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TITLE OF THESIS Bark beetle nematodes in British Columbia with emphasis on the biology and host-parasite relationship of Contortylenchus reversus.

UNIVERSITY Simon Fraser University

DEGREE FOR WHICH THESIS WAS PRESENTED Ph.D.

YEAR THIS DEGREE GRANTED 1973

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BARK BEETLE NEMATODES IN BRITISH COLUMBIA WITH EMPHASIS ON THE BIOLOGY AND HOST-PARASITE RELATIONSHIP OF CONTORTYLENCHUS REVERSUS

by

CYRIL HOW SIK THONG

B.Sc. Honours, University of Singapore, 1969

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

December 1973

APPROVAL

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Degree: Doctor of Philosophy

Title of Thesis: Bark Beetle Nematodes in British Columbia with Emphasis on the Biology and Host-Parasite Relationship of Contortylenchus reversus.

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January 18, 1974

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ABSTRACT

British Columbia populations of Dendroctonus brevicomis, D. rufipennis, D. pseudotsugae and Trypodendron lineatum were examined for nematode parasites and the incidence of infection was recorded. Two populations of D. brevicomis were 32.0% and 17.4%, respectively, infected with Contortylenchus brevicomi. Two populations of D. rufipennis were 13.6% and 45.5%, respectively, infected with either Sphaerulariopsis dendroctoni or C. reversus in the hemocoel. Ektaphelenchus sp. larvae were found under the elytra of all specimens, and Parasitorhabditis obtusa larvae occurred in the gut. Populations of D. pseudotsugae from either sites in B.C. had infections of C. reversus ranging from 0% to 50.0%. Gut parasitism by P. obtusa ranged from 0% to 77.4%. Four species of subelytral nematodes were found. Two populations of T. lineatum were 13.0% and 2.4% infected by a tylenchid larva.

C. barberus is synonymized with C. brevicomi from D. brevicomis and the designation of the larval stages of C. brevicomi is revised. A new species of an aphelenchoid nematode, belonging to either the genus Bursaphelenchus or the genus Parasitaphelenchus, is described from the galleries of D. pseudotsugae. A first report of a tylenchid nematode in the hemocoel of T. lineatum is also presented but its biology and specific identity are unknown.

C. reversus from the hemocoel of D. pseudotsugae is redescribed including the first description of the free-living, infective female.

The post-embryonic development of C. reversus indicates four larval staggs within the host hemocoel. In the galleries, the L_4 larvae molt into sexually mature males and females, copulation occurs, and the females migrate up the larval galleries and reinfect primarily second or possibly early third stage larvae of the next host generation.

Unlike mermithids, the sex ratio of C. reversus apparently is unaffected by either host stage, sex, or the number of parasites present.

Adult D. pseudotsugae were examined for the physiological effects of parasitism. C. reversus depletes the hemolymph protein of mature but not of callow adult females. Parasitism also reduces the oocyte size by 20%. Two protein fractions increase significantly over the controls in the infected callow males but not in the infected mature males. However, total hemolymph protein levels in male hosts are unaffected. Hemolymph carbohydrate and amino-acid levels are not significantly altered by parasitism in all four adult host categories.

C. reversus alters the behaviour and fecundity of D. pseudotsugae, effecting the formation of 20 - 30% shorter egg galleries by infected than by uninfected females and 40 - 50% fewer eggs laid by infected than by uninfected females. No directional change in gallery construction occurred. Egg viability was unaffected by parasitism. Mating with infected males affected neither the gallery length nor the fecundity of uninfected females. However, infected males were good dispersal agents for C. reversus, carrying it into galleries that might otherwise be parasite free.

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Dr. J. M. Webster, for suggesting the research topic and for his advice and encouragement throughout this study. My thanks are also due to Dr. R. Gordon, at present with the Department of Biology, Memorial University, Newfoundland, for helpful suggestions in the physiological studies, Dr. J. H. Borden and Dr. J. P. M. Mackauer for advice and assistance in various phases of the research and for critical review of the thesis manuscript, Mr. R. Long for photography, Dr. J.A. Chapman of the Forestry Service, Canada Department of the Environment, Victoria, for specimens of D. rufipennis, Dr. C. L. Massey of the U.S. Forest Service, Albuquerque, New Mexico, for the loan of the type specimens of C. brevicomi, and Messrs. D. Henkelman and R. Holmberg for technical assistance.

I am also grateful to the Department of Biological Sciences for providing facilities for this study.

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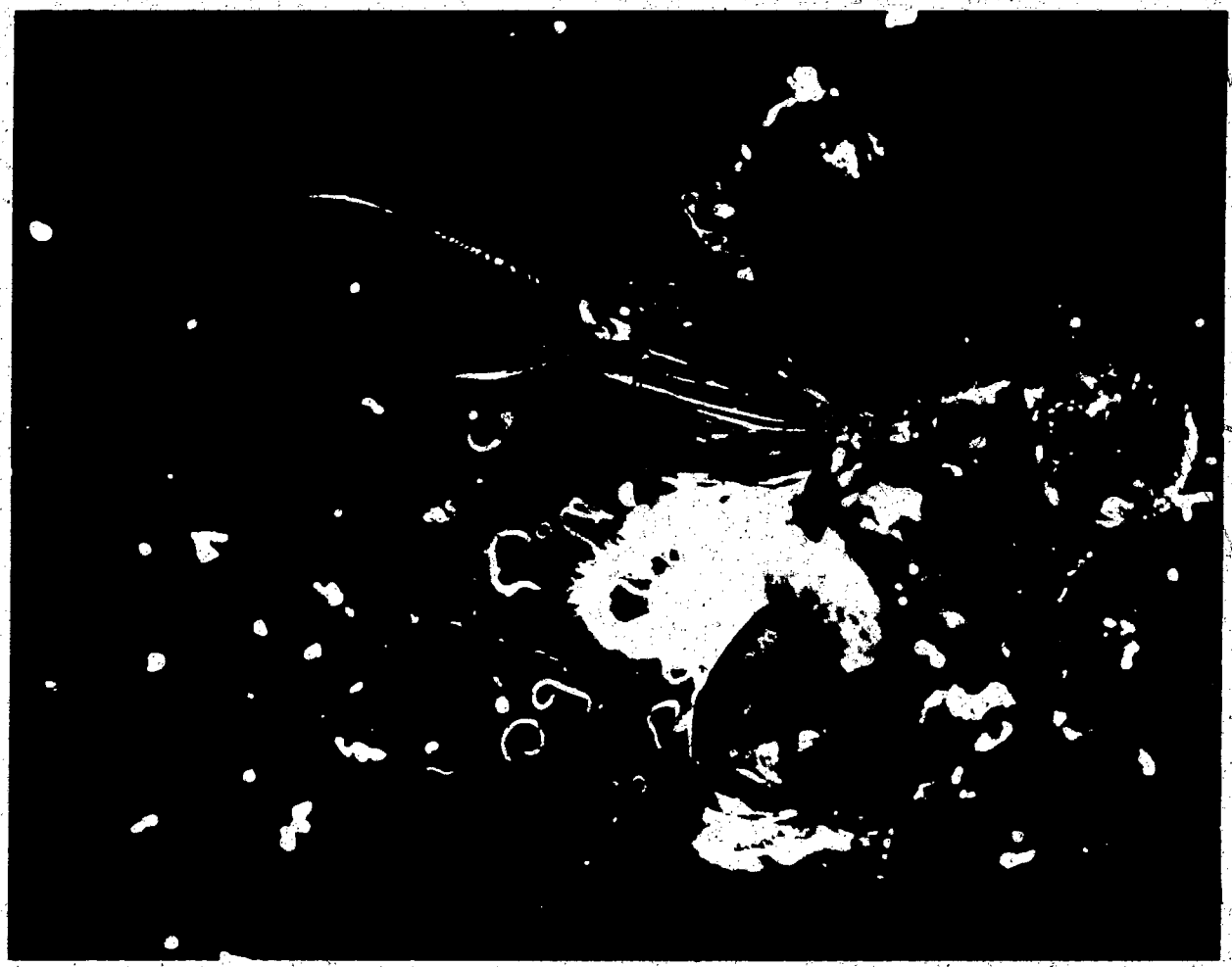


GENERAL INTRODUCTION

Bark beetles are common pests of timber, attacking both standing and fallen trees. Between 1960 and 1970, approximately 1,164 million cubic feet of standing timber was destroyed mainly by bark beetles in British Columbia (Cottrell and Fiddick, 1968, 1972).

In North America, most of the bark beetle pests belong to the genus Dendroctonus. Species of this genus may overwinter in almost any stage of development. Emergence from infested timber begins in April or May when temperatures rise above 10°C (50°F), and continues to late September or October with peaks around June and July. The emerged female initiates the attack on new timber and attracts the male for mating. After mating, the male either leaves in search of another female or dies within the gallery. The female then commences egg laying and may eventually die within the gallery or, alternatively, she may emerge and attack a second or third tree (Wood, 1963). The larvae that hatch from the eggs tunnel laterally from the main gallery within the bark. Pupation usually occurs in the outer bark. The number of generations per year varies from one to five or more, with fewer generations in colder areas.

Nematodes are frequently found in association with bark beetles but very little is known of their effect on the biology and population characteristics of their hosts. The first report of nematodes parasitizing bark beetles was by von Linstow (1890) who reported the presence of Allantonema in the hemocoel of Ips typographus. By 1963, a total of 250 species of nematode were recorded from 60 bark beetle species (Nickle, 1963a). The degree of association ranged from casual phoresy to obligate endoparasitism.

Steiner (1932) hypothesized an evolutionary sequence in the parasitism of bark beetles from the use of the beetle as an occasional carrier to its function as a host to ectoparasites and to true endoparasites.

Fuchs (1915) described the effects of bark beetle nematodes as killing or weakening the host, causing a reduction in host fecundity and prevention of a second host generation in the same year. In Russia, Yatsentkowsky (1924) noted that bark beetles of the genus Blastophagus infected with small numbers of nematodes were castrated, while those with large numbers of nematodes were killed. RÜhm (1956) believed that the influence of parasitic nematodes upon bark beetle populations is minor and could not be considered as a cause of decline in host populations.

The number of nematode species and the abundance of each species changes when bark beetles attack different host trees or different parts of the same tree (Rühm, 1956). The number of nematode species and their abundance decreases and later the survivors produce dwarf nematodes and dauer larvae. This suggests that bark beetle nematode survival may depend on their adaptability to the effects of changing environmental conditions on the hosts.

Rühm (1956) reported that interspecific competition occurs amongst nematodes within the beetle gallery. Aphelenchoides piniperda, for example, occurs in lower numbers in the galleries of Blastophagus piniperda when present together with Parasitorhabditis piniperda and Panagrolaimus tigrodon. Under such conditions, most of the aphelenchoids are juveniles rather than adults. Competition between parasite species within a gallery occasionally leads to the premature invasion of the host, or alternatively, to a total cessation of nematode development.

Bark beetle nematodes occur in one of three sites on the beetle. Externally, they inhabit mainly the subelytral position, occurring usually as clumps of ensheathed third stage larvae, commonly called dauer larvae. Only the genus Ektaphelenchus exhibits true ectoparasitism (Massey, 1956). Most Rhabditoidea, Tylenchoidea and Aphelenchoidea are phoretic when they occur subelytrally. Internally, the parasites may be found in the gut or in the hemocoel. Many rhabditids enter the host via the anus and migrate up to the midgut while the beetle is overwintering. Nickle (1963a) stated that larval parasitorhabditids caused abrasion of the epithelial layer of the ventriculus in Ips paraconfusus. Those nematodes that parasitize the hemocoel enter either by direct cuticular penetration or by being ingested by the host and subsequently penetrating the host gut. All hemocoel parasites possess a buccal stylet which may be used to aid in host tissue penetration.

Observations on the physiological effects of nematode parasitism on bark beetles have been few. Rühm (1956) stated that larval and adult hemocoel parasites obtained nutrients from the fat body of bark beetles but did not support his theory with evidence from biochemical and physiological studies. It has also been reported that I. paraconfusus heavily infected with Contortylenchus elongatus had fewer fat cells than uninfected individuals (Nickle, 1963b), and that Sphaerulariopsis ungulacauda interferes with the growth and function of organs in Dendroctonus pseudotsugae (Khan, 1957b). The physiological effects of parasitism may sometimes manifest themselves as a reduction in host fecundity. Sphaerulariopsis dendroctoni has been reported to cause a decrease in egg production in Dendroctonus rufipennis

from an average of 76.5 to 28.8 eggs per adult female (Massey, 1956). Reduced fecundity has also been observed in Scolytus ventralis infected with Sulphuretylenchus elongatus (Ashraf and Berryman, 1970) and in I. paraconfusus infected with C. elongatus (Massey, 1962). Nematodes are known to induce cellular defence reactions in some host beetles. Eggs and larvae of S. elongatus, for example, are encapsulated by first to third instar larvae of S. ventralis but not by other stages (Ashraf and Berryman, 1970).

Nematode parasitism can affect bark beetle behaviour. Atkins (1961) reported that the duration of initial flight of D. pseudotsugae was reduced by up to 50% by nematode parasitism but that total flight duration and the number of rest periods remained unaffected. Impaired flight capacity has also been demonstrated for S. ventralis heavily infected with S. elongatus (Ashraf and Berryman, 1970) and D. brevicomis infected with C. brevicomi (Nickle, 1963a). Reid (1945) observed the D. ponderosae heavily infected with Sphaerulariopsis hastata could be detected by their lethargic movements and the impaired "escape" behaviour when removed from their galleries. Tremors in the antennae and legs of infected beetles were also observed. Aberrant gallery construction is displayed by female S. ventralis infected with S. elongatus (Ashraf and Berryman, 1970), and by female Scolytus rugulosus infected with Neoparasitylenchus rugulosi (Nickle, 1971).

In this study of the nematode parasites and associates of economically important scolytid beetles in British Columbia, there were three objectives - (i) to assess and describe their nematode parasite fauna, (ii) to study the biology and development of the hemocoel parasite C. reversus in the Douglas-fir beetle, D. pseudotsugae, and (iii) to elucidate the host-parasite relationship between C. reversus and D. pseudotsugae.

I. THE NEMATODE ASSOCIATES OF SOME SCOLYTID BEETLES IN BRITISH COLUMBIA

Introduction

Little previous work has been done on nematode parasites of bark beetles in British Columbia. Reid (1945) reported that of the seven nematode species associated with D. ponderosae Hopkins, S. hastata Khan was the only endoparasite. Khan (1957a,b) described S. hastata and S. ungulacauda from the Douglas-fir beetle, D. pseudotsugae Hopkins, in British Columbia. Both these nematodes have now been placed in the genus Sphaerulariopsis (Nickle, 1963c). Ektaphelenchus macrostylus Khan has been described from specimens found under the elytra of Douglas-fir beetles (Khan, 1960).

This section on the nematode associates of four species of British Columbia scolytids includes: (i) the synonymy of Contortylenchus barberus (Massey) with C. brevicomi (Massey) from the hemocoel of the western pine beetle, D. brevicomis LeConte; (ii) a description of a new species of aphelenchoid nematode belonging to either the genus Bursaphelenchus or the genus Parasitaphelenchus found under the elytra and in the galleries of the Douglas-fir beetle; (iii) a redescription of the gravid female and mature male of C. reversus Thorne with a description of the free-living, infective female from the galleries of the Douglas-fir beetle, (iv) a report of the incidence of nematode infection of two populations of the spruce beetle, D. rufipennis Kirby, and (v) a report and description of a tylenchid nematode larva from the hemocoel of the striped ambrosia beetle, Trypodendron lineatum Olivier. Redescriptions of previously described nematode species are in Appendices I and II.

Materials and Methods

Bark was stripped from logs or trees infested with bark beetles, placed in large polythene bags for transport to the laboratory, transferred to emergence cages and the emergent beetles collected twice daily. Frass that was scraped from beetle galleries was placed in Baermann funnels for nematode extraction (Southey, 1970).

Beetles were dissected in insect saline after examination for subelytral parasites. Nematodes obtained from subelytral, midgut and hemocoel positions were kept separate. The nematodes were killed and fixed in hot (50°C) triethanolamine/formalin (T.A.F.) and stored in fresh fixative. Stained preparations were made with 0.01% cotton blue in lactophenol, processed through Baker's solutions (Southey, 1970), and mounted in glycerine.

Drawings and measurements were made under transmitted light and phase-contrast microscopy. Measurements follow the de Man formula except for G_1 (length of female gonad expressed as a percentage of body length) and V_t (distance of vulva from tail end). All values are given as Means $\pm$ one Standard Error.

Descriptions of bark beetle nematodes

1. Host: DENDROCTONUS BREVICOMIS LeConte

Host beetles were collected from Lytton on two occasions. In December 1969, 117 beetles were examined of which 59 were males. The hemocoel of 32% of the beetles contained the nematode C. brevicomi, with 28% of the females and 36% of the males being infected. In December 1970, 46 beetles, of which 23 were males, were examined. On this occasion, 17.4% had C. brevicomi in the hemocoel with 4.3% of the females and 30.4% of the males being infected. No subelytral or gut parasites were found on either occasion.

Contortylenchus brevicomi (Massey, 1957) Rühm 1960

synonym Contortylenchus barberus (Massey, 1957) Rühm 1960.

