Journal of Nematology 18(4):575–580. 1986.


# Pinewood Nematode, *Bursaphelenchus xylophilus*, Associated with Red Pine, *Pinus resinosa*, in Western Maryland[1]

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*Abstract:* Red pines *Pinus resinosa* in Garrett and Allegany counties, Maryland, were examined during 1982–84 to determine distribution of the pinewood nematode, *Bursaphelenchus xylophilus*, within and among trees. Approximately 25-year-old (younger) and 47-year-old (older) trees were subdivided into the following categories: 1) trees with mostly green needles; 2) trees with mostly reddish-brown needles; 3) trees lacking needles but with bark intact; 4) trees lacking both needles and bark; and 5) trees with chlorotic, bleached-green needles. *Bursaphelenchus xylophilus* was found infecting 68% of younger red pines and 77% of older red pines. Nematodes were not evenly distributed in trees within any given tree decadence category or in trees of the same age. Nematodes were recovered from 20% of wood samples from trunks and primary and secondary branches in younger pines and from 15% of older red pines. On the basis of tree decadence category, the highest incidence of infection in younger trees (31%) was in bleached-green needled trees (category 5), whereas in older trees the highest infection (25%) occurred in green needled trees (category 1). At both sites trunks were infected more often than branches.

*Key words: Bursaphelenchus xylophilus,* pinewood nematode, pinewilt disease, *Pinus resinosa,* red pine.

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970, is associated with severe pinewilt disease in Japan in Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) and Japanese black pine (*Pinus thunbergii* Parl.). In the United States pines may vary in susceptibility to pinewood nematode infestations. Scotch pine (*Pinus sylvestris* L.), especially those growing as ornamentals, appear to be highly susceptible. *Bursaphelenchus xylophilus* has been isolated from dying red pines (*Pinus resinosa* Ait.) from several states, including Maryland (8). Red pines showing symptoms of pinewilt disease are usually under stress, such as drought or off-range planting.

Recently, this nematode has been responsible for a loss of millions of dollars worth of wood chips exported from the United States to Scandinavia (9). People are becoming more aware of *B. xylophilus* damage to ornamental and forest coniferous trees, both direct and indirect, as more scientists are able to diagnose this nematode problem.

Received for publication 11 February 1986.

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In previous studies, pine seedlings have been used to determine pinewood nematode distribution within trees. Histological sections revealed concentrations of nematodes in axial and radial resin canals, in the bark, and, in lesser numbers, in various other parts of pine seedlings (1,7). Though Malek and Appleby (5) referred briefly to locations of nematodes in various parts of field-grown Scotch pines of different sizes in Illinois, the distribution of pinewood nematodes in older field-grown trees has received little attention. Our objective was to study in field-grown red pine the occurrence and distribution of pinewood nematodes 1) within pines of two age groups and 2) among five pinewilt disease categories.

## Materials and Methods

Two off-range planted red pine plantations suffering from decline and mortality for several years were chosen for study in the two westernmost (Appalachian) counties of Maryland. One of these, Savage River State Forest Compartment 42 (Allegany County, site 1, elevation 792 m), consisted of young trees, ca. 25 years old. The other, Garrett State Forest (Garrett County, site 2, elevation 731.2 m), consisted of older trees, ca. 47 years old.

During 1982–84, 25 trees from site 1 and 17 trees from site 2 were felled for sampling. Three to five trees were selected from each of the following tree decadence

