity. These nematodes congregate in the ovary (Fig. 10) just before their exit from the insect, causing sterility to the host. Instead of laying eggs, the fly mock oviposits a batch of infective stage nematodes on the cow patties.

Phoretic Nematodes

There are many nematodes in several miscellaneous groups which are found associated with insects. These nematodes are often found in the frass, under the wing covers, adhering between the body segments by a sticky substance, or in some form of resting stage and attached to insects. They use the insect to transport them from one medium which is drying out to another fresh medium. They cause the insect no harm. The only reason for mentioning them here is because they sometimes confuse entomologists who may think they are parasites.

Habitat Manipulation and Insect Parasitic Nematodes

There is currently much interest in the utilization of nematodes for reducing populations of selected pest insects. I would hazard a guess that in the next ten years, more manipulations and introductions will be made with mermithids, neoaplectanids, sphaerulariids, and aphelenchids than all the earlier work combined. Pest management methodologies will be worked out to enhance the nematode self-perpetuating biological control agents because they play a significant role in reducing numbers of certain pest insects. I feel that the potential of insect parasitic nematodes is greatest in areas where chemical pesticides are not used or are most noxious to man and the environment. These situations, such as: large wild areas; near water, streams, ponds; near livestock and other animals; near man's dwellings; and where concentrated irrigated or river bottom agricultural production occur, are also fortunately ideal habitats for high nematode parasitism of insects.

One role that is available for researchers in the area of insect parasitic nematodes is to attempt manipulations of parasites from a closely related insect to a pest species. Often this is not possible in nature but is feasible in the laboratory.

Conservation of Existing Insect Parasitic Nematodes

The role of insect parasitic nematodes in pest management is appreciated when you consider each pest insect and the influence of the nematode parasites on insect population levels. For instance, we know that the saltmarsh mosquito, *Aedes sollicitans*, is widely distributed along the Atlantic and Gulf Coasts of North America from Nova Scotia to Texas. We have records that show that a mermithid nematode, *Perutilimermis culicis* (Fig. 1B), kills up to 65 percent of this pest from at least New Jersey to Florida and Louisiana. Factors influencing the habitat which increases or decreases the effectiveness of this nematode has great bearing on the levels of this pest species. Pesticides used for control of this mosquito in Mosquito Abatement work, that have nematicidal side effects, would reduce the effectiveness of this natural control agent.

Mass Rearing of Insect Parasitic Nematodes

1. *Neoaplectana dutkyi* (DD-136)

Dutky, Thompson and Cantwell (1964) described the technique for mass rearing of this nematode. It has been used successfully in dozens of laboratories throughout the world. The nematode is reared on the greater wax moth, *Galleria mellonella* (L.) as per the technique also developed by Dutky et al (1962). Ensheathed second stage nematode larvae are preserved for at least four months in a solution of 0.1 percent formalin in water. A stream of air flows continuously through the fluid, keeping the nematodes in suspension and providing them with oxygen. These nematodes are stored in a suspension of 50,000 infective stages per ml of solution at 7.1°C (45°F). They can have a shelf life of over 5 years. Last larval stage wax moth larvae are infected by placing them and about four ml of nematode suspension into a petri dish containing two sheets of filter paper (9 cm in diameter). Up to 20 wax moth larvae are anesthetized with CO_2 and distributed over the moist filter paper

The insect larvae die within 24 hours and after 5-8 days the insect cadavers are transferred to a G. F. White trap (White 1929). The second stage infective nematodes migrate from the disintegrating insect larvae into the fluid from which they can be transferred to the storage flasks containing the 0.1 percent formalin solution.

2. *Reesimermis nielseni* (Mermithidae)

Petersen and Willis (in press) describe the mass rearing of the mermithid from mosquitoes as follows. Preparasitic stages of *Reesimermis nielseni* were obtained by flooding cultures with tap water 16-24 hours prior to use. The inoculum of nematodes desired was obtained by diluting the water containing the preparasitic nemas, counting the nemas in 0.1 ml samples, and extrapolating for the undiluted preparasitics.

Southern house mosquitoes, *Culex pipiens quinquefasciatus* Say, were exposed to the nematodes and reared in 136 x 52 x 5 cm galvanized trays. For maximum production 20,000 hosts (about three per sq cm of surface area) were exposed to about 240,000 preparasitic nematodes (1:12 ratio). The optimum host diet that would produce the largest yield of female nematodes and the most uniform growth of infected hosts was determined. Seven days after exposure, host mosquitoes were collected and placed into specially built nema-collecting containers.

