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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.

TABLE 1. Response of jack pine inoculated with *Bursaphelenchus xylophilus* at Cloquet, Minnesota, and Black River Falls, Wisconsin, after 4 months.

Treatment	Cloquet				Black River Falls			
	Number of branches	Symptoms			Number of trees	Symptoms		
		Healthy	Dying	Dead		Healthy	Dying	Dead
Inoculated*	80	59	21	0	20	15	5†	0
Not inoculated	80	76	4‡	0	20	20	0	0
Inoculated/girdled	80	0	72	8	20	0	4	16
Not inoculated/girdled	80	8	64	8	20	3	6	11

* Branches each inoculated with 10,000 nematodes.
† Only tree parts above inoculation point showed symptoms.
‡ Four dying branches apparently girdled during inoculation.

Pathogenicity of the isolate used in Cloquet and Zimmerman has previously been shown on red and Scots pine seedlings where 8 of 30 and 12 of 20 seedlings died, respectively (22).

Forest inoculations—Minnesota: No symptoms developed within 4 months at Zimmerman. Inoculation of jack pines at Cloquet resulted in death of 21 inoculated branches and 4 control branches within 4 months (Table 1). Death of control branches appeared to have been due to excessive damage during inoculation. The majority of branches on girdled trees were either dead or dying at 4 months. There were no symptoms of disease on branches of red pine regardless of treatment.

Of the 10 inoculated Austrian pine branches sampled in Zimmerman, 9 contained nematodes with an average of 45 nematodes per branch sample (Table 2). Nematodes were extracted from all red pine branches sampled at this time with an average of 47 nematodes per branch. After one year, 15 of the remaining 30 inoculated Austrian and 14 of the 32 red pine branches contained nematodes. The maximum number of nematodes extracted from any Austrian and red pine branch sample was 167 and 277, respectively. No nematodes were recovered from control branches from either species. At 10 months, 31 of 40 red pine branches inoculated in Cloquet during 1981 contained nematodes, whereas 51 of 80 inoculated branches on girdled trees contained nematodes (Table 3). No nematodes were extracted from branch samples of girdled or nongirdled control trees. Sixteen of forty inoculated jack pine branches from trees inoculated but not girdled contained nematodes at 10 months, whereas 36 of 80 branches from trees which had been girdled contained nematodes. *Bursaphelenchus xylophilus* also was found in four branches from girdled trees which had not

TABLE 2. Numbers of nematodes extracted from Austrian and red pine branches in Zimmerman, Minnesota, 4 and 12 months after inoculation with *Bursaphelenchus xylophilus.**

Months after inoculation	Pine type	Treatment	Number of trees sampled	Nematodes present in branches sampled	Numbers of nematodes		
					Per branch†		Per gram of wood sampled‡
					Mean	Maximum	
4	Austrian	Inoculated	5	9/10	44.6	111	ND§
	Red	Inoculated	5	10/10	46.9	173	ND§
12	Austrian	Inoculated	10	15/20	13.6	167	0.29
		Control	5	0/20	0	0	0
	Red	Inoculated	10	14/32	36.3	277	0.6
		Control	5	0/20	0	0	0

* Branches each inoculated with approximately 10,000 nematodes.
† Samples for each nematodes extraction included branch tissue 60 cm long (30 cm on either side of the inoculation point).
‡ Average weight of wood samples extracted was 108.6 g.
§ No data.

TABLE 3. Numbers of nematodes extracted from red pine and jack pine branches in Cloquet, Minnesota, 10 months after inoculation with *Bursaphelenchus xylophilus*.*

		Nematodes present		Number of nematodes		
				Per branch†		Per gram of wood sampled‡
Pine type	Treatment	Trees	Branches	Mean	Maximum	
Red	Inoculated	10/10	31/40	76.1	958	14.0
	Not inoculated	0/10	0/40	0	0	0
	Inoculated/girdled	19/20	51/80	111.0	1,256	16.0
	Not inoculated/girdled	0/20	0/80	0	0	0
Jack	Inoculated	8/10	16/40	87.9	1,757	11.4
	Not inoculated	0/10	0/40	0	0	0
	Inoculated/girdled	15/20	36/80	57.3	2,871	5.9
	Not inoculated/girdled	2/20	4/80	2.0	73	0.2

* Branches each inoculated with approximately 10,000 nematodes.
† Samples for nematodes extraction included branch tissue 30 cm long including the inoculation point.
‡ Average weight of wood samples extracted was 27.1 g for red pine and 31.2 g for jack pine.

been inoculated. No nematodes were found in samples from trees that had not been girdled. Greater numbers of nematodes were extracted from branches of girdled than nongirdled trees.

Wisconsin: There were no symptoms at 4 months on any red pines at Black River Falls; however, the tops of five nematode-inoculated jack pines were dead above the inoculation site (Table 1). All trees that were inoculated and girdled were either dead or dying, and only three trees that were girdled but not inoculated remained healthy. No symptoms developed on nongirdled control trees.