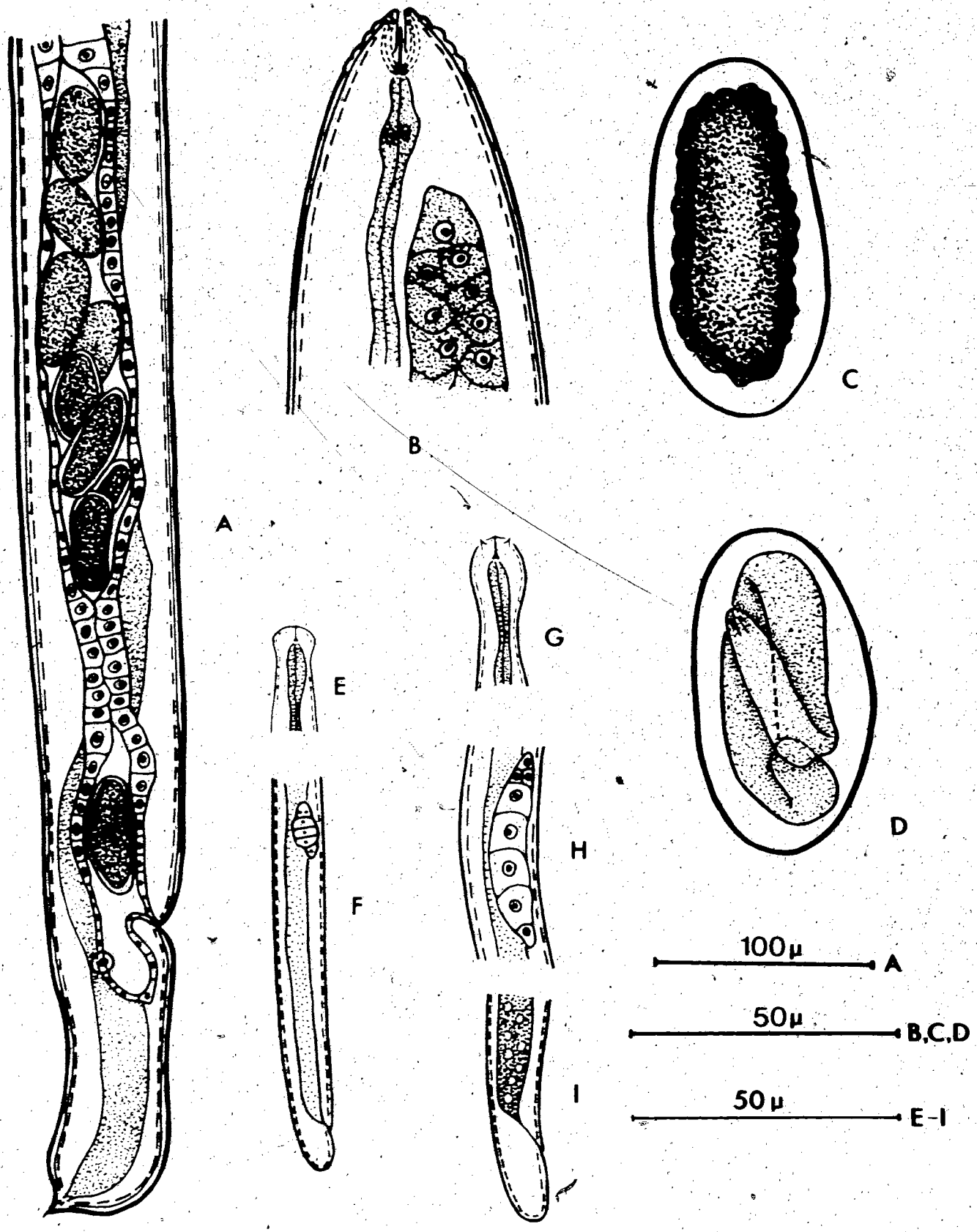
Females (n = 16): L = 3.61 ± 0.14 (2.82-4.82) mm ; w = 86.9 ± 2.69 (70-100)μ; a = 41.8 ± 1.6 (32.8-60.0); V = 95.9 ± 0.4 (94.7-97.0)%; G_1 = 93.5 ± 0.6 (88.5-96.5)%; V_t = 147.2 ± 5.0 (110-180)μ.

The mature female nematodes are found only in the hemocoel. Female long and slender, with body slightly bent dorsally. Cuticle finely striated except at extreme anterior where it becomes annulated (Fig. 1,B). Buccal stylet very distinct in egg producing females but degenerate in older specimens. Stylet short and slender with small basal knobs. Esophagus straight, non-bulbous; esophageal glands indistinct. Excretory pore and nerve ring not visible. Posterior end of female broad at vulva, with rounded terminus ending abruptly in a mucro (Fig. 1,A). Mucro present in all specimens. Anal opening not visible. Intestine appears to end in mucro. Phasmids present just posterior to vulva. Ovary monodelphic, prodelphic, and occasionally reflexed. Length of gonad variable, occasionally reaching head end of nematode. Post-uterine sac present only in some specimens. Vulva a transverse slit at posterior end of body. The more posteriorly positioned eggs in the uterus have a thick wall. Spermatozoa not observed in uterus.

No males were found in the hemocoel. However, males of the genus Contortylenchus generally remain in the galleries of the host and inseminate females prior to their entry into the beetle host. Massey (1957) found males of C. barberus but not of C. brevicomi in the beetle galleries.

Figure 1.

Contortylenchus brevicomi A. Tail of female showing terminal mucro and postuterine sac. B. Head and anterior end of ovary of female. C. Unembryonated egg. D. Unhatched first stage larva. E. Head of second stage larva. F. Posterior end of second stage larva. G. Head of third stage larva. H. Genital primordium of third stage larva. I. Tail of third stage larva.


A
B
C
D
E
F
G
H
I
100µ
A
50µ
B,C,D
50µ
E-I

Eggs: (58-66) x 32μ. Eggs from the beetle hemocoel showed various stages of development up to the formation of the first larval stage (Fig. 1, C,D). The coiled first stage larva is about 135μ long, with a rounded tail and body tapering toward the anterior end. Buccal stylet very small and cuticle very finely striated.

Second stage larva (L_2): L = 170μ; w = 10μ. Larva minute and free in host hemocoel. Cuticle very finely striated. Head distinctly offset from rest of body by a narrower neck region; head expanded and rounded anteriorly (Fig. 1,E). Stylet very small and esophagus not easily discernible. Genital primordium in posterior of body (Fig. 1,F). Tail rounded. Anal opening 6-7μ from tail terminus. This stage has been designated L_2 because it is the smallest larval stage found in the hemocoel of the host, and differs from the unhatched L_1 larva in its head shape, and development of the genital primordium.

Third stage larva (L_3): L = 290μ; w = 16μ. Larva slender and short with finely striated cuticle. Club-like head region offset from rest of body by narrower neck region (Fig. 1,G). Head with prominent cephalic papillae when examined under phase contrast microscopy. Spear slender and short. Esophagus straight and non-bulbous. Genital primordium conspicuous (Fig. 1,H). Tail rounded, anal opening 12-13μ from tail terminus (Fig. 1,I). This larval stage has been designated L_3 as distinct from the L_2 because of its larger size, general cephalic structure, and its greater tail length. This was the largest larval stage found in the host hemocoel.

Synonymy: The taxonomy of the genus Contortylenchus was revised by Nickle (1967), who retained C. brevicomi and C. barberus as separate species. Massey (1957) first described these two nematodes and gave the range of the body

length for mature parasitic females of C. barberus as 2.1 - 3.9 mm and that for females of C. brevicomi as 3.2 - 4.2 mm. Mature female C. brevicomi from my collection ranged in size from 2.8 to 4.8 mm. This large variation demonstrates the unreliability of body length as a criterion for the separation of the two species. The greatest width in the specimens examined by me ranged from 70 to 100μ which conforms with Massey's description of C. barberus, but not with that of C. brevicomi. In my opinion, the greatest width, like the body length, varies with the degree of development of the female gonad and the number of eggs present.

In the original description, the distance of the vulva from the tail terminus was used by Massey as a feature for differentiating the two species. This distance was 70μ for C. barberus and 90μ for C. brevicomi, but since neither the number of specimens observed nor the range of the measurements was given, it is a weak criterion for the separation of these two species. The difference of 20μ between the two measurements is small compared to the 70μ range for V_t recorded in B.C. specimens of C. brevicomi. Despite the difference between the original measurements of V_t given for C. barberus and C. brevicomi, and those for the Lytton populations of C. brevicomi, agreement in other characters identifies the Lytton populations as C. brevicomi.

Massey used the presence of a mucro in the tail of C. barberus, and its occasional presence in the tail of C. brevicomi as a criterion in the separation of these species. However, examination of the type specimens of C. brevicomi showed the mucro to be present in all cases. Similarly, it was present in all specimens of C. brevicomi obtained in the present study.

The eggs recovered in the present study were larger than those of either C. barberus or C. brevicomi as originally described. Two larval stages

of C. brevicomi were found within the host hemocoel, and have been designated L_2 and L_3 for reasons mentioned above. Massey's designation of the larva of C. barberus found in the host hemocoel as a L_1 is possibly erroneous as the appearance and description of this larva is similar to the designated L_3 by virtue of its size and head shape. Furthermore, the prominent cephalic papillae (observed on L_3 larvae in the present study and on the type larval specimens) are characteristic of the L_3. The other larval stage observed, designated L_2, is smaller than, and has a different cephalic structure from that of the L_3 and is differentiated from the unhatched larva by its head and tail shape and development of the genital primordium.

The synonymy of the hosts D. barberi with D. brevicomis (Wood, 1963) lends further support to the argument for synonymizing the two parasitic nematodes. The synonymy of C. barberus with C. brevicomi is now supported by Dr. C. L. Massey (personal communication).

2. Host: DENDROCTONUS PSEUDOTSUGAE Hopkins

Host beetles were collected from eight sites throughout B.C. at various times and an indication as to their incidence of infection is presented in Table I. Four species of subelytral associates, namely Ektaphelenchus macrostylus, a new species of aphelenchoid belonging to either the genus Bursaphelenchus or the genus Parasitaphelenchus, Cryptaphelenchus latus and Panagrolaimus dentatus, were present in all beetles examined. E. macrostylus, C. latus and P. dentatus are redescribed in Appendix I, and in each instance, more detailed morphometric measurements and descriptions are presented than were given in the original descriptions, and supplementary notes on their biology have been added. Other nematodes that have previously been found

Table 1. Incidence of nematode infection of D. pseudotsugae from collections in southwestern British Columbia.*

Collection site	Date of collection	Host sex	No. beetles examined	% with gut infection by P. obtusa	% with hemocoel infection by C. reversus	
					By sex	Total
Boston Bar	Dec. 1969	♀	28	2.3	3.6	4.5
		♂	16		6.3	
	Apr. 1970	♀	17	77.4	29.4	41.9
		♂	14		57.1	
Hixon	Nov. 1970	♀	15	0	26.7	17.2
		♂	14		7.1	
North Bend	Apr. 1971	♀	21	20.5	4.8	6.8
		♂	23		8.7	
Pemberton	May 1970	♀	11	14.3	36.4	42.9
		♂	10		50.0	
Point Roberts, Washington	Apr. 1970	♀	10	60.0	10.0	25.0
		♂	10		20.0	
U.B.C. Forest, Maple Ridge	May 1970	♀	9	0	33.0	38.0
		♂	7		43.0	
	Apr. 1971	♀	7	26.7	0	0
		♂	8		0	
Whistler Mtn.	Apr. 1971	♀	5	10.0	0	15.2
		♂	15		20.0	
Williams Lake	May 1971	♀	3	21.4	0	50.0
		♂	11		45.5	

*One collection at Point Roberts, Washington, 1 mile south of Canadian/U.S.A. border.

associated with the Douglas-fir beetle but were absent in B.C. specimens are Ektaphelenchus obtusus and a species of Micoletzkya (Furniss, 1967).

Wide variations in the incidence of gut and hemocoel parasitism were observed between sites and between different periods of sampling at the same site. The only gut parasite found was P. obtusa which was present in all samples except those obtained from Hixon and from the U.B.C. Forest, Maple Ridge, in May 1970. A redescription of P. obtusa is presented in Appendix I.

C. reversus was the main hemocoel parasite in B.C. samples of D. pseudotsugae. It was present in all samples except that from the U.B.C. Forest, Maple Ridge, in April 1971. The degree of infection in single populations ranged from 4.5 - 50% and generally, male beetles had a higher incidence of infection than female beetles with the exception of the sample from Hixon. Female beetles from two samples obtained from Whistler Mountain and Williams Lake were free of hemocoel infections while their male counterparts had 20% and 45.5% infection, respectively. However, the differences in degree of infection between the sexes of the host may have been due to chance.

Larvae of an unidentified Parasitaphelenchus sp. occurred in the hemocoels of a few specimen of D. pseudotsugae. They had a very low incidence of occurrence and only one larval stage of this parasite was found. Furniss (1967) reported finding C. reversus and a Parasitaphelenchus sp. in the hemocoel of D. pseudotsugae from Idaho and Utah, U.S.A. In the former area, Parasitaphelenchus sp. occurred three to four times more frequently than did C. reversus, whereas in the latter area, C. reversus occurred twice as frequently as did Parasitaphelenchus sp.

Other species of nematode previously found inhabiting the hemocoel of D. pseudotsugae in B.C. but were not present in my samples are Sphaerulariopsis hastata (Khan, 1957a) and S. ungulacauda (Khan, 1957b).

2.1 A description of a new species of aphelenchoid belonging to either the genus Bursaphelenchus or the genus Parasitaphelenchus.

Holotype female: L = 0.78 mm; a = 27.9; b = 9.8; c = 19.0; c' = 2.6; V = 74.4%; G_1 = 59.0%; tail length = 41μ; stylet = 15μ.

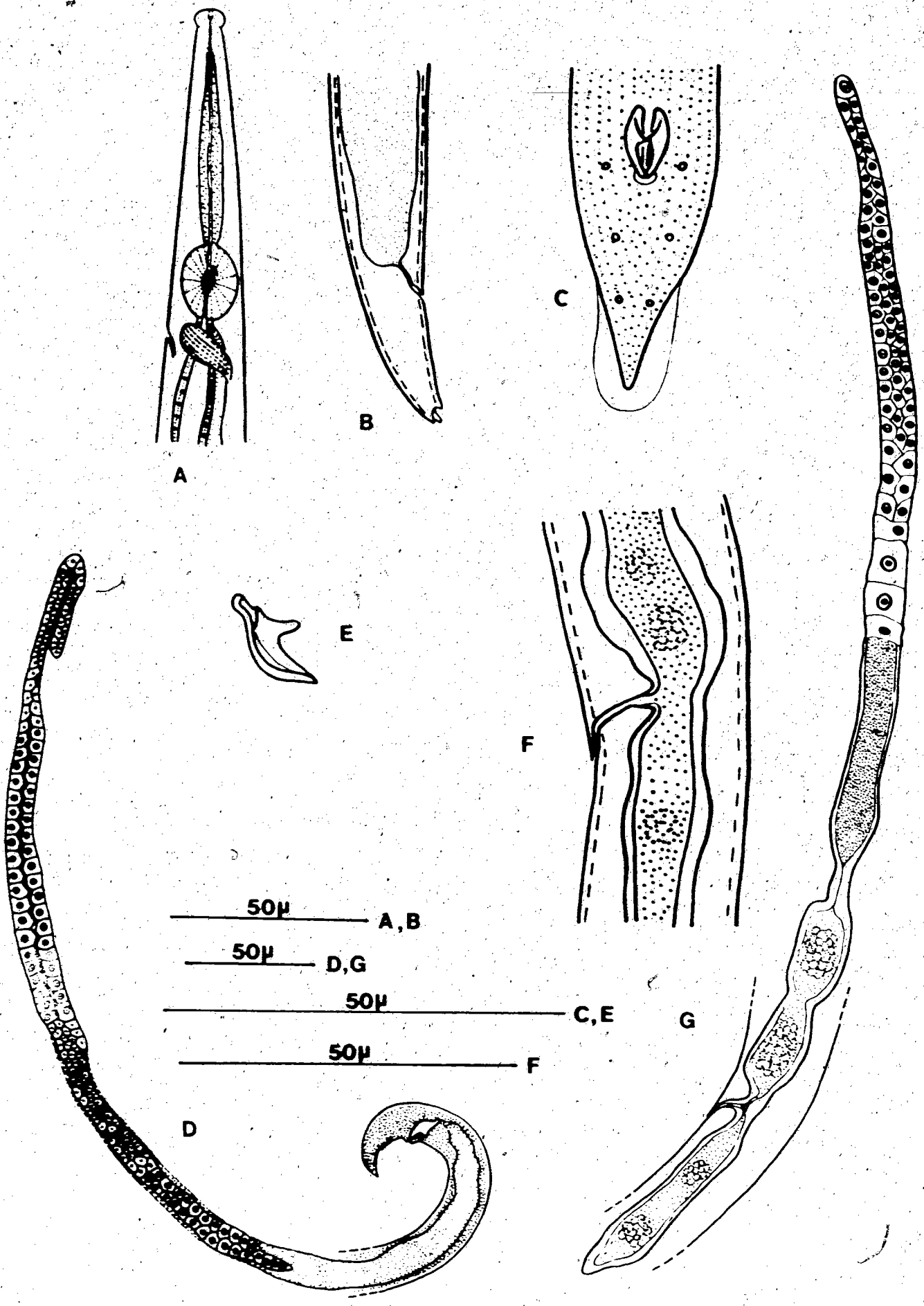
Paratype females (n = 7): L = 0.79 ± 0.02 (0.71-0.89) mm; a = 30.3 ± 1.2 (26.4-35.8); b = 10.1 ± 0.2 (9.6-10.9); c = 19.5 ± 1.1 (15.4-24.7); c' = 2.7 ± 0.1 (2.5-3.2); V = 76.0 ± 1.1 (72.7-82.4)%; G_1 = 60.6 ± 1.8 (53.9-69.8)%; tail length = 41.7 ± 2.7 (32-50)μ; stylet = 14.9 ± 0.4 (12-17)μ.

Paratype males (n = 6): L = 0.72 ± 0.04 (0.54-0.84) mm; a = 35.7 ± 1.6 (29.7-41.1); b = 8.8 ± 0.3 (7.4-9.6); c = 19.7 ± 1.0 (15.3-23.0; c' = 2.2 ± 0.1 (1.8-2.7); T = 69.3 ± 2.1 (64.7-80.0)%; tail length = 36.7 ± 0.9 (33-40)μ; spicule = 15.1 ± 0.6 (13-17)μ; stylet = 16.0 ± 0.1 (15-17)μ.

Female body long and thin, with body inflexion at the vulva. Cuticle finely striated. Head rounded and offset from rest of body, flattened slightly at the top (Fig. 2,A). Stylet long and aphelenchoid with small basal knobs. Procorpus of esophagus narrow at base of stylet and widening posteriorly, ending in a constriction at the median bulb. Median bulb large and oval in shape, with prominent valvular apparatus; anterior duct of esophageal gland opens just in front of valve apparatus;

Figure 2

New species of aphelenchoid nematode. A. Anterior end of female. B. Tail of holotype female. C. Tail of male. D. Male gonad and tail. E. Male spicule. F. Vulval region of female. G. Female gonad and vulval region.


A
B
C
D
E
F
G
50µ
A,B
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D,G
50µ
C,E
50µ
F

posterior esophageal gland opening not discernible. Esophageal glands long. Intestine straight, ending in posterior anal opening 32 - 50μ from tail terminus. Excretory pore situated about 10μ behind the median bulb. Position of nerve ring just posterior to median bulb/intestine junction. Tail of female conical in shape, ending in three finger-like processes (Fig. 2,B). In the female, the shape of the tail terminus varies intraspecifically (Fig. 3), the terminal processes sometimes being absent.

