The Douglas fir bark beetle, *Dendroctonus pseudotsugae* Hopk. (Scolytidae) is an important forest pest which is difficult to control. A study of the nematode parasites and associates of this beetle was done near Corvallis, Oregon, to determine the possibility of using a nematode as a biological control for these beetles.

The only endoparasitic nematode found was *Contortylenchus reversus* (Thorne 1935) Rühm 1956. This nematode was found in 5.5% of the adult beetles, never in larvae or pupae. The five nematodes found associated with the beetles in their galleries were identified as: *Parasitorhabditis obtusa* (Fuchs 1915) Dougherty 1953; *Mikoletzkya pinicola* (Thorne 1935) Baker 1962; *Ektaphelenchus obtusus* Massey 1956; *Panagrolaimus judithi* Massey 1964; and an undescribed species of *Plectonchus*. *Parasitorhabditis obtusa* and *Mikoletzkya pinicola* were the most abundant of the associated nematodes.
averaging about 10,000 per gallery. All of these associated nematodes were recovered and cultured from the body of the adult beetles. They occurred either externally or as larval parasites of the gut, in the case of P. obtusa. Cultures were obtained using a mixture of ground inner bark and agar. All the cultured nematodes grew and multiplied on these plates.

Control of the beetles using two neoplectanid nematodes designated DD136 and DD517 by Dr. S. R. Dutky of Beltsville, Maryland, was attempted. These nematodes carry a bacterium which was lethal to the beetles experimentally; however, the nematodes were not able to reach the beetles under the bark in the field tests that were conducted. It was concluded that DD136 cannot be used as an agent to carry a pathogen into the beetle galleries.
NEMATODE ASSOCIATES
AND PARASITES OF THE DOUGLAS FIR BEETLE
DENDROCTONUS PSEUDOTSUGAE HOPKINS
WITH NOTES ON BIOLOGICAL CONTROL

by

GORDON WYATT MARTIN

A THESIS
submitted to
OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of
MASTER OF ARTS

June 1964
APPROVED:

Redacted for Privacy

Professor of Zoology

In Charge of Major

Redacted for Privacy

Chairman of Department of Zoology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented May 2, 1964

Typed by Dolores Martin
ACKNOWLEDGMENT

Most of the research for this thesis was supported by the United States Forest Service research contract number 12-11-196-005 for which support I gratefully express my appreciation.

My appreciation and thanks are further expressed to Dr. C. G. Thompson and Dr. Roger Ryan of the Forest Service for their valuable advice and help in obtaining material. I am also very grateful to Dr. Calvin L. Massey of the United States Forest Service and Dr. Harold Jensen of Oregon State University for identifying several of the nematodes.

My thanks are extended to my two major professors: Dr. A. G. Canaris for his helpful encouragement and advice at the beginning of this research, and Dr. Ivan Pratt for his guidance and suggestions during the writing of this manuscript.

Finally to my wife goes my grateful appreciation for her encouragement and the typing of this thesis.
# TABLE OF CONTENTS

I. Introduction ........................................ 1

II. Methods and Materials .......................... 3

III. Parasitic Nematodes .............................. 10

IV. Associated Nematodes ............................ 13

V. Culturing ........................................... 21

VI. Control Attempts .................................. 24

VII. Summary ........................................... 36

VIII. Bibliography .................................... 37

IX. Appendix ........................................... 41
NEMATODE ASSOCIATES
AND PARASITES OF THE DOUGLAS FIR BEETLE
DENDROCTONUS PSEUDOTSUGAE HOPKINS
WITH NOTES ON BIOLOGICAL CONTROL

INTRODUCTION

Much work has been done on the biology of the Douglas fir beetle, *Dendroctonus pseudotsugae* Hopk., (32, p. 156-167) mostly aimed at developing a method for control of this serious forest pest. Various methods of control have been tried, but none has been too successful, mainly because of the inaccessibility of the beetle. Work with nematodes of *D. pseudotsugae* has been done in British Columbia where Khan (13, p. 519-523; 14, p. 635-639; 15, p. 91-97) described three new species: *Sphaerularia hastata*, *S. unguilacauda* and *Ektaphelenchus macrostylus* which parasitize the beetles. The two *Sphaerularia* species have since been transferred to the genus *Stictylus* (16, p. 225-226) and then again to the genus *Sphaerulariopsis* (25, p. 218-223) where they remain at present. Other species of bark beetles in the United States and Europe have been investigated extensively for nematode parasites and associates (18, p. 14-24; 19, p. 29-34; 20, p. 14-22; 21, p. 95; 22, p. 67-75; 27, p. 221-242; 28, p. 1-437; 29, p. 200-213; 30, p. 437; 31, p. 131-144). Massey (20, p. 14-22) determined that the egg laying potential of *Ips confusus* was reduced 52 percent by the presence of *Aphelenchulus elongatus*. Work on the Japanese beetle by Girth, *et al.* (8, p. 1-29) has shown
that an internal nematode parasite, Neoaplectana glaseri, can be used as a biological control with up to 85 percent success.

The above mentioned research has led to the opinion that perhaps a nematode control for D. pseudotsugae could be discovered. With this in mind as a long-range goal, a study of the nematode parasites and associates of this beetle was undertaken. In this thesis, the nematodes that were found to be parasites of, or associated with, the Douglas fir beetle are listed, and work with nematodes as controlling agents of the beetles is described.
METHODS AND MATERIALS

The material used in this study was all collected from a relatively small area in the Coast Range mountains of Oregon about 20 miles west of Corvallis. "Blow-down" Douglas fir (Pseudotsugae menzisei) logs were located and examined for Dendroctonus galleries which appeared normal and in use. These galleries were located by finding patches of "frass" in the bark crevices which indicate an opening where the beetles have cleared out the gallery by pushing the "sawdust-like" material to the outside. The bark was then removed with an ax and chisel, and the inner bark containing the galleries was carved or stripped away from the heavy outer bark and placed in plastic bags. If the nematodes were to be counted, the entire gallery was taken back to the laboratory; however, usually only a portion of the gallery was collected. The trails of "frass" left on the wood under the bark were also carefully scraped off and placed in plastic bags. The larvae and adult beetles found were also collected in vials for later examination.

