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Comparison of the pine wood nematode, Bursaphelenchus xylophilus from pine and balsam fir[1]

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Abstract

The pine wood nematode, *Bursaphelenchus xylophilus* was found on balsam fir in Minnesota and Wisconsin but was apparently not the primary cause of tree mortality. Unlike *B. xylophilus* from pine, adult females from balsam fir had mucronate tails similar to those of *B. mucronatus*. However, *B. xylophilus* from balsam fir mated with *B. xylophilus* from pine and not with *B. mucronatus*. The balsam fir isolate of *B. xylophilus* was pathogenic on greenhouse grown balsam fir seedlings and did not kill Scots or red pine seedlings. The converse was true for a pine isolate of *B. xylophilus* from Minnesota which killed pine and not balsam fir seedlings. Pine and balsam fir isolates of *B. xylophilus* reproduced equally well on cultures of *Botrytis cinera*, but the balsam fir isolate reproduced minimally on cultures of *Ceratocystis ips*.

1 Introduction

The pine wood nematode, *Bursaphelenchus xylophilus* Steiner and Buhrer has caused severe mortality of native pine species (*Pinus thunberghii* Parl. and *P. densiflora* Sieb. et Zucc.) in Japan (MAMIYA 1972, 1976; MAMIYA and ENDA 1972). *B. xylophilus* is transmitted to branches of healthy pines during maturation feeding by cerambycid beetles (Coleoptera: Cerambycidae) (MAMIYA and ENDA 1972; MORIMOTO and IWASAKI 1972). The biology and etiology of the pine disease caused by *B. xylophilus* in Japan has been reviewed (DROPKIN et al. 1981; MAMIYA 1972, 1976; WINGFIELD et al. 1982 a). *B. xylophilus* was first found in the United States in 1979 (DROPKIN and FOUDIN 1979) and has since been widely reported throughout the country on pines and other conifers (ROBBINS 1982). The biology of *B. xylophilus* in the United States appears to be similar to that described in Japan although its role as a primary pathogen of native North American pines has been questioned (WINGFIELD 1982 a; WINGFIELD et al. 1982 b).

Japanese researchers have suggested that *B. xylophilus* is native to the United States and that it was introduced into Japan (WINGFIELD et al. 1982 a). Evidence that *B. xylophilus* was present in this country during the early part of this century (NICKLE et al. 1980) supports this hypothesis. Isolates of *B. xylophilus* from Japan and the United States have been compared and appear similar in morphology, however, the Japanese population was more pathogenic (KONDO et al. 1982). Differences between populations of *B. xylophilus* within the United States have not been reported. This paper presents evidence of a variant of *B. xylophilus* on balsam fir [*Abies balsamea* (L.) Mill.] in Minnesota and Wisconsin, USA.

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2 Materials and methods

2.1 Field observations

Dead and dying (30–50 years old) balsam fir located on the Cloquet Forestry Center (Carlton Co. MN, USA) and the Nicolet State Forest, Eagle River, WI, USA were examined for the apparent cause of mortality. In Cloquet, symptoms were typical of those of a root disease, with dead trees in the center of a pocket surrounded by trees at various stages of decline (Fig. 1). Fifteen recently killed trees, two with early crown symptoms and five healthy trees at the periphery of the disease center were examined. Root systems of trees were exposed and examined for signs of disease. Isolations from diseased roots were made on Difco malt extract agar (MEA) containing 0.01% Streptomycin sulphate and 0.4% lactic acid on a selective medium for the isolation of basidiomycetes (BLANCHETTE and SHAW 1978). Trees were felled and two-inch thick discs of wood were removed from the bottom, middle and top thirds of the bole for nematode extractions.

In Eagle River, approximately 14,000 ha of balsam fir and black spruce (*Picea mariana* Mill. B. S. P.) were dead or dying (Fig. 2). Mortality of these trees was attributed to attack by spruce budworm, *Choristoneura fumiferana* (Clemens). Ten recently killed balsam fir and five black spruce were felled and examined. Three bolts, 15 cm long were removed from the bottom, middle and top thirds of the bole of each tree for nematode extractions.