Eight of nine red pines that had been nematode-inoculated and not girdled and 9 of 10 trees both inoculated and girdled contained *B. xylophilus* in branches surrounding the inoculation site 3 months after inoculation (Table 4). At 9 months, 7 of the remaining 10 inoculated and girdled trees and 5 of 17 trees that were girdled and not inoculated contained *B. xylophilus*. Maximum number of nematodes extracted from any inoculated tree was considerably smaller (less than 5%) than the 10,000 nematodes inoculated. No nematodes were extracted from noninoculated and nongirdled trees.

At 9 months, eight of the remaining nine trees that had been girdled and inoculated contained nematodes. The average number of nematodes extracted from trees that

TABLE 4. Numbers of nematodes extracted from red pine and jack pine trees in Black River Falls, Wisconsin, 3 and 9 months after inoculation with *Bursaphelenchus xylophilus*.*

Pine type	Months after inocula-tion	Treatment	Trees with nematodes present	Number of nematodes†		
				Mean	Maximum	Per gram of wood sampled‡
Red	3	Inoculated	8/9	97.1	369	0.4
		Inoculated/girdled	9/10	113.5	399	0.6
	9	Not inoculated	0/10	0	0	0
		Inoculated/girdled	7/10	65.7	220	0.4
		Not inoculated/girdled	5/17	2.4	17	0
Jack	3	Inoculated	10/10	83.9	7,620	7.6
		Inoculated/girdled	9/10	52,764.0	143,600	435.7
	9	Not inoculated	0/10	0	0	0
		Inoculated/girdled	8/9	48,724.7	138,398	753.2
		Not inoculated/girdled	9/19	340.5	1,523	4.4

* Trees each inoculated with approximately 10,000 nematodes.
† Samples from tree stem 60 cm long (30 cm on either side of the inoculation point).
‡ Average weight of samples extracted was 130 g in red pine and 128.8 g in jack pine.

had been inoculated but not girdled was considerably lower (less than 2%) than the average for inoculated and girdled trees. At 9 months, 9 of 19 trees girdled but not inoculated also contained *B. xylophilus.* The average number of nematodes extracted per tree in this case was less than that from trees inoculated, whether girdled or not. No nematodes were extracted from trees that were not inoculated or girdled.

Discussion

Bursaphelenchus xylophilus has been found throughout much of the United States and is pathogenic on seedlings of many pine species in the greenhouse (4,8,9,22). Pathogenicity on greenhouse seedlings does not, however, imply pathogenicity on established trees in the forest. In view of the extensive losses of native pines attributed to pinewood nematode infestation in Japan, it is important to establish whether *B. xylophilus* can kill native North American conifers under natural conditions. Results presented here suggest that, while *B. xylophilus* may kill seedlings of particular pine species, established trees may not die following inoculation. Red pine inoculated in this study appeared to be unaffected in the forest, even though seedlings of this species died following inoculation with the same nematode isolate in the greenhouse (22). These observations are similar to those of Mamiya (12) who indicated that inoculation of potted seedlings tends to give variable results. They also may explain differences in reports by Mamiya (12) and Dropkin et al. (4) of susceptibility and resistance in pine species following seedling inoculations.

Evidence suggests that drought stress results in increased susceptibility of pines to infection by *B. xylophilus* infestation (18,19). Nematode inoculations of trees during the summer in Japan resulted in rapid tree death, whereas inoculations during cooler periods were less effective (5). Minnesota and Wisconsin inoculations were initiated during early summer which should have favored nematode infestation. However, nongirdled trees were growing under near optimum conditions and did not appear stressed. Application of artificial stress conditions to these trees may have produced different results.

There was a distinct difference between red and jack pines in response to girdling. Many jack pines but no red pines, whether inoculated or not, died during the first summer after girdling. Girdling of red pine did not appear to influence the development of *B. xylophilus* within inoculated trees. The numbers of nematodes recovered from inoculated and girdled jack pine trees were considerably greater than those for inoculated nongirdled trees at Black River Falls but not at Cloquet during the same year. Trees at Black River Falls were, however, considerably smaller than those at Cloquet and died more rapidly after girdling. As trees died, they were infested by bark beetles (Coleoptera: Scolytidae) carrying blue stain fungi. Since *B. xylophilus* is primarily a mycophagous nematode which reproduces on fungi (2,6,7), nematode populations in girdled trees apparently increased rapidly once the trees were colonized by fungi.

Bursaphelenchus xylophilus was recovered from jack pines that had been girdled but not nematode inoculated, probably a result of nematode transmission into the dying trees during cerambycid beetle oviposition. Recent studies have demonstrated this mode of nematode transmission to dying trees and cut timber (20,21). Therefore, the nematode can be present in dying trees (24) without necessarily being the primary cause of tree death.

Results of this study in Minnesota and Wisconsin have not shown an extreme susceptibility to *B. xylophilus* in red, jack, or Austrian pines comparable to that reported for native Japanese pines. Jack pine, however, appeared to be more susceptible than Austrian or red pine to the nematode. It is important to establish whether native North American pine species, as well as exotic pines grown in the United States, are threatened by *B. xylophilus.* The status of the nematode as a potential pathogen of pines in North America can be clarified only by further tests on established forest trees under differing climatic conditions utilizing techniques that most closely approximate natural infection.