At the laboratory the carved-off galleries were either examined directly with a dissecting binocular microscope or placed in tap water in glass dishes and soaked overnight, usually under refrigeration. The larger pieces of bark were then removed, and the excess water was decanted off. The residue was then pipetted into petri
dishes for examination.

It was found that the nematodes could best be concentrated with very little frass material to obscure the examination if the bark material was first placed in two layers of gauze or placed on fine mesh copper screening in a funnel filled with non-chlorinated water. The nematodes crawled through the mesh and sank through the water into a glass vial affixed to the bottom of the funnel; the rubber connection between the vial and the funnel can then be clamped off, the vial removed, and emptied into a petri dish for microscopical examination.

The preliminary examination was done under a binocular dissecting microscope. When a nematode was observed, it was removed with a probe made of a bamboo splinter or a hair glued onto a probe handle. Once transferred to a slide, the nematodes were heated over a flame (overheating badly distorts the specimens) or hot plate. The cover glass had to be supported with glass rods so that the nematode would not be crushed. The cover glass was then ringed with either candle wax, for very temporary mounts, or Zut, a commercial ringing compound, if a more permanent mount was desired. All this was necessary since all of these nematodes must be examined under oil immersion. If a count of the nematodes in a gallery was to be made, the residue material of an entire gallery was spread out in a large enamel pan which was marked off in numbered squares. Sample
squares were withdrawn at random, using a device of either clay or rubber which would enable the total residue covering the one inch square to be removed. The total number of nematodes was then tabulated; by calculation, the approximate total number of nematodes in the gallery could be estimated. Due to the steps involved in preparing these slides for counting, only enough was done to obtain a rough estimate of the nematode populations in the galleries.

The nematodes found in the space beneath the elytra of the beetles (referred to in this paper collectively as sub-elytral nematodes) were obtained by removing the elytra and washing them off the beetle into a petri dish containing water. Adults, pupae, and larvae were all examined for internal parasitic nematodes by dissection of the entire body under a dissecting microscope.

The specimens of nematodes collected for the survey samples were either examined in temporary water mounts, preserved in two to four percent formalin, or killed, fixed, and prepared for permanent mounting in desiccated glycerin.

Limited culturing of naturally occurring nematodes was conducted with several species on agar plates. Although Massey (18, p. 22) had raised Parasitorhabditis obtusa on malt agar, this was not used in the present study. Fresh, inner bark and plain agar served well. Initially the bark was ground quite fine and after mixing with sugar and agar was sterilized at 15 pounds pressure for 15 minutes.
These plates did not grow the nematodes well and were shortly discontinued in favor of a much more coarsely ground bark plate containing no sugar.

Most of the cultures were general, containing all the nematodes found on or in the body of the *D. pseudotsugae* adults. These cultures were started by placing live *D. pseudotsugae* adults on the bark-agar plates and allowing the nematodes to leave the beetle and develop as they did in the galleries in the field.

Cultures were also readily started by concentrating nematodes by the funnel method previously described and pipetting them onto a fresh bark-agar plate. Several good cultures were also obtained by making regular bark-agar plates, allowing fungi to develop, autoclaving the plates, then pipetting on the nematodes. All cultures were kept at room temperature in normal lighting.

The direct attempts at biological control were carried out using two unnamed nematodes obtained from Dr. S. R. Dutky of Beltsville, Maryland. These nematodes are designated by Dutky as DD136 and DD517 and probably belong to the genus *Neoplectana*, being similar to *N. glaseri* (12, p. 16-17). These nematodes can be reared in large numbers using the techniques of Glaser, *et al.* (9, p. 614-615; 10, p. 1-34; 11, p. 512-514).

For the study of these nematodes, shipments of approximately five million second stage ensheathed larvae were obtained from
Dutky in thermos bottles containing an atmosphere of oxygen. The nematodes survived the trip quite well and were placed in aerated flasks in a 16°C coldroom where they remained alive and infective for at least a month.

DD136 and DD517 nematodes are normally either reared in Galleria sp. (wax moth) hosts or on nutrient agar medium consisting of beef kidney, agar, and other constituents.

The procedure for rearing them in Galleria sp. larvae is as follows: Approximately 50 larvae are placed on filter paper dampened with two ml. of water in a petri dish. About four drops of the larval nematode suspension are also placed in the petri dish, usually before the wax moth larvae are inserted. The larval nematodes can be seen crawling over the surface of the wax moth larvae; however, no actual penetration of the cuticle was ever observed. The nematodes probably either enter through the host's cuticle or via the spiracles. The nematodes are not normally ingested since the non-feeding pupae are also infected, usually to a greater degree than the larvae.

After one day a few nematodes were observed in the coelom and fatty tissues of the Galleria larvae. The larvae start dying after one day and are almost all dead by the second and third day after exposure. After about one week, the nematodes molt and metamorphose into adults, and soon the females begin producing eggs which are retained within the body until they have hatched into larvae. The
females quickly become nothing more than "sacks" of larvae which obliterate all the internal organs of the moth. Soon the female nematode bursts, and the larvae are released, probably as second stage ensheathed forms. The nematodes can apparently pass through more than one generation in a host and probably develop generation after generation until the host tissue is consumed.

