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The Pine Wood Nematode in Jack Pine Infected with Dwarf Mistletoe

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ABSTRACT. *Bursaphelenchus xylophilus* was found only in declining or dead jack pine infested with *Arceuthobium americanum*. All trees containing *Bursaphelenchus xylophilus* were also infested with cerambycid beetles, bark beetles, and blue stain fungi. *Arceuthobium americanum* appeared to be the primary cause of tree mortality. *Bursaphelenchus xylophilus* was extracted from a dead jack pine, then increased on *Botrytis cinerea* and inoculated into 3-year-old seedlings

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of jack pine, red pine, Japanese red pine, and eastern white pine. After 6 weeks, 87, 13, 23, and 10 percent of the inoculated seedlings were dead or declining for those species, respectively. FOREST SCI. 31:866–870.

ADDITIONAL KEY WORDS: *Arceuthobium americanum, Bursaphelenchus xylophilus, Pinus banksiana.*

THE PINE WOOD NEMATODE *Bursaphelenchus xylophilus* (Steiner and Buhrer), (*B. lignicolus* Mamiya and Kiyohara) (Nickle and others 1980), causes a wilt disease of Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) and Japanese black pine (*P. thunbergii* Parl.) in Japan (Mamiya 1976, 1983; Mamiya and Enda 1972). Extensive mortality has occurred in Japan for the past 30 years and the disease threatens pine forests of that country (Mamiya 1983).

The pine wood nematode is vectored by certain cerambycid beetles (Coleoptera: Cerambycidae) which develop in dead trees, some of which are infested with nematodes. After emergence, these vectors undergo maturation feeding on young shoots of healthy pines. During this feeding, the nematodes leave the insect and enter newly created feeding wounds (Mamiya and Enda 1972). Once *B. xylophilus* are inside the tree, they infest the resin canals and multiply rapidly reducing oleoresin flow, and trees become symptomatic. Chlorotic symptoms appear in infested trees within 2 weeks and mortality may occur within 3 months (Mamiya 1972). Details on the biology of this disease have been previously reviewed (Mamiya 1976, 1983; Dropkin and others 1981; Wingfield and others 1982).

Bursaphelenchus xylophilus was first associated with pine mortality in the United States by Dropkin and Foudin (1979). This nematode has since been found in many areas of the United States on pines and other conifers (Robbins 1982). Recent studies suggest native pines may be less susceptible than pines in Japan (Wingfield 1982, Wingfield and others 1982), but that exotic pines such as Scots pine (*P. sylvestris* L.) and Japanese black pine grown in the United States are susceptible to *B. xylophilus* (Adams and Morehart 1981, Malek and McClary 1981).

Bursaphelenchus xylophilus was recently found in Manitoba, Canada, on dead jack pine (*P. banksiana* Lamb.) infected with the dwarf mistletoe *Arceuthobium americanum* Nutt.: Engelm. (Knowles and others 1983). The objective of this study was to examine the association of *B. xylophilus* with jack pine infested with *A. americanum* and to evaluate the ability of the nematode isolate from Manitoba to cause symptoms in seedlings of several species of pines.

METHODS

Field Observations.—Jack pine infested with dwarf mistletoe in Grand Beach Provincial Park, Manitoba, were sampled for *B. xylophilus*. The sampling categories were: (1) healthy trees: no dwarf mistletoe; (2) infected trees: vigorous with living mistletoe brooms; (3) infected trees: vigorous with living and dying mistletoe brooms; (4) infected trees: declining with living, dying, and dead mistletoe brooms; (5) dead trees infected with dwarf mistletoe. All trees sampled were 30–52 years old with an average diameter of 11 cm at 1.4 m and an average height of 9 m.