categories: 1) predominantly green needled with some reddish-brown needles present; 2) predominantly reddish-brown needled; 3) no needles but with bark; 4) lacking needles and bark; and 5) bleached-green needled. Diameter at breast height (DBH), length of each tree, and tree age were recorded. Trees were felled 25.5 cm from ground with 5-cm disks (trunk disk samples) taken every 3 m from stump to top of tree. A 3-cm-long terminal sample was also taken from trunks. Primary branches, defined as limbs growing from the trunk, were sampled at 3-m intervals the length of the trunk, and secondary branches, defined as growing from primary branches, were sampled at 1.5-m intervals the length of the primary branch. Branch selections were based on predominant foliage coloration, lack of foliage, or lack of both foliage and bark. Disk samples (3 cm long) were cut from the base (butt) and at 1.5-m intervals the length of each branch. Terminal samples were taken from each primary and secondary branch. All samples were placed in airtight plastic bags for transport to the laboratory. Samples were freed of bark by surfaces being scraped or trimmed, rinsed, then soaked for at least 24 hours in tap water. Additionally, trunk radial (horizontal) samples were prepared by sectioning trunk disks at 5-ring intervals from perimeter to center. If samples could not be examined immediately for *B. xylophilus*, they were fixed in 3% formaldehyde for later examination. The occurrences of other nematodes, protozoans, rotifers, and blue-stain fungi were also recorded. Percentages of samples having nematodes are rounded to nearest whole numbers.

## Results

The 25 medium-sized, younger red pines from site 1 were 7.5–12.5 m tall with DBH of 10.4–16.9 cm. The larger, older red pines of site 2 were 21.3–24.4 m tall with DBH of 22.9–34 cm. Seventeen of the 25 trees sampled in site 1 (68%) and 13 of 17 (77%) in site 2 were positive for *B. xylophilus* in at least one sample (Table 1). More trunk disks were positive for *B. xylophilus* than primary and secondary branches (Table 1).

By tree decadence category, site 1 had the most total samples positive for *B. xylophilus* in trees with mostly reddish-brown needles (27%) and in trees with bleached-green needles (31%), whereas site 2 had the most total samples positive in trees with mostly green needles (25%) and in trees lacking both needles and bark (19%) (Table 2). Site 2 had more positive trunk disk samples in trees with mostly green needles (81%) and in trees lacking both needles and bark (62%), whereas site 1 had more positive trunk disk samples in trees with bleached-green needles (58%) than in trees in other categories (Table 3). At site 1, more butt branch samples were positive for *B. xylophilus* than other limb areas. Also more branches with bleached-green needles at site 1 were positive than branches with foliage in other conditions.

More primary branch samples were positive for *B. xylophilus* in three tree decadence categories—trees with mostly green needles, trees with primarily reddish-brown needles, and trees with bleached-green needles—than in the other two categories.

Table 1. Average percentage of samples positive for *Bursaphelenchus xylophilus* in red pine for site 1* (25 medium-sized, younger pines) and site 2 (17 large, older pines).

| | | Trunk† | | | Branches | |
|---|---|---|---|---|---|---|
| | Whole tree | Disks | Radials | Terminals | Primary‡ | Secondary§ |
| Site 1 | 68 | 38 | 35 | 13 | 11 | 4 |
| Site 2 | 77 | 39 | 20 | 24 | 14 | 7 |

* Site 1, Savage River State Forest (trees ca. 25 years old); and site 2, Garrett State Forest (trees ca. 47 years old).

† Trunk disks were taken at 3-m intervals the length of tree; radials were taken from trunk at 5-ring intervals from perimeter to center; terminals were tips of trees.

‡ Primary branches defined as limbs growing from main stem or trunk.

§ Secondary branches defined as limbs growing from primary branches.

Table 2. Percentages of total samples positive for *Bursaphelenchus xylophilus* in red pines by tree decadence category and by site.*

| Tree decadence category† | No. trees | Site 1 | Site 2 | Total |
|---|---|---|---|---|
| Mostly green needles | 8 | 7 | 25 | 14 |
| Mostly reddish-brown needles | 9 | 27 | 13 | 18 |
| No needles, with bark | 8 | 14 | 12 | 13 |
| No needles or bark | 8 | 6 | 19 | 13 |
| Bleached-green needles | 9 | 31 | 11 | 24 |

* Sites as defined in Table 1.

