THE UNIVERSITY OF MANITOBA

# NEMATODE PARASITES OF BARK BEETLES (COLEOPTERA: SCOLYTIDAE) IN MANITOBA: TAXONOMY AND PATHOGENICITY.

by

Marek Tomalak

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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Canada,

the second country

to which I owe so much.

TABLE OF CONTENTS

PAGE

## ABSTRACT

In the 1980 - 83 studies, 32 species of bark beetles (Scolytidae), and some 56 species of parasitic nematodes were found in southern Manitoba. Parasites belonged to the families Rhabditidae, Aphelenchidae, and Allantonematidae. Six general types of host - parasite relationships were established based on the time of invasion of the bark beetle by the parasite, life cycle of the nematode, and the niche occupied in the beetle body. Four out of twelve species of nematodes selected for detailed studies were considered new, and described as Parasitylenchus caudapapilli sp. nov., Sulphuretylenchus pseudoundulatus sp. nov., S. nopimingi sp. nov., and S. posteruteri sp. nov.

Histological studies revealed a wide range of pathological changes in the nematode infected beetles. Histopathology usually was parasite specific. Juveniles of Parasitorhabditis autographi did not induce any apparent pathological effects in the beetle host, while P. obtusa caused lesions in the intestinal epithelium. Juvenile Aphelenchoides pityokteini parasitized Malpighian tubules of the beetles and caused partial, or complete degradation of the cuboidal epithelium in the tubules. Synergistic effects of coexistence of rod-shaped bacteria and juveniles of Parasitaphelenchus oldhami, in the hemocoel of D.E.D. vector, Hylurgopinus rufipes, led to the rapid depletion of the beetle fat body, and significantly reduced the beetle population prior to, and during hibernation. Juveniles of Bursaphelenchus fraudulentus caused local lesions in the fat body tissue of the host.

Allantonematids were the most pathogenic parasites. Nematodes caused abnormal development of the fat body tissue and gonads, during parasitism of the female nematodes, and led to the partial or complete degradation of the beetle tissues, during the parasitism of juveniles. Parasitism caused sterilization and premature death of the beetle hosts. The sequence of pathological processes in Polygraphus rufipennis infected with Sulphuretylenchus pseudoundulatus was considered as a general model, and compared with the pathology caused by other allantonematids. "Crowding" of parasites in the host led to reduction of sizes, and fecundity of female nematodes, or stimulated immune responses of the beetles.

Present findings emphasize the role of nematode parasites as important, natural factors controlling populations of bark beetles.

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LIST OF FIGURES PAGE

CHAPTER IV.



CHAPTER V.

CHAPTER VI.

Histology of noninfected bark beetle (e.g. P. sparsus).



Gonads of noninfected bark beetle (e.g. P. rufipennis).

LIST OF TABLES



LIST OF APPENDICES

PART A

# GENERAL INTRODUCTION

Many species of bark beetles (Scolytidae) are serious pests of forests. Each year scolytids are responsible for the loss of billions of board feet of timber (Bright, 1976). The Economic importance of bark beetles is related to the massive killing of hosts by direct feeding under the bark, or to the spreading of fungal tree pathogens, carried on the insect body. Members of the genus Dendroctonus Erichson devastate wide areas of spruce and pine forests in North America. Several species of Ips De Geer cause chronic problems in Europe. Scolytus scolytus Fab., Scolytus multistriatus Marsh., and Hylurgopinus rufipes Eichoff are major vectors of Ceratocystis ulmi (Buisman) Moreau, the causative agent of Dutch Elm Disease. Many other species of bark beetles can cause serious losses through their feeding in cones, on seedlings, or on various parts of adult trees.

Nematodes are regarded as one of the major biotic factors influencing the dynamics of bark beetle populations (Massey, 1974), as they significantly reduce the fecundity of female beetles (Massey, 1956; 1960; Schvester, 1957; Reid, 1958; MacGuidwin et al., 1980). Massey (1964) and Ashraf and Berryman (1970a) found that Sulphuretylenchus elongatus (Massey, 1958) Nickle, 1967, caused some mortality in its host. Various changes in the behavior of parasitized beetles were reported by several authors (Atkins, 1961; Nickle, 1971; Poinar and Caylor, 1974).

Despite the recognized role of nematodes as pathogenic agents in bark beetles, only a few detailed studies described the mechanisms involved (Nickle, 1963; Ashraf and Berryman, 1970b, 1971; Thong and

Webster, 1975). Host-parasite dynamics are complex as both parasite and host are developing at the same time as parasitism occurs. Since limited information is available on the histopathological processes in the beetle body, the external effects of parasitism, which are manifested by the abnormal behavior, reduced fecundity, and higher mortality of insects, remain inexplicable.

Various types of associations between nematodes and insects were reviewed by Bovien (1937), Welch (1963, 1965), Poinar (1972, 1975). Nematodes associated with bark beetles occupy numerous niches on the surface and inside the insect body. The latter group adapted to exploit specific sites in the beetle e.g. intestine, Malpighian tubules, or the insect hemocoel. The ecological role of some nematodes, especially those occupying the host intestine and Malpighian tubules remains unclear. In these groups, both commensalism and parasitism were suggested by different authors (Rühm, 1956; Lazarevskaya, 1965).

"Crowding" of parasites in the host can affect development of both species. This phenomenon and its influence on the nematodes infecting insects were investigated by only a few authors (Petersen, 1972, 1977; Craig and Webster, 1982). Despite the practical consequences of crowding as a factor limiting biocontrol potential of the parasite, this problem was not studied in bark beetles.

The main objectives of this study were to:

1. identify and if necessary describe new species of nematode parasites found in selected species of bark beetles;
2. study the bionomics of selected nematode parasites;
3. establish general criteria and classify bark beetles and parasitic nematodes into distinctive types of host-parasite

systems;

4. describe histopathological processes in bark beetles infected with nematodes;
5. correlate histopathological processes and actual changes in reproductive potential and viability of parasitized beetles;
6. examine "crowding effects" and selected factors influencing nematode intensity in the host beetle.

## MATERIALS AND METHODS

During the preliminary study of 1980 and 1981, material was collected from 35 localities in the southern part of Manitoba (south of 53° 00'N). All species of bark beetles sampled were examined for the presence of nematode parasites. In the subsequent studies of 1982 and 1983, 8 species of bark beetles, each from a single locality were selected for detailed examination (Appendix I). Distribution of all collection sites is shown on Fig. 1.

### Collection technique.

Only dying, but standing or recently fallen trees infected with bark beetles were examined in the present study. Four to six sections of the trunk or branches were cut from each tree with Craftsman 2.0 12" chain saw. Collected logs were 70 cm in length and their diameters varied from 8 to 30 cm.

Most of the field collections were made in the spring and early summer (May - July), shortly after the infestation of trees by bark beetles. Additional logs infected with various stages of developing beetles were collected in August and September. Only logs infected with one species of bark beetle were used in the study.

Logs cut in the field were transported to the laboratory and processed further within 24 hours after collection. Each sample, consisting of 4-6 logs, was divided into two groups. One or two logs were analysed immediately, while the remaining 3 to 4 logs were deposited in polyethylene bags and stored at room temperature (20±2°C), to allow further development of the new beetle generation. Bags were opened once a week for 2-3 hours to remove excess moisture and reduce development of saprophytic fungi.

Routine analysis of logs included stripping off the bark, general examination of the gallery patterns, hand removal of beetles from the galleries, and examination of the frass for the presence of nematodes.

Pieces of bark containing galleries of individual insects were examined separately to find relationship between individual bark beetles and the nematode composition in their galleries.

Extraction of nematodes from the frass was carried out as follows: pieces of the bark containing galleries of bark beetles were placed with cambium down in 9 cm petri dishes. Then, dishes were filled with distilled water up to the level of 3 mm from the bottom. After 30 min., the bark was removed, and nematodes were pipetted from the water under a dissecting microscope.

Bark beetles removed from galleries were dissected in cold (4°C) phosphate buffer, pH 7.2 and their organs and nematode parasites were processed further.

Preparation of nematodes for taxonomic studies.

Adult and juvenile nematodes recovered from galleries and from the insect body were deposited in a drop of distilled water on a cavity

glass slide, and heat killed over an alcohol lamp flame. Nematodes were then transferred by pipette to 2 ml. vials containing T.A.F. fixative (Triethanolamine-Formaldehyde fixative, Courtney, Polley, and Miller, 1955). After 10 min. the solution was replaced by the fresh T.A.F. fixative. Vials were capped and nematodes fixed overnight at room temperature (20±2°C). Specimens fixed in T.A.F. were washed in one change of distilled water for 15 min., and processed through the glycerol-ethanol method to glycerine for about 48 hrs., at 40°C. (Seinhorst, 1959). Dehydrated and cleared nematodes were mounted in glycerin and slides were sealed with Glyceel or nail polish.

Slides were examined, and drawings and measurements of nematodes were made using Leitz-Wetzlar microscope equipped with Labourlux Drawing Apparatus.

Preparation of tissue for histopathological studies.

Samples of 50-100 larvae ($L_3$, $L_4$), pupae and immature adults were taken for each species of bark beetles. During construction of egg galleries by adult beetles, samples were collected at 3-5 day intervals.

Adult and larval beetles were dissected in cold (about 4°C), 0.1 M phosphate buffer, pH 7.2, and their organs i.e., intestine, Malpighian tubules, gonads and sometimes ganglia were removed. Dissected organs were immediately fixed in cold (about 4°C) modified Karnovsky's fixative (Huebner and Anderson, 1972) for at least 2 hrs. After fixation, the material was divided into two parts and processed further for whole mounts and for sectioning.

Preparation of whole organs, for mounting in glycerin was carried out using the Seinhorst's method, as described for dehydration of nematodes.

Fixed organs prepared for a serial sectioning were washed (3 changes in 0.1 M phosphate buffer at 4°C, pH 7.2) and gradually dehydrated in an ethanol series at 4°C (70% - 1 min., 80% - 1 min., 95% - 1 min., and 3 changes, each 1 hr. in the absolute ethanol). Ethanol series was followed by 3 changes, 15 min. each in propylene oxide at 20°C. Infiltration was carried out in 1:1 solution of propylene oxide and Epon-Araldite mixture, for 2 days, at temperature of 20°C, followed by a brief vacuum infiltration to ensure complete removal of the propylene oxide. Individual organs were then embedded in Epon-Araldite mixture (Anderson and Ellis, 1965) and polymerized at 60°C for 48 hrs.

Semi-thin sections (2μ thick) were cut on the Sorvall JB-4 microtome, placed on ethanol cleaned slides and heat dried. Sections were stained with 1% toluidine blue in 1% borax for 2-4 min., over an alcohol lamp flame. The material was mounted in immersion oil and slides were sealed with nail polish.

In order to obtain cross and longitudinal sections of whole beetles, live specimens were placed in cold Karnovsky's fixative (about 4°C). The body was punctured around the inter-segmental region between the prothorax and mesothorax with a fine needle. Fixative rapidly penetrated through the holes and killed the beetle. After the initial period of fixation of about 1 hr., the insect's wings were cut off close to their base to avoid trapping of air bubbles during subsequent processing. Adult insects were cut with a scalpel into two parts through the intersegmental region between the prothorax and mesothorax.

Specimens remained in a cold fixative overnight and were then washed, dehydrated, infiltrated and embedded in the Epon-Araldite mixture as described for preparation of dissected organs.

Slides were examined under the Leitz-Wetzlar Photomicroscope, Zeiss Photomicroscope I, and Zeiss Photomicroscope II, using the bright field microscopy and Nomarski differential interference contrast optics. Micrographs were taken on the Zeiss Photomicroscopes I, and II, on Kodak Panatomic-X film.

Rearing of nematodes.

Juvenile nematodes recovered from dissected beetles were reared xenically in vitro by the hanging drop method. (Poinar, 1975). Samples consisted of 50 nematodes. Groups of 10 juveniles each, were deposited on a cover slip in a drop of Yeager's dextrose, and placed in an inverted position over the cavity of the glass slide. Edges of cover slips were sealed with vaseline to prevent desiccation. Nematodes were transferred to a fresh solution every 2-3 days, until maturity.

Calculation of nematode volume and fecundity.

Mature parasitic females were heat killed when they reached the stage of endotokia matricida. A simple formula for the calculation of the volume of a cylinder was used to determine nematode volume with length and average width applied in the calculations.

After calculation of the volume, parasitic females recovered from the same host were placed in the cavity glass slide in a drop of water, and dissected with fine needles under a dissecting microscope. All released juveniles and eggs were washed into a 100 ml. beaker with 30

ml. of 0.16% agar in 0.85% saline, liquified by heating to 60°C. After a thorough stirring on Pyro-Magnestir, 5 samples of 1.0 ml. each were withdrawn from the suspension, and placed on cavity glass slides. Individual nematodes were pipetted and counted in cooled, partially solidified agar with the aid of a dissecting microscrope. To find the total number of nematode progeny (i.e., eggs and juveniles) produced per host, the mean value obtained from five 1.0 ml. counts was multiplied by 30 ml. i.e., the original volume of the nematode suspension.

Isolation and culturing of bacteria.

In the study on Hylurgopinus rufipes (Eichoff) attempts to establish bacterial cultures from the insect tissues were as follows: after the wings had been removed, adult insects were surface sterilized by placing the beetles in 96% ethanol for 2-3 sec. Insects were then washed twice in sterilized water. Surface sterilized beetles were deposited in a drop of sterilized water and partially dissected, avoiding breakage of the intestine. Ovaries were removed from the insects, washed 3 times in sterilized water and placed in the test tube with bacteriological tripticase soya broth. In the broth, ovaries were squashed with a sterile bacteriological loop. Bacteriological plates with blood agar, chocolate agar, and MacConkey's agar were inoculated with the prepared broth. Plates were incubated at 20°C and 37°C.

Gramm tests were carried out on the tissue of insect ovaries squashed in a drop of distilled, sterilized water.

## Results

During the preliminary studies (1980-1981), a survey of 35 collection sites in southern Manitoba (south of 53°00'N.) revealed 32 species of bark beetles and about 56 species of parasitic nematodes. In the collected material, many parasites were represented only by juvenile stages, thus an exact determination of nematode species was impossible. The list of bark beetle species examined during the preliminary study, nematode parasites determined to the level of family, and niches occupied by the parasites in the insect body are shown in Table 1.

Three families of parasitic nematodes were found; namely, Rhabditidae, Aphelenchidae, and Allantonematidae, and they presented two significantly different life cycles (Appendix II). In the Rhabditidae and Aphelenchidae only juvenile nematodes were parasitic. In the Allantonematidae adult females and juvenile nematodes parasitized the insect.

Six general types of host-parasite relationships were established. The classification was based on the life cycle of the nematode, time of invasion of the bark beetle by the parasite, and niche occupied in the host body.

The general types of the host-parasite relationships (Fig. 2) are as follows:

A. Parasitism in the individual organ of the host.

I. Juvenile nematodes invaded immature adult beetles, and occupied the host intestine.

II. Juvenile nematodes invaded larval insects and occupied the host intestine.

III. Juvenile nematode invaded larval beetles and occupied Malpighian tubules of the host.

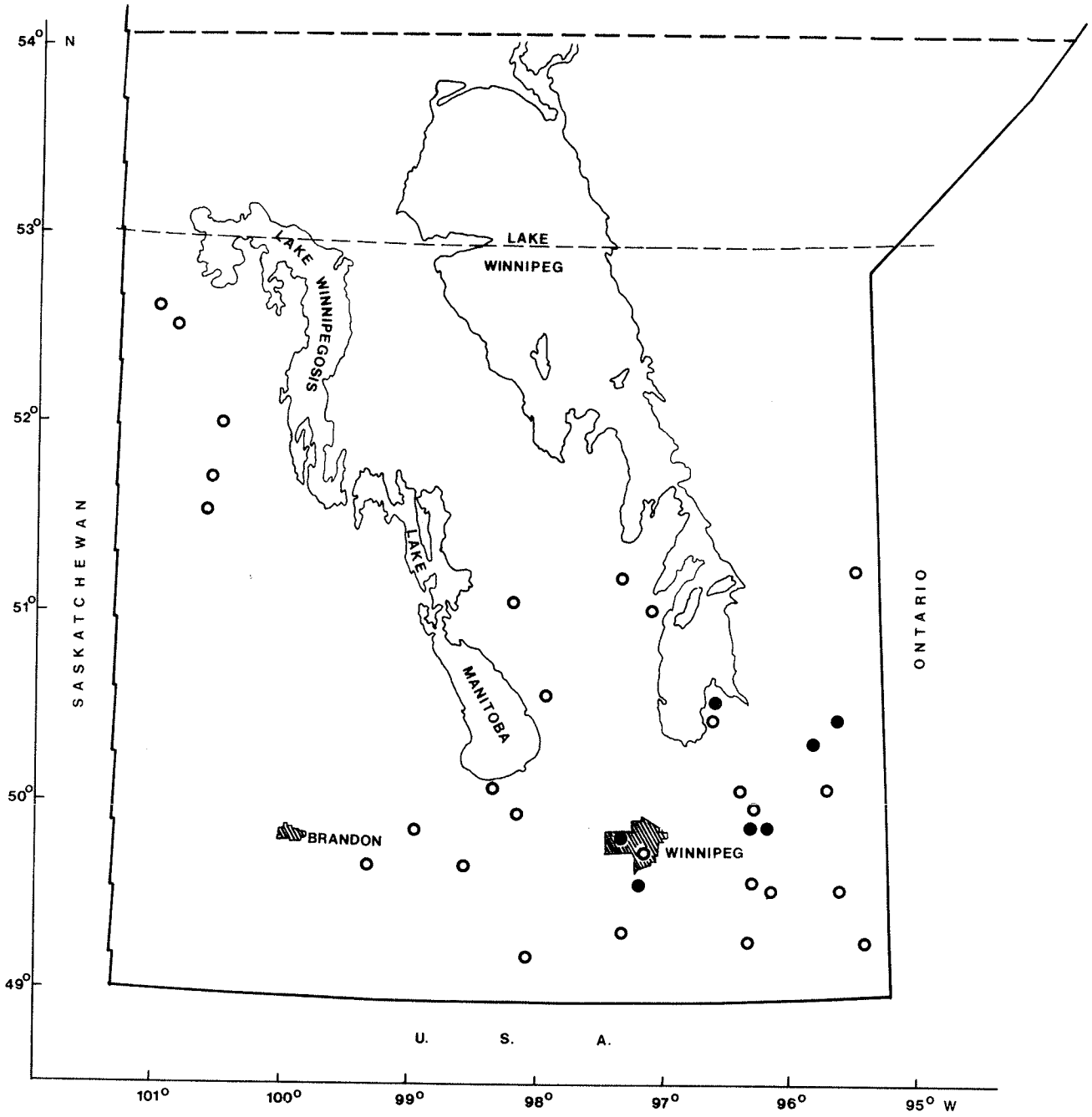
B. Parasitism in the host hemocoel.

IV. Juvenile nematodes invaded immature adult beetles.

V. Juvenile nematodes invaded larval insects.

VI. Female nematodes invaded larval beetles and reproduced in the host hemocoel.

Nematodes emerged from the host during construction of egg galleries by the adult beetles. One representative of each of the above relationships i.e., nematode parasite and its insect host, was selected for further, detailed study. A wide range of pathological effects associated with allantonematids was found. Thus, in the type no. VI, 7 species of nematodes infecting 6 species of bark beetles were studied in detail. Nematodes and bark beetles selected for detailed studies, and prevalence of infection are shown in Table 2. Details on exact locations of collection sites, species of host trees, and biology of bark beetles are given in Appendix I.




o Collection sites for preliminary survey in 1980-81.

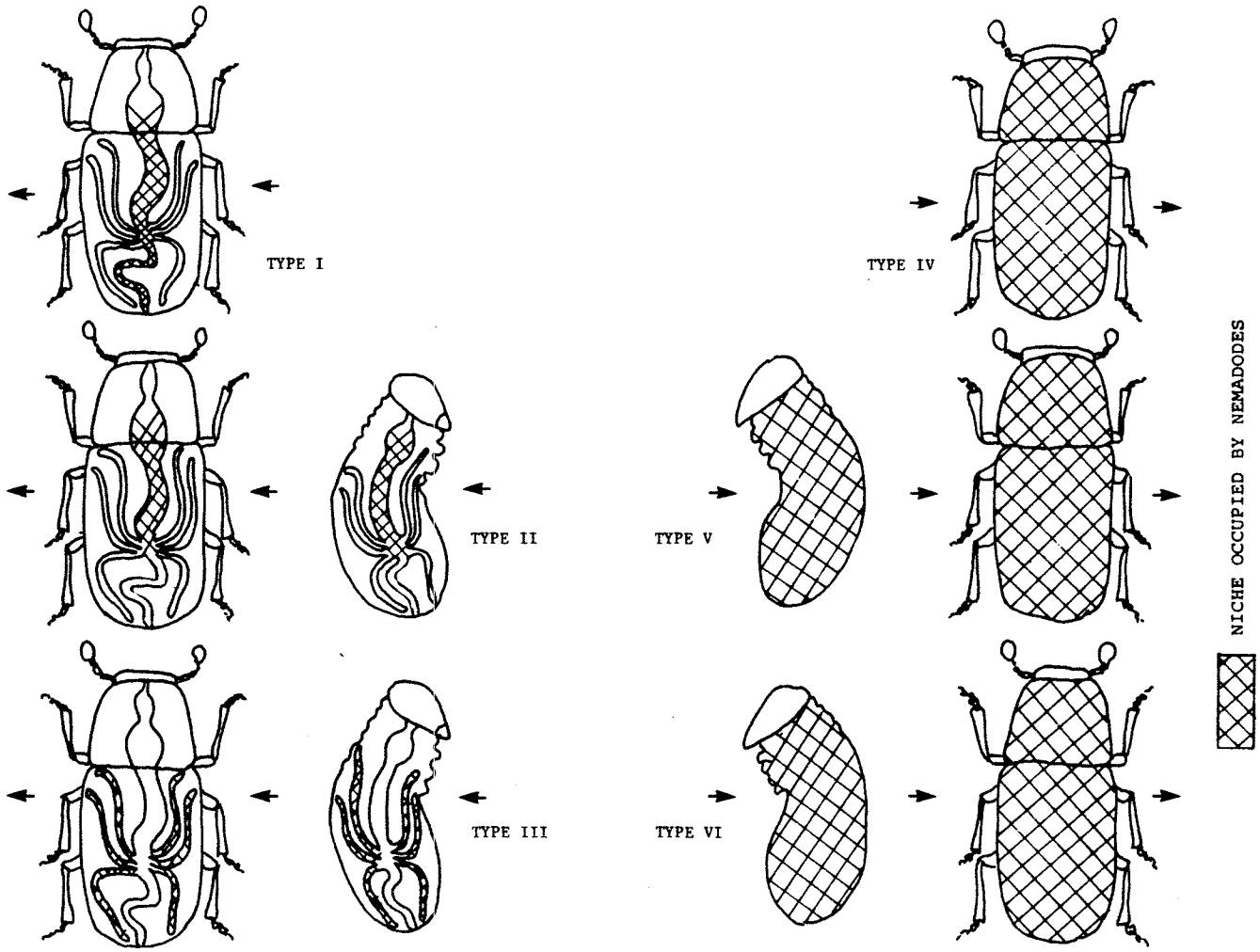
● Collection sites for main study in 1980-83.

Fig. 1. Locations of collection sites in southern Manitoba.

TABLE 1. BARK BEETLES AND THEIR NEMATODE PARASITES FOUND IN SOUTHERN MANITOBA DURING 1980-83.

| BARK BEETLE SPECIES | HOST TREE | PARASITE NICHE OCCUPIED IN THE BEETLE INTESTINE, MALP. TUB., HEMOCOEL | | |
|---|---|---|---|---|
| 1. Scolytus piceae (Swaine) | Larix sp. | - | - | A |
| 2. Hylurgops pinifex (Fitch) | Picea sp. | B | - | A(3),B,C |
| 3. Hylastes porculus Erichson | Picea sp. | B | | A(3),C |
| 4. Dendroctonus valens LeConte | Pinus sp. | B | | C |
| 5. Dendroctonus murrayanae Hopkins | Pinus sp. | - | - | - |
| 6. Dendroctonus rufipennis (Kirby) | Picea sp. | - | - | A(2) |
| 7. Dendroctonus simplex LeConte | Larix sp. | B | - | A |
| 8. Hylurgopinus rufipes (Eichhoff) | Ulmus sp. | - | - | C |
| 9. Leperisinus aculeatus (Say) | Fraxinus sp. | - | - | A |
| 10. Phoeosinus canadensis Swaine | Thuja sp. | - | - | A |
| 11. Phloeotribus laminaris (Harris) | Padus sp. | - | - | - |
| 12. Phloeotribus piceae Swaine | Picea sp. | - | - | - |
| 13. Chaetophloeus heterodoxus (Casey) | Padus sp. | - | - | - |
| 14. Polygraphus rufipennis (Kirby) | Picea sp. | B | - | A |
| 15. Trypophloeus populi Hopkins | Populus sp. | - | - | C |
| 16. Cryphalus ruficollis Hopkins | Picea sp. | - | - | - |
| 17. Crypturgus borealis Swaine | Picea sp. | B | C | - |
| 18. Trypodendron betulae Swaine | Betula sp. | - | - | - |
| 19. Trypodendron retusum (LeConte) | Populus sp. | - | - | - |
| 20. Trypodendron lineatum (Olivier) | Abies sp. | - | - | - |
| 21. Dryocoetes autographus (Ratzeburg) | Picea sp. | B | - | A(2) |
| 22. Dryocoetes affaber (Mannerheim) | Picea sp. | B | - | A(2) |
| 23. Pityogenes plagiatus plag. (LeConte) | Pinus sp. | - | - | A(2),C |
| 24. Pityokteines sparsus (LeConte) | Abies sp. | B | C | A |
| 25. Orthotomicus caelatus (Eichhoff) | Picea sp. | B | - | C |
| 26. Ips pini (Say) | Pinus sp. | B | - | A(3),C |
| 27. Ips perroti Swaine | Pinus sp. | B | - | A,C |
| 28. Ips perturbatus (Eichhoff) | Picea sp. | B | - | A(4),C |
| 29. Ips grandicollis (Eichhoff) | Pinus sp. | B | - | A,C |
| 30. Gnothotrichus materiarius (Fitch) | Picea sp. | - | - | - |
| 31. Pityophthorus sp. 1 | Picea sp. | - | - | - |
| 32. Pityophthorus sp. 2 | Pinus sp. | - | - | - |

A - Allantonematidae, B - Rhabditidae, C - Aphelenchidae. One species unless indicated otherwise in a bracket.




A. Parasites in individual organs.

B. Parasites in the hemocoel.

Fig. 2. Host - parasite relationships in bark beetles infected with nematodes.

TABLE 2. BARK BEETLES AND NEMATODES SELECTED FOR DETAILED STUDIES.

| TYPE* | BARK BEETLE SPECIES | NEMATODE SPECIES | NUMBER OF SAMPLED BEETLES | PREVALENCE OF INFECTION ** |
|---|---|---|---|---|
| I | Ips perturbatus (Eichhoff) | Parasitorhabditis obtusa (Fuchs, 1915, 1937) | 192 | 76.6% |
| II | Dryocoetes autographus (Ratzeburg) | Parasitorhabditis autographi (Fuchs, 1937) | 182 | 35.7% |
| III | Pityokteines sparsus (LeConte) | Aphelenchoides pityokteini Massey, 1974 | 648 | 57.7% |
| IV | Hylurgopinus rufipes (Eichhoff) | Parasitaphelenchus oldhami Rühm, 1956 | 396 | 92.5% |
| V | Trypophloeus populi Hopkins | Bursaphelenchus fraudulentus Rühm, 1956 | 206 | 82.2% |
| VI a | Polygraphus rufipennis (Kirby) | Sulphuretylenchus pseudoundulatus sp. nov. | 249 | 20.3% |
| b | Pityokteines sparsus (LeConte) | Sulphuretylenchus nopimingi sp. nov. | 242 | 26.7% |
| c | Ips perroti Swaine | Sulphuretylenchus posteruteri sp. nov. | 263 | 15.4% |
| d | Ips perturbatus (Eichhoff) | Parasitylenchus caudapapilli sp. nov. | 513 | 12.5% |
| e | Ips perturbatus (Eichhoff) | Neoparasitylenchus ipinius Massey, 1974 | 513 | 15.6% |
| f | Dryocoetes autographus (Ratzeburg) | Allantonema paramorosum Massey, 1974 | 308 | 14.5% |
| g | Dendroctonus simplex LeConte | Contortylenchus reversus Rühm, 1956 | 196 | 13.8% |

* Type of host - parasite relationship (see Fig. 2).

** Prevalence of nematode infection in immature adult beetles, shortly after pupation.

PART B

# CHAPTER I

## FOUR NEW ALLANTONEMATIDAE (Nematoda) PARASITES OF BARK BEETLES (Coleoptera, Scolytidae).

## Introduction

Many species of nematodes were found in the galleries and on or in the body of bark beetles (Fuchs, 1915, 1937; Rühm, 1956; Massey, 1974; Poinar, 1975). Studies carried out in 1980-1983 on nematode parasites of bark beetles in Manitoba revealed 56 species of nematodes in 32 species of scolytids. Sixteen species of bark beetles were infected with Allantonematidae. After examination of the available literature and type specimens from Dr. C. Massey's collection, four out of seven species of allantonematids selected for detailed study were considered new.

## Materials and Methods

The collection technique, preparation of nematodes for taxonomic study, and rearing of nematodes were as described previously in Part A, Materials and Methods.

Descriptions include the morphological characteristics of nematodes. Biological characteristics and pathogenicity are described in Chapter VI. Free-living females and males, as well as immature and mature adult parasitic individuals are described for each species. Measurements are presented according to de Man's Formula (Goodey, 1963). Variations about means are expressed as standard deviation. Length (L), greatest width (W), and ranges of these measurements are in millimeters. All other measurements are in $\mu$.

Range is abbreviated as R.

Generic nomenclature used in this paper is according to the classification of Nickle (1967).

## Results and Discussion

### Parasitylenchus caudapapilli sp. nov.

#### Description

Free-living female. Fig. 1 A,B. Holotype: L - 0.366; W - 0.012; a - 30.5; c - 14.6; V - 89%. Paratypes: (n - 10) L - 0.355 ± 0.011 (R: 0.343 - 0.372); W - 0.012 ± 0.001 (R: 0.011 - 0.013); a - 26.9 - 34.3; c - 12.5 - 20.7; V - 89 - 90%.

Body straight, cylindrical. Cuticle 0.6 - I.0 thick with fine transverse striae (0.8 wide), more pronounced at anterior end. Lip region slightly set off. Short stoma present. Stylet 9 - 10 long, without basal thickening. Nerve ring 64 - 70 from anterior end. Excretory pore 10 - 15 posterior to nerve ring. Oesophageal glands not discernible. Anus visible, but in some specimens indistinct, located 17 - 28 from posterior end. Rectum 3 long. Gonads prodelphic, monodelphic, about 1/4 length of body. Vagina 2 - 3 long. Lips of vulva not protruded. Tail narrowly conical, rounded at tip.

Free-living male. Fig. 1 C,D,E. Allotype: L - 0.330; W - 0.011; a - 30.0; c - 12.2; T - 51%. Paratypes: (n - 10) L - 0.316 ± 0.016 (R: 0.295 - 0.341); W - 0.011 ± 0.001 (R: 0.010 - 0.012); a - 24.9 - 33.2; c - II.3 - 15.5; T - 48 - 72%.

Body cylindrical, slender, ventrally arcuate, especially in tail region. Cuticle 0.6 - 0.8 thick with distinct transverse striae (0.8 wide) along entire body length, but more pronounced on head and tail. Lips not set off. Short stoma present. Stylet 8 - 9 long; slender; without basal thickening. Nerve ring 56 - 65 from anterior end. Excretory pore 6 - 10 posterior to nerve ring. Oesophageal glands

degenerated or overlapped by intestine and gonads. Intestine usually filled with large granules and partially overlapped by testis. Anus 19 - 26 from tail end. Testis outstretched posteriorly. Spicules 10 long. Gubernaculum small. Tail terminus narrowly rounded. Bursa absent.

Parasitic female of partially free-living generation. Fig. 2.

Paratypes: (n - 9) L - 1.485 ± 0.223 (R: 1.200 - 1.822); W - 0.122 ± 0.008 (R: 0.117 - 0.133); a - 10.3 - 14.2; c - 80.0 - 137.9; V $^{69\%}_{87}$ -$^{82\%}_{100\%}$.

Body cylindrical, ventrally arcuate. Cuticle 4 thick, with distinct transverse striae (2 - 6 wide). Head rounded with little mouth cone. Stoma not visible. Stylet 9 long, slender, without basal thickening. Swollen oesophagus present. Nerve ring and excretory pore not visible. Anus and rectum present, located 0 - 17 from posterior end. Ovary monodelphic, prodelphic, reflexed usually twice in the anterior region. Juveniles hatch in female uterus. Vagina prominent, 28 long. Vulva distinct, lips not protuberant. Tail rounded to slightly conical.

Fertilized female juvenile ($L_4$) of completely parasitic generation. Fig. 3A.

Paratypes: (n - 11) L - 0.567 ± 0.021 (R: 0.524 - 0.609); W - 0.030 ± 0.003 (R: 0.025 - 0.032); a - 17.5 - 21.5; c - 21.8 - 31.2; V - 92 - 94%.

Body cylindrical, ventrally arcuate. Cuticle 1 thick with fine striae in head region. Lips not set off. Stoma short. Oesophagus

swollen. Stylet 9 - 10 long without basal thickening. Intestine inconspicuous and difficult to trace. Nerve ring 63 - 68 from anterior end. Excretory pore not visible. Anus 18 - 24 from tail terminus. Ovary monodelphic, prodelphic in various stages of development. Spermatheca and uterus usually filled with sperm. Vagina prominent, 11 long. Lips of the vulva protruded. Anus and rectum often inconspicuous. Tail conical.

Adult female of completely parasitic generation. Fig. 4 A,B,C.

Paratypes: (n - 10) L - 1.078 ± 0.103 (R: 0.922 - 1.267); W - 0.075 ± 0.008 (R: 0.062 - 0.088); a - 12.4 - 16.5; c - 60.8 - 95.5; V - 96 - 97%.

Body ventrally arcuate, cylindrical. Cuticle 2 thick with fine transverse striae (2 wide). Head rounded with prominent nipple-like mouth cone. Stoma invisible. Stylet 9 long, slender, without basal thickening. Swollen oesophagus present. Intestine obscure and difficult to trace. Anus and rectum present, often inconcpicuous. Anus 11 - 20 from posterior end. Ovary prodelphic, monodelphic, usually 2-3 times reflexed in anterior region. Juvenile nematodes hatch in female uterus. Vagina distinct, 15 - 18 long. Lips slightly swollen. Tail conical with transverse foldings and nipple-like terminus.

Adult male of completely parasitic generation. Fig. 3B.

Paratypes: (n - 10) L - 0.456 ± 0.016 (R: 0.422 - 0.471); W - 0.018 ± 0.001 (R: 0.018 - 0.019); a - 22.4 - 26.2; c - 13.6 - 17.6; T - 66 - 81%.

