Decline and Death of *Pinus* spp. in Delaware Caused by *Bursaphelenchus xylophilus*[1]

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Abstract: Etiological studies to determine the cause of decline and death of *Pinus* spp. in Delaware were initiated in 1980. The pinewood nematode, *Bursaphelenchus xylophilus,* was found to be the major cause of mortality in Japanese black pine (*Pinus thunbergii*). When inoculated into healthy 5-yr-old Japanese black pines, *B. xylophilus* produced typical decline symptoms observed in the field. The xylophilous fungi most often associated with declining trees, *Rhizosphaera pini, Fusarium* spp., and *Pestalotia funerea,* were not pathogenic to Japanese black pine in greenhouse tests. Mineral analyses of soil and foliage showed no significant differences between healthy and infested trees. *B. xylophilus* was also found on loblolly pine (*P. taeda*), scrub pine (*P. virginiana*), Scots pine (*P. sylvestris*), red pine (*P. resinosa*), Eastern white pine (*P. strobus*), and pitch pine (*P. rigida*). *Key words:* pinewood nematode, Japanese black pine.

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Bursaphelenchus xylophilus (Steiner and Buhrer 1934) Nickle 1970 (syn. *B. lignicolus,* Mamiya and Kiyohara 1972), the pinewood nematode, induces a rapid decline and death of several *Pinus* species and other conifers (1,13). The nematode is a problem in Japanese forests as evidenced by epidemics on Japanese black and Japanese red pines (*P. thunbergii* Parl. and *P. densiflora* Sieb. & Zucc., respectively) (5,9). Japanese black pine is planted widely along Delaware beach areas because it stabilizes dunes, is aesthetically attractive, and is tolerant of the coastal environment. Decline and death of Japanese black pine has been observed for 10 yr in Delaware and was generally attributed to poor species adaptation (R. B. Carroll and A. L. Morehart personal communcation). Repeated isolations and tissue analyses failed to yield phytopathogenic agents or fertility problems. Reports of *B. xylophilus* in several states (13) prompted a further evaluation of the problem in Delaware. The pinewood nematode was found on Japanese black pine in the fall of 1980, and the identification was confirmed by Dr. Lorin Krusberg, University of Maryland, College Park (L. Krusberg personal communication). This report summarizes a survey designed to determine the incidence and severity of pinewood nematode in Delaware.

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MATERIALS AND METHODS

To determine the incidence of pinewood nematode, random collections were made in New Castle and Sussex counties from symptomatic pines during a 6-wk period from January to February 1981. Several branches were removed at breast height from each tree. On Japanese black pine sites, needles and soil samples from symptomatic and asymptomatic trees were taken for study. No samples were taken in Kent County because of the limited distribution of *Pinus* spp. in that part of Delaware (4).

Bursaphelenchus xylophilus was extracted from the samples by the Baermann funnel technique (3). After removing the bark, branches were cut into ca. 5-cm^3 pieces. Three replicate 100-cm^3 samples were placed in distilled water in plastic funnels and left overnight. To quantify populations, triplicate 3-ml aliquots were taken from each replicate and nematodes were counted under a dissecting microscope.

Pathogenicity of *B. xylophilus* recovered from survey samples was tested using 5-yr-old Japanese black pines growing at Newark, Delaware. Nematodes were extracted as described and centrifuged in distilled water at 1,000 × g to concentrate inoculum. A 1.0-ml-capacity syringe fitted with a 26-gauge needle was used to introduce ca. 10^3 nematodes/tree into terminal buds of healthy trees. The wounded area was then sealed with Parafilm. Control trees were similarly inoculated with distilled water. Trees were harvested 6 wk after inoculation, divided in 5-cm sections, and nematode determinations were made, as previously described.

To determine the presence of pathogenic

and/or associated micro-organisms, small chips of wood from each sample were surface disinfested in 10% clorox v/v (0.5% sodium hypochlorite) followed by 70% ethanol v/v, rinsed in sterile distilled water, and incubated in the dark at 28 ± 1 C on acidified potato dextrose agar. Fungi most frequently recovered (*Pestalotia funerea*, *Fusarium* sp., and *Rhizosphaera pini*) were tested individually for pathogenicity to 8-wk-old Japanese black pines in the greenhouse. In the first test, fungal inoculum was introduced into steam-pasteurized soil, followed by planting of pine seedlings. Inoculum was prepared from 2-wk-old fungal cultures grown on potato dextrose agar at 28 ± 1 C. Four cultures were mixed with 400 ml sterile distilled water in a Waring Blendor, and 100 ml of inoculum was hand mixed into the soil of each replicate. Control soil was treated with an identical volume of an agar slurry. In the second test, pine seedlings growing in pasteurized soil were wounded at the base of the stem with a sterile scalpel and agar plugs of fungal mycelium were placed in the wound, which was then wrapped with Parafilm. Control trees were wounded and inoculated only with agar plugs. Both experiments were complete randomized block designs with six replications per treatment. Seedlings were observed for symptom development for 12 wk, then harvested, disinfested, and incubated at 28 ± 1 C on acidified potato dextrose agar.