Nematodes were extracted from 60–90 g sub-samples of the wood and bark separately, using Baermann funnels (SOUTHEY 1970). Nematodes were identified and concentrated in a clinical, swing bucket centrifuge. After two washes in sterile water, sterilization in 0.1% Streptomycin sulphate and two post-sterilization washes in sterile water, nematodes were placed on mycelial mats of *Botrytis cinerea* Pers. ex Fr. growing on Difco potato dextrose agar (PDA) in 125 ml flasks.

2.2 Nematode identification

Adult male and female nematodes resembling *B. xylophilus* from the original extractions and from cultures on *B. cinerea* were identified and compared morphologically with isolates of *B. xylophilus* from pines in Minnesota and *B. mucronatus* from Japan. A culture of one isolate of the nematode in balsam fir from Cloquet was retained. This isolate was transferred to fresh cultures of *B. cinerea* every two months and the morphology of the nematodes re-examined after every transfer for one year.

Nematodes from the isolate of *B. xylophilus* from balsam fir were mated with *B. xylophilus* from Scots pine (*P. sylvestris* L.) in Missouri and with *B. mucronatus* from Japan. Mating combinations included: 1. female *B. xylophilus* from balsam fir with males of the same isolate; 2. females from balsam fir with males from pine; 3. females from pine with males from balsam fir; 4. females from balsam fir with males of *B. mucronatus* (Table 2).

Five, late fourth stage (final moulting stage) female nematodes and ten adult male nematodes of each combination were placed on a drop of water on a sterile coverglass. The larval females were allowed to moult to the adult stage after which nematodes were re-examined microscopically on the coverglass to confirm that five adult females were present. Coverglasses were inverted onto the mycelia of *B. cinerea* cultures on PDA in 6 cm Petri dishes. After incubation at 25 C for 14 days, nematodes were extracted in plastic weighing boats and counted. Each mating combination was replicated between three and six times (Table 2).

2.3 Seedling inoculations

An isolate of *B. xylophilus* from Austrian pine growing in Zimmerman, MN, USA, and the isolate from balsam fir from Cloquet, MN maintained on cultures of *B. cinerea* in one litre flasks for 4 weeks at 25°C were used for inoculations. Control inoculations were with a

slurry from a nematode free culture of *B. cinerea* blended with distilled water. The top 1 cm of each seedling was excised and a receptacle was made around the cut surface with waterproof tape (Fig. 3). The inoculum was placed in contact with the cut surface of the seedling and the receptacle was closed.

Inoculations with the two isolates of *B. xylophilus* were as follows: 1. 3-year old nursery grown red pine (*P. resinosa* Ait.) and balsam fir (30 of each species) inoculated with 2000 nematodes while an equal number of each species served as controls; 2. 3-year old nursery grown Scots pine and approximately 3-year old field-collected balsam fir seedlings inoculated with 2500, 5000 or 10000 nematodes from the two isolates of *B. xylophilus* (20 seedlings per treatment including controls); 3. 3-year old black spruce inoculated with 5000 nematodes (30 seedlings with each of the two nematode isolates and 30 control seedlings).

Inoculated seedlings were maintained in the greenhouse (approximately 25°C) and observed at weekly intervals for 4 months. Red pine and balsam fir which died after inoculation with 2000 *B. xylophilus* were uprooted, washed and placed in Baermann funnels to extract nematodes. Nematodes present in these dead seedlings were identified microscopically but were not counted. Scots pine and balsam fir inoculated with 2500, 5000 or 10000 nematodes which had died or had dead tops were divided into root (approximately 1 g dry weight) and top (approximately 3 g dry weight) samples and placed in plastic weighing boats to extract nematodes. In addition, nematodes were extracted from the roots and tops of 5 asymptomatic seedlings from each treatment including the controls and from the roots and above ground parts of 10 (5 inoculated and 5 controls) black spruce in a similar manner (Table 5). All nematodes extracted using plastic weighing boats were identified and counted.