Nematodes resemble males of free-living generation, but longer and more strongly arcuate, especially in tail region. Cuticle 1 thick. Head and tail with fine transverse striae. Lips not set off. Short stoma and oesophagus present. Stylet 7 - 8 long without basal thickening. Nerve ring 62 - 65 from anterior end, but in many specimens difficult to distinguish. Excretory pore inconspicuous, 7 - 10 posterior to nerve ring. Position of oesophageal glands obscure. Anus 26 - 32 from tail end. Testes expanded throughout most of body length. Anterior part of gonads with large, distinct nuclei. Posterior region filled with sperm. Spicules 13 long. Gubernaculum small. Tail terminus narrowly rounded. Bursa absent.

Diagnosis.

Parasitic males and females of both generations larger than P. dispar, (Fuchs, 1915) Filipjev, 1934, P. curvidentis (Fuchs, 1914) Micoletzky, 1922 and P. diplogenus Welch, 1959. Cuticle of parasitic females of both generations with distinct transverse striae, which separates this new species from P. curvidentis with cuticle structured in parasitic females of partially free-living generation only, and from P. dispar with cuticle smooth in females of both generations. Oesophagus, anus and vulva visible in all adult parasitic forms. Juvenile nematodes of both generations hatch in female uterus. Distinct mouth cone present in parasitic females of both generations. Females of the completely parasitic generation possess peculiar conoid tails with several transverse foldings and nipple-like terminus which separate this species from three other species belonging to the genus

Parasitylenchus. Tail of parasitic male narrowly rounded and similar to P. curvidentis, but without bursa.

Type host: Parasitic nematodes in hemocoel of larvae, pupae and adults of Ips perturbatus (Eichhoff). Free-living stages in galleries of host beetles, under bark of spruce Picea mariana (Mill). B.S.P.

Type locality: Agassiz Provincial Forest, near Rd. 15, 5 km east of Hazel, Manitoba, Canada.

Type material: Type specimens are deposited in the National Collection of Nematodes, Ottawa.

Sulphuretylenchus pseudoundulatus sp. nov.

Description

Free-living female. Fig. 5 A,B,C. Holotype: L - 0.669; W - 0.013 a - 51.5; c - 29.1; V - 93%. Paratypes: (n - 10) L - 0.831 ± 0.066 (R: 0.661 - 0.889); W - 0.014 ± 0.001 (R: 0.012 - 0.017); a = 50.0 - 65.4; c - 22.8 - 35.9; V - 93 - 95%.

Body cylindrical, slender. Cuticle 1 thick with fine transverse striae. Lips slightly set off. Stoma 5 - 6 deep. Stylet weak and slender, 12 long, slightly thickened at base. Nerve ring 77 - 84 from anterior end. Excretory pore 9 - 12 posterior to nerve ring. Two distinct oesophageal glands visible; their anterior ends located 22 - 25 and 41 - 51 from head; extend posteriorly to half body length. Intestine only partially visible, with large granules. Anus 24 - 29

from tail tip. Ovary monodelphic, prodelphic; extend anteriorly to about 1/2 body length. Spermatheca and uterus filled with sperm. Lips of vulva only slightly swollen. Tail narrowly conical pointed at terminus.

Free-living male. Fig. 5 D,E. Allotype: L - 0.888; W - 0.013; a - 68.3; c - 27.8; T - 61%. Paratypes: (n - 10) L - 0.800 ± 0.052 (R: 0.689 - 0.892); W - 0.012 ± 0.001 (R: 0.011 - 0.014) a - 53.3 - 73.7; c - 23.2 - 31.1; T - 58 - 74%.

Body cylindrical, slender. Cuticle I thick with fine transverse striae. Lips slightly set off. Stoma 4 deep. Nerve ring 70 - 74 from anterior end. Excretory pore 18 - 20 posterior to nerve ring. Oesophageal glands usually indistinct, obscured by intestine and testis. Intestine only partially visible. Anus 28 - 35 from tail terminus. Testes straight. Spicules 16 - 17 long. Gubernaculum 1/4 length of spicules. Tail long and narrow, pointed at tip. Leptoderan bursa present.

Parasitic female from pupae and just moulted adult beetles. Fig. 6 A,B. Paratypes: (n - 10) L - 3.111 ± 0.371 (R: 2.667 - 3.655); W - 0.171 ± 0.019 (R: 0.156 - 0.208); a - 16.2 - 23.4.

Body cylindrical, slender, ventrally arcuate. Head region rounded. Cuticle 11 - 14 thick with peculiar structure, composed of internal layer of ridge-like thickenings and external layer of lighter cuticle, smooth on surface. Short stoma present. Stylet 13 - 14 long, stout, with basal thickening, retracted or overgrown by thick cuticle. Part of oesophagus visible. Intestine distinct, partially obscured by gonads.

Rectum visible. Ovary prodelphic, monodelphic, reflexed several times. Uterus filled with unembryonated and partially embryonated eggs. Vulva and anus not clearly visible, located in cuticular depression. Tail rounded.

Mature parasitic female from adult beetles. Fig. 7. Paratypes: (n - 10) L - 2.079 ± 0.368 (R: 1.514 - 2.694); W - 0.148 ± 0.033 (R: 0.111 - 0.222); a - 8.8 - 18.7.

Body cylindrical to sac-like. Cuticle 6 - 8 thick, undulating with numerous irregular ridges over body length. Head outline irregularly rounded, often with numerous foldings. Stylet 14 long, with basal thickening, retracted and displaced by developing gonads; visible in some specimens only. Almost entire body filled with eggs and developing juveniles. Intestine not visible. Anus and vulva not discernible.

Diagnosis.

Size and body shape of parasitic females similar to that of S. posteruteri sp. nov. Peculiar structure of cuticle of immature parasitic females unique in genus. Free-living males most slender of all Sulphuretylenchus species and show greatest length to width ratio. Narrowly conical tail of free-living females separates this species from S. elongatus with tail cylindrical and obtuse at tip. Lack of short postuterine sac in free-living females separates this species from S. posteruteri. Two large oesophageal glands visible in free-living females distinguish this species from S. nopimingi sp. nov. with only

one prominent gland, and S. posteruteri with obscured oesophageal glands.

Type host: Parasitic nematodes in hemocoel of larvae, pupae and adults of Polygraphus rufipennis (Kirby). Free-living stages in galleries of host beetles, under bark of spruce Picea mariana (Mill). B.S.P.

Type locality: Whiteshell Provincial Forest, near Rd. 315, 1 km south to bridge on Bird River, Manitoba, Canada.

Type material: Type specimens are deposited in the National Collection of Nematodes, Ottawa.

Sulphuretylenchus nopimingi sp. nov.

Description

Free-living female. Fig. 8 A,B,C. Holotype: L - 0.648; W - 0.015 (24%) a - 43.2; c - 28.2; V - 92%. Paratypes: (n - 10) L - 0.631 ± 0.033 (R: 0.578 - 0.667); W - 0.014 ± 0.001 (R: 0.013 - 0.017); a - 37.2 - 50.9; c (16%) (29%) - 21.4 - 33.2; V - 92 - 94%.

Body cylindrical, slender. Cuticle 1 thick, with fine transverse striae. Lips not set off. Distinct stoma present, 5 deep. Stylet prominent, 13 long, with basal thickening. Nerve ring 76 - 82 from anterior end. Excretory pore adjacent to nerve ring, located 5 - 7 posteriorly to it. Only one prominent oesophageal gland visible, anterior end reaching 50 - 52 from head end and often extended posteriorly to half of body length. Intestine with large granules,

partially overlapped by oesophageal gland and gonads. Anus 20 - 26 from tail terminus. Ovary monodelphic, prodelphic, extended to 1/3 - 1/2 length of body. Spermatheca and often uterus filled with sperm. Vulva with slightly swollen lips. Tail conical, narrowly rounded at tip.

Free-living male. Fig. 8 D,E. Allotype: L - 0.669; W - 0.015; a - 44.5; c - 26.7; T - 85%. Paratypes: (n - 10) L - 0.639 ± 0.018 (R: 0.620 - 0.672); W - 0.015 ± 0.001 (R: 0.014 - 0.017); a - 36.5 - 46.1; c - 22.6 - 30.1; T - 81 - 87%.

Body cylindrical, slender. Cuticle 0.8 - 1.0 thick with fine transverse striae. Lips slightly set off. Stoma present, 4 deep. Stylet slender 8 - 9 long, with basal thickening. Nerve ring often indistinct, 59 - 64 from anterior end. Excretory pore not visible. Oesophageal glands indistinct, partially obscured by overlapping testes. Intestine overlapped by testis. Anus 21 - 28 from tail terminus. Spicules 14 long. Gubernaculum small, 1/4 length of spicules. Testes extended anteriorly almost to nerve ring. Tail ventrally arcuate. Peloderan bursa present.

Immature parasitic female from larval beetles. Fig. 9. Paratypes: (n - 11) L - 0.725 ± 0.113 (R: 0.590 - 0.915) W - 0.069 ± 0.015) (R: 0.055 - 0.092); a - 8.6 - 12.3; c - 54.1 - 70.4; V - $\overset{40\%}{96}$ - $\overset{89\%}{97}$%.

Body cylindrical to sac-like. Cuticle 1.5 - 2.5 thick. Mouth region in depression caused by overgrowth of adjacent cuticle and hypodermis. Short stoma present. Stylet 13 long, stout with basal thickening. Nerve ring and excretory pore not visible. Intestine distinct. Anus 13 - 16 from tail end. Ovary prodelphic, monodelphic,

rarely reflexed. Spermatheca filled with sperm. Vulva in shallow depression. Tail rounded. Large hypodermal cells present in some specimens.

Mature parasitic female from adult beetles. Fig. 10. Paratypes (n - 17) L - 1.862 ± 0.385 (R: 1.208 - 2.572); W - 0.172 ± 0.031 (R: 0.106 - 0.233); a - 8.6 - 14.6.

Body cylindrical to sac-like, tapered at both ends, irregularly ventrally acruate. Cuticle 3 - 4 thick. Head rounded. Lip region partially enveloped by overgrowing cuticle and hypodermis. Stylet 12 - 13 long, 1 thick, with basal knob, not retracted. Nerve ring, oesophagus and excretory pore not visible. Only small portion of intestine visible, pushed to head region. Ovary monodelphic, reflexed several times in posterior region. Gonads occupy most of body, reaching almost to base of stylet. Juvenile nematodes hatch in female uterus. Anus and vulva not descernible.

Diagnosis.

Mature parasitic females small, similar to S. grossmannae (Rühm, 1954) Nickle, 1967 and S. escherichi (Rühm, 1956) Nickle, 1967. Absence of discernible anus or vulva separates this species from S. grossmannae. Stylet is neither retracted nor distorted by developing gonads distinguishing this species from S. escherichi. Characteristic invagination of mouth part in parasitic females separates S. posteruteri from other Sulphuretylenchus. Free-living males are shorter and stouter than males of remaining species and have lowest length to width ratio. In free-living females only one large oesophageal gland visible; extends

posteriorly to half body length, separating this species from S. posteruteri, with obscured glands, and from S. pseudoundulatus with two distinct oesophageal glands.

Type host: Parasitic nematodes in hemocoel of larvae, pupae and adults of Pityokteines sparsus (LeConte). Free-living stages in galleries of host beetle, under bark of Balsam fir Abies balsamea (L.) Mill.

Type locality: Nopiming Provincial Park, near Bird Lake, 1 km south to Rd. 315, Manitoba, Canada.

Type material: Type specimens are deposited in the National Collection of Nematodes, Ottawa.

Sulphuretylenchus posteruteri sp. nov.

Description

Free-living female. Fig. 11 A,B,C. Holotype: L - 0.851; W - 0.017; a - 50.1; c - 25.8; $V^{29\%}_{1\%}$ - 91%. Paratypes: (n - 12) L - 0.833 ± 0.056 (R: 0.755 - 0.947); W - 0.015 ± 0.001 (R: 0.014 - 0.017); a - 48.8 - 63.1; c - 22.4 - 31.5; $V^{22-34\%}_{1\%}$ - 91 - 92%.

Body cylindrical, slender, ventrally arcuate. Cuticle 0.8 thick with fine transverse striae. Lips slightly set off. Stoma present, 5 deep. Stylet 10 - 11 long and 1 wide, with basal thickening. Nerve ring 75 - 83 from posterior end. Excretory pore 8 - 14 posterior to nerve ring, but in some specimens indistinct. Oesophageal glands only

partially visible, obscured by granules in intestine. Anus indistinct, 28 - 38 from tail end. Ovary monodelphic, prodelphic, about 1/3 length of body. Vagina short, 7 - 8 . Gonads form short, 8 long, postuterine sac, posterior to vagina. Lips of vulva slightly swollen. Tail conical; narrowly rounded terminus.

Free-living male. Fig. 11 D,E. Allotype: L - 1.010; W - 0.019; a - 53.2; c - 30.6; T - 83%. Paratypes (n - 12) L - 0.982 (S.D. ± 0.060) (range 0.883 - 1.055); W - 0.018 (S.D. - ± 0.002) (range 0.014 - 0.021); a - 43.1 - 64.1; c - 27.9 - 37.2; T - 70 - 86%.

Body cylindrical, slender, ventrally arcuate. Cuticle 0.8 thick with distinct transverse striae (1 wide). Lips slightly set off. Stoma present 5 deep. Stylet 9 - 10 long and 1 wide, with distinct basal knob. Nerve ring 74 - 77 from anterior end. Excretory pore 16 - 18 posterior to nerve ring. Intestine filled with large granules obscuring position and extent of oesophageal glands. Anus 27 - 36 from tail terminus. Anal lips slightly protruded. Testis extended to region of nerve ring. Spicules 15 long, gubernaculum 1/3 length of spicules. Tail conical with acute terminus. Peloderan bursa present.

Immature parasitic female from larval beetles. Fig. 12. Paratypes: (n - 11) L - 1.286 ± 0.149 (R: 1.056 - 1.438); W - 0.087 ± 0.006) (0.079 - 0.092); a - 12.6 - 16.2; V - 95 - 98%.

Body cylindrical. Cuticle 4 - 7 thick, undulating, with numerous villus-like projections. Head region rounded. Weak mouth cone present in some specimens. Stoma, stylet and oesophagus obscure. Nerve ring and excretory pore not visible. Intestine distinct, filling most of pseudocoelom. Anus visible. Vulva indistinct in many specimens. Ovary monodelphic, prodelphic in various stages of development, not reflexed;

extend to 1/3 - 1/2 length of body. Thick walled spermatheca filled with sperm. Large hypodermal cells present in some specimens. Tail rounded to slightly conical.

Mature parasitic female from adult beetles. Fig. 13. Paratypes: (n - 12) L - 3.084 (S.D. - ± 0.405) (range 2.569 - 3.853); W - 0.210 S.D. - ± 0.045 (range 0.167 - 0.314); a - 11.5 - 17.2.

Body cylindrical. Head and tail regions broadly rounded. Cuticle 13 - 18 thick, with distinct ridges. Mouth structures not distinct. Stylet 13 long with distinct basal thickening, retracted into body. Intestine obscured by mass of gonads. Ovary monodelphic, prodelphic, reflexed several times. Juvenile nematodes hatch in female uterus. Posterior part of uterus poorly defined in many specimens. Anus and vulva not discernible.

Diagnosis: One of larger species of Sulphuretylenchus. Mature parasitic females shorter than S. elongatus (Massey, 1958) Nickle, 1967, S. pilifronus (Massey, 1958) Nickle, 1967 and S. stipatus (Massey, 1966) Poinar, 1975; narrower than S. scrutillus (Massey, 1964) Poinar, 1975. Broadly rounded tail and no discernible anus or vulva of mature parasitic females separate this species from S. elongatus, with protuberant vulva, and from S. stipatus with conical tail and anal opening at terminus. Thick cuticle of mature parasitic females has distinct ridges, which separates this species from S. pilifronus with characterstically translucent and finely striated cuticle. In free-living females, gonads have short postuterine sac which separates this species from four other species for which free-living females are

described (S. grosmannae, S. elongatus, S. nopimingi, and S. pseudoundulatus. Conical tail of free-living females narrowly rounded at tip and different from cylindrical tail of S. elongatus, obtuse at terminus. Immature parasitic female has cuticle with numerous villus-like projections, feature that was not described in any other Sulphuretylenchus.

Type host: Parasitic nematodes in hemocoel of larvae, pupae and adults of Ips perroti Swaine. Free-living stages in galleries of host beetle, under bark of Jack pine Pinus banksiana Lamb.

Type locality: Belair Provincial Forest, near Belair, Rd. 59, Manitoba, Canada.

Type material: Type specimens are deposited in the National Collection of Nematodes, Ottawa.

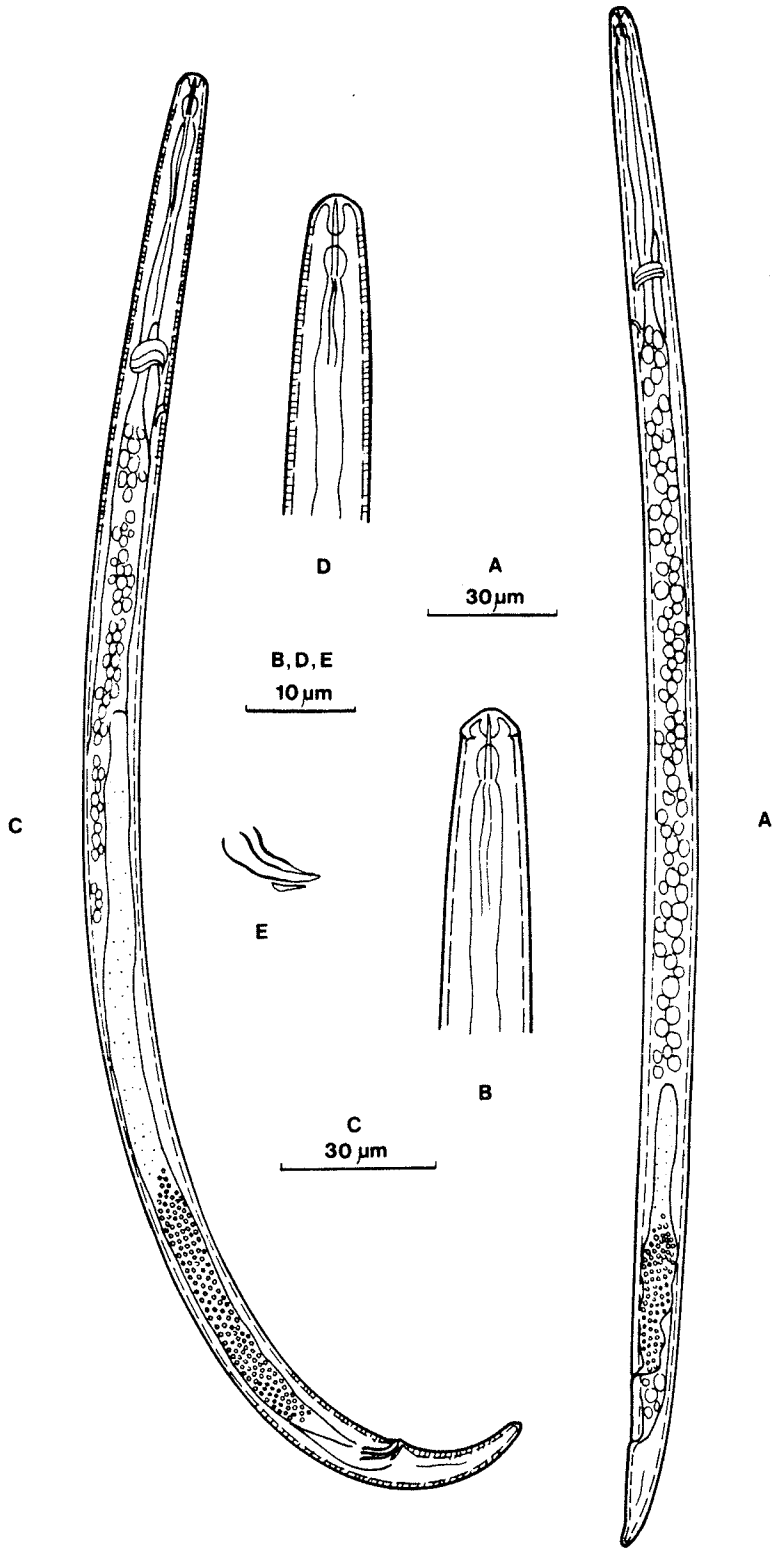



Fig. 1. Parasitylenchus caudapapilli sp. nov.: A. Free-living female; B. free-living female, head; C. free-living male; D. free-living male, head; E. free-living male, spicules.

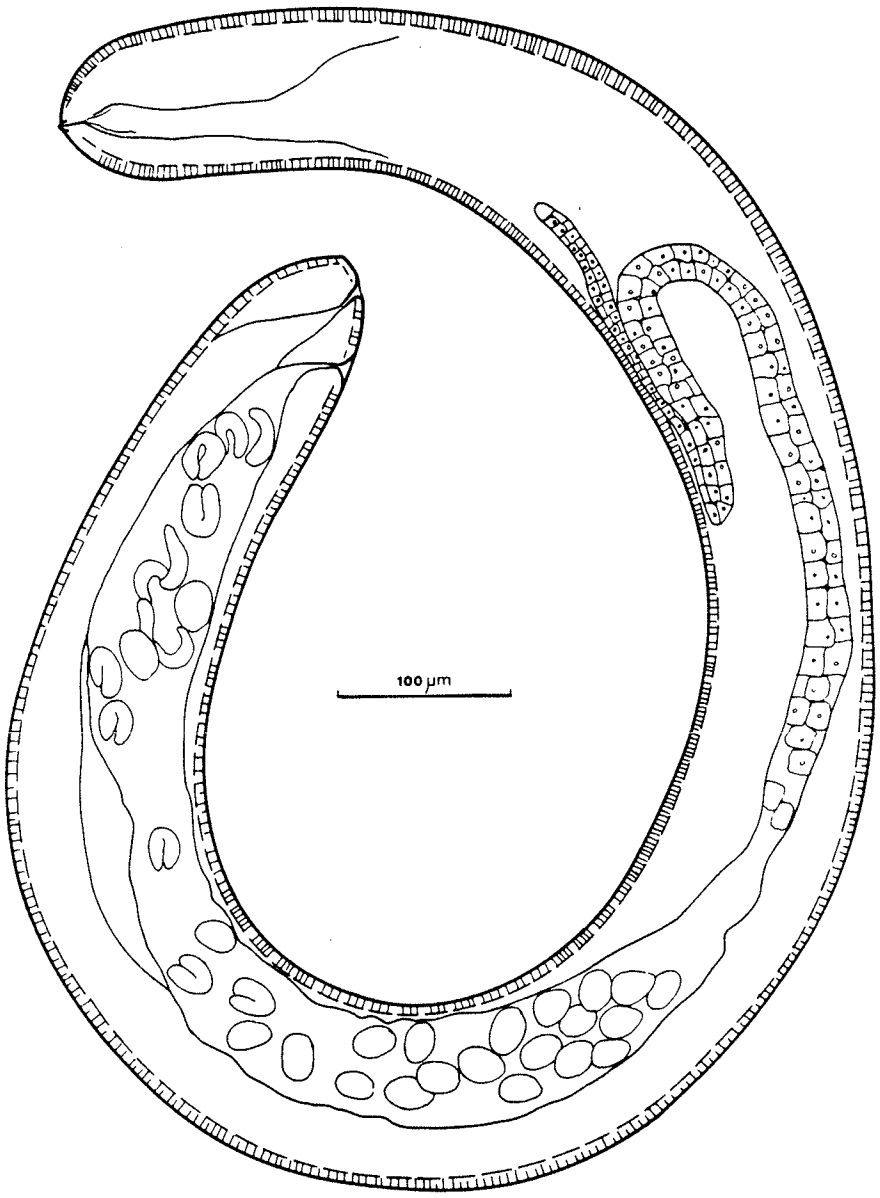



Fig. 2. Parasitylenchus caudapapilli sp. nov. Parasitic female of partially free-living generation.

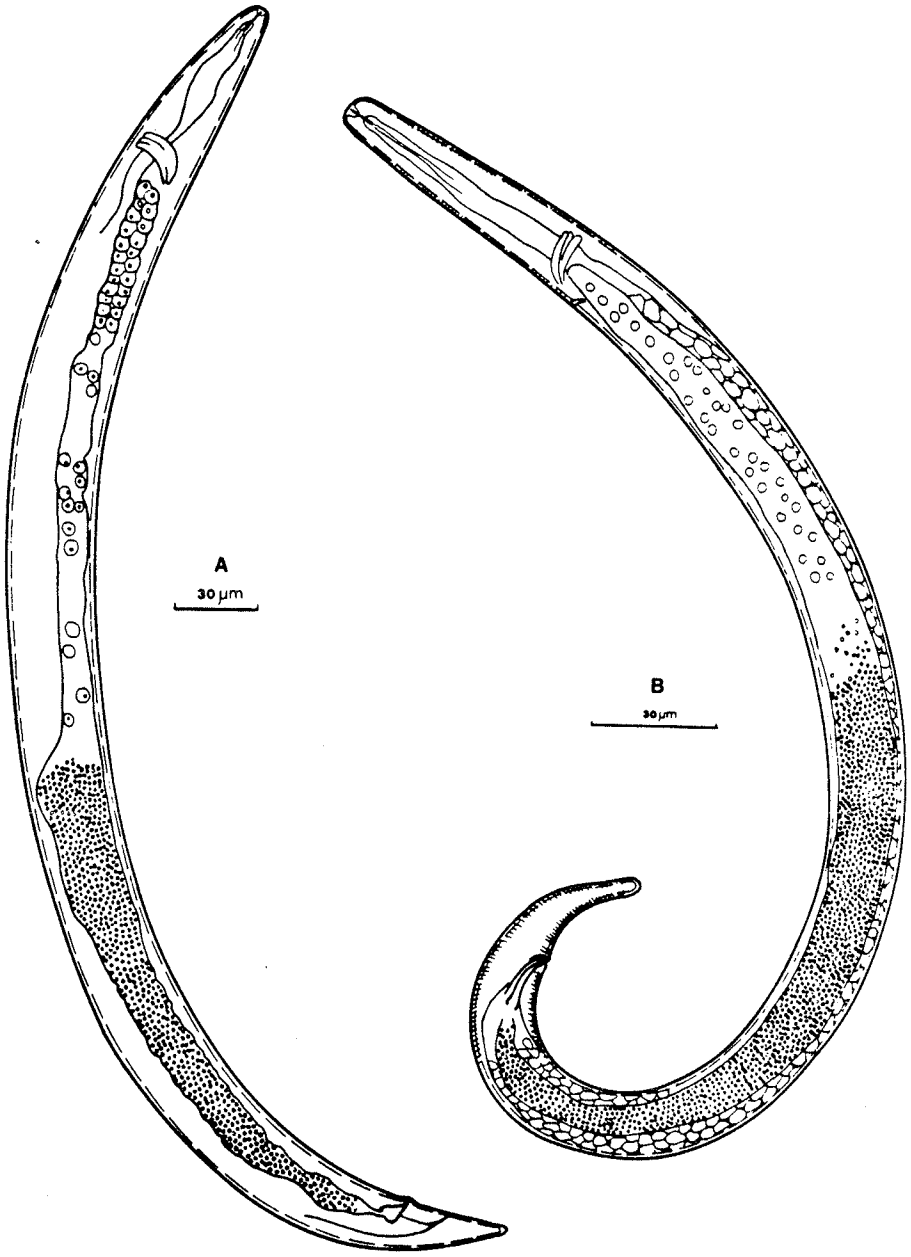



Fig. 3. Parasitylenchus caudapapilli sp. nov.: A. Fertilized female juvenile ($L_4$) of completely parasitic generation; B. male of completely parasitic generation.

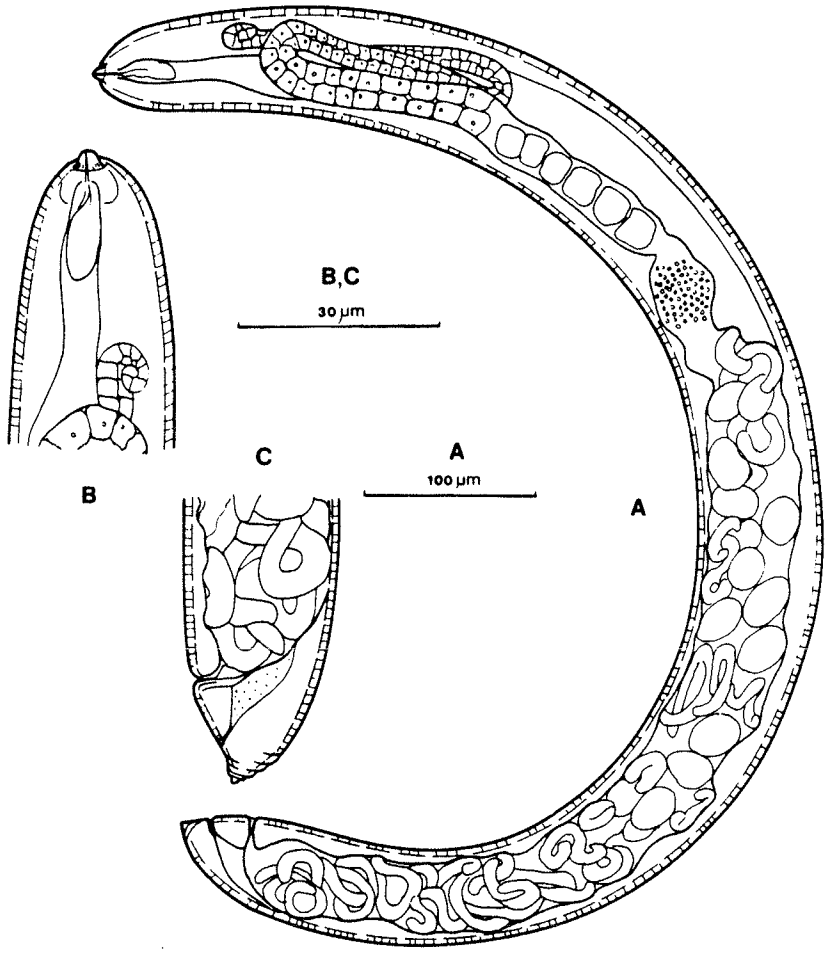



Fig. 4. Parasitylenchus caudapapilli sp. nov.: A. Adult female of completely parasitic generation; B. head; C. tail.

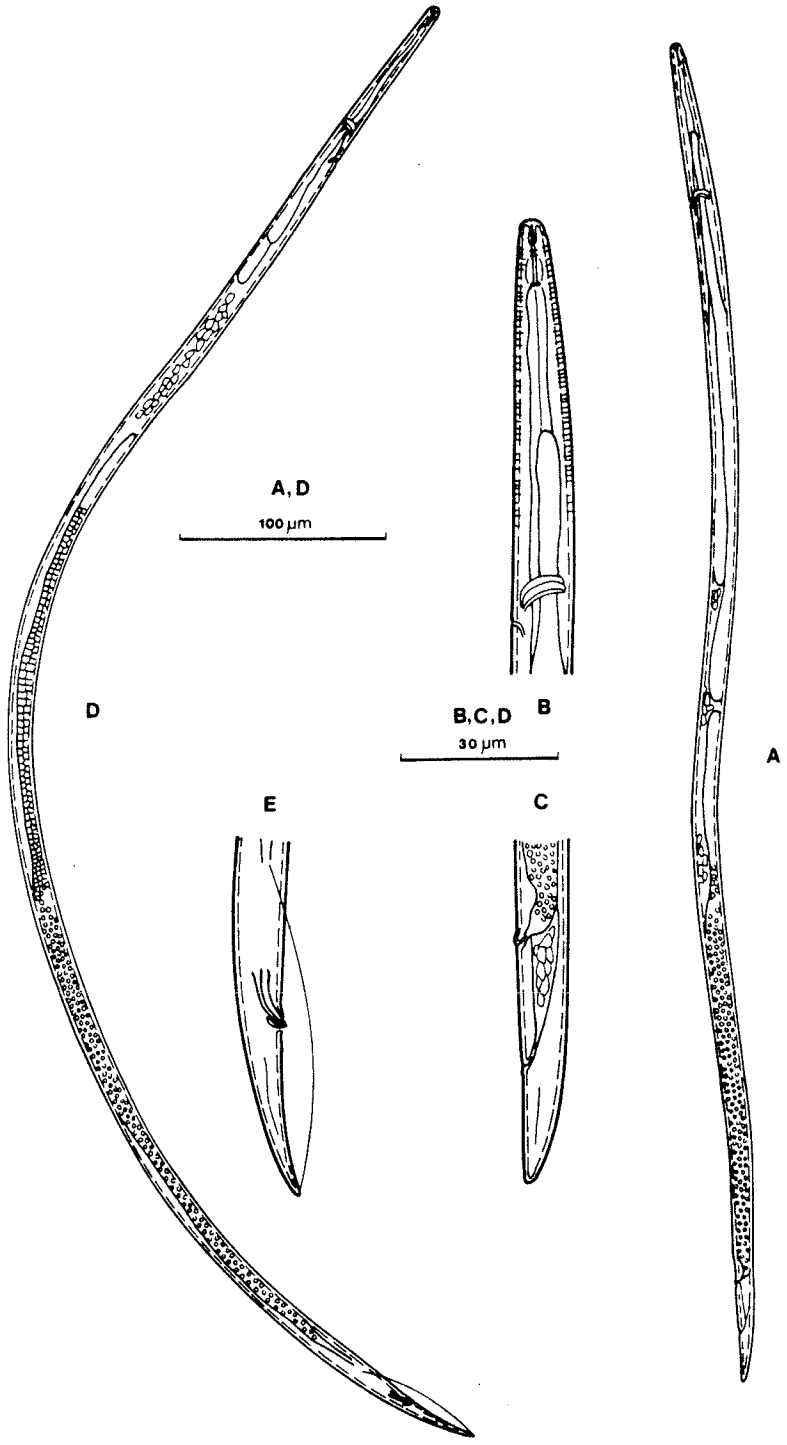



Fig. 5. Sulphuretylenchus pseudoundulatus sp. nov.: A. Free-living female; B. free-living female, head and neck; C. free-living female, tail; D. free-living male; E. free-living male, tail.

