

## Association of Pine Wood Nematode with Stressed Trees in Minnesota, Iowa, and Wisconsin

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### ABSTRACT

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Trees infected with the pine wood nematode *Bursaphelenchus xylophilus* in Minnesota, Iowa, and Wisconsin were stressed by various forest diseases and insects. Nematodes were only found in trees already colonized by secondary insects such as bark beetles (Scolytidae) and borers (Cerambycidae). *B. xylophilus* was also found in dead tops or dead branches of otherwise healthy trees that appeared to have died as a result of fungal infection or insect attack. Typical wilt symptoms associated with *B. xylophilus* infection in Japan were not observed.

The pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhler) Nickle (formerly *B. ligniculus* Mamiya and Kiyohara [13]) causes a wilt disease of pines in Japan. Extensive losses to Japanese black pine (*Pinus thunbergii* Parl.) and Japanese red pine (*P. densiflora* Sieb. et Zucc.) have occurred in southwestern Japan (17). *B. xylophilus* is vectored primarily by longhorn beetles (Coleoptera: Cerambycidae) that emerge from dead trees carrying the dauer larvae to healthy trees where they enter feeding wounds caused by the beetle (9,11). The nematodes mature and reproduce rapidly

in the resin canals, resulting in reduced oleoresin flow, arrest of transpiration, chlorosis, and death within 3 mo after initial infection (7). Cerambycid beetles oviposit in dying trees, and the life cycle of the nematode, closely coordinated with the beetle vector (18), is repeated.

*B. xylophilus* was reported from the United States in 1979 (4) where it was first found on Austrian (*P. nigra* Arnold) and Scots (*P. sylvestris* L.) pines in Columbia, MO. The nematode has now been reported in 33 states on 20 pine species, atlas cedar (*Cedrus atlantica* Manetti), deodar cedar (*C. deodara* (Roxb.) Loud.), European larch (*Larix decidua* Mill.), tamarack (*L. laricina* (Du Roi) K. Koch), and white spruce (*Picea glauca* (Moench) Voss) (18). Since the first report, concern has been expressed that *B. xylophilus* poses a threat to forests of the United States similar to that apparent in Japan (18). This report documents the results of a 2-yr field investigation made to establish the relative importance of the pine wood nematode in a number of

study sites in Minnesota, Iowa, and Wisconsin.

### MATERIALS AND METHODS

Areas in which *B. xylophilus* was found in a preliminary survey to be associated with a considerable number of dead or dying trees were chosen for further examination. The following areas were examined during the summers of 1980 and 1981: Zimmerman, MN (Sherburne County), 18-yr-old Austrian and red pine (*P. resinosa* Ait.) in an old Christmas tree plantation; Cloquet, MN (Carlton County), 30-yr-old eastern white pine (*P. strobus* L.), Cloquet Forestry Center, University of Minnesota; Durand, WI (Pepin County), 15-yr-old Austrian and red pine in an old Christmas tree plantation; Black River Falls, WI (Jackson County), 20-yr-old jack pine (*P. banksiana* Lamb.), Gordon plantation; Black River Falls, WI (Jackson County), 15-yr-old red pine, Shamrock plantation; Farmington, IA (Lee and Van Buren counties), 20-yr-old Scots and red pine, Shimek State Forest.

Healthy as well as dead or dying trees were examined for root, stem, branch, and needle disease symptoms. Isolations for fungi from diseased portions of the tree were made on 2% malt extract agar (20 g of Difco malt extract and 20 g of Difco Bacto agar per 1,000 ml of water). Samples for nematode extractions were taken from roots; bottom, middle, and top thirds of the bole; lower branches; upper branches; and small twigs of each tree sampled. Disks of wood about 15 cm

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thick were removed with a chain saw from the root and bole samples; branches and twigs were randomly removed using pruning shears. Nematodes were extracted from 60- to 90-g (fresh weight) subsamples using Baerman funnels. The general health of surrounding trees and the presence of insects on sample trees were documented.