Ten trees chosen randomly from each sampling category were felled and 5 cm thick disks were cut from the top, middle, and bottom of the boles. In addition, twigs (3.2 mm average diameter) were randomly collected from mistletoe brooms. All samples were examined for the presence of cerambycid beetles, bark beetles, and blue stained wood. Samples were debarked, wood was cut into approximately 1 cm pieces, and approximately 80 gm (fresh weight) subsamples were placed in Baermann funnels (Southey 1970) for nematode extractions. After 24 h the funnels were drained and samples examined for *B. xylophilus*.

Pathogenicity Tests.—Nematodes used for inoculations were extracted from the bole of a dead jack pine (infected with dwarf mistletoe) from the stand in which field observations were made. After extraction (using a Baermann funnel) the nematodes were concentrated in a clinical, swingbucket centrifuge. Ten late fourth stage larvae were surface sterilized in 0.1 percent streptomycin sulfate for 5 minutes and washed in sterile water. Larvae were

TABLE 1. Bursaphelenchus xylophilus, *Cerambycidae, Scolytidae and* Ceratocystis *stain fungi in* Aceuthobium-*infested* Pinus banksiana.

Tree category	Tree part sampled	Number of samples with			
		Cerambyc-idae	Scolytidae	*Cerato-cystis*[a]	*B. xylophilus*
Healthy trees with no mistletoe	Live twigs[b]	0	0	0	0
	Bole[c]	0	0	0	0
Infected trees: vigorous with living mistletoe brooms	Live twigs	0	0	0	0
	Bole	0	0	0	0
Infected trees: vigorous with living and dying mistletoe brooms	Live twigs	0	0	0	0
	Dead twigs[b]	0	8	8	0
	Bole	0	0	0	0
Infected trees: declining with living, dying and dead mistletoe brooms	Live twigs	0	0	0	0
	Dead twigs	1	5	8	1
	Bole	0	0	2	0
Dead trees infected with mistletoe	Dead twigs	0	1	7	6
	Bole	10	10	9	9

[a] Blue stain fungi of the genus *Ceratocystis.*
[b] Forty twigs sampled per tree category.
[c] Thirty bole samples per tree category.

then placed on mycelium of *Botrytis cinerea* Pers. : Fr. on Difco potato-dextrose agar to increase nematode numbers. Once established in the fungus culture, *B. xylophilus* were multiplied on additional cultures of *B. cinerea.* Each of thirty 3-year-old seedlings of jack pine, Japanese red pine, red pine (*P. resinosa* Ait.), and eastern white pine (*P. strobus* L.), were inoculated with 5,000 nematodes. Wounds were made by removing a piece of bark (approximately 1 cm long) from the top third of each seedling. Waterproof tape was placed around the wound, nematodes were placed in the wound with a syringe and the tape closed. An additional 30 seedlings of each species were inoculated with a water slurry of *B. cinerea* as controls.

Inoculated seedlings were maintained in a greenhouse at approximately 20°C and observed weekly. Jack pine seedlings were harvested after 4 weeks; the remaining seedlings were harvested after 6 weeks. All seedlings inoculated with *B. xylophilus* and 10 control seedlings from each species were prepared for extraction of nematodes. Seedlings were divided into three sections: Top: stem above inoculation point, Middle: stem from inoculation point to root collar, and Root: stem below root collar. Bark was removed, and nematodes were extracted in plastic weighing boats (Wingfield and others 1983). After 24 h the presence of *B. xylophilus* was noted and nematodes were counted.

Results

Field Observations.—*Bursaphelenchus xylophilus* was only found in dead parts of trees (Table 1). *Bursaphelenchus xylophilus* was extracted from the dead twigs of a declining tree but not from other living trees. Nine of ten dead trees contained *B. xylophilus* in the boles and six of ten from twigs of dead mistletoe brooms.

Cerambycid and scolytid galleries were observed in the boles of dead trees. Scolytid galleries were also observed in dead twigs of mistletoe-infested trees. Blue stain fungi were only present in dead pine tissues which were often colonized by scolytid bark beetles.