Female gonad monodelphic, prodelphic, occasionally reflexed, beginning at the level of the esophageal glands. Oviduct and uterus filled with spermatozoa (Fig. 2,G). Walls of uterus thickened. Vagina slopes posteriorly to vulval opening; vulval opening covered by a characteristic anterior cuticular flap (Fig. 2,F). Post-uterine sac long extending 70 - 120μ posteriorly from the vulva; post-uterine sac thick-walled and filled with spermatozoa.

Males generally shorter than females and with similar cephalic morphology. Tail of male conoid, 33 - 40μ in length, and sharply curved ventrally. Small bursa present terminally, rounded around tail terminus. Three pairs of caudal papillae present (Fig. 2,C), the first pair just anterior to the cloaca, the second pair mid-way between the cloaca and the tail terminus, and the third pair at the level of the beginning of the bursa. Male testis single, usually reflexed at the anterior extremity (Fig. 2,D). Spicules paired, unfused along most of their length but possibly fused at the posterior extremity, with strong ventral incurvation, and prominent ventral process at the proximal end (Fig. 2,E). Gubernaculum absent.

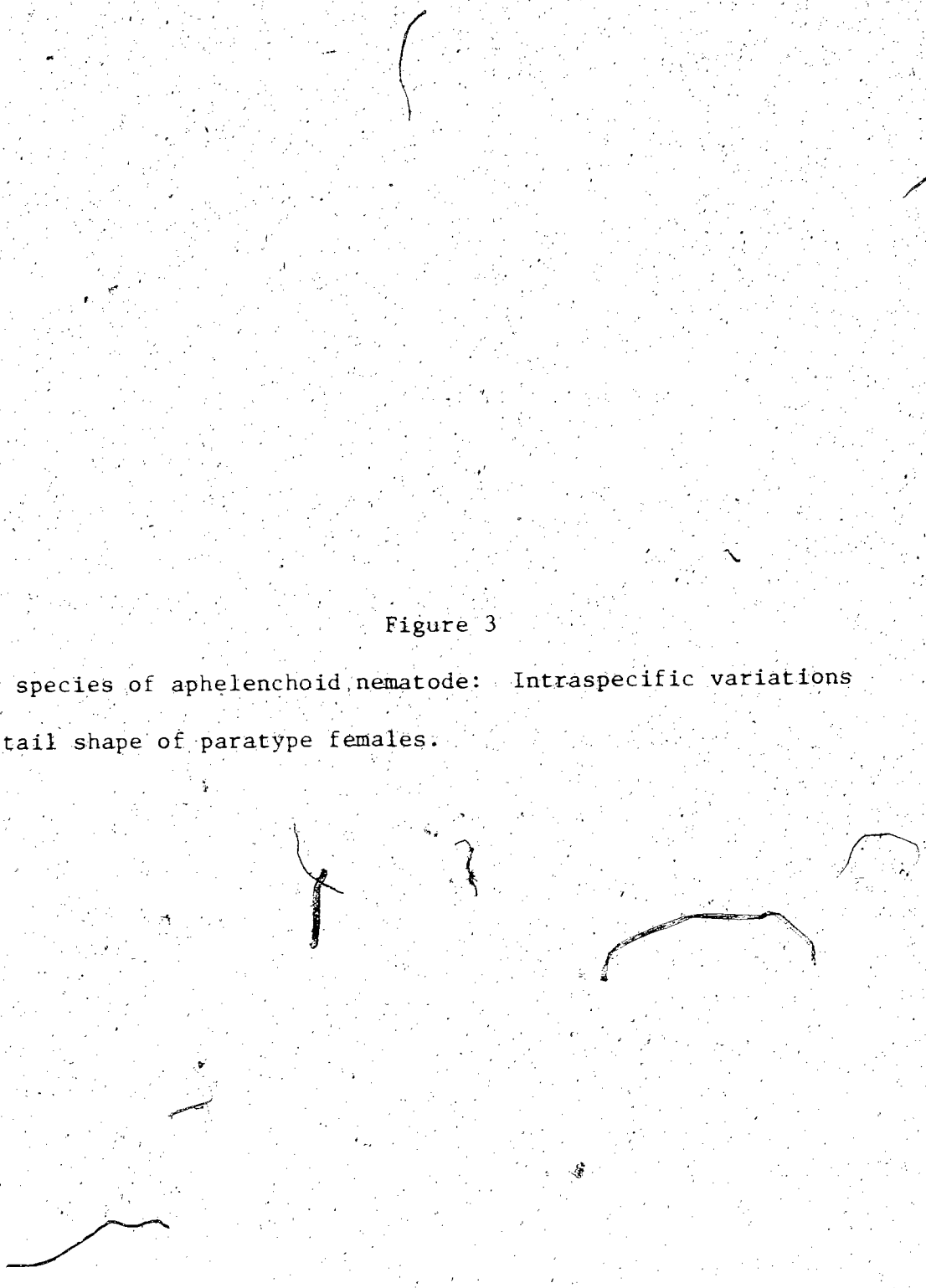
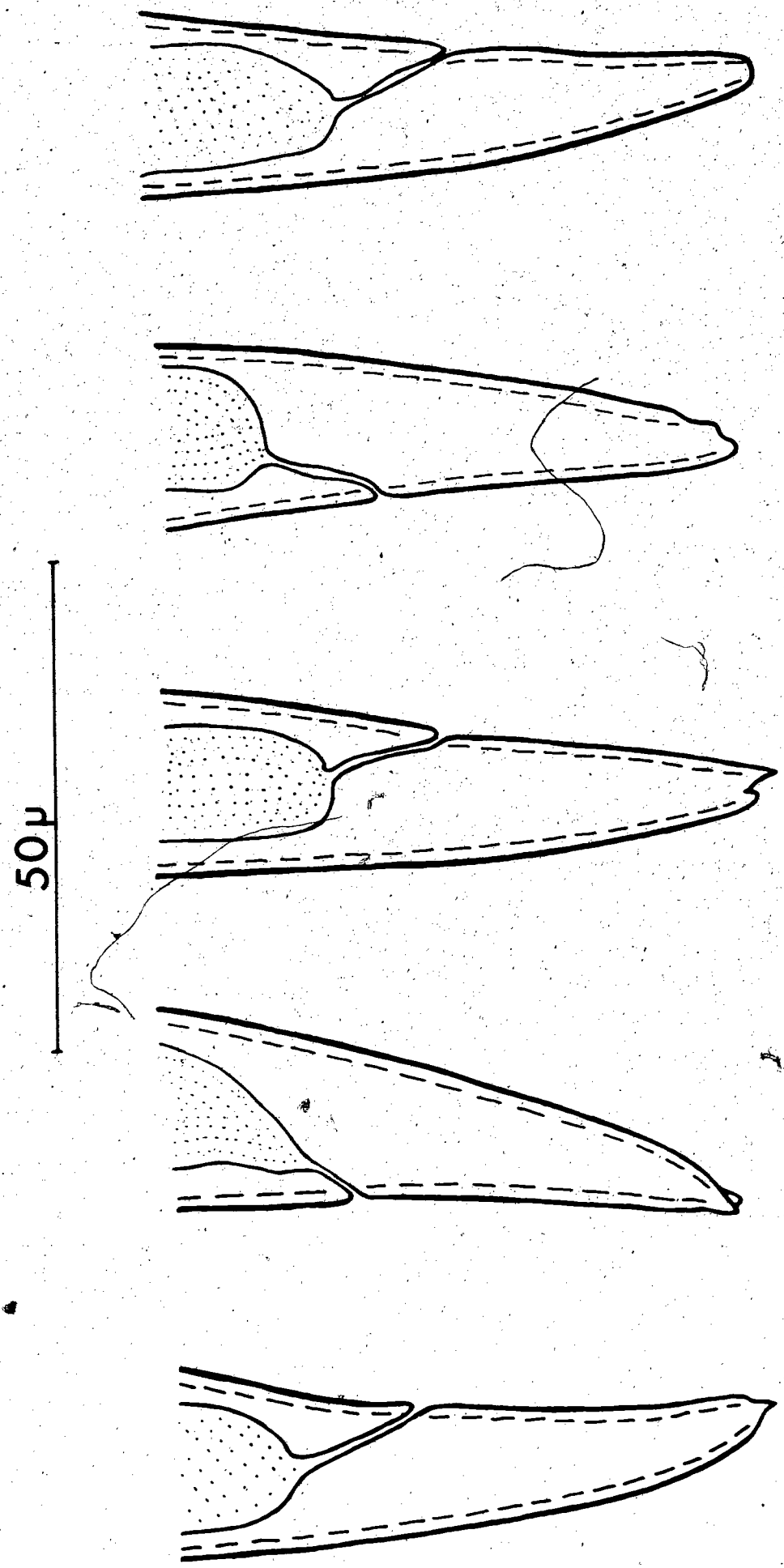


Figure 3

New species of aphelenchoid nematode: Intraspecific variations in tail shape of paratype females.


50μ

Diagnosis: There is uncertainty as to whether this nematode belongs to the genus Bursaphelenchus or to the genus Parasitaphelenchus. The morphological characters that separate these two genera are insufficient to separate the two genera conclusively. The males of the genus Bursaphelenchus have spicules that are either separate or partially fused, whereas those of the genus Parasitaphelenchus have spicules that are always fused (Rühm, 1956). There is doubt as to whether the spicules in my specimens are fused, but even if they were partially fused, this character alone is insufficient to place them into one or other of these two genera. In addition, males of the genus Bursaphelenchus have two pairs of caudal papillae with an occasional third pair present, whereas those of the genus Parasitaphelenchus usually have three pairs of large caudal papillae (Goodey, 1963). Nevertheless, from diagrams presented by Rühm (1956), there does not appear to be any significant difference between males of these two genera with respect to the size and the number of caudal papillae present, thus making this criterion unsatisfactory.

It appears, therefore, that the only satisfactory criterion to separate these two genera is their respective biologies. Bursaphelenchus spp. are phoretic and do not invade the bark beetle hemocoel, whereas Parasitaphelenchus spp. are parasitic in the hemocoel as larvae but free-living as adults (Rühm, 1956). Since no studies on the life cycle of this new species of aphelenchoid were undertaken, further biological data is unavailable. However, the fact that these nematodes were receovered in all frass samples collected

from beetle galleries, even from those galleries initiated by beetles that were free of hemocoel infection by either C. reversus or Parasitaphelenchus sp. larvae, prompts me to favour the placement of these nematodes into the genus Bursaphelenchus.

This species of Bursaphelenchus differs from all other species of the genus except B. lignicolus Mamiya and Kiyohara, 1972, in the presence of a distinct vulvar flap in the female, and in the shape of the bursa and spicules, and the number and position of the caudal papillae in the male. It differs from B. lignicolus in the length of the female gonad and post-uterine sac and the slope of the vagina, which is perpendicular to the ventral body wall in B. lignicolus but sloping posteriorly towards the vulva in this new species. The males of the two species differ in spicule shape and size, bursa shape, and number of caudal papillae. The spicule of male B. lignicolus is twice as long and more arcuate than that of this new species, and the bursa of the former has a pointed terminus whereas that of the latter is rounded terminally. In this new species there are three pairs of caudal papillae in the male tail but in B. lignicolus there are only two pairs present.

2.2 Contortylenchus reversus (Thorne, 1935) Rühm, 1956

Egg-laying females (n = 18): L = 1.32 ± 0.05 (0.93-1.66) mm; a = 18.3 ± 0.6 (14.9-23.7); V = 93.8 ± 0.3 (92.5-95.8)%; G_1 = 113.7 ± 5.3 (93.5-196.8)%; V_t = 80.6 ± 2.5 (70-100)µ.

Egg-laying females are present only in the beetle hemocoel and have been isolated from all stages of the beetle except for the eggs and first larval instar.

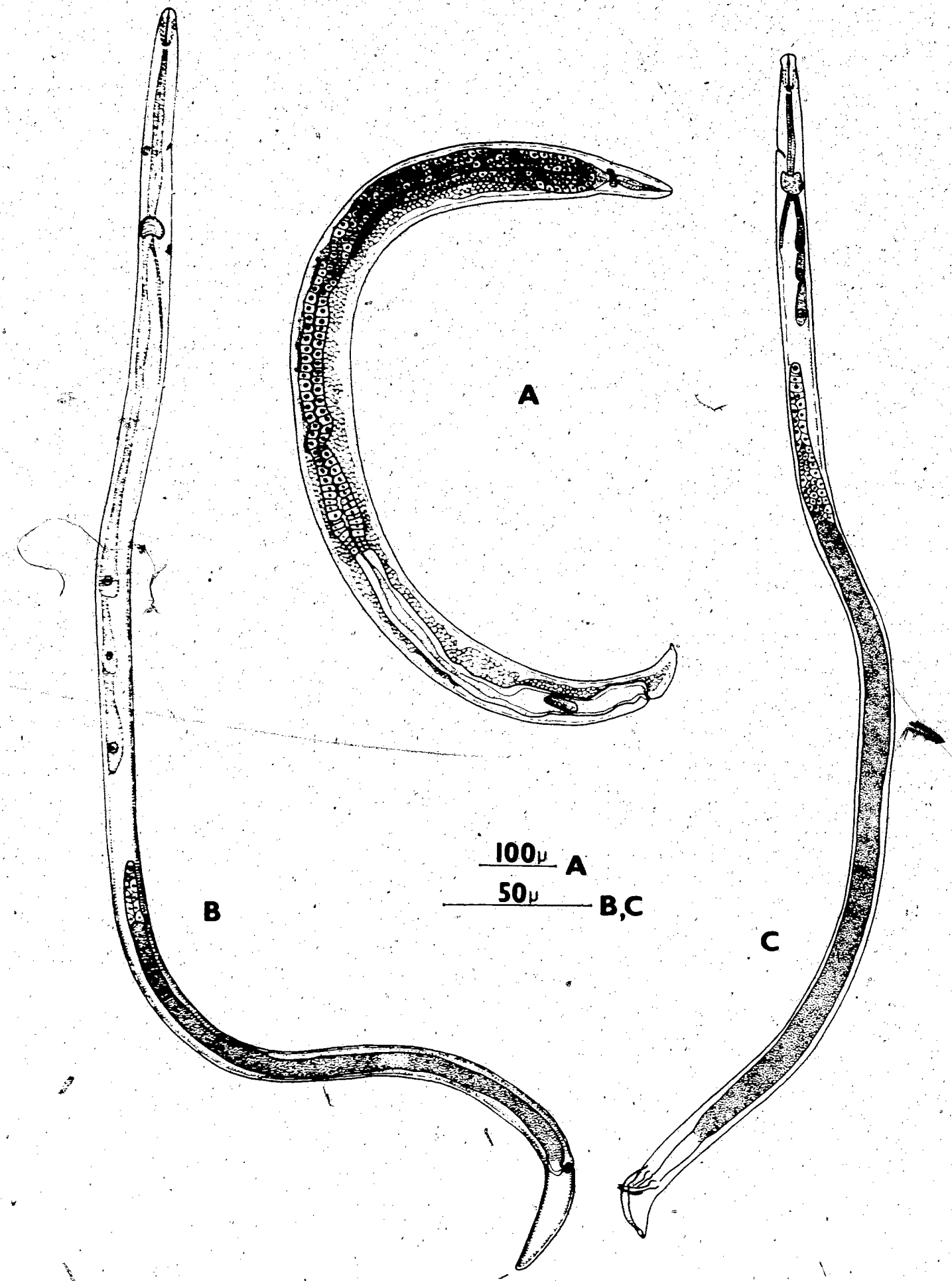
Nematode body long and stout, dorsally flexed (Fig. 4,A) and tapering anteriorly to the head, which may be annulated in some specimens. Stylet shows signs of degeneration in older specimens. Esophagus short, with nerve ring about one body width behind head end. Intestine simple, running whole length of body. No anus visible. Tail conoid from vulva, ending in a mucro. Body slightly constricted at vulva. Gonad single and reflexed, reaching level of esophago-intestinal junction. Length of entire gonad often longer than length of body. Oviduct and spermatheca situated in middle of body; vulva is a transverse slit. Eggs and sperm often visible in uterus.

Free-living females (n = 14): L = 0.40 ± 0.01 (0.34-0.43)mm; a = 31.0 ± 0.6 (28.6-35.8); V = 91.0 ± 0.2 (89.8-92.4)%; G_1 = 25.3 ± 1.2 (17.2-32.3)%; V_t = 35.9 ± 0.6 (30-40)µ; stylet = 14.6 ± 0.3 (13-17)µ.

The free-living female has not been previously described. Nematode body long and thin, dorsally flexed when fixed (Fig. 4,B). Head rounded, tail conoid. Stylet well developed with prominent basal knobs; ratio of length of forestylet to aftstylet averaging 0.6. Esophagus relatively straight, narrowing slightly as it passes through the nerve ring, which is situated

Figure 4

Contortylenchus reversus A. Gravid egg-laying female. B. Free-living, infective female. C. Free-living male.


A
B
C
100µ A
50µ B,C

75μ from the head end. Excretory pore located approximately mid-way between head and nerve ring. Hemizonid located slightly behind nerve ring. Intestine straight, running from nerve ring to tail end. No discrete anus visible. Three dorsally situated esophageal glands extremely long, with cell bodies extending to middle of nematode body. Gonad simple, unreflexed, occupying posterior half of body. Ovary and oviduct very short, about 20μ in length. Uterus long, extends rest of way to vulva, filled with spermatozoa. Body wall at region of vulva not constricted as in egg-laying female. Vagina short and muscular. Vulva 30 - 40μ from tail end. No eggs are produced at this stage. Free-living males (n = 12): L = 0.40 ± 0.01 (0.35-0.45)mm; a = 31.7 ± 0.7 (28.9-35.8); c = 24.3 ± 0.6 (20.8-28.0); c' = 2.0 ± 0.1 (1.5-2.5); T = 61.3 ± 3.7 (40.7-77.3)%; tail length = 16.3 ± 1.0 (15-18)μ; stylet = 14.3 ± 0.6 (11-17)μ; spicules = 14.5 ± 0.4 (12-17)μ; gubernaculum = 5.7 ± 0.1 (5-6)μ.