## RESULTS

**Zimmerman, MN.** Red pine were healthy, whereas many Austrian pine on this site were dead and dying (Fig. 1). Austrian pine had severe Dothistroma needle blight caused by *Dothistroma pini* Hulb. and held only current-year needles. About half the Austrian pine in the area had dead tops or dead branches in the top third of the crown (Fig. 2) associated with severe infestations of the Zimmerman pine moth, *Diorystria zimmermani* (Grote), which results in copious resin exudation at the base of the dead parts (Fig. 3). Four living Austrian pine trees were sampled and found free of *B. xylophilus* and other disease-causing organisms. *B. xylophilus* was only found in dead portions of Austrian pine with dead tops or branches and in all dead trees or trees with chlorotic needles that were infested by bark beetles (Coleoptera: Scolytidae) and wood borers (Coleoptera: Cerambycidae) (Table 1).

**Cloquet, MN.** A large number of the white pine had dead branches or dead tops resulting from white pine blister rust (*Cronartium ribicola* J. C. Fischer ex Rhabd.) infection. In some cases, trees infected with blister rust were chlorotic and dying. These trees had *Armillariella mellea* (Vahl) ex Karst. and *Verticiladiella procerata* Kendrick root infections (6). *B. xylophilus* was not isolated from healthy trees or trees with blister rust infection. The nematode was, however, present in dead tops and in the bole of trees that were chlorotic and infested by insects (Table 1).

**Durand, WI.** No mortality occurred in

red pine, whereas about 70% of the adjacent Austrian pine trees were dead or dying (Fig. 4). Austrian pine needles were heavily infected with *Dothistroma pini*, and branches only held the current-year needles. In addition, most of the trees had advanced pine root collar weevil (*Hylobius radicis* Buch.) infestation, resulting in resin exudation in the root collar area (Fig. 5). *B. xylophilus* was not extracted from trees with green needles but was present in all dead or dying trees colonized by insects (Table 2).

**Black River Falls, WI (Gordon plantation).** About 95% of the 2,000 jack pine in the study area were dead or dying (Fig. 6). All living and dead or dying jack pine had pine root weevil (*H. rhizophagus* M. B. W.) infestation. Red pine in surrounding stands were healthy. Smaller twigs and branches of trees were damaged by hail and insect feeding and were infected by *Diplodia pinea* Kickx. (Fig. 7). Dead twigs of otherwise healthy trees were free of *B. xylophilus*. Dead or dying trees, some with early bark beetle and borer attack, contained *B. xylophilus*. Otherwise healthy trees with early root weevil infestation, without crown symptoms and before bark beetle and borer infestation, did not contain *B. xylophilus* (Table 2).

**Black River Falls, WI (Shamrock plantation).** About 50% of the 800 red pine in the study area had dead tops (Fig. 8) caused by *Diplodia pinea* infection. In three out of 10 cankered trees sampled, dead tops contained *B. xylophilus* and healthy parts of the same trees were free of nematodes (Table 2). *B. xylophilus*, bark beetles, and borers were not detected in any of the healthy trees sampled.

**Farmington, IA.** Red pine and Scots pine had hail-initiated *Diplodia pinea* cankers on the boles and branches. Cankers on Scots pine were larger and more numerous, causing death of smaller branches. About 50% of the 1,200 Scots pine in the area examined were dead or

dying, whereas the red pine appeared healthy (Fig. 9). Bark beetles and borers had attacked severely cankered trees. These trees contained *B. xylophilus* (Table 2). Dead twigs and smaller branches that had not been infested by insects were free of *B. xylophilus*. In one otherwise healthy tree, however, a single dead branch had been colonized by insects and also contained *B. xylophilus*.