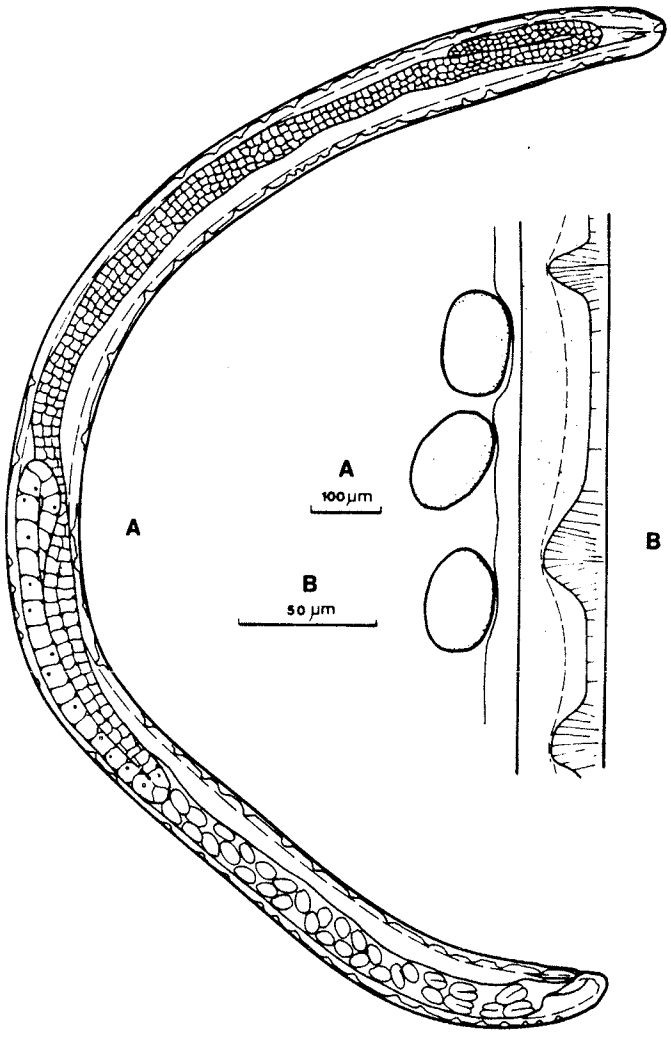



Fig. 6. Sulphuretylenchus pseudoundulatus sp. nov.: A. Parasitic female from insect pupa; B. Section of hypodermis and cuticle.

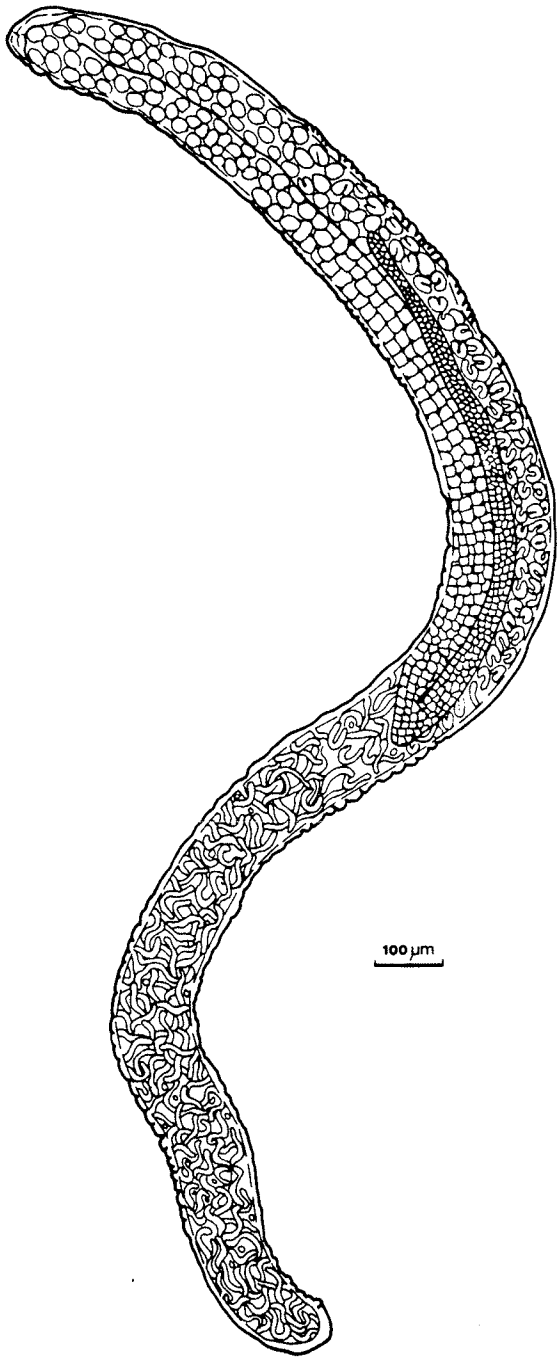



Fig. 7. Sulphuretylenchus pseudoundulatus sp. nov.: Mature parasitic female from adult beetle.

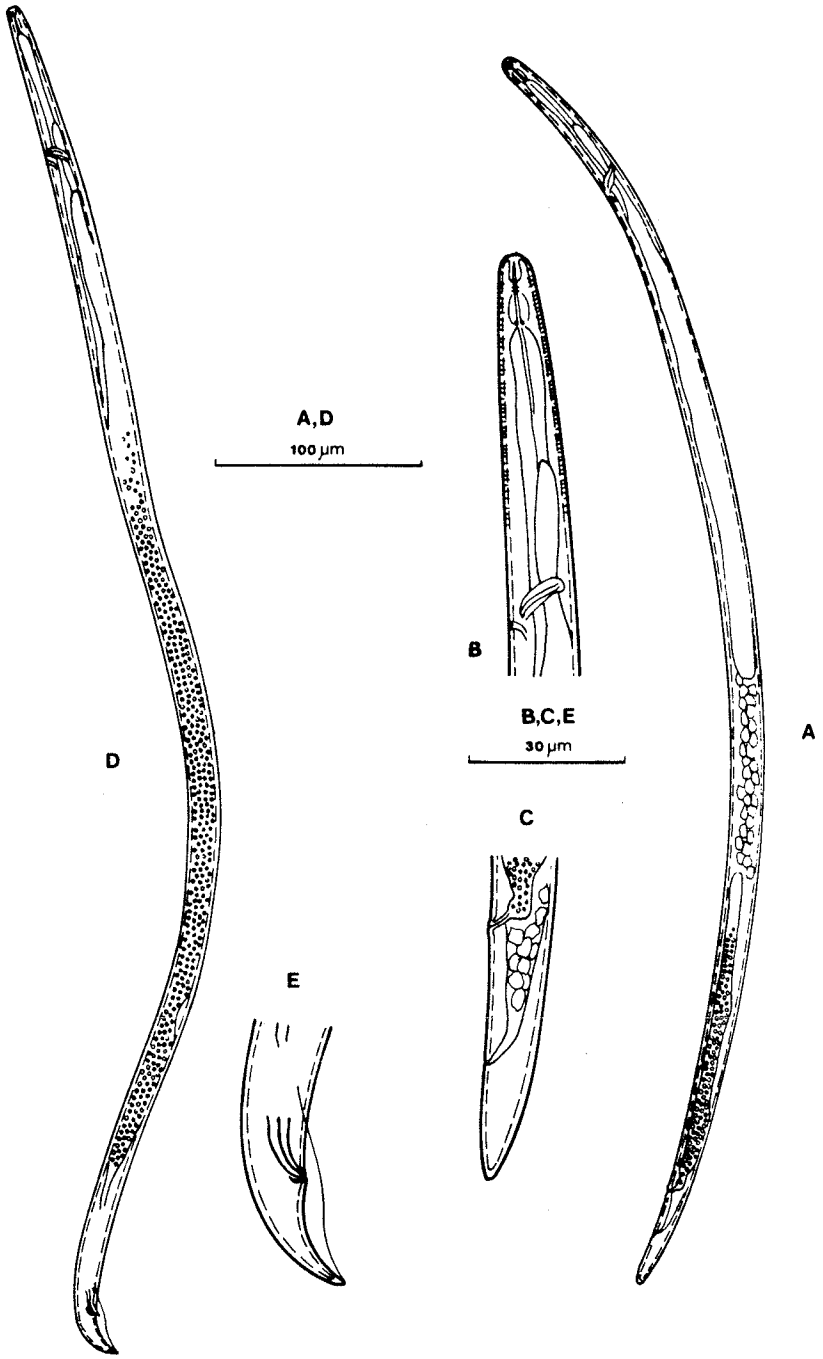



Fig. 8. Sulphuretylenchus nopimingi sp. nov.: A. Free-living femal; B. free-living female, head and neck; C. free-living female, tail; D. free-living male; E. free-living male, tail.

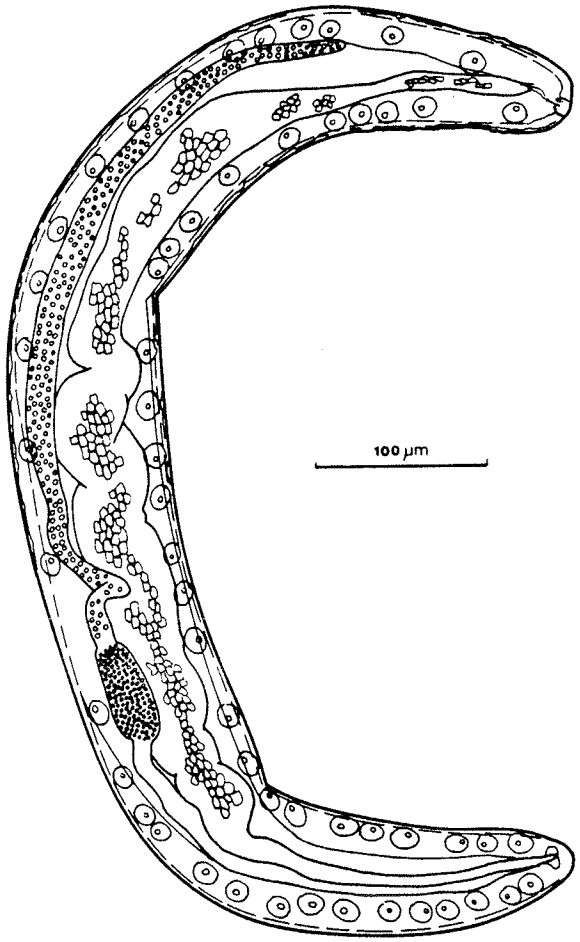



Fig. 9. Sulphuretylenchus nopimingi sp. nov.: Immature parasitic female from larval beetle.

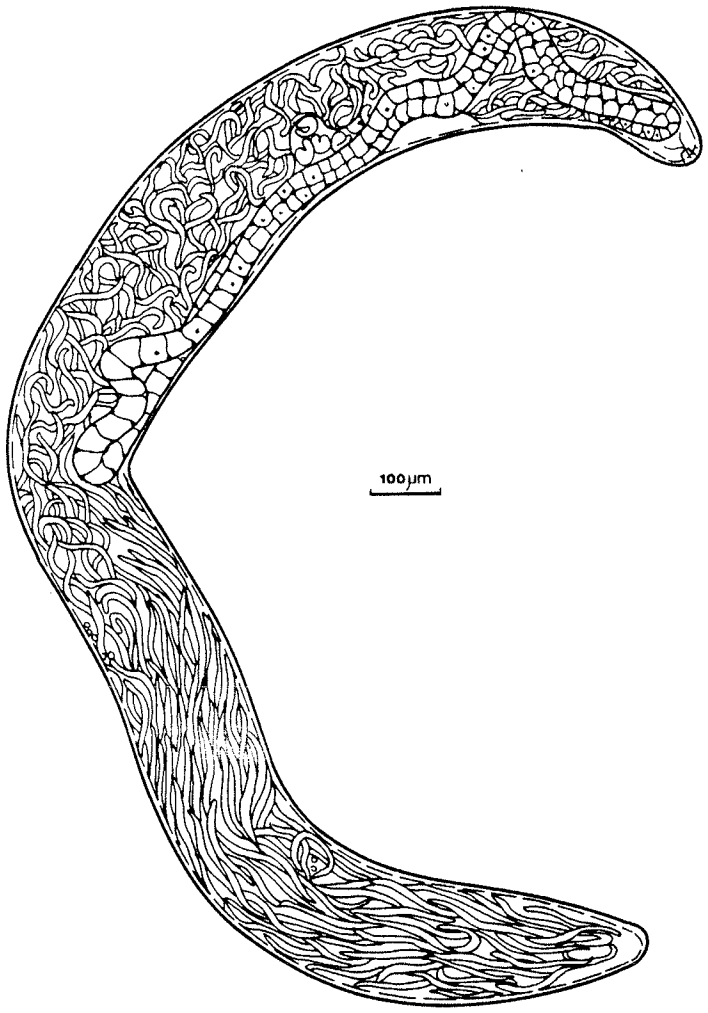



Fig. 10. Sulphuretylenchus nopimingi sp. nov.: Mature parasitic female from adult beetle.

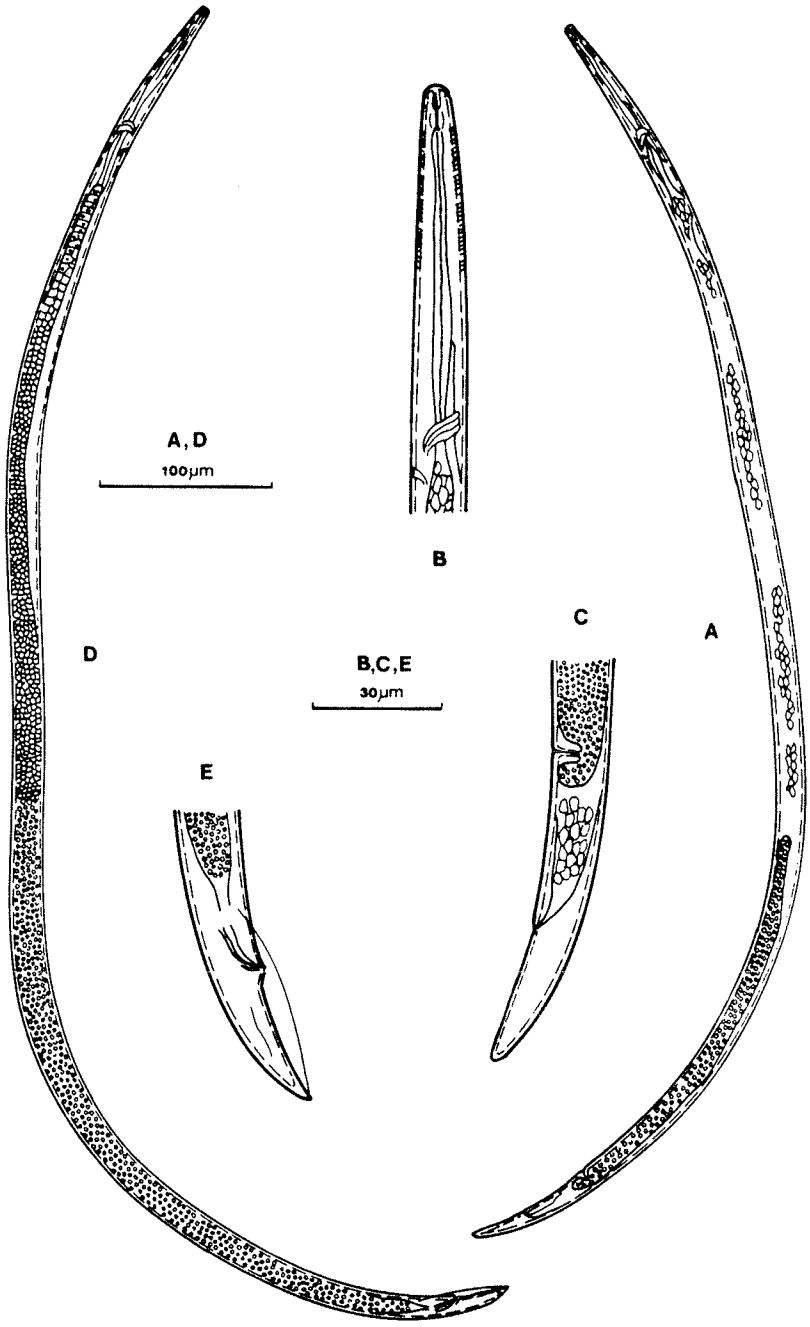



Fig. 11. Sulphuretylenchus posteruteri sp. nov.: A. Free-living female; B. free-living female, head and neck; C. free-living female, tail; D. free-living male; E. free-living male, tail.

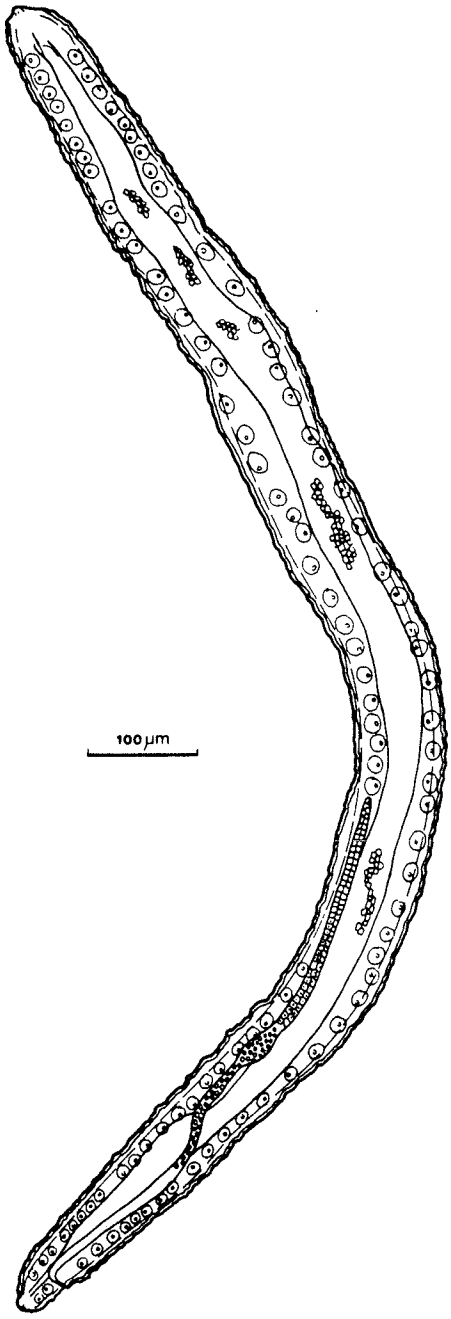



Fig. 12. Sulphuretylenchus posteruteri sp. nov.: Immature parasitic female from larval beetle.

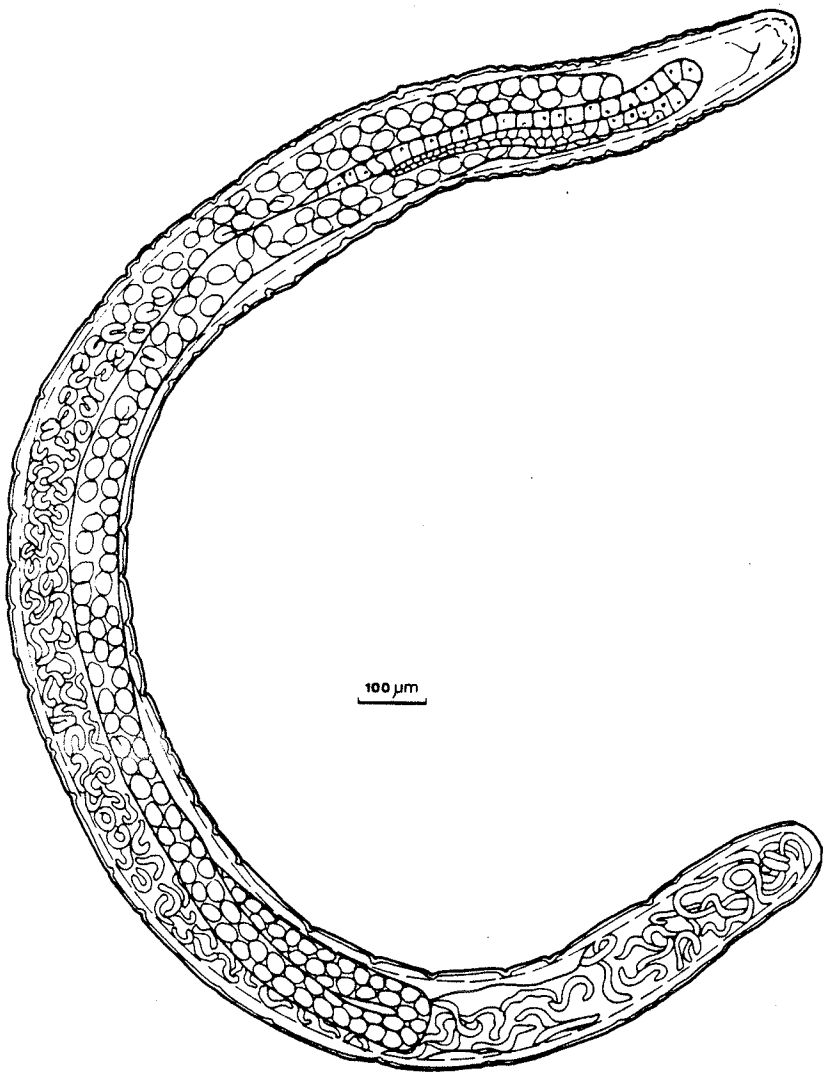



Fig. 13. Sulphuretylenchus posteruteri sp. nov.: Mature parasitic female from adult beetle.

# CHAPTER II

## PARASITISM OF *PARASITORHABDITIS OBTUSA* (Fuchs, 1915, 1937) AND *P. AUTOGRAPHI* Fuchs (1937) (Nematoda, Rhabditidae) IN THE INTESTINE OF BARK BEETLES (Coleoptera, Scolytidae).

## Introduction

Members of the genus Parasitorhabditis (Fuchs, 1937) Chitwood, 1950 are usually associated with bark beetles (Scolytidae), and less often with other wood-boring insects; Cerambycidae and Curculionidae. Biology of these nematodes was discussed by several authors, with the greatest contributions made by Fuchs (1937), Ruhm (1956), Lazarevskaya (1965), and Poinar (1972). Adult nematodes live in beetle frass, and second or third and fourth stage juveniles occur in the beetle body. The niches occupied in the insect separate the genus Parasitorhabditis into three ecological groups i.e., nematodes inhabiting insect's intestine, Malpighian tubules, or hemocoel.

Pathogencity of P. obtusa (Fuchs, 1915, 1937) and P. autographi Fuchs (1937)in bark beetle hosts is examined in the present study.

## Materials and Methods

Collection technique, preparation of nematodes for taxonomic studies, preparation of tissue for histopathological studies, and rearing of nematodes were as described earlier in Part A, Materials and Methods. Locations of collection sites are described in Appendix I.

## Results

P. obtusa in the intestine of I. perturbatus.

Prevalence of nematode infection was 76.6% (N* = 192). Intensity ranged from g - 5g nematode juveniles per intestine with a mean of 24.2 ± 14.4 nematodes per infected beetle. Juvenile nematodes invaded the intestine of immature adult beetles shortly after the pupa/imago moult, or rarely during the last larval or pupal stages. Parasites occupied

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* Number of beetles examined

both the midgut and hindgut (Fig. 1,2). Numbers of nematodes found in both sections of intestine varied significantly and ratios of intensity of infection in the midgut to that in the hindgut ranged from 0.11 - 18.0. Parasites left the host intestine when insects started construction of new egg galleries. Juveniles moulted twice and initiated a new generation in the bark beetle frass.

Histological structure of the midgut of noninfected I. perturbatus is shown in Fig. 3. Bark beetle midgut did not have a peritrophic membrane. The intestinal wall was composed of a layer of columnar epithelium with microvilli, and covered with a basement membrane. The muscle layer outside the epithelium was poorly developed and inconspicuous.

The parasite caused severe injuries to the epithelial tissue of the host intestine. Nematodes often penetrated the midgut wall and this led to the formation of numerous, long canals inside the epithelium, and lesions on the surface of this tissue (Fig. 4-7). Microvilli in the striated border of the midgut were locally degraded in various regions. Parasites penetrated epithelial cells only, while the external layer of the intestinal basement membrane remained intact. Nematodes did not penetrate tissue of the hindgut, which was lined with a thick intima (Fig. 8). In the hindgut, cellular and connective tissue structures remained unchanged. The severity of degradation of intestinal epithelium in the midgut depended on the intensity of nematode infection and increased with the number of parasites. In the hindgut no injuries were observed regardless of the numbers of nematodes present.

Histopathological examination of other organs of infected insects (i.e., gonads, fat body tissue, and muscles) did not show any

differences in structure compared to noninfected beetles. No differences in the number of eggs deposited nor in the general pattern of egg galleries were observed between galleries of infected and noninfected beetles.

Parasitic juvenile nematodes developed in bark beetles' intestines. Nematodes from immature adult beetles were smaller than those from ovipositing beetles (L = 444.5$\mu$ ± 32.6, W = 13.7$\mu$ ± 1.7 and L = 514.0$\mu$ ± 38.8, W = 16.2$\mu$ ± 1.9 respectively). No direct evidence of moult was observed, but all juvenile nematodes recovered from the intestine of adult beetles during oviposition were enveloped in double cuticle, which had started to separate at the tail region.

P. autographi in the intestine of D. autographus.

Juvenile nematodes infected the intestine of larvae ($L_3$, $L_4$), pupae or immature adult beetles shortly after the pupa/imago moult. Prevalence of nematode infection was 35.7% (N =182). Intensity ranged from 2 - 56 nematode juveniles per infected host with a mean of 21.8 ± 14.0. Parasites aggregated in the midgut (Fig. 9). Individual nematodes were occasionally found in the hindgut, especially in the earlier developmental stages of the beetles. Therefore the route of infection was probably through the anus. Nematodes emerged from the hosts to their galleries during the period of beetle oviposition. Free-living juveniles passed two moults before they reached maturity.

Histological structures of the midgut of noninfected D. autographus were as those observed in I. perturbatus (Fig. 10).

The parasite caused no detectable injury to the intestine (Fig. 11,12). Epithelial tissue remained unchanged throughout the entire

period of parasitism, and microvilli of the striated border of the midgut were unaffected. Examination of other organs (i.e., gonads, fat body tissue and muscles) showed no apparent pathological changes in their structure. Intensity of nematode infection did not influence histopathology. No effect of parasitism on egg gallery construction and oviposition was observed.

Juvenile nematodes developed in the insect intestine. Measurements of parasites from larvae and immature adult beetles and from mature adults during oviposition were not significantly different in length (L = 452.0μ± and L = 461.3μ ± 42.6, respectively), but width of nematodes differed (W = 15.2μ ± 1.3 and W = 19.3μ ± 1.6, respectively).

## Discussion

Histopathology of bark beetle intestine, caused by P. hectographi Rühm et Chararas, 1957, was reported by Rühm and Chararas (1957). Nickle (1963) also observed reduction of the epithelial layer on the ventriculus of Ips confusus (Lec.) caused by an unidentified Parasitorhabditis sp.

Parasitism of P. hectographi caused thinning of the intestinal epithelium, vacuolation of the cytoplasm of epithelial cells, and disappearance of the brush border.

Parasites probably competed with the epithelium for nutrients, and nutritional deficiences may have led to degeneration or disruption of the regeneration processes, normally taking place in the intestine of noninfected insects. Histolytic enzymes or waste products excreted by the nematodes to the lumen of the insect gut may also account for severe degradation of cells immediately adjacent to the intestinal lumen. Rühm

and Chararas (1957) did not observe direct penetration of nematodes to the intestinal epithelium of D. hectographi.

Different types of histopathological changes were observed in the present study. P. obtusa directly penetrated the intestinal wall of I. perturbatus. Lysis of cells occurred locally, only at sites of intimate contacts between the parasite head and the host tissue. Numerous histolytic enzymes are supplied by pharyngeal glands to the lumen of digestive canal of nematodes (Lee and Atkinson, 1977). In Rhabditidae, the duct of the dorsal esophageal gland opens to the lumen of the procorpus, posterior to the glottoid apparatus; subventral glands open to the lumen of metacorpus (Chitwood and Chitwood, 1974). Repeated release of previously ingested food materials through the mouth of feeding rhabditids was observed by Doncaster (1962).

A simple stoma with fused rhabdions does not facilitate an active penetration of the host tissue by means of boring or puncturing it. Localization of injuries around the nematode head supports the hypothesis of extracorporeal digestion by P. obtusa. Nematode excretions were apparently able to lyse cells, but did not act on the basement membrane of the intestine. Absence of any pathological effects in D. autographus infected with P. autographi suggests a lack of histolytic excretions of the parasite.

Individuals of both species of nematodes developed during the period of parasitism in the intestine, and nutrient was likely taken from the host intestine. This observation supports findings of Lazarevskaya (1965), who in contrast to Rühm (1956) claimed development of intestinal forms of Parasitorhabditis in the host.

Juveniles of P. autographi occupied a specific niche in the midgut; P. obtusa was less specific, occurring in both midgut and hindgut. Nematodes in both sections of the intestine developed at the same rate, but only individuals living in the midgut caused lesions in the epithelium. This fact suggests that feeding on intestinal tissue was not essential for the parasite to continue growth. It is probable that both species of Parasitorhabditis fed mainly on the contents of the host intestine. The significance of the ability of P. obtusa to lyse epithelial tissue and penetrate the intestinal wall of the host remains to be answered.

Most members of the genus Parasitorhabditis are restricted to the hindgut of their host. Only several species of Parasitorhabditis were reported from the midgut (e.g., P. curvidentis (Fuchs, 1937) Rühm, 1956, P. hectographi, P. obtusa and P. autographi). Rühm (1956) listed three species invading Malpighian tubules of bark beetles of which, according to Lazarevskaya (1965), parasitic forms were morphologically more similar to the forms from midgut than to those from hindgut. Only juveniles of P. piniperdae (Fuchs, 1937) Rühm, 1956, and P. minoris (Fuchs 1937) Rühm, 1956 penetrate the intestinal wall and develop in the hemocoel of their hosts (Fuchs, 1915, 1937; Rühm, 1956).

If we accept the hypothesis of Lazarevskaya (1965) of progressive specialization of Parasitorhabditis from intestinal forms living in the hindgut to the most specialized forms living in the hemocoel, P. obtusa would be the most advanced of intestinal forms. It lacks the final characteristic necessary to gain entry into the host hemocoel, which is the ability to penetrate the intestinal basement membrane.

Rühm and Chararas (1957) recorded high mortality of beetles infected with P. hectographi. We could not directly show a correlation between beetle mortality and the presence of parasites in the intestine, but severe injuries to the intestinal epithelium caused by the parasite may disrupt food absorption by the insect and cause death.

Histopathological studies of Rühm and Chararas (1957), Nickle (1963) and our present results showed that even closely related species of parasites, occurring in similar niches but in different hosts, can present significantly different biological characteristics.

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Figs. 1 - 8 *I. perturbatus*

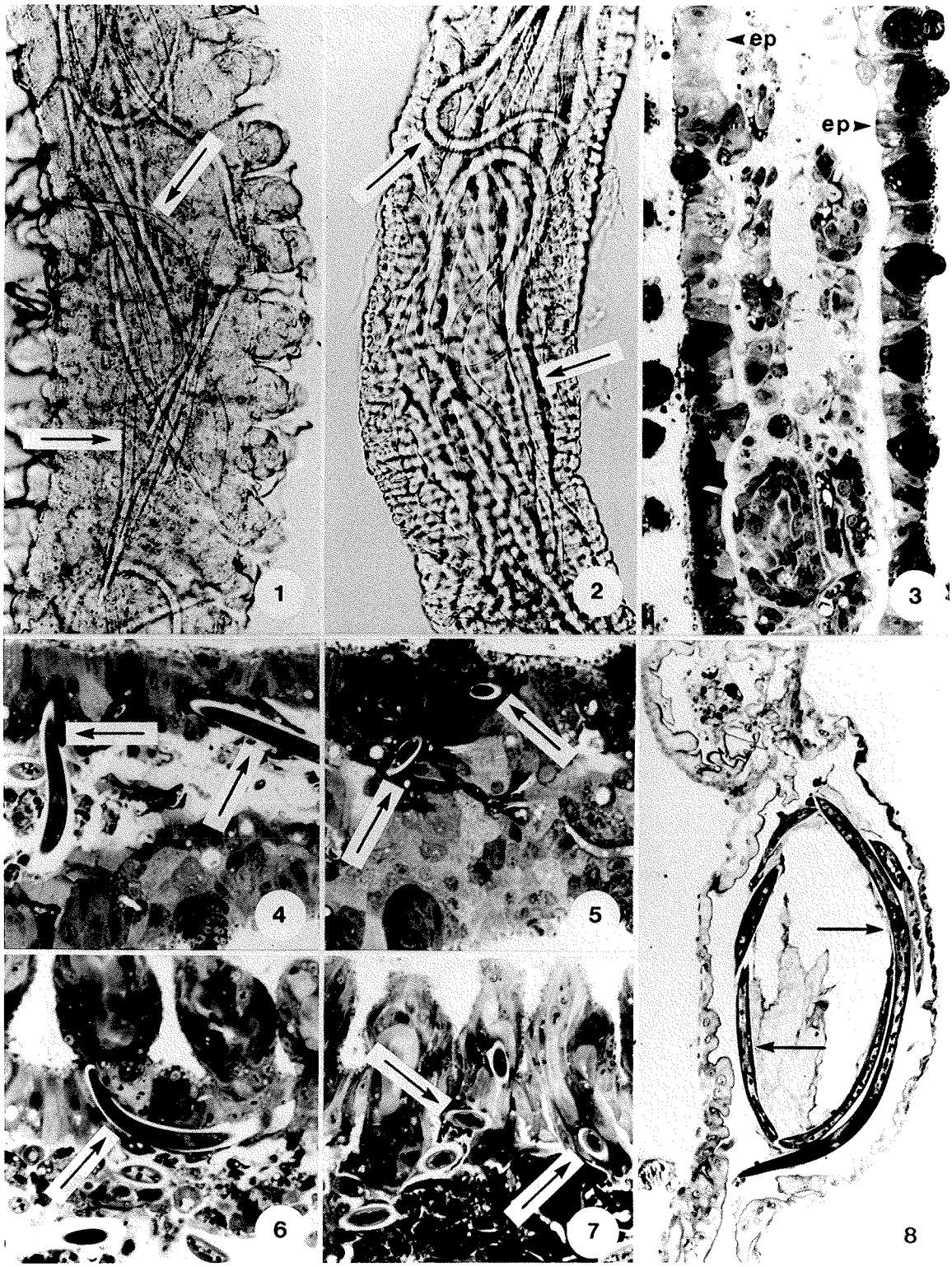
Fig. 1. Juveniles of *P. obtusa* (arrows) in midgut. Glycerin mount; x 190.

Fig. 2. Juveniles of *P. obtusa* (arrows) in hindgut. Glycerin mount; x 250

Fig. 3. Midgut of noninfected beetle; ep - columnar epithelium with microvilli. Longitudinal section; x 190.

Figs. 4 - 7 Juveniles of *P. obtusa* (arrows) in columnar epithelium of midgut. Note distinct lesions in tissue. Section; x 300.

Fig. 8. Juveniles of *P. obtusa* (arrows) in hindgut. Note lack of injuries to cuboid epithelium. Longitudinal section; x 250.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Figs. 9 - 12. *D. autographus*

Fig. 9. Juveniles of *P. autographi* (arrows) in midgut. Glycerin mount; x 300.

Fig. 10. Midgut of noninfected beetle; ep - columnar epithelium with microvilli. Longitudinal section; x 300.

Figs. 11 - 12. Juveniles of *P. autographi* (arrows) in midgut. Note lack of injuries to columnar epithelium. Longitudinal section; x 300.