Male long and thin (Fig. 4,C) like free-living female. Head end with narrower neck region. Stylet slender and basal knobs well developed. Ratio of length of forestylet to aftstylet on average 0.5. Esophagus narrow and straight, narrowing slightly when passing through the nerve ring which is located approximately 45μ from anterior end. Excretory pore located about one body width in front of nerve ring. Intestine a straight tube from level of nerve ring to cloacal opening, which is situated about 15-18μ from tail end. Three dorsally situated esophageal glands overlap esophagus but not as outstretched as in female. Tip of most posterior gland situated about 50μ from nerve ring. Tail of male approximately conoid, ending in a mucro-like process. Bursa stretching from just anterior of the cloaca to the tail terminus. Testis

well developed, 50 - 60μ long, reaching up to one body width behind last dorsal gland cell body. Vas deferens long and packed with spermatozoa, opening ultimately at the cloaca. Copulatory spicules paired and curved ventrally; anterior part of spicules broad and cup-like, while median portion is uniformly thick, tapering posteriorly into a fine point. Gubernaculum trough-shaped, 5 - 6μ in length.

The egg-laying female was described first from the mountain pine beetle, D. ponderosae (Thorne, 1935), and later from the spruce beetle, D. rufipennis, and D. terebrans (Massey, 1956). Thorne also found the eggs and two larval stages of this parasite in the hemocoel of D. ponderosae, and Massey found the free-living male in the galleries of D. rufipennis and D. terebrans.

3. Host: DENDROCTONUS RUFIPENNIS Kirby

Two samples collected from Prince George and Pemberton were obtained by courtesy of the Department of Fisheries and Forestry, Victoria, B.C., in November 1970. Fourty-four beetles, 22 of which were males, were examined from Prince George; 13.6% of the beetles had hemocoel infections, with 9.5% of the males and 17.4% of the females infected. From Pemberton, 33 beetles, of which sixteen were males, were examined with an incidence of hemocoel infection of 37.5% among males and 52.9% among females. This was the only host species that was attacked by two different species of hemocoel parasite though never together. These parasites were C. reversus, which has been described above, and Sphaerulariopsis dendroctoni, which is described in Appendix II, with the latter species occurring more frequently than the former. Larval stages of a species of Ektaphelenchus were found under the elytra of all beetles examined, but further identification of these was not attempted. Larvae of Parasitorhabditis obtusa were found in the midgut of

50% of the beetles from Prince George and in 35% of those from Pemberton.

4. Host: TRYPODENDRON LINEATUM Olivier

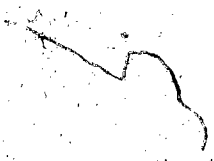
There have been no previous reports on the occurrence of nematodes associated with this beetle either in Western Europe or in North America. The incidence of infection of T. lineatum was very low. Larval nematodes were obtained from the hemocoel of two females and one male out of 23 beetles collected from the U.B.C. forest in October 1969 and from only one female out of 41 beetles collected from the same site in December 1969. Between 20 and 100 larvae were found in the hemocoel of individual beetles, all apparently in the same stage of development.

Tylenchid larva: L = 0.37 mm; w = 12μ. Larva long and thin (Fig. 5). Head rounded with prominent cephalic frame. Tail pointed, approximately 45μ long. Stylet short, 5μ long, with prominent basal knobs. Procorpus of esophagus straight, ending in a narrow, elliptically-shaped median bulb 5μ wide; valvular apparatus not well developed. Esophageal glands overlap intestine to a distance of 20μ behind the median bulb. Nerve ring just behind median bulb. Intestine straight and undifferentiated, opening at anus. Genital primordium two-thirds of way down body length, consisting of three cells, the central cell being larger than the proximal and distal ones.

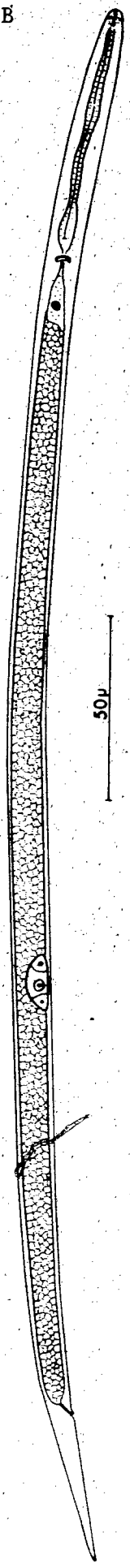
This apparently is the first stage larva as indicated by the structure of the genital primordium. No other larval stages were found. The nematode larvae did not have any obvious effect on the beetles.

Figure 5

Tylenchid larva from the hemocoel of T. lineatum.



27B



II. LIFE HISTORY AND POST-EMBRYONIC DEVELOPMENT OF *CONTORTYLENCHUS REVERSUS* IN *DENDROCTONUS PSEUDOTSUGAE*.

Introduction

In his original description of C. reversus, Thorne (1935) included descriptions of the egg and two larval stages, and suggested that the second stage larva was the infective stage. Massey (1956) in describing this nematode from D. rufipennis and D. terebrans, included a description of the free-living male and stated that the first stage larva was the infective stage.

Massey (1962) reported that the closely related species C. elongatus, parasitic in I. paraconfusus, had two larval stages in the hemocoel of its host and two in the galleries, and that the spermatised immature female was the infective stage. Nickle (1963b) confirmed the status of the spermatised female, but found that all four larval stages of the nematode occurred within the host hemocoel and that the fourth stage larvae left the host and molted into free-living male and female nematodes within the galleries.

The present study was undertaken to elucidate the development of C. reversus in relation to that of its host, the Douglas-fir beetle, and to compare the life cycle of C. reversus with that of C. elongatus.

Materials and Methods

Colonies of D. pseudotsugae were maintained in Douglas-fir logs in the laboratory. Females were introduced into the logs through pre-drilled entry holes; males were introduced on the onset of frass production by the female beetle. Thereafter, bark was removed from the logs at weekly intervals for 2 months, and all larval, pupal and adult stages of the beetle were collected separately. Nematodes were extracted from gallery frass by the Baermann funnel technique (Southey, 1970).

All stages of the beetle were checked for parasitism by dissecting in insect saline. Some of the larval nematodes obtained from the hemocoel of infected hosts were placed in cultures of damp frass at room temperature. Nematodes were extracted from these cultures 3 and 7 days after inoculation.

Nematodes were killed, fixed, stained and processed as per methods described in the previous section.

Life History and Post-Embryonic Development

The life cycle of C. reversus is represented diagramatically in Fig. 6. Unembryonated eggs (60 x 25μ) are laid into the hemocoel of the host. The first larval stage is formed within the egg and, prior to hatching, is about 140μ long (Fig. 7,A). Head and tail ends are rounded and the head bears small cephalic papillae. The stylet is formed but the components are not readily distinguishable. The rudiments of the esophagus are the only recognizable parts of the digestive system. The genital primordium is clearly visible and consists of three cells, of which the central one has a much larger nucleus than the other two. Hirschmann (1962) observed these three nuclei in the genital primordium of the molting first stage larva of Ditylenchus triformis and called the largest the germinal nucleus and the two smaller ones the somatic nuclei.

After hatching, the first larval stage (L_1) undergoes a short period of growth before molting into the second stage larva (L_2). Specimens were obtained showing the cast-off cuticle of the L_1 covering the head of the L_2 (Fig. 7,B). This is the common indicator of a molt in all the larval stages as the old cuticle elsewhere on the body of the nematodes adheres closely to the new

Figure 6

The life cycle of C. reversus in the Douglas-fir beetle, D. pseudotsugae.

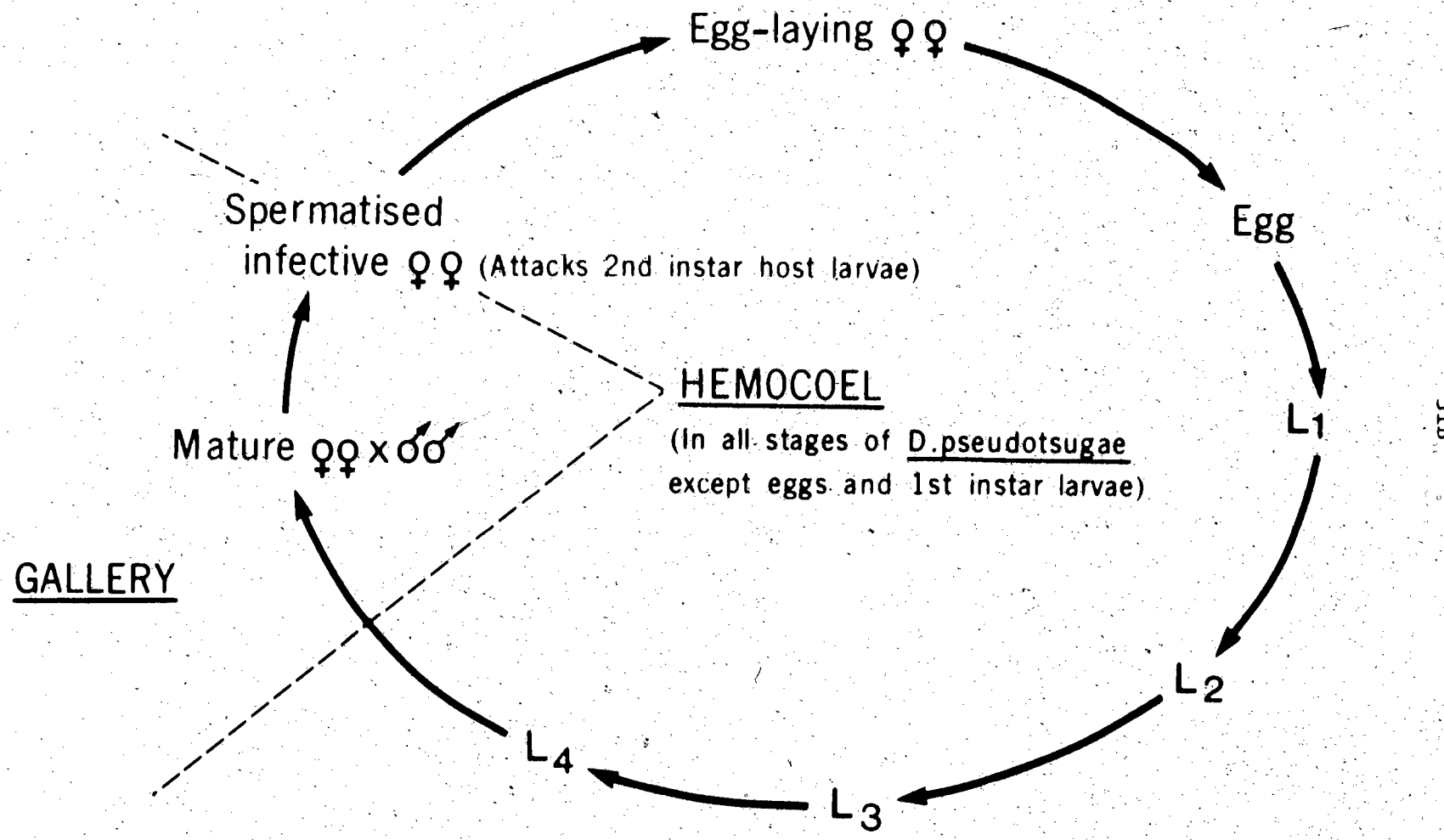
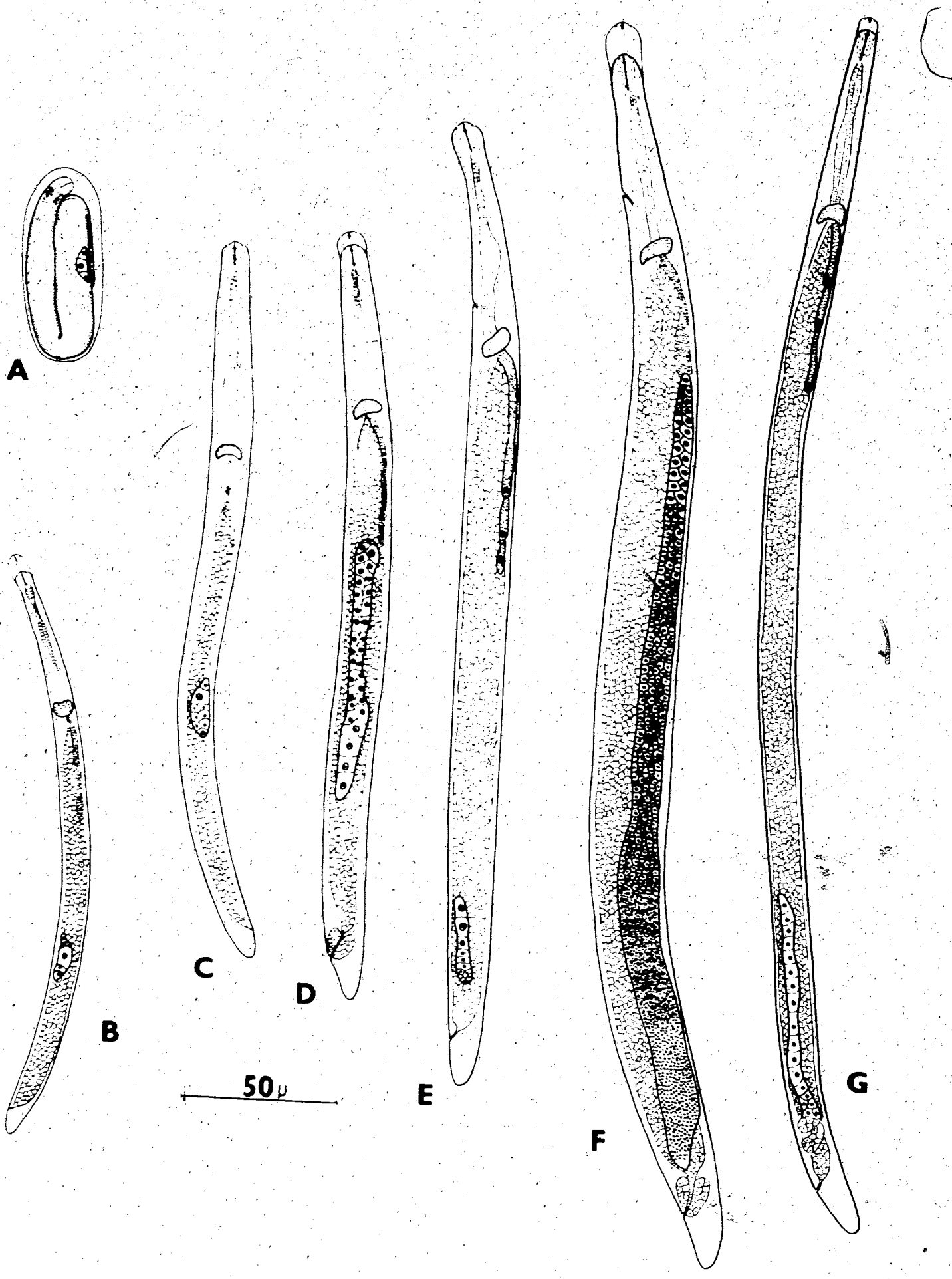

Egg-laying ♀♀
Egg
L1
L2
L3
L4
Mature ♀♀ x ♂♂
Spermatised infective ♀♀ (Attacks 2nd instar host larvae)
HEMOCOEL
(In all stages of D.pseudotsugae except eggs and 1st instar larvae)
GALLERY

Figure 7

Contortylenchus reversus larvae. A. Unhatched first stage larva. B. Ensheathed L_2 male larva. C. Female L_2 larva. D. Ensheathed L_3 male larva. E. Female L_3 larva. F. Ensheathed L_4 male larva. G. Ensheathed L_4 female larva.


A
B
C
D
E
F
G
50μ

cuticle and cannot be easily discerned. The male L_2 larva (Fig. 7,B) is about 185μ long and has a rounded head with small cephalic papillae. The stylet is well formed and shows definite basal knobs. The esophagus is relatively straight, narrowing as it passes through the nerve ring, which is located 45μ from the anterior end. The intestine begins at the level of the nerve ring and runs posteriorly to the anus. The rudiments of the dorsally situated esophageal glands are present but their cell bodies are not visible. The tail of the L_2 is conoid and rounded terminally. The structure of the genital primordium, located about two-thirds of the way along the body at this stage, identifies the sex of the larvae. In the male, the anterior somatic cell divides and produces a few small cells. The posterior somatic cell also divides but produces just two cells, the more posterior one of which probably remains as a cap cell throughout larval development as in D. triformis (Hirschmann, 1962). The germinal cell is still evident in the centre of the primordium.

The female L_2 is about 40μ longer than the male L_2 and a little stouter (Fig. 7,C). The nerve ring is situated about 60μ from the anterior end. The stylet knobs are poorly developed. The genital primordium differs from that of the male in that the posterior somatic cell has divided to form a number of small cells, whereas the anterior somatic cell has not yet divided. Eventually, the latter will divide and give rise to two cells, very much as in the female larva of D. triformis. The germinal cell with its large nucleus remains just anterior to the group of small cells that originate from the posterior somatic cell. The ventral chord nuclei seen in developing female

larvae of D. triformis are not noticeable in C. reversus.

The male third stage larva (L_3) is 240 - 250μ in length and 15μ wide (Fig. 7,D), and retains much of the body form of the L_2 except for the growth of the genital primordium and the presence of the spicule primordium. The anterior cap cell is clearly visible at the anterior of the genital primordium. Behind this, the cells, which are arranged in two parallel rows, form the initials of the vas deferens. Posteriorly, there is a single row of four to five cells. The length of the primordium is now 80 - 100μ. During the period before the L_3 molts into the fourth stage larva (L_4), the primordium differentiates into testis and vas deferens. Meanwhile, around the anus of male L_3 larvae a group of small cells appears that forms the spicule primordium. This group of cells persists through to the L_4 and gives rise to spicules and gubernaculum only after the last molt.

The L_3 female larva is about 70μ longer than the L_3 male, but equally broad (Fig. 7,E). The excretory pore is visible 10 - 15μ anterior to the nerve ring, which is 70μ from the anterior end. The genital primordium of the female larva develops slower than that of the male. At this stage, the primordium moves to a more posterior position, occupying the posterior one-quarter of the body. In comparison with the male larva there is little development of the female primordium between the L_2 and L_3. The posterior cap cell has a group of smaller cells just anterior to it. The anterior part of the primordium consists of a single row of larger cells and, at this stage, it is difficult to distinguish the germinal cell from the other cells. The genital primordium at this stage is only 30μ long.

The fourth stage male larva (L_4) becomes rather stout and is about 400μ long and 25μ wide (Fig. 7,F). The nerve ring is situated about 60μ from the anterior end, with the excretory pore about 20μ in front of it. The undifferentiated spicule primordium appears as a group of small cells surrounding the rectum. The gonad is nearly fully developed. Spermatogenesis has commenced, and sperm are visible in the posterior part of the vas deferens. The anterior end of the gonad extends forward to within a body width of the nerve ring. There is no connection between the vas deferens and the anus.

