INSECT-NEMATODE-RED PINE ASSOCIATIONS IN WESTERN MARYLAND WITH MAJOR EMPHASIS ON BURSAPHELENCHUS XYLOPHILUS AND MONOCHAMUS SPP.

by
Amy Susan Litten Harman

Dissertation submitted to the Faculty of the Graduate School
of the University of Maryland in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
1985

Copy 1

APPROVAL SHEET

Title of Dissertation: Insect-Nematode-Red Pine Association in Western Maryland with Major Emphasis on *Bursaphelenchus xylophilus* and *Monochamus* spp.

Name of Candidate: Amy Susan Litten Harman
Doctor of Philosophy, 1985

Thesis and Abstract Approval: Lorin R. Krusberg
Dr. Lorin R. Krusberg
Professor of Plant Pathology
Department of Botany

Date Approved: August 7, 1985

ABSTRACT

Title of Dissertation: Insect-nematode-red pine associations in Western Maryland with major emphasis on Bursaphelenchus xylophilus and Monochamus spp.

Amy Susan Litten Harman, Doctor of Philosophy, 1985

Thesis directed by: Dr. Lorin R. Krusberg
Professor, Department of Botany
University of Maryland

Western Maryland red pines, Pinus resinosa Ait. were examined over three years, 1982-1984, to determine the distribution of Bursaphelenchus xylophilus, the pinewood nematode. Ca. 47-year-old (older) and ca. 25-year-old (younger) trees were subdivided into the following categories: (1) trees with mostly green needles; (2) trees with mostly reddish-brown needles; (3) trees with no needles but with bark intact; (4) trees with no bark; and (5) trees with chlorotic, bleached-green needles.

The pinewood nematode infected 76.5% of older red pines and 68% of younger red pines. The nematode was not evenly distributed in trees in any tree decadence category or by tree age. Bursaphelenchus xylophilus infected 15.1% of the samples from trunk, primary and secondary branches in older red pines and 18.5% in younger red pines. By tree decadence category older trees had the highest infection (25.2%) in green needled trees (category 1) whereas younger trees had the highest infection (28.7%) in bleached-green needled trees (category 5).

Trees collected in late May-early June 1982 lacked Monochamus rearings and belonged to categories 1, 2, 3 and 4. Trees from these categories had emergences of Ips spp., Tetropium schwarzianum Casey, Pissodes approximatus Hopkins and Otiorhynchus spp. in summer 1982. From bleached-green needled red pines (category 5) collected in late July-early August 1983 two Monochamus species emerged in 1984, M. carolinensis (Olivier) and M. scutellatus (Say), as well as other insects including Neacanthocinus pusillus (Kirby) and Amniscus collaris Haldemann. Chrysobothris scabripennis Cast. and Gory emerged from trees cut in 1983, but which remained uncaged in the field until 1984.

Bursaphelenchus xylophilus was present in 94% of tracheal systems of both M. scutellatus and M. carolinensis. Pinewood nematode was found infesting 4.2% of N. pusillus specimens. One specimen each of P. approximatus, Ips spp. and C. scabripennis were positive for B. xylophilus.

Dolichomitus tuberculatus tuberculatus (Geoff.), an ichneumonid parasite, was reared from Monochamus spp. larvae. Two deutonymph mites, Dendrolaelap isodentatus (Hurlbutt) and Trichouropoda hirsuta Hirschmann, were located externally on Monochamus spp. beetles.

ACKNOWLEDGMENTS

The author thanks Dr. Lorin R. Krusberg for his advice, assistance and encouragement throughout this project and in the preparation of this dissertation.

The author thanks Dr. William R. Nickle for his assistance, his willingness to help and his donations of materials and information in support of this project.

Appreciation goes to the following taxonomists from the Systematic Entomology Laboratory, IIBIII, United States Department of Agriculture who contributed to this study by identifying insects: P. M. Marsh, W. F. Barr, G. Steyskal, D. M. Anderson, J. M. Kingsolver, D. R. Whitehead, R. D. Gordon, T. J. Spilman, Department of Entomology, Smithsonian Institution and D. N. Kinn who identified the mites. Appreciation also goes to V. K. Gupta, Entomology and Nematology Department, University of Florida who identified ichneumonids and braconids.

Much appreciation goes to her two sons, Eric W. and Benjamin A., who assisted in cutting several trees, making insect cages and in doing chores around the house so that time could be spent on this dissertation research. The author's husband, Dan M., who tirelessly gave of his time and efforts to make this mission possible, is owed a great debt of gratitude.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

INTRODUCTION AND OBJECTIVES

I. Literature Review.

In 1934 Steiner and Buhrer first described the timber nematode or pinewood nematode as *Aphelenchoides xylophilus*. This nematode was collected from three localities in the United States: (1) a small piece of blue-stained wood from a green pole of longleaf pine harvested either from southeastern Texas or western Louisiana but observed in Orange, Texas, in 1929; (2) wood collected from a sawmill in Bogalusa, Louisiana; and (3) a shortleaf pine collected near Fairfax, Virginia.

In 1969 a "pinewood nematode" was identified from wood of dead pines collected from many localities in Kyushu, Japan (Tokushige and Kiyohara 1969). It was named *Bursaphelenchus lignicolus* by Mamiya and Kiyohara in 1972. In 1970 William R. Nickle proposed the name *Bursaphelenchus xylophilus* for *Paraphelenchoides xylophilus* (Steiner and Buhrer 1934) Kahk, 1967 (= *Aphelenchoides xylophilus* Steiner and Buhrer 1934). *B. lignicolus* and *B. xylophilus* were determined to be one and the same species (Nickle et al. 1981).

The pinewood nematode causes the pine wilt disease in

Japan with billions of board feet of lumber destroyed. This disease reached epidemic proportions in 1948 with losses that year in volume of timber estimated at 1,280,000 cubic meters (Mamiya 1972). Pine wilt causes the rapid death of trees. Trees healthy in the spring may die in late summer or autumn and are characterized by a reddish-brown discoloration of the needles. Trees often die within 30-50 days following reduction in oleoresin flow in mid-July to early August (Mamiya 1972).

In Japan two major species of trees susceptible to this wilt are Japanese black pine, _Pinus thunbergiana_ Franco (+_P. thunbergii_ Parl.) and Japanese red pine, _P. densiflora_ Sieb. and Zucc. (Kiyohara and Tokushige 1971).

The nematode was rediscovered in the United States in February 1979, when a Japanese plant pathologist, Dr. S. Ouchi, visiting the University of Missouri, spotted a dead Austrian pine, _Pinus nigra_, on the campus and suspected that it was infected by pinewood nematode. The nematode was extracted and identified by Dr. V. H. Dropkin (Dropkin and Foudin 1979) as _Bursaphelenchus lignicolus_.

Since its rediscovery in the United States investigators have been surveying to determine the distribution of _B. xylophilus_ by states and counties, and by tree species infected. By September 1980 the USDA Forest Service had reported occurrences of the pinewood nematode in 121 counties and 28 states from the following _Pinus_ species (Robbins 1980):

P. banksiana - jack pine

P. cembra - Swiss stone pine

P. clausa - sand pine

P. contorta var. murrayana - lodgepole pine

P. echinata - shortleaf pine

P. elliottii - slash pine

P. mugo - Swiss mountain pine

P. nigra - Austrian pine

P. palustris - longleaf pine

P. ponderosa - ponderosa pine

P. radiata - Monterey pine

P. resinosa - red pine

P. strobus - eastern white pine

P. sylvestris - Scotch pine

P. taeda - loblolly pine

P. thunbergiana - Japanese black pine

P. virginiana - Virginia pine

All species of pines in the United States are potential hosts for the pinewood nematode. Of the 17 *Pinus* species listed above, 12 are native species (9 eastern and 3 western) and 5 are exotic species (4 European and 1 Japanese). Four of the exotic species belong to the same *Pinus* subsection Sylvestres, as the three very susceptible Japanese pines. The most susceptible species in most states seems to be Scotch pine growing as ornamentals.

By 1981 *B. xylophilus* was known to occur in 32 states and 221 counties, and nematodes had been recovered from 24 species of trees including 19 species of pines. Additional conifer species found infected with this nematode included Allepo (P. halepensis); Japanese red pine (P. densiflora); Eastern larch or tamarak (Larix laricina); European larch (L. decidua); deodar cedar (Cedrus deodara); Atlas cedar (C. atlantica) and white spruce (Picea glauca).

II. Pinewilt Problem in Maryland.

By early 1981 pinewood nematode infected trees, especially in exotic ornamental pine species, had been found in 20 of Maryland's 23 counties (Dekker 1981). Since then all 23 counties in Maryland have reported detections of B. xylophilus (R. A. Dekker, pers. comm.). Affected pines include: Scotch, Japanese black, Austrian, Virginia, white and red pines.

Red pine plantations planted in the 1930's and 1940's have consistently shown decline and mortality in central and western Maryland. Stress in these red pines had been diagnosed as caused by off-site planting (since red pine's natural southernmost range is Pennsylvania) fostering Ips spp. attack and high summer temperatures. Red pines are now established in plantations at elevations above 1,600 feet in Maryland.

Three Garrett County Maryland red pine plantations, Big Run, Pleasant Valley 4-H Center and Herrington Manor State Park have been examined and trees at all these locations have contained B. xylophilus. Known planting dates go back to 1932 for Herrington Manor (actually Garrett State Forest which lies adjacent to the park) and 1938 for Big Run. Tree mortality was observed in the early 1960's at Garrett State Forest near Herrington Manor. Stresses have led to low vigor in the pines making them attractive to woodboring cerambycids and other insect species which may introduce pinewood nematodes into the trees as they feed and oviposit. Populations of both the beetle and nematode increase under such conditions (Dekker 1981).

In Japan the Japanese pine sawyer, Monochamus alternatus, Hope. has been identified as the main insect vector with the average number of nematodes carried per beetle being about 15,000 (Mamiya and Enda 1972) although some other Cerambycids contained a few pinewood nematodes.

III. Bursaphelenchus xylophilus Associations with Insects.

More than 70 insect species, mostly in the families Cerambycidae, Curculionidae and Scolytidae, have been found associated with weakened, suppressed and dead Japanese red and black pine trees in Japan (Mamiya and Kiyohara 1972). Little is known of the associations between most of these insects and B. xylophilus.

As early as 1934 it was suggested that the pinewood nematode may have been carried by Ips spp. beetles and by Dendroctonus frontalis, the southern pine beetle, and that the nematode was found in association with blue-stain fungi in trees (Steiner and Buhrer 1934).

Japanese investigators have recovered B. xylophilus from the following 8 species in 4 sub-families within the Cerambycidae, order Coleoptera, the family commonly known as the long-horned beetle (Mamiya 1976):

ASEMINAE - Arhopalus rusticus L.

LAMIINAE - Acalolepta fraudatrix Bates

- Acanthocinus griseus F.
- Monochamus alternatus Hope.
- M. nitens Bates
- Uraecha bimaculata Thomson

SPONDYLINAE - Spondylis buprestoides L.

CERAMBYCINAE - Corymbia succedanea Lewis

Of the 10 North American Monochamus species, dauerlarvae of the pinewood nematode have been recovered from the following four: M. carolinensis (Olivier) (Florida, Missouri, Illinois); M. obtusus Casey (California); M. scutellatus (Say) (Maryland) (white-spotted sawyer); and M. titillator Fabricius (Iowa) (southern pine sawyer). In addition to the Monochamus species, Arhopalus rusticus obsoletus (Randall) (Missouri) has been shown to carry this nematode in the United States (Holdeman 1980). According to W. R. Nickle (pers. comm.) there are at least three possible vectors of pinewood nematode in Maryland: M. scutellatus, M. carolinensis and M. titillator. M. titillator was collected from declining Austrian pines in Finksburg, Maryland (Nickle 1981) while M.

scutellatus was collected from Virginia pine (Nickle et al. 1981). The first record of pinewood nematode transmission by cerambycid adults to red pine occurred in Iowa (Williams 1980).