2.4 Reproduction on fungi

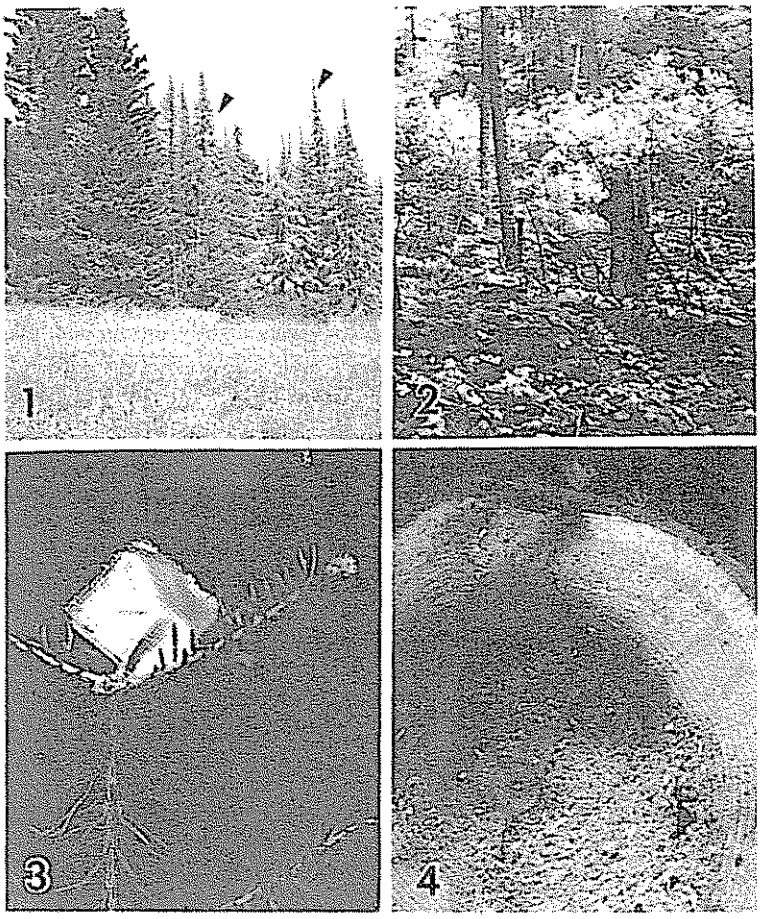
Reproduction of nematodes from the isolates of *B. xylophilus* from balsam fir and pine used in seedling inoculations were compared at 15, 20 and 25°C on cultures of *B. cinerea* on PDA. Ten late fourth stage female and five adult male nematodes were placed on a drop of distilled water on a sterile glass coverslip which was inverted onto the mycelium of *B. cinerea*. Five replicates of each treatment were maintained in Petri dishes for 30 days.

Reproduction of the two nematode isolates were also compared on Petri dish cultures of *B. cinerea*, *Diplodia pinea* (Desm.) Kickx, *Verticicladiella procera* Kendrick, and *Ceratocystis ips* Rumbold. Fifty larvae (second stage) were placed in a drop of sterile distilled water on a sterile coverslip which was inverted onto the mycelium of the various fungi. Ten replicates of each treatment were maintained at 25°C for 33 days. Nematodes were extracted from fungal cultures using Bearmann funnels and counted.

3 Results

3.1 Field observations

All dead and dying trees in Cloquet had white mycelial mats, typical of *Armillaria mellea* (Fr.) Kummer root rot under the bark of the roots and root collar. Roots of trees at the periphery of the infection centers had signs of *A. mellea* under the bark and the fungus was isolated from the lesions. In addition to root disease, trees had heart rot (Fig. 4) from which *Odontia bicolor* (Alb. and Schw.) Bres. was isolated. The 15 dead trees examined were all infested with the bark beetle, *Pityokteines sparsus* (LeC.) (Coleoptera: Scolytidae). Trees with early symptoms of root disease as well as healthy trees had no signs of insect attack. Small numbers (less than 10 adults) of *B. xylophilus* were extracted from the wood but not from the bark of all dead trees colonized by insects (Table 1).