† Tree decadence category was based on needle and bark condition of individual trees.

TABLE 3. Percentage of samples positive for *Bursaphelenchus xylophilus* in red pine trunks and primary and secondary branches, by tree decadence category and by site.*

| Tree decadence category§ | Trunk† | | | Primary branch‡ | | | Secondary branch | | |
|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Total | Site 1 | Site 2 | Total | Site 1 | Site 2 | Total |
| Mostly green | 13 | 81 | 35 | 6 | 24 | 14 | 4 | 10 | 7 |
| Mostly reddish-brown | 39 | 21 | 28 | 16 | 17 | 17 | 8 | 5 | 6 |
| No needles, with bark | 33 | 5 | 18 | 0 | 10 | 6 | 0 | 16 | 10 |
| No needles or bark | 5 | 62 | 35 | 5 | 2 | 3 | 0 | 6 | 3 |
| Bleached-green | 58 | 42 | 52 | 16 | 11 | 14 | 5 | 1 | 3 |

* Sites as defined in Table 1.
† Trunk samples included disks and terminals.
‡ Primary and secondary branches as defined in Table 1.
§ Tree decadence category as defined in Table 2.

In comparison with other anatomical structures, secondary branches had the lowest percentage of positive samples; however, the highest percentage of positive samples for secondary branches (16% at site 2) occurred in trees in the decadence category lacking needles but having bark (Table 3).

The trend in trunks of older pines (site 2) was an increase in percentage of positive samples from bottom (19%) to top (75%) of trees (Table 4). The upper 20% of trunks in trees of site 1 had fewer positive samples than the lower 80%. More primary branches were positive for *B. xylophilus* at tree heights of 3–6 m and 9–12 m for site 1 and at heights of 6–15 m and 21–24 m for site 2 than branches at other heights. Secondary branches on trees at site 1 had more positive samples (9%) at 0–3-m trunk height, whereas those of site 2 had more positive samples at trunk heights 0–3 m (18%) and 9–12 m (21%) than secondary branches at other heights (Table 4).

In older pines the first (outer) five rings of wood disks had the highest percentage of positive samples (44%) for *B. xylophilus*; the numbers of positive samples decreased toward the center of the trunk with the central 10 rings lacking nematodes. In younger trees all ring intervals were positive, although rings 6–15 had the highest percentage positive samples (38–40%) for *B. xylophilus* (Table 5).

Among all decadence categories at site 1, 11 trees lacked infecting nematodes. Two trees were infected in all but the terminals. Nematodes were recovered from only the basal trunk sample in one tree. Variations in the distribution of infecting nematodes were seen in each decadence category.

An unknown species of Aphelenchoidea was discovered in trees at both sites. Of the 742 samples in site 1, 124 (16.7%) con-

TABLE 4. Percentage of samples positive for *Bursaphelenchus xylophilus* as related to trunk height (in meters) by trunk and primary and secondary branches and by site.*

| Disk samples† | 0–3 | 3–6 | 6–9 | 9–12 | 12–15 | 15–18 | 18–21 | 21–24 |
|---|---|---|---|---|---|---|---|---|
| Trunk | | | | | | | | |
| Site 1 | 40 | 44 | 40 | 36 | 18 | | | |
| Site 2 | 19 | 25 | 38 | 44 | 47 | 50 | 43 | 75 |
| Primary branch | | | | | | | | |
| Site 1 | 2 | 10 | 4 | 15 | 0 | | | |
| Site 2 | 7 | 0 | 23 | 21 | 17 | 12 | 6 | 20 |
| Secondary branch | | | | | | | | |
| Site 1 | 9 | 3 | 4 | 0 | 0 | | | |
| Site 2 | 18 | 0 | 9 | 21 | 13 | 2 | 0 | 0 |
| Total | | | | | | | | |
| Site 1 | 12 | 12 | 14 | 17 | 8 | | | |
| Site 2 | 15 | 7 | 21 | 24 | 17 | 11 | 9 | 31 |

* Sites 1 and 2 as defined in Table 2.
† Trunk and primary and secondary branches as defined in Table 1.