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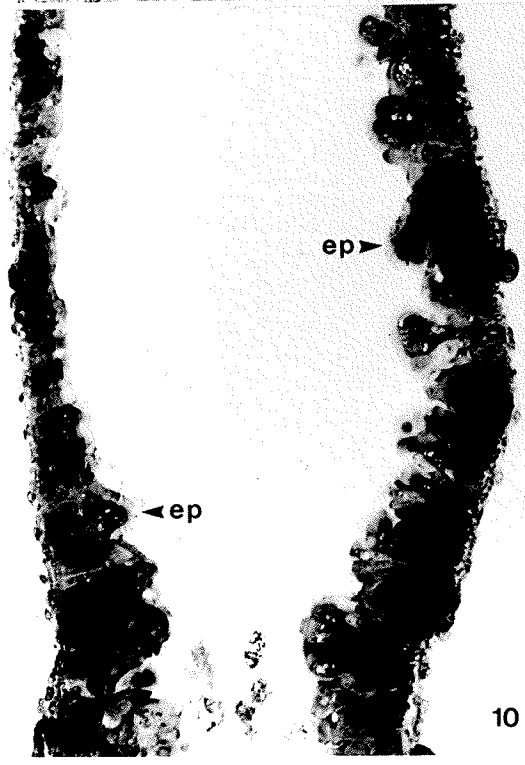


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# CHAPTER III

## PATHOGENICITY OF *APHELENCHOIDES PITYOKTEINI* Massey, 1974 (Nematoda, Aphelenchidae) IN MALPIGHIAN TUBULES OF *PITYOKTEINES SPARSUS* (LeConte) (Coleoptera, Scolytidae)

## Introduction

Nematode parasites and associates of bark beetles occupy a large variety of niches on, or in the insect body. Several species of Cryptaphelenchus (Fuchs, 1937) Rühm, 1956, and Parasitorhabditis (Fuchs, 1937) Chitwood, 1950 were reported from the Malpighian tubules of bark beetles (Rühm, 1956). Our studies revealed a high incidence of infection of Malpighian tubules in Pityokteines sparsus by juvenile Aphelenchoides pityokteini Massey, 1974. Massey (1974) classified this species as an associate of Pityokteines spp. The biology of nematodes living in Malpighian tubules of scolytids, and their ecological role remain obscure. The main objective of this study was to examine relationships between the beetle, and the nematodes living in its tubules.

## Materials and Methods

The collection technique, preparation of nematodes for taxonomic studies, rearing of nematodes, and preparation of tissue for histopathological studies were as described in the Part A, Materials and Methods. Location of the collection site is described in Appendix I.

## Results

Juveniles of A. pityokteini invaded Malpighian tubules of third instar larvae of P. sparsus. Prevalence of nematode infection was 57.7% (N = 648). Intensity ranged from 1 - 152 ($\bar{x}$ = 31.0 ± 28.6) nematodes per infested beetle.

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$\bar{x}$ = mean ± S.D.

Nematodes formed compact aggregations, usually in the distal and central portions of the tubule and they occurred throughout the whole organ only in heavily infected beetles. In individual tubules, intensity ranged from 1 - 76 nematodes. Nematodes emerged from P. sparsus during the oviposition of beetles. Juveniles moulted twice in the insect galleries before they reached maturity.

Nematodes tended to aggregate in some tubules only, while other tubules of the same host remained unoccupied. Striking differences occurred between distribution of nematodes in Malpighian tubules of larvae, and adult beetles (Fig. 1). In 9% of infected larvae, nematodes were found in all six tubules. In adult insects, juveniles of A. pityokteini were usually present in 2 - 3 tubules, and no individuals were found with all six tubules infected.

The Malpighian tubules infected with A. pityokteini showed severe pathological changes compared with tubules of noninfected beetles.

Histology of uninfected tubules.

The major part of excretory system of P. sparsus consisted of six Malpighian tubules, each ranging between 2.4 - 2.8 mm. in length. The individual tubule was composed of a circular layer of simple cuboidal epithelium covered with a basement membrane (Fig. 2,3). In cross sections of the tubule, one epithelial cell usually bordered 1/3 - 2/3 of the circumference of the lumen.

Each tubule consisted of two histologically different regions. The first, proximal to the intestine was short, and constituted 6-8% of the total length of the tubule. In this region epithelial cells had a typical honeycomb border with closely packed microvilli projecting into

the lumen of the tubule (Fig. 4,5). Cytoplasm of these cells was homogeneous, and not vacuolated. The distal part of the tubule had epithelial cells with less regularly arranged microvilli, which composed the brush border of the organ (Fig. 6,7). The cytoplasm of these cells had numerous, large vacuoles, and the nuclei were larger than in cells of the proximal part of the tubule. In the transitional region of the organ, both types of the cuboidal epithelium were sharply delineated. The tubules had approximately the same diameter throughout their length.

Histology of nematode infected tubules.

The severity of pathological changes in Malpighian tubules of P. sparsus, caused by A. pityokteini was directly related to the intensity of nematode infection. The diameter of localized regions of the tubules increased 3-5 times in the presence of aggregations of nematodes.

Tubules, where only one or a few nematodes were present, showed usually only partial degradation of the tissue, and individual epithelial cells or nuclei were still present (Fig. 8). Degradation of the epithelium was always local. Nematodes were surrounded by a large, lysed area, while more distant tissues remained intact.

The most dramatic effect of nematode parasitism in Malpighian tubules was a complete degradation of the cellular epithelium leaving only the basement membrane filled with nematodes (Fig. 9,10,11). Severe injuries occurred primarily in the distal region of tubules, where nematodes aggregated most frequently.

Epithelial cells did not regenerate. Beetles examined on different days after nematode emergence had tubules with the epithelium partially or completely absent.

No pathology was observed in the intestine, fat body tissue, gonads, and muscles of nematode infected beetles. The numbers of eggs deposited and the general pattern of galleries were also unchanged.

A. pityokteini developed in the Malpighian tubules. Measurements of nematode juveniles recovered from the larval, pupal, and adult insects varied. The sizes of nematodes from larvae and pupae were similar (l = 320μ ± 18.6, and w = 9μ ±0.5), but juveniles from adult insects were larger (l = 408μ ±13.3, and w = 15μ ±0.7). No traces of shed cuticle were found in the tubules, but both groups of nematode juveniles differed in the shape of lips and oesophagus.

Larval P. sparsus did not show any immunological responses to the presence of juvenile A. pityokteini, but 38.8% of infected adult beetles melanized the parasites. Immune reactions usually affected only a few nematodes, while the remaining parasites were intact (Fig. 12). The number of melanized nematodes increased during the infection, reaching 17-38% of the original size of the parasite infrapopulation. Melanized nematodes did not disintegrate, but remained in the tubules after the living juveniles emerged to the galleries (Fig. 13). Cross sections of melanized A. pityokteini showed that the parasites were impregnated with dark brown or black pigment.

## Discussion

Representatives of the genus Aphelenchoides Fisher, 1894 are regarded as phytophagous or mycetophagous nematodes, inhabiting soil or plant tissue (Nickle, 1970). Timm and Franklin (1969) found two marine species of Aphelenchoides. Several authors reported numerous species of this genus which are associated with bark beetles, live in their

galleries, and have a phoretic relationships with these insects (Fuchs, 1937; Rühm, 1956; Massey, 1974; Poinar, 1972). Our study showed that juveniles of A. pityokteini inhabit the Malpighian tubules of P. sparsus.

Similarities in sizes of nematodes from larval and pupal beetles, and differences between these and sizes of juveniles from adult beetles suggest that the most rapid development of nematodes occurred during the transitional period from pupae to the imago of P. sparsus. The moulting of nematodes in the host was uncertain as no shed cuticle was found in the tubules. Two moults were observed after the nematodes emerged from the host. Thus, the infective stage of A. pityokteini was probably $L_3$ juvenile.

Nematode development in the insect usually depends on uptake of nutrient from the host. Information concerning feeding of nematodes in Malpighian tubules of bark beetles is limited. Poinar (1972) stated that juveniles of Cryptaphelenchus spp. could receive nourishment from tubules, but provided no further details. Rühm (1956) suggested that Cryptaphelenchus probably lived on insect excretions. Tubular fluid consists of uric acid, urate, inorganic ions, and small amounts of amino acids and sugars (Stobbart and Shaw, 1974). Organic substances could be a potential source of nutrient. Juveniles of A. pityokteini caused extensive injuries to the Malpighian tubules of P. sparsus. Epithelial cells were degraded only in regions immediately adjacent to nematodes, and the severity of injuries was related to the numbers of parasites present. These observations suggest that juvenile A. pityokteini probably feed directly on the insect tissue, or that the nematode products are cytotoxic to the cuboidal epithelial cells.

Malpighian tubules are the prime organs carrying out excretion of uric acid and urate from the insect body. Formation of the tubule fluid and the flow of urine is dependent on the active transport of potassium and sometimes sodium ions (Ramsay, 1953, 1954, 1955; Stobbart and Shaw, 1974). Partial or complete lysis of epithelium in the tubules of P. sparsus may lead to cessation of the active transport of excretions. In injured tubules the flow of substances is probably less selective, and depends mainly on factors such as concentration gradient and properties of basement membrane.

Distribution of nematodes in Malpighian tubules differed significantly in larval and adult beetles. The life cycle of A. pityokteini was synchronized with the development of its host. None of the parasites left the beetle before the beetle matured and attacked a new tree. Hence the absence of adult beetles with all tubules infested suggests premature mortality of a part of the population of P. sparsus.

All injuries were limited to the cellular epithelium in the tubule. No lesions nor penetration of nematodes through the basement membrane were observed. Bradley (1981) found that the basement membrane in Malpighian tubules of Rhodnius prolixus (Stal.) was insoluble in sodium hydroxide and in most enzymes normally digesting vertebrate connective tissue, but was readily removed by elastase. The basement membrane consisted of long filamentous strands (Bradley, 1981). These findings suggest that elastin or elastin-like molecules are present in the basal membrane of Rhodnius tubules. If the same components occur in the basement membrane of P. sparsus, stretching of elastin filamentous strands may account for the unusual increase in the diameters of parasitized tubules.

Information concerning immune reactions of insects to their nematode parasites is abundant and was reviewed by Salt (1963) and Poinar (1974). In bark beetles, defence reactions were stimulated by several nematodes living in the host hemocoel (Fuchs, 1915; Rühm, 1956; Ashraf and Berryman, 1970a; and Nelmes and Hussain, 1972). Immune responses against parasites infecting Malpighian tubules are rare, and were observed only in insects infected with spirurids (Roubaud and Descazeaux, 1921; Alicata, 1935), and filariids (Yen, 1938; Kartman, 1953). Parasites were found in thin-walled capsules originating from the cells of the host tubular epithelium.

Defence reactions of P. sparsus were of the humoral type. Dead and melanized nematodes lay free in the lumen of tubules without any capsule or attachment to the epithelium. Origin of this reaction remains unclear. No penetration of hemocytes into tubules was observed. Nematodes were melanized in the later stage of infection, when the epithelium adjacent to the parasite was usually completely degraded. Thus defence reactions induced by the epithelium of tubules or hemocytes directly contacting with parasites can be excluded. A possible explanation would be diffusion of humoral factors from the insect hemocoel to the lumen of the tubules.

Melanization of A. pityokteini by P. sparsus differed from reactions of this type described by other authors, where the pigment was deposited only on the surface of parasites. In juveniles of A. pityokteini the brown or black pigment impregnated the nematode body.

Manifestations of the immune responses by adult beetles and not by larvae can be related to the change in the insect developmental stage as well as to the change in characteristics of nematode cuticle.

Malanization was first observed shortly after the last pupal/imago moult of beetles and coincided with rapid development of parasites. Insect immune reactions to only some developmental stages of nematodes were reported by Ashraf and Berryman, (1970) and Poinar (1974).

The defence reactions produced by P. sparsus were efficient only to some extent. Usually one or a few nematodes were melanized, while the rest remained unaffected. Welch and Bronskill (1962) observed that immune responses of Aedes aegypti (L.) to juvenile Neoaplectana carpocapsae Weiser, 1955 ceased when intensity of infection exceeded 6-7 parasites per host. In P. sparsus, immune responses occurred most often in heavily infected beetles, and the number of melanized nematodes increased during infection. This may be related to the low sensitivity of the beetle immune system to stimuli from the parasite, and probably factors such as the number of parasites and the period of infection contribute to the stimulation or the defence reaction.

The development of nematodes in the beetle, severe pathogenicity to the host organ, and beetle immune reactions presented in this study indicate that juvenile A. pityokteini are parasitic in P. sparsus.

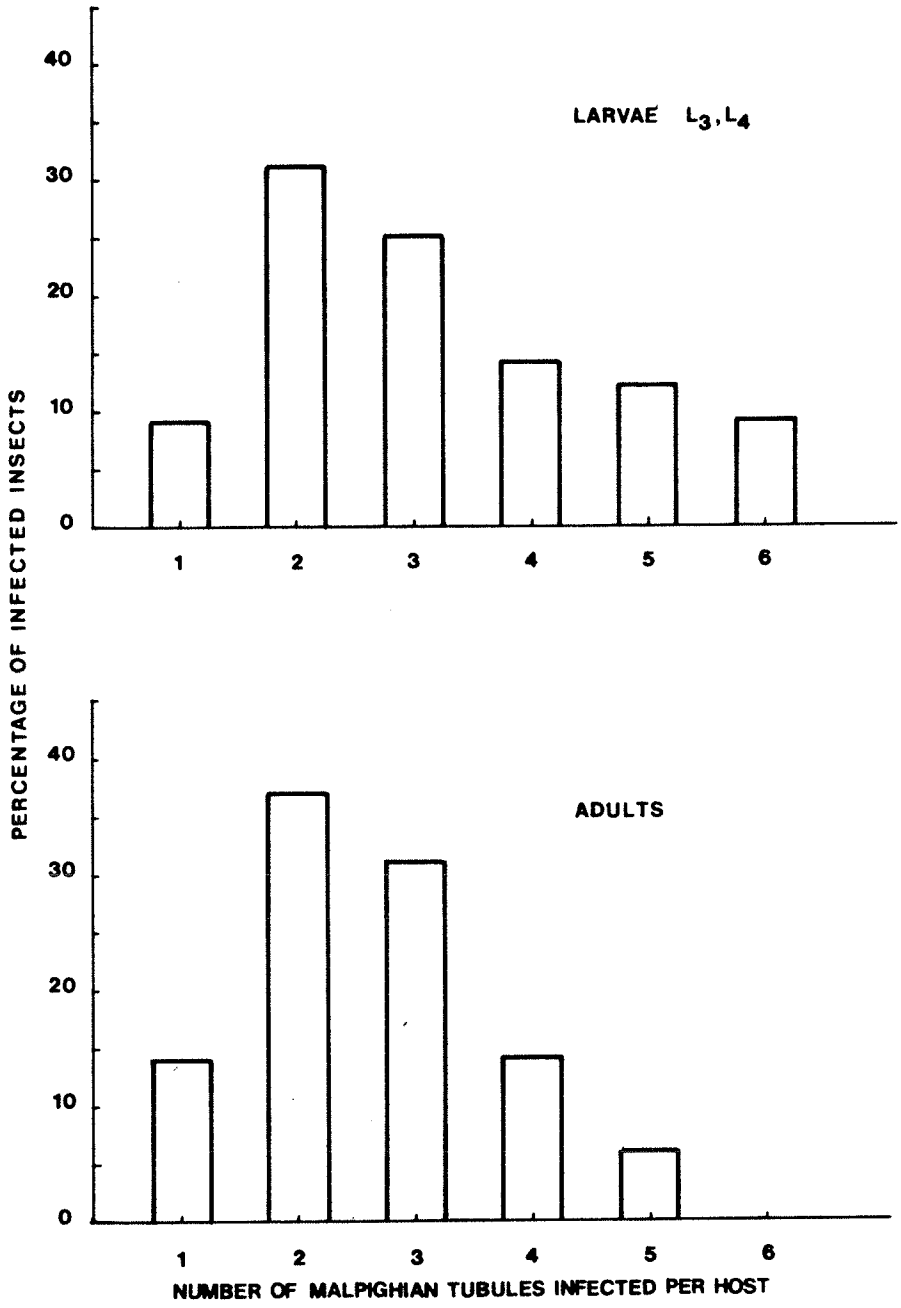


Fig. 1. Distribution of juveniles of Aphelenchoides pityokteini in Malpighian tubules of Pityokteines sparsus.

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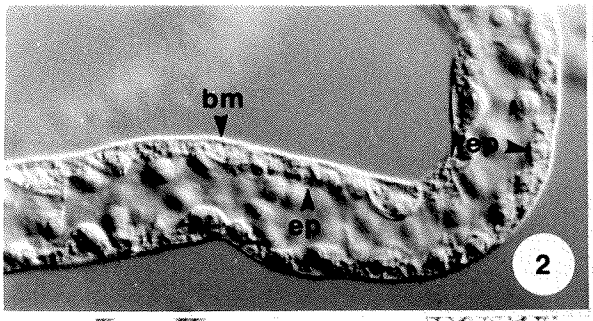
Figs. 2 - 7. *P. sparsus* - Malpighian tubules of noninfected beetles.

Figs. 2 - 3. Central (2) and terminal (3) portions of tubule; ep - cuboidal epithelium, bm - basement membrane. Glycerin mount; differential interference contrast micrograph; x 400.

Figs. 4 - 5. Longitudinal (4) and transversal (5) sections of tubule in region with honeycomb border; v - microvilli on cuboidal epithelium, n - nucleus of epithelial cell; x 400.

Figs. 6- 7. Longitudinal (6) and transversal (7) sections of tubule in region with brush border; v - microvilli on cuboidal epithelium, n - nucleus of the epithelial cell; x 400.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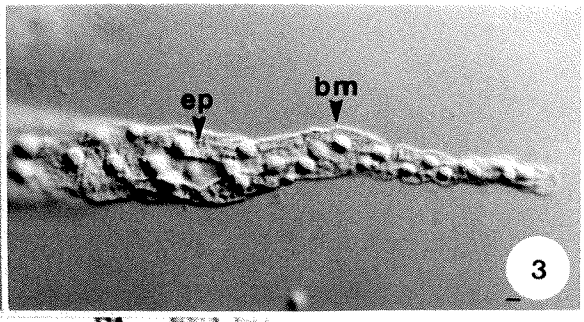
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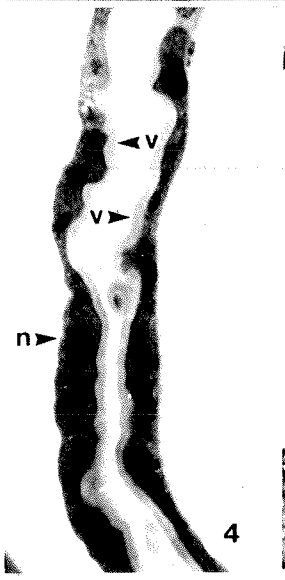
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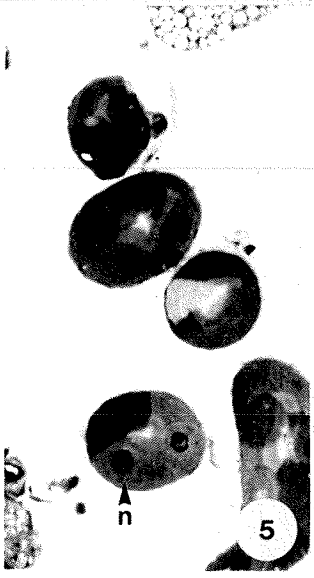
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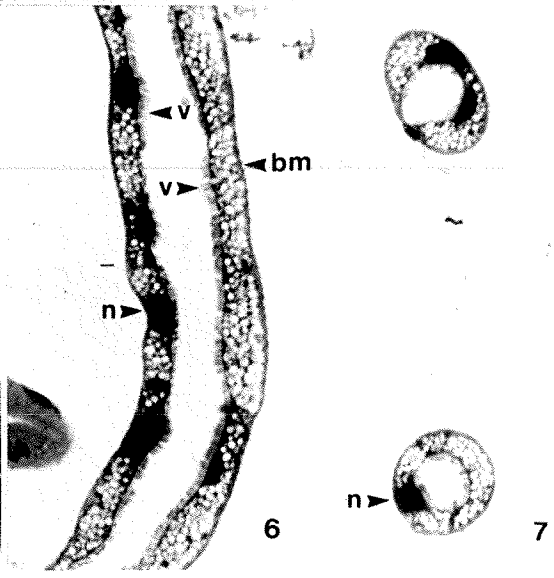
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Figs. 8 - 13. P. sparsus - Malpighian tubules infected with A. pityokteini.

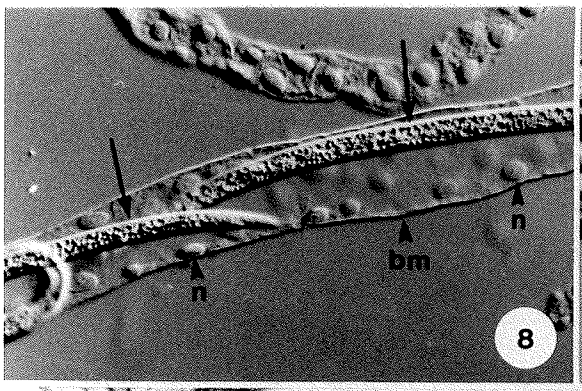
Fig. 8. Juvenile nematodes (arrows) in tubule - low intensity of infection; bm - basement membrane, n - nucleus of epithelial cell. Note distinct nuclei and lack of membranes of epithelial cells. Glycerin mount; differential interference contrast micrograph; x 230.

Fig. 9. Juvenile nematodes (arrows) in tubule - high intensity of infection. Note increased diameter of tubule and complete absence of cellular structures. Glycerin mount; differential interference contrast micrograph; x 230.

Fig. 10 - 11. Longitudinal (10) and transversal (11) sections of tubule infected with juvenile nematodes (arrows); bm - basement membrane. Note complete lack of cuboidal epithelium; x 560.

Fig. 12. Juvenile nematodes (arrows) in tubule of adult beetle prior to oviposition. Note viable (light) and melanized (black) individuals. Glycerin mount; x 230.

Fig. 13. Melanized nematodes (arrows) in tubule of ovipositing beetle; bm - basement membrane. Glycerin mount; x 230.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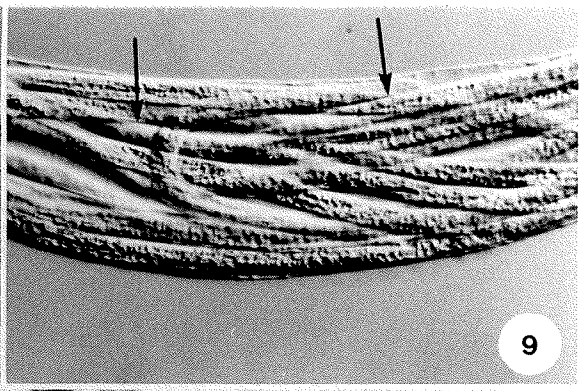
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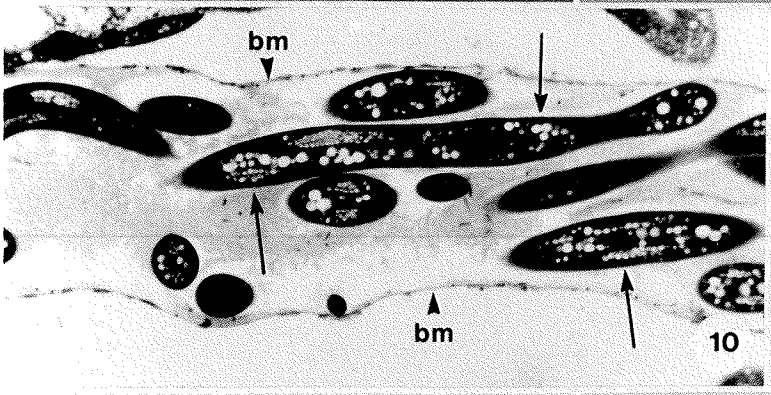
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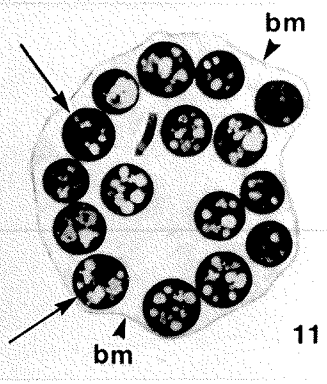
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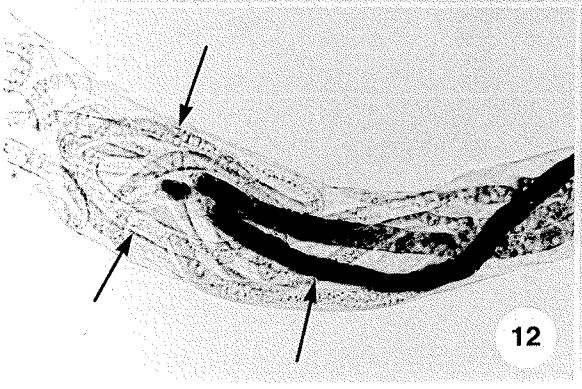
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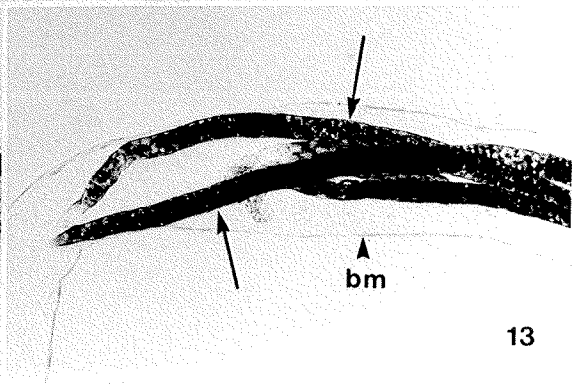
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# CHAPTER IV

## PARASITIC NEMATODE AND BACTERIA INTERACTION IN THE DUTCH ELM DISEASE VECTOR *HYLURGOPINUS RUFIPES* (Eichhoff) (Coleoptera, Scolytidae).

## Introduction

The smaller European elm bark beetle, Scolytus multistriatus (Marsh.), and the native elm bark beetle, Hylurgopinus rufipes (Eichhoff) are major vectors of Ceratocystis ulmi (Buisman) Moreau, the causative agent of Dutch Elm Disease in North America (Bright, 1976). Parasites and predators of S. multistriatus were studied by several authors (Saunders and Norris, 1961; Hunt and Hague, 1974), but little is known about the natural agents controlling H. rufipes.

In Manitoba, only H. rufipes is regarded as an important vector of Dutch Elm Disease (Buth and Ellis, 1981). Our investigations on nematode parasites of bark beetles in Manitoba included pathogens of H. rufipes, and were intended to determine the impact they have on the beetle population.

## Materials and Methods

Collection technique, preparation of nematodes for taxonomic studies, rearing of nematodes, preparation of tissue for histopathological studies, and isolation and culturing of bacteria were as described in the Part A, Materials and Methods. Location of the collection site is described in Appendix I.

## Results

Juvenile ($L_3$) Parasitaphelenchus oldhami Rühm, 1956 invaded immature adults of H. rufipes 1 - 2 days after the imaginal moult. In Manitoba this occurs in August and September. Parasites occupied the insect hemocoel. Infective juveniles were 314$\mu$± 35 in length and

16μ± 2 in width. Nematodes moulted in a few days, and measurements of the parasites that were examined 5 to 6 days after pupation were 496μ± 43 and 25μ ± 2 respectively. Nematodes overwintered in the host, remaining for about 9 months in the hemocoel. During this period, only a slight increase in nematode size, and no apparent changes in its morphology were observed. Parasites left the host in early summer during oviposition. In the insect galleries nematode juveniles moulted once before they reached maturity.

Prevalence of nematode infection changed with time. In samples of immature beetles collected when they emerged from larval galleries, 92.5% of these insects were infected with P. oldhami (N = 396). During the winter this number declined significantly, and only 26.2% of beetles from the same population were infected with P. oldhami when examined next spring, during oviposition (N = 340).

Intensity of infection ranged from 1 to 113 juveniles per host in immature adult beetles, and 1 to 28 nematodes per host in mature adults during oviposition.

Histology of noninfected H. rufipes.

All larval, pupal and adult H. rufipes had rod- shaped bacteria in their tissues. The bacteria was transmitted from the female beetle to its progeny. Large aggregations of these microorganisms were found in syncytial cytoplasm of the tropharia in female gonads (Figs. 1 & 2). During further oogenesis and vitellogenesis clumps of bacteria were found inside developing oocytes (Fig. 3). In regions occupied by rods,

cytoplasm was locally altered, while in remaining parts of oocytes normal deposition of proteins and lipids occurred. The shape of developing oocytes and all characteristics of follicular epithelium were unchanged when compared with other bark beetles.

Rod-like bacteria recovered from gonads of ovipositing female beetles were Gramm negative (Fig. 4). Repeated attempts of culturing of these microorganisms in the bacteriological Tripticase soya broth, blood agar, chocolate agar, and MacConkey's agar incubated at both 20°C and 37°C were unsuccessful. This made it impossible to identify the species under study.

In the insect hemocoel, most of the spaces not occupied by other organs were filled with regularly formed fat body tissue (Fig. 5). Bacteria were localized in separate sites in this tissue (Fig. 6). Rods occurred inside the cells of fat body, forming small clumps. No bacteria were found outside the cells, circulating free in the hemocoel or invading other organs. Despite the presence of bacteria, cytoplasm of cells infected with these microorganisms did not differ from the cytoplasm of noninfected cells, and had numerous granules of lipids, glycogen and proteins. No other organs (intestine, muscles and Malpighian tubules) showed any changes that could be related to the presence of bacteria.

Mature adult beetles examined during oviposition had reduced total volume of fat bodies but large clusters of this tissue were still present. In all specimens aggregations of bacteria remained in the fat body tissue cells, and their form was unchanged.

Histopathology of H. rufipes infected with P. oldhami.

Fat body tissue was rapidly altered in bark beetles infected with nematodes. Parasites occupied the hemocoel of the host and they directly penetrated fat body (Fig. 7). Nematodes moving in the tissue had ruptured cell membranes which led to the release of bacteria from their normal sites. During early stages of infection, numerous rods occurred inside the cells and in the intercellular spaces, especially in the regions surrounding nematodes (Fig. 8). Active movement of parasites led to spreading the bacteria from a few limited sites to the entire fat body tissue. The total volume of this tissue was rapidly reduced (Fig. 9), and characteristics of the cells changed. Numerous lipid and protein granules, typical in noninfected cells disappeared at this stage of infection (Fig. 10). The cytoplasm of the remaining fat body cells was packed with bacterial rods.

The extent and rate of degradation of fat body tissue was related to the number of nematode parasites. The most rapid process of tissue degradation was observed in the beetles heavily infected with nematodes, shortly after parasites invaded the host. Fat body was almost completely lacking in the beetles recovered from overwintering sites in October.

## Discussion

Oldham (1930) gave the first outline of the biology of P. oldhami. This author incidently described immature stages of P. oldhami as juveniles of Parasitylenchus scolyti Oldham, 1930. Rühm (1956) established P. oldhami as a separate species, but based his description on juvenile stages. More detailed studies on the biology of this species were carried out by Saunders and Norris (1961), and Hunt and

Hague (1974). The latter redescribed P. oldhami, including characteristics of free-living adult nematodes.

In all previous studies P. oldhami was recorded from elm bark beetles S. scolytus, and S. multistriatus. In our investigations this nematode was found to infect another elm bark beetle, H. rufipes.

Rühm (1956) stated that Parasitaphelenchus Fuchs, 1929, without exception parasitized larval bark beetles. Parasitism of P. oldhami. only in adult H. rufipes does not support that statement.

Significant differences in nematode bionomics were observed in both groups of hosts. The present studies show that juvenile nematodes invade immature adults H. rufipes. In S. scolyti and S. multistriatus parasites were already present in the beetle larvae (Hunt and Hague, 1974). Bionomics of hosts apparently influenced bionomics of the nematode.

Species of the genus Scolytus Geoffrey differ from most other bark beetles in that they always overwinter as larvae. H. rufipes usually overwinter as immature adults. In both groups of hosts, nematodes developed rapidly for a few days after infection, moulted to the $L_4$ juvenile stage, and ceased development. They remained almost unchanged for the rest of their life in the insect hemocoel. Thus, during this period of suspended development, which usually lasted for about 9 months, nutritional dependence of the parasite on the host was probably insignificant.

Penetration into the host hemocoel prior to the adverse winter conditions establishes the parasite in a more stable environment than the beetle gallery. Internal mechanisms of insects control water

crystalization in the beetle tissue, prevent it from frost damage, and may also have a protective value for the parasite. Thus, infection of earlier developmental stage of S. scolytus and S. multistriatus, than those of H. rufipes may be an adaptation to improve overwintering conditions for the nematode.

All nematode parasites of bark beetles, even those with long free-living phases in their life cycles, overwinter in the host. Altered bionomics of P. oldhami, which ensured overwintering of the nematode in the host hemocoel, was probably a necessary adaptation permitting the exploitation of several host species with different life cycles. This emphasizes the ecological significance of the host body as an overwintering site.

Life cycle of P. oldhami infecting larval beetles, as described by Saunders and Norris (1961) was considered as typical for the genus Parasitaphelenchus (Poinar, 1972). Significant differences in the parasite life cycle, depending on the biology of the host infected suggest, however, that it cannot be regarded as typical.

The normal role of rod- shaped bacteria in the fat body tissue of H. rufipes remains unclear. In our investigations none of 14 other species of bark beetles studied contained similar bacteria. The well established route of vertical transmission of the bacteria from one generation of beetles to the next indicates a long coexistence of these organisms. The absence of pathological effects in the host, occupation of restricted sites in the fat body tissue, and 100% prevalence of infection suggest a high degree of adaptation by the bacteria to H. rufipes. Mutalistic associations of bacteria and fungi with insects were recorded by several authors (Koch 1955; Brooks, 1963). These

organisms were often localized in special cells i.e. mycetocytes of the fat bodies. They probably supply the host with essential vitamins, amino acids, and other substances (Brooks, 1963; Chapman, 1971). This type of relationship between rod- shaped bacteria and H. rufipes is possible, but further studies are necessary to substantiate this.

The addition of the nematode to the H. rufipes - bacteria system dramatically changes a hitherto harmless coexistence. Juveniles of P. oldhami fed and depleted the insect fat body tissue. Parasites ruptured cell membranes in the fat body releasing bacteria from the original, limited sites. Movements of nematodes facilitated spreading of these microorganisms throughout the entire tissue. The remaining fat body tissue was degraded by the bacteria. Rapidly disappearing granules of fat, glycogen and proteins, in cells densely packed with bacteria, indicated a progressive pathological processes.

Depleted volume of fat body tissue and changes in structure of individual cells influenced viability of insects. Significant differences between the prevalence of nematode infection of beetles in the autumn and during the following spring suggest a dramatic decline in the population of H. rufipes. The fat body is the prime tissue of storage and metabolism of energetic and organogenic substances in the insect (Wigglesworth, 1965; Chapman, 1971). Heavily infected beetles, partially or completely deprived of this tissue seemed to have a limited potential to fly to new sites and survive the overwintering period. The part of H. rufipes population, that had successfully overwintered, consisted of noninfected insects and of insects with low nematode burden. Thus the population of H. rufipes was controlled by synergistic interaction of normally harmless rod bacteria and parasitic nematodes.