In Missouri besides the species mentioned above, the following insects had B. xylophilus recovered from specimens (Linit et al. 1983):

Cerambycidae

Asemem striatum (L.)

Amniscus sexguttata (Say)

Curculionidae

Pissodes approximatus Hopkins

Hylobius pales (Herbst.)

Buprestidae

Chrysobothris sp.

Bursaphelenchus xylophilus is carried phoretically under the elytra and in the tracheae of long-horned beetles and nematodes drop off randomly. Approximately 80% of the nematodes eventually drop off (Morimoto and Iwasaki 1972). Dauerlarvae gain entrance into trees while the young beetles carry on maturation feeding on young soft shoots, needles and bark, and during oviposition in the bark by mature females. Monochamus alternatus breeds in the upper part of the trunk and main stem pine crowns (Kanehori, Yasui and Ono 1975, 1976). Adult M. alternatus examined just after emergence contained most of the dauerlarvae in the tracheae directly connected with the metathoracic spiracles with a few also found in the tracheae of the head. Only the dauerlarval stage was found externally under the elytra or internally in the tracheal system (Mamiya and Enda 1972).

Once the xylem is exposed by the beetle through feeding or oviposition the nematodes move into this tissue where they molt into adults and infection occurs (Morimoto and Iwasaki 1972). *B. xylophilus* moves into both the axial and radial resin ducts damaging and destroying some of the parenchyma cells of these ducts (Mamiya and Kiyohara 1972).

IV. Objectives of This Study.

To date many aspects of the pinewood nematode problem remain unstudied in Maryland. Although decline in planted red pines has been common for many years, the insect-nematode-tree relationships have been little investigated. My study focuses on two sites in western Maryland exhibiting dying and dead red pines to determine the distribution of the pinewood nematode in these diseased trees and to determine which insects reared from these trees transport *B. xylophilus*. Samples of various portions of red pine trees were analyzed for pinewood nematodes and red pine bolts were caged for rearing insects to determine pinewood nematode associations.

METHODS AND MATERIALS

Two different study sites in Appalachian Maryland were utilized in the study. Study site 1, in Compartment 42 of Savage River State Forest, consisted of red pine trees ca. 25 years old; whereas study site 2, located in Garret State Forest, near Herrington Manor State Park, consisted of trees ca. 47 years old.

In the spring and summer of 1981 a general survey of the two most western counties in Maryland was conducted to determine if the pinewood nematode, Bursaphelenchus xylophilus, was present in any dying pines. In that investigation sickly eastern white pines, slash pines and Virginia pines were sampled. Sections of collected wood were soaked in water for twenty-four hours and the water then examined for nematodes. Scotch pines with reddish needles typical of trees harboring pinewood nematode were also found dying on the edge of a strip mine operation. Several of these trees containing B. xylophilus were recorded for future reference as sources of nematode specimens. Christmas tree plantations, such as Custer's near Deep Creek Lake, were inspected for trees with symptoms of pinewood nematode disease. Scotch pines, balsam fir and white pines in these plantations lacked symptoms and were not further studied. Slash sites of red pine plantations were visited where logs which showed insect attacks were split to expose borer larvae. Chipped wood block samples with insect larvae were taken to the laboratory to attempt insect rearing. Adult insect specimens were collected for species identification.

In the meantime foresters were contacted to determine the existence of diseased or dying pines on state forest lands. Jerry Sword, manager of Herrington Manor State Park supplied discs of wood from 40-50-year-old red pines. These trees were from a tract of red pines in which clumps of trees were dying and had been felled recently. Upon soaking this wood, vast numbers of B. xylophilus emerged.

In April 1982 a plantation of large red pines, ca. 47 years old, designated as site 2, was selected to begin the insect-nematode association study. Trees were divided into the following four decadence categories (1) predominantly green needles but with some reddish-brown needles; (2) predominantly reddish-brown needles (Fig. 1); (3) no needles but most of the bark intact (Fig. 2); (4) no needles and no bark (Fig. 2); and (5) predominantly bleached needles (Fig. 3). Three to four trees were selected in each category for a total of 17 trees felled in 1982-1983. Diameter at breast height (DBH) and total length of each tree were measured. Sections about 5 cm thick were sawed with a chain saw 25.5 cm from the ground and every 1.5 m thereafter to the top of the tree. Every other 1.5 m log was taken to the laboratory or the cage area set up in the open portion of a hardwood forest on Little Savage Mountain. Moreover, wood samples from the felled trees were taken from

Figure 1. Dying, large red pines exhibiting reddish needles as in tree decadence category 2 from Herrington Manor State Park.

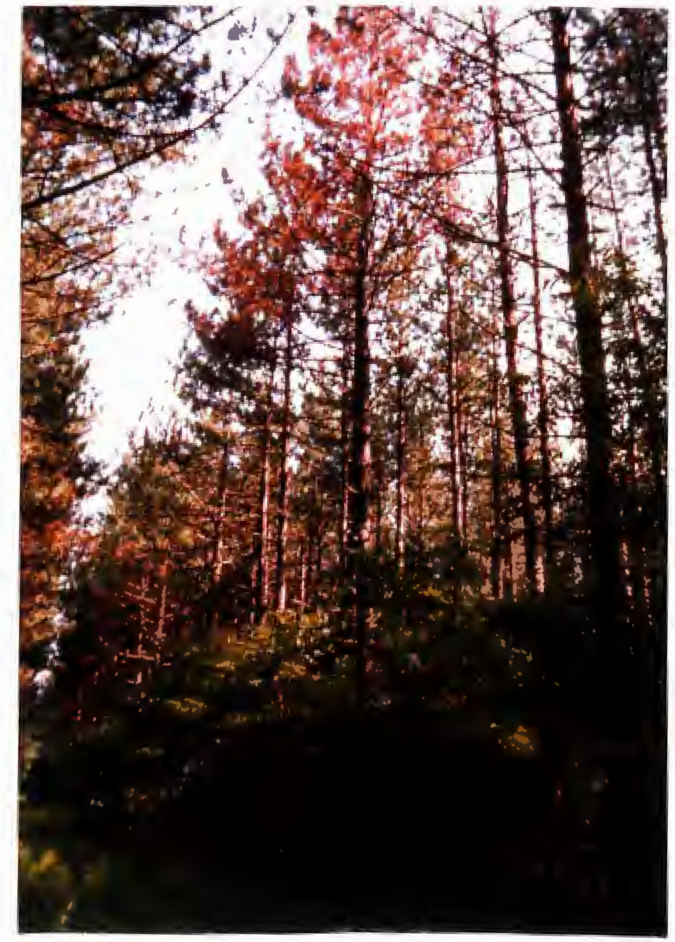


Figure 2. Large, dead red pines lacking needles illustrating bark versus barkless conditions as described for tree decadence categories 3 and 4.

Figure 3. Medium-sized red pine exhibiting bleached-green needles as described in tree decadence category 5 from Compartment 42, Savage River State Forest.

limbs. Primary limbs and secondary limbs were selected from each 3 m section of the tree starting with the bottom and continuing to the top of the tree. Primary limbs were defined as those growing from the main trunk of the tree whereas secondary limbs were limbs growing from the primary limb. In most cases it was possible to use foliage coloration to classify individual limbs as used for tree categories, i.e., mostly green needles, reddish-brown needles, bleached-green needles (a light bluish-green) or no needles. Cylindrical sections about 3 cm long were cut from the following positions on branches: (1) the base of the branch (2) at 1.5 m intervals thereafter until the end of the branch and (3) the terminal of each branch. Numbers were put on each cylindrical sample as it was placed in a plastic bag for transportation to the laboratory (Fig. 4).

Bolt sections of the main stem or trunk were caged whereas the 5 cm disc samples of the trunk and 5 cm cylindrical sections of the primary and secondary branches were taken to the laboratory where samples were soaked to release any nematodes. Several preliminary procedures were followed before placing the specimen wood in water: (1) rings were counted; (2) insects present were recorded and identified to species when possible; (3) presence of blue-stain fungus was recorded; (4) the bark was peeled off; (5) the specimen wood was washed under running tap water to eliminate rhabditid nematodes on the surface of the wood; (6) the wood was cut into longitudinal pieces with

Figure 4. Trunk sections from various heights on red pines, as used in sampling for the pinewood nematode <u>Bursaphelenchus xylophilus</u>.

pruning shears or if a trunk disc radial sample it was cut into 5-ring subsamples from the disc perimeter to the disc center (referred to as trunk radial samples and representing the horizontal dimension of trees) and; (7) wood placed in a clean petri dish or plastic pill cup and covered with tap water. After 24 hours the water was poured into a counting dish for examination. If the sample could not be examined immediately, it was preserved in a 3% formaldehyde solution.

Nematodes were counted using a binocular dissecting microscope at 45 X and were placed on microscope slides for species identifications. Nematodes were placed in either water, or for longer storage, cotton blue-lactophenol solution.

In summer 1982, only mature trees from site 2 (Garrett State Forest) were cut and caged for insect rearing. The cages for holding sample logs, or bolts, were constructed so that the top which was shaped like a tent could be lifted completely or tilted to collect emerging insects (Fig. 5). The triangular ends were covered with wire insect screen for ventilation and the other two sides of the tent were covered with plastic rolled on the edge fastened to the wooden frame to prevent escape of insects. The rectangular bottom of the cage had 1.83 m x 0.91 m dimensions as did the sides of this tent constructed cage. A total of 26 cages were built to hold the sample log sections from the pines of site 2. Bolts were grouped sequentially and by category with three bolts from three different trees of adjacent sections

Figure 5. Tent-shaped cages built for horizontal placement of red pine bolts and for the capturing of emerging insects.

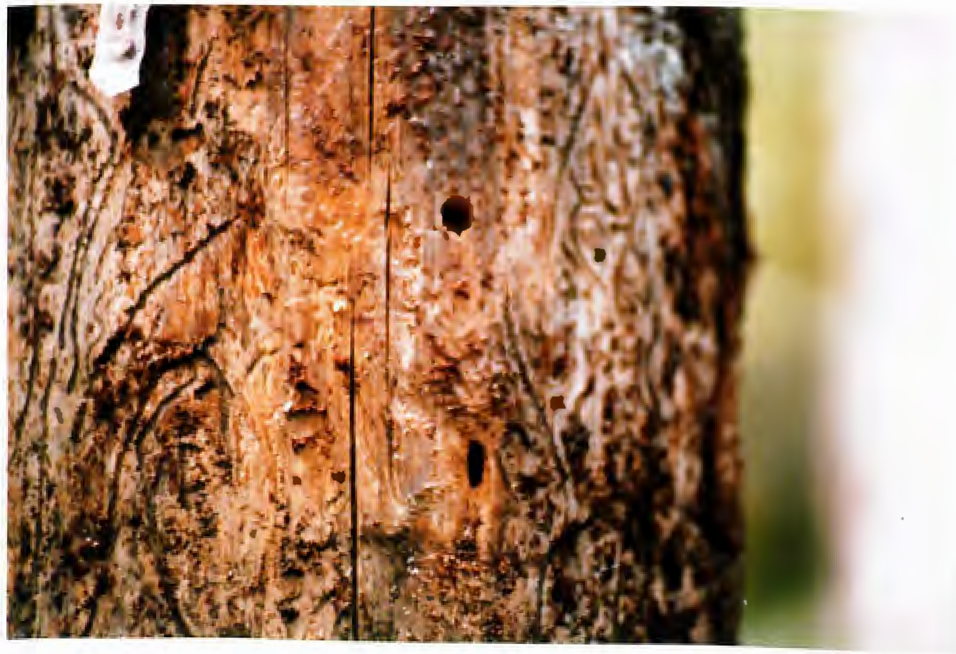
occupying the same cage. Starting at 1.5 m, every other 1.5 m section of tree trunks were caged.