After about one and one-half to two weeks, the second stage nematodes can be "trapped." This is accomplished by wrapping a block of wood, plastic, etc. in filter or thin blotting paper and placing it in a petri dish. The dish is then filled with dechlorinated water up to within about one-sixteenth of an inch from the top of the "platform." The dead Galleria larvae are then placed on this platform where they are dampened by the water in the filter paper. Soon the nematode larvae can be seen pouring out of the host in slow moving streams visible to the unaided eye. After approximately 12 hours, thousands of larvae can be poured out of the petri dish into a flask which is then either aerated or refrigerated. This procedure can be repeated with the same larval hosts until their supply of nematodes is exhausted. If the culture is allowed to develop over two weeks, the adult nematodes will emerge with the ensheathed larvae. This is not desirable since the adults soon die and decompose, contaminating and killing the more resistant ensheathed stages.

This method of application was used with all the preliminary
work done on *D. pseudotsugae* adults, pupae, and larvae to determine the lethal effect of the DD136 and DD517 nematodes on these beetles.

Crude attempts at control of the beetle populations while still encased beneath the host tree bark were carried out simply by pouring a suspension of these nematode larvae over the bark of a log that was observed to contain a good population of the beetles.
PARASITIC NEMATODES

During the survey for nematodes occurring in the galleries, Dendroctonus pseudotsugae—adults, pupae, and larvae—were examined for parasitic and symbiotic nematodes. The symbionts, which are considered by Rähm (28, p. 1-437) as "semi-parasitic," were not identified. Therefore, these nematodes which occurred mainly in the sub-elytral space are referred to collectively as sub-elytral nematodes.

Contortylenchus reversus (Thorne 1935) Rähm 1956

Syn. Aphelenchulus reversus Thorne 1935

(Plate I, Fig. 1)

Only one truly endo-parasitic species was found, that being Contortylenchus reversus (Thorne, 1935) Rähm, 1956. This nematode was described as Aphelenchulus reversus by Thorne in 1935 (31, p. 132-134); however, Rähm (28, p. 437) considered this and other "contortus" type of nematodes to be distinct from the type species Aphelenchulus mollis Cobb, 1920, and therefore erected the genus Contortylenchus in 1956. Nickle (25, p. 218-223) studied specimens and drawings of Aphelenchulus mollis from Cobb's 1917 collection and determined that A. mollis is not a "contortus" type of nematode but considered Contortylenchus to be a valid genus including all other
species of *Aphelenchulus* thus far described.

The following description is taken from Thorne (31, p. 133-134):

**FEMALES FROM GRUBS AND ADULT BEETLES**

Length 1.0 to 1.8 mm; width 50 to 180 μ. Vulva 94 to 96 percent. Body bent dorsally, more or less cylindrical throughout greater part of its length but tapering conspicuously at the very narrow lip region, which is not set off in any manner. Cuticle annulated near the head and at the terminus; on some specimens annulus conspicuous, on others almost invisible. Body constricted at vulva, especially ventrally. Tail broad, dorsal, hornlike, annulated terminal projection which actually is the upturned original tail of the immature nema.... The four labial papillae almost invisible even from a face view. The amphids lie close to oral opening. Four large glands are prominent feature of head region. Spear 12 to 14 long, slender, with short ventrally located aperture. Knobs of the spear vary from obscure to distinct. Lumen of esophagus can be traced only a short distance from the spear. A series of 15 to 18 pairs of conspicuous lateral structures distributed throughout the body. Vulva a broad transverse slit. Three glands lie opposite vulva, causing constriction of the organs. Anus and rectum absent. Ovary extending forward about three-fourths the length of body, then reflexed a distance equal to 1 to 2 body lengths. Oviparous...

Massey (18, p. 18) also found and described the male of this worm from the frass of both *Dendroctonus engelmanni* Hopk. and *Dendroctonus terebrans* Oliv. No males were found during my work with *D. pseudotsugae*. Other hosts in which *C. reversus* has been found include: *Dendroctonus borealis* Hopk., *D. ponderosae* Hopk., *D. monticola* Hopk., *Ips pilifrons* Sw., and *I. borealis* SW. (18, p. 20). Massey (18, p. 20) considered this to be
a common parasite of the genus *Dendroctonus*, probably occurring in all species. *C. reversus* was found only in the adult *D. pseudotsugae* contrary to the condition in *D. engelmanni* where the worm occurred in all stages except the egg (18, p. 20).

The results of the examinations for these parasitic and symbiotic nematodes are tabulated in Table I.

**TABLE I**

<table>
<thead>
<tr>
<th></th>
<th>No. of D. pseudotsugae exam.</th>
<th>No. with sub-elytral nematodes</th>
<th>% with sub-elytral nematodes</th>
<th>No. with Aphelenchulus</th>
<th>% with Aphelenchulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>308</td>
<td>213</td>
<td>69.1</td>
<td>17</td>
<td>5.5</td>
</tr>
<tr>
<td>Larvae</td>
<td>213</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pupae</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

As can be seen, the percentage of *D. pseudotsugae* adults carrying sub-elytral nematodes is quite high. In some populations it approaches 100 percent; however, this study was primarily confined to the Mary's Peak, Corvallis area and may not indicate the infestation rate over the entire range of *D. pseudotsugae*. The parasitic *Contortylenchus* adults are not found in great numbers, usually only two to three per beetle. Larvae of this species were also observed in the body cavity of the beetles.
ASSOCIATED NEMATODES

Only five nematodes have been found associated with Dendroctonus pseudotsugae in this area. They have all been isolated in various developmental stages from the galleries and from the beetles themselves, either being under the elytra or on other parts of the body.

