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Pathogenicity of *Bursaphelenchus xylophilus* on Pines in Minnesota and Wisconsin[1]

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Abstract: The pinewood nematode, *Bursaphelenchus xylophilus,* was inoculated into established native jack and red pines (*Pinus banksiana* and *P. resinosa*) and exotic Austrian pine (*P. nigra*) in Minnesota and Wisconsin forests during summer 1981. The nematode isolates did not kill established nonstressed pine trees growing in the forest. However, the same nematode isolates killed pine seedlings under greenhouse conditions. Girdling the main stem of some trees to induce stress resulted in the death of the majority of inoculated and noninoculated branches of Austrian and jack pines, but no branch death was observed on red pine. Greater numbers of nematodes were extracted from branches of inoculated, girdled trees than from nongirdled trees. The mean number of nematodes extracted from branches of inoculated, nongirdled trees was 0.3–14 nematodes per gram of wood.

Key words: pinewood nematode, *Pinus banksiana, P. resinosa, P. nigra.*

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer), causes a serious wilt disease of the native pines *Pinus densiflora* Sieb. et Zucc., *P. luchensis* Mayr, and *P. thunbergii* Parl. in Japan (11–13). Trees become infected during maturation feeding of cerambycid beetles (Coleoptera: Cerambycidae), the vectors of the nematode (13,14). Subsequent to infection, the nematodes enter resin canals and multiply, causing wilt symptoms such as lowered oleoresin flow, reduced transpiration, and tree death within 3 months. Several reviews of the disease have described the biology and pathogenicity of *B. xylophilus* in Japan (4,10–12,23).

Bursaphelenchus xylophilus was first found in the United States during the early part of this century (17). At that time, it was known as *Aphelenchoides xylophilus* (15). Only recently, however, was the nematode recognized as a pathogen in this country (3). Concern has been expressed that *B. xylophilus* may pose a threat to forests of the United States as it has in Japan (4,23). Pathogenicity of *B. xylophilus* on seedlings of many conifer species has been documented in the United States (1,4,8,9,22), but its ability to kill established pine trees in forests, particularly native North American pines, has received little attention. Our objectives were to determine the effects of inoculations of *B. xylophilus* on native jack pine (*P. banksiana* Lamb.) and red pine (*P. resinosa* Ait.) and exotic Austrian pine (*P. nigra* Arnold) in forests of Minnesota and Wisconsin and to examine the role of the nematode in tree death.

MATERIALS AND METHODS

Greenhouse inoculations: Before forest trees were inoculated, the pathogenicity of the nematode isolates was tested on seedlings in the greenhouse. Two different isolates of *B. xylophilus* were used. An isolate collected from a dying jack pine near Black River Falls, Wisconsin, was used for Wisconsin inoculations, and an isolate from a dying Austrian pine in Zimmerman, Minnesota, was used for inoculations in Minnesota.

Nematodes were extracted from wood using Baermann funnels (16) and concentrated in a clinical swinging bucket centrifuge. They were washed twice in sterile distilled water, surface disinfected in 0.1% streptomycin sulfate, rinsed twice in sterile distilled water, and placed on *Botrytis cinerea* Pers. : Fr. growing on potato dextrose agar in 125-ml Erlenmeyer flasks.

The Minnesota isolate of *B. xylophilus* was increased for greenhouse inoculations on *Botrytis cinerea,* and the Wisconsin isolate was increased on *Ceratocystis ips* Rumbold. Isolates were maintained on the fungi in

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1-liter Erlenmeyer flasks for 4 weeks at 25 C. Control inoculations were made with a slurry from nematode-free cultures of *B. cinerea* or *C. ips* blended with distilled water.

Twenty 3-year-old Scots pine (*P. sylvestris* L.) seedlings were inoculated with the Wisconsin isolate at 5,000 nematodes per seedling. Seventeen noninoculated seedlings served as controls. The top centimeter of each seedling was excised, and a receptacle was made around the cut surface with waterproof tape. Inoculum was placed in contact with the cut surface, and the receptacle was closed. The pathogenicity of the Minnesota isolate on greenhouse-grown seedlings of red and Scots pine was examined in a previous study (22).

Forest inoculations: Trees were inoculated in 1981 as follows: Cloquet (Minnesota) Forestry Center, 5-year-old jack and red pines the first week in June; Zimmerman, Minnesota, 15-year-old Austrian pines and 15-year-old red pines the second week in June; Black River Falls, Wisconsin, approximately 12-year-old naturally regenerated jack pines and 10-year-old red pines the second week in July.

Nematode isolates for inoculations in the forest were increased on *B. cinerea.* A slurry from nematode-free cultures of this fungus blended with distilled water was used for control inoculations.

Minnesota: At Zimmerman, 15 Austrian and 15 red pines were inoculated, and five trees of each species were used for noninoculated controls. At Cloquet, 40 jack and 40 red pines were inoculated, and an equal number of trees served as noninoculated controls. Half of the inoculated and half of the control trees at Cloquet were completely girdled 30 cm above ground level at the time of nematode inoculation.

Inoculation techniques used in Zimmerman and Cloquet were similar. Four branches in the top third of each tree were inoculated 60 cm from the bole with 10,000 nematodes per branch. Holes were drilled 5 mm deep into the upper side of the branches using a hand drill with a 3-mm-d bit. The inoculum was placed in the holes, and the wounds were wrapped with waterproof tape.

Inoculated trees were examined every 2 weeks for the first 4 months after inoculation. Five Austrian and five red pine branches that had been inoculated with nematodes were removed 4 months after treatment at Zimmerman. At 12 months, the remainder of the nematode-inoculated and all control branches were removed for laboratory examination. Nematodes were extracted from branch samples 15 cm long with the inoculation site in the center.

Red and jack pine at Cloquet were sampled 10 months after inoculation. Inoculated branches were removed from all trees that had been girdled and from 10 nematode-inoculated and 10 control trees of each species that had not been girdled. Branch sections, 30 cm long with the inoculation site in the center, were taken from each branch for nematode extraction. Prior to extraction, bark was removed from all sections, wood was chopped into 2-cm^3 pieces, and samples were weighed. Nematodes were extracted from samples in Baermann funnels, concentrated by centrifugation, and counted.

Wisconsin: Forty jack and forty red pines at Black River Falls were inoculated with the nematode, and an equal number of each species served as controls. A hole, 5 mm deep and 3 mm diameter, was drilled into the stem of each tree 90 cm from the top, and 10,000 nematodes or a nematode-free slurry of the fungus were introduced. Inoculation sites were then sealed with waterproof tape. Half of the nematode-inoculated and half of the control trees were girdled completely 60 cm from the base at the time of inoculation.

Three months later, 10 of the 20 girdled and nematode-inoculated jack and red pines and an equal number of nongirdled inoculated trees were sampled. Nine months after inoculation, the remaining 10 nematode-inoculated and 20 control trees that had been girdled and 10 control trees that had not been girdled or inoculated with nematodes were removed. Samples 60 cm long were taken from the stem of each tree for nematode extractions. The sample zone extended to 30 cm on either side of inoculation sites.

RESULTS

Greenhouse inoculations: Bursaphelenchus xylophilus isolated from jack pine, grown on *C. ips,* and used in the inoculation in Black River Falls killed 7 of 20 inoculated Scots pine seedlings in 3 months in the greenhouse. No control seedlings died.