For the 25 smaller trees of site 1 (Compartment 42 of Savage River State Forest), the above cages were modified as follows: (1) instead of lifting the top off for insect collection, one side was fitted with a piece of wire insect screen so that it could be rolled toward the apex of the tent to collect insects, (2) the sides of this so-called door could be hooked over nails on either end of the cage for securing and, (3) the lower edge of the "door" consisting of wire stapled to a horizontal strip of wood could be wedged between stakes driven in the ground for securing. Trunk sections of all trees of category 5 (bleached-green and chlorotic needles) bore Monochamus entrance holes (Fig. 6) and were the only trees used for insect rearings in 1983-84. Cages held the following bolts from these trees: (1) from the base up to 1.5 m (2) 1.5-3 m (3) 3-4.5 m (4) 4.5-6.0 m (5) 6.0-7.5 m (6) 7.5-9 m.

Trees selected in late May-early June (categories 1, 2, 3 and 4) from the younger stand (site 1) were examined for nematodes but were not caged to capture emerging insects because the experience of the previous year indicated no Monochamus would be emerging. Other emerging insects were not of sufficient interest to merit a second year of investigation.

Several trees in site 1 were felled and left lying in the field until February 1984 at which time these trees were

Figure 6. Entrance (oval) and exit (round) holes made by Monochamus species in red pine trees.



brought into the laboratory and placed in various cages for insect rearings. One professionally-built cage made entirely of metal and screen was ideal for capturing Monochamus but was too small for large-scale insect rearing. Another rather satisfactory cage was constructed of an elongated trough-like sink to which a wooden, screened frame fitted securely. Several bolts were labeled and placed in this cage. As insects emerged they were collected in storage vials, labeled and if they were Monochamus the lengths and widths of exit holes were measured. Two plastic barrels with 1.5 cm holes in the snuggly fitting lids were used to store several bolt sections but were unsatisfactory because of excessive condensation.

Three large cages were constructed in summer 1983 to hold the bolts collected in late July-early August from only bleached-green needled mature pines of site 2. Since materials were costly, only three cages could be built and those shared common walls. Pine studs were used for framing these cages. Posts anchored the four corners of the main structure. Sides and roof were completely screened and the floor on which the bolt samples rested consisted of soil covered by a 2-3 inch layer of sand. Each compartment cage had hinged doors (Fig. 7).

Insects were collected 2 to 3 times per week using large plastic vials with snap-on lids for easy handling. When insects could not be dissected on the day of collection, they were refrigerated.

Figure 7. Large cages built for the vertical placement of red pine bolts and for the easy capturing of emerging insects.

In early June 1983 six ca. 7-year-old Scotch pines, six ca. 25-year-old Scotch pines and six ca. 25-year-old red pines were inoculated with approximately 32,000 larval and adult B. xylophilus. Nematodes soaked from 45-year-old Scotch pine wood were injected into a boring made in the trunk. Trees were observed weekly until the end of summer.

I. Insect Dissection for Nematodes.

After the shape of the apices of the elytra were recorded as either round, slightly protruded or dentate, woodborers were dissected into the following body parts and placed in individual Syracuse watch glasses: (1) head (2) prothorax (3) meso-metathorax and (4) abdomen. The body parts were forcep macerated in watch glasses which were stored for three to four hours to allow nematodes to emerge. For longer storage (2-3 days), samples were refrigerated. When nematodes were too numerous for direct counting, dilutions were made to facilitate counting.

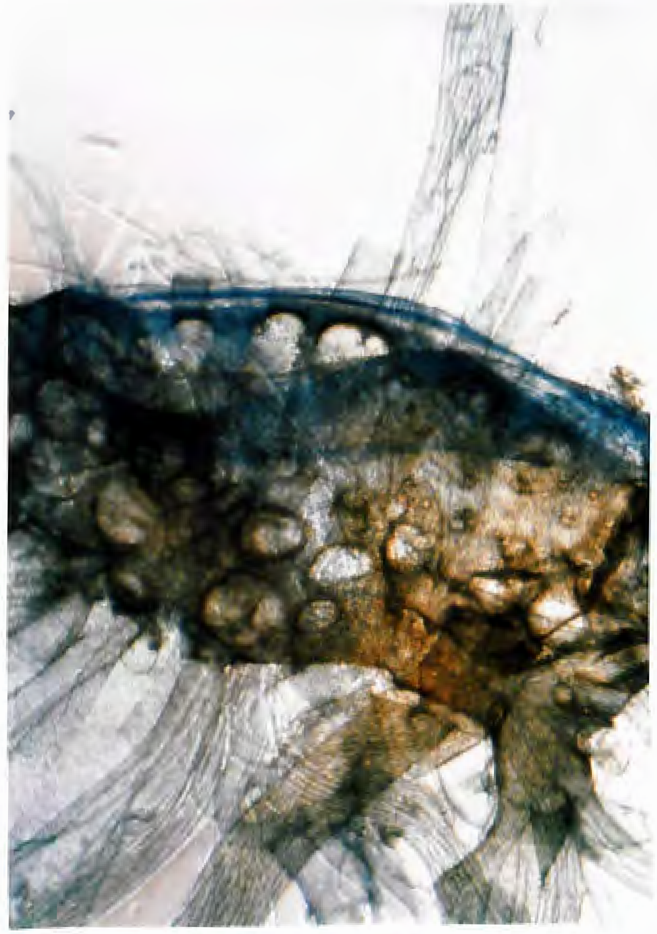
When only knowing the presence or absence of the nematode in insects was desired, the third, right leg was pulled from the body and the following leg parts were examined in sequence for the presence and counting of nematodes therein: (1) metacoxa-metatrochanter (2) metafemur and (3) metatibia. If the leg lacked nematodes, then the whole body by parts as indicated above were examined for nematodes. Specimens of nematodes present on the outside of the insect body were placed on slides for identification. From time to time clusters of these nematodes were cultured on Botrytis cinerea. Locations of nematodes found externally on insects were recorded.

Dauerlarvae found in the tracheal system of Monochamus (Fig. 8) were cultured on Botrytis cinerea. The distinctive vulval flap in adult females and the typical spicules with expanded tips in males were used to identify B. xylophilus (Nickle et al. 1981).

Several Monochamus larvae from infested bolts felled July 1983 and overwintered in the field were dissected in February 1984. Larvae were examined both externally and internally for nematodes with a dissecting microscope at 45 X. Moreover, measurements of Monochamus larval tunnels were made from this infested wood.

5a

Figure 8. Dauerlarvae of the pinewood nematode *Bursaphelenchus xylophilus* in the tracheal system of an adult *Monochamus scutellatus*.



RESULTS

I. Nematode Distribution In Medium-Sized Red Pines, Study Site 1.

The sample from site 1 located in Compartment 42 of Savage River State Forest (elevation 792 m) consisted of 25 trees with an average height of 10.1 m and mean DBH of 14.0 cm. Heights ranged from 7.5 m to 12.5 m and diameters from 10.4 to 16.9 cm. The average age was 22.7 years and ranged from 13 to 25 years.

Seventeen (68%) of the 25 red pines sampled were infected with _B_. _xylophilus_. Infection ranged from 0 to 50% with an average of 16.3%. Of the 610 trunk and branch disc samples 82, or 13.4%, were positive for _B_. _xylophilus_. Trunk disc samples had the highest infection rate (38.0%) followed by primary branch base (butt) samples (15.7%). Per decadence, category 5 (bleached needled trees) had the highest infection (30.6%) followed by category 2 (reddish needled trees), (27.1%). Among radial (horizontal) samples (34.8%) were infected with _B_. _xylophilus_. Table 1 shows the occurrence of _B_. _xylophilus_ by anatomical position on trees by decadence category at site 1.

Distribution of _B_. _xylophilus_ in discs taken every 3 m in trunk ranged from 18.2% to 44.0%. The highest percentage infection of trunk samples was in 3 m. Primary branch samples were not as commonly infected (2.0% to 15.4%). Highest primary branch infection occurred between 6 and 12

Table 1. Occurrence of Bursaphelenchus xylophilus (Bx) in red pines by tree decadence category a/ by anatomical position b/ at site 1, Savage River State Forest, Maryland.

Anatomical	Tree decadence category					
	1	2	3	4	5	
Position	+/total	+/total	+/total	+/total	+/total	%Bx
Trunk						
disc	3/26	7/15	5/15	1/15	25/37	38.0
terminal	1/6	0/3	1/3	0/4	1/8	12.5
Primary Branches						
base	4/34	6/20	0/12	1/11	9/50	15.7
1.5 m	1/11	0/4	0/2	0/1		5.6
terminal	0/34	1/19	0/12	0/7	7/50	6.6
Secondary Branches						
base	1/26	2/18	0/13	0/6	1/42	3.8
terminal	1/29	1/18	0/12	0/6	3/41	4.7
Sub-total	11/166	17/97	6/69	2/50	46/228	13.4
Trunk						
radial	6/65	18/32	9/35	3/29	50/86	34.8
Grand-total	17/231	35/129	15/104	5/79	96/314	
% Bx	7.4	27.1	14.4	6.3	30.6	19.6

Table 1. (cont'd). Footnotes.

---

a/ Decadence categories are defined as follows: 1. mostly green needled trees, 2. mostly reddish-brown needled trees, 3. no needled trees, 4. trees lacking needles and bark, 5. bleached-green needled trees.

b/ Anatomical position denotes various sections of trees as follows: 1. trunk disc samples cut at 3 m intervals the length of the tree ; 2. trunk radials were annual growth rings sampled every 5 rings from the trunk perimeter inward to the trunk center; 3. trunk terminals were the tops of trunks; 4. primary branches were those growing from the main trunk of the tree whereas secondary limbs were limbs growing from the primary limb. Branches were sampled from the base, at 1.5 m intervals the length of the branch and at terminal end of branch.

m. Secondary branch samples had an infection ranging from 2.9% to 8.7% and highest infection occurred from the 0-3 m trunk region (Table 2).

By foliage coloration of limbs, bleached needled branches had the highest B. xylophilus infection (26.4%). In decadence category 1 (green needled trees) the branches with the highest infection were those of green foliage (11.1%) although green needled branches of bleached needled trees lacked B. xylophilus (Table 3).

A higher percentage of primary branch base samples were infected than branches in other sample categories. Branch terminal and base samples were infected at higher percentages with B. xylophilus in bleached-green needled branches than in branches with other foliage characteristics. Collectively, branch samples of all foliage conditions had an increasing incidence of infection from no needles (4.0%) to green needles (7.9%) to reddish needles (9.1%) to bleached-green needles (26.4%) (Table 4). High infection by trunk height occurred in the 6-9 m (22.2%) and 9-12 m (20.0%) parts of the trees (Appendix A).

The distribution of B. xylophilus horizontally in trunk samples by 5-ring interval from the trunk perimeter to trunk center ranged from 26% to 40%. The lowest infection occurred in the 5 rings in the center and the highest in the 6-10 (38.2%) and 11-15 (40.0%) ring intervals. See Appendix B which compares occurrences of B. xylophilus by radial ring

Table 2. Incidence of Bursaphelenchus xylophilus (Bx) infection by anatomical position a/ and by trunk interval b/ at site 1.