Parasitorhabditis obtusa (Fuchs 1915) Dougherty 1953

Syn. Rhabditis obtusa Fuchs 1915

(Plate I, Fig. 2, 3, and 4)

Many Parasitorhabditis obtusa were found in the frass of every beetle gallery examined. They were usually the most abundant of all the nematode associates. According to Massey (18, p. 22) the larvae of this species occur parasitically in the gut of D. pseudotsugae and is probably cosmopolitan in distribution. This species was found very commonly in the galleries, both as larvae and adults. The larvae were also found in the gut of the insect.

The following description is given by Thorne (31, p. 142):

Female = 0.8 - 1.1 mm; a = 25, b = 5, c = 5.5, v = 64/95
Male = 0.6 - 0.8 mm; a = 30, b = 4.5, c = 23, t = 60

Bodies of both sexes almost cylindrical between esophagus and genital opening. Neck tapering uniformly to lip region, which is about one-third as wide as base of neck. Female tail short, bluntly conoid. Vulva exceedingly far back. Striae about 1µ wide at mid body, slightly wider near head. Lip
region almost continuous with neck contour. Six conical, forward-pointing, labial papillae were visible but other details of head were always obscured by clinging debris. Amphids minute. Pharynx about three times as deep as wide. Cheilorhabdions and proto-rhabdions slightly convex. Telostom absent. Esophagus: Corpus cylindrical; medial bulb slightly wider than corpus; isthmus same length as corpus and half as wide; terminal bulb ovate, two-thirds as wide as neck. Nerve ring midway of isthmus. Excretory pore slightly posterior to nerve ring. Female prodelphic. Vulva elevated. Vagina extending almost straight forward. Uterus one-third as long as body. Ovary extending forward from uterus, then reflexed until the blind end reaches one-half to three-fourths the distance back to the vulva. Posterior uterine branch absent. Testis single, extending nearly to esophagus, then reflexed a short distance. Spicula and gubernaculum as shown (Fig. 4). Bursa enveloping the tail, with 2 pairs of preanal ribs, then 3 pairs grouped close together just posterior to anus, followed by 4, rarely 3 or 5, pairs; general bursal formula being 2(3)1,1,2.

In addition to being associated with D. pseudotsugae, Massey (18, p. 22) lists the following associated host beetles: D. ponderosae, D. monticola, D. engelmanni, D. borealis, Ips pilifrons, and I. borealis.

Mikoletzkya pinicola (Thorne 1935) Baker 1962

Syn. Diplogaster pinicola Thorne 1935

Fuchsia pinicola (Thorne 1935) Paramonov 1952

(Plate I, Fig. 5 - 7)

This large nematode was found to be the second most abundant species, at times even more abundant than P. obtusa. The following
description is in part from Massey's 1956 paper (18, p. 23):

Female = 1.0 mm; a = 25, b = 7, c = 15, v = 20/51/22
Male = 1.0 mm; a = 30, b = 6.2, c = 15, t = 52

Cuticle finely annulated, finely striated; body moderately slender, tapering gradually toward the anterior end; pharynx bearing six visible teeth; isthmus with terminal bulb slightly longer than corpus with median bulb; nerve ring midway of the isthmus; excretory pore slightly posterior to the nerve ring; vulva with protuberant lips; female tail convex, conoid with an acute terminus; male tail curved with a spicate terminus; spicula arcuate; gubernaculum with a trough-like distal extension in which the spicula glides; testes reflexed, single; 8 pair of caudal papillae.

The larvae of this species have been observed in large masses in the beetle's sub-elytral space. Other associate hosts have been listed by Massey (18, p. 23) as: *D. engelmanni, D. ponderosae, D. monticolae, D. borealis, D. brevicomis Lec, Ips pilifrons,* and *I. borealis.

Plectonchus sp.

(Plate II, Fig. 1 and 2)

According to Massey¹ this is probably a new species of nematode which occurs infrequently in the galleries. Usually this nematode is one of the most frequent in the general cultures.

A tentative description is given here:

Female = 0.67 mm; a = 28.1, b = 4.5, c = 15.3, v = 82.2%
Male = 0.67 mm; a = 27.2, b = 4.7, c = ?, t = 73.2%

¹ Personal communication from Dr. Calvin L. Massey, USFS, Albuquerque, New Mexico, December 2, 1963, by letter.
Cuticle with very fine annulations; neck tapering to the lip region. Female tail pointed, slightly curved backwards with a small swelling near the tip. Prominent vulva near posterior. Buccal cavity about as wide as deep. Esophagus cylindrical, with a single terminal oval bulb containing a valve. Nerve ring midway of isthmus. Female monodelphic, ovary once reflexed. Vulva elevated strongly. Uterus extending anteriorly, ovary reflexed once, backward to a point a little over midway between vulva and anterior part of ovary. Testis single, extending nearly to the esophagus. Spicula and gubernaculum as in figure 1. Four pair of caudal papillae.

The larvae of this species occur under the elytra of *D. pseudotsugae*. Both the male and female adults are found in the galleries of the beetle.

This species has apparently been recovered only from the galleries of *D. pseudotsugae* in Oregon.

*Panagrolaimus judithi* Massey 1964

(Plate II, Fig. 3 - 5)

This nematode which occurred rather rarely in the galleries examined, has recently been described by Massey (23). The following description is from his paper.