Pathogenicity Tests.—Seedling mortality was first noticed in jack pines 10 days after inoculation with *B. xylophilus.* Most jack pines inoculated developed symptoms within 4 weeks (Table 2). Seedlings became chlorotic and soon wilted and died. Seven Japanese red pine and four red pine died after inoculation, no eastern white pines died, although the tops of three seedlings died; and living nematodes were found in asymptomatic trees. No eastern white pine, no red pine, one jack pine, and two Japanese red pine controls died.

TABLE 2. Survival and condition of pine seedlings inoculated with an isolate of Bursaphelenchus xylophilus *from Manitoba, Canada.*

Species	Tree condition[a]					
	Nematode inoculated			Control inoculation		
	Dead	Declining	Healthy	Dead	Declining	Healthy
	Percent			*Percent*		
Pinus densiflora	23	0	77	6	0	94
P. resinosa	13	0	87	0	0	100
P. banksiana	57	30	13	3	0	97
P. strobus	0	10	90	0	0	100

[a] *Pinus banksiana* were harvested 4 weeks after inoculation; the other species were harvested after 6 weeks.

Nematodes were only found in seedlings inoculated with *B. xylophilus*. The numbers of nematodes in seedlings varied but the greatest number of nematodes were extracted from jack pine seedlings (Table 3). Inoculated eastern white pine had more nematodes than did Japanese red pine or red pine.

Discussion

In the field, the primary cause of tree death appeared to be dwarf mistletoe infection followed by an infestation of scolytid bark beetles and cerambycid beetles which commonly attack declining trees. *Bursaphelenchus xylophilus* was found in dead trees and in dead portions of trees which had been colonized by cerambycid beetles. *Bursaphelenchus xylophilus* can be transmitted to dead or dying trees during oviposition by cerambycid beetles (Wingfield 1982, 1983; Wingfield and Blanchette 1983) and we postulate that the nematodes entered in this way. This is unlike the situation in Japan where *B. xylophilus* is transmitted during maturation feeding of cerambycid beetles and colonizes healthy pines (Mamiya and Enda 1972).

Blue stain in pines is most commonly caused by *Ceratocystis* spp. and is transmitted to dying trees by scolytid beetles (Upadhyay 1981). In this study, blue stain was present in all trees with evidence of scolytid bark beetle attack. It has been postulated that *B. xylophilus* feed on blue stain fungi in dead trees (Wingfield 1982).

TABLE 3. Average number of Bursaphelenchus xylophilus *per gram fresh weight extracted from seedlings 4–6 weeks after inoculation with* Bursaphelenchus xylophilus.

Tree species	Tree part	Nematodes
Pinus banksiana	Top[a]	163
	Middle[b]	278
	Root[c]	226
P. resinosa	Top	12
	Middle	208
	Root	43
P. densiflora	Top	126
	Middle	84
	Root	23
P. strobus	Top	53
	Middle	181
	Root	218

[a] Top: Stem above inoculation point.
[b] Middle: Stem from inoculation point to root collar.
[c] Root: Stem below root collar.

This is the first report of a pathogenicity test with an isolate of *B. xylophilus* from Canada. Jack pine seedlings were more susceptible to the Canadian isolates than seedlings of the other pines tested. Japanese red pine is reported to be highly susceptible to *B. xylophilus* in Japan (Mamiya 1983), but did not show similar susceptibility in this study. This isolate of *B. xylophilus* from jack pine may represent a physiological variant which is more virulent on jack pine. Physiological variation was observed in an earlier study of an isolate of *B. xylophilus* from balsam fir (*Abies balsamea* (L.) Mill.) (Wingfield and others 1983).

It is important to note that these inoculation studies were made on seedlings and that results may not be the same if established trees in forests are inoculated. This view is supported by Mamiya's (1983) suggestion that inoculation of potted seedlings tends to show variable results which are not consistent with results obtained under natural conditions. The pathogenicity of *B. xylophilus* on jack pine seedlings may not necessarily relate to the role of the nematode in the events leading to mortality of mistletoe-infested jack pines.

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