Figs. 1–4. Dying balsam fir and seedling inoculated with *B. xylophilus*. 1. Balsam fir and spruce (arrows) in Eagle River, WI dying from spruce budworm infestation. 2. Pocket of dying balsam fir in Cloquet, MN with *Armillaria* root disease and *Odontia bicolor* butt rot. 3. Balsam fir seedling inoculated with *B. xylophilus*. 4. Section of balsam fir bole showing rot associated with *O. bicolor*

Table 1

Association of bark beetles (Coleoptera: Scolytidae), borers (Coleoptera: Cerambycidae) and B. xylophilus on balsam fir and black spruce in Minnesota and Wisconsin

Location	Tree species	Tree health	No of trees examined	Number of trees with				Other forest insects and diseases
				Bark beetles	Borers	*B. xylophilus*		
						In bark	In wood	
Eagle River	balsam fir	dead	10	10	10	1	1	SBW
Wisconsin	black spruce	dead	5	5	5	0	0	SBW
Cloquet	balsam fir	healthy	5	0	0	0	0	–
Minnesota		dying	2	0	0	0	0	ARR
		dead	15	15	15	0	15	ARR OBBR

ARR = *Armillaria* root rot; OBBR = *Odontia bicolor* butt rot; SBW = Spruce bud worm.

In Eagle River WI, balsam fir and black spruce killed by the spruce budworm had also been infested by bark beetles (*P. sparsus*) and wood borers. *B. xylophilus* was extracted from the wood and bark of only one balsam fir tree and was not found in black spruce (Table 1). Less than 10 adult *B. xylophilus* were present in any sub-sample of the single balsam fir containing the nematode.

3.2 Nematode identification

Adult male *B. xylophilus* extracted from balsam fir were indistinguishable from those extracted from pines in Minnesota and Wisconsin. The tails of the adult females were distinctly mucronate (Fig. 5), unlike those from pines and more like *B. mucronatus* (MAMIYA and ENDA 1979). The mucronate tails of *B. mucronatus* were, however, slightly shorter and less pointed than those of *B. mucronatus* were, however, slightly shorter and less pointed than those of *B. xylophilus* from balsam fir (Fig. 5). Tails of adult female *B. xylophilus* from balsam fir maintained their distinct morphology after six transfers at 2-month intervals.

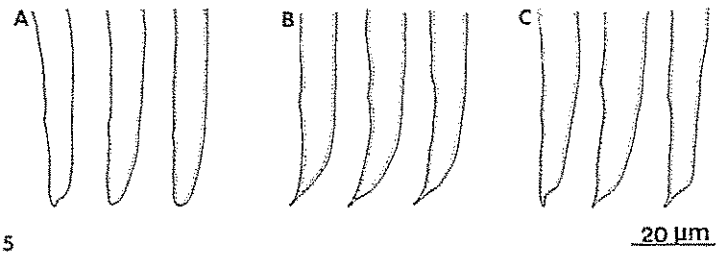


Fig. 5. Tails of adult female *B. xylophilus* and *B. mucronatus*. *A. B. xylophilus* from pine in Minnesota. *B. B. xylophilus* from balsam fir in Minnesota. *C. B. mucronatus*

Female *B. xylophilus* from balsam fir mated with male *B. xylophilus* from balsam fir and with male and female *B. xylophilus* from pine. The average number of nematodes resulting from the cross between the nematodes from pine and balsam fir were considerably lower than those from the cross of the nematodes from balsam fir with itself (Table 2). The nematodes from balsam fir did not mate with *B. mucronatus* from Japan (Table 2).