Literature Cited

1. Bergdahl, D. R. 1982. Occurrence of the pine wood nematode in eastern larch. Pp. 47–55 *in* J. E.

Appleby and R. B. Malek, eds. Proceedings National Pine Wilt Disease Workshop, Chicago, Illinois. Illinois Department of Energy and Natural Resources.

2. Dozono, Y., and N. Yoshida. 1974. Application of the logistic curve for the population growth of pine wood nematode, *Bursaphelenchus lignicolus* on the cultures of *Botrytis cinerea.* Journal of Japanese Forest Society 56:146–148.

3. Dropkin, V. H., and A. S. Foudin. 1979. Report of the occurrence of *Bursaphelenchus lignicolus*-induced pine wilt disease in Missouri. Plant Disease Reporter 63:904–905.

4. Dropkin, V. H., A. S. Foudin, E. Kondo, M. J. Linit, M. T. Smith, and K. Robbins. 1981. Pine wood nematode: A threat to U.S. forests? Plant Disease 65: 1022–1027.

5. Kiyohara, T., and Y. Tokushige. 1971. Inoculation experiments of a nematode, *Bursaphelenchus* sp., onto pine trees. Journal of Japanese Forest Society 53:210–218.

6. Kobayashi, T., K. Sasaki, and Y. Mamiya. 1974. Fungi associated with *Bursaphelenchus lignicolus,* the pine wood nematode. I. Journal of Japanese Forest Society 56:136–145.

7. Kobayashi, T., K. Sasaki, and Y. Mamiya. 1975. Fungi associated with *Bursaphelenchus lignicolus,* the pine wood nematode. II. Journal of Japanese Forest Society 57:184–193.

8. Kondo, E., A. S. Foudin, M. J. Linit, M. T. Smith, R. Bolla, R. E. K. Winter, and V. H. Dropkin. 1982. Pine wilt disease—Nematological, entomological and biochemical investigations. University of Missouri Special Bulletin SR 282.

9. Malek, R. B. 1982. Symptomatology of pine wilt in Scotch pine. Pp. 14–16 *in* J. E. Appleby and R. B. Malek, eds. Proceedings National Pine Wilt Disease Workshop, Chicago, Illinois. Illinois Department of Energy and Natural Resources.

10. Mamiya, Y. 1972. Pine wood nematode, *Bursaphelenchus lignicolus* Mamiya and Kiyohara, a causal agent of pine wilting disease. Review of Plant Protection Research (Japan) 5:46–60.

11. Mamiya, Y. 1976. Pine wilting disease caused by the pine wood nematode, *Bursaphelenchus lignicolus,* in Japan. Japanese Agriculture Research Quarterly 10:206–211.

12. Mamiya, Y. 1983. Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus.* Annual Review of Phytopathology 21:201–220.

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

14. Morimoto, K., and A. Iwasaki. 1972. Role of *Monochamus alternatus* (Coleoptera: Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoidae). Journal of Japanese Forest Society 54:177–183.

15. 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. Journal of Nematology 3:385–392.

16. Southey, J. F., ed. 1970. Laboratory methods for working with plant and soil nematodes. Ministry of Agriculture Fisheries and Food (Great Britain) Technical Bulletin No. 2. London: Her Majesty's Stationery Office.

17. Steiner, G., and E. M. Buhrer. 1934. *Aphelenchoides xylophilus* n. sp., a nematode associated with blue stain and other fungi in timber. Journal of Agricultural Research 48:949–951.

18. Suzuki, K., and T. Kiyohara. 1978. Influence of water stress on development of pine wilting disease caused by *Bursaphelenchus lignicolus.* European Journal of Forest Pathology 8:97–107.

19. Takashita, K., Y. Hagihara, and S. Ogawa. 1975. Environment analysis to pine damage in western Japan. Bulletin Eukuokaken Forest Experiment Station 24:1–45.

20. Wingfield, M. J. 1982. Transmission of pine wood nematode to cut timber and girdled trees. Plant Disease 66:35–37.

21. Wingfield, M. J., and R. A. Blanchette. 1983. The pine wood nematode, *Bursaphelenchus xylophilus* in Minnesota and Wisconsin: Insect associates and transmission studies. Canadian Journal of Forest Research 13:1068–1076.

22. Wingfield, M. J., R. A. Blanchette, and E. Kondo. 1983. Comparison of the pine wood nematode *Bursaphelenchus xylophilus* from pine and balsam fir. European Journal of Forest Pathology 13:360–372.

23. Wingfield, M. J., R. A. Blanchette, T. H. Nicholls, and K. Robbins. 1982. The pine wood nematode: A comparison of the situation in the United States and Japan. Canadian Journal of Forest Research 12:71–75.

24. Wingfield, M. J., R. A. Blanchette, T. H. Nicholls, and K. Robbins. 1982. Association of the pine wood nematode with stressed trees in Minnesota, Iowa and Wisconsin. Plant Disease 66:934–937.