	Trunk Interval (m)					
	0-3		3-6		6-9	
Anatomical position	No. of samples	% with Bx	No. of samples	% with Bx	No. of samples	% with Bx
Trunk						
Disc	25	40.0	25	44.0	25	40.0
Radial	91	33.0	65	32.3	53	41.5
Terminal					2	0
Primary Branches						
Base	24	4.2	37	13.5	45	22.2
1.5 m	3	0	12	8.3	3	0
Terminal	24	0	35	5.7	43	9.3
Secondary Branches						
Base	23	8.7	34	2.9	37	2.7
Terminal	23	8.7	35	2.9	38	5.3
Totals	213	21.1	243	17.3	246	19.9

Table 2. (cont'd.)

	Trunk Interval (m)			
	9-12		12-15	
Anatomical position	No. of samples	% with Bx	No. of samples	% with Bx
Trunk				
Disc	22	36.4	11	18.2
Radial	27	40.7	11	18.2
Terminal	10	10.0	12	16.7
Primary Branches				
Base	20	20.0	1	0
1.5 m	-	-	-	-
Terminal	19	10.5	1	0
Secondary Branches				
Base	11	0	-	-
Terminal	10	0	-	-
Totals	119	21.8	36	16.7

a/ Tree anatomical positions defined as in Table 1.

b/ Trunk interval designates samples from every 3.0 m section of trunk from bottom to apex excluding 3 cm trunk terminal samples.

Table 3. Occurrence and percentages of *Bursaphelenchus xylophilus* (Bx) infection by tree decadence category a/ by branch foliage condition b/, site 1.

	Green		Reddish		No Needles		Bleached	
Decadence category	Total samples	%Bx	Total samples	%Bx	Total samples	%Bx	Total samples	%Bx
1	45	11.1	31	3.2	58	1.7		
2			34	14.7	45	11.1		
3					51	0		
4					31	3.2		
5	18	0	23	8.7	89	4.5	53	26.4

a/ Tree decadence category defined as in Table 1.

b/ Branch foliage condition indicates the needle coloration of the particular branch sampled and does not necessarily represent the tree as a whole which may belong to another category dependent on the condition of most of the branches on that particular tree.

Table 4. Incidence of infection by Bursaphelenchus xylophilus (Bx) in primary and secondary branches a/ by branch foliage condition b/ and by anatomical position c/, site 1.

	Condition of Needles on Branches							
	Mostly Green		Reddish		None		Bleached	
Anatomical position	Bx/ Total	%Bx	Bx/ Total	%Bx	Bx/ Total	%Bx	Bx/ Total	% Bx
Primary Branches								
Base	3/15	20.0	5/24	20.8	5/72	6.9	7/16	43.8
1.5 m	1/4	25.0	0/3	0	0/11	0		
Term	0/16	0	2/24	8.3	2/67	3.0	4/15	26.7
Secondary Branches								
Base	1/13	7.7	0/18	0	2/62	3.2	1/12	8.3
Term	0/15	0	1/19	5.3	2/62	3.2	2/10	20.0
Total	5/63	7.9	8/88	9.1	11/274	4.0	14/53	26.4

a/ Primary branch defined as in Table 1.

b/ Branch foliage condition defined as in Table 3.

c/ Anatomical position defined as in Table 1.

interval in older and younger trees.

Among all decadence categories, eleven trees had no nematode infection. Two trees had infection in all but the terminal samples. One tree had nematodes in the basal trunk sample only. Variable distributions of infection were seen in each decadence category.

The largest number of nematodes (8,550) was recorded from tree 7 (category 2) in the 3 m trunk interval. Of the pinewood nematodes counted 50.3% were from the 3 m section of the trunk; 32.8% from the basal section (0 m); 13.1% from 6 m and 3.9% from the 9 m trunk interval.

An unknown species of Aphelenchoidea was discovered in trees at both study sites. In study site one 16.7%, of 742 samples were positive for the unidentified nematode. Trees in all decadence categories except number one (green needles) had a mixture of B. xylophilus and the Aphelench. All trees contained at least one sample with the unidentified Aphelench. Wood samples from 8 of 25 (32.0%) trees sampled contained both B. xylophilus and the unidentified nematode. Of these 8 trees 13.8% of samples contained both B. xylophilus and the unknown Aphelench having a range of association of 3.4% to 33.0% (Table 5). These trees represented all decadence categories except category 1 which had three trees containing only the Aphelench. Half of the trees in category 3 (lacking needles) and category 4 (lacking needles and bark) contained the Aphelench alone. Table 6 shows the association of this unknown species and B.

xylophilus by tree decadence category. Of the 124 times this unknown species was found, 14.5% were in association with *B. xylophilus*. Of the samples with *B. xylophilus* and/or the unknown Aphelench, 6.6% contained both nematodes.

Trunk sections including trunk terminals contained both *B. xylophilus* and the unknown Aphelench in 10 out of 73 samples. The association of these two nematodes in trunks and branches is shown in Table 7. Primary branch sections 1.5 m distal and primary and secondary terminals lacked association of the two nematodes. The association by branch foliage conditions is shown in Table 8.

In addition to the unidentified Aphelench another Aphelenchoid, smaller with a c-shaped body, was found in some samples. Other tylenchids were found but in relatively small numbers. One tylenchid was found in 29% of the samples, rhabditid nematodes in 52% of the samples, and several other unidentified nematodes in 52 of 742 samples (7%).

Blue-stain occurred in 458 out of 742 tree samples (61.7%). Protozoans were in 39% of the samples and rotifers in 18%.

Table 5. Association of an unknown species of Aphelenchoidea with <u>Bursaphelenchus</u> <u>xylophilus</u> (Bx) and its occurrence by individual tree at site 1.

Tree specimen	Bx only	Aphelench only	Bx + Aphelench	% Association
5	11	17	1	3.4
7	13	3	5	23.8
9	6	1	1	12.5
10	0	10	2	20.0
12	2	3	1	16.7
18	7	3	5	33.3
19	10	2	1	7.7
21	21	3	2	7.7
Totals	70	42	18	13.8

Table 6. Occurrence of an unknown Aphelenchoidea by tree decadence category a/ and in association with *Bursaphelenchus xylophilus* (Bx) at site 1.

	Tree Decadence Category					
	1	2	3	4	5	Total
Both b/	0	6	3	1	8	18
Bx only c/	17	29	12	44	87	149
Unk only d/	29	23	23	14	17	106
Total	46	58	38	19	112	273
% Assoc.	0	10.3	7.9	5.3	7.1	6.6

a/ Tree decadence defined as in Table 1.

b/ Occurrence of unknown Aphelench and *B. xylophilus* in the same sample.

c/ *B. xylophilus* occurrence alone.

d/ Aphelench occurrence alone.

Table 7. Association of unknown species of Aphelenchoidea with Bursaphelenchus xylophilus (Bx) by tree anatomical position a/, site 1.

	Trunk		Branches				
			Primary			Secondary	
	disc	term	base	1.5 m distal	term	base	term
Both b/	9	1	5	0	0	3	0
Bx only c/	31	2	15	1	8	1	5
Unk only d/	27	3	29	4	17	17	9
Total	67	6	49	5	25	21	14
% Assoc.	13.4	16.7	10.2	0	0	14.3	0

a/ Anatomical position defined as in Table 1.

b/ Both defined as in Table 6.

c/ Bx defined as in Table 6.

d/ Unk defined as in Table 6.

Table 8. Association of unknown species of Aphelenchoidea with Bursaphelenchus xylophilus (Bx) by branch foliage condition a/, study site 1.

	Branch Foliage Condition				
	Green Needles	Reddish Needles	No Needles	Bleached Needles	Total
Both b/	0	2	5	1	8
Bx only c/	5	8	11	14	38
Unk only d/	1	16	54	5	76
Total	6	26	70	20	122
% Assoc.	0	7.7	7.1	5.0	6.6

a/ Branch foliage condition defined as in Table 3.

b/ Both defined as in Table 6.

c/ Bx defined as in Table 6.

d/ Unk defined as in Table 6.

II. Nematode Distribution in Large Red Pines at Site 2, Herrington Manor State Park.

Mature red pines of site 2, Herrington Manor State Park (elevation 731.5 m) contained trees that ranged from 21.3 to 24.4 m tall and were ca. 47 years old. DBH ranged from 22.9 to 35 cm. A total of 17 trees were examined at 3 m intervals along their trunks and at various sites along primary and secondary branches. Of these trees 13 of the 17 (76.5%) were positive for B. xylophilus in at least one sample. From a total of 708 samples, 107 (15.1%) were positive for B. xylophilus. In categories 4 and 5, all trees were positive for the pinewood nematode.

As shown in Table 9, infection by B. xylophilus varied widely at intervals along the trunk. In general, nematode infection increased from the bottom to top of the trunk. The lowest infection (18.8%) was in base samples where 3 of 16 trees were infected with the nematode. A total of 111 samples from trunk discs were analyzed of which 43 (38.7%) yielded B. xylophilus. The range of B. xylophilus infection in trunk samples was from 4.8% in tree decadence category 3 to 81.3% in tree decadence category 1.

Infection at various sites along primary branches is given in Table 10. B. xylophilus was found in 45 of the 318 (14.2%) primary branch samples. Pinewood nematode was found in 22% of primary branch base samples and only 1.3% of the primary branch terminals.

Table 11 shows the incidence of primary branch samples

Table 9. Incidence of *Bursaphelenchus xylophilus* (Bx) infection in trunk discs per trunk height interval per tree decadence category a/ at site 2.

	Category					
Height on trunk (m)	1 Trees +/total samples	2 Trees +/total samples	3 Trees +/total samples	4 Trees +/total samples	5 Trees +/total samples	%Bx
0	2/3	0/4	0/3	1/3	0/3	18.8
3	2/3	0/4	1/3	1/3	0/3	25.0
6	3/3	1/4	0/3	2/3	0/3	37.5
9	2/3	1/4	0/3	3/3	1/3	43.8
12	2/2	1/4	0/3	2/3	2/3	46.7
15	1/1	1/4	0/3	3/3	2/3	50.0
18	1/1	1/4	0/3	1/3	3/3	42.9
21		1/1			2/3	75.0
Totals	13/16	6/29	1/21	13/21	10/24	
% Bx	81.3	20.7	4.8	61.9	41.7	

a/ Tree decadence category defined as in Table 1.

infected with *B. xylophilus* at branch origin above ground (m) at site 2. Primary branch samples differed in the percentages of infection along the trunk. Interval 3-6 m had no nematodes but the next interval, 6-9 m, had the highest infection (22.6%) recorded for primary branches at any trunk height interval.

Analyzing primary branches per tree decadence category, the number of samples containing *B. xylophilus* ranged from 2.3% in tree category 4 to 24.1% in tree category 1 (Table 11).

Analysis of the percentages of primary branch samples infected with *B. xylophilus* by branch foliage condition and tree decadence category is shown in Table 12. The "no needle" branches of tree decadence categories 1 and 2 exhibited the highest infection with *B. xylophilus*, i.e., 52% and 22%, respectively.

Occurrence of *B. xylophilus* in secondary branch samples 1.5 m, 2.0 m and 4.5 m distal to the trunk is shown by tree decadence category for site 2 in Table 13. Altogether these secondary branches had the highest infection (15.9%) in trees lacking needles but with bark (category 3) but overall were only 6.8% infected with *B. xylophilus*.

Secondary branch base samples were infected with *B. xylophilus* in 12 of 143 samples (8.4%), whereas terminals had *B. xylophilus* in 6 out of 129 samples (4.7%).

Table 10. Incidence of infection by Bursaphelenchus xylophilus (Bx) per tree anatomical position on primary branches a/ at site 2.