Despite its long recognition, Dutch Elm Disease remains one of the most devastating diseases affecting trees, especially those of city parks and streets. For decades numerous attempts to control both the pathogenic fungus and its insect vectors were undertaken in Europe and North America. Studies on biological control agents were conducted by many authors (Doane, 1959; Barson, 1976; Doberski, 1981; Strobel and Myers 1982; Weber, 1982, and others). Nonspecific nematode parasites Neoplectana carpocapsae Weiser, 1955 were tested against S. scolytus (Finney and Mordue, 1977; Finney and Walker, 1979), and against H. rufipes (Tomalak and Welch, 1982). No radical cure or control of Dutch Elm Disease was found. Small scale tests yielded promising results, but wider application usually were less successful. Although our present findings do not solve this problem, they show the great potential of natural, biological agents controlling pest population. Naturally occurring parasitic nematode - bacteria synergism appears to play an important role suppressing (or controlling) populations of H. rufipes. If we elucidate and manage the ecological factors limiting proliferation of the nematode component of this pathological complex, effective control of H. rufipes, the important vector of D.E.D. will be more probable.

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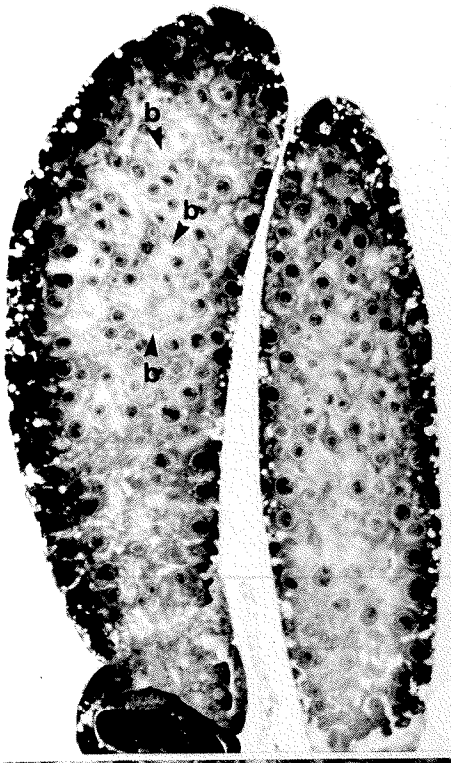
Figs. 1 - 4. *H. rufipes* - Adult Beetles

Fig. 1. Tropharia of mature beetles; b - aggregations of bacteria (light areas) in cytoplasm. Longitudinal section; x 210.

Fig. 2. Aggregations of bacteria (light areas) in syncytium of the tropharium; b - bacteria, n - nurse cell nucleus. Section; x 560.

Fig. 3. Aggregations of bacteria (arrows) in cytoplasm of developing oocytes. Longitudinal section of vitellarium x 240.

Fig. 4. Rod-shaped bacteria (b) isolated from beetle oocytes. Smear; Gramm stain; x 740.

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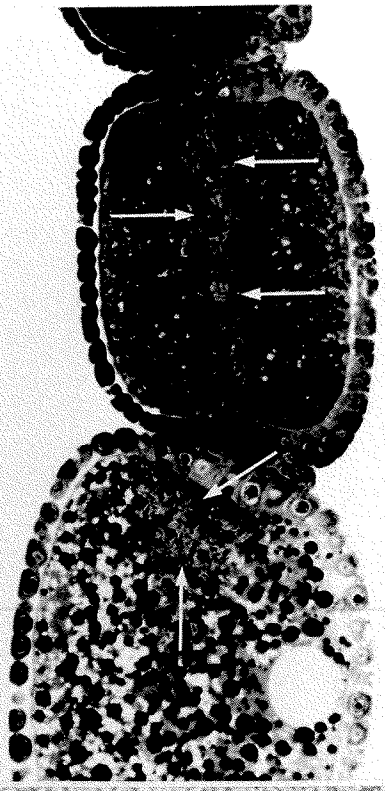

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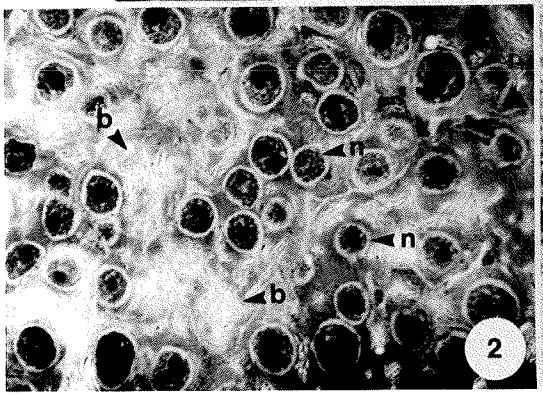


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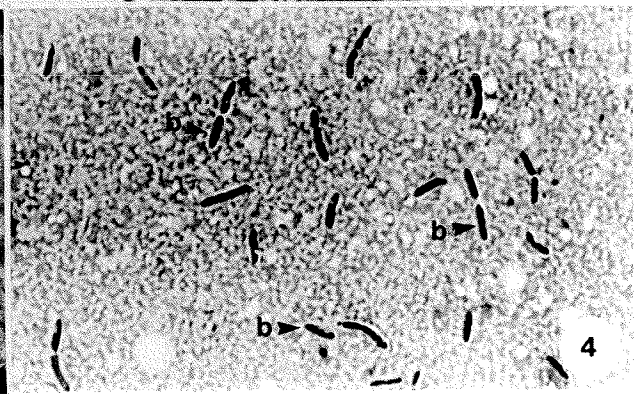


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Figs. 5 - 10. H. rufipes - Adult Beetles

Fig. 5. Sagittal section of abdomen of beetle not infected with nematodes. Note fat body tissue present in spaces between other organs. x 190.

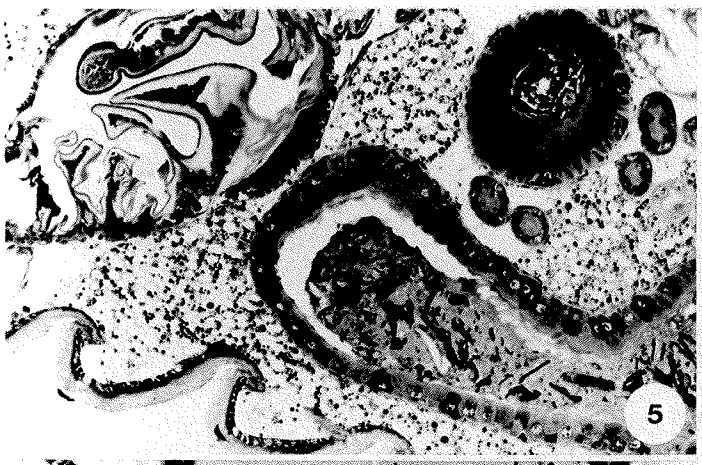
Fig. 6. Fat body tissue in abdomen of beetle not infected with nematodes. Note small aggregations of bacteria (b) in the cytoplasm of trophocytes. Section; x 500.

Fig. 7. Juveniles of P. oldhami (arrows) in beetle abdomen - early stage of infection. Transversal section; x 190.

Fig. 8. Juveniles of P. oldhami (arrows) in fat body tissue - early stage of infection. Note numerous aggregations of bacteria (b) surrounding nematodes. Section; x 500.

Fig. 9. Juveniles of P. oldhami (arrows) in beetle abdomen - late stage of infection. Note almost complete reduction of fat body tissue (ft). Sagittal section; x 190.

Fig. 10. Fat body tissue (ft) in late stage of nematode infection Note cytoplasm of fat body cell being almost completely filled with rod-shaped bacteria (b). Arrow indicates juvenile of P. oldhami.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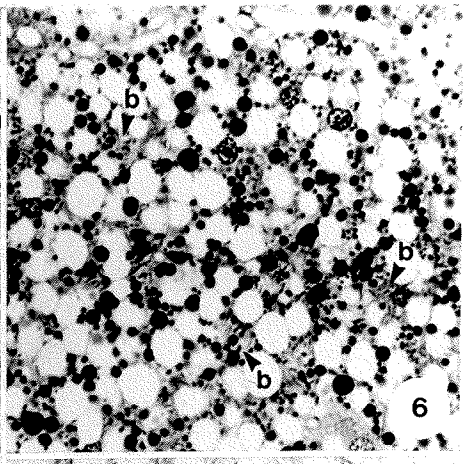
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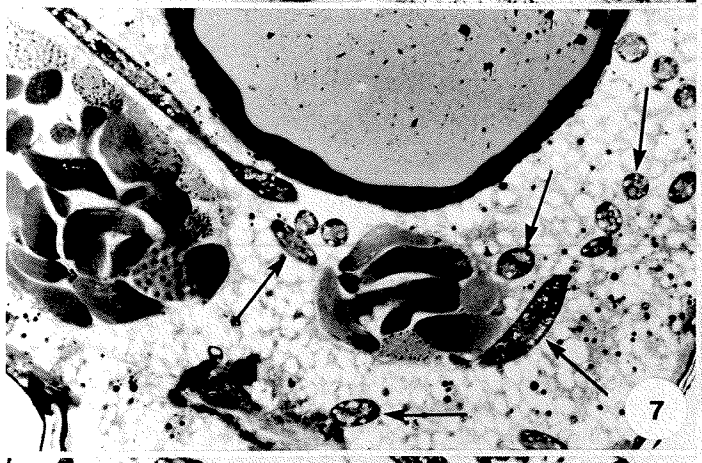
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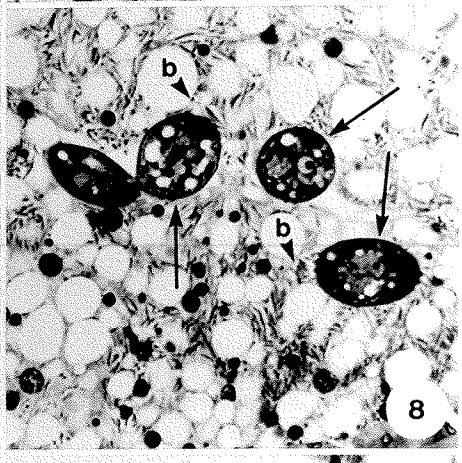
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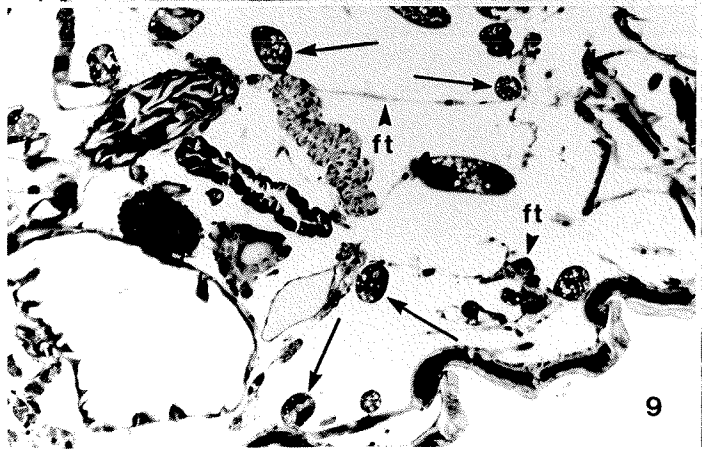
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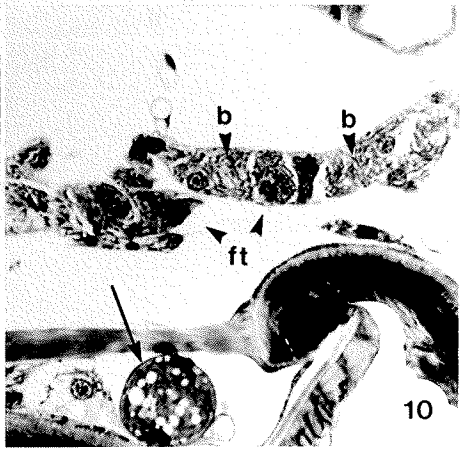
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# CHAPTER V

## ENDOPARASITISM OF

## *BURSAPHELENCHUS FRAUDULENTUS* Ruhm, 1956

## (Nematoda, Aphelenchidae)

## IN *TRYOPOHLOEUS POPULI* Hopkins

## (Coleoptera, Scolytidae)

## Introduction

Nematodes belonging to the genus Bursaphelenchus Fuchs, 1937 are often found in galleries, or on the body of bark beetles and other wood inhabiting insects (Fuchs, 1937; Rühm, 1956; Nickle, 1970; Poinar, 1972; Massey, 1974). According to Rühm (1956) only "dauerlarvae" of these nematodes are directly associated with beetles. They were frequently observed on the surface of the beetle body, forming small white aggregations in folds between abdominal segments, or in sack-like structures under the elytra of beetles. Free-living forms are probably mycetophagous (Nickel, 1970).

Juveniles of Bursaphelenchus fraudulentus Rühm, 1956 frequently infected the hemocoel of larval, pupal, and adult Trypophloeus populi Hopkins. Further histological studies to elucidate the ecological role of this nematode were undertaken.

## Materials and Methods

Collection technique, preparation of nematodes for taxonomic studies, rearing of nematodes, and preparation of tissue for histopathological studies were as described in the Part A, Materials and Methods. Location of the collection site is described in Appendix I.

## Results

Juvenile nematodes invaded third instar larvae of the beetle. Invasive juveniles were 246$\mu$ ± 10 long, and 12$\mu$ ± 1 wide. In some $L_3$, all $L_4$ larvae, pupae and adult insects, nematodes were 330$\mu$ ± 13 long and 16$\mu$ ± 1 wide. No traces of shed cuticle were found, but the sudden change of nematode sizes probably followed a moult which took place

shortly after parasites invaded the host. Nematodes left the beetle during oviposition. They moulted once before reaching maturity.

Prevalence of nematode infection was 82.2% (N = 206). Intensity varied significantly and usually ranged between 10 - 30 nematodes per host.

Histology of noninfected insects did not differ from that of other bark beetles. The regions of the hemocoel of larvae, pupae and immature adult beetles which were not occupied by the intestine, muscles and other organs, were filled with fat body tissue. In mature adult beetles, examined just prior to, or during oviposition, the amount of fat body was reduced, and large clusters of this tissue occurred only in the abdomen and sometimes in the metathorax. In these insects, especially in ovpositing females, the gonads occupied most of the hemocoel.

Numerous parasites were observed in the hemocoel of nematode infected insects. In beetle larvae, pupae and immature adult nematodes penetrated fat bodies by pushing and possibly digesting their way through the tissue (Fig. 1). Lesions of the fat body tissue were always local, in the regions immediately adjacent to the nematode.

In mature beetles, where the volume of fat bodies was reduced, nematodes moved freely in the hemocoel, usually occupying spaces between the remaining fat body tissue and other organs (Fig. 2,3). In the sites of close contact between nematodes and fat bodies, this tissue was characteristically invaginated or locally digested (Fig. 2). Parasites rarely penetrated deeper in this tissue causing local lesions (Fig. 4). The total reduction of the fat body caused by parasites was insignificant despite the long period of existence of B. fraudulentus in the hemocoel of T. populi (usually 10 months).

Tissues of other organs (intestine, muscles, and gonads) were not altered when compared with noninfected insects. No apparent differences were found in the development and reproduction of parasitized and nonparasitized beetles.

## Discussion

Nematodes belonging to the genus Bursaphelenchus are classified as insect associates living on the surface of their body and in the frass of larval galleries (Fuchs, 1937; Ruhm, 1956; Massey, 1974). The insect hemocoel is a hitherto unknown niche occupied by members of this genus. Attainment of this niche by B. fraudulentus suggests the possession of undescribed yet in Bursaphelenchus ability to penetrate the intestinal wall or integument of insect larva. Direct passage of the nematode into the hemocoel of T. populi was not observed. This could have occurred through either the intestine or the integument since both routes are utilized by other nematode parasites of insects.

Members of the genus Bursaphelenchus are probably mycetophagous when free-living in the bark beetle galleries (Nickle, 1970). The method of food uptake by phoretic forms, which usually live on the beetle body is unknown. Among nematodes normally occurring on the body of scolytids the uptake of nutrients from the host was documented only in Ectaphelenchus dendroctoni Ruhm, 1956 (Kurashvili et al., 1964). Besides external parasites the genus Ectaphelenchus (Fuchs, 1937) Skrjabin et al., 1954 includes also E. larici Lazarevskaya, 1963 which penetrates the intestine of bark beetles (Lazarevskaya, 1963), and E. skrjabini Lazarevskaya, 1962, which parasitizes the beetle hemocoel (Lazarevskaya, 1962). B. fraudulentus is the only species of

Bursaphelenchus known to occur in the bark beetle hemocoel. Nematodes developed in the host. Parasites increased in size after a short period of living in the beetle hemocoel which suggests uptake of nutrient from the host.

The most rapid growth of the nematodes occurred in the beetle larvae where most of the hemocoel was filled with fat body tissue. Local lesions in fat body were caused by direct contact with B. fraudulentus and occurred around the nematode.

The overall extent of injuries to the host was limited. Only fat body was affected and no apparent influence of the nematode activity on other organs and the rate of development of infected beetles was observed. This pathology corresponds to injuries caused by other aphelenchids, where mainly fat body was depleted in the hemocoel (Ruhm, 1956). Oldham (1930) recorded significant reduction in the size of gonads of Scolytus scolytus Fabr., and Scolytus multistriatus Marsh., as a result of infection with Parasitaphelenchus oldhami (Ruhm, 1956), but other authors did not confirm that finding.

The present observations of nematode development in the beetle hemocoel suggest that B. fraudulentus should be classified as a parasite of T. populi.

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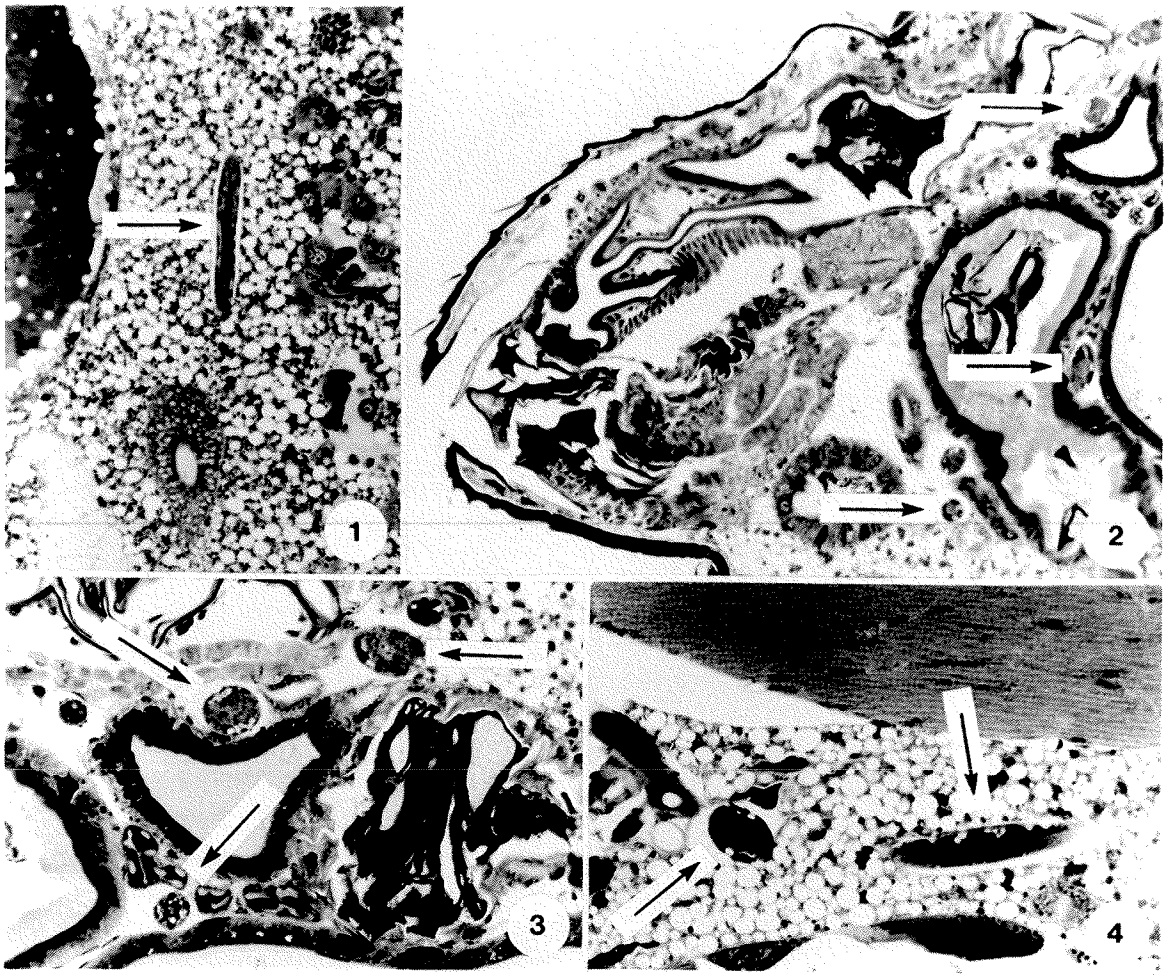
T. populi infected with juveniles of B. fraudulentus.

Fig. 1. Juvenile nematode (arrow) in fat body tissue of beetle pupa. Frontal section; x 210.

Fig. 2. Juvenile nematodes (arrows) in abdomen of adult beetle. Sagittal section; x 210.

Fig. 3. Juveniles of B. fraudulentus (arrows) in thorax of adult beetle. Note position of nematodes in spaces between organs. Section; x 410.

Fig. 4. Juveniles of B. fraudulentus (arrows) in fat body tissue of adult beetles. Section; x 410.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# CHAPTER VI

## PATHOGENICITY OF ALLANTONEMATIDAE (Nematoda)

## INFECTING BARK BEETLES (Coleoptera, Scolytidae).

## Introduction

Nematodes of the Allantonematidae are the most common obligate parasites of bark beetles (Fuchs, 1937; Ruhm, 1956; Massey, 1974). Female nematodes invade beetles and after a period of a rapid growth, reproduce in the host hemocoel. These parasites may affect host flight (Atkins, 1961; Ashraf and Berryman, 1970a) reproductive potential (Massey, 1964; MacGuidvin et al., 1980), and can cause the death of infected beetles (Ashraf and Berryman, 1970a).

As only a few physiological and histopathological studies on parasitism of allantonematids in bark beetles were conducted (Nickle, 1963; Ashraf and Berryman, 1970b; Thong and Webster, 1975), mechanisms involved in host and parasite relations remain obscure.

The main objectives of this study were to determine the histopathology in selected species of bark beetles infected with allantonematids, to establish their connection with sterilization and death of the insect host, and to examine "crowding effects" on these parasites.

## Materials and Methods

The collection technique, preparation of nematodes for taxonomic studies, rearing of nematodes, preparation of tissue for histopathological studies, and calculation of nematode volume and fecundity were as described in Part A, Materials and Methods. Locations of collection sites are described in Appendix I.

## Results

Fertilized free-living female allantonematid nematodes usually infected the third instar larvae of beetles, and developed in the hemocoel into sausage-shaped parasitic females. Mature females produced numerous eggs which hatched in the uterus or in the host hemocoel. The time of hatch was characteristic for each species and commenced when the insect hosts were still in larval galleries or shortly before the beetles left their overwintering sites. Juvenile nematodes developed in the insect body cavity, and usually left the host in the $L_3$ or $L_4$ stage, when beetles constructed their egg gallery. Only Parasitylenchus caudapapilli sp. nov. had an additional second parasitic generation. Nematodes matured and mated in the larval galleries. After copulation, males died and females infected third instar larvae of the new beetle generation.

Nematodes examined in this study were represented by three groups with different bionomics of parasitic forms. Species of parasites classified into each group are listed in Table 3.

Group I. Sulphuretylenchus type.

Eggs hatched in the ovaries of the female parasite, and early juvenile stage fed on maternal tissue, leading to the development of endotokia matricida. Large numbers of juveniles were released to the insect hemocoel when the maternal cuticle ruptured. Consequently, parasites started feeding on the host tissue and reached the next developmental stages ($L_3$ and occasionally $L_4$). When most of the host tissue was exhausted, nematodes emerged from the beetle into the galleries. Parasites normally left the host via the mouth opening. Due to the large numbers of nematodes simultaneously developing in the host

hemocoel, the process of tissue degradation was rapid and the beetle was killed prior to oviposition.

Nematodes of this group caused the most dramatic pathological changes in the host. Parasitism of S. pseudoundulatus sp. nov. in P. rufipennis was used as a model to show the sequences of pathological events occurring during the development of parasitemia.

Group II. Parasitylenchus type

A few parasitic females (usually 1 - 4) of the first generation produced juveniles, and gradually released them into the host hemocoel. These nematodes matured and mated in the insect body cavity. Males of this generation died after copulation, while females (usually 70 - 120 per host) developed further, increasing in size and depositing a second generation of juveniles in the beetle hemocoel. Juveniles of this generation developed, and after reaching the $L_3$ stage they gradually left the host through the base of gonads; thus two generations of nematodes developed in the insect hemocoel.

Group III. Neoparasitylenchus type

Parasitic female nematodes deposited eggs or juveniles into the insect hemocoel. As reproduction occurred over a long period of time, different developmental stages of nematodes could be simultaneously found in the insect. Nematodes that reached the third juvenile stage gradually emerged from beetles to the galleries via the host anus. This led to elimination of overcrowding, and fewer nematodes were present in the beetle hemocoel at any one time.

Two distinctive phases in the development of parasitism in the host were observed.

The first phase started with the beginning of infection, and lasted until juvenile nematodes were released into the hemocoel of the host. During this period only parasitic females were present in the beetle body cavity. Nematodes increased about 500-800 times in size, but no local lesions were found internally on the host organs. During the first phase, most pathological effects in the host were expressed as an abnormal development of some organs e.g., gonads, and fat body. Nematodes probably fed on hemolymph and changes in the hemolymph influenced host organogenesis.

The second phase lasted from the release of nematode juveniles in the bark beetle body cavity until they emerged from the host. During this period, nematodes developed in the insect hemocoel and fed on hemolymph and directly on other tissues. At this stage partial or complete degradation of previously formed internal structures occurred.

The rate of development of histopathological processes and their severity were related to the biology of both parasite and host. Only limited numbers of juvenile nematodes were found in the host hemocoel of immature adult beetles, examined during the autumn. The massive presence of juvenile parasites in the beetle body cavity was observed during insect attack on new host trees, the following spring.

Histology of noninfected bark beetles.

Serial sections of noninfected adult beetles showed that in the insect hemocoel most spaces between intestine, muscles and gonads were occupied by regularly formed fat body (Figs. 1,2,3). Numerous bundles of microfibrillar muscles were found joining abdominal segments (Fig. 4) around the base of legs and wings in the thorax (Fig. 5) and filling

most of the volume of the head capsule. Masses of fibrillar muscles (indirect flight muscles) were present in the meso- and metathorax (Fig. 6). In mature female beetles, developing gonads took over part of the volume of the hemocoel previously occupied by fat body tissue.

Telotrophic ovaries of mature female bark beetles consisted of two pairs of ovarioles, each connected with the lateral oviduct (Fig. 7). Two lateral oviducts joined the median oviduct. The spermatheca, bursa copulatrix, and accessory glands were connected with the lower part of the median oviduct. The ovariole consisted of the tropharium and vitellarium. The terminal filament was the most distal part of the ovariole. The syncytial tropharium had an apical zone with numerous small germ cell compartments, and main region with large nurse cell nuclei (Fig. 8). The tropharium base abutted a differentiating zone, where oocytes became morphologically distinguishable from nurse cells. Oocytes entering the zone of previtellogenic growth were covered with epithelial cells from the prefollicular tissue. In the previtellogenic zone, oocytes had homogeneous cytoplasm and follicular epithelial cells formed compact layer on their surface (Fig. 9). The zone of vitellogenic growth was in the lower region of the ovariole. Vitellogenic oocytes contained numerous granules of proteins and lipids (Fig. 10). Follicular cells decreased in size and degenerated at the end of vitellogenesis. The entire ovary was covered with a basement membrane and outer epithelial sheath. An additional layer, the inner epithelial sheath, was observed between the basement membrane and tropharium germ cells. Usually one or two eggs were present in the oviduct.

Oogenesis in adult insects, just after pupation, was in an early stage. The region of prefollicular epithelium was almost adjacent to the lateral oviduct, joined with it by a short pedicel (Fig. 11).

Testes of nonparasitized beetles consisted of two follicles (Fig. 12). In each follicle a sequence of zones could be distinguished (Fig. 13). These were zones of spermatogonia, of growth with cysts of spermatocytes, and of maturation where spermatids were produced as a result of meiosis. Finally, there was a zone of transformation where spermatids were converted into spermatozoa. All cells were in cysts. In testes of mature adult beetles, cysts with spermatozoa were most abundant.

Histopathology of bark beetles infected with allantonematids.

1. P. rufipennis infected with S. pseudoundulatus.

During the first phase of infection female nematodes occupied a substantial volume of the insect body cavity, often pushing host organs to new positions (Figs. 14,15,16). Fat body was not developed as well as those in noninfected insects, but distinct clusters of this tissue were still present in the beetle abdomen and thorax. No local lesions or injuries on the surface of any insect organs were noted. During the first phase of parasitism beetle gonads developed abnormally.

Testes of parasitized beetles were smaller, often 1/4th or less the size of normal testes (Fig. 17,18). Only a few cysts of spermatozoa occurred in the follicles of infected beetles (Fig. 19). The remaining spermatogonia differentiated into several types of cells, but existed as separate units, never enclosed in cysts as in noninfected insects. Various types of cells were scattered throughout the follicles of

infected beetles, and no apparent direction in their transformation was observed.

In some specimens development of all germ cells stopped at an early stage (Fig. 20). Follicles were filled with undifferentiated spermatogonia and other cells. No sperm were produced by these beetles and sterilization was complete.

The ovaries of parasitized beetles were often 1/6th the size of those in normal beetles (Figs. 21,22). The tropharia had significantly fewer cells, only some of which were viable (Fig. 23). These formed irregular syncytia containing nuclei of different sizes. The remaining cells degenerated. The zone of prefollicular epithelium was narrow and had a small number of cells. None of the oocytes acquired follicular cells on their surface and none developed further. Tissue degeneration occurred at this stage, thus no vitellogenesis took place in ovarioles of parasitized insects, and sterilization was complete. No organs other than fat body tissue and gonads, showed abnormal structure and size during the first phase of infection.

In the second phase of parasitism, rapid degradation of the host tissue occurred upon the release of juvenile nematodes into the beetle hemocoel. Fat body was affected first. A few lesions at the surface of this tissue were observed during the early stage of infection. Muscles and other organs remained unaffected. At this stage juvenile nematodes occupied the beetle abdomen, thus most of injuries were localized in the posterior region of the body. As the process of tissue degradation progressed, fat bodies were reduced to a few irregular clusters with distinct lesions (Fig. 24). Parasites rapidly spread throughout the abdomen and thorax. Numerous lesions on insect muscles appeared with

the gradual exhaustion of fat body. Fibrillar indirect flight muscles were injured first. Juvenile nematodes fed on this tissue causing local lesions (Fig. 25). Further feeding led to almost complete degradation of fibrillar muscles (Fig. 26). A small amount of tissue debris remained where indirect flight muscles existed previously (Fig. 27).

Initial injury to microfibrillar muscles in the abdomen and the thorax corresponded with almost complete degradation of fat body and with partial lysis of indirect flight muscles (Fig. 28). At this stage of parasitemia, basement membrane of the intestine, gonads and Malpighian tubules remained intact, and no penetration of parasites into these organs was observed. (Fig. 29). Legs were not invaded by the juvenile parasites with exception of the coxae, where a few nematodes were occasionally observed at the late stage of infection (Fig. 30). At this stage the beetle nervous system was also often injured (Fig. 31). Local lesions were observed in abdominal and thoracic ganglia.

Penetration of the insect prothorax and head by the parasites began when degradation of tissue in the abdomen and thorax was well advanced. At the onset of infection of the head, most injuries were localized in the central region, surrounding the alimentary canal (Fig. 32). In the head, selective degradation of tissues was not as easy to observe as in the abdomen and thorax. Fat body tissue, numerous microfibrillar muscles, and the central nervous system were injured almost simultaneously. In the brain and suboesophageal ganglion, the layer of glial cells was penetrated first.

At the later stage of parasitemia, nematodes were found in all regions of the head causing local lesions in the muscles of mandibles,

maxillae, labium, labrum and other parts of the insect head (Figs. 33,34).

Partial destruction of the head tissues accompanied by almost complete degradation of the beetle tissues in the abdomen and thorax led to the inevitable death of parasitized hosts (Fig. 35). The parasitic part of the nematode life cycle was completed by penetration of the anterior region of the midgut and emergence of juveniles into beetle galleries.

Infection of P. rufipennis by S. pseudoundulatus led to almost complete degradation of cellular structures in the beetle body. Summary of pathology is shown on Figs. 55a, and 56a.

2. P. sparsus infected with S. nopimingi.

Histopathological processes were similar to those described for P. rufipennis. Some differences were observed during the first phase of parasitism.

Ovarioles of infected beetles were often 1/4th the size or less of nonparasitized insects. Tropharia were filled with spindle-like cells similar to prefollicular epithelium in noninfected beetles (Fig. 36). Nuclei of these cells were small. Neither the formation of a syncytium nor differentiation of cells into trophocytes and oocytes was observed. The zone of prefollicular epithelium was composed of numerous cells, much smaller than those in noninfected insects. No further development of oocytes took place and ovarioles degenerated during the second phase of nematode parasitism.

Testes of parasitized beetles had fewer cysts than noninfected insects, but produced viable spermatozoa.

Noticeable malformations of genitalia were recorded in all parasitized male beetles (Fig. 37,38). All parts of the copulatory organ were small and of unusual form, which made identification of individual components normally present in the beetle genitalia impossible. Pathology is summarized on Figs. 55b, and 56b.

3. I. perroti infected with S. posteruteri.

Processes of tissue degradation were similar to those described in previous species (Fig. 39). Differences occurred in the size and structure of gonads.

Ovaries of infected beetles were often 1/2 the size of those in normal insects. During the first phase of parasitism all zones in the tropharium developed normally. Nuclei in the syncytium of tropharium were smaller, but their numbers corresponded with tropharia of noninfected beetles.

In the second phase, oocytes passing through the zone of prefollicular epithelium were surrounded by follicular cells. Early oogenesis appeared to be unaltered. A rapid process of degeneration began in the vitellarium (Fig. 40). On the surface of degenerating oocytes follicular cells lost their regular arrangement. Oocytes shrank and detached from the follicular layer, and cytoplasm gradually broke down. Degeneration of oocytes occurred in all ovarioles, and neither vitellogenesis nor production of eggs were observed. Thus, sterilization of female beetles was complete.

Histological structure of testes and spermatogenesis in parasitized insects appeared similar to that of noninfected beetles. Pathological changes are summarized on Figs 55c, and 56c.