TABLE 5. Percentage of trunk radial samples positive for *Bursaphelenchus xylophilus* in younger (ca. 25 years, site 1) versus older (ca. 47 years, site 2) red pines.

| Trunk radial samples* | Site 1 | Site 2 |
|---|---|---|
| 0–05 | 30 | 44 |
| 6–10 | 38 | 30 |
| 11–15 | 40 | 27 |
| 16–20 | 29 | 29 |
| 21–25 | 26 | 12 |
| 26–30 | | 10 |
| 31–35 | | 5 |
| 36–40 | | 4 |
| 41–45 | | 0 |
| 46–50 | | 0 |

* Trunk radial samples as defined in Table 1.

tained this unidentified nematode. Some trees in decadence category 1 (green needled), category 3 (lacking needles), and category 4 (lacking both needles and bark) contained this unidentified nematode but lacked *B. xylophilus*. Thirty-two percent of all trees from site 1 contained both *B. xylophilus* and the unidentified nematode.

At site 1, in addition to the unidentified aphelench, another aphelenchoid nematode, smaller and with a c-shaped body, was found in some samples. Some tylenchids were found, but in relatively small numbers. One tylenchid was found in 29% of the 742 samples, rhabditid nematodes in 52%, and several other unidentified nematodes in 7%.

Blue-stain occurred in 458 samples (62%) from site 1. Protozoans were found in 39% and rotifers in 18% of the samples.

## DISCUSSION

In the two westernmost counties of Maryland, red pine plantations established during the 1940s, such as Garrett State Forest (site 2), have exhibited tree decline and mortality since the early 1960s. In the 1980s 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 of trees were diseased in this plantation of approximately 100 acres established in the 1950s and 1960s. The disease in Compartment 42 seemed to have spread radially from a central location, with new trees dying annually.

At the beginning of this study, sampling 12 declining ca. 47-year-old red pines with a single increment boring detected no *B. xylophilus*. Early in this study it became evident that increased knowledge of nematode distribution in field-grown pines was a necessary prerequisite to further investigation. In some studies of Japanese pine seedlings, *B. xylophilus* apparently moved rapidly from inoculation sites throughout the trunk and branches, so that nematodes were distributed throughout the seedlings (6,7). Conversely, in older Scotch and Austrian pines, nematodes were frequently found localized rather than distributed throughout trees (5).

Diseased (declining and dying) red pines examined for pinewood nematodes showed that in the older stand of this study (trees ca. 47 years old), 77% of the trees were infected with *B. xylophilus*, whereas in the younger stand (trees ca. 25 years old), 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 numbers of positive samples from bottoms to tops of trees, whereas the nematode appeared more evenly distributed in the lower 80% of trunks of younger trees. Apparently in older trees it may take a longer time after initial infection for *B. xylophilus* to appear in lower portions of trunks (5) than it does in younger trees. Trunk terminals of older red pines had a higher percentage of infection with *B. xylophilus* (24%) than did those of younger trees (13%).

Red pines in four of the five tree decadence categories were observed and samples collected in early spring. The fifth category, bleached-green needled red pines, was not observed or sampled until July and August and was the only tree category containing developing *Monochamus* spp. (Coleoptera: Cerambycidae) larvae. Similarly, in Scotch pines symptoms of light grayish green needles were reported during warm or hot summer periods (5).