After emergence of the nematodes was complete, 10-15 grams of the wet weight mermithids were placed into paraffin-coated aluminum pans (22 x 33 x 5 cm) which contained clean, coarse, sterile sand covered to a depth of one cm with water. The cultures were covered with loose fitting plastic lids and stored. After about 3 weeks, visible dead nematodes were removed, the water was carefully decanted, and the excess water was absorbed with paper towels. The cultures were stored for an additional 4-15 weeks before use.

Under these conditions preparasitic nematodes can be produced at a cost of about 7-10 cents per million: if one technician can rear one million mosquito larvae per 40 hour week, if 80 percent parasitism yields 7.2-7.5 x 10^5 female nematodes, and if female nematodes produce a mean of 2500 eggs.

Field Introductions of Insect Parasitic Nematodes

Neoaplectana dutkyi (DD-136) has been used extensively on many pest insects under field conditions. Figure 11A shows pressure spraying of this biological insecticide for codling moth control in apple orchards.

Fig. 11. Field experiments with *Neoaplectana dutkyi* (DD-136). A. Pressur spraying of nematodes on apple trees for codling moth control. B. and D. Th banding technique used to kill codling moth larvae in pupation sites. (A. B. an D. courtesy of Dutky). C. Shows white-fringed beetle larva with typical infection o *Neoaplectana dutkyi* (DD-136). (Courtesy of Harlan).

Good controls, up to 99 percent were obtained by these field application and they were found to be comparable to the chemical spray tests. An other method of using these nematodes is shown in Figs. 11B and 11D Wax moth larvae, replete with tens of thousands of infective stage nema todes, were placed in the heavy bandage-like band, which was place around the trunks of the apple trees. When the codling moth larva came down the trunk of the trees seeking places to pupate, they congre gated under the bands and were killed by the nematodes. These tech niques and others were developed by Dutky.

Dutky (1967) found that this nematode could enter stems and othe structures and seek out and kill insects deep within the plant tissues Nematodes applied to peach trees killed borers deep in the trunks, an when sprayed on cotton plants can enter the cotton square and kill bo weevil larvae within the squares. In tests in Florida in which th nematode was applied in chicken houses to control house fly larva breeding in the manure, fully 40 percent of the larvae were entered an killed by the nematodes within 48 hours after the application. Thre months later no fly breeding was observed in treated houses although consistent fly breeding was found at nearby chicken farms.

During my visit to the Soviet Union in 1968, Artyukhovsky told me that they are using larvae of a species of *Hexamermis* to control a weevil on oak and that they are spraying eggs of *Mermis nigrescens* on vegetable crops to control grasshoppers. The Soviets have had problems with pesticides on their collective farms, because they found that after applying these pesticides to the fields fairly high levels of these poisons in the water, they pumped out of the ground water for their own use and for the use of their livestock. They have a strong program on cultural control of pests in the Soviet Union.

The mosquito parasite, *Reesimermis nielseni* has been developed by the Lake Charles, Louisiana Laboratory of the USDA as a biological control agent for larvae of culicines and anophelines. Very large numbers of preparasitics can be produced in the laboratory on the house mosquito for inoculation into mosquito larval habitats. In Louisiana, 50-64 percent parasitism of *Anopheles crucians* and certain culicines was induced by treatment of ten sites and the levels of parasitism subsequently established have reached 49 percent. In California, approximately 50-80 percent control of *A. freeborni* was achieved in rice fields. In Taiwan, inoculations at the rate of one preparasitic *Reesimermis* per ml of water resulted in 95 percent parasitism of the house mosquito in ricefield water. Further work with *Reesimermis* in Bangkok and elsewhere is planned within the WHO program on Alternative Methods of Vector Control.

A natural introduction of the face fly nematode, *Heterotylenchus autumnalis*, into the Eastern United States helped to reduce the pest factor caused by this fly on cattle and horses. As the fly moved westward, the first wave of flies was healthy and it took about two summers before the nematodes and other parasites and predators caught up to the flies. At that time we reared the face fly nematode and a predaceous staphylinid beetle in a USDA laboratory in Nebraska and leap-frogged them to the areas at the front of the face fly westward migration. The nematode now sterilizes about 30 percent of them and along with other parasites and predators, including birds, we are not as concerned about this fly as we used to be.

Conclusions

Nematodes are important parasites of insects. Evidence is presented showing that they cause epizootics to insects in nature and that currently, increasing effort is being made to utilize and enhance their role in pest insect management.

One of the problems facing this effort is the lack of expertise and, along with this, there is a constant need for more preliminary work in this area. Therefore progress seems to be slow in developing.

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