4. I. perturbatus infected with P. caudapapilli.

In this and the following species, histopathological processes were slower and not as severe as in beetles infected with Sulphuretylenchus spp.

During the first phase of parasitism, gonads of both male and female beetles showed no significant abnormalities in either their size or histological structure.

Development of oocytes appeared normal at the beginning of the second phase of parasitism. Only the number of oocytes developing in ovaries was smaller, usually ranging between 4 - 5 oocytes per ovariole (Fig. 4), while in noninfected insects usually 8 - 9 oocytes developed per ovariole. During the late second phase of parasitism, most oocytes in the vitellarium degenerated.

Tissue degradation was observed only in the abdomen and thorax, and was not as extensive as in previously described species (Fig. 42,43). In many specimens partially lysed fragments of muscles were still present upon the death of the beetles. Beetles were killed in various stages of oviposition. Nematodes left the host through the anus. Summary of pathology is shown on Figs. 55d, and 56d.

5. I. perturbatus infected with N. ipinius.

In the first phase of parasitism, gonads of both male and female insects were slightly smaller compared to noninfected beetles, and the histology of the ovaries differed. Tropharia of parasitized insects contained greater numbers of nuclei and less cytoplasm than in noninfected beetles (Fig. 44,45). Nuclei were closely packed and

irregular in shape. At this stage remaining parts of gonads were unchanged.

Oogenesis and vitellogenesis appeared normal early in the second phase of parasitism. Only the number of oocytes developing in each ovariole was smaller, usually 4 - 5 oocytes per ovariole, than in noninfected insects, where usually 8 - 9 oocytes developed in each ovariole. At the late second phase most oocytes in ovarioles degenerated (Fig. 46).

Juvenile nematodes were found only in the abdomen and meta- and mesothorax of the host. The head and prothorax were not penetrated by parasites. Fat body tissue and fibrillar muscles in the abdomen and thorax were degraded almost completely until the late second phase, while the microfibrillar muscles were injured only partially (Figs. 47,48). Emerging nematodes ruptured the host oviduct or ductus ejaculatoris and left the insect through the anus. Beetles were killed during late oviposition. Histopathology is summarized on Figs. 55e, and 56e.

6. D. autographus infected with A. paramorosum.

Gonad size of parasitized insects was not significantly different from that of noninfected insects. In ovaries of some parasitized females, one or more individual ovarioles degenerated at the early stage of development. Remaining ovarioles in the same ovary appeared normal.

During the second phase of parasitism oogenesis and vitellogenesis in most ovarioles remained unaltered, and only the number of maturing oocytes was lower than in normal ovaries. At the late stage of parasitism the most developed oocytes degenerated.

In parasitized insects, most of the fat body tissue was degraded (Fig. 49). Only limited injuries to the fibrillar and microfibrillar muscles were observed (Fig. 50). Parasites did not penetrate the host prothorax and head. Beetles ovisposited and lived in the egg galleries for several months.

Nematodes gradually left the host through the anus. Infected beetles usually died at the end of the gallery. Generalized pathology is shown on Figs. 55f and 56f.

7. D. simplex infected with C. reversus.

In parasitized insects, during the first phase both male and female gonads were unchanged compared with noninfected beetles. During the second phase of parasitism the number of developing oocytes in individual ovarioles (usually 8 - 10) and the proportion of oocytes in late vitellogenesis (about 30%) was lower than in nonparasitized insects, where these numbers ranged from 14 - 16, and up to 50% respectively.

Parasites occupied the beetle abdomen and were less abundant in the host thorax. Juvenile nematodes caused extensive injuries to the fat body tissue of the beetle (Fig. 51), while muscles were only occasionally affected. Parasites left the host through the anus. Beetles died in various stages of oviposition.

Histopathological changes in bark beetles are summarized on Figs. 55g, and 56g.

Effect of allantonematid parasitism on gallery construction by the beetles.

The intesification of pathological processes in the second phase of parasitism usually corresponded to the period of construction of egg gallery and beetle oviposition.

Oviposition carried out by I. perturbatus, D. autographus, and D. simplex infected with allantonematids was usually slower when compared with noninfected beetles. Length of egg galleries varied significantly, and was always shorter than in nonparasitized insects. Beetle cadavers were found in various locations throughout galleries.

In P. rufipennis, P. sparsus, and I. perroti infected with Sulphuretylenchus spp., complete sterilization of beetles and rapid tissue degradation influenced the most severe changes in gallery patterns. Tunnels of infected insects did not contain egg niches (Fig. 53). The total length of these galleries seldom exceeded 1.0 - 1.5 cm. While in noninfected beetles it ranged between 4 - 10 cm. Frass was expelled from the egg galleries of nonparasitized insects, while in infested beetles it remained in the gallery.

Tunnels of noninfected P. sparsus were engraved in the sapwood of the tree throughout the entire length. Galleries of females parasitized by S. nopimingi led from the initial point in the sapwood to the softer parts of the bark. The cadavers of beetles infected with Sulphuretylenchus spp. were always found at the end of the short galleries. In I. perroti parasitized with S. posteruteri, juvenile nematodes leaving the host through the mouth formed characteristic caps

on the front of the insect cadaver, and remained in this position for several days (Fig. 54).

Short galleries of infected insects were connected with other branches of the whole gallery system through the nuptial chamber (Fig. 53). Nematodes emerging from parasitized beetles penetrated galleries of noninfected insects and invaded their offspring.

Intensity and prevalence of nematode infection.

The initial intensity of allantonematid infection was low and usually ranged between 1 - 2 parasitic females per host beetle. Occasionally these numbers were higher with a maximum of 11 parasitic females per host in P. sparsus.

Parasitic juveniles of the new generation were numerous and ranged between 2 - 4 thousand per host.

Prevalence of nematode infection in immature adults of each bark beetle species is shown in Table 2. Additional data were obtained from mature adults of selected species during oviposition. There was a dramatic decline in proportions of parasitized insects during the period of overwintering (Table 4).

Most species of the examined bark beetles overwintered in the forest litter, or in the short galleries in bark at the base of the host tree. Thus, collection of bark beetles from overwintering sites, in numbers sufficient for evaluation of prevalence of infection, was almost impossible. Only P. sparsus, which overwintered as adults in larval galleries, could be easily collected from those sites. Changes in the prevalence of nematode infection in adult beetles are shown on Fig. 57. There was 41.5% reduction of the ratio of parasitized beetles after seven months of overwintering. Further, rapid reduction of 29% of

nematode infected P. sparsus occurred during one month (April/May), when beetles left overwintering sites and flew to new trees.

"Crowding effects" on allantonematids infecting bark beetles.

Effects on parasite size and fecundity:

Significant differences in sizes of parasitic female nematodes occurred between individuals in all examined species. Detailed analysis of volumes and fecundity related to the intensity of infection was conducted on S. pseudoundulatus infecting P. rufipennis.

Mean volumes of individual female parasites, and mean total volumes of parasites per host were plotted against the intensity of infection (Fig. 58). Mean volume of individual nematodes declined steadily along with increasing intensity of infection from 0.086 ± 0.012 $mm^3$ in single infection to 0.016 ± 0.011 $mm^3$ in multiple infection with 8 nematodes per host. Mean total volume of parasites per host increased from 0.086 ± 0.013 $mm^3$ in infection with one parasite to 0.161 ± 0.005 $mm^3$ in multiple infection with 6 parasites per host, which is only a twofold increase of the total volume over sixfold increase of the number of parasites per host. Above the intensity of 6 nematodes per beetle the total volume of parasites per host declined.

Significant differences in fecundity of female parasites, related to the intensity of nematode infection were observed (Fig. 59). Total number of nematode progeny produced per beetle increased from 1933 ± 350 juveniles per host in the single infection to 3316 ± 350 juveniles per host in infections with 5 parasitic females. Above the limit of 5 parasites per host, the total number of progeny produced declined. Numbers of progeny produced by individual female nematodes declined steadily with increasing intensity of infection, and ranged from 1933 ±

350 juveniles per female in a single infection to about 309 juveniles per female in simultaneous infection with 8 parasites.

Effects on the viability of S. posteruteri infecting I. perroti.

Forty- one per cent of infected I. perroti contained normal, viable as well as dead, melanized female nematodes (Fig. 54). Numerous clusters of hemocytes were attached to the dead nematode. Melanization did not occur in beetles parasitized with 1 or 2 nematodes which was the most frequent situation (Fig. 60). In heavily infected hosts (3 or more nematodes per beetle) some of the parasites were dead and melanized. Never more than four female S. posteruteri were observed to develop normally in the hemocoel of I. perroti.

Melanized nematodes usually had similar sizes to those of infective females. Consequently the immune reactions must have occurred shortly after the parasites had invaded the host.

## Discussion

Parasitism of allantonematids in the hemocoel of bark beetles causes severe pathology to insect organs.

Infective female allantonematids are small and usually range between 343μ and 947 μ in the length, and 11μ and 17μ in width (see Part B, Chapter I). As only one or a few nematodes invade the host, injuries to the insect organs are insignificant at the early stage of infection. In the host hemocoel, parasitic females grow rapidly. During the period of parasitism in the beetle body cavity, females of S. pseudoundulatus increased in size about 800 times.

Concurrent with growth, the nematode stylet is retracted into the body mass and apparently becomes non-functional. The digestive canal degenerates as well. These changes influence the method of nutrient uptake by the parasites. Several routes other than per os have been suggested for nutrient uptake by nematode parasites of insects. Nematodes can absorb nutrients through the everted uterine membrane (Poinar and Hess, 1972); numerous hypodermal microvilli projecting from the body surface (Riding, 1970; Bedding, 1967; 1968); cuticular canals in the outer body wall (Nicholas, 1972); pores in the cuticle (Poinar and Hess, 1977), or over the general body surface (Webster and Gordon, 1974; Rutherford and Webster, 1974).

In the present study there were no direct observation on the method of feeding of parasitic female nematodes, but with the exception of nutrient uptake through the everted uterine membrane, characteristic of Sphaerularia bombi (Dufour), all other ways of food ingestion listed above are possible in the species examined. Ingestion through the body wall of the parasite suggest uptake of substances from the hemolymph of the host, and can also explain absence of direct injuries to insect tissues.

Parasitic juvenile nematodes of the new generation have both, a functional stylet and digestive canal. These characteristics allow direct feeding of parasites on various tissues of the host.

Differences in feeding activity found between parasitic females and juvenile nematodes account for the occurrence of two distinctive phases in the development of pathological processes in the bark beetle. The first phase corresponds with the exclusive presence of parasitic females in the beetle hemocoel, and it is distinguished by a lack of direct

injuries to the host organs. An uptake of nutrients, essential for the insect, causes abnormal development of its organs e.g. fat bodies and gonads. The second phase of host-parasite relationships corresponds with large numbers of juvenile nematodes present in the beetle body cavity. It is distinguished by direct injuries, and often complete degradation of tissues in various organs of the host e.g., fat body, fibrillar muscles, microfibrillar muscles, gonads, and nervous tissue.

Changes in the host, P. rufipennis infected with S. pseudoundulatus can be considered as a model to show the sequence of pathological events taking place during development of parasitemia. Development of pathological processes was as follows:

The first phase of parasitism:

i. partially impaired development of fat body tissue,

ii. abnormal development of gonads.

The second phase of parasitism:

iii. gradual degradation of fat body tissue in abdomen and later in the thorax,

iv. gradual degradation of indirect flight muscles (fibrillar muscles); occasional injuries to the microfibrillar muscles,

v. gradual degradation of microfibrillar muscles of the abdomen and thorax; occasional penetration of prothorax; complete or partial degeneration of gonads and oocytes,

vi. gradual degradation of microfibrillar muscles of prothorax and head; penetration of ganglia,

vii. penetration of the intestine; partial degradation of intestinal epithelium,

viii. continuation of the degradation of cellular structures in

the insect body; death of the beetle; emergence of nematodes to the bark beetle gallery.

Pathology of P. rufipennis, P. sparsus, and I. perroti parasitized by nematodes belonging to the genus Sulphuretylenchus spp. fits the described model completely. In these species histopathological processes showed the widest range of effects of parasitism when compared with other bark beetles infected with allantonematids. In the remaining species these processes differed in detail, were often slower and stopped at an early stage, usually because of death of the insect host. Juvenile parasites emerged from the host throughout most of the period of oviposition.

Parasites developing in the insect body compete with the host organs for essential nutrients. In parasitized insects, significant changes were recorded in the content of hemolymph carbohydrates (Gordon et al., 1978; Rutherford and Webster, 1978; Schmidt and Platzer, 1980a) amino acids and proteins (Thong and Webster, 1975; Schmidt and Platzer, 1980b; Gordon and Webster, 1971); lipids (Gordon et al., 1979; Dutky et al., 1967), and major and trace elements (Levy et al., 1979). The substances taken up from the hemolymph by parasites are partially compensated by increased catabolism and/or decreased anabolism in the fat bodies, often leading to significant reduction of this tissue (Gordon et al., 1971; Schmidt and Platzer, 1980a). These changes in the hemolymph content caused by female allantonematids and compensation for them by the insect homeostatic mechanisms may possibly explain the significant reduction in the total amount of the bark beetle fat body tissue observed during the first phase of parasitism.

Bark beetles gonads are the most sensitive organs to parasitism by female allantonematids. Significant differences occurred between the severity of pathology in male and female reproductive organs. The rapid development of parasitic female nematodes and the subsequent oogenesis causes the greatest demand for nutrients. These processes correspond with the larva ($L_4$) - pupa - imago moults in bark beetles. By this time spermatogenesis in many testicular cysts is almost complete, while in female beetles, ovaries are still in an early stage of development. Rapidly decreasing nutrient content in the insect body apparently conflicts with further organogenesis in the beetle. Thus, the development of insect ovaries and of individual oocytes, normally active at this stage, becomes impaired.

Quantity as well as quality of nutrients influence insect organogenesis (Engelmann, 1970). Withdrawal of nutrients from the host hemocoel by nematode parasites is selective and is species specific. Significant variations were found in changes of the host haemolymph content caused by the same parasite in different hosts (Gordon et al., 1978), and by different parasites in the same species of the host (Gordon et al., 1979). Depending on the species of parasite or host, proportions of only some individual amino acids and lipids were altered, while other components of the hemolymph remained unchanged. In our study members of the genus Sulphuretylenchus were more pathogenic to their hosts than any other species of parasite. During the first phase of infection S. pseudoundulatus and S. nopimingi severely affected both the oogenesis and spermatogenesis of their hosts. The remaining species of parasites did not change development of insect testes, and had only limited influence on the development of ovaries. Females of S.

pseudoundulatus and S. nopimingi, which grew rapidly in the host hemocoel, apparently depleted hemolymph components e.g. specific amino acids or proteins, which were crucial in the early development of the insect gonads, while the quality and/or quantity of components ingested by the other parasites were less important with regards to spermatogenesis and early oogenesis.

The role of the insect endocrine system should also be regarded as an alternative or supplemental factor possibly involved in the development of pathological effects in the beetle. Essential physiological reactions e.g., amino acid uptake and protein release from the fat bodies are regulated by the insect endocrine system (Highnam and Hill, 1969). Nematode parasitism can interfere with the activity of this system. Rajulu et al., (1972) observed production of an ecdysone-like hormone by the nematode Phocanema sp. Toxins produced by Sphaerularia bombi seriously damaged the corpora allata and their control of vitellogenesis in Bombus spp. (Palm, 1948). Condon and Gordon (1977) found significant changes in nuclear DNA/RNA activity in the corpus allatum of Simulium venustum Say infected by Mesomermis flumenalis Welch, 1962. Pleiotrophic actions of juvenile hormone produced by the insect corpus allatum include stimulation of development of rough endoplasmic reticulum in fat bodies, induction of transcription of the coded information for vitellogenin, control of transport of vitellogenin from the fat bodies and uptake by growing oocytes, and various other functions (Engelmann, 1980). Thus, possible influences of parasites on the activity of corpus allatum may have grave consequences for the developing gonads.

During the second phase of parasitism, rapid degradation of fat body intensifies the pathology of gonads. As the synthesis of vitellogenin occurs in the fat bodies (Engelmann, 1980) further reduction of the total amount of this tissue, normally observed during the massive release of allantonematid juveniles to the beetle hemocoel, deprives the host of the major source of vitellogenins. When juvenile parasites had lysed beetle fat bodies, hitherto limited influences of female S. posteruteri on early oogenesis of I. perroti were altered almost instantaneously and rapid degeneration of all oocytes in vitellogenesis took place. In I. perturbatus, D. autographus, and D. simplex, degeneration of all, or only some oocytes entering vitellogenesis also corresponded with reduction of fat body by juvenile nematodes feeding directly on this tissue. In these species degradation of fat body was slower and beetles usually were able to oviposit for some period.

Several authors recorded significant reduction of bark beetle reproductive potential caused by allantonematid nematodes. Complete sterilization of Scolytus ventralis LeConte by Sulphuretylenchus elongatus (Massey) Nickle 1967 was reported by Massey (1964). Schvester (1957) observed a similar effect of parasitism on Scolytus rugulosus Ratzeburg. Partial sterilization related to nematode infection was reported in Dendroctonus ponderosae Hopkins (Reid, 1958), Ips confusus LeConte (Nickle, 1963), Dendroctonus pseudotsugae Hopkins (Thong and Webster, 1975), S. ventralis (Ashraf and Berryman, 1970a,b).

Changes in the fecundity of nematode infected bark beetles were often attributed to the reduced sizes of insect ovaries (Fuchs, 1937; Rühm, 1956; Massey, 1964, 1966). In the available literature, only

Ashraf and Berryman (1970b) described histopathology of gonads in beetles infected with allantonematids. S. elongatus caused degeneration of entire ovarioles during the vitellogenesis in S. ventralis, and frequently ruptured the epithelial sheath surrounding ovaries and testes. These changes were related to the presence of parasitic females and juvenile nematodes in the host hemocoel.

In our studies, pathological processes led to the sterilization of all infected insects. The degree of sterilization was specific for each parasite and for the stage of infection. Parasitism of S. pseudoundulatus and S. nopimingi altered early phases of oogenesis, causing complete sterilization of beetles. Germ cell cluster formation was suppressed and individual germ cells degenerated at early stages of their development. No differentiation of trophocytes and oocytes and further development of previtellogenic and vitellogenic zones occurred in infected beetles.

Remaining species of nematodes did not significantly affect differentiation of oocytes in early oogenesis. Nematode parasitism completely or partially suppressed the host vitellogenesis.

Reproduction of bark beetles takes place in galleries constructed by parent insects. The general pattern of gallery system is characteristic for each species (Bright, 1976). Nematode parasitism affects insect reproductive behavior and often results in aberrant gallery pattern. Noninfected Scolytus rugulosus Ratzeburg constructed galleries parallel to the stem of the tree, while beetles parasitized by Neoparasitylenchus rugulosi Schv. 1957 made horizontal galleries and did not oviposit (Nickle, 1971). Ashraf and Berryman (1970a) observed a wide spectrum of gallery forms and variable numbers of eggs deposited by

S. ventralis infected with S. elongatus. Heavily infected females tended to bore short, sometimes elongate entrance holes, while short egg galleries were formed by moderately infected insects. Lightly infected females constructed longer galleries and occasionally laid fertile eggs. The latter effect was also observed in other bark beetle species (Thong and Webster, 1975; MacGuidwin et al., 1980).

Except for the reduced fecundity, no other explanations for altered gallery patterns by nematode infected bark beetles were suggested. Degradation of muscle tissue and direct injuries to the insect nervous system indicate that sterilization may only partially account for the changes in construction of galleries. Sterilization seems to be responsible mainly for reduction in numbers of egg niches and deposited eggs. Extensive injuries to the microfibrillar muscles of the insect thorax and head probably have detrimental influences on insect boring behavour. This was clearly shown in bark beetles infected with members of the genus Sulphuretylenchus. Rapid degradation of muscles in the thorax and severe lesions in the muscles of the mandibles and other mouthparts led to the construction of galleries in softer bark tissue by P. sparsus, or to the construction of very short egg galleries by I. perroti, which are unusual behaviours for these species.

Progressive degradation of the beetle tissue finally led to the complete cessation of boring behaviour. Slower pathological processes in beetles parasitized by other nematodes resulted in only partial reduction of the length of galleries.

In bark beetles, where individual gallery systems are usually separated from each other, complete sterilization of a female would limit nematode dispersion. In our studies three species of bark beetles

were completely sterilized by parasites. All these species were polygamic and parasites could freely penetrate galleries of other, noninfected females. The polygamic habits of P. rufipennis, P. sparsus, and I. perroti assure survival and continuing dispersal of the parasite population.

Adverse winter conditions can cause mortality in overwintering insects (Berryman, 1970; Johnson, 1967). A decline in the prevalence of nematode infection in overwintering beetles may be explained by disproportional reduction of infected insects during hibernation. Depletion of fat body tissue and reduction of storage material by parasites may have accounted for this situation. In many bark beetle species examined, a number of nematode juveniles were released to the hemocoel of immature adult beetles. Direct feeding on host tissue could significantly accelerate the process of fat body degradation prior to onset of winter. Fedorko (1971) observed that most mortality of Leptinotarsa decemlineata Say caused by Pristionchus uniformis Fedorko et Stanuszek 1971 occurred during early spring, when nematode parasitized hibernating beetles.

In the spring and early summer bark beetles fly from their overwintering sites to the new host tree, and start reproduction. Effects of nematode parasitism on the dispersal and flight potential of insects were reported by many authors. Brugia pahangi Buck et Ede. caused severe pathology of indirect flight muscles of Aedes aegypti (L.), and influenced the length of flight of these insects (Hockmeyer et al., 1975). Changes in the fight activity caused by the nematode parasitism were also observed in Chironomus spp. (Wülker, 1961), Schistocerca gregaria Forsk (Weis-Fogh, 1956), Bombus spp. (Poinar and

Van der Laan, 1972), and among bark beetles in Conophthorus monophyllae Hopk. (Poinar and Caylor, 1974) Dendroctonus pseudotsugae (Atkins, 1961), and S. ventralis (Ashraf and Berryman, 1970a). Ashraf and Berryman (1970a) reported that parasitism of S. elongatus severely impaired dispersal of the beetle host. Parasitized insects flew significantly shorter distances and only lightly infected beetles could reach potential breeding sites. Based on their ultrastructural studies, Ashraf et al. (1971) suggested that changes in organization of mitochondria in flight muscles could contribute to the lowering of flight efficiency.

In our investigations, no specific studies were carried out on beetle dispersal. Rapid decline in prevalence of nematode infection observed in P. sparsus during the spring emergence and the insect flight suggest that a large number of parasitized beetles did not reach new breeding sites. Fat bodies and indirect flight muscles were easily lysed by juvenile parasites. Release of juvenile nematodes to the hemocoel of some beetle individuals commenced before, or during the period of overwintering, and in these insects rapid processes of tissue degradation started in the overwintering sites. Individuals with severely affected fat bodies and flight muscles could not fly far enough to find potential host trees. Thus, the low percentage of infected insects recorded in new breeding trees represents only that part of the beetle population in which juvenile parasities emerged to the beetle hemocoel and started feeding on the host tissue later in the spring.

"Crowding effects" on parasities were extensively studied in vertebrates (Read, 1951; Ghazal and Avery, 1974). Little information is available on these phenomena occurring in nematode infections of

insects. Multiple parasitism of mermithids in the insect hosts influences the sex ratio and mortality of parasites (Christie, 1929; Petersen, 1972, 1977; Craig and Webster, 1982). Crowding of neoaplectanids in Lucilia sp. partially or completely supressed nematode reproduction (Molyneux et al., 1983).

Crowding of allantonematid parasites in the bark beetle host caused reduction of the body size and of fecundity in female S. pseudoundulatus, and led to melanization of some nematodes in infrapopulations of S. posteruteri.

There are at least three factors influencing these changes: nutritional deficiences, limited space in the host, and the insect immune responses.

Potential nematode nutrients, present in the beetle body are limited. Increased numbers of parasites in the host hemocoel increase the competition of nematodes for food resources. Thus, in multiple infections inadequate quality and/or quality of nutrients available in the insect hemolymph may have accounted for reduced growth and lower fecundity of parasites.

The disproportionally large size of parasitic female allantonematids, when compared with the size of bark beetle may result in inadequate space in the host hemocoel and can influence "crowding effects" on nematodes. Even in the low intensity of infection, parasites occupied most of the volume of beetle abdomen and thorax. Increased numbers of parasites per host, and unchanged volume of the insect hemocoel apparently prevent female nematodes from reaching maximum size.

Mechanisms of stimulation of immune responses to multiple infection with S. posteruteri remains obscure. The most striking feature is the

apparent fixed level of insect tolerance to the parasite burden. Mechanisms of foreign body recognition and an initiation of the defence reactions are not completely understood in insects. Hemocyte chemotaxis stimulated by the substances released by the parasite was suggested by Nappi and Stoffolano (1971). Vinson (1974) found hemocyte response to electrostatic charges on the parasite surface. The presence of carbohydrate specific molecules, which can bind selectively to hemocytes and to the parasite was postulated by Parish (1977). It is probable that in I. perroti accumulation of "non-self factor" in the insect haemolymph may reach some level detectable by the insect immune system and triggers a reaction. When melanization of a certain number of nematodes reduces the level of this "factor" in the beetle hemocoel, the reaction ceases. This problem requires further investigation.

Diversity of pathological effects observed in insect infections with allantonematids makes this group of nematodes worthy of further study exploring the host-parasite relationships on the biochemical and ultrastructural levels. Much is to be learned about the properties of the host-parasite interface, selective uptake of nutrients, and parasite toxins released to the host hemocoel.

This group of nematodes also has a practical value. Severe pathology leading to the partial or complete sterilization and death of parasitized bark beetles, shown in the present study emphasizes again that allantonematid nematodes are an important, natural, biological agent controlling populations of bark beetles.

TABLE 3.

BIOLOGICAL GROUPS OF NEMATODES EXAMINED

(Classification based on the bionomics of parasitic forms.)

| Biological Group | Species of Nematode |
|---|---|
| I. *Sulphuretylenchus* type | *Sulphuretylenchus pseudoundulatus* *sp. nov.* |
| | *Sulphuretylenchus nopimingi* *sp. nov.* |
| | *Sulphuretylenchus posteruteri* *sp. nov.* |
| II. *Parasitylenchus* type | *Parasitylenchus caudapapilli* *sp. nov.* |
| III. *Neoparasitylenchus* type | *Neoparasitylenchus ipinius* Massey, 1974 |
| | *Allantonema paramorosum* Massey, 1974 |
| | *Contortylenchus reversus* Rühm, 1956 |

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Histology of noninfected bark beetle (e.g. P. sparsus).

Fig. 1. Frontal section of beetle abdomen and thorax; fm - fibrillar muscles (indirect flight muscles), mm - microfibrillar flight muscles, ft - fat body tissue, in - intestine. x 90.

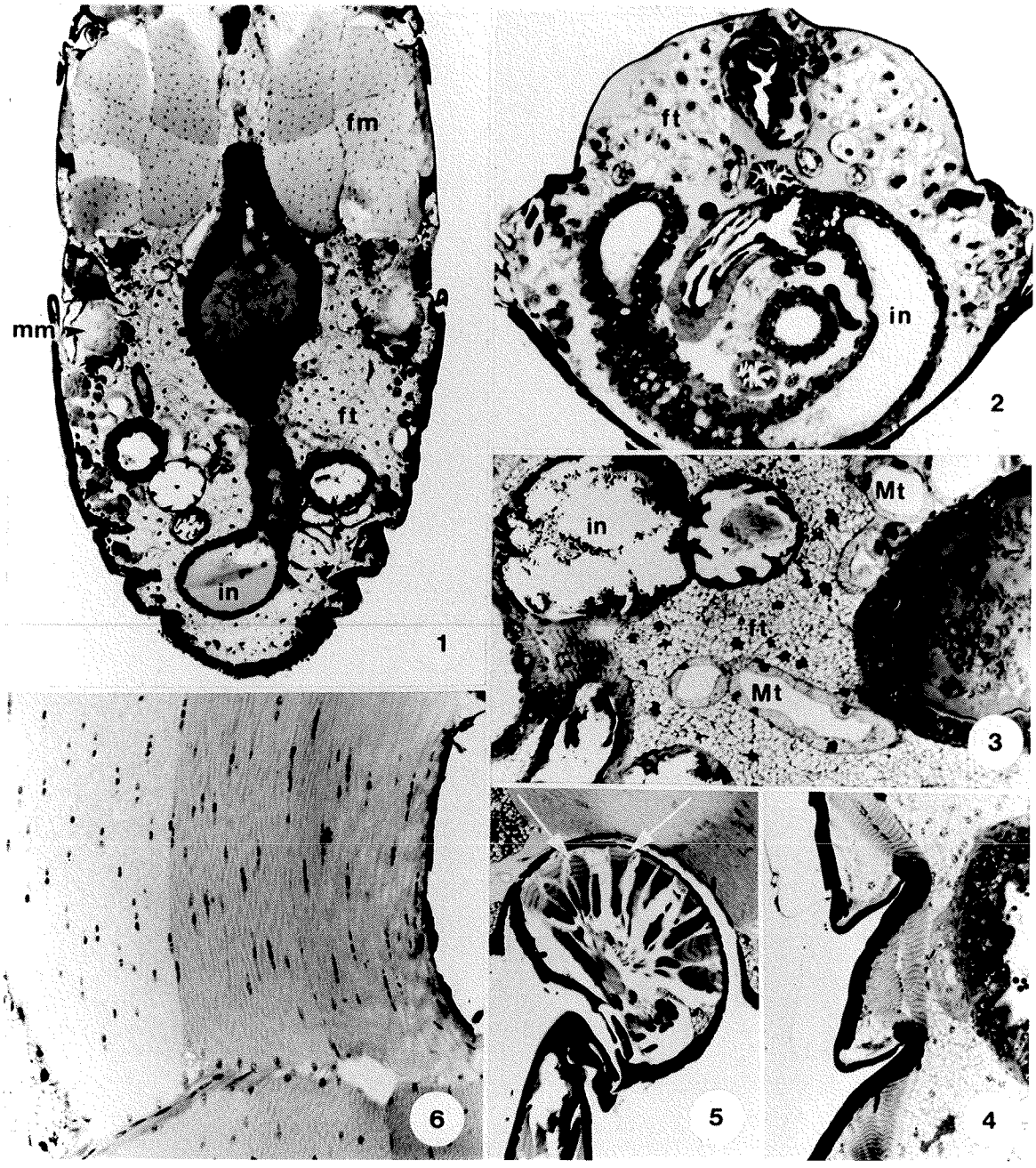
Fig. 2. Transversal section of beetle abdomen. Note regularly formed fat body tissue (ft). x 90.

Fig. 3. Section of beetle abdomen. Note fat body tissue (ft) filling spaces between other organs i.e. Malpighian tubules (Mt), and intestine (in). x 210.

Fig. 4. Microfibrillar muscles joining segments of beetle abdomen. Note distinct transversal striae. Sagittal section; x 210.

Fig. 5. Microfibillar muscles of coxa (arrows). Note distinct transversal striae on muscles. Section; x 210.

Fig. 6. Indirect flight muscles (fibrillar muscles) in insect thorax. Sagittal section; x 210.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mm
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ft
in
2
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ft
Mt
3
6
5
4

Gonads of noninfected bark beetle (e.g. P. rufipennis).

Fig. 7. Reproductive system of mature female beetle; T - tropharium, V - vitellarium, t - terminal filament, Lo - lateral oviduct, Mo - median oviduct, s - spermatheca, co - bursa copulatrix, O - oocyte. Glycerin mount; x 50.

Fig. 8. Tropharium of mature female beetle; g - germ cells, which usually form syncytium. Note differences in sizes of cells in upper and lower part of tropharium. Apical zone and zone of prefollicular epithelium are not shown here. Longitudinal section; x 110.

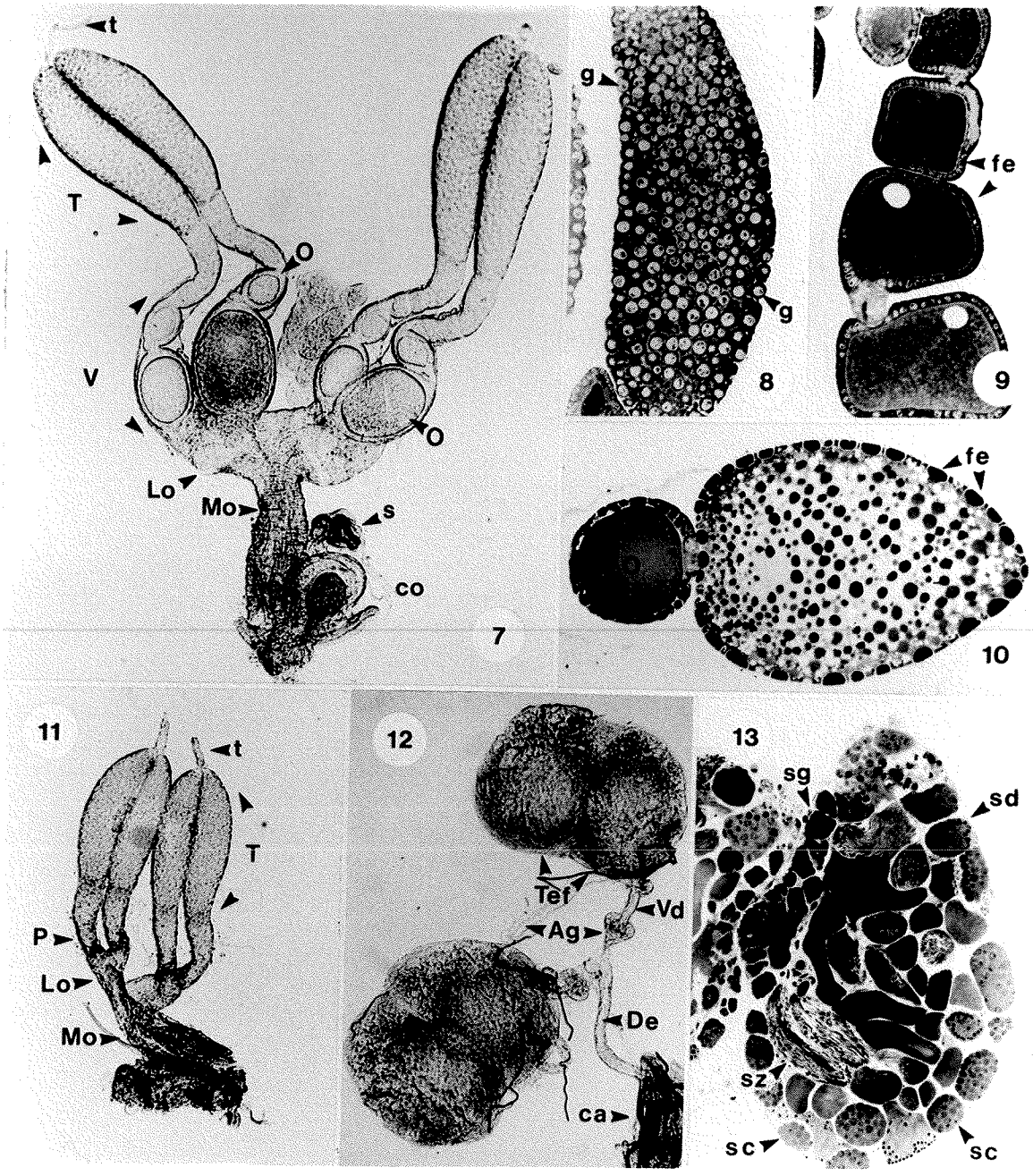
Fig. 9. Oocytes in central region of ovariole (previtellogenic zone). Note compact layer of follicular epithelium (fe) surrounding each oocyte. Longitudinal section; x 110.