Dilutions of soil samples from Japanese black pine sites were performed as described by Johnson and Curl (6), using dilutions of 10^{-4} and 10^{-5} on acidified potato dextrose agar and OAES medium (17). Plant tissue and soil mineral analyses were performed by the Soil Testing Laboratory, University of Delaware, Newark.

RESULTS

Bursaphelenchus xylophilus was found in tissue of seven species of pine in Delaware: red (*P. resinosa* Ait.,), Scots (*P. sylvestris* L.), scrub (*P. virginiana* Mill.), Eastern white (*P. strobus* L.), loblolly (*P. taeda* L.), pitch (*P. rigida* Mill.), and Japanese black (*P. thunbergii*). Distribution of these species is outlined in Fig. 1. Ninety-seven percent of 67 Japanese black pines

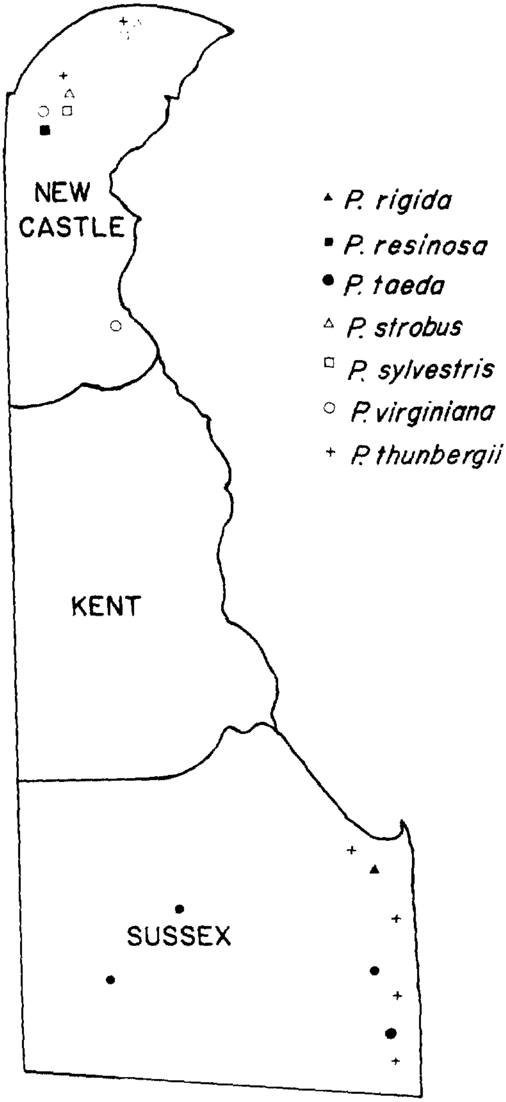

Fig. 1. Distribution in Delaware of *Pinus* spp. infected with *Bursaphelenchus xylophilus*.

and sixty-nine percent of 13 symptomatic loblolly pines examined were positive for *B. xlyophilus*. All samples from other species (sample numbers were 5, 12, 5, 3, and 8 for *P. resinosa*, *P. sylvestris*, *P. virginiana*, *P. strobus*, and *P. rigida*, respectively) were positive. Nematodes extracted from Japanese black pine, inoculated into terminal buds of healthy Japanese black pines growing in Newark, Delaware, produced decline symptoms (severe chlorosis followed by needle necrosis) within 3 wk. Although symptoms were severe, death did not occur before harvest. Large populations of *B. xylophilus* were recovered from these trees several centimeters below the point of inoculation. No nematodes were recovered from controls.

Population levels of *B. xylophilus* were compared between loblolly, Japanese black, Scots, and pitch pine. Populations of nematodes from Japanese black pine were significantly higher ($P = 0.05$) than other species, none of which differed significantly (Table 1).

Table 1. Estimated populations of *Bursaphelenchus xylophilus* on declining *Pinus* spp.*

Species	No. of *B. xylophilus*	S.D.
P. thunbergii	112.8 A†	±27.6
P. radiata	28.0 B	± 5.8
P. sylvestris	29.5 B	±10.7
P. taeda	31.5 B	±11.8

*Populations are expressed as the mean of three 3-ml aliquots taken from a Baermann funnel determination of a 100-cm³ sample.

†Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

Several xylophilous fungi were found associated with nematode-infested Japanese black pine (Table 2). These included *Botrytis cinerea, Ceratocystis* sp., *Cladosporium* sp., *Fusarium* spp., *Gliocladium roseum, Pestalotia funerea, Rhizoctonia solani,* and *Rhizosphaera pini.* Of these fungi, *Pestalotia funerea, Fusarium* spp., and *Rhizosphaera pini* were most frequently isolated, appearing in 80.0, 66.1, and 44.6% of the samples, respectively. Isolates of these three fungi were not pathogenic when inoculated into soil around roots or into stems of 8-wk-old Japanese black pines growing in the greenhouse. Dilutions of soil samples from Japanese black pine sites failed to yield any phytopathogenic fungi, with the exception of one site, which was negative for the presence of *B. xylophilus.*

Table 2. Xylophilic fungi associated with *Bursaphelenchus xylophilus*-infested *Pinus thunbergii.*

Species	Frequency*	Sample Percentage*
Botrytis cinerea	1.3	7.7
Ceratocystis sp.	1.8	4.6
Cladosporium sp.	0.9	7.7
Fusarium spp.	4.9	66.1
Gliocladium roseum	2.7	16.9
Pestalotia funerea	8.8	80.0
Rhizoctonia solani	0.6	4.6
Rhizosphaera pini	3.9	44.6

*Percent isolation based on a total of 1,040 attempts.

†Percent isolation from 65 samples (100% = occurrence on at least one isolation from all 65 samples).

Textural and nutritional analyses of soils indicated that soil factors have little or no effect on nematode infestation of Japanese black pine. A large variation existed in elemental and organic matter content for both infested and noninfested sites. Tissue analysis of Japanese black pine foliage on the same sites yielded no significant differences in element concentrations between infested and noninfested trees.

DISCUSSION

Of 18 pine species listed as hosts of *B. xylophilus* in the U.S. (13), 16 are from the subgenus Diploxylon (12); six of seven host species found in Delaware were Diploxylon pines. It is not immediately evident whether this indicates a greater susceptibility of two- and three-needle pines, reflects the greater number and distribution of these pine species, or results from specificity in feeding habits of the vector Cerambycid beetles. Our results, based on population levels of the nematode, suggest a difference in susceptibility among Diploxylon species. Japanese black pine consistently yielded higher nematode populations than the other three species tested, all of which were Diploxylon (Table 1). Suzuki and Kiyohara (15) observed that small initial *B. xylophilus* populations (one male and one virgin female) inoculated into Japanese black pine seedlings produced large nematode numbers in one month. The same authors (7) also reported a guantitative relationship between nematode number and symptom development in affected pines. The possibility, however, of greater frequency in feeding by the vector (hence large initial *B. xylophilus* populations) on Japanese black pines should not be excluded. We are presently using greenhouse and laboratory screeing methods to determine if field observations can be reproduced with controlled inoculum levels.

Our observations of fungi associated with *B. xylophilus* infestations are similar to those of previous reports (8,10). Several of the fungi isolated have been reported as pathogens of Japanese black pine (14), but are probably not involved in the etiology of pine decline, based on greenhouse pathogenicity tests. *Pestalotia funerea, Fusarium* spp., and *Rhizosphaera pini* were isolated frequently, but are typically associated with secondary invasion of diseased tissue (16). Species in the genus *Bursaphelenchus* are mycophagous (11); we often observed feeding activity of nematodes on mycelium from surface-disinfested wood chips, particularly with *Gliocladium roseum* and *Rhizosphaera pini*. These fungi could possibly enhance nematode survival in drying tissue.

Inconclusive results from soil and tissue analyses were expected, considering the wide distribution of *B. xylophilus* on several species growing in a variety of soil types. We did not include a nitrogen analysis in our study because previous work showed no correlation between nitrogen levels and decline incidence (R. B. Carroll personal communication).

Pinewood nematode has reached epidemic proportions on Japanese black pine in several parts of southern Delaware. Using color and false-color infrared aerial photography, verified by ground-truth studies, we have estimated a 35% incidence of *B. xylophilus* on *Pinus* spp. at Cape Henlopen State Park, in Sussex County. Scots pines growing in northern New Castle County have sustained equal damage in 15–25-yr-old stands.

We have not, however, observed any significant increase in the incidence of *B. xylophilus* from May to August 1981. According to U.S. Department of Commerce data for 1980 and 1981 (2), mean temperatures were lower, and total precipitation greater, during May–August 1981, compared to the same period in 1980. Studies (7,15) have shown that water stress greatly increases symptom severity on nematode-infested Japanese black pines. Possibly the high incidence of *B. xylophilus* infestations observed in the beginning of our survey were a result of drought-induced predisposition of *Pinus* spp. during the 1980 growing season.

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