Anatomical position	Samples analyzed	No. + for Bx	% + for Bx
base	109	24	22.0
1.5 m distal	90	13	14.4
3.0 m distal	32	5	15.6
4.5 m distal	7	1	14.3
6.0 m distal	3	1	33.3
terminals	76	1	1.3
Totals	317	45	14.2

a/ Anatomical position defined as in Table 1.

Table 11. Incidence of infection by *Bursaphelenchus xylophilus* (Bx) in primary branch a/ samples by trunk height interval per tree decadence category b/, site 2.

Branch origin above ground	Decadence category 1 +/ total	2 +/ total	3 +/ total	4 +/ total	5 +/ total	%Bx
0 - 3	0/6	1/8				7.0
3 - 6	0/11	0/9			0/4	0.0
6 - 9	3/10	3/12	0/1		1/8	22.6
9 - 12	5/10	5/16	1/11	0/4	2/20	21.3
12 - 15	2/7	8/31	0/6	0/15	3/16	17.3
15 - 18	2/8	1/20	2/3	0/12	2/15	12.1
18 - 21	2/6	0/14	0/8	1/11	0/10	6.1
21 - 24		1/5				20.0
Totals	14/58	19/115	3/29	1/43	8/73	
% Bx	24.1	16.5	10.3	2.3	11.0	14.2

a/ Primary branch samples defined as in Table 1.

b/ Tree decadence category defined as in Table 1.

Table 12. Percentages of infection by _Bursaphelenchus xylophilus_ in primary branch a/ samples per branch condition b/ per tree decadence category c/ at site 2.

Tree decadence category	Branch Condition: Green needles	Reddish needles	No needles	Bleached needles	No bark
Green needles	7.0%	11.0%	52.0%		
Reddish needles		11.0%	22.0%		20.8%
No needles			7.0%		0.0
Bleached needles		10.5%	10.0%	12.0%	
Barkless			0.0%		5.0%

a/ Primary branch defined as in Table 1.

b/ Branch condition defined as in Table 3.

c/ Tree decadence defined as in Table 1.

Disc-shaped cross sections from intervals along tree trunks were analyzed further for B. xylophilus. Sub-samples of each cross section were taken radially toward disc center at intervals of 5 growth rings. The percentage of infection decreased from the outer rings (44.4) to the inner sub-samples (0.0) (Appendix B). The highest number of nematodes for any radial sub-sample was 2,550 in the 15 m cross section of category 2. Of the B. xylophilus counted 45.5% were found in the 15 m section of the trunk.

Two of the 4 trees in decadence category 1 had B. xylophilus in the basal disc and one of these contained the nematode in every interval.

In tree decadence category 2, B. xylophilus occurred in 4 trees at intervals 9 m, 12 m, 15 m, 18 m and 21 m. Category 3 had nematodes in only one tree at only one level (3 m). Collectively, 3 trees in category 4 had nematodes at all intervals. One tree from category 4 had nematodes in all but the highest level (18 m). In decadence category 5, nematodes infected intervals 9 m, 12 m, 15 m, 18 m, and 21 m in 3 trees. Intervals 0 m, 3 m and 6 m lacked nematodes.

The ca. 25-year-old and ca. 7-year-old Scotch pines and ca. 25-year-old red pines each inoculated with ca. 32,000 B. xylophilus mixture of adults and larvae did not succumb or show any visible symptoms of pinewilt disease.

Table 13. Occurrence of Bursaphelenchus xylophilus (Bx) in secondary branches a/ 1.5 m, 3.0 m and 4.5 m distal to trunk by anatomical position b/ per tree decadence category c/ at site 2.

Anatomical position	1	2	3	4	5	Total Bx	% Bx
1.5 m Distal from Trunk							
Base	1/15	1/24	1/14	0/5	1/22	4/80	5.0
1.5	0/ 3					0/3	0.0
3.0	0/1					0/1	0.0
Term	1/15	1/24	1/13	0/6	0/20	3/78	3.8
Sub-total	2/34	2/48	2/27	0/11	1/42	7/162	
% Bx	5.9	4.2	7.4	0	2.4	4.3	

Anatomical position	1	2	3	4	5	Total Bx	% Bx
3.0 m Distal from Trunk							
Base	2/5	3/17	0/6	1/4	0/12	6/44	13.6
1.5	0/1					0/1	0.0
3.0	1/1					1/1	0.0
6.0	0/1					0/1	0.0
Term	0/4	0/16	2/5	0/2	0/12	2/39	5.1
Sub-total	3/12	3/33	2/11	1/6	0/24	9/86	
% Bx	25.0	9.1	18.2	16.7	0.0	10.5	

Table 13. (cont'd.)

4.5 m Distal from Trunk							
Base	0/3	0/6	2/3		0/7	2/19	10.5
Term	0/3	0/6	1/3			1/12	8.3
Sub-total	0/6	0/12	3/6		0/7	3/31	
% Bx	0.0	0.0	50.0		0.0	9.7	
Grand total	5/52	5/93	7/44	1/17	1/73	19/279	
Grand % Bx	9.6	5.4	15.9	5.9	1.4	6.8	

a/ Secondary branches defined as in Table 1.

b/ Anatomical position defined as in Table 1.

c/ Tree decadence defined as in Table 1.

III. Distribution of Insects at Sequential Heights in Ca. 47-Year-Old Red Pine at Herrington Manor State Park.

Total insect emergences from bolts of ca. 47-year-old trees collected May 24-June 2, 1982 and caged every other 1.5 m section were estimated at 67,992 for Ips sp. and 3,814 for Pissodes sp., and by direct count were 19 for Monochamus sp. and 371 for other Cerambycids. Monochamus sp. were found in trees from 6 m up but were most numerous in 15-18 m bolts (Table 14).

Insects emerging from caged red pine bolts in 1982-1984 represented four families, i.e., Scolytidae, Curculinonidae, Buprestidae and Cerambycidae (Table 15). Tetropium schwarzianum Casey, Pissodes approximatus Hopkins, Ips grandicollis (Eichhoff) and Ips pini (Say) dissected in 1982 lacked B. xylophilus. However, these same insects contained haemocoel, intestinal and elytral (phoretic) nematodes (Table 16). No Monochamus sp. were reared from caged bolts of categories 1, 2, 3 or 4 in 1982.

IV. Distribution of Insects Reared from Red Pine Bolts from Herrington Manor and Compartment 42, Savage River State Forest in 1984.

Trees of categories 1, 2, 3 and 4 collected May 17-July 14, 1983 were not caged for insect rearing as these trees lacked evidence of Monochamus sp. infestation. However, bleached-green trees collected from sites 1 and 2 July 26-August 12, 1983 had woodborings on understory vegetation

Table 14. Total insects reared from 21.3-24.4 m Pinus resinosa. Logs taken May 24-June 2, 1982 at sequential heights.

Height above ground in m	Ips sp.	Pissodes sp.	Monochamus sp.	Other Cerambycidae
0-3	11,198	166	0	154
3-6	10,404	226	0	104
6-9	8,424	784	1	24
9-12	13,034	1,108	2	46
12-15	4,818	1,104	3	11
15-18	13,766	326	10	22
18-21	6,348	100	3	10
Totals	67,992	3,814	19	371

Table 15. Coleoptera species by family of insects reared from red pine in western Maryland.

CERAMBYCIDAE	CURCULIONIDAE
Monochamus scutellatus (Say)	*Pissodes approximatus* Hopkins
Monochamus carolinensis (Olivier)	*Otiorhynchus ovatus* (Linnaeus)
	Otiorhynchus rugostriatus (Goeze)
Tetropium schwarzianum Casey	
Amniscus collaris Haldeman	**BUPRESTIDAE**
Neacanthocinus pusillus (Kirby)	*Chrysobothris scabripennis* Cast. and Gory
Rhagium inquisitor (L.)	

SCOLYTIDAE
Ips pini (Say)
Ips grandicollis (Eichoff)

Table 16. Percentage of haemocoel, elytral and intestinal nematodes in insects reared from bolts of 21.3-24.4 m trees, site 2.

Nematodes	*Ips* sp.	*Pissodes* sp.	*Tetropium* sp.
B. xylophilus	0	0	0
% with none	67.5	46	39
Elytral phoretics	12	49	17
Intestinal	12.5	4	12
% with nematodes	32	54.5	61

and were thus caged. These trees yielded the insects listed in Table 17. Two *Monochamus* species, i.e., *M. scutellatus* (Say) and *M. carolinensis* (Olivier) were recovered from tree bolts collected from sites 1 and 2 but altogether more *M. scutellatus* were reared than *M. carolinensis*. *M. carolinensis* was more abundant from site 1 than from site 2; whereas *Chyrsobothris scabripennis*, a buprestid, was collected from only site 1. More *Neacanthocinus pusillus*, another cerambycid, were collected from site 2 than from site 1. Site 1 lacked both cerambycids *Rhagium inquisitor* and *T. schwarzianum*.

Those insects from both sites carrying *B. xylophilus* are listed in Table 18. Five species were positive for pinewood nematode, i.e., *M. scutellatus*, *M. carolinensis*, *N. pusillus* (Kirby), *P. approximatus* and *Ips* sp.. Ninety-four percent of *Monochamus* sp. collected carried *B. xylophilus* dauerlarvae in tracheae. In site one 87.3% and in site two 98.6% of the *Monochamus* sp. carried dauerlarvae. Ninety-six percent of all *M. scutellatus* and 90% of all *M. carolinensis* transported *B. xylophilus*. In site one 90% and in site two 100% of *M. scutellatus* carried *B. xylophilus* dauerlarvae. The three *M. carolinensis* collected from site 2 lacked *B. xylophilus* but at site one 92.3% of the *M. carolinensis* collected carried the dauerlarvae. The other insects transporting dauerlarvae in tracheae were *N. pusillus* (4.2%, site 1; 27.7%, site 2); 1 of 332 *P. approximatus*; 1 of 2,667 *Ips*. spp. and 1 of 8 *C. scabripennis*

Table 17. Numbers of insects reared from *Pinus resinosa* in Maryland, in summer 1984; trees cut in summer 1983, sites 1 and 2.

Taxon	Site 1 7.6-9.1 m trees	Site 2 21.3-24.4 m trees
CERAMBYCIDAE		
Monochamus scutellatus	124	96
Monochamus carolinensis	29	3
Neacanthocinus pusillus	18	144
Amniscus collaris	9	15
Rhagium inquisitor	0	8
Tetropium schwarzianum	0	9
CURCULIONIDAE		
Otiorhynchus spp.	4	7
Pissodes approximatus	0	332
BUPRESTIDAE		
Chyrsobothris scabripennis	8	0

Table 18. Occurrence of *Bursaphelenchus xylophilus* by insect species, study site and height in red pine trees.

Number of insects (Pos) and (Neg) for *B. xylophilus*

Study Site 1; 7.6-9.1 m trees

Taxon	0-1.5 m		1.5-3.0 m		3.0-4.5 m	
	Pos	Neg	Pos	Neg	Pos	Neg
M. scutellatus	2	0	27	0	41	0
M. carolinensis	0	0	1	0	2	0
N. pusillus	0	0	1	1	1	0
A. collaris	0	0	0	0	0	0
T. schwarzianum	0	0	0	0	0	0
P. approximatus	0	0	0	0	0	0
Otiorhynchus spp.	0	0	0	1	0	2
R. inquisitor	0	0	0	0	0	0
Ips spp.	0	304	0	346	0	345

Table 18. (cont'd.)