Table 2

Mating between B. xylophilus from balsam fir in Minnesota with B. xylophilus from pine in Missouri and B. mucronatus from pine in Japan [1]

Mating combination [2]		Number of replicates	Average number of nematodes per Petri dish	% adults	% females with mucronate tails
Male	Female				
Bxb	Bxb	5	19280	13.8	100
Bxb	Bxp	5	8992	27.0	68.2
Bxp	Bxb	3	6147	27.0	68.2
Bxb	Bm	6	21	70.6	–

[1] Data published in KONDO et al. 1982. [2] Bxb = *B. xylophilus* from balsam fir; Bxp = *B. xylophilus* from pine (Missouri); Bm = *B. mucronatus* from pine (Japan).

3.3 Seedling inoculations

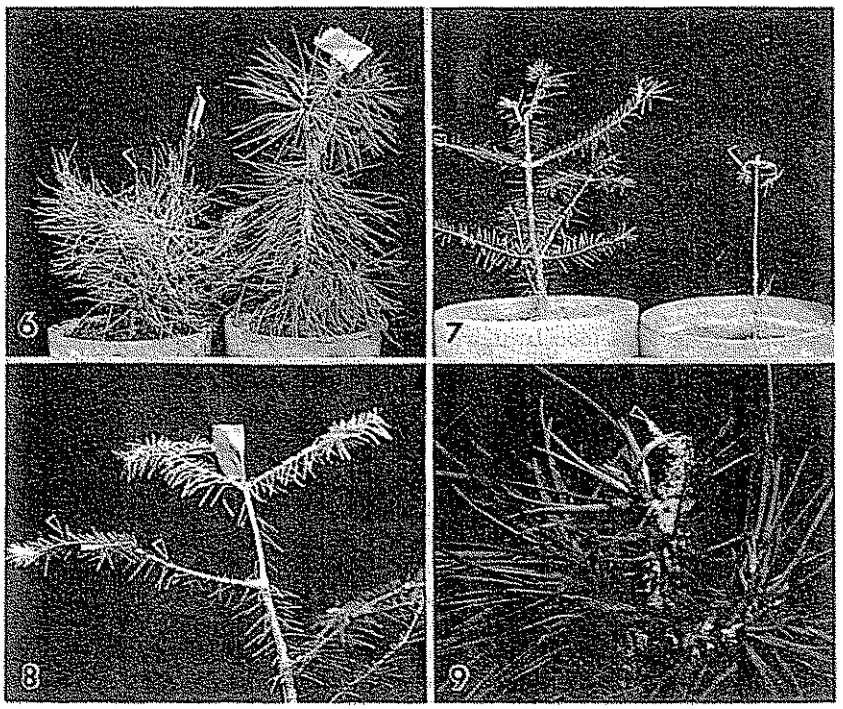
Eight of 30 red pine seedlings inoculated with 2000 *B. xylophilus* from pine died within four months while no seedlings inoculated with the balsam fir isolate of *B. xylophilus* died (Table 3). Similarly, nine balsam fir seedlings inoculated with *B. xylophilus* from balsam fir died (Fig. 6) whereas no seedlings inoculated with the pine isolates died. Control red pine and balsam fir seedlings remained healthy (Table 3).

Table 3

Mortality of 3-year-old red pine and balsam fir seedlings inoculated with B. xylophilus from balsam fir and pine after 4 months

Species inoculated	Inoculum source	Number of trees	
		Dead	Healthy
Red pine	balsam fir	0	30
	pine	8	22
	no nematodes	0	30
Balsam fir	balsam fir	9	21
	pine	0	30
	no nematodes	0	30

Mortality of Scots pine inoculated with *B. xylophilus* from pine (Fig. 7) varied depending on the inoculum load (Table 4). The terminal shoots of two seedlings inoculated with 10000 were killed. Scots pine seedlings inoculated with *B. xylophilus* from pine (Table 4) appeared to be more susceptible than red pine inoculated with the same nematode isolate (Table 3). Two Scots pine seedlings inoculated with the balsam fir isolate of *B. xylophilus* died. This was unlike the results obtained from inoculation of this nematode on red pine seedlings (Table 3) and may indicate a greater degree of susceptibility of Scots pine to *B. xylophilus*.