## DISCUSSION

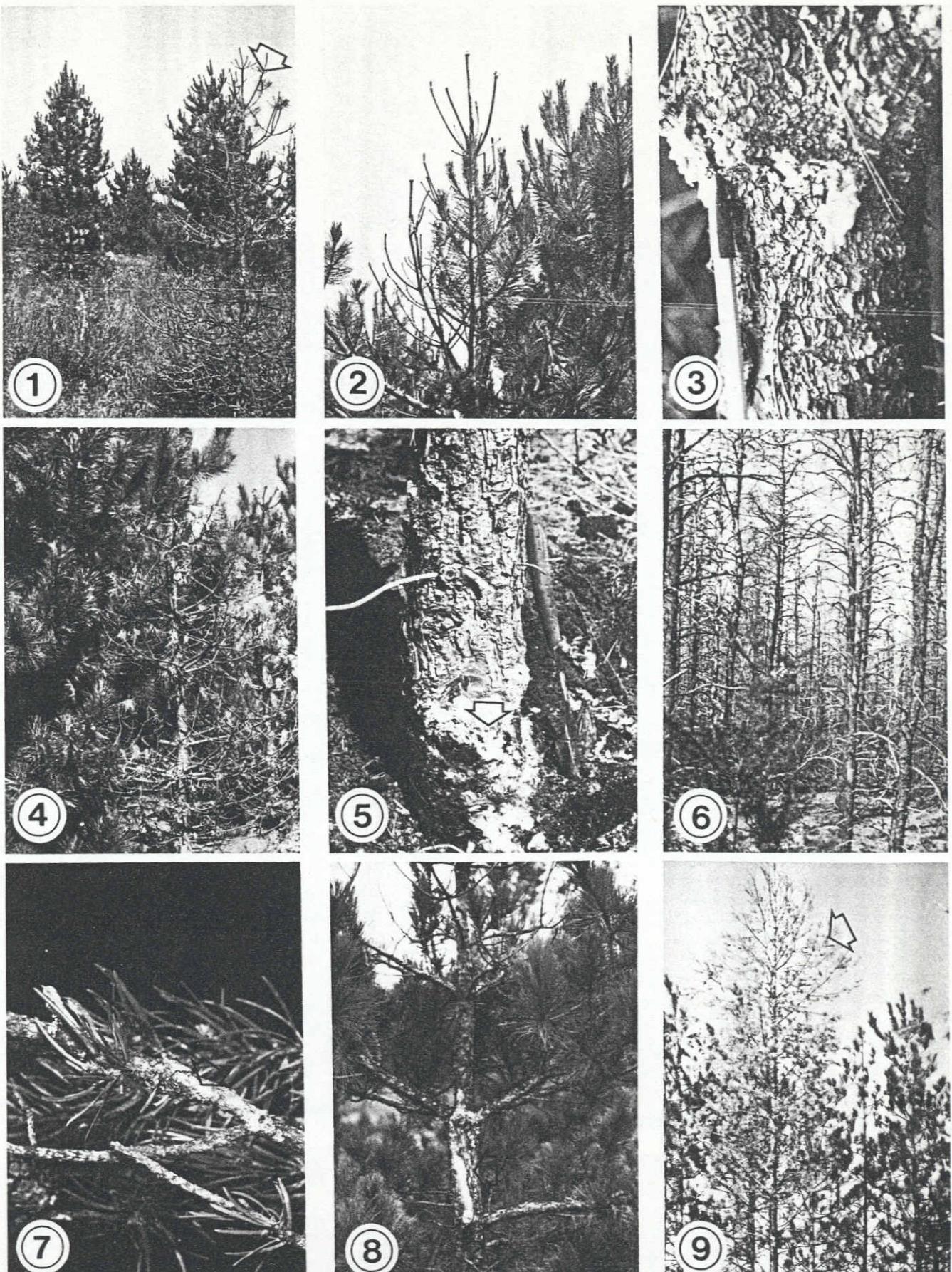
In Japan, trees infected with *B. xylophilus* die within 3 mo after typical wilt symptoms (8). Trees infected with *B. xylophilus* in this investigation did not appear to die of a wilt disease. Tree death was gradual and associated with various fungal pathogens and insects. Pine species in the study areas that are not susceptible to the forest pathogens and insects observed did not die. For instance, Austrian pine is highly susceptible to Dothistroma needle blight, whereas red pine is resistant to this disease (12) and less susceptible to root collar weevil infestation. (L. T. Wilson, personal communication). In our study, only the Austrian pine had died and contained *B. xylophilus*. Similarly, the extreme mortality of the jack pine in Black River Falls has been attributed to the effects of *H. rhizophagus* (5,10). Trees with initial root weevil infestation that still held green needles and had not been infested with bark beetles and borers did not contain nematodes. The observations imply that the nematodes colonize trees at the same time as bark beetles and borers.