Taxon	Portion of tree by 1.5 m section, upward from ground					
	4.5-6.0		6.0-7.5		7.5-9.0	
	Pos	Neg	Pos	Neg	Pos	Neg
M. scutellatus	22	0	27	0	5	0
M. carolinensis	13	2	7	0	1	0
N. pusillus	0	10	2	2	1	0
A. collaris	0	2	0	4	0	3
T. schwarzianum	0	0	0	0	0	0
P. approximatus	0	0	0	0	0	0
Otiorhynchus spp.	0	0	0	0	0	0
R. inquisitor	0	0	0	0	0	0
Ips spp.	1	189	0	453	0	136

Table 18. (cont'd.)

Study Site 2; 21.3-24.4 m trees

	Portion of Tree					
	Lower		Middle		Upper	
Taxon	Pos	Neg	Pos	Neg	Pos	Neg
M. scutellatus	2	1	31	5	50	3
M. carolinensis	0	0	0	0	0	3
N. pusillus	3	60	2	29	1	49
A. collaris	0	1	0	4	0	10
T. schwarzianum	0	19	0	20	0	6
P. approximatus	1	46	0	109	0	176
Otiorhynchus spp.	0	2	0	0	0	2
R. inquisitor	0	0	0	0	0	1
Ips spp.	0	380	0	338	0	176

collected from one tree cut in 1983 and caged in 1984.

Monthly insect emergences from site 1 bolts (Table 19) show that M. scutellatus emerged in June from all sections of trees but most emerged from 3 m-7.5 m bolts. The bottom 1.5 m and top 1.5 m had the fewest beetle emergences. M. scutellatus was the only insect that emerged from the bottom 1.5 m sections of trees. The tree sections with the highest emergences of M. carolinensis were the fourth 1.5 m sections.

In bolts from site 2 only 3 specimens of either Monochamus species were collected from the bottom 1/3 of trees. The 3 M. carolinensis that emerged from red pines of site 2, Herrington Manor, were from the top 1/3 of trees (Table 20). Most of the N. pusillus emerged from the bottom and top thirds of the trees.

M. scutellatus and M. carolinensis emergence dates during 1984 (Table 21) differed in several ways. M. scutellatus emerged 6 days before M. carolinensis. Before the first emergence of M. carolinensis, 40.5% of M. scutellatus had emerged. By the peak emergence date of M. carolinensis, June 19, 64% of M. scutellatus had emerged. The peak emergence date for M. scutellatus was June 11. The data show that 99.5% of M. scutellatus emerged in June but only 45.2% of M. carolinensis emerged in June. The greatest emergence of M. carolinensis (54.8%) occurred in July.

A total of 92 specimens of Monochamus sp. were dissected and examined for B. xylophilus. Most B.

Table 19. Monthly insect emergence by 1.5 m log section, August 1984. Study site 1, 7.6-9.1 m red pines. Trees cut in summer 1983.

Taxon	Section of stem, upward from base								
	1.5 m			3.0 m			4.5 m		
	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
M. scutellatus	4			24			39		
M. carolinensis					2		1	1	
N. pusillus					2				
A. collaris								1	
Otiorhynchus spp.				1					
	6.0 m			7.5 m			9.1 m		
M. scutellatus	16			23			5		
M. carolinensis	4	11		7	1		2		
N. pusillus	6	1		3			1		
A. collaris	1	1		3		1			
Otiorhynchus spp.				2					

Table 20. Monthly insect emergence by bottom, middle and top thirds of tree stems sampled during summer 1984. Study site 2, 21.3-24.4 m red pine; cut in summer 1983.

	Bottom 1/3				Middle 1/3				Top 1/3			
Taxon	May	Jun	Jul	Aug	May	Jun	Jul	Aug	May	Jun	Jul	Aug
M. scutellatus		3				41				54	1	
M. carolinensis											3	
N. pusillus		47	1	1		22	2			47	2	
A. collaris				1			1	3			3	5
Otiorhynchus spp.			1	2				1			1	2
R. inquisitor	5				1				1	1		
T. schwarzianum		2	5			1	1					
P. approximatus			1									

Table 21. Peak emergence dates for *Monochamus scutellatus* and *Monochamus carolinensis* from red pine in western Maryland.

	Monochamus scutellatus			*Monochamus carolinensis*			
	Study Sites			Study Sites			
Date	1	2	Sub-Total	1	2	Sub-Total	Total
June 6	1		1				1
7	22	4	26				26
8	4		4				4
9	17	5	22				22
11	31	13	44a/				44
12	3	6	9	1		1	10
13	7	4	11	1		1	12
14	5	13	18				18
18	5	14	19				19
19	3	3	6	4		4b/	10
20	3	5	8	3		3	11
23	2	14	16	1		1	17
25	1	4	5				5
26	4	4	8				8
27	1		1	1		1	2
28	1	2	3	3		3	6

Table 21. (cont'd.)

	Monochamus scutellatus			*Monochamus carolinensis*			
	Study Sites			Study Sites			
Date	1	2	Sub-Total	1	2	Sub-Total	Total
July 2		1	1	1	1	2	3
3				3		3	3
7							
11				1		1	1
12				1		1	1
16				3		3	3
17				1		1	1
21				3	1	4	4
23							
25							
27				1	1	1	
31				1		1	1

a/ Peak emergence date of *Monochamus scutellatus*.

b/ Peak emergence date of *Monochamus carolinensis*.

xylophilus were recovered from the meso-metathoracic regions but head, prothorax, abdomen and leg tracheae also contained dauerlarvae. Eighty-four percent of M. scutellatus and 77% of M. carolinensis carried dauerlarvae in their meso-metathoracic regions. The area with fewest tracheal B. xylophilus was the head (35%). Rhabditids were in the tail tip in 83% of the insects dissected (Table 22).

In the 26 Monochamus sp. specimens dissected to determine actual numbers of dauerlarvae per body part, the highest number of B. xylophilus was 15,158 found in the meso-metathorax (mean number in this area was 2,918 dauerlarvae). The greatest number of B. xylophilus for any insect for the head was 101; prothorax, 329; and abdomen, 1,172. Only dauerlarvae of B. xylophilus were seen in the tracheal system. Insect tail tips averaged 210 recovered rhabditids (Table 23).

Tunnels made in red pine by Monochamus larvae were measured (Table 24). The average total distance mined was 53.9 mm. Entry hole directions were both clockwise and counterclockwise. One larva was parasitized probably by an ichneumonid.

An ichneumonid species emerging from red pines was identified as Dolichomitus tuberculatus tuberculatus (Geoff.). Two prevalent species of deutonymph mites found on Monochamus sp. were Dendrolaelaps isodentatus (Hurlbutt) and Trichouropoda hirsuta Hirshmann. Several deutonymph specimens were identified as D. armatus Hirschmann.

Table 22. Occurrence of Bursaphelenchus xylophilus per body part per tree section for Monochamus spp.and occurrence of rhabditids.

Monochamus scutellatus

Study Site 1	Head		Thorax				Abdomen		Rhabditids	
			Pro		Meso-Meta					
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
0-1.5 m	3	1	3	1	4	0	4	0	3	1
1.5-3.0 m	0	3	1	2	3	0	0	3	3	0
3.0-4.5 m	5	1	6	0	6	0	4	2	2	4
4.5-6.0 m	3	1	3	1	4	0	3	1	2	2
6.0-7.5 m	2	4	5	1	6	0	6	0	4	2
7.5-9.1 m	0	1	0	1	1	0	0	1	0	1
Miscellaneous	0	4	1	3	4	0	4	0	1	3
Study Site 2										
Lower	1	1	1	1	2	0	1	1	1	1
Middle	3	12	3	12	9	6	4	11	11	4
Upper	3	9	2	10	9	3	2	10	8	4
% Positive	35		44		84		49		61	

Table 22. (cont'd.)

<u>Monochamus</u> <u>carolinensis</u>

Study Site 1	Head		Thorax				Abdomen		Rhabditids	
			Pro		Meso-Meta					
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
0-1.5 m	1	3	2	2	3	1	2	2	3	1
1.5-3.0 m	0	2	0	2	2	0	2	0	2	0
3.0-4.5 m	1	1	1	1	2	0	1	1	0	2
4.5-6.0 m	0	11	0	11	8	3	5	6	9	2
6.0-7.5 m	4	7	4	7	8	3	8	3	10	1
7.5-9.1 m	1	0	0	1	1	0	1	0	1	0
Miscellaneous	0	4	0	4	3	1	2	2	4	0
% Positive	20		20		77		60		83	

Table 23. Distribution of Bursaphelenchus xylophilus dauerlarvae in Monochamus spp. by body part and by height in tree and occurrence of rhabditids.a/

Monochamus scutellatus

Tree Section	Head	Thorax		Abdomen	Rhabditids
		Pro	Meso-Meta		
0-3.0 m	4	40	726	101	400
	101	329	2,976	1,172	+
	46	104	309	457	-
	0	0	6,760	3	23
3.0-6.0 m	30	144	2,227	1,084	1,265
	0	129	15,158	411	+
	0	0	1,037	3	+
6.0-9.1 m	0	0	270	36	-
	2	23	1,200	9	532
	0	69	822	934	33
	0	7	5,269	20	+
Miscellaneous	0	18	6,760	3	23

Table 23. (cont'd.)

Monochamus carolinensis					
0-3.0 m	0	0	34	0	+
	2	3	9,253	3	40
	0	0	0	6	-
3.0-6.0 m	0	0	0	0	+
	0	0	0	124	+
	0	0	1,037	3	+
6.0-9.1 m	0	0	0	0	+
	0	0	0	0	+
	3	0	7,020	3	+
	0	2	10,028	4	+
	4	2	1,808	15	+
	0	0	0	0	+
Miscellaneous	0	0	0	0	+
Av. Nema/ Body Part	7.7	34.8	2,918	176.8	210.5

a/ The symbols (+) and (-) indicate the presence or absence of nematodes when actual counts were not made.

Table 24. Measurement from dissection of *Monochamus* sp. larval tunnels in one red pine , decadence category 5, site 1, cut summer 1983, dissected March 1984.

	Larva:		Total	Mine Terminal Point		
	Present	Entry	Dist.	Vert.		Tangential
Mine	Yes	direc-	mined	from	from	centrally
no.	No	tion a/	mm	entry	horiz.	
1	yes	clock	32	2 neut	3 neut	32
2	yes	counter	82	76 down	0 neut	25
3	yes	counter	74	52 down	9 rt.	15
4	no	clock	61	3 down	11 rt.	41
5b/	no	clock	35	3 neut	9 rt.	12
6	no	counter	30	16 down	11 lt.	20
7	yes	counter	63	23 up	3 rt.	10

Table 24. (cont'd.)

Mine no.	Farthest mine distance from entry pt. (mm) c/ vertical	from horizontal	central
1	22 down	2 lt.	32
2	76 down	13 rt.	34
3	52 down	12 rt.	21
4	11 down	10 rt.	41
5	0 neut	11 rt.	15
		17 lt.	
6	16 down	10 rt.	20
7	30 up	13 rt.	21
		0 lt.	

a/ Clockwise or counter-clockwise entry, looking down from above.

b/ Mine number 5 had a parasitized larva.

c/ Horizontal and vertical distances from tangential described as up, down, right, left and neutral.

DISCUSSION

In western Maryland red pine plantations established during the 1940's, such as Herrington Manor, have exhibited decline and mortality since the early 1960's. Dying trees of Herrington Manor were scattered in clumps. In the 1980's new areas of decline and mortality were detected in Compartment 42 of the Savage River State Forest, site 1 of this study. About 10-15 acres were diseased in this plantation of approximately 100 acres established in the 1950's-1960's. The disease in Compartment 42 seemed to have spread radially from a centralized point with more trees dying each year.