Figs. 6–9. Scots pine and balsam fir seedlings inoculated with *B. xylophilus*. 6. Dead Scots pine seedling (arrow) inoculated with *B. xylophilus* from pine and noninoculated seedling. 7. Balsam fir seedling inoculated with *B. xylophilus* from balsam fir (arrow) and noninoculated seedling. 8. Field collected balsam fir seedling inoculated with *B. xylophilus* from balsam fir showing only top mortality. Living branch with new growth (arrows) at base of dead top. 9. Adventitious buds (arrow) at base of inoculation point of Scots pine seedling which did not die after *B. xylophilus* infestation

Table 4

Mortality of 3-year old Scots pine, balsam fir and black spruce inoculated with isolates of B. xylophilus from Austrian pine and balsam fir

Species inoculated	Inoculum source	No. of nematodes	Number of trees[1]		
			Healthy	Dead top	Dead
Scots pine	pine	2500	12	0	8
		5000	8	0	12
		10000	7	2	11
	balsam fir	2500	19	0	1
		5000	19	0	1
		10000	20	0	0
	no nematodes	–	20	0	0
Balsam fir	pine	2500	20	0	0
		5000	20	0	0
		10000	20	0	0
	balsam fir	2500	18	2	0
		5000	13	4	3
		10000	13	7	0
	no nematodes	–	20	0	0
Black spruce	pine	5000	20	0	0
	balsam fir	5000	20	0	0
	no nematodes	–	20	0	0

[1] 20 seedlings inoculated for each treatment.

Field collected balsam fir seedlings inoculated with the pine isolate of *B. xylophilus* did not die irrespective of the number of nematodes applied (Table 4). Although some balsam fir seedlings inoculated with 5000 nematodes from balsam fir died (Fig. 6), a more common symptom observed was death of the seedling tops (Fig. 8). More seedlings with dead tops resulted when greater numbers of nematodes were applied (Table 4). All seedlings inoculated as controls remained healthy. Seedlings inoculated as controls and those that did not die as result of nematode inoculation developed adventitious buds just below the point of inoculation approximately two weeks after inoculation (Fig. 9).

Black spruce seedlings inoculated with the two isolates of *B. xylophilus* remained asymptomatic. Similarly, no mortality was observed in the control inoculations of the spruce seedlings (Table 4).

Nematodes were extracted from all red pine and balsam fir seedlings which died after inoculation with 2000 *B. xylophilus* of the two different isolates. Adult male and female nematodes were identical to those used in the inoculations. The mucronate tails of the female *B. xylophilus* from balsam fir remained present in the nematodes after extraction from dead seedlings. Nematodes extracted from tops and roots of dead and living balsam fir, Scots pine and black spruce were also examined and found to be morphologically similar to those used in the inoculations. The average number of nematodes extracted from the tops and roots of these seedlings are presented in Table 5. Higher numbers of nematodes were extracted from the root samples than from the tops. Balsam fir seedlings with dead tops after inoculation with *B. xylophilus* from balsam fir, most commonly contained nematodes only in the dead tissue. One seedling with a dead top inoculated with 2500 nematodes from balsam fir contained higher numbers of *B. xylophilus* in the roots (Table 5) than above ground parts of the seedling.

Although spruce seedlings showed no symptoms after inoculation with the two nematode isolates, samples from the tops of these seedlings yielded small numbers of *B. xylophilus*. No nematodes were present in the control seedlings (Table 5).

Table 5

Numbers of B. xylophilus extracted from roots and tops of Scots pine, balsam fir and black spruce seedlings 4 months after inoculation with 0, 2500, 5000, 10000 nematodes