The newly molted female L_4 larva is as long as but only half as wide as the male L_4 (Fig. 7,G). The nerve ring is situated 60μ from the anterior end. The three dorsally situated esophageal glands are clearly visible, the most posterior one extending about 60μ behind the nerve ring. These glands undergo considerable elongation to reach almost halfway down the length of the body before the last molt occurs. The genital primordium, though not as well developed as in the male L_4, is about 80μ long. The anterior four-fifths consists of a single row of large cells, which eventually form the ovary and oviduct. The posterior one-fifth contains a group of smaller cells which will form the uterus. The genital primordium abuts posteriorly upon a group of small cells arranged in a cup-like fashion, with the mouth pointing towards the ventral body wall. This is the vaginal primordium that will ultimately link the uterus to the vulva. These cells are present only in the L_4 female, as is the case with D. triformis (Hirschmann, 1962).

The development of the nematode ceases at this stage until a transfer of habitats occurs. The larvae escape from the host hemocoel through the hindgut wall and out with the feces. Fourth stage larvae of C. reversus were

observed within the hindgut of both sexes of the adult beetle (Fig. 8) as early as 24 hours after entry of the host beetle into a log, and also in the frass of the egg galleries excavated by the female beetles. However, fourth stage larvae were not always found in the hindgut at the same time they occurred in the galleries (Table II), which suggests that the larvae leave the insect periodically rather than all at once. This is probably an adaptation to ensure a better distribution of infective stages in different parts of the gallery. This release obviously occurs throughout the boring activity of the parent beetle as fourth stage larvae are still present within the hemocoel and hindgut of parent beetles close to the cessation of boring. It is also apparent from Table II that male hosts can act as effective agents for the dissemination of fourth stage larvae of C. reversus, by releasing them into the galleries of uninfected female beetles.

The final molt of the nematodes occurs very shortly after the L_4 larvae are released into the galleries. Adult males and females were obtained from frass cultures after 3 days but only a few nematodes could be recovered from the cultures after a week. The host searching period of the nematode, therefore, lasts for approximately one week, after which they perish in the galleries if no host is found. The advanced gonad development in the male larvae ensures that fertilization of the female occurs soon after she molts into an adult in the galleries. The male then dies within the galleries. A comparison of the gonads of the free-living male, free-living female, and egg-laying female shows that whereas the male gonad achieves its full maturity in the adult nematode in the galleries, the female gonad reaches full maturity only after she enters into another host. The adult female of C. reversus infects primarily second or possibly early third

Figure 8

Fourth stage larvae of C. reversus (►) within the hindgut of an adult D. pseudotsugae. Magnification x 80. (Lower left arrow indicates rectal end.)

Table II. Occurrence of fourth stage larvae of C. reversus in the hemocoel, hindgut, and gallery of adult D. pseudotsugae and its relationship to the length of the egg gallery.

Length of egg gallery (cm) and sex of infected parent(s)		Nematode present or absent			No. of D. pseudotsugae offspring produced*
		Hemocoel	Hindgut	Gallery	
3.2	♀	+	+	+	2
3.7	♂	+	+	+	5
4.5	♀	+	+	+	8
5.3	♂	+	-	+	12
6.0	♀	+	-	-	2
7.2	♀	+	-	-	4
7.7	♂	+	+	+	13
8.4	♂	+	-	+	12
9.0	♀ + ♂	+	-	-	8
9.0	♀	+	-	-	15
9.5	♀	+	-	-	22L
12.2	♀ + ♂	+	-	-	29
12.3	♀	+	+	+	9
13.5	♀ + ♂	+	-	-	18L
15.0	♀	+	-	-	33 + 8L
15.6	♀	+	+	+	14
17.5	♀	+	-	+	24
18.0	♀	+	-	-	15
18.5	♀	+	-	-	6 + 40L
19.3	♀	+	-	+	33L
19.4	♂	+	-	+	41L
20.0	♀	+	-	-	42
20.0	♀	+	-	-	50L
20.2	♀	+	+	+	32L
21.4	♀ + ♂	+	-	+	28L
23.0	♀ + ♂	+	-	+	37L
24.5	♀ + ♂	+	-	-	32L

*Eggs and larvae (L).

instar larvae of D. pseudotsugae. Egg-laying females together with larval stages of C. reversus were found in all stages of the host except the eggs and first instar larvae (Table III).

Discussion

Although Thorne (1935) and Massey (1956) were working with different host species, it is unlikely that the life cycle of C. reversus in D. pseudotsugae is radically different from that in D. rufipennis, D. ponderosae or D. terebrans. The specimens of the free-living male in the present study are similar with those of Massey's (1956) except for the position of the excretory pore. Thorne (1935) described two larval stages of C. reversus from the hemocoel of D. ponderosae, and stated that the ovary of the second stage larva develops from a rudimentary genital primordium until it extends to about half the body length and its terminus is flexed a distance equal to three to five body widths. The probability of the ovary developing so rapidly within a single larval stage is small.

Thorne's designated L_2, in its most advanced stage of development, is stated by him to leave the host and transfer to the next generation. This is presumably what I believe to be the L_4. Thorne did not find any other stages in the galleries, possibly because the gallery phase of the life cycle lasts about one week and because mortality is high during this period. Massey (1956) found the male in the galleries of D. rufipennis and D. terebrans and stated that "the infective stage of the species is evidently the first larval stage" without explaining the presence of the L_2 (Thorne, 1935) and his free-living male. I have shown that all four larval stages of both male and female C. reversus occur in the host hemocoel and that sperm production begins in the

Table III. Occurrence of C. reversus infection in larval instars and pupae of D. pseudotsugae in the laboratory.

Host stage and head capsule width (mm)		Nematode infection*		
		No. hosts examined	No. infected	%
Larva I	≤ 0.65	139	0	0
II	0.90 - 1.06	214	8	3.74
III	1.22 - 1.30	31	9	29.03
IV	≥ 1.38	115	21	18.26
Pupa		113	19	16.81

*Gravid females, eggs and larvae.

L_4 male larva before the spicules and gubernaculum are formed, and a connection with the anus is established.

The post-embryonic development of C. reversus is similar to that of D. triformis as described by Hirschmann (1962). However, since differential staining of nuclei was not attempted, the derivatives of the germinal and somatic cells could not be traced beyond the L_2. The ventral chord nuclei present in the female larvae of D. triformis were not observed in C. reversus. Hirschmann states that from the second molt, male and female larvae can be distinguished by the differential development of the genital primordium and by the presence of the spicule primordium in the male larva. The sex of the L_2 C. reversus was distinguishable by the development of the genital primordium even at this early stage.

The life cycle of C. reversus resembles that of C. elongatus as shown by Nickle (1963b). The gravid female lays unembryonated eggs in the hemocoel of the host, but whereas it is the L_2 that hatches from the egg in C. elongatus, it is the L_1 which hatches from the egg in C. reversus. In general, the larval stage that hatches from the egg is variable in the Sphaerulariidae, e.g. Tripius sciarae hatches as an L_1 (Poinar, 1965) and Sphaerularia bombi hatches as an L_3 (Poinar and Van der Laan, 1972). There are four molts in the life cycle of C. reversus and males develop more quickly than females. Rühm (1956) believed that the L_3 larvae of the genus Contortylenchus leave the beetle and that there are two molts to maturity in the galleries. My findings indicate that it is the L_4 larvae that leave the host in C. reversus and molt into adults within the galleries where copulation occurs almost immediately after which the males die. The spermatised female then reinfects a second or early third instar beetle larva. Both sexes of parent D. pseudotsugae are capable of spreading the infection to the subsequent generation of offspring. It has been shown, however, that progeny of I.

paraconfusus are more likely to be infected with C. elongatus when the female parent was infected than when the male alone was infected (Massey, 1962). This difference between the two bark beetle hosts may be explained by the fact that the males of I. paraconfusus unlike those of D. pseudotsugae do not follow the females into the egg galleries.

The development and egg production of the female nematode is equally successful in all life stages of the Douglas-fir beetle in which it is found. Ashraf and Berryman (1970) found that the nematode Sulphuretylenchus elongatus was encapsulated by first to third instar larvae of the beetle Scolytus ventralis but not by later stages of the beetle. This was not observed in D. pseudotsugae infected by C. reversus which suggests that C. reversus is a better adapted parasite than is S. elongatus. The wide host range of C. reversus, which includes four different species of Dendroctonus, may also be advantageous for the survival of this parasite.

The route of infection of larval instars of D. pseudotsugae by the female C. reversus is unknown. Attempts to observe the penetration process of D. pseudotsugae larvae by the parasite females on either plain agar plates or in frass cultures were unsuccessful. I suspect that penetration is cuticular rather than oral or anal because bark beetle larvae are constantly chewing into fresh phloem tissue and passing out a steady stream of frass through the anus. Nematodes are unlikely to be present in unpenetrated phloem tissue and thus would not be ingested, and nematodes entering anally would have to migrate against the current of frass being produced. Furthermore, the infective female nematode has a well developed buccal stylet and digestive glands which are structures that conceivably could aid penetration of the host cuticle.

Young females of C. reversus do not feed during the short period spent in the galleries, and it is reasonable to assume that large amounts of host nutrients are removed by the female nematode after her entry into the second stage beetle larva because of a fourfold increase in her body size, and the development of her gonad to fill almost her whole pseudocoelom. The host, therefore, supplies the nutritional requirements for the growth and gonad development of the parasitic female C. reversus.

III. HOST-PARASITE RELATIONSHIP BETWEEN C. REVERSUS AND D. PSEUDOTSUGAE.

A. THE INFLUENCE OF HOST AGE, HOST SEX AND PARASITE BURDEN ON THE SEX RATIO OF C. REVERSUS

Introduction

Sex in nematodes is determined by genetic and environmental factors. Little is known of the genetic aspects since in most bisexual nematode species, the male chromosomal composition has not been well studied. In some nematode species, e.g. Anguina tritici and the genus Heterodera, both sexes have equal numbers of chromosomes. In the rhabditids, the male has one chromosome less than the female. In hermaphroditic nematodes which produce sperm and eggs with the same chromosomal complement, sex chromosomes have not been observed (Triantaphyllou, 1971).

Environmental influences on the direction of sex differentiation have been demonstrated in insect and plant parasitic nematodes. Parasite burden and host species affect the sex ratio of some mermithid parasites, with higher numbers of parasites and smaller hosts producing higher proportions of male parasites (Christie, 1929; Parenti, 1965). Petersen (1972), however, found that the size of the host larva did not affect the sex ratio of Reesimermis nielseni. Host diet influences the sex ratio of R. nielseni in that starved hosts produce relatively more male parasites than unstarved hosts (Petersen, 1972). Strelkov (1964) suggested that the sex of chironomid hosts influences the sex of the parasite Filipjevimermis singularis, and Parenti (1965) reported that the sex of the first penetrating larva of Paramermis contorta influences the sex of other nematodes that subsequently enter and develop to maturity.

Hence, although the sex of mermithids has been genetically determined in embryogenesis, the direction of sex differentiation may be altered by environmental factors after the nematode larvae enter the host insect.

In plant-parasitic nematodes, sex may be influenced by nutritional or physical factors. In species of Meloidogyne, sex reversal occurs in response to a sudden onset of unfavourable conditions after the initiation of sex differentiation (Triantaphyllou, 1971). The duration of feeding of second stage larvae is an important factor in the sex determination of M. incognita (Trudgill, 1972). Females of H. rostochiensis exhibit sex reversal when they invade nematode resistant varieties of potato, thus increasing the proportion of males in the population (Trudgill et al., 1967). However, increased proportions of males are sometimes due to the differential death rates of males and females as has been shown for H. glycines (Koliopanos and Triantaphyllou, 1972) and for H. schachtii (Johnson and Viglierchio, 1969). Parasitic female larvae require more space and nutrients than do male larvae to mature. Therefore, under adverse conditions such as crowding, nutrient deficiencies, removal of leaves and stem, and resistant hosts, many female larvae die before maturity whereas male larvae manage to become adults (Johnson and Viglierchio, 1969). In H. glycines and H. schachtii, sex is genetically determined, and only the sex ratio, but not the sex expression, is modified by environmental influences.

In view of the high larval density of C. reversus in the hemocoel of the Douglas-fir beetle, it was decided that the influence of host age, host sex, and parasite burden on the sex ratio of the parasite should be studied to determine the effect, if any, of environmental factors on sex differentiation in C. reversus.

Materials and Methods

Different life stages of D. pseudotsugae obtained from log cultures in the laboratory were dissected, and, in all cases, the number of larval and gravid female nematodes per host was counted. Thirty nematode larvae picked at random from each insect were used for each sex ratio determination. They were fixed and stained by the methods mentioned before. Differentiation between the sexes was based on the differential growth of the genital primordia in male and female larvae. The sex ratio was calculated as the number of female larvae to the total number of nematodes examined.

Results and Discussion

The results obtained in this study are presented in Table IV. Of the twenty larvae of the Douglas-fir beetle examined, twelve were fourth instar larvae, four were third instar larvae, and four were second instar larvae. Since the results indicated no obvious differences between the larval instars with respect to sex ratio of the parasites as a function of nematode burden, all twenty samples were treated as a single group. Parasite burden also did not affect the sex ratio of nematode populations from pupal and adult hosts, and the results were grouped for each of these host categories. No significant differences were found in the sex ratio of samples obtained from male and female hosts, and those from larvae and pupae of the beetles. Therefore, neither host stage, host sex, nor nematode burden affects the direction of sex differentiation in C. reversus, which normally produces a greater number of female than male offspring.

Table IV. The sex ratio of populations of C. reversus in different stages of D. pseudotsugae.

Host stage and sex	n	Sample size	Sex ratio * ($\bar{X}$)	S.E.	Range
Larvae	20	30	0.61	0.01	0.57 - 0.67
Pupae	14	30	0.62	0.02	0.57 - 0.73
Adult ♀	17	30	0.59	0.01	0.43 - 0.67
Adult ♂	17	30	0.58	0.01	0.43 - 0.67

* Sex ratio = $\frac{♀}{♀ + ♂}$

It is reasonable to assume that host stage, sex and parasite burden could affect the sex ratio of C. reversus since these factors are shown to affect the sex ratio of mermithids in other insects (Christie, 1929; Petersen, 1972; Strelkov, 1964). However, the apparent independence of sex determination in C. reversus from host conditions and parasite burden may be due indirectly to three factors. Firstly, C. reversus has a very short free-living, non-feeding phase of about one week, after which another host must be located or death ensues. Therefore, the amount of nutrients that must be stored to enable the nematode to survive this non-feeding phase is very small. In mermithids, however, there is characteristically a comparatively long free-living, non-feeding period in the life cycle, e.g. M. nigrescens takes 1 to 2 years, and as such these nematodes must store large amounts of nutrients during the parasitic phase to enable survival of the free-living stage. Hence, the "stress" on the host exerted by C. reversus is proportionately less than that exerted by mermithids on their host during the parasitic phase. Secondly, the phenomenal increase in size of mermithids from the time of entry into their hosts up till the time of emergence of the parasite implies a great demand for host nutrients for parasite growth. The increase in size of C. reversus during its parasitic phase is small compared with that of the mermithids and, consequently, less host nutrient is required. This indirectly suggests that there are probably sufficient host nutrients available for all the developing larvae of C. reversus, and, therefore, less "stress" on the nematode population, so precluding the shift towards increased proportions of males. Thirdly, the larvae of C. reversus, because they are very small, do not occupy as much space in the insect hemocoel as do mermithids in their

hosts. This "spatial stress", which has been shown to be a factor affecting sex determination in H. schachtii (Johnson and Viglierchio, 1969) may be important in the sex determination of entomophilic nematodes.

Mermithids have been shown to cause large depletions in hemolymph and fat body nutrients in locusts (Gordon and Webster, 1971, 1972; Gordon et al., 1971) and decrease host weight, deplete host storage products, and inhibit imaginal disc development in mosquitoes (Bailey and Gordon, 1973). Mermithids invariably kill their hosts at emergence. The effects of C. reversus on the Douglas-fir beetle are less pronounced, and although the host physiology and behaviour are affected, the host is not killed and nutrient depletion is less drastic than that caused by mermithid infections in locusts. Hence, by being a less demanding parasite, C. reversus has probably adapted itself to being present in high numbers without jeopardizing its own survival.

B. EFFECTS OF C. REVERSUS ON HEMOLYMPH COMPOSITION, OOCYTE DEVELOPMENT, GALLERY CONSTRUCTION, FECUNDITY, AND EGG VIABILITY IN D. PSEUDOTSUGAE

Introduction

There have been few studies on the physiology of the host-parasite relationship between insects and nematodes. Strickland (1911) observed that pupal histoblast development in Simulium larvae was retarded by a small nematode. This resulted in abnormal respiration and a cessation of maturation processes. At that time, he proposed that the juvenile nematode secreted a substance that inhibited the development of pupal and adult histoblasts. Nematodes parasitic in the hemocoel of insects generally cause a reduction in fecundity or sterility or death. Female D. rufipennis parasitized by either S. dendroctoni or C. reversus laid fewer eggs than healthy beetles (Massey, 1956), and female I. paraconfusus infected by C. elongatus had smaller broods than healthy individuals (Massey, 1962). Scolytus destructor and S. multistriatus have been reported to be sterilized by Parasitylenchus scolyti (Oldham, 1930), and S. ventralis is sterilized when heavily infected with S. elongatus (Ashraf and Berryman, 1970). When infections were light or medium, the host ovaries were smaller and the germaria translucent producing smaller and malformed oocytes.

The development of nematode parasites usually is closely synchronised with that of their hosts. The egg development of the nematode Heterotylenchus autumnalis is affected by the diapause of its host, the facefly Musca autumnalis, and this is attributed to the effect of either the host juvenile hormone

level or the level of sterol esters in the host hemolymph (Stoffolano, 1967). The possibility of a hormonal control by the insect of its parasitic fauna is also demonstrated by the reduction in the number of adult nematode parasites in the gut of Blatta orientalis after removal of the median neurosecretory cells (Gordon, 1968, 1970). A similar operation performed on the cockroach Periplaneta americana, however, brought about an increase in the number of nematode parasites in the gut (Hominick and Davey, 1972). It also seems that some nematode parasites are unable to either develop to maturity or begin egg-laying until the host insect attains sexual maturity. C. elongatus females in I. paraconfusus pupae have fully developed eggs in their uteri but do not oviposit until the beetle becomes adult (Massey, 1962). In addition, Parasitylenchus stipatus larvae are produced only in sexually mature D. adjunctus (Massey, 1966b).