At the beginning of this study, the sampling of 12 declining, ca. 47-year-old red pines with a single increment boring detected no B. xylophilus although their presence was probable. Early in this study it became evident that increased knowledge of nematode distribution in large red pines was a necessary prerequisite to further investigation. In Japanese pine seedlings apparently B. xylophilus rapidly moved from inoculation sites throughout the trunk, branches and roots in some studies, so that nematodes were evenly distributed throughout the seedlings (Mamiya and Kiyohara 1972, Mamiya 1984). But, conversely, in older Scotch and Austrian pines, nematodes were frequently found localized rather than distributed through trees (Malek and Appleby 1984).

Diseased, declining and dying red pines examined for pinewood nematode showed that in the older stand of this study stand (ca. 47-year-old trees, site 2) 76.5% of the trees were infected by *B. xylophilus* whereas in the younger stand (13- to 25-year-old trees, site 1) 68% of the trees were infected. Also numerous trunk, and primary and secondary branch samples from declining, dying red pines in western Maryland showed that *B. xylophilus* was not evenly distributed in these trees. Older tree trunks had a progressive increase in the population density of the pinewood nematode from bottom to top of the tree whereas the nematode was more evenly distributed throughout the trunks of younger trees. Apparently in older trees it may take longer for *B. xylophilus* to appear in the lower portion of the trunk after initial infection (Malek and Appleby 1984). Trunk terminals in older red pines had more *B. xylophilus* (23.5%) than younger trees (12.5%).

Four of the five tree decadence categories of red pines, all but the bleached-green needle category, were observed early in spring. The fifth category, bleached-green needled red pines, were not observed until July and August. Similarly, in Scotch pines symptoms of light grayish green needles occurred in summer during warm or hot periods (Malek and Appleby 1984).

In young red pines percentages of infected samples varied among the decadence categories. Bleached-green needled trees contained the highest percentages of infection by *B. xylophilus* followed by reddish needled trees.

Progression of pinewilt symptoms in Scotch pines proceeds similarly (Malek and Appleby 1984). Reddish needled trees had more *B. xylophilus* than green needled trees possibly relating to the length of time since infection by *B. xylophilus*. Reddish needled trees were probably originally bleached-green needled trees dying in summer-fall which lose their needles the summer of the following year. Dead, barkless, needleless, young red pines had small percentages of infection by *B. xylophilus*. Apparently, populations of *B. xylophilus* may increase with the progression of the disease but as soon as water content decreases population density starts to decrease (Futai 1980). This seems to be further supported in standing Scotch pines dead up to 3 years in which pinewood nematode population densities decreased (Malek and Appleby 1984).

By tree category per total samples, the older trees of study site 2 had higher percent samples infected by *B. xylophilus* in tree category 1, (mostly green needled) followed by category 4 (lacking needles and bark) than bleached-green needled trees. These results are difficult to explain. Green needled trees are thought to be diseased a shorter length of time than reddish needled pines and therefore one would expect fewer *B. xylophilus* in the former trees. It was expected that the barkless category 4 of older red pines would exhibit more complete infection than other categories since trees in this category apparently had been infected the longest. Since population density of *B.*

xylophilus is dependent on water content of trees an adequate supply of water was also assumed. Population density and distribution of B. xylophilus may also depend on how many Monochamus spp. feed on the tree and where feeding occurs plus the number of nematodes carried by the insect. Monochamus sp. may also be attracted to trees in one category in preference to trees in another just as this insect has certain pine feeding preferences in the laboratory (Walsh and Linit 1985). Some older pine trees appeared to be dead at the tops while alive in lower portions. Large trees may thus represent several different disease and dying states introducing additional factors which could influence the distribution of B. xylophilus populations.

Older and younger red pines differed as to the percentage infection in primary branches per decadence category. Primary branches of older trees in the green needled category had a higher percentage infection (24.1) than younger trees (6.3) in the same category. Branches of younger trees were found in other studies to have small percentages of infection. In 20-year-old Scotch pines, B. xylophilus was frequently absent from branch wood at any stage of symptom development in spring mortalities (Malek and Appleby 1984).

Primary branch base samples (butts) from older and younger red pines had higher incidences of B. xylophilus infection than other areas of branches. Primary and secondary branch terminals had a very low percentage infection (1.3) especially in older red pines (1.3). Again, low water content of these branch parts may have suppressed the incidence of B. xylophilus.

The percentage infection of trunk discs of both old and young red pines were similar. However, the distribution of B. xylophilus in the trunk radials (horizontal distribution) were different in old red pines (19.9%) versus young red pines (34.6%). Perhaps the difference in trunk diameters between these two groups of trees were responsible for this difference, or perhaps the percentage infection by the nematode is related to the position of Monochamus spp. tunnels. In trunks with small diameters Monochamus spp. larvae may tunnel to the center of the trunk but not in trunks of large diameter.

B. xylophilus found in trees were adults and larvae. In red pines some samples contained only larvae and others contained molting larvae in different developmental stages. Percentages of adult vs. larval stages in tree samples has been studied in Japan (Ishibashi and Kondo 1977). Malek and Appleby (1984) observed only the dispersal larvae in the lower trunks of old Scotch pines.

This investigation showed that some green needled trees of category 1, 3 and 4 lacked B. xylophilus but contained the unidentified species of Aphelenchoidea and Ips spp. indicating that death of these trees was not caused by B. xylophilus. There are other reports of declining western Maryland red pines exhibiting infestations with Ips beetles and the fungus, Armillaria melea. Symptoms similar to pinewilt are also found in trees suffering from Diplodia dieback. Large numbers of an Aphelenchoides sp. thought to feed on fungi were recovered from branches of blighted Austrian pines (Malek and Appleby 1984).

In western Maryland Scotch pines (7 to 10 years old and 20 to 25 years old) and red pines (ca. 20 years old and 25 years old) developed no symptoms of pinewilt after inoculation with B. xylophilus. In Wisconsin and Minnesota red pines, jack pines and Austrian pines were inoculated with 40,000 nematodes per tree during late spring with no apparent damage to trees (Wingfield et al. 1984). Perhaps timing of the inoculation with state of development of candles and needles caused this failure of infection as was attributed to inoculated 20-year-old Scotch pines in Illinois (Malek and Appleby 1984).

All of the bleached-green needled trees of both old and young red pines had Monochamus spp. emergences. Since the trees of all decadence categories collected in late April, May and early June of 1982 contained no Monochamus spp., apparently these trees lacked oviposition exposure before being caged. Future investigations might further compare categories of diseased trees and seasonal timing of death for preference by Monochamus spp..

Insects associated with red pines infected with B. xylophilus represented orders Coleoptera, Hymenoptera and Diptera, primarily collected from May to September. Four families of Coleopterans reared from red pine bolts included 6 species of Cerambycidae, 3 species of Curculionidae, 2 species of Scolytidae and 1 species of Buprestidae. Those insects phoretically capable of transmitting B. xylophilus to red pines included primarily two species, M. scutellatus and M. carolinensis. In Missouri and Illinois the primary vector of the pinewood nematode was only one species, M. carolinensis, and the tree host studied was Scotch pine (Linit et al. 1983, Appleby 1982). Western Maryland probably represents an overlap in range of these two species of woodborers. It is not unusual to have two species living together, as in eastern Canada both M. scutellatus and M. notatus (Drury) live in the same host (Gardiner 1954).

The average number of B. xylophilus dauerlarvae per individual Monochamus spp. was low in comparison to findings in other Monochamus species (Mamiya and Enda 1972, Linit et al. 1983). Monochamus species on red pines may contain fewer dauerlarvae in their tracheal systems because red pines are not as favorable a substrate for the nematode as certain other pines. The Monochamus species containing large numbers of B. xylophilus were reared on very susceptible pines, i.e., Japanese red and black pines and Scotch pines. Other factors relating to numbers of B. xylophilus in these beetles may include: (1) feeding preference of Monochamus (Walsh and Linit 1984); (2) high reproductive rate of B. xylophilus in certain pines (Futai 1980); and (3) elevation, as damage to trees by pinewilt infection was not evident above 700 m in

Japan (Mamiya 1984).

In red pines 96% of Monochamus scutellatus carried the pinewood nematode whereas for M. carolinensis it was 90%. In Missouri 94% of the M. carolinensis carried the nematode (Linit et al. 1983).

Monochamus spp. collected from red pines had B. xylophilus dauerlarvae in the tracheae of head, pro-, meso-, and metathorax, and abdomen but dauerlarvae were most prevalent in the meso-metathoracic area. Similarly in Japan and in Missouri nematodes were most prevalent in the thoracic tracheae (Mamiya and Enda 1972, Linit et al. 1983). The present study did not differentiate between the mesothorax and metathorax but most of the dauerlarvae seemed to be associated with the metathoracic spiracle and connected tracheae as was found in Monochamus alternatus (Mamiya and Enda 1972).

Notable differences between the Missouri study (Linit et al. 1983) and this one included (1) occurrence here of two Monochamus species; (2) the occurrence of N. pusillus in western Maryland versus its absence in Missouri and the presence of the pinewood nematode in N. pusillus in this study; (3) the occurrence of Amniscus collaris only in Maryland and Amniscus sexguttatus (Say) only in Missouri; and (4) lack of B. xylophilus in Amniscus collaris in Maryland but presence of B. xylophilus in Amniscus sexguttatus in Missouri. C. scabripennis emergences were few probably because oviposition occurred after late July-early August and only three trees studied were exposed to its oviposition during this time.

Monochamus scutellatus in young red pines emerged from every section of the red pine trunk although few emerged from the bottom and top 1.5 m sections. In older, taller red pines of this study the bottom 1/3 (ca. 8 m) of the tree had few Monochamus spp. emergences. Only three Monochamus carolinensis emerged from the older pines and these emerged from the upper 1/3 of the trees. It appears that Monochamus carolinensis may attack smaller trees or smaller parts of large trees preferentially. However, Walsh and Linit (1985) found that the thickness of the bark was not correlated to oviposition density by M. carolinensis. In 20-year-old Scotch pines in Missouri M. carolinensis was not collected from the base of tree trunks up to 3 m above ground but in 7- to 11-year-old pines entire trunks were attacked by this insect (Linit et al. 1983). Monochamus carolinensis oviposition occurred in the middle to upper portions in Scotch pines (Walsh and Linit 1985).

Most M. scutellatus emerged before M. carolinensis, indicating that their oviposition might occur earlier than for M. carolinensis. The late July-early August transfer of trees from the field into cages and earlier oviposition of M. scutellatus may also account for larger numbers of M. scutellatus recovered. Trees lying in the field all winter before caging exhibited about equal emergences of M. scutellatus and M. carolinensis supporting the suggestion of late oviposition by the latter beetle.

Field emergence of sawyers in western Maryland occurred during June-July with development requiring only ca. 11 months. In Ontario, Canada, emergence of M. scutellatus from balsam firs occurred in June-July but development required 23 months (Rose 1957).

M. scutellatus emerged from caged red pine bolts and a single pair was observed to mate immediately. The pair was placed in a bottle with fresh red pine twigs, and were observed mating and feeding. Eventually the antennae and legs of both insects were torn from their bodies. After death the female was dissected and eggs were present. There is evidence that some M. scutellatus do not require maturation feeding and that oviposition could occur if suitable substrate is available (Gosling 1981). However, an obligatory sexual maturation period upon emergence for Monochamus spp. has been reported (Linsley 1959). The percentage of Monochamus sp. adults which are able to oviposit immediately after emergence, not requiring sexual maturation feeding, is unknown. These and other behavioral features of Monochamus sp. should be further investigated. B. xylophilus were observed to leave the insect pair while kept in the feeding jar.