Percentages of nematode-infected samples in young red pines varied among tree decadence categories. Bleached-green needled trees contained the highest per-

centages of infection by *B. xylophilus,* followed by reddish needled trees. Progression of pinewilt symptoms in Scotch pines proceeded similarly (5). Reddish needled trees contained more *B. xylophilus* than green needled trees, possibly correlated with the length of time from initial infection with *B. xylophilus.* Reddish needled trees probably were originally green needled trees that died in summer–fall and lost their needles the following summer. Dead, barkless, needleless, young red pines had low percentages of infection with *B. xylophilus.* Apparently, population densities of *B. xylophilus* may increase with the progression of the disease, but as soon as water content decreases, population density also decreases (3). This phenomenon seems to be further supported by the finding that in standing Scotch pines, dead up to 3 years, pinewood nematode population densities decreased (5).

By tree decadence category, the older trees of study site 2 had a higher percentage of samples infected by *B. xylophilus* in tree category 1 (mostly green needled trees) followed by category 4 (trees lacking needles and bark) than in category 5 (bleached-green needled trees). These results are difficult to explain. Because green needled trees are thought to have been diseased for a shorter length of time than reddish needled pines, one would expect fewer, not more, *B. xylophilus* in green needled trees than in the reddish needled pines. Assuming an adequate water supply, one might expect the barkless category 4 of older red pines to exhibit more complete infection than other categories, since trees in this category apparently had been infected the longest. Population density and distribution of *B. xylophilus* may also depend on how many *Monochamus* sp. are attracted to trees to feed and oviposit on trees in one category in preference to trees in another. Some older pine trees appeared dead at the tops but alive in lower portions. Large trees may thus represent several different disease and dying states, introducing additional factors that could influence the distribution of *B. xylophilus* in trees.

Older and younger red pines differed in percentages of infection in primary branches according to tree decadence category. Older trees in the green needled category had a higher percentage of infection (24%) of primary branches than did younger trees (6%) in the same category (Table 3). Branches of younger trees were found in other studies to have low percentages of infection. In 20-year-old Scotch pines, *B. xylophilus* was frequently absent from branch wood at any stage of symptom development in trees that died in spring (5).

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 very low percentages of infection, especially in older red pines (1%). Again, low water content of these branch parts may have suppressed the incidence of *B. xylophilus.*

The trunk disk infection percentages of both old and young red pines were similar. However, the distributions of *B. xylophilus* in the trunk radials (horizontal distribution) of old red pines (20%) and young red pines (35%) were different (Table 1). Perhaps the difference in trunk diameters between these two groups of trees was responsible for this difference in distribution, or perhaps the percentage of infection by the nematode is related to the position of *Monochamus* spp. tunnels. *Monochamus* spp. larvae may tunnel to the trunk center in small-diameter trunks but not in larger trunks.

*Bursaphelenchus xylophilus* found in red pines were both adults and juveniles. Some samples contained only juveniles, and others contained nematodes in different developmental stages. Percentages of nematode adult versus juvenile stages in tree samples were studied in Japan (4). Malek and Appleby (5) observed only the dispersal juveniles in the lower trunks of old Scotch pines.

This investigation demonstrated that some trees of categories 1, 3, and 4 lacked *B. xylophilus* but contained an unidentified species of Aphelenchoidea and *Ips* spp. (Scolytidae: Coleoptera), indicating that death of these trees was not caused by *B. xylophilus.* There are other reports of declining red pines in western Maryland exhibiting infestations with *Ips* spp. beetles and the fungus *Armillaria mellea* (2). Symptoms similar to pinewilt are also found in

trees suffering from Diplodia dieback and large numbers of an *Aphelenchoides* sp. thought to feed on fungi were recovered from branches of Diplodia blighted Austrian pines (5).

In summary we found that nematodes were present in only 15–20% of all wood samples examined and were not uniformly distributed within trees in any given tree decadence category or in trees of the same age. Therefore, one core sampling may not be adequate to determine whether a tree actually has *B. xylophilus.* This study indicates that the best places to sample for this nematode are the trunk and the butts of branches. Most *B. xylophilus*-infected samples occurred in bleached-green needled trees in these off-range planted red pines. Only trees exhibiting bleached-green needles contained currently developing *Monochamus* spp. larvae.

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