Mermis nigrescens infection decreases the rate of excretion (as measured by the dye amaranth) in Schistocerca gregaria but not the host hemolymph volume, indicating a probable indirect effect on excretion by the suppression of the hormonal balance in the host (Gordon and Webster, 1971). M. nigrescens also depletes hemolymph carbohydrates owing to a progressive depletion of glycogen phosphorylases and reduced glycogenesis in the host fat body (Gordon et al., 1971). Parasitism did not affect hemolymph protein and amino-acid levels, but fat body protein and amino-acid levels were reduced. Since neither signs of fat body tissue degeneration were detected nor the presence of proteolytic enzymes in homogenates of the nematode demonstrated, it was concluded that the nematode was altering protein metabolism

within the fat body tissue rather than feeding directly upon it. The nematodes probably stimulate protein catabolism within the fat body and the release of amino-acids into the host hemolymph furnishes the nematode with sufficient protein nitrogen for active uptake (Gordon and Webster, 1971). It was later found that M. nigrescens was capable of incorporating labelled amino-acids (Gordon and Webster, 1972). Since the functions affected are hormonally controlled, it is possible that the parasite may affect them by altering the host hormonal regime. More recent studies have demonstrated differential effects of M. nigrescens parasitism on protein fractions in the hemolymph of the desert locust (Gordon et al., 1973).

Nematodes are known also to affect insect behaviour (Ashraf and Berryman, 1970; Atkins, 1961; Massey, 1960; Nickle, 1963a; Reid, 1945).

This study was undertaken to determine the effects of C. reversus on the physiology and behaviour of the Douglas-fir beetle. To accomplish this, hemolymph analysis was undertaken and oocyte development, gallery construction, fecundity and egg viability were observed.

Materials and Methods

For the experiments, naturally infected Douglas-fir beetles were used. Infected beetles contained fifty or more nematodes in the hemocoel. All data were analysed using Student's t-test, and significant differences between control and C. reversus infected groups are expressed at levels of $P < 0.05$ (*), $P < 0.02$ (**), and $P < 0.01$ (***).

(a) Hemolymph Analysis

Analyses were performed on the hemolymph of both sexes of callow unemerged and mature emerged adult beetles prior to oviposition. Hemolymph was drawn from beetles through a wound made by the removal of a metathoracic leg. The body of the beetle was squeezed gently with a pair of forceps to eject hemolymph through the wound. Withdrawal of hemolymph and the measurement of hemolymph volume was done using 1 µl disposable micropipettes (Drummond "Microcaps"). Hemolymph that was not used immediately was stored in vials at -30°C with a crystal of phenylthiourea.

Hemolymph trehalose assay: This was performed using thin layer chromatography (TLC). A newly formulated adsorbant mixture consisting of 24g of MN Kieselguhr and 6g of Celite analytical filter aid (Johns-Manville, U.S.A.) was slurried with 80 ml of 0.02 M sodium acetate solution and layered to a thickness of 250 µ on 20 x 20 cm glass plates. Hemolymph from individual beetles and two serial dilutions of a trehalose standard solution were spotted on each TLC plate 2 cm apart. The plates were developed to a distance of 15 cm using a mixture of 60 ml ethyl acetate and 40 ml isopropanol/water (2:1) as solvent. The compounds separated out were detected using Stahl's anisaldehyde/sulphuric acid reagent (50 ml glacial acetic acid with 0.5 ml anisaldehyde and 1 ml conc. sulphuric acid) and by heating plates at 110°C in an oven till the spots attained maximum intensity. The plates were then removed from the oven and cooled for half an hour at room temperature. Quantification was done with a Zeiss chromoscan spectrophotometer for TLC using a wavelength of 370 mµ. Readings were calibrated against a trehalose standard curve.

Total hemolymph protein assay: Hemolymph from individual beetles was spotted on 2 x 2 cm Whatman 42 filter paper. Assay was carried out essentially according to the method of Bramhall et al. (1969), using xylene brilliant cyanin G (Michrome 1224) as a stain. Samples were read in a spectrophotometer at 620 mμ wavelength and calibrated against a bovine serum albumin (BSA) standard curve.

Disc acrylamide gel electrophoresis of hemolymph proteins: Hemolymph from eight insects was pooled and 5 μl used for each sample run. Acrylamide gels were made by the method of Davis (1964) using 7% running and 3% spacer gels (Smith, 1968). A pH 8.9 Tris-HCl buffer was used and bromophenol blue was added to the upper buffer chamber as a marker. Upon completion of the electrophoretic run, the gels were fixed in 10% trichloroacetic acid for one-half hour, and stained for one hour at 37°C in a 19:1 solution of 10% TCA and 1% Coomassie blue RL (Michrome 100). Gels were destained over several days with several changes of 7% acetic acid and were quantitated using the Joyce-Loebl chromoscan.

Total hemolymph amino-acid assay: Hemolymph from individual insects was used. The method employed was essentially that of Rosen (1957) using the ninhydrin reagent, but volumes of reagents were decreased to compensate for the small volume of hemolymph obtainable from each insect. Samples were read in a spectrophotometer at 570 mμ and standardised against a glycine standard curve.

(b) Oocyte Development

The rate of development of oocytes can be estimated by measuring the length of the largest terminal oocyte in adult female insects prior to oviposition (Thomsen, 1948; Thomsen and Moller, 1959a,b, 1963). In this experiment, thirteen uninfected and thirteen infected mature adult D. pseudotsugae from the same colony were used, and the length of the largest terminal oocyte of each beetle was measured. Measurements were taken under 375x magnification on the dissecting microscope using a calibrated micrometer eyepiece.

(c) Gallery Construction, Fecundity and Egg Viability

Beetle colonies collected from Williams Lake district that showed at least 20% infection by C. reversus were used. Female beetles were introduced into Douglas-fir logs in the laboratory through pre-drilled entrance holes; males were introduced after frass production by the female began. Entrance holes were made approximately 20 cm apart to avoid subsequent overlap of larval mines by offspring of different parent beetles. Bark was removed from some logs at intervals of 7, 14 and 23 days following the introduction of the parent beetles into the logs. At each of these periods, gallery length, gallery shape, the number of unhatched eggs, and the number of larvae per parent beetle were recorded. Six replicates were observed at 7 and 14 days and four at 23 days. Unhatched eggs were placed in Petri dishes on damp pieces of filter paper with a cap-full of water in each dish to maintain dampness within the dish. The dishes were covered and left at room temperature (22°C) for one week and at the end of this period, unhatched eggs, if

any, were counted and designated as non-viable. In situations where larvae were dislodged during the process of bark removal, a larval count was done by counting the number of larval mines leaving the primary egg gallery. Parent beetles that were still alive at the time of bark removal were dissected to determine whether or not they were infected.

Results

(a) Carbohydrates

A preliminary experiment showed that the level of glucose in the hemolymph was extremely low and often undetectable by thin layer chromatography. Trehalose appeared to be the main carbohydrate component, making up more than 90% of the total hemolymph carbohydrates. The normal physiological level of hemolymph trehalose in D. pseudotsugae does not change significantly ($P > 0.05$) in the maturation of callow female adults, but mature male adults had 25% more trehalose than callow male adults ($P < 0.02$) (Table V). Mature male adults also had 25% higher hemolymph trehalose levels than mature female adults ($P < 0.02$). No significant differences ($P > 0.05$), however, were detected in the level of hemolymph trehalose between control and infected groups in each adult category.

(b) Total hemolymph protein

The total hemolymph protein levels in normal D. pseudotsugae decreases significantly during the maturation of both sexes of callow adults (Table VI). There was no significant difference ($P > 0.05$), however, between total hemolymph protein levels in male and female beetles. No significant difference ($P > 0.05$) in total hemolymph protein was detected between control and infected callow females. However, infected mature females had 20% less hemolymph protein

Table V. Hemolymph trehalose levels in control and C. reversus infected adult D. pseudotsugae (Mean ± 1 S.E.).

Host stage and sex	n	Trehalose concentration †	
		Control	Infected
Callow ♀	6	10.7 ± 0.9	10.1 ± 0.9
Mature ♀	8	10.6 ± 1.0	11.3 ± 1.4
Callow ♂	6	11.0 ± 0.7	9.7 ± 1.2
Mature ♂	8	14.9 ± 1.2	14.5 ± 1.0

† µg trehalose/µl hemolymph.

Table VI. Total hemolymph protein levels in control and C. reversus infected adult D. pseudotsugae (Mean ± 1 S.E.).

Host stage and sex	n	Total protein concentration	
		Control	Infected
Callow ♀	6	19.8 ± 1.2	19.2 ± 1.6
Mature ♀	8	14.3 ± 1.2	11.0 ± 0.7*
Callow ♂	6	21.2 ± 2.1	17.6 ± 1.3
Mature ♂	6	14.4 ± 1.6	16.1 ± 2.3

µg BSA equivalents/µl hemolymph.

* Significantly different from controls at $P < 0.05$.

($P < 0.05$) than control insects of the same group. No significant differences ($P > 0.05$) between total protein levels of control and infected callow and control and infected mature males were detected, although the difference in levels between control and infected callow males approached the 5% significance level.

(c) Individual proteins

Nine protein fractions were separated from the hemolymph of callow female beetles, with fractions I and VIII being the major fractions (Table VII; Fig. 9). No significant differences ($P > 0.05$) between control and infected beetles were observed with respect to the percentage composition of these nine fractions. Fraction IV, which is always present in the hemolymph of mature females was undetectable in callow females, and fractions VIIIB and IX that were sometimes present in mature females were totally absent in all the callow females. In mature females, a total of twelve fractions were detected in the hemolymph but fractions VIIIB, IX, and X were absent from some of the samples analysed. There was a lower concentration of fractions V and VI and a higher concentration of fraction III in mature as compared to callow females (Table VII). Despite there being no significant differences ($P > 0.05$) in percentage composition of protein fractions recorded between control and infected groups of mature female beetles, the gels showed that the total level of protein (according to the intensity of stain retained) was higher in the control groups (Fig. 10), supporting the results obtained in the total protein analysis. Parasitism, therefore, caused a proportional decrease in all protein fractions.

Table VII. Percentage concentration of protein fractions in the hemolymph of control and C. reversus infected adult D. pseudotsugae. Proteins separated by acrylamide gel disc electrophoresis. Concentrations presented as Mean + 1 S.E.

Fraction No.	R_f	Callow ♀ (n=5)		Mature ♀ (n=5)		Callow ♂ (n=6)		Mature ♂ (n=5)	
		Control	Infected	Control	Infected	Control	Infected	Control	Infected
I	0.05-0.10	27.7±1.4	23.7±1.9	27.5±1.0	28.5±1.6	22.4±2.1	20.2±1.9	26.3±1.9	23.2±2.3
II	0.09-0.17	3.3±0.5	3.9±0.8	1.3±0.2	2.5±1.2	2.7±0.9	2.0±0.4	1.3±0.5	1.3±0.4
III	0.14-0.21	2.9±1.1	4.0±0.8	8.8±2.2	7.4±1.0	8.0±1.7	5.9±2.0	6.8±1.6	7.9±2.1
IV	0.20-0.24	-	-	3.3±1.0	3.8±1.3	14.2±1.9	8.0±3.8	5.7±0.5	6.5±1.2
V	0.24-0.30	11.7±2.4	14.7±2.4	6.2±2.1	5.3±1.3	4.8±1.2[a]	8.9±0.7[a]	8.8±1.0	10.6±1.3
VI	0.30-0.38	10.9±0.7	10.5±1.8	6.4±0.5	6.5±0.8	14.0±1.8	9.6±1.5	6.9±0.7	7.0±0.6
VII	0.36-0.47	13.2±2.3	9.1±1.8	11.0±1.3	11.0±1.5	9.4±0.9[b]	15.9±1.2[b]	10.6±1.3	11.7±1.3
VIII	0.49-0.57	23.7±0.8	25.8±2.0	28.3±2.4	27.1±2.5	22.9±2.3	24.4±1.3	24.0±1.6	25.1±1.5
VIIIB	0.64-0.70	-	-	2.3±0.1[†]	1.9±0.2[†]	-	-	2.1±0.3	1.5±0.7[†]
IX	0.78-0.86	-	-	0.8±0.2[†]	1.8±0.7[†]	0.6±0.3[†]	1.2±0.3[†]	1.5[†]	0.6[†]
X	0.90-0.95	2.0±0.8	1.6±0.2	2.2±0.3[†]	1.9±0.2[†]	2.4±0.8	3.4±0.7	3.5±0.5	2.3±0.5[†]
XI	1.0	4.9±0.8	6.1±0.3	3.7±0.5	4.2±0.4	3.6±1.2	3.6±0.7	4.2±1.2	3.8±1.2

† Fraction not present in all samples.

[a] Significantly different from each other at $P < 0.05$.

[b] Significantly different from each other at $P < 0.01$.

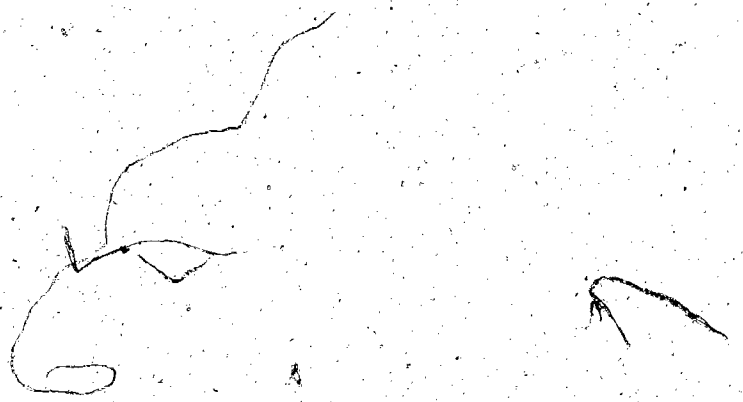


Figure 9

Electropherograms of callow adult female D. pseudotsugae.

Left - Control; Right - Infected. The major fractions are marked.

No significant differences were detected between the protein fractions of control and infected hemolymph with respect to percentage composition.

I

II

III

V

VII

VIII

X

XI

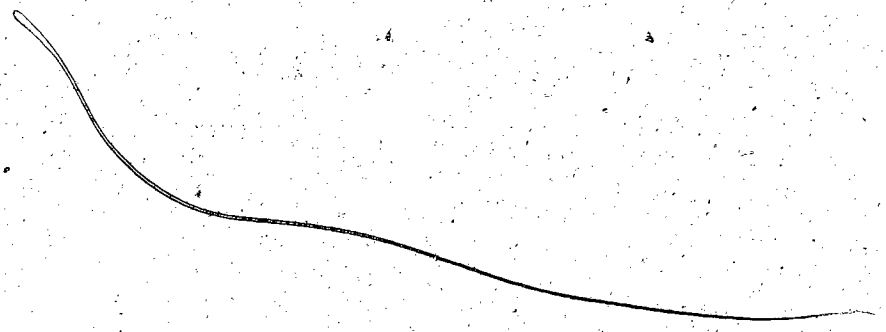
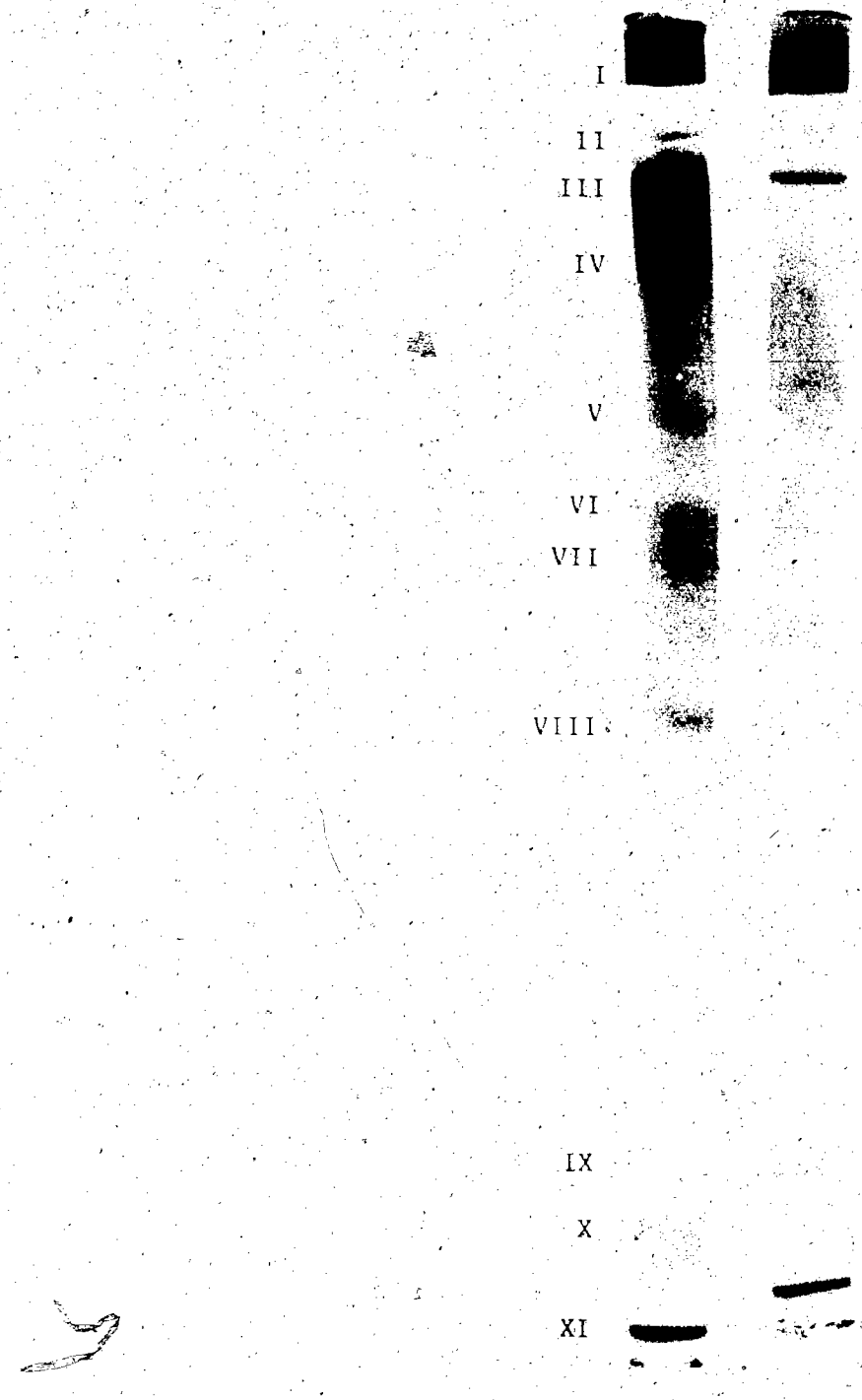


Figure 10

Electropherograms of mature adult female D. pseudotsugae. Left - Control; Right - Infected. The major fractions are marked. Although no significant differences in the percentage composition of protein fractions over total protein concentration was recorded between control and infected hemolymph, infected electropherograms retained less stain than control electropherograms indicating less total protein.