Female (6): 0.75-0.78 mm; a = 17, b = 5, c = 8, v = 58%
Male (4): 0.64-0.70 mm; a = 20, b = 4.4, c = 13
FEMALE: Cuticle with fine transverse and longitudinal striations. Lips distinct, each with a minute apical papillae. Depth of pharynx and width of head about equal. Cheilostom about one-half the depth of prostom, the cheilorhabdions distinct, convex in lateral view; meso, meta, and telostom fused, joined directly to lumen of esophagus. Corpus of esophagus equal in length to isthmus and terminal bulb combined; terminal bulb valvate. Nerve ring at middle of isthmus. Excretory pore adjacent to anterior end of terminal bulb. Hemizonid immediately anterior to excretory pore. Ovary single, reflexed, its terminus extending beyond the anal opening. Vulva transverse, lips protuberant. Uterus with stored sperm cells at the anterior flexure. Tail elongate, conoid; terminus subacute.

MALE: Testis single, reflexed, one to two body widths. Spicules paired, cephalated, ventrally arcuate. Gubernaculum one-third the length of the spicules. Three pair of caudal papillae: 1 preanal ventro-submedian, 2 postanal ventro-submedian, 3 postanal ventro-submedian. Tail elongate, terminus spicate, subacute.

In addition to *D. pseudotsugae*, *P. judithi* has been found associated with *Dryocoetes confusus* Sw. in subalpine fir, *Abies lasiocarpa* var. *lasiocarpa* (Hook.) Nutt.

_Ektaphelenchus obtusus_ Massey 1956

(Plate II, Fig. 6 and 7)

This nematode was found in most of the galleries examined, usually occurring in small numbers. The following description is from Massey's 1956 paper (18, p. 20-21):

Female: 0.8 mm; a = 30, b = 8, c = ?, v = 41/78
Male: 0.7 mm; a = 23, b = 7, c = ?, t = 34
Cuticle with moderately fine annulations; lip region flattened, definitely set off; face view reveals four prominent labial papillae and the amphids which are similar in size and shape to the papillae; spear moderately slender, three times as long as the width of lip region, without basal knobs; esophageal bulb ovate, elongate, approximately twice as long as wide; esophageal glands very prominent, elongate, extending dorsally several body widths along anterior end of intestine; nerve ring one bulb length behind bulb; ovary outstretched, posterior uterine branch very short; anus and rectum not observed; tail length undetermined; lumen of intestine broad and conspicuous throughout its length; tail convex conoid, almost blunt; male similar in shape and conformation to that of female; testes short, not reflexed; spicula mitten-shaped; three pairs of prominent caudal papillae, one pair preanal, two pairs postanal.

_E. obtusus_ was originally described from _Dendroctonus engelmanni_. It has also been collected from _D. pseudotsugae_ in New Mexico and _D. borealis_ in Alaska.

This nematode resembles _Ektaphelenchus macrostylus_ Khan (15, p. 91-93) described from _D. pseudotsugae_ in Canada. The differences listed by Khan are: a notched spicule, knobs on the stylet, and one pair of caudal papillae. This nematode has a notched spicule, resembling that of _E. macrostylus_ however; it was identified as _E. obtusus_ by Massey¹.

The total number of free-living nematodes encountered in the galleries is only roughly known. Counting methods have yielded as

¹ Personal communication by letter from Dr. Calvin L. Massey dated December 2, 1963, Albuquerque, New Mexico.
high as 26,000 *Parasitohabditis obtusa*, a high percentage of which were larvae, in a short (ten inch) gallery. This gallery had many fewer of the other species. It contained only about 2,000 unknown larvae, no *Mikoletskya pinicola* were found, and no *Ektaphelenchus obtusus* were present. *M. pinicola* and *P. obtusa* were usually present in similar numbers; however, *P. obtusa* was the most common form of all. The results of the counts are tabulated here.

**TABLE II**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Approximate Number Per Gallery</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>P. obtusa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult male</td>
<td>4860</td>
<td>720</td>
</tr>
<tr>
<td>Adult female</td>
<td>4320</td>
<td>720</td>
</tr>
<tr>
<td>Immature</td>
<td>16900</td>
<td>2500</td>
</tr>
<tr>
<td><em>M. pinicola</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult male</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adult female</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Immature</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. obtusus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult male</td>
<td>180</td>
<td>540</td>
</tr>
<tr>
<td>Adult female</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Immature</td>
<td>1080</td>
<td>720</td>
</tr>
<tr>
<td><em>P. judithi</em> and <em>Plectonchus sp.</em></td>
<td>500</td>
<td>180</td>
</tr>
<tr>
<td>Unknown Larvae</td>
<td>2600</td>
<td>180</td>
</tr>
</tbody>
</table>
All of the galleries included in Table II were about 10 to 12 inches. P. obtusa appears to be universally distributed in all galleries that have been quantitatively examined. M. pinicola has been present in a greater number of the galleries than is indicated in Table II, due to the fact that most of the galleries were not examined in a quantitative manner.
CULTURING

All of the nematodes mentioned in this thesis except the endoparasitic Contortylenchus reverus have been cultured. Parasitorhabditis obtusa was the easiest to culture; Mikoletskya pinicola and Panagrolaimus judithi also grew well on the bark-agar medium. Most of the cultures were general, i.e., including all the nematodes available. Pure cultures of P. obtusa and M. pinicola were developed, both reproducing in large numbers.

Adult beetles also have survived quite well on the bark-agar plates. One of the first successful cultures was found to have one of the four beetle adults alive after 65 days. The beetles tunnel down into the medium and build galleries similar to those found in the logs.

The first general culture plate examined for approximate numbers of nematodes was found to contain the following numbers of nematodes previously listed.