Fig. 10. Oocytes in lower region of ovariole (vitellogenic zone). Note distinct cells of follicular epithelium (fe) and numerous protein and lipid granules in cytoplasm of oocyte. Longitudinal section; x 130.

Fig. 11. Reproductive system of immature female beetle. Note absence of developing oocytes and short pedicel (P) between tropharium (T) and lateral oviduct (Lo); t - terminal filament, Mo - median oviduct. Gylcerin mount; x 50.

Fig. 12. Reproductive system of male beetle. Note two follicles in each testis; Tef - testis follicle, Ag - accessory glands, Vd - vas deferens, De - ductus ejaculatorius, ca - copulatory organ. Glycerin mount; x 70.

Fig. 13. Section of testis follicle. Spiral, counterclockwise arrangement of cysts of germ cells. sg - spermatogonia, sc - spermatocytes, sd - spermatids, sz - spermatozoa. x 210.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*P. rufipennis* infected with *S. pseudoundulatus*.

Figs. 14 - 20. First phase of infection.

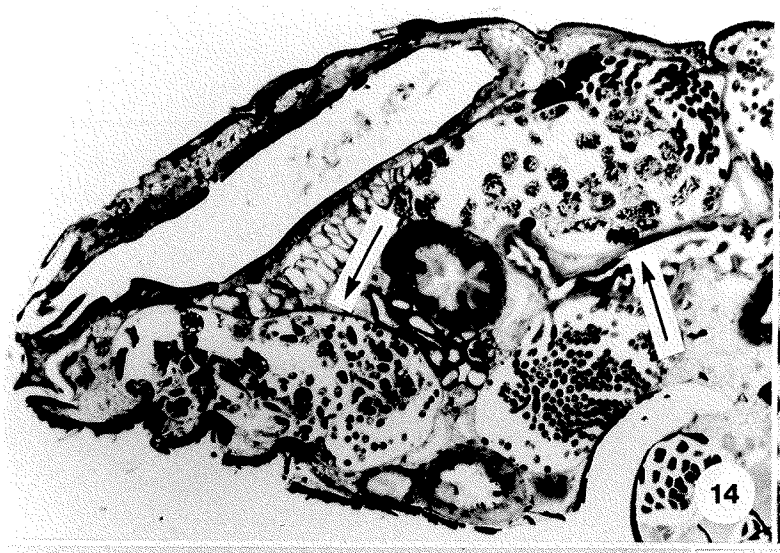
Figs. 14 - 15. Parasitic female nematodes (arrows) in abdomen and thorax of adult beetle. Note numerous juveniles developing inside female nematode. Sagittal section; x 120 (14), x 160 (15).

Fig. 16. Transversal section of abdomen of nematode infected beetle. Arrows indicate parasitic female nematodes. Note juveniles inside body of female nematode. x 120.

Figs. 17 - 18. Testes of noninfected (17) and infected (18) beetle. Note differences in sizes of these organs. Gylcerin mount; x 130.

Fig. 19. Testis of nematode infected beetle. Note small number of cysts with spermatozoa (arrows). Section; x 550.

Fig. 20. Testis of nematode infected beetle. Note only undifferentiated germ cells present in follicle. Section; x 550.



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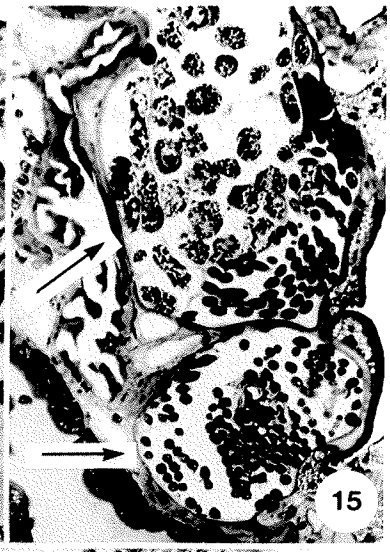

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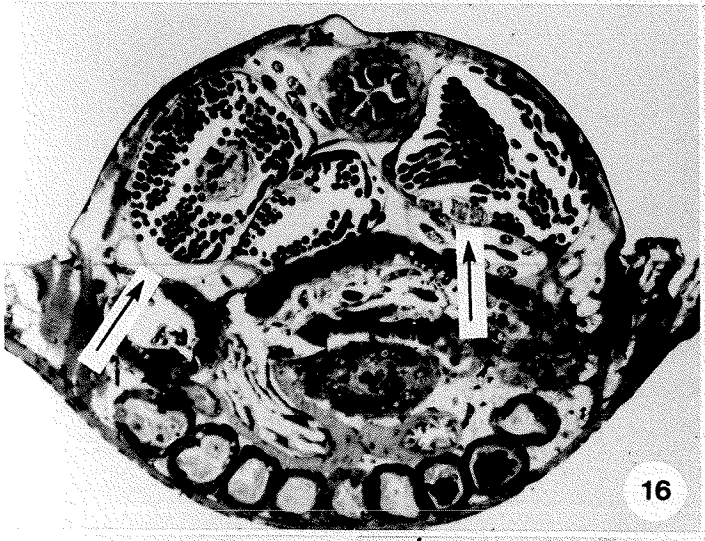

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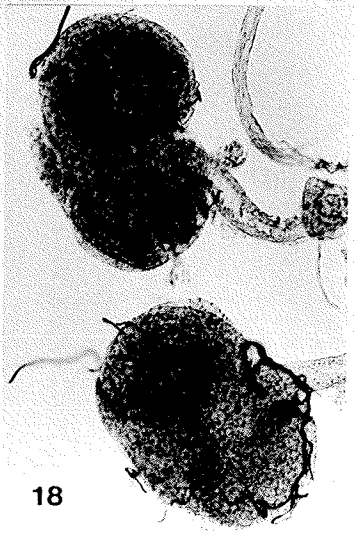

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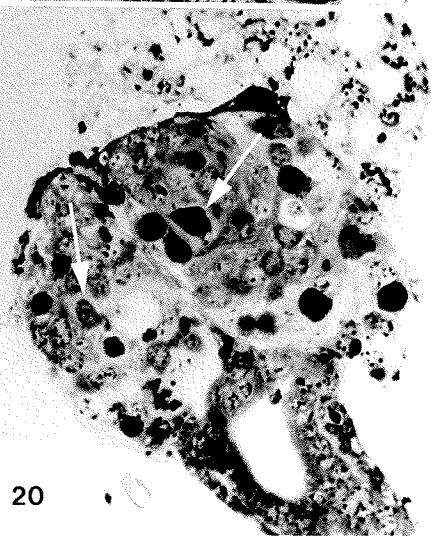

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P. rufipennis infected with S. pseudoundulatus.

Figs. 21 - 23. First phase of infection.

Figs. 21 - 22. Ovary of noninfected (21) and infected (22) immature female beetle. Note differences in sizes of ovarioles; T - tropharium. Glycerin mount; x 80.

Fig. 23. Tropharium of nematode infected beetle. Note distinct regions with viable germ cells (vg) and degenerated germ cells (dg) in the organ. Longitudinal section; x 740.

Figs. 24 - 29. Second phase of infection.

Fig. 24. Debris of beetle fat body tissue (ft) with distinct lesions caused by nematode juveniles. Section; x 400.

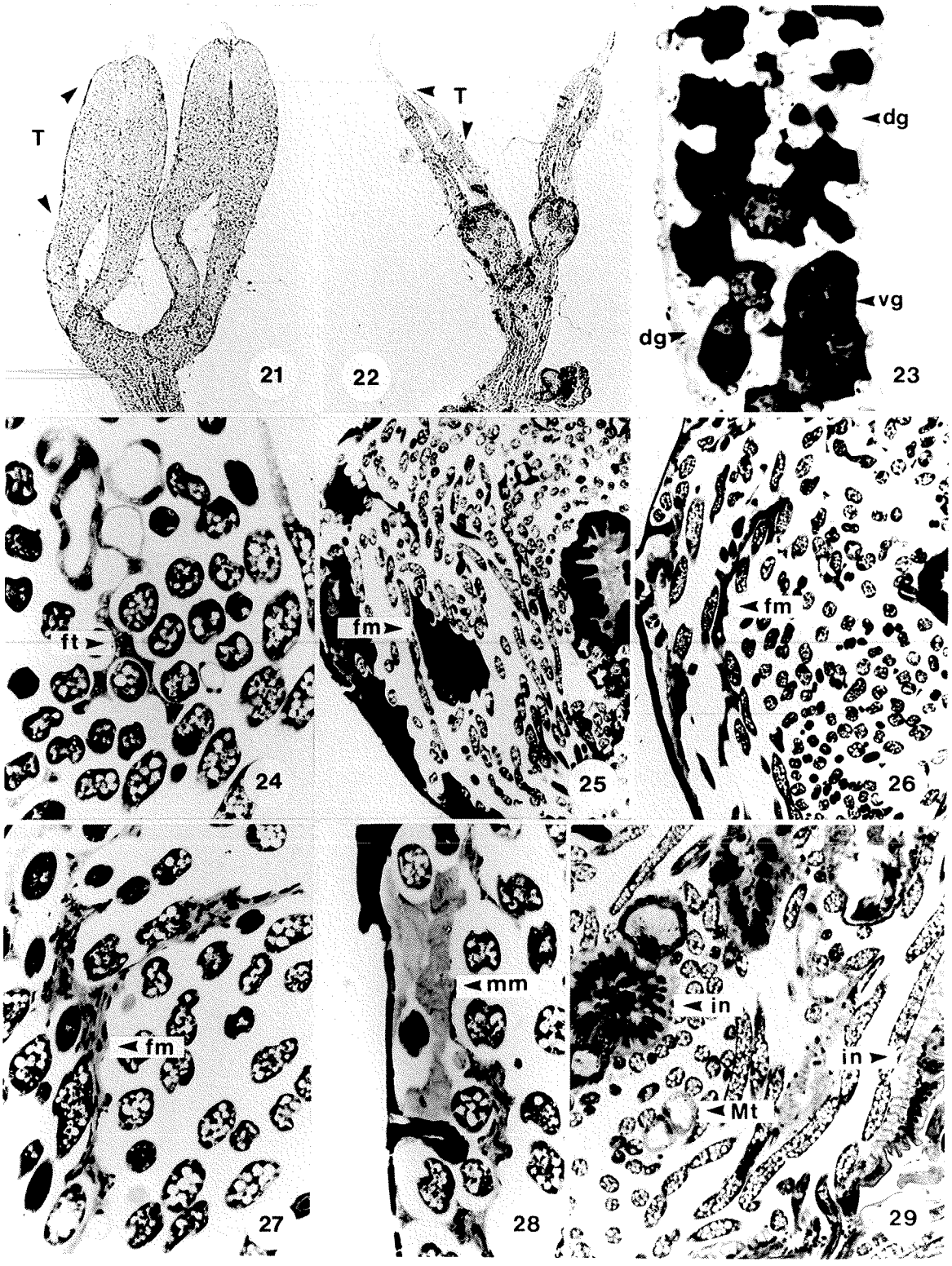
Fig. 25. Transversal section of beetle thorax with indirect flight muscles (fibrillar muscles) (fm) partially injured by nematode juveniles. x 100.

Fig. 26. Transversal section of beetle thorax with almost completely degraded fibrillar muscles (fm). x 150.

Fig. 27. Debris of indirect flight muscles (fibrillar muscles) (fm) with distinct lesions caused by nematode juveniles. Section; x 400.

Fig. 28. Microfibrillar muscles (mm) of the beetle thorax locally injured by juvenile nematodes. Section; x 400.

Fig. 29. Nematode juveniles in abdomen of beetle. Note complete absence of fat body tissue and hitherto unaffected intestine (in) and Malpighian tubules (Mt). Section; x 170.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Figs. 30 - 35. P. rufipennis infected with S. pseudoundulatus. Second phase of infection.

Fig. 30. Transversal section of beetle coxa with distinct lesions in microfibrillar muscles caused by nematode juveniles (arrows). x 400.

Fig. 31. Section of thoracic ganglion with lesions caused by nematode juveniles (arrows). x 400.

Fig. 32. Frontal section of beetle prothorax and head. Note distinct lesions in microfibrillar muscles and nervous tissue of ganglia (arrows). x 130.

Fig. 33. Section of beetle head. Note numerous nematode juveniles (arrows) in microfibrillar muscles. x 100.

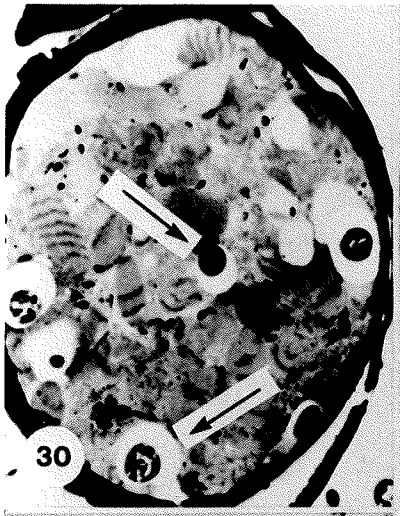
Fig. 34. Sagittal section of beetle head. Note lesions in microfibrillar muscles of insect mouthparts and in brain (br) caused by parasites (arrows). x 100.

Fig. 35. Transversal section of beetle thorax, in final stage of infection. Note numerous nematode juveniles and complete absence of tissues in insect hemocoel. x 100.

Figs. 36 - 38. P. sparsus infected with S. nopimingi.

Fig. 36. Tropharia of nematode infected beetle. Note small number of undifferentiated germ cells. Longitudinal section. x 450.

Figs. 37 - 38. Copulatory organ of noninfected (37) and infected (38) male beetles. Note differences in shape and sizes of chitinous structures. Glycerin mount. x 90.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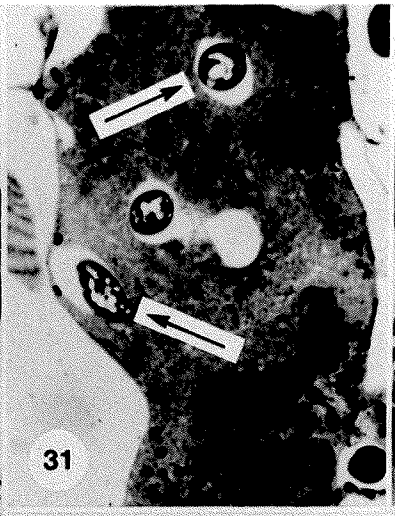
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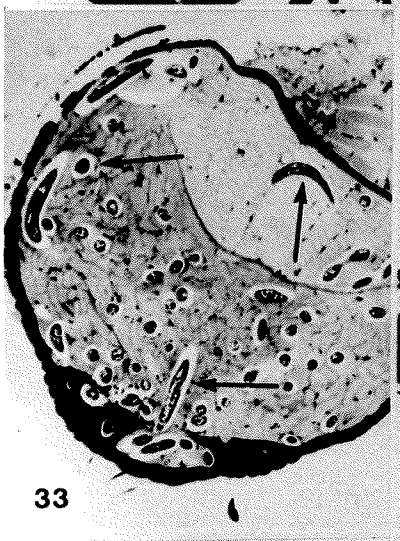
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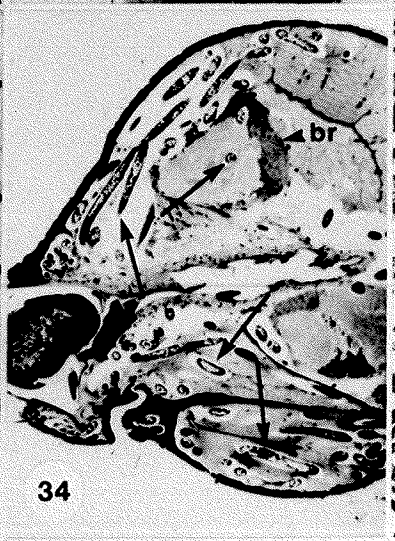

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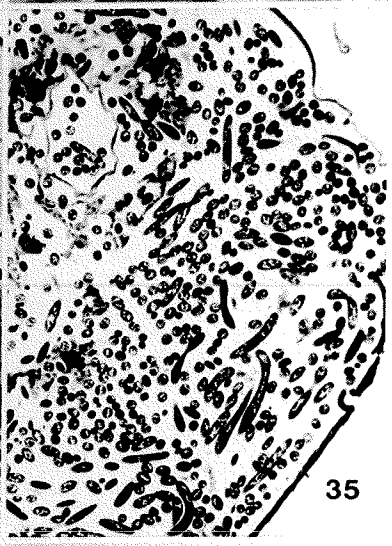
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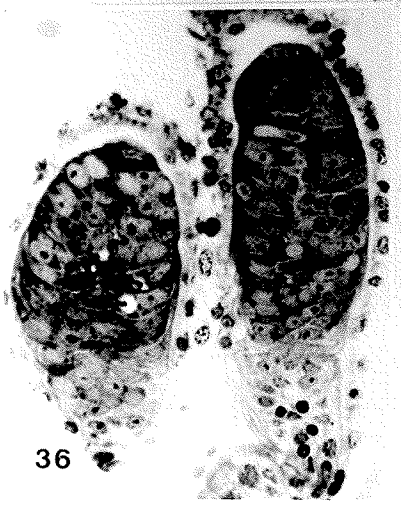
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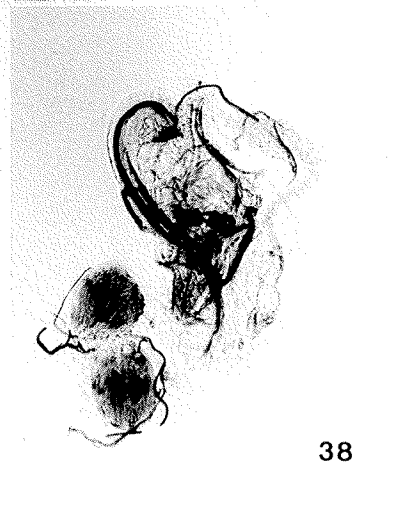
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Figs. 39 - 40. I. perroti infected with S. posteruteri

Fig. 39. Transversal section of beetle thorax. Note lack of fat body tissue and lesions in microfibrillar muscles caused by juvenile nematodes. x 170.

Fig. 40. Degenerating ovarioles of nematode infected beetle. Note break down of oocyte (O) cytoplasm and disintegration of follicular epithelium (fe). Longitudinal section; x 140;

Figs. 41 - 43. I. perturbatus infected with P. caudapapilli.

Fig. 41. Ovary of nematode infected beetle. Note reduced numbers of simultaneously developing oocytes in individual ovarioles. Glycerin mount. x 30.

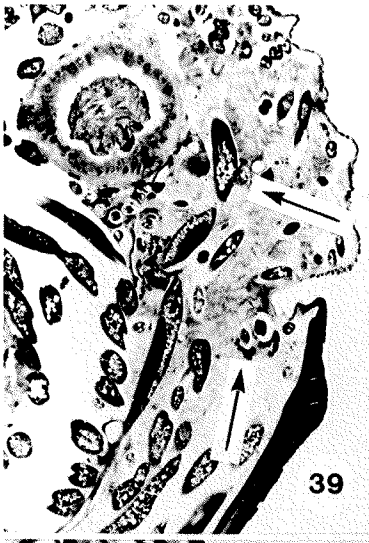
Fig. 42. Transversal section of beetle thorax in early stage of infection. Nematode juveniles (arrows) present in fat body tissue. x 180.

Fig. 43. Section of beetle thorax in late stage of infection. Parasitic females and juvenile nematodes (arrows) present in hemocoel. Note partially degraded fibrillar muscle tissue (fm). x 180.

Figs. 44 - 46. I. perturbatus infected with N. ipinius.

Figs. 44 - 45. Syncytium in tropharium of noninfected (44) and infected (45) beetle. Note differences in shape of germ cell nuclei. Longitudinal section; x 210.

Fig. 46. Degenerated ovariole of nematode infected beetle. Longitudinal section; x 140.



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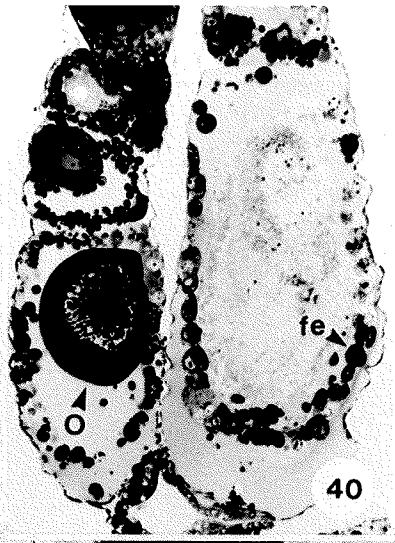


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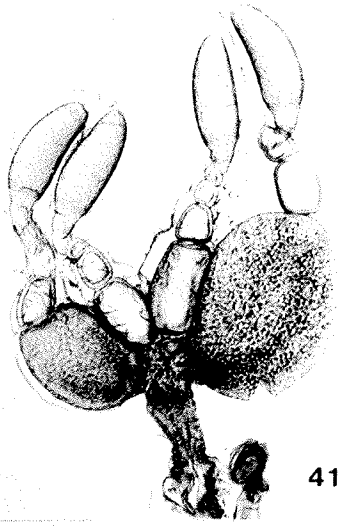

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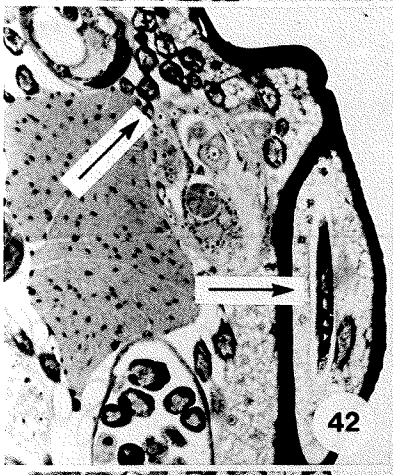

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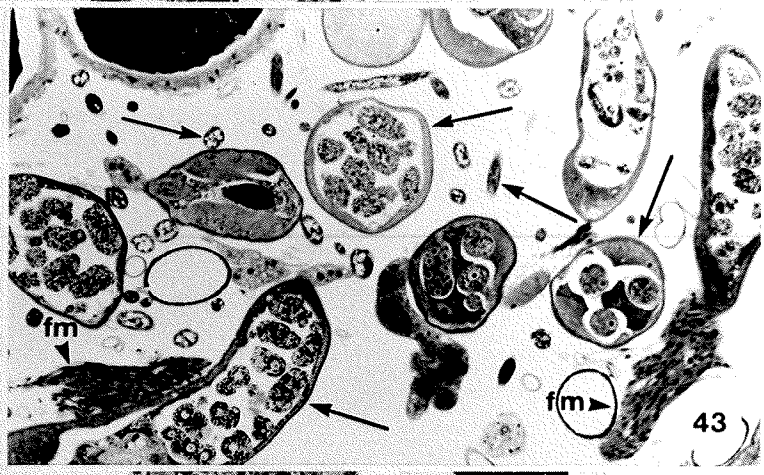


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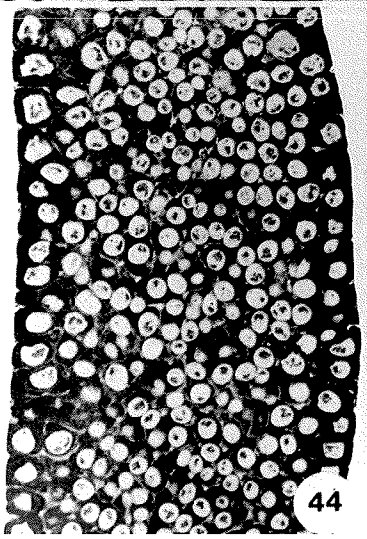

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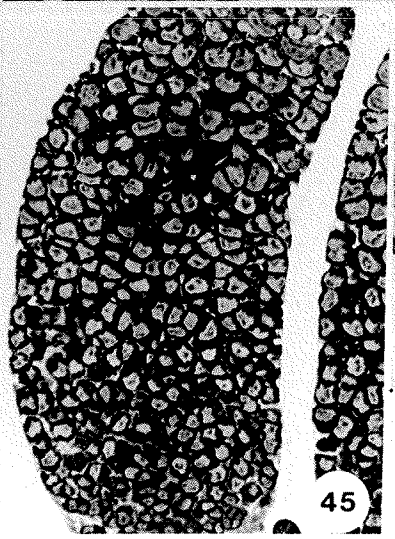

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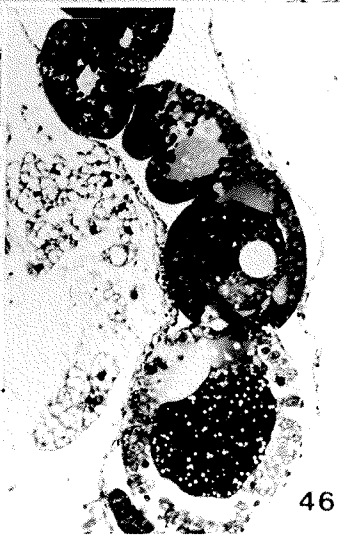

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Figs. 47 - 48. I. perturbatus infected with N. ipinius.

Fig. 47. Nematode juveniles in beetle abdomen - early stage of infection. Note absence of fat body tissue and hitherto unaffected microfibrillar muscles (mm). Sagittal section; x 150.

Fig. 48. Nematode juveniles in beetle abdomen - late stage of infection. Note partially degraded microfibrillar muscles (mm) with distinct lesions caused by parasites (arrows). Sagittal section; x 150.

Figs. 49 - 50. D. autographus infected with A. paramorosum.

Figs. 49 a,b. Juvenile nematodes in beetle hemocoel. Note lesions (arrows) in fat body tissue. Transversal section; x 130.

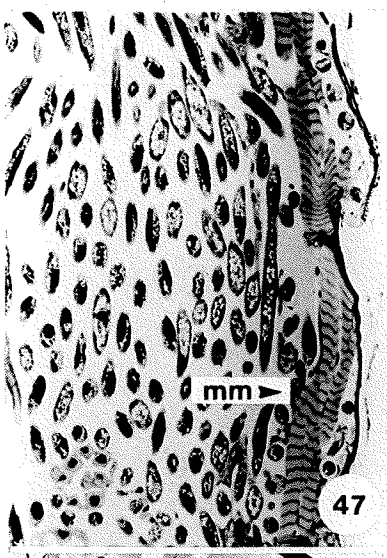
Fig. 50. Local lesions (arrows) in microfibrillar muscles of beetle abdomen caused by nematode juveniles. Transversal section; x 250.

Fig. 51. Juveniles of C. reversus in hemocoel of D. simplex. Note limited injury to beetle fat body tissue. Section; x 130.

Fig. 52. Gallery system of polygamic P. sparsus. Note galleries of noninfected females with distinct egg niches, and galleries of sterilized females parasitized with S. nopimingi (arrows). x 0.6.

Fig. 53. Aggregation of juvenile S. posteruteri (arrow) emerged from body of I. perroti. x 1.2.

Fig. 54. Melanized parasitic female of S. posteruteri recovered from hemocoel of I. perroti. Glycerin mount. x 240.

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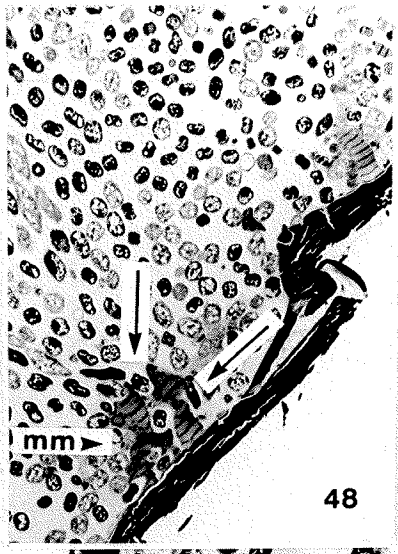


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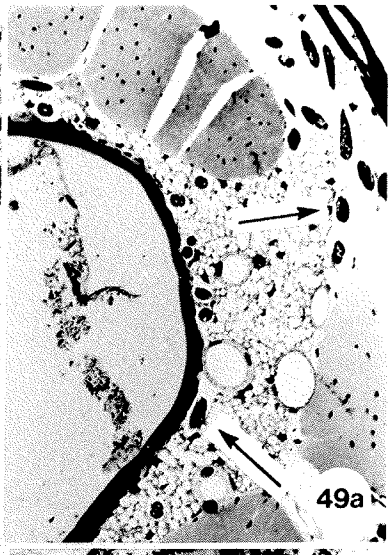

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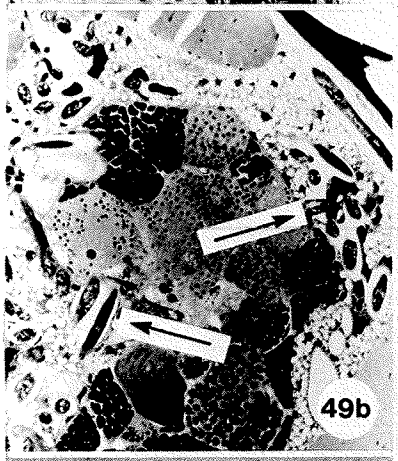

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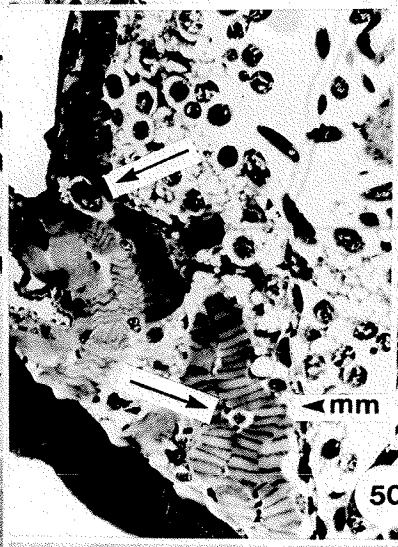
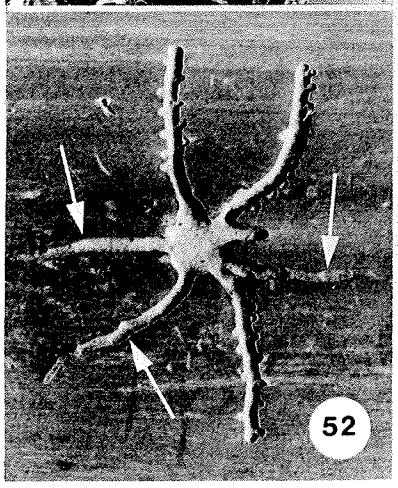
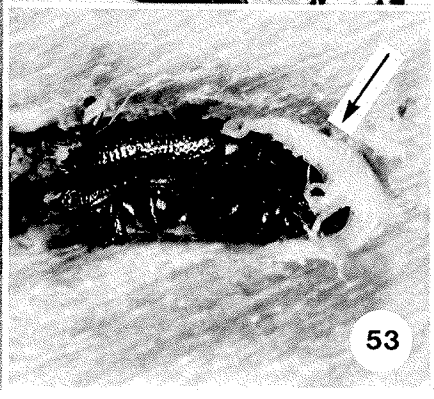



50

51



52



53

54

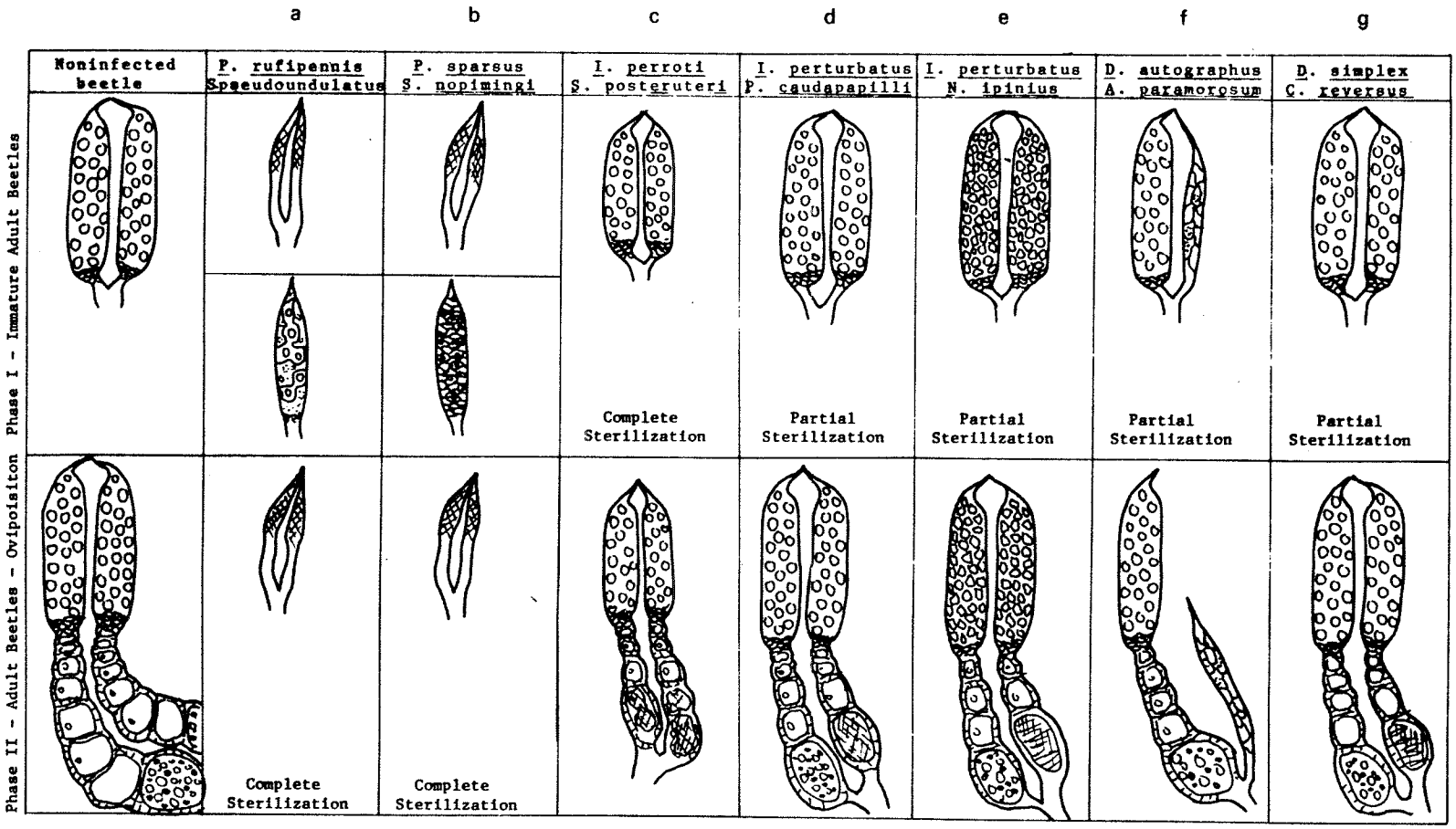



Fig. 55. Influence of nematode parasitism on the development of bark beetle gonads - summary of pathology.