There was some evidence that hybridization of M. scutellatus and M. carolinensis might be occurring in western Maryland as small apical elytral spines were observed on some M. scutellatus. In Monochamus, males of one species have been reported in attendance upon females of another and behave as one population, occurring on the same host at the same time and place (Linsley 1959). Hybridization in these species has been indicated (T. J. Spilman, pers. comm.).

This is the first time Monochamus sp. larvae have been observed infested with B. xylophilus. The occurrence of B. xylophilus larvae on the abdominal tip of Monochamus spp. larvae is unusual in that rhabditids usually occur on the abdominal tip in the adult sawyer. Hundreds of rhabditids were on the Monochamus spp. larvae just behind the head capsule. In Japan nematode dauerlarvae occurred only on adults just after they emerged from pupae (Mamiya 1984). These preliminary results suggest that further investigation into nematode-insect larvae associations is needed.

Several ichneumonid species emerged from red pines in western Maryland. Only one D. tuberculatus tuberculatus (Geoff.) was a parasitoid of Monochamus sp. (V. K. Gupta, pers. comm.). Several deutonymph mites were found on Monochamus spp. Of these D. isodentatus and T. hirsuta were the most numerous. One mite species, possibly D. isodentatus, was often found in the metathoracic spiracles and possibly was consuming nematodes. Preliminary investigation indicated that D. isodentatus might be a predator of other acarines (D. N. Kinn, pers. comm.).

In conclusion, B. xylophilus was not evenly distributed in the large red pines of this study. Because of this nematode distribution, at least in some stands, survey accuracy may depend upon sampling technique. For best results in rearing Monochamus spp. diseased trees (bleached-green needles) should be cut starting in late summer or until weather prohibits collections in the fall allowing adequate time for ovipositions. As soon as woodborings are noticed on the ground or ground cover or if the chewed depressions of oviposition are observed on tree trunks, Monochamus spp. rearings are possible (Fig. 9). Further investigations are needed to determine the primary disease agents of red pines and other pines in Maryland.

Figure 9. Woodborings by Monochamus spp. on tree trunks and understory vegetation.

APPENDIX

Appendix A. Occurrence of Bursaphelenchus xylophilus (Bx) per total number of samples in red pine in base samples of primary branches a/ by trunk interval by tree decadence category b/ and by branch foliage condition c/ at site 1.

Decadence Category	Trunk Interval in Height (m) 0-3	3-6	6-9	9-12	12-15	Total	% Bx
I Needles Green (Tree)							
Branch Condition							
1.Green Needles	-	1/3	2/6	0/1	-	3/10	30.0
2.Reddish Needles	-	0/4	1/5	0/1	-	1/10	10.0
3.No Needles	0/6	0/5	0/3	-	-	0/14	0
II Needles Reddish (Tree)							
Branch Condition							
2.Reddish Needles	-	1/2	1/3	1/3	-	3/8	37.5
3.No Needles	1/3	1/3	1/3	0/3	-	3/12	25.0

Appendix A. (cont'd.)

Decadence Category	Trunk Interval in Height (m) 0-3	3-6	6-9	9-12	12-15	Total	%Bx
III No Needles But Bark (Tree)							
Branch Condition							
3.No Needles	0/3	1/4	0/4	0/1	-	0/12	0
IV No Needles; No Bark (Tree)							
Branch Condition							
3.No Needles	0/4	0/4	0/3	-	-	1/11	9.0
V Bleached Needles (Tree)							
Branch Condition							
1.Green Needles	0/1	-	0/2	0/2	-	0/5	0
2.Reddish Needles	-	0/1	1/4	0/1	-	1/6	16.7
3.No Needles	0/7	0/8	0/5	1/3	-	1/23	4.3
5.Bleached Needles	-	1/3	4/7	2/5	0/1	7/16	43.8
Total Bx/Samples	1/24	5/37	10/45	4/20	0/1	20/127	
% Bx	4.2	13.5	22.2	20.0	0	15.7	

a/ Primary branches defined as in Table 1.

b/ Tree decadence categories defined as in Table 1.

c/ Branch condition defined as in Table 3.

Appendix B. Comparisons of older (ca. 47-year-old) and younger (13- to 25-year-old) red pines as percent *Bursaphelenchus xylophilus* infection by trunk radial samples a/.

Part infected	Percent infected	
	Older	Younger
Trunk radial samples		
0-5	44.4	30.2
6-10	30.4	38.2
11-15	26.8	40.0
16-20	28.6	29.0
21-25	11.7	26.0
26-30	10.2	
31-35	5.4	
36-40	4.0	
41-45	0.0	
46-50	0.0	

a/ Trunk radial samples defined as in Table 1.

LITERATURE CITED

Appleby, J. E. 1982. Insects associated with the pinewood nematode in Illinois. Pp. 84-85. Proc. Nat. Pine Wilt Disease Workshop, ed. J. E. Appleby and R. B. Malek. Ill. Nat. Hist. Surv., Champaign.

Dekker, R. A. 1981. Maryland Forest Pest Report. Vol. 4, No. 2.

Dropkin, V. H. and A. S. Foudin. 1979. Report of the occurrence of Bursaphelenchus lignicolus-induced pine wilt disease in Missouri. Plant Dis. Rep. 63(11):904-905.

Dropkin, V. H., A. Foudin, E. Kondo, M. Linit, M. Smith and K. Robbins. 1981. Pinewood nematode-a threat to United States forests? Plant Dis. 65(12):1022-1027.

Futai, K. 1980. Population dynamics of Bursaphelenchus lignicolus (Nematoda: Aphelenchoididae) and B. mucronatus in pine seedlings. Appl. Entomol. Zool. 15(4):458-464.

Gardiner, L. M. 1954. differential growth as evidence of the relationship of Monochamus notatus (Drury) and Monochamus scutellatus (Say) (Coleoptera:Cerambycidae) Can. Ent. 86:465-470.

Gosling, D. C. L. 1981. Ecology of the Cerambycidae in a Southern Michigan Woodland. Ph. D. Thesis. University of Michigan. 142 pp.

Holdeman, Q. L. 1980. The pinewood nematode (Bursaphelenchus lignicolus Mamiya and Kiyokara, 1972) and the associated pine wilt disease of Japan. Prepared for the Depart. of Food and Agri. and the Agri. Commissioners of California. Sacramento, CA. 45 pp.

Ishibashi, N. and E. Kondo. 1977. Occurrence and survival of the dispersal forms of pine wood nematode, Bursaphelenchus lignicolus Mamiya and Kiyohara. Appl. Entomol. Zool. 12(4):293-302.

Kanehori, F. and K. Yasui, S. Ono. 1975. Studies on the ecology and eradication of pine-bark beetles. I. Changes of Bursaphelenchus sp. and adults and larvae of pine-bark beetles in wood. Sci. Rep. Fac. Agr. Okayama Univ. 46:72-78.

Kanehori, F. and K. Yasui, S. Ono. 1976. Studies on the ecology and eradication of pine-bark beetles. II. Emergence time of Monochamus alternatus and effects of insecticide spray. Sci. Rep. Fac. Agr. Okayama Univ. 48:77-83.

Kondo, E., A. Foudin, M. Linit, M. Smith, R. Bolla, R. Winter, V. Dropkin. 1982. Univer. Mo. Agric. Exp. Sta. SR 282.

Kiyohara, T. and Y. Tokushige. 1971. Inoculation experiments of a nematode, Bursaphelenchus sp., onto pine trees. For. Soc. 53:210-218.

Linit, M. J., E. Kondo, and M. T. Smith. 1983. Insects associated with the pinewood nematode, Bursaphelenchus xylophilus (Nematoda:Aphelenchoididae) in Missouri. Environ. Entomolo. 12:467-470.

Linsley, E. G. 1959. Ecology of Cerambycidae. Annu. Rev. Entomol. 4:99-137.

Malek, R., N. R. Nicol, M. C. Shurteleff, R. D. McClary and J. E. Appleby. 1982. Pine wilt disease. Univ. of Ill. Report Plant Disease No. 1104. 9 pp.

Malek, R. B. and J. E. Appleby. 1984. Epidemiology of pine wilt in Illinois. Plant Dis. 68(3):180-186.

Mamiya, Y. 1972. Pine wood nematode, Bursaphelenchus lignicolus Mamiya and Kiyohara, as a causal agent of pinewilting disease. Rev. Plant Protection Res. 5:46-60.

Mamiya, Y. 1976. Pinewilting disease caused by the pinewood nematode, Bursaphelenchus lignicolus. J. Jap. Agric. Res. Q. 10:206-211.

Mamiya, Y. 1984. The pine wood nematode. Pp. 589-626 in W. R. Nickle, ed. Plant and insect nematodes. New York:Marcel Dekker.

Mamiya, Y., and N. Enda. 1972. Transmission of Bursaphelenchus lignicolus (Nematoda:Aphelenchoididae) by Monochamus alternatus (Coleoptera:Cerambycidae). Nematologica 18:159-162.

Mamiya, Y. and T. Kiyohara. 1972. Description of Bursaphelenchus lignicolus n. sp. (Nematoda: Aphelenchoididae) from pine wood and histopathology of nematode-infested trees. Nematologica 18(1):120-124.

Morimoto, K. and A. Iwasaki. 1972. Role of Monochamus alternatus (Coleoptera:Cerambycidae) as a vector of Bursaphelenchus lignicolus (Nematoda:Aphelenchoididae). J. Jap. For. Soc. 54(6):177-183.

Nickle, W. R. 1970. A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda: Tylenchida). J. Nematol. 2:375-392.

Nickle, W. R. 1981. Research on the pine wood nematode in the United States. IUFRO World Congr. Proc. 179(Div.2):269-271.

Nickle, W. R., W. Friedman and T. J. Spilman. 1980. Monochamus scutellatus:vector of pinewood nematode on Virginia pine in Maryland. USDA Coop. Plant Pest Rep. 5:383.

Nickle, W. R., A. M. Golden, Y. Mamiya, and W. P. Wergin. 1981. On the taxonomy and morphology of the pine wood nematode, Bursaphelenchus xylophilus (Steiner and Buhrer, 1934) Nickle 1970. J. Nematol. 13:385-392.

Robbins, K. 1982. Distribution of the pine wood nematode in the United States. Pp. 3-6 in Proc. Natl. Pine Wilt Disease Workshop, ed. J. E. Appleby and R. B. Malek. Ill. Nat. Hist. Surv. Champaign.

Rose, A. H. 1957. Some notes on the biology of Monochamus scutellatus (Say) (Coleoptera:Cerambycidae) Can. Entomol. 89(12):547-553.

Steiner, G. and E. M. Buhrer. 1934. Aphelenchoides xylophilus, n. sp., a nematode associated with blue-stain and other fungi in timber. J. Agri. Res. 48(10):949-951.

Tokushige, Y. and Kiyohara, T. 1969. Bursaphelenchus sp. in the wood of dead pine trees. J. Jap. For. Soc. 51:193-195.

Walsh, K. D. and M. J. Linit. 1984. Feeding preferences of the adult pine sawyer, Monochamus carolinensis (Coleoptera:Cerambycidae), for four pine species. Environ. Entomol. 13:1164-1166.

Walsh, K. D. and M. J. Linit. 1985. Oviposition biology of the pine sawyer, Monochamus carolinensis (Coleoptera: Cerambycidae) Ann. Entomol. Soc. Am. 78:81-85.

Wingfield, M. J., R. A. Blanchette and T. H. Nicholls. 1984. Is the pine wood nematode an important pathogen in the United States? J. For. 82(4):232-235.

Williams, D. J. 1980. Forest and shade trees - diseases. Coop. Plant Pest Rep. 5(33):627.