<table>
<thead>
<tr>
<th>Nematode Type</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panagrolaimus judithi larvae</td>
<td>720</td>
</tr>
<tr>
<td>Plectonchus sp (all stages)</td>
<td>6300</td>
</tr>
<tr>
<td>Mikoletskya pinicola (juvenile)</td>
<td>360</td>
</tr>
<tr>
<td>Unidentifiable (dead, distorted, etc.)</td>
<td>1080</td>
</tr>
<tr>
<td>Approximate total</td>
<td>8460</td>
</tr>
</tbody>
</table>

This culture was allowed to develop for 63 days after four live adult D. pseudotsugae were placed on it. Therefore, all the nematodes present came from some place on or in the beetle.

As can be seen from the data, there were a large number of
Plectonchus sp. and relatively few other species. No P. obtusa were observed in counting samples of the previously mentioned culture; however, a pure culture of P. obtusa was maintained for 62 days. The nematodes were washed off the plate, and 4,500 to 5,000 P. obtusa in all stages of development were counted. The initial inoculation was 20 P. obtusa, therefore at least a 150-fold increase was obtained. Other cultures examined yielded large numbers of M. pinicola nematodes, a few Ektaphelenchus obtusus, and a few Panagrolaimus judithi. Therefore, all of the free-living adult nematodes have been cultured.

Apparently Parasitorhabditis obtusa is not carried under the elytra as are the other species, since it was not recovered from cultures started using the dissected elytra. It is probably carried elsewhere on the body or as larvae in the intestine of the adult beetles.

Since all the described nematodes have been recovered from the sub-elytral space or from the body itself, they are therefore most likely transported from tree to tree in this manner. If this is the only way these nematodes get into the galleries, they can be considered useless as vectors of a controlling pathogen; however, this work is by no means conclusive. It is possible that some of these nematodes also enter the galleries in other ways.

From the data available from this study, it appears that the general cultures eventually become dominated by one of the species
of nematodes. *P. obtusa* and *M. pinicola* apparently can develop at almost equal rates in the same culture; however, the *Plectonchus* sp. develops rapidly and can take over a culture if it is inoculated in sufficient numbers at the beginning. The only nematode that did not become dominant in any of the general cultures was *E. obtusus*.

Most of these nematodes, except *E. obtusus*, reproduce at rates sufficiently high to make them practical in biological control work if a method of useful application could be developed.
CONTROL ATTEMPTS

Upon obtaining *Dendroctonus pseudotsugae*, experiments were conducted to determine the lethal effects of their exposure to the DD136 nematode. The initial test of the effectiveness of DD136 to inject the *Dendroctonus* beetle was carried out in the same way as previously done with the *Galleria* larvae. Five pupae and three adult *Dendroctonus* were placed on dampened filter paper along with the four drops of nematode larvae suspension. (Each drop contained between 200 and 300 nematode larvae, depending upon the concentration of the suspension.) After six days they were examined and found to be infected as indicated in table III.

**TABLE III**

<table>
<thead>
<tr>
<th>BEETLE</th>
<th>DD136 INFECTION</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupa #1</td>
<td>One female, no larvae</td>
<td>Dead</td>
</tr>
<tr>
<td>Pupa #2</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>Pupa #3</td>
<td>Several adults with larvae</td>
<td>Dead</td>
</tr>
<tr>
<td>Pupa #4</td>
<td>Several adults with larvae</td>
<td>Dead</td>
</tr>
<tr>
<td>Pupa #5</td>
<td>Several adults with larvae</td>
<td>Dead</td>
</tr>
<tr>
<td>Adult #1</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>Adult #2</td>
<td>Several dead ensheathed larvae, no adults</td>
<td>Dead</td>
</tr>
<tr>
<td>Adult #3</td>
<td>Several male and female adults, eggs of female not mature</td>
<td>Dead</td>
</tr>
<tr>
<td>TOTALS</td>
<td>Six infected, two negative</td>
<td>Six dead, two alive</td>
</tr>
</tbody>
</table>

Twelve wax moth larvae were exposed to the same dosage using the same nematodes, i.e., original culture from Dutky,
49 days after arrival from Maryland, and kept aerated at 16°C. It was found that ten of them had the normal heavy infections while two had only a light population of nematodes. This suggests a possible decline in the infectability of the nematodes during this period.

Since the susceptibility of *Dendroctonus* to the nematode was reasonably well established in the first attempt, a "time development study" was undertaken to determine the length of time it takes the DD136 to complete its life cycle in the bark beetle. Therefore, to accomplish this, seven petri dishes each containing 20 *Dendroctonus pseudotsugae* larvae were prepared in the usual manner with four drops (800 - 1200 nematodes) of DD136 suspension which had been stored for seven days with aeration at 16°C. All the *Dendroctonus* larvae were healthy specimens mostly in the prepupal stage. All were extracted from the same log.

The dishes were examined at daily intervals to determine the extent of development of the nematodes up to the time of dissection. The results were as follows:
<table>
<thead>
<tr>
<th>Days of exposure</th>
<th>Dead</th>
<th>Alive</th>
<th>No.</th>
<th>% Inf.</th>
<th>% Dead</th>
<th>Stage of Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>7</td>
<td>13</td>
<td>65</td>
<td>65</td>
<td>Ensheathed larvae mostly about double entry size</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>1</td>
<td>18</td>
<td>95</td>
<td>95</td>
<td>Mature adults, one with eggs in uterus</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>100</td>
<td>Twelve gravid females, nemas with few to many eggs</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>Females with eggs, to hatched larvae, one with many larvae</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>Females all with larvae, some only incubation sacks, many hatched larvae</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td>100</td>
<td>100</td>
<td>Many free larvae about double hatching size, most female nematodes disintegrated</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A few ensheathed larvae</td>
</tr>
</tbody>
</table>

**TOTALS:**
- **Dendroctonus larvae examined** . . 108
- **(12 dried up)**
- **Dead (100 infected)** . . . . . . . 100
- **Alive** . . . . . . . . . . . . . . 8

Several of the smaller *Dendroctonus* larvae dried up, apparently from lack of moisture before it was time to dissect them. Since the larvae of corresponding size in the control dishes also dried up, it was assumed that the drying up was not due to the effects of the parasitism. Several of the dried specimens were examined, however,
and found to contain nematodes. The filter paper was dampened nearly every day but did not seem to retard the desiccation of the smaller beetle larvae. The much larger prepupae survived the seven day test quite well in both the controls and the dead parasitized specimens.