I
II
III
IV
V
VI
VII
VIII
IX
X
XI

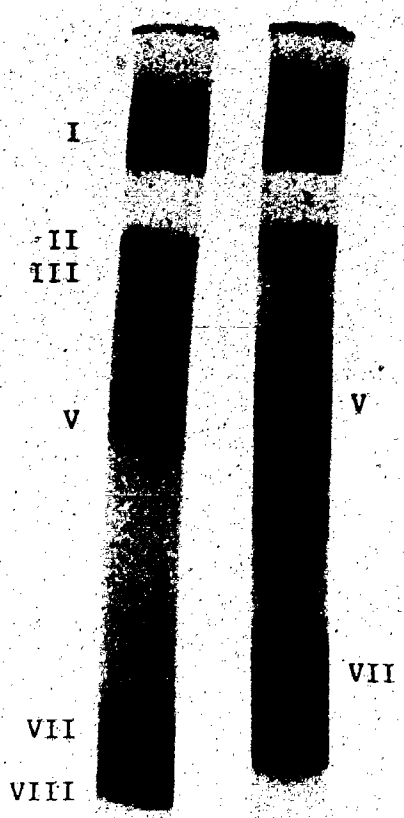
In the callow males, eleven fractions were separated. Fraction VIIIB was absent from all samples and fraction IX was detectable in only some samples. Fraction IV, which was not found in the hemolymph of callow females, accounted for about 10% of the total protein in callow males. Two fractions increased significantly in infected groups (Table VII; Fig. 11). These two fractions, V and VII, increased approximately 4% and 6% respectively in percentage of total protein, which was a 30 - 40% increase over the controls. Fractions IV and VI decreased slightly in percentage of total protein composition, but these changes were not significant at the 95% confidence limit. In the mature male beetles, twelve fractions were recognised, but, as in the mature females, fractions VIIIB, IX, and X were absent from some samples. No significant differences ($P > 0.05$) were detected in the level of the protein fractions between control and infected mature male beetles. Notably, the higher level of fractions V and VII that occurred in infected than control callow males does not occur in the mature males (Table VII; Fig. 12). Fractions IV and VI were significantly lower ($P < 0.05$) in control groups of mature males than in control groups of callow males. Fraction VIIIB, which was detected in some samples of hemolymph from mature males, was not detected in callow males. As in all the other groups analysed, fractions I and VIII were the two major fractions in the hemolymph.

(d) Total hemolymph amino-acids

The level of amino-acids in the hemolymph was approximately the same in callow and mature female beetles, and in callow and mature male beetles

Figure 11

Electropherograms of callow adult male D. pseudotsugae. Left - Control; Right - Infected. The major fractions are marked. Fractions V and VII were significantly higher in infected hemolymph than in healthy hemolymph ($P < 0.02$ and $P < 0.01$ respectively).



X

XI

Figure 12

Electropherograms of mature adult male D. pseudotsugae. Left - Control; Right - Infected. The major fractions are marked. No significant differences were detected between the protein fractions of control and infected hemolymph.


I
II
III
IV
V
VII
VIII
IX
X
XI

(Table VIII), and no significant differences in amino-acid levels were detected between control and infected members of each of the four adult categories studied.

(e) Oocyte development

Infected female beetles had significantly smaller oocytes than did uninfected ones (Table IX). Before oviposition, the oocytes were 20% smaller in infected females than in the controls.

(f) Gallery construction, fecundity and egg viability

At 7, 14, and 23 days after the initiation of gallery construction, the infected female beetles had significantly ($P < 0.05$) shorter egg galleries than uninfected females (Fig. 13). At 7, 14, and 23 days, the gallery of infected females was 25%, 28% and 27% respectively shorter than that of the controls. Despite these differences, the slopes of the regression lines for control and infected beetles are not significantly ($P > 0.05$) different.

The number of eggs laid by infected females by 7, 14, and 23 days after the initiation of gallery construction were significantly ($P < 0.05$) lower than those laid by the control females (Fig. 14). The difference at 7, 14, and 23 days was 50%, 33%, and 45% respectively. Further, the infected groups had a significantly ($P < 0.02$) lower rate of egg-laying than did the uninfected insects as indicated by the relative slopes of the regression lines. The depicted linear relationship between the number of eggs laid by infected bark beetles and the length of infection is perhaps not a satisfactory representation of the assumed functional relationship. The time relationship is probably curvilinear (the curve reaching a plateau) since the number of eggs available is finite.

Table VIII. Total amino-acid levels in the hemolymph of control and C. reversus infected adult D. pseudotsugae (Mean + 1 S.E.).

Host stage and sex	n	Total amino-acid concentration[1]	
		Control	Infected
Callow ♀	7	10.4 + 0.7	10.3 + 0.7
Mature ♀	7	9.6 + 0.6	10.5 + 0.8
Callow ♂	7	11.5 + 0.7	12.0 + 1.3
Mature ♂	7	10.9 + 0.7	11.7 + 1.4

[1] µg Glycine equivalents/µl hemolymph.

Table IX. The effect of C. reversus on the size of the terminal oocyte in mature female adult D. pseudotsugae (Mean + 1 S.E.).

Group	n	Length of largest terminal oocyte (mm)
Control	13	0.54 + 0.02
Infected	13	0.44 + 0.02***

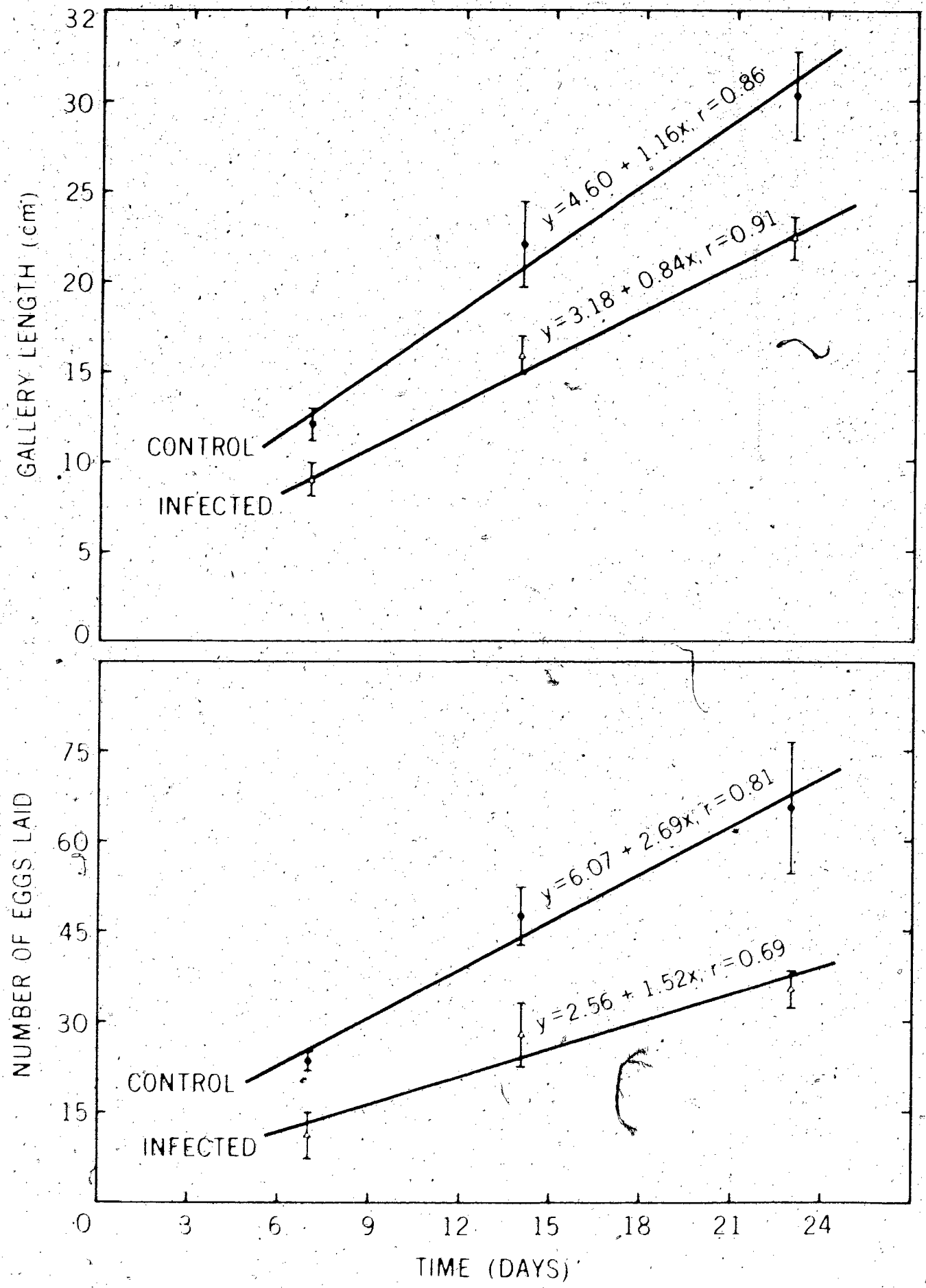
*** Significantly different from controls at P < 0.01.

Figure 13

Regression lines for the gallery length of normal and C. reversus infected female adult D. pseudotsugae. Points represent Mean + 1 S.E. at each time period.

Figure 14

Regression lines for the number of eggs laid by normal and C. reversus infected female adult D. pseudotsugae. Points represent Mean + 1 S.E. at each time period.


GALLERY LENGTH (cm)
32
30
25
20
15
10
5
0
y=4.60 + 1.16x, r=0.86
y=3.18 + 0.84x, r=0.91
CONTROL
INFECTED
NUMBER OF EGGS LAID
75
60
45
30
15
0
y=6.07 + 2.69x, r=0.81
y=2.56 + 1.52x, r=0.69
CONTROL
INFECTED
3
6
9
12
15
18
21
24
TIME (DAYS)

Nematode infection affects neither the shape of the gallery nor the egg viability of female D. pseudotsugae (Table X). Measurement of egg viability at 23 days was not possible owing to the difficulties in distinguishing between larval mines from neighbouring galleries and the deterioration of unhatched eggs.

Discussion

Trehalose is the major blood sugar of insects and its concentration in the hemolymph is maintained by hormonal action. If hemolymph trehalose is depleted by the energy demands of the insect, it is reconstituted at the expense of glycogen stored in the fat body (Gilmour, 1965). Trehalose is broken down by the enzyme trehalase to two molecules of glucose, which normally is only a minor constituent of insect hemolymph and there is no evidence of any regulatory mechanism controlling its concentration (Gilmour, 1965). In both control and infected beetles, the level of glucose was undetectable. The level of trehalose in D. pseudotsugae (10.6 µg/µl in mature females and 14.9 µg/µl in mature males) is high when compared to that of other Coleoptera previously studied, e.g. 5 - 7 µg/µl in adult Dytiscus marginalis and 3 - 5 µg/µl in adult Hydrophilus piceus (Duchateau and Florkin, 1959). It is comparable, however, with the levels found in the hemolymph of more active fliers, e.g. 6 - 12µg/µl in adult Apis mellifera (Duchateau and Florkin, 1959) and 14 µg/µl in adult Periplaneta americana (Treherne, 1960) and adult Melanoplus differentialis (Randall and Derr, 1965). Notably, D. pseudotsugae is also considered as an active flier (Atkins, 1961; Bennett and Borden, 1971).

Table X. Viability of eggs laid by normal and C. reversus infected female adult D. pseudotsugae (Mean + 1 S.E.).

Days after start of culture	n	Egg viability (%)	
		Control	Infected
7	6	62.3 + 6.1	72.4 + 6.3
14	6	83.1 + 3.4	78.7 + 9.1

Some nematode species are capable of glucose uptake from the surrounding medium. This ability has been demonstrated for M. nigrescens (Gordon and Webster, 1972) and Ascaris lumbricoides (Entner and Gonzalez, 1959; Castro and Fairbairn, 1969). It is possible, therefore, that although no significant change in the level of hemolymph trehalose in C. reversus infected beetles was detected, host glucose from the catabolism of trehalose may be removed by the nematode and the consequent tendency to lower the insect hemolymph trehalose level would be compensated for by the hormonal control mechanism releasing trehalose from the fat body. M. nigrescens changes the carbohydrate metabolism in both the hemolymph and the fat body of its locust host (Gordon and Webster, 1971; Gordon et al., 1971). However, the biochemistry of the fat body of C. reversus infected bark beetles was not studied and so it is not known whether parasitism affects carbohydrate metabolism in the fat body of these insects in a similar way to that in M. nigrescens infected desert locusts.

In insects the corpora allata directly regulate protein synthesis in the fat body and indirectly control hemolymph protein levels. Protein may be released by the fat body into the hemolymph and taken up by the oocytes for incorporation into ovarial proteins (Wigglesworth, 1970). The amino-acid level in the hemolymph, however, is not under hormonal control but varies with the nutritional state and metabolic activities of different tissues (Gilmour, 1965). Although the average protein level in the hemolymph of Coleoptera is 30 - 40 µg/µl (Florkin and

and Jeuniaux, 1964), the 14 - 20 µg/µl level of protein found in adult female D. pseudotsugae agrees with that found by Sahota (1970) for the same insect. The fact that protein depletion caused by C. reversus in the beetle hemolymph is detected only in mature but not in callow adult female beetles suggests that during the maturation process of the latter, when active incorporation of hemolymph proteins into ovarial proteins occurs, the nematode is also drawing from the host hemolymph protein source and the homeostatic mechanism for the maintenance of hemolymph protein levels is unable to compensate for the relatively large quantity of protein removed. The nematode probably removes hemolymph proteins also from the callow female, but the hormonal control of hemolymph proteins is able to cope with this rate of depletion, without the developing eggs drawing from the available proteins. Alternatively, during beetle diapause, the nematode also may enter a state of inactivity and reduce its rate of metabolism and, hence, of protein utilization. Such effects have been observed in Heterotylenchus autumnalis parasitizing the facefly (Stoffolano, 1967).

The protein depletion measured in the hemolymph of the infected mature female D. pseudotsugae may possibly be an indirect consequence of changes in the host fat body caused by the nematode. M. nigrescens, for example, has been shown not to affect total hemolymph protein levels in the desert locust but depletes both fat body protein and amino-acids (Gordon and Webster, 1971). This was attributed to the nematode's

ability to stimulate protein catabolism in the fat body of its host and to take up amino acids released by this process into the hemolymph. The precise pathway by which the nematode could affect host protein synthesis is unknown, but it may be directly or indirectly mediated through the host hormonal system. It is also possible that the nematode is capable of the bioconversion of carbohydrates into amino-acids in the hemolymph. This ability has been demonstrated for A. lumbricoides (Pollak, 1957) and Caenorhabditis briggsae (Rothstein and Tomlinson, 1961). The reduction in the size of the oocytes in infected female beetles is probably a consequence of the hemolymph protein depletion in D. pseudotsugae. The fact that infected female beetles have oocytes that are 20% smaller than those of uninfected female beetles suggests that either the nematode competes with the developing oocytes for hemolymph protein or the nematode affects the host hormonal control system for vitellogenesis.

Sahota (1970) was able to separate eight fractions from the hemolymph of mature female adult D. pseudotsugae using a 6% running gel. I found nine fractions in all samples using a 7% running gel, with three other fractions detectable only in some samples. Electrophoretically, the depletion in total hemolymph protein in infected mature female beetles can be observed in differences in the staining intensity of gels of control and infected beetles. The percentage composition of various protein fractions within the hemolymph of infected mature females does

not change over the controls, indicating a non-selective uptake of proteins by the parasite. However, despite the fact that total levels of hemolymph proteins in callow males do not change significantly, electrophoresis shows significant increases in the percentage of two protein fractions in the hemolymph of infected beetles over that of controls. This may be due either to a selective uptake of specific amino-acids, or to a selective utilization of specific proteins, or to a direct or indirect effect on protein catabolism in the fat body of the beetle and a subsequent disproportionate release of the various amino-acids into the hemolymph. M. nigrescens depletes some protein fractions in the hemolymph of desert locusts while causing an increase in certain protein fractions in the insect fat body (Gordon et al., 1973).

Despite the fact that no significant changes were detected in total amino-acid levels in the hemolymph of infected beetles, the possibility of uptake of host amino-acids by the parasite cannot be ignored. The beetles may be able to replace their amino-acids by increased food intake. A selective uptake of amino-acids was demonstrated for M. nigrescens (Gordon and Webster, 1972). Alternatively, parasite induced catabolism of fat body protein may be supplying the nematode with sufficient free amino-acids in the hemolymph for uptake.

C. reversus, like M. nigrescens, does not appear to feed directly on host fat body tissue. The nematode family Sphaerularlidae, to

which C. reversus belongs, includes the genus Sphaerularia where nutrient absorption occurs through the epithelia of the extruded vagina and uteri (Pofnar and Bess, 1972). Although members of the genus Contortylenchus do not exhibit such behaviour, nevertheless, it is reasonable to speculate that nutrient uptake in this genus is through the cuticle. Nicholas (1972) reported, for example, that the parasitic females of the closely related genus Heterotylenchus have numerous canals ramifying through the cuticle, running from the hypodermis to the outer surface, and suggested that these canals are concerned with nutrient uptake.

Parasitism in D. pseudotsugae by C. reversus results in a reduction in the length of the primary gallery built by the invading female, but does not affect its shape. The closely related C. elongatus has a similar effect on the beetle I. paraconfusus (Massey, 1960) and S. elongatus also causes this condition in S. ventralis (Massey, 1964; Ashraf and Berryman, 1970). In both S. ventralis and S. rugulosus, nematode parasitism also affects the shape of the gallery, which suggests an effect on the nervous coordination and vigour of the beetles that may be caused directly or indirectly by excretory or secretory products of the nematode parasites or indirectly through nutrient depletion of the hosts. Ashraf and Berryman (1970) have shown that parasitized S. ventralis females sometimes constructed normal galleries but ceased to oviposit long before the cessation of boring. This resulted in a decrease in the average number of eggs laid per centimeter of gallery. In D. pseudotsugae, however, egg laying proceeds with gallery construction

and infected females may build shorter galleries because there are fewer developing oocytes available for egg laying. This effect of C. reversus on egg production has also been observed in D. rufipennis (Massey, 1956). The slower rate of egg production by infected D. pseudotsugae may be indirectly due to the protein depletion in the hemolymph caused by the nematode or may indicate an upset in the vitellogenic processes. There was no significant difference in viability between eggs laid by infected and those laid by healthy female beetles. Thus unlike S. elongatus which sterilizes 90% of infected female S. ventralis (Ashraf and Berryman, 1970), C. reversus only reduces by 40 - 60% the fecundity of D. pseudotsugae.