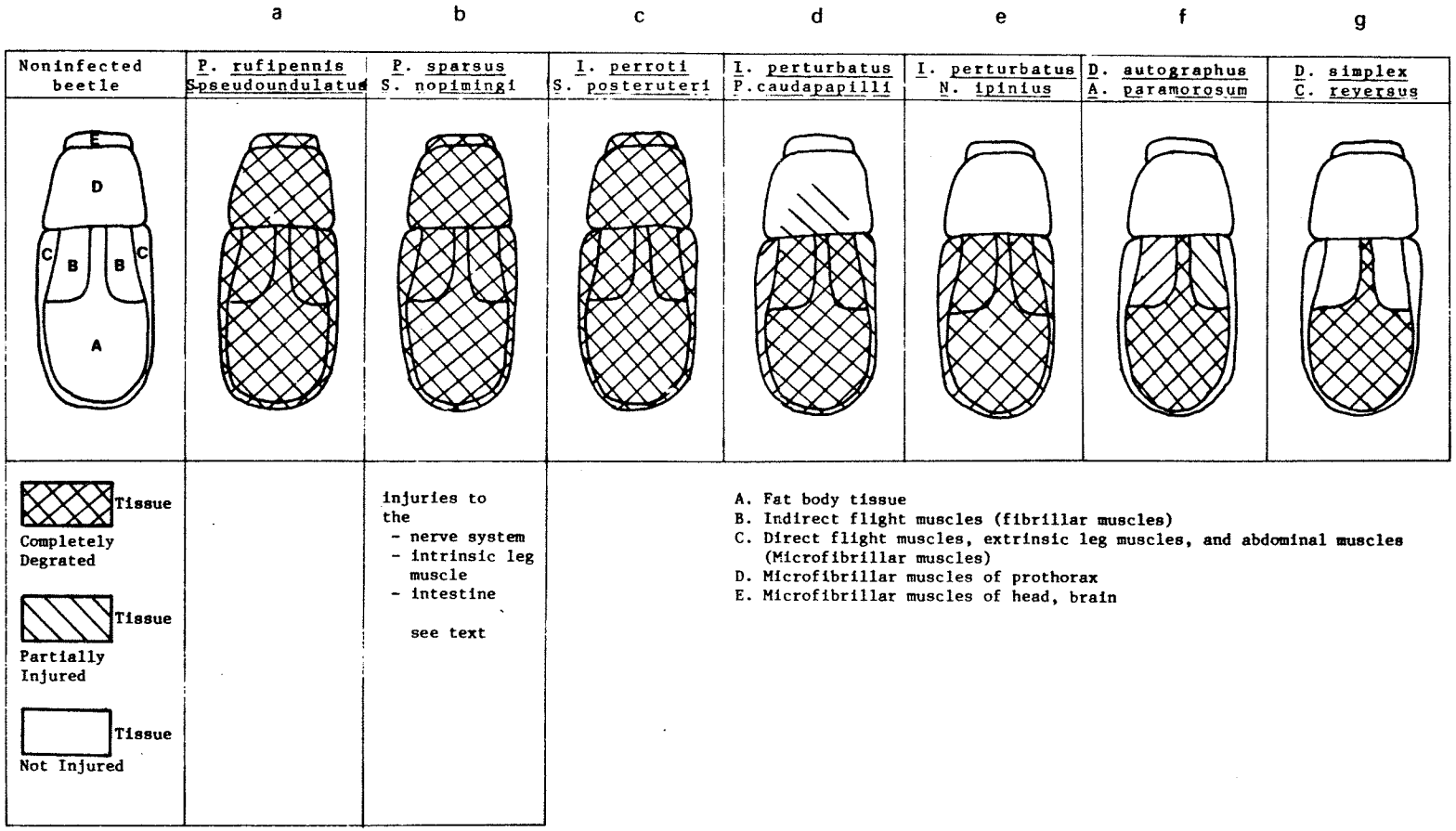



Fig. 56. Tissue degradation in bark beetles infected with allantonematids - summary of pathology.

TABLE 4. SEASONAL CHANGES IN PREVALENCE OF NEMATODE INFECTION IN ADULT BARK BEETLES.

| BARK BEETLE SPECIES | NEMATODE SPECIES | LATE SUMMER 1981 IMMATURE ADULTS IN LARVAL GALLERIES | | SPRING 1982 MATURE ADULTS DURING OVIPOSITION | |
|---|---|---|---|---|---|
| | | NO. EXAMINED | PREVALENCE % | NO. EXAMINED | PREVALENCE % |
| *Ips perroti* Swaine | *Sulphuretylenchus posteruteri* sp. n. | 311 | 15.4 | 388 | 2.1 |
| *Polygraphus rufipennis* (Kirby) | *Sulphuretylenchus pseudsundulatus* sp. n. | 312 | 20.3 | 774 | 3.2 |
| *Pityokteines sparsus* (LeConte) | *Sulphuretylenchus nopimingi* sp. n. | 330 | 26.7 | 328 | 7.8 |

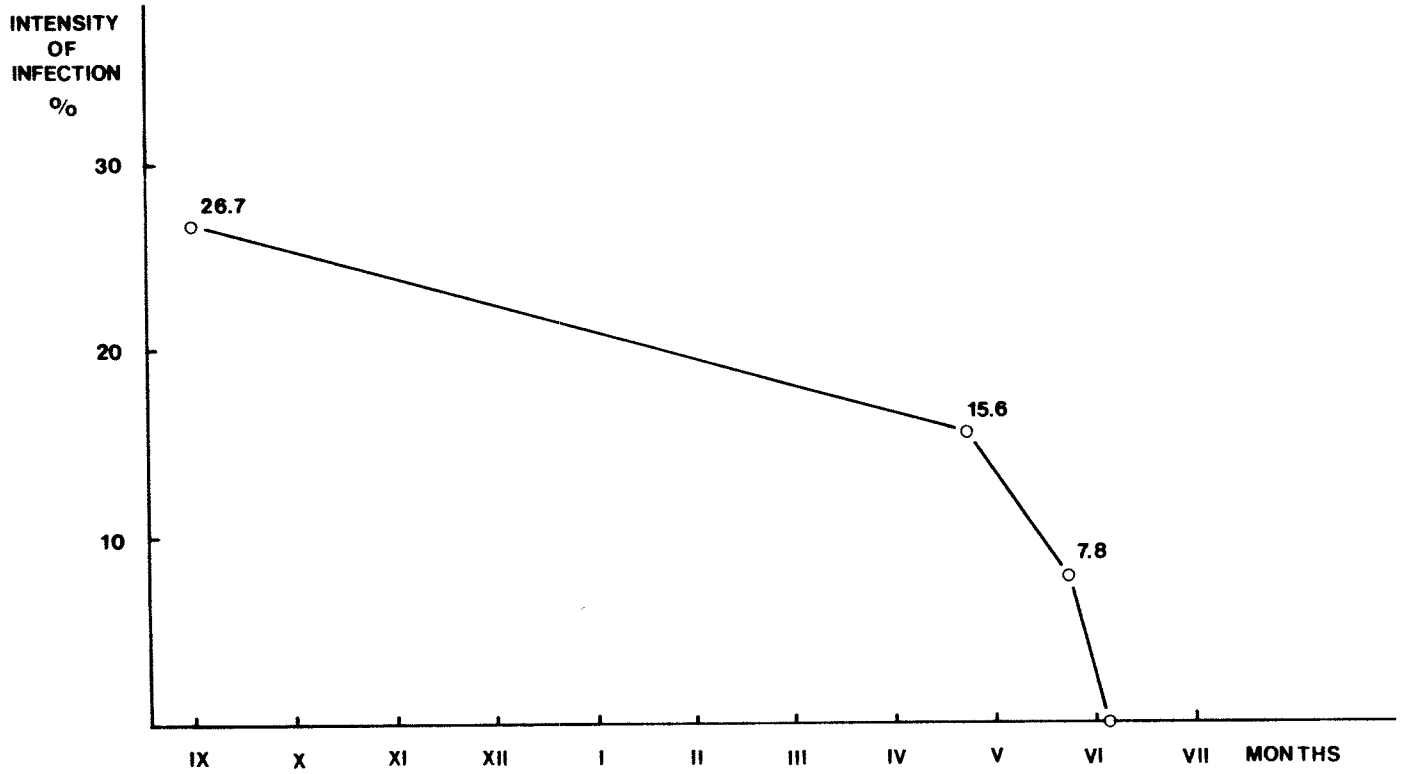



Fig. 57. Seasonal changes in prevalence of infection of Pityokteines sparsus by Sulphuretylenchus nopimingi.

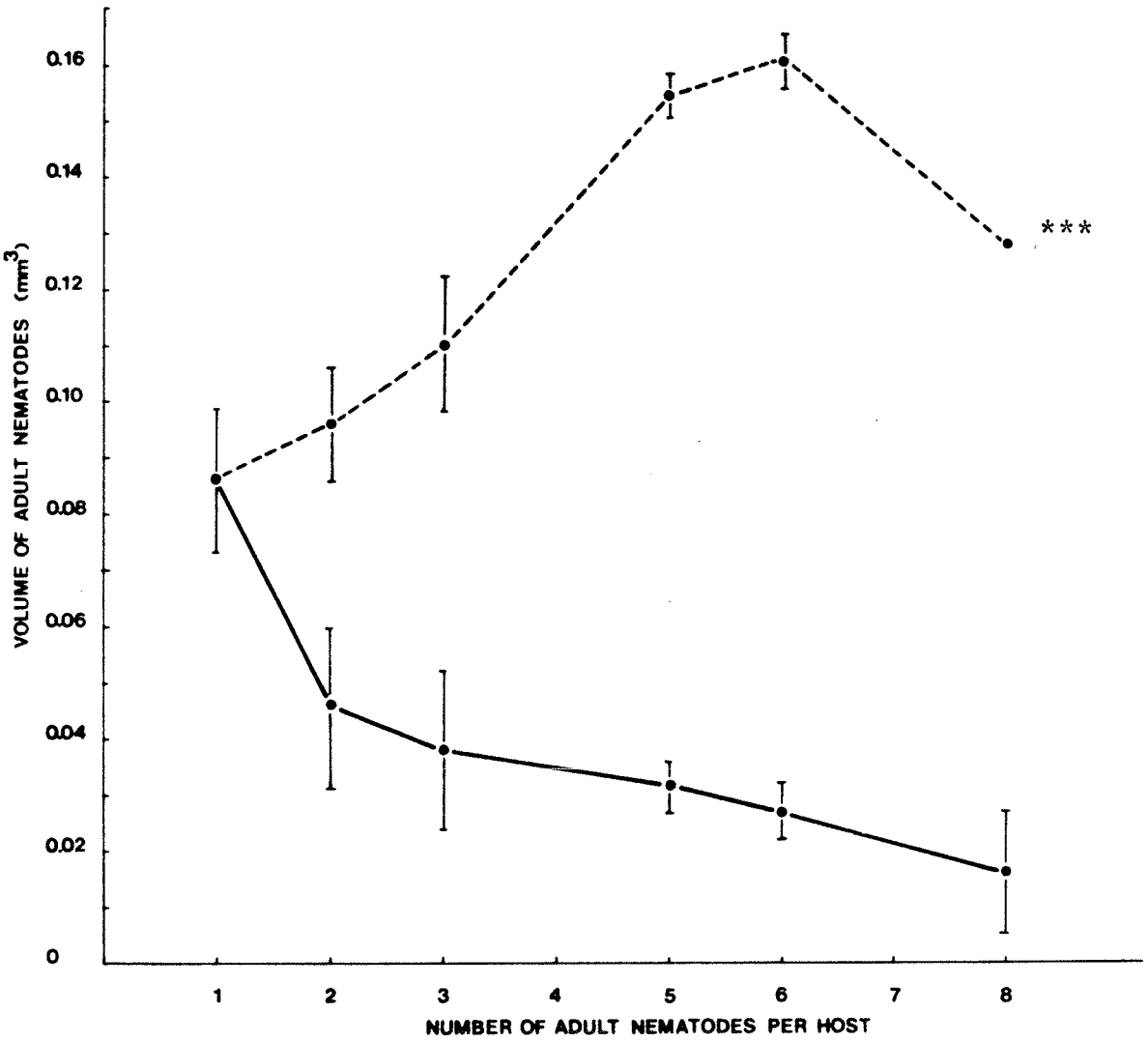



Fig. 58. Relationship between intensity of infection and volumes of parasitic females, Sulphuretylenchus pseudoundulatus in Polygraphus rufipennis.
(----) Mean of total volume of parasites per host.
(——) Mean volume of individual parasites.
*** Only one sample.

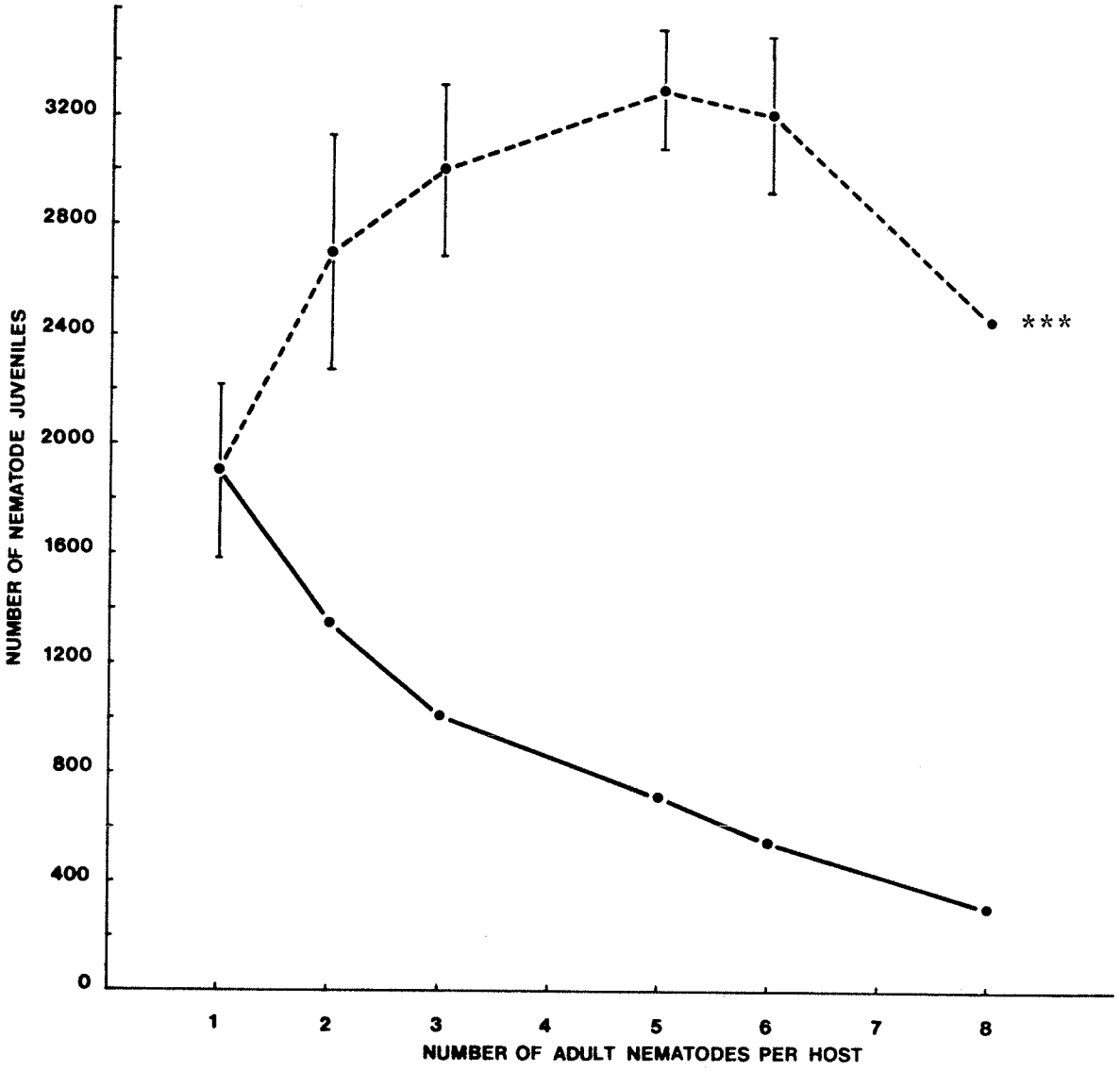



Fig. 59. Relationship between intensity of infection and fecundity of Sulphuretylenchus pseudoundulatus in Polygraphus rufipennis.

(----) Mean number of juvenile nematodes per host.
(——) Number of juvenile nematodes per parasitic female nematode (calculated as mean number of juveniles per host / intensity of infection).
*** Only one sample.

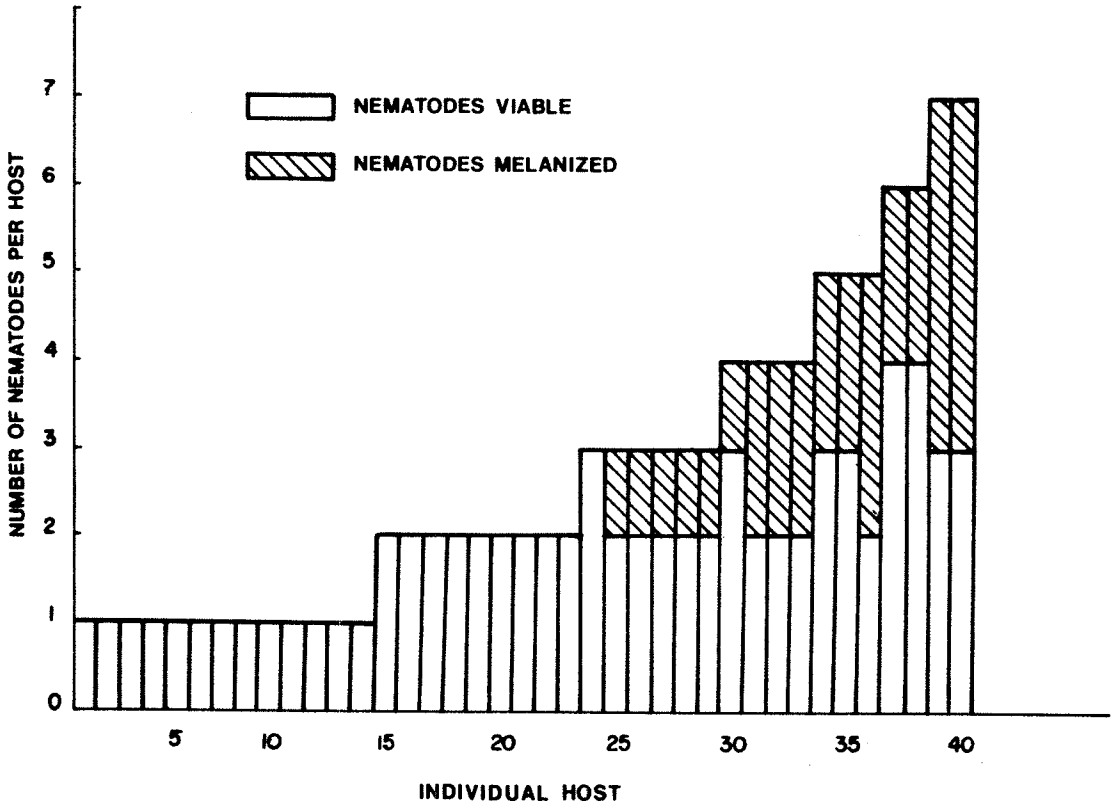



Fig. 60. Relationship between intensity of infection and numbers of melanized female parasites, Sulphuretylenchus posteruteri in Polygraphus rufipennis.

## GENERAL DISCUSSION

Histopathology of nematode infection has been given only limited attention in entomological studies. Thus a detailed comparison of effects caused by different groups of parasites is difficult. From available literature and our investigations three general classes of pathology related to the method of food ingestion by the parasite can be distinguished. The most unusual, histopathologically, are infections with neoaplectanids, in which changes of the host tissue are caused mainly by bacterial septicemia, and are expressed as gradual break down of the insect tissue (Poinar and Himsworth, 1967; Tomalak and Welch, unpublished). Mermithids, take up nutrients from the host hemolymph through the body wall and cause abnormal development and atrophy of the insect organs (Bailey and Gordon, 1973). Local, direct injuries to the insect tissue are caused by filariids (Hockmeyer et al., 1975; Bartlett, 1984), spirurids (Jilek and Crites, 1980), rhabditids and aphelenchids (described in the present study). Pathology caused by allantonematids represents an intermediate position between the last two classes. Abnormal development of host organs during the first phase of parasitism, and direct injuries to various tissues during the second phase are related to the presence of female nematodes and juveniles respectively, and make Allantonematidae unique among entomophilic parasites.

Histopathology caused by nematodes in the bark beetles examined was parasite specific. The severity of injuries ranged from no apparent pathological changes in the host, observed in D. autographus infected

with P. autographi, to almost complete degradation of the insect tissues in beetles infected with Sulphuretytenchus spp. Differences in pathogenicity were related to several biological characteristics presented by the nematodes studies.

Life cycles separated the parasites into two groups. Only juvenile Rhabditidae and Aphelenchidae infected the host beetle. Nematodes usually developed to the next ($L_3$, or $L_4$) juvenile stage and suspended their growth for the remaining period in the insect body. Thus, no reproduction and only limited growth of the parasites occurred in the beetle.

The infection of earlier developmental stages of the host and consequently longer period of parasitism did not influence the severity of injuries caused by Rhabditidae and Aphelenchidae. Nematodes which infected larval ($L_3$) beetles, (P. autographi and B. fraudulentus) were not more pathogenic than species which infected immature adult beetles (P. obtusa and P. oldhami). This was probably related to the suspension of the parasite development after some period in the host.

Allantonematids infected the host as fertilized females and grew through the entire period in the insect hemocoel. Also juveniles of a new generation developed in the beetle. Thus, a significant increase in the parasite size and reproduction occurred in the host.

The severity of injuries caused by rhabditids and aphelenchids increased with the numbers of nematodes infecting the beetle. The relationship between intensity of nematode infection and severity of injuries was not easily observed in beetles parasitized by allantonematids. "Crowding" of nematodes led to the significant reduction of sizes and fecundity of parasitic females, or to the

reduction of parasites by the insect immune responses. Thus, the high intensity of infection was controlled by intraspecific competition between nematodes, or by the insect immunity, which significantly affected the sizes of parasite infrapopulations.

Rhabditidae and Aphelenchidae were niche specific. Nematodes remained in the infected organs (intestine, Malpighian tubules) or tissues (fat body, hemolymph) and most of the direct injuries were limited to those sites. Allantonematidae occurred in the host hemocoel, and during an early stage of infection, mainly fat body was affected. As parasitism progressed, other tissues also were injured by developing juveniles.

Finally, the nematode pathogenicity was related to the specific ability of parasites to rupture and/or to lyse the host tissues. Juveniles of P. autographi were apparently lacking the ability to penetrate the host intestinal epithelium. P. obtusa penetrated the intestinal epithelium and caused local lesions in that tissue. Parasitism of P. pityokteini led to the partial or complete degradation of cuboidal epithelium in Malpighian tubules. Those species, however, lacked the ability to rupture the basement membranes of occupied organs and to enter the host hemocoel.

All remaining species of the parasites examined, readily entered the beetle hemocoel through the intestine or probably through the integument (Rühm, 1956). Juveniles of aphelenchids: B. fraudulentus and P. oldhami caused lesions only in the fat body, while the other tissues remained intact.

Juvenile allantonematids presented significant differences in ability to lyse the host tissues. All the species examined caused

degradation of fat body. Usually no other tissues were injured by C. reversus. Parasitism of A. paramorosum caused partial degradation of the indirect (fibrillar) flight muscles, and only occasionally local lesions were observed in microfibrillar muscles.

N. ipinius and P. caudapapilli almost completely lysed the indirect (fibrillar) flight muscles and caused partial degradation of microfibrillar muscles. Juveniles of Sulphuretylenchus spp. lysed all tissues of the host.

Despite the individual differences between effects of parasitism caused by all examined nematodes, pathogenicities of Rhabditidae and Aphelenchidae have many similar characteristics, and significantly differ from those of Allantonematidae.

# GENERAL CONCLUSIONS

1. During the 1980 - 1983 study, 32 species of bark beetles and some 56 species of nematode parasites of these insects were found in the southern part of Manitoba (south of 53°00'N.).

2. Four new species of nematode parasites of scolytids were found and described taxonomically, namely;

   *Parasitylenchus caudapapilli sp. nov.*

   *Sulphuretylenchus pseudoundulatus sp. nov.*

   *Sulphuretylenchus nopimingi sp. nov.*

   *Sulphuretylenchus posteruteri sp. nov.*

3. Six general types of host - parasite relationships between bark beetles and nematodes were established based on differences in the bionomics of the parasites, the time of invasion of the host and the niche occupied in the insect body.

4. Juveniles of *Parasitorhabditis* can severely injure the intestinal epithelium of bark beetles. Pathogenicity was parasite specific: *P. obtusa* caused local lesions in the intestinal epithelium, while *P. autographi* did not induce any apparent pathological effects in the host.

5. Juveniles of *A. pityokteini* parasitized Malpighian tubules of *P. sparsus*.

a. Nematodes developed in the host.

b. Parasitism led to partial or complete degradation of the cuboidal epithelium in infected tubules.

c. Superparasitism and severe injuries to the Malpighian tubules led to premature death of heavily infected insects.

d. Adult P. sparsus responded immunologically to the nematode infection and caused melanization of some of the parasite individuals.

6. Synergistic effects of the coexistence of rod-shaped bacteria and juvenile P. oldhami in the hemocoel of H. rufipes caused severe pathological changes in the host.

   a. Bacteria were spread from local sites throughout the insect fat body by nematodes feeding on this tissue.

   b. Partial or complete breakdown of fat body by nematodes and bacteria lowered the insect potential to overwinter normally, and caused significant reduction of the beetle population prior to and during the period of hibernation.

   c. The bionomics of P. oldhami in H. rufipes differed in details from that described in other beetle hosts. In H. rufipes only immature adult beetles were invaded by the parasites.

7. Juvenile B. fraudulentus parasitized larval, pupal and adult T. populi, and caused local lesions in the host fat body.

8. Allantonematids were the most pathogenic nematode parasites of bark beetles.

a. Two phases: the first, abnormal development of beetle organs, and the second, degradation of previously formed tissue were characteristic of the allantonematid infections in bark beetles.

b. During the first phase of parasitism female nematodes probably fed on the host haemolymph, and by changing the content of hemolymph impaired the development of fat body tissue. This caused abnormal oogenesis, and in some species disrupted spermatogenesis of the insects.

c. In the second phase, juvenile nematodes fed directly on the beetle tissues, and caused partial or complete degradation of the host organs.

d. Juvenile nematodes affected the fat body tissue first, and later fibrillar muscles, microfibrillar muscles and nervous system.

e. Histopathological processes in bark beetles infected with allantonematids reduced the insect reproductive potential, altered the behaviour in construction of galleries, and usually caused premature death of infected beetles.

f. Pathogenicity was parasite specific, and the severity of pathological processes was greatest in beetles infected with Sulphuretylenchus spp.

g. Overcrowding of allantonematids in the beetle caused a reduction of body size and fecundity of parasitic female nematodes, and in I. perroti it stimulated the host immune responses.

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# Appendix I

## LOCALITIES OF COLLECTIONS AND BIOLOGY OF INVESTIGATED BARK BEETLES (COL., SCOLYTIDAE).

1. Ips perturbatus (Eichhoff).

Locality: Agassiz Provincial Forest, near Rd. 15, about 5 km east to Hazel.

Host: Black spruce, Picea mariana (Mill.) B.S.P.

Biology: Adult insects infested trunks of older fallen or dying trees. First attacks were observed in early May. General pattern of galleries was X, or Y-shaped with 3 to 4 egg galleries leading from the nuptial chamber. Beetles of a new generation emerged in the late July and August. Additional attacks on the host trees were observed in late July. There is probably a second generation or "sister generation" of I. perturbatus in Manitoba. Beetles overwintered as imagines, probably in the litter.

2. Dryocoetes autographus (Ratzeburg).

Locality: Agassiz Provincial Forest, near Rd. 15, about 10 km east to Hazel.

Host: Black spruce, Picea mariana (Mill.) B.S.P.

Biology: Adult insects infested the host tree in May and June. Construction of egg galleries occurred under the bark of trunks and larger limbs of dying and injured elms. Beetles of a new generation emerged in September. Immature adult beetles usually flew to healthy trees where they fed and later overwintered under the bark near the base of the trunk. Before the initiation of oviposition in the spring, beetles fed for a short period in the branches of still living trees. Part of the beetle population may overwinter as larvae (Bright, 1976), but it was not observed in our research. H. rufipes is one of the major vectors of Dutch Elm Disease in Canada.

5. Trypophloeus populi Hopkins.

Locality: Winnipeg, a small forest stand near the intersection of Saskatchewan Ave. and Summit Rd.

Host: Aspen, Populus tremuloides Michx.

Biology: Monogamic species. Beetles infested thin branches and twigs of a living host tree. The main attack took place in July. Insects overwintered as larvae and continued their development during spring. Immature insects emerged in June. Maturation feeding was carried out on aspen twigs. One generation per year was observed in Manitoba.

6. Polygraphus rufipennis (Kirby).

Locality: Whiteshell Provincial Forest, near Rd. 315, about 1 km south of the bridge on Bird River.

Host: Black spruce, Picea mariana (Mill.) B.S.P.

Biology: Beetles overwintered as adults, larvae and occasionally as pupae. Insects that overwintered as adults infested a new host tree in May, while beetles that overwintered as larvae matured during the spring and attacked hosts in July. Irregular, star-shaped gallery systems consisted of 3 - 5 egg galleries, each made by one female. Insects infested trunks and branches of dead or dying trees. One or one and a half generation per year was observed in Manitoba.

7. Ips perroti Swaine.

Locality: Belair Provincial Forest, near Belair, Rd. 59.

Host: Jack pine, Pinus banksiana Lamb.

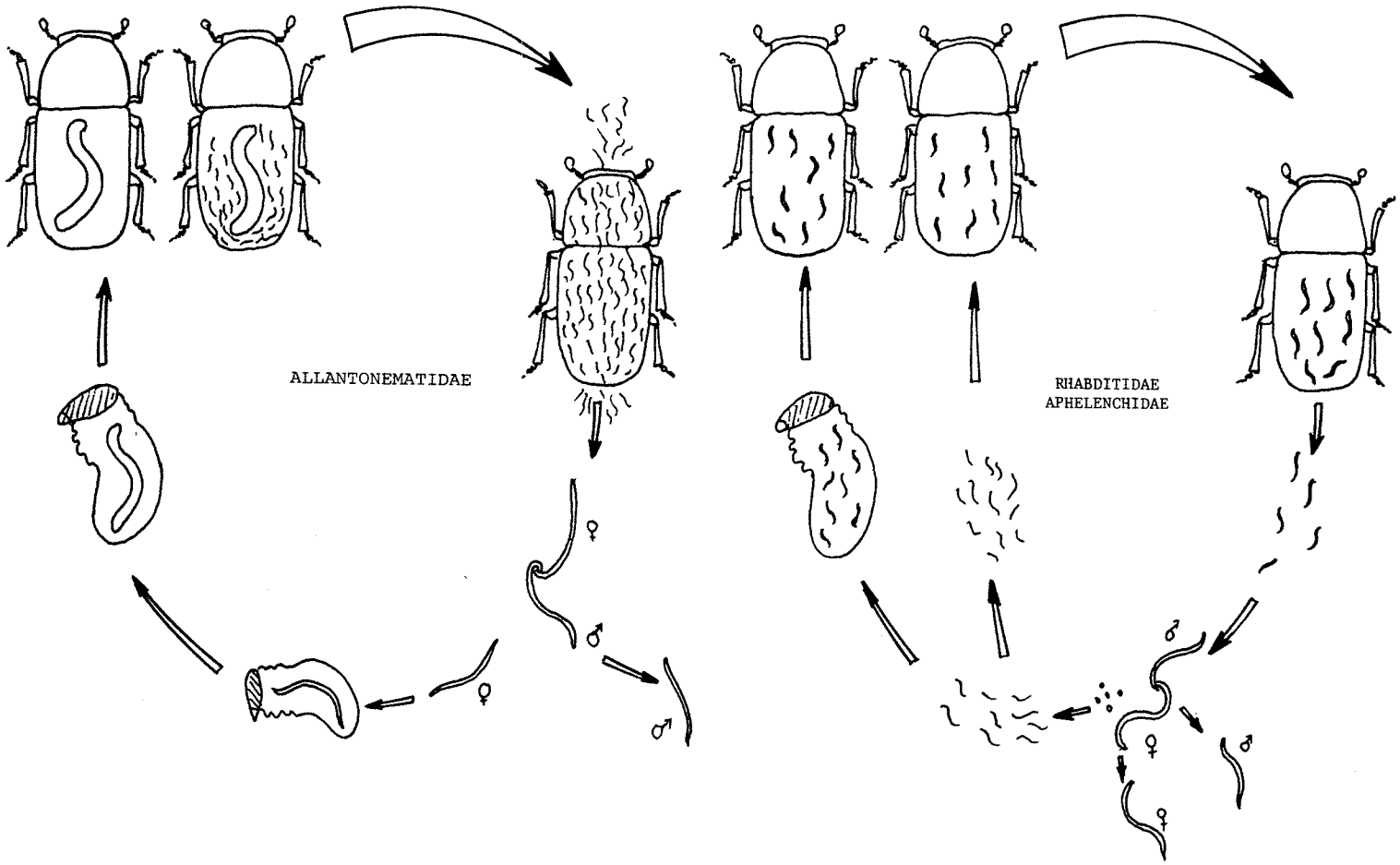
Biology: Adult beetles infested thin-barked portions of trunks and limbs of fallen and dying trees. The main attack on the host occurred in the late May and early June. Each system of galleries consisted of 3 - 4 egg galleries radiating from the nuptial chamber. Beetles apparently carried spores of blue fungi causing characteristic blue coloration of the sapwood adjacent to the galleries. A new generation emerged from the host tree by the end of August. Beetles overwintered as adults, probably in the litter. One generation per year was observed in Manitoba.

8. Dendroctonus simplex LeConte.

Locality: Agassiz Provincial Forest, near Rd. 15, about 5 km east to Hazel.

Host: Tamarack Larix laricina (Du Roi) K. Koch.

Biology: Monogamic species. Adult insects attacked trunks of injured and dying trees. Infestation of the host occurred in late June and July. Beetles of a new generation overwintered as immature adults in the brood sites or emerged by the late summer and hibernated in short galleries, usually in the bark near the base of the tree. One generation per year was observed in Manitoba.




APPENDIX II. LIFE CYCLES OF NEMATODES, PARASITES OF BARK BEETLES.