As can be seen from Table IV, 13 of the 20 beetle larvae were already infected and dead after one day. Apparently only one nematode in the body cavity is needed to cause death. This would seem to correlate the "potency" of the lethal organism with body size since the wax moth larvae, which are larger, did not start dying until about 48 hours after exposure to infection. At any rate one nematode carries enough bacteria into the hosts examined in this study to eventually kill it. This hypothesis was verified in a later experiment where about 50 Dendroctonus larvae were placed in petri dishes and exposed to infection as before. After about 30 minutes, however, the larvae were all removed to clean petri dishes with dampened filter paper but no DD136. Therefore, upon examination, many were found to be infected with only one nematode. Those so infected died almost as quickly as others more heavily infected while many that had not yet been invaded by the nematodes lived for about ten days, about as long as control prepupae will live under the same conditions.

Six of the thirteen beetle larvae infected at day "one" were found to be invaded with more than 50 nematode larvae, the rest
containing between one and ten; all were dead. The number of nematodes per larvae remained about the same until the second generation of larvae was released by the parent.

At day "two" the adult nematodes were mature, males with spicules were seen and one female was found with eggs in the uterus. Many ensheathed larvae were found which had either recently entered or had not developed beyond the larval stage.

The day "three" beetle larvae contained mostly mature females and males, the females containing eggs in the two to eight cell stage.

By day "four" the entire uteri of the females were packed with eggs in all stages of development up to the larval stage. A few of the larvae had apparently hatched but might have been pressed out of the female upon dissection.

Day "five" females were for the most part nothing more than squirming "sacks" of larvae. All of the internal organs of these females were obliterated. Several of the females had begun to break up and release the larvae into the coelom of the insect.

Some of the six day larvae were apparently maturing into a new generation of adults. Several males were seen with the spicule in evidence. This would seem to indicate that these nematodes will go through more than one complete cycle if the host has sufficient food material to support the parasites.

On the seventh day some of the smaller beetle larvae were
seen releasing ensheathed larvae. The filter paper had no excessive moisture, such as caused the "traps" to function; therefore, it was hypothesized that the nematodes had run out of room and were leaving the spent host in their resistant ensheathed stage because of the lack of space and food. No other evidence of this was observed, however.

It can be stated, therefore, that this species of nematode, DD136, completes its life cycle in as little as six and one-half to seven days and can be trapped from the bark beetle larvae after this time. This study was carried out at room temperature, about 23°C. Nothing was done with respect to the effect of various temperatures on the length of time development. It was assumed that the cycle would be longer in colder environments.

This preliminary work with the bark beetle larvae in petri dishes was mainly concentrated on DD136; however, similar experiments were carried out with DD517. The results obtained with DD517 were almost identical with those of DD136. All the trials yielded 100 percent infection and death with the Dendroctonus larvae, pupae, and adults exposed to it. No time development study was carried out with DD517; however, it was assumed to be closely similar to that of DD136.

The attempts made to produce infections of the bark beetles while enclosed in their galleries protected by the bark have all been negative; however, a discussion of methods used might be useful to
other investigators.

The first attempt at this was a field experiment. Logs were found which had a good population of bark beetles. Nematode larvae carried in flasks were poured upon the bark, the logs were marked, and the bark was later dissected (after about one and one-half to two weeks). None of the hundreds of larvae, pupae, or adults examined had any DD136 or DD517 nor were any of these nematodes found in the galleries. This was admittedly a rather haphazard initial trial, the nematodes being applied to marked off areas on the log with no regard to presence of air or frass-elimination holes made by the beetles. It was also quite warm and dry; and even though the galleries were quite moist, the nematodes probably did not survive a great length of time.

After this, similar studies were attempted in the lab. Several logs experimentally infested with bark beetles were "saturated" with the aqueous nematode suspension as was done previously in the field. The same negative results were obtained. Three other infested logs were first dampened extensively with water before the nematodes were poured on. The same negative results were obtained. Still other infested logs were used, placing the DD136 larvae in depressions carved through the bark down to the cambium at places where "beetle holes" were observed. In this latter experiment two beetle larvae were observed to poke their heads out of their tunnels into the carved depression which had been saturated with DD136 larvae.
Eight days later they were both dead with their bodies literally packed with nematodes. The nematodes had not, however, migrated from the place where the beetle larvae were observed. At the outset of this research project it was hypothesized that if these nematodes once got into the galleries they would rapidly spread throughout the whole system. This does not seem to be the case, probably because of the frequent barriers of frass packed into the galleries. Several of the infested logs were "injected" with DDl36 to test this. This "injection" was carried out with a drawn out pipette which was inserted into the entry and air holes. The solution was pushed in and the surrounding area was saturated. The same negative results were obtained; apparently the nematodes won't or can't navigate the galleries to any great degree. Only one larvae out of about 200 bark beetle larvae, pupae, and adults observed was found infected, and it was only about one inch from the site of injection.