Tree species	Tree health	Number of trees sampled	Number of nematodes inoculated	Nematode source	Average number of nematodes extracted	
					Roots	Tops
Scots pine	dead	4	2000	pine	289.0	139.8
		4	5000	pine	245.5	17.5
		5	10000	pine	311.8	113.8
	dead	1	2500	fir	234	30.0
		1	5000	fir	0	6.0
	living	5	2500	pine	3.2	17.6
		5	5000	pine	25.6	6.0
		5	10000	pine	6.6	31.8
	living	5	2500	fir	0	0
		5	5000	fir	1.8	2.5
		5	10000	fir	0.8	2.8
	living	5	0		0	0
Balsam fir	dead top	1	2500	fir	39.0	23.0
	dead	2	5000	fir	30.5	3.0
	dead top	2	5000	fir	0	2.0
	dead top	4	10000	fir	0	0
Balsam fir	living	5	2500	fir	2.6	0
		5	5000	fir	0	0
		5	10000	fir	0	0
	living	5	2500	pine	0	0
		5	5000	pine	0	0
		5	10000	pine	0	0
	living		0		0	0
Black spruce	living	5	5000		0	3.8
		5	5000		0	1.2
	living	5	0		0	0

3.4 Reproduction on fungi

Reproduction of the two nematode isolates did not occur at 15 °C (Table 6). The number of nematodes recovered from plates maintained at 25 °C was approximately the same for the two isolates. Reproduction of *B. xylophilus* from balsam fir was considerably slower at 20 °C than that of the pine isolate of *B. cinerea* (Table 6).

Nematodes of both isolates reproduced best on cultures of *B. cinerea* (Table 7). Approximately the same number of nematodes were recovered from plates representing the two different isolates of *B. xylophilus*. The pine isolate of *B. xylophilus* reproduced well on

Table 6

Reproduction of B. xylophilus from balsam fir and pine on Botrytis cinerea at 15, 20 and 25 °C

Nematode isolate	Number of nematode[1]		
	15 °C	20 °C	25 °C
Pine	2.0	12884	35470
Fir	1.2	5250	35309

[1] Average number of nematodes recovered from 5 Petri dishes after 30 days.

Table 7

Reproduction of B. xylophilus from balsam fir and pine on Botrytis cinerea, Diplodia pinea, Verticicladiella procera and Ceratocystis ips

Fungus	Number of nematodes [1]	
	Pine isolate	Fir isolate
B. cinerea	29607	28904
D. pinea	368	185
V. procera	5025	1580
C. ips	13405	94

[1] Average number of nematodes recovered from 10 Petri dishes maintained at 25 °C for 33 days.

cultures of *C. ips* while the balsam fir isolate did not appear to reproduce on this fungus. Both nematode isolates reproduced on *V. procera* although better reproduction occurred with the pine isolate. Comparatively low numbers of nematodes were recovered from cultures of *D. pinea* although numbers of *B. xylophilus* from pine were more than double those of the balsam fir isolate on this fungus.

4 Discussion

B. xylophilus did not appear to be the primary cause of balsam fir mortality in either field location. Only trees that had been colonized by bark beetles and borers contained *B. xylophilus*. These observations are similar to those previous reports associating *B. xylophilus* with stressed pines in Minnesota, Iowa and Wisconsin (WINGFIELD et al. 1982 b). Nematodes were absent from dying trees not attacked by insects. It is probable that the nematodes were transmitted to these trees during Cerambycid oviposition. This has been shown to occur in the United States (LUZZI and TARJAN 1982; WINGFIELD 1982 a) and is thought to occur in Japan (MAMIYA personal communication).

The nematode isolate from balsam fir was morphologically similar to *B. mucronatus* (MAMIYA and ENDA 1979). The major morphological characteristic separating *B. mucronatus* from *B. xylophilus* is the mucronate tail in adult female of *B. mucronatus* (MAMIYA and ENDA 1979). The mucronate tails of adult female *B. xylophilus* from balsam fir were, however, more pointed than those of *B. mucronatus*. *B. mucronatus* does not mate with *B. xylophilus* (MAMIYA and ENDA 1979; KONDO et al. 1982). The fact that the nematodes from balsam fir mated with *B. xylophilus* and not *B. mucronatus* suggests that the former nematode is a morphological variant of *B. xylophilus* regardless of its similarity with *B. mucronatus*.

Mating combinations between pine isolates and balsam fir isolates of *B. xylophilus* yielded lower numbers of nematodes than did the cross of the balsam fir isolate with itself (Table 2). This indicates some differences between the pine and the balsam fir isolates of *B. xylophilus*. The matings between the balsam fir isolate of *B. xylophilus* and *B. mucronatus* produced sterile hybrids which did not give rise to established populations of the nematode.