When male beetles were infected, the size of the generation that resulted from their mating with healthy females was no different from that resulting from matings between uninfected insects. It would appear, therefore, that parasitism does not affect fertility in male D. pseudotsugae. This has also been reported for S. ventralis infected with S. elongatus (Ashraf and Berryman, 1970) and for the ichneumonid wasps, Rhyssa spp., infected with the nematode Deladenus sp. (Hocking, 1967).

Parasites that cause little or no pathological effects on their hosts are thought to have been associated with their hosts for a long time (Read, 1970). However, some evolutionists do not agree with this theory and quote instances where parasites have been introduced into new hosts without gross pathogenic effects (Ball, 1943). There is also a hypothesis that host specificity in parasites has evolved as a result

of overspecialization of the latter in their requirements for particular ecological or physiological conditions (Read, 1970). C. reversus is relatively avirulent to its host, reducing its fecundity but not sterilizing, killing or causing detectable cellular responses to its presence. S. elongatus is more antagonistic towards its host S. ventralis, causing sterility and inducing cellular reactions in certain host larval instars. Encapsulation may be interpreted as an indicator of poor host-parasite compatibility, and the fact that C. reversus has been found in all stages of the Douglas fir beetle except the egg and first larval instar may indicate a long association between this parasite and the host. C. reversus has been found in three other species of Dendroctonus, namely D. rufipennis, D. ponderosae and D. terebrans, but no cellular reactions to its presence were reported. This indicates that C. reversus is a parasite well adapted to its host(s), but not so overspecialised in its ecological and physiological requirements as to be narrowly host specific.

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

This taxonomic and biological study has encompassed only a limited number of species of bark beetles in British Columbia, and the biology of all of the associated nematode species has not been studied in detail. Several inconsistencies have been found between my results and those of others especially in studies on morphology, life cycle and post-embryonic development of bark beetle nematodes. From the present study, a few areas for future research can be defined. Further knowledge of the biology and life history of the hemocoel parasite from T. lineatum should prove both useful and interesting because it may have an important influence on the host's biology and is the only known nematode parasite of T. lineatum. Also a closer study of the post-embryonic development of S. dendroctoni from D. ruffipennis may elucidate the number of larval stages present in the host hemocoel and clarify previous findings.

This study has brought out several interesting features of C. reversus. Firstly, it has shown that the nematode spends only a short free-living period in the galleries of the Douglas-fir beetle. In preparation for this brief free-living period, it develops to the fourth larval stage in the host hemocoel and becomes adult in the galleries immediately after leaving the beetle. The male gonad produces gametes in the fourth larval stage so that insemination of the female is possible shortly after molting into the adult. This study also demonstrates that the infective stage of C. reversus is the spermatised female. We also now know that the nematodes leave the beetle via the gut and are released at intervals to facilitate distribution along the length of the egg gallery. However,

we still do not know how the nematode enters the host larvae. Although cuticular penetration has been suggested, actual observations of the infective process are needed. The reason that the first instar larva of D. pseudotsugae is not parasitized by C. reversus has still to be established. It is not known whether this particular stage is "immune" to the parasite, although cellular reactions have not been observed in any stage of the beetle in response to this nematode. It has been shown also that both sexes of the parent beetle are able to spread the nematode infection to the subsequent generation of offspring.

The physiological studies attempted, although preliminary, indicate that C. reversus causes disturbances in the host that manifest themselves as changes in hemolymph composition, oocyte development and behaviour. The effects of C. elongatus on I. paraconfusus were believed to be "mechanical rather than pathological" because of the large numbers of parasitic larvae and eggs (Massey, 1966a). My findings indicate that C. reversus causes a nutrient depletion which is the result of the parasite's particular nutrient demands and/or changes induced by parasitism affecting the hormonal control of metabolism in the beetles. It is essential that the fat body of the beetles should be analysed to enable correlation of changes within it with those occurring in the hemolymph. It has been assumed that the method of nutrient uptake by C. reversus is through the digestive system. Electron microscope studies on the cuticle of parasitic stages of C. reversus may show anatomical features that may support the theory of cuticular nutrient uptake.

The behaviour of D. pseudotsugae has been shown to be altered by the parasite C. reversus. However, unlike S. elongatus and N. rugulosi which cause aberrant directional changes in the gallery of their female hosts (Ashraf and Berryman, 1970; Nickle, 1971), C. reversus only reduces the length of the egg gallery of its female host without altering its direction. Reduction in fecundity of the host is a widespread phenomenon caused by bark beetle nematodes. C. reversus, unlike mermithids, does not kill its host and, unlike S. elongatus, does not cause sterility.

This study has identified in detail some of the effects of bark beetle nematodes on their hosts. It is reasonable, therefore, to envisage that further research into the interactions between nematodes and bark beetles would lead to a better understanding of the effects of these parasites on the natural populations of bark beetles and so to their utilization as biological control agents.

APPENDICES

APPENDIX I

Nematodes associated with the Douglas-fir beetle, D. pseudotsugae, in British Columbia.

These nematodes have been previously described by other workers. However, in several instances there is new information on the life histories and in every instance a more detailed morphological description is given. Measurements presented are from B.C. specimens.

Ektaphelenchus macrostylus Khan, 1960.

Females (10): L = 0.78 ± 0.01 (0.71-0.81)mm; a = 28.4 ± 1.1 (25.1-36.7); b = 8.4 ± 0.1 (7.8-9.1); V = 78.0 ± 0.3 (76.0-79.8)%; G_1 = 44.2 ± 1.6 (37.1-52.2)%; stylet = 13.4 ± 0.8 (11-19)μ.

Males (10): L = 0.69 ± 0.02 (0.61-0.78)mm; a = 33.5 ± 1.3 (26.6-41.6); b = 7.6 ± 0.1 (6.9-8.3); c = 19.6 ± 0.5 (17.0-22.3); c' = 2.3 ± 0.1 (2.0-2.6); T = 28.0 ± 0.7 (24.1-30.6)%; stylet = 9.6 ± 0.4 (7-11)μ; spicule = 21.0 ± 0.3 (20-22)μ.

Adult nematodes were found in the gallery frass of the Douglas-fir beetle. Nematodes long and stout. Cuticle regularly annulated. Head offset by slight cephalic constriction. Stylet long and pointed with small inconspicuous basal knobs. Procorpus of esophagus long and cylindrical with slight constriction at junction with median bulb, which is oval, twice as long as wide. Nerve ring approximately 10μ behind median bulb. Excretory pore about 10-15μ posterior to nerve ring. Intestine

undifferentiated running from median bulb to posterior end of nematode. Anus not discernible in female specimens. Tail in both sexes conoid with rounded terminus.

Female gonad monodelphic, prodelphic and unreflexed. Post-uterine sac present, 90-130μ long, extending approximately two-thirds way to tip of tail.

Male gonad single, extending half way down body length. Spicules very prominent, mitten-shaped, with a prominent ventral process. Distal end of spicule adjacent to cloaca with well defined hook-like process. Gubernaculum absent. Single pair of acudal papillae present just behind tail terminus.

This species was originally described by Khan (1960) who presented measurements of "L", "a", "b", "c", "V", and "T" for only one male and one female obtained from the Douglas-fir beetle in B.C. His "b" ratio for the female specimen was 4.0, half that of those obtained in the present study. Khan also mentioned that he found females of E. macrostylus under the elytra and males in the bark. In the present study, only ensheathed L_3 larvae were found under the elytra; male and female adult nematodes occurred only in the gallery. The life cycle occurs almost entirely in the galleries and only the L_3 larvae reattach to adult beetles prior to emergence of the latter for transfer to another habitat.

Panagrolaimus dentatus (Thorne, 1935) Rühm, 1956.

Females (10): L = 0.49 ± 0.02 (0.42-0.57)mm; a = 15.5 ± 0.5 (13.1-18.0); b = 4.6 ± 0.2 (4.0-5.4); c = 11.1 ± 0.3 (10.1-13.3); c' = 2.5 ± 0.1 (2.0-2.8); V = 61.7 ± 0.6 (58.5-64.9)%; G_1 = 69.5 ± 2.5 (59.6-82.2)%; tail length = 43.8 ± 1.2 (40-50)μ.

Males (10): L = 0.44 ± 0.01 (0.39-0.49)mm; a = 17.9 ± 0.8 (14.3-22.8); b = 4.5 ± 0.1 (3.9-5.1); c = 10.6 ± 0.3 (9.2-12.5); c' = 2.3 ± 0.1 (1.8-2.7); T = 66.9 ± 1.9 (59.5-76.3)%; tail length = 41.8 ± 1.6 (35-49)μ; spicule = 22.2 ± 0.6 (20-25)μ; gubernaculum = 12.4 ± 0.3 (11-14)μ.

Larval nematodes were found under the elytra. Adults were obtained from frass of the beetle galleries.

Adult nematodes short and stout. Head end with six lips. Buccal apparatus panagrolaimid, with cheilorhabdions fused with pro- and mesorhabdions forming a wide chamber. Tooth present on dorsal segment of mesorhabdion. Procorpus of esophagus cylindrical and wide, narrowing posteriorly into an isthmus equal in length to the procorpus; median bulb round in shape with prominent valvular apparatus. Intestine opens into anus approximately two body widths in front of tail terminus. Tail in both sexes conoid with attenuated terminus which occupies one-third to one-half of total tail length. Male tail with one pair of pre-anal papillae, one pair of post-anal papillae just behind the cloaca, and two pairs of caudal papillae at base of attenuation of tail. The last two pairs of caudal papillae are one pair dorsal and one pair ventral in position.

Ovary monodelphic, prodelphic, and reflexed, the terminus sometimes extending to the rectum or into the tail. Post-uterine sac rudimentary, approximately one body width in length. Vulva a transverse slit with slightly elevated lips.

Male gonad single, with terminal portion reflexed. Spicules club-shaped, paired, ventrally arcuate, with an expanded proximal portion. Gubernaculum present, 11 - 14μ long.

P. dentatus was first described by Thorne (1935) from D. ponderosae in Utah. Originally placed in the genus Panagrodontus, it was later transferred to the genus Panagrolaimus, with which the former genus has been synonymized (Rühm, 1956). Thorne presented measurements in the Cobb formula for one male and one female specimen. His specimens were slightly longer than those obtained in the present study and had longer tails. He did not measure the full length of the reflexed female gonad and, therefore, a comparison with my specimens is not possible. Thorne, like myself, found the ensheathed larvae only under the elytra of D. ponderosae and the adult nematodes in the galleries.

Cryptaphelenchus latus (Thorne, 1935) Rühm, 1956

Females (12): L = 0.31 ± 0.01 (0.26-0.38)mm; a = 20.0 ± 0.4 (18.7-22.2); b = 7.5 ± 0.2 (6.5-8.6); V = 78.5 ± 0.3 (76.8-80.3)%; G_1 = 48.7 ± 2.0 (37.2-63.5)%; stylet = 8.9 ± 0.3 (8-10)μ.

Males (7): L = 0.26 ± 0.01 (0.23-0.29)mm; a = 20.6 ± 0.7 (19.9-24.1); b = 6.6 ± 0.1 (6.1-7.0); c = 10.7 ± 0.7 (8.9-11.8); c' = 2.1 ± 0.1 (1.9-2.4); T = 39.3 ± 1.3 (35.1-

44.3)%; tail length = 24.3 + 1.2 (21-29)μ; stylet = 9.1 + 0.4 (8-11)μ; spicules = 15.9 + 0.4 (14-17)μ; gubernaculum = 9.7 + 0.4 (8-11)μ.

Adult nematodes were recovered from gallery frass. Body of nematode short and tapering towards both ends. Head offset from body by narrower neck region. Spear short, about 10μ long, with small basal knobs. Procorpus of esophagus narrow and about one to two body widths long; median bulb oval to spherical with conspicuous valvular apparatus. Nerve ring situated approximately one body width behind median bulb. Excretory pore not easily visible. Intestine with granular inclusions; anus not discernible in female specimens. Female tail slightly arcuate, conoid and tapering to a point. Male tail ventrally arcuate, conoid and pointed; two pairs of caudal papillae present, one pair pre-anal and the other close to tail terminus.

Female gonad monodelphic, prodelphic, and unreflexed. Post-uterine sac present extending half-way down tail from vulva. Vulva a transverse slit with the anterior lip slightly raised and overlapping the posterior lip.

Male gonad single and outstretched to about two-fifths the body length. Spicules paired and mitten-shaped with a prominent ventral process in the proximal part. Gubernaculum absent.

C. latus was originally described from D. ponderosae (Thorne, 1935) and named Aphelenchoides latus, but was transferred to the genus Crypt-

aphelenchus by Rühm (1956). Thorne's original description presented the Cobb formula for one male and one female specimen. The male in Thorne's description was larger than those in the present study, and his male and female specimens had "a" ratios one-half that of my specimens. Thorne's specimens also had slightly longer esophaguses in proportion to their body length than did my specimens, and although the lengths of the tails in the two descriptions are similar, the tails of Thorne's specimens were wider.

Parasitorhabditis obtusa (Fuchs, 1915) Dougherty, 1953

Females (10): L = 0.70 ± 0.04 (0.54-0.88)mm; a = 19.7 ± 0.5 (18.1-22.7); b = 4.2 ± 0.4 (3.4-4.6); c = 32.3 ± 1.0 (26.8-38.5); c' = 1.1 ± 0.04 (0.9-1.2); V = 93.6 ± 0.3 (92.7-95.4)%; G_1 = 73.2 ± 2.2 (61.1-82.2)%; tail length = 21.8 ± 1.0 (18-27)μ.

Males (13): L = 0.72 ± 0.03 (0.54-0.91)mm; a = 20.8 ± 0.8 (16.1-24.5); b = 4.5 ± 0.2 (4.0-6.1); c = 28.3 ± 1.3 (23.0-39.6); c' = 1.2 ± 0.1 (0.9-1.4); T = 78.2 ± 1.3 (71.8-85.5)%; tail length = 25.7 ± 0.9 (22-30)μ; spicule = 45.1 ± 0.9 (38-55)μ; gubernaculum = 23.3 ± 0.6 (20-28)μ.

Larval stages of the nematode are found living free in the midgut and occasionally in the hindgut of adult beetles. The nematodes are in constant movement, working against the flow of food material in the gut. Adult nematodes are found in the frass of the galleries.

Adult worms short and stout. Stoma narrow, approximately three times as deep as wide; cheilorhabdions and prorhabdions slightly convex, telostom absent. Corpus of esophagus cylindrical with narrower isthmus which is as long as the corpus; median bulb not well developed in both sexes; terminal bulb ovate with well developed valvular apparatus. Nerve ring present half way down isthmus. Excretory pore slightly posterior to nerve ring.

Female gonad monodelphic, prodelphic, reflexed. Post-uterine sac absent. Vulva less than one body width in front of the anus, with lips slightly elevated. Female tail conoid ending in a rounded terminus.

Male approximately the same size as the female. Male gonad single, outstretched and reflexed. Tail of male conoid and ending in a pointed terminus. Bursa present, enveloping tail, with ten pairs of supporting rays, two of which are pre-anal and the rest arranged in groups of 3, 1, 1, 3 post-anally. One pair of pre-anal and one pair of post-anal papillae present close to anal opening. Spicules of male paired and fused distally, approximately 45μ long, slightly bent ventrally, with proximal terminal knobs. Gubernaculum present, 23μ in length, slightly wider in central portion than at both ends.

Fuchs (1915) first described P. obtusa from I. typographus and placed it in the genus Rhabditis. It was later transferred to the genus Parasitorhabditis of which P. obtusa is now the type species (Dougherty, 1953). Thorne (1935) described specimens of P. obtusa from D. ponderosae.

The measurements of his specimens, presented in the Cobb formula, showed females with tails one-half the length of those in my specimens and consequently "c" ratios twice those of my specimens. Nickle (1963a) stated that the larval parasitorhabditids reduced the epithelial layer of the ventriculus of I. paraconfusus. However, as these nematodes do not attach to the mucosa but are free in the lumen, it is difficult to envisage such an effect unless the nematodes are numerous enough to cause gut occlusion. The mode of re-infection of these nematodes is unknown, but it is probably either oral during feeding or anal during the quiescent overwintering period of the adult beetle.

APPENDIX II

Nematode parasite from the hemocoel of the spruce beetle, *D. rufipennis*.

Sphaerulariopsis dendroctoni (Massey, 1956) Nickle, 1963

Stages occurring in the hemocoel were studied. Mature gravid females, eggs, and at least three larval stages were obtained. The eggs (80 x 40μ) are deposited in the hemocoel after initial cleavage. At least one molt occurs within the egg. No detailed study was undertaken of the post-embryonic development of *S. dendroctoni* but fourth stage female larvae were observed by virtue of the presence of a vaginal primordium.

The female is unusual in that, early in development, after the female larvae become sexually mature, the uterus, ovary and oviduct are extruded through the vulva. These structures then enlarge to more than a hundred times their original size, dwarfing the original body of the female nematode to which they are still attached. The size of these protruded reproductive organs may reach a length of 1.6 mm and a width of 0.25 mm. The surface of the everted uterus is covered with rounded projections containing enlarged nuclei of the uterine cells. The body of the female at this stage is approximately 0.4 mm long and 30μ wide.

The original description of this nematode from the same host species collected from Colorado and northwestern Montana included two larval stages (Massey, 1956). Nevertheless, fourth stage larvae of *S. dendroctoni* were observed in the host hemocoel in the present study, and, therefore,

it is possible all four larval stages occur in the hemocoel of D. rufipennis. Massey also found males together with the females in the beetle hemocoel, which is an interesting report that needs further examination since in other members of the Sphaerulariidae previously studied, the male is free-living and does not enter the host.

Egg production of female beetles parasitized by S. dendroctoni was reported to have been reduced (Massey, 1956). Poinar and Hess (1972) in their studies on the related Sphaerularia bombi observed that nutrients were taken up by the nematode through the walls of the extruded uterus.

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