Fungal diseases (eg, *Diplodia pinea* canker and Dothistroma needle blight) and insects (eg, pine root collar and pine root weevils) may stress trees, predisposing them to nematode infestation. Stresses such as poor site conditions and drought have previously been associated with *B. xylophilus*-killed trees in Japan (14–16). In other parts of the United States, such as Missouri (V. H. Dropkin,

**Table 1.** Insects and diseases associated with *Bursaphelengus xylophilus* infection of trees from two locations in Minnesota during the summers of 1980 and 1981

Tree species and location	Tree health	Trees sampled (no.)	Number of trees infested with			
			Bark beetles (Scolytidae)	Borers (Cerambycidae)	<i>B. xylophilus</i>	Other diseases and insects <sup>a</sup>
Austrian pine, Zimmerman	Living	4	0	0	0	DNB
	Dead top	8	8	8	6	DNB ZPM
	Dead branches	4	4	4	3	DNB ZPM
	Dead or dying	4	4	4	4	DNB ZPM
White pine, Cloquet	Living	2	0	0	0	...
	Dead top	6	6	6	4	WPBR
	Dead branches	2	2	0	0	WPBR
	Dead or dying	3	3	3	3	WPBR ARR VRD

<sup>a</sup>ARR = Armillaria root rot, DNB = Dothistroma needle blight, VRD = Verticiladiella root disease, WPBR = white pine blister rust, ZPM = Zimmerman pine moth.



Figs. 1-9. Trees stressed by various forest diseases and insects. (1) Dead Austrian pine (arrow) infested with *Bursaphelenchus xylophilus*, with healthy red pine in the background. (2) Dead top of Austrian pine containing *B. xylophilus*. (3) Resin exudation resulting from Zimmerman pine moth infestation associated with dead top of Austrian pine. (4) Dead Austrian pine infested with *B. xylophilus* next to healthy red pine. (5) Resin associated with pine root collar weevil infestation (arrow) on Austrian pine infested with *B. xylophilus*. (6) Stand of dead jack pine infested with *B. xylophilus*. (7) *Diplodia pinea* infection of hail wounds (arrow) on jack pine. (8) *D. pinea* canker (arrow) at the base of a red pine top infested with *B. xylophilus*. (9) Dying Scots pine (arrow) surrounded by healthy red pine.

**Table 2.** Insects and diseases associated with *Bursaphelenchus xylophilus* infection of trees from four locations in Wisconsin and Iowa during the summers of 1980 and 1981

Tree species and location	Tree health	Trees sampled (no.)	Number of trees infested with			
			Bark beetles (Scolytidae)	Borers (Cerambycidae)	<i>B. xylophilus</i>	Other diseases and insects <sup>a</sup>
Austrian pine, Durand, WI	Living	2	0	0	0	DNB
	Dead or dying	5	5	5	5	DNB PRCW
Jack pine, Black River Falls, WI	Living	4	0	0	0	DPTB PRW
	Dead or dying	5	5	5	5	DPTB PRW
Red pine Black River Falls, WI	Living	2	0	0	0	...
	Dead top	10	10	10	3	DPC
Scots pine, Farmington, IA	Living	3	0	0	0	DPC
	Dead or dying	4	4	4	4	DPC

<sup>a</sup> DNB = Dothistroma needle blight, DPC = *Diplodia pinea* canker, DPTB = *D. pinea* tip blight, PRCW = pine root collar weevil, PRW = pine root weevil.

(personal communication) and Delaware (1), however, Scots pine and Japanese black pine are thought to be dying primarily of *B. xylophilus* infection. This apparent difference may be explained by the fact that Japanese black pine is extremely sensitive to nematode infection (7), whereas both species are exotic to the United States and may be stressed.

*B. xylophilus* has been reported in single dead branches of otherwise healthy trees in Louisiana (V. H. Dropkin, personal communication). In this study, *B. xylophilus* was found in dead tops and branches. Mortality of branches or tops was, however, associated with an insect or pathogen (blister rust on white pine, Zimmerman pine moth on Austrian pine, *D. pinea* cankers on red pine) with the capacity to kill portions of a tree (2,3; Nicholls, unpublished). It is possible that *B. xylophilus* is only able to establish itself on stressed parts of a tree. However, cerambycid vectors of *B. xylophilus* may also transmit nematodes to dead and dying trees or parts of trees during oviposition. Further studies, including field inoculations with *B. xylophilus* and sampling of trees over an extended time period, are necessary to elucidate the significance of the pine wood nematode

in Minnesota, Iowa, and Wisconsin.

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