This phase of the work was more or less abandoned at this point to wait for rainy weather which was hoped would keep the nematodes alive longer and allow them more time to find their way down the holes the beetles had made.

In late September and early October, 15 Douglas fir logs were brought into the lab and experimentally infested with *Dendroctonus pseudotsugae*. The infested logs were kept in the lab until November 26, 1962 when they were taken out and placed in an exposed place
about one-third of the way up Mary's Peak, Benton County, Oregon. The logs were separated into three groups--five control logs to remain free of DD136, five logs in group one to be dissected in late December after application of DD136, and five logs in group two to be dissected in April.

The logs were allowed two days to acclimatize, during which time they were rained upon at least twice and then saturated with about five million DD136 ensheathed larvae obtained from Dr. C. G. Thompson. This would be roughly 300,000 larvae per log. This large number was used to insure penetration if it were going to occur at all. The nematode solution was poured slowly and carefully in all cracks and crevices of the logs to insure good survival of the nematodes. After returning to the lab, the nematode stock was checked and found to be alive after the trip.

On December 18, 1962 the five logs in group one plus one of the control logs were brought into the laboratory. This had been after an exposure to the DD136 of 48 days. The experimental logs were debarked and examined for dead beetles; all dead specimens were examined first as was the area of the gallery in which they were laying. The live beetles were examined secondly since it was assumed that they were not infected anyway. And lastly the bark which was soaked overnight was examined for DD136 in the galleries. The total number of Dendroctonus examined was tabulated in Table V.
Included in the number of dead *Dendroctonus* are those which were smashed upon debarking the logs since it was not known if they were already dead prior to debarking. The only nematodes found in the bodies of the dead insects were not DD136. Three of the dead adults harbored *E. obtusus* which was not observed to be parasitic. Two dead larvae contained two *P. judithi*.

**TABLE V**

<table>
<thead>
<tr>
<th>Log</th>
<th>Adults</th>
<th>Dead</th>
<th>Larvae</th>
<th>Dead</th>
<th>Pupae</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>1</td>
<td>34</td>
<td>10</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>117</td>
<td>18</td>
<td>13</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>88</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>374</td>
<td>29</td>
<td>58</td>
<td>20</td>
<td>49</td>
</tr>
</tbody>
</table>

| Average Per Log | 74.8 | 11.6 | 9.8 |

As can be seen from Table V, 481 *D. pseudotsugae* of various stages were examined after their respective host-logs had been exposed to infestation of DD136 nematodes for 48 days under naturally occurring conditions. No DD136 were found in the larvae, pupae, or adult bark beetles, either dead or alive. Also no DD136 were found in the galleries after extensive searching.

In April the second group of logs was brought in from the field
site and examined as before. The results are found in Table VI.

**TABLE VI**

<table>
<thead>
<tr>
<th>Log</th>
<th>Adults</th>
<th>Dead</th>
<th>Larvae</th>
<th>Dead</th>
<th>Pupae</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>92</td>
<td>79</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Average Per Log 18.4

As the table indicates, most of the beetles were dead. However, the high moisture content of the logs and the coldness of the weather kept them in excellent shape. All were examined for DD136 and found to contain none. The galleries were examined extensively but nothing except the normally associated nematodes were found.

Admittedly the evidence thus far is not conclusive; however, it is my opinion that the DD136 larvae will not penetrate into the galleries of these bark beetles when applied to the bark in the manner described.

As an alternate hypothesis, it may be suggested here that perhaps it would be possible to infect the adult bark beetles as they emerge from the galleries in the spring. This would no doubt require precise timing if, in fact, it could be accomplished at all. Infection
would depend on the amount the adult beetle moves about on the bark after emergence, upon the survival of the nematode after application, and upon application of the nematodes at the exact time of beetle emergence. If the bark remained moist during emergence, this means of infection might prove successful and either DD136 or DD517 might be used. Other than this, no practical way of using DD136 or DD517 to control *Dendroctonus pseudotsugae* can at present be envisioned.
SUMMARY

Five nematodes were found to be associated with the Douglas fir bark beetle. All occur on or in the beetle in the larval stage and are therefore carried from tree to tree by the beetle. One endoparasitic nematode was found in five percent of the adult beetles examined.

All of the associated nematodes were readily cultured on a mixture of ground inner bark and agar.

Attempts at controlling the bark beetles with nematode DD136 obtained from Dr. S. R. Dutky of Beltsville, Maryland were carried out. Experimentally the bacterium carried by the nematodes into the beetles was very lethal to them; however, all field tests proved negative because the nematode could not reach the beetles while they were in their galleries beneath the bark.


PLATE I

Figure 1. Adult female Contortylenchus reversus.  
(After Nickle, 24, p. 261)

Figure 2. Parasitorhabditis obtusa, anterior end.  
(After Thorne, 31, p. 142)

Figure 3. P. obtusa, female posterior end.  
(After Thorne, 31, p. 142)

Figure 4. P. obtusa, male posterior end, showing bursa.  
(After Thorne, 31, p. 142)

Figure 5. Mikoletzkya pinicola, anterior end.  
(After Thorne, 31, p. 141)

Figure 6. M. pinicola, posterior end of female.  
(After Thorne, 31, p. 141)

Figure 7. M. pinicola, posterior end of male.  
(After Thorne, 31, p. 141)
PLATE II

Figure 1. Posterior end of male *Plectonchus* sp.

Figure 2. *Plectonchus* sp. female.

Figure 3. Anterior end of *Panagrolaimus judithi*.

Figure 4. Posterior end of female *P. judithi*.

Figure 5. Posterior end of male *P. judithi*.

Figure 6. *Ektaphelenchus obtusus* female.

Figure 7. Posterior end of *E. obtusus* male.