Initial inoculations on red pine and balsam fir seedlings indicated that *B. xylophilus* originating from balsam fir was selectively pathogenic on fir and the pine isolate pathogenic only on red pine seedlings. Subsequent inoculations using Scots pine and balsam fir seedlings, however, resulted in limited mortality of Scots pine inoculated with *B. xylophilus* from balsam fir. This result reinforces previous observations that Scots pine seedlings are more susceptible to infection by *B. xylophilus* than are red pine seedlings (FUTAI and FURANO 1979; DROPKIN et al. 1981; KONDO et al. 1982). Field collected balsam fir seedlings appeared to be more resistant to infection by *B. xylophilus* from balsam fir than greenhouse grown seedlings. This may be due to the presence of more succulent tissue in the greenhouse maintained seedlings.

Dead top symptoms were most common when seedlings (balsam fir and pine) were inoculated with 10000 nematodes. It is possible that the greater number of nematodes caused more damage to the tops of the seedlings. The resulting dead tissue may then have prevented nematode movement into the remaining portion of the seedling. This theory is supported by the small number of nematodes extracted from balsam fir seedlings with dead tops (Table 5).

Considerably greater numbers of nematodes were extracted from Scots pine seedlings inoculated with nematodes from pine than were extracted from balsam fir seedlings inoculated with nematodes from balsam fir. This may be attributed to the fact that Scots pine contains resin canals through which nematodes are reported to move (MAMIYA 1972, 1976). Balsam fir lacks resin canals (PANSHIN and DE ZEEUW 1964) which may limit the movement and development of the nematodes in the seedlings. The reason that higher numbers of nematodes were extracted from the roots than from the tops of seedlings may be due to the tops drying out. Although the tops of the seedlings die, the roots remain moist and better suited to nematode survival. Similarly, the relatively low number of nematodes extracted from dead seedlings may be due to a lack of food and moisture in dead tissue.

Several living balsam fir and pine seedlings contained low numbers of nematodes four months after inoculation in spite of the fact that no symptoms were evident. Fewer nematodes were found in living Scots pine seedlings inoculated with *B. xylophilus* from balsam fir than those from pine. This supports the hypothesis that the pine isolate of *B. xylophilus* is better suited to living in pine. Nematodes were never found in balsam fir seedlings inoculated with *B. xylophilus* from pine. This supports the evidence that balsam fir is a less susceptible host of *B. xylophilus* perhaps attributable to the absence of resin canals as mentioned above.

B. xylophilus has been reported to occur in black spruce (ROBBINS 1982). The fact that neither of the nematode isolates used in these inoculations were able to kill black spruce seedlings suggests that different physiological races of *B. xylophilus* exist. This study presents evidence of an isolate of *B. xylophilus* from balsam fir apparently better suited to colonization of its host of origin. We may speculate that isolates of *B. xylophilus* found in black spruce will be selectively pathogenic on seedlings of that host. On the contrary, however, isolates of *B. xylophilus* from larch *Larix laricina* (DuRoi) K. Koch and pine are equally pathogenic on pine and larch seedlings (BERGDAHL 1982).

Certain cerambycid species such as *Monochamus scutellatus* Say. are associated with *B. xylophilus* in the North Central United States (WINGFIELD and BLANCHETTE 1983). This insect is known to infest many pine species as well as balsam fir, spruce and larch in the area (WILSON 1962). The capacity for *B. xylophilus* to be transmitted to conifers other than pine by beetles emerging from pine therefore exists. Whether *M. scutellatus* emerging from one conifer species feeds and oviposits on other species and in turn transmits *B. xylophilus* is unknown.

Differences in growth and reproduction of the two nematode isolates on different fungi may be related to their ecological origins. *Botrytis cinerea* is a weakly pathogenic fungus associated with the grey mould disease of many plants (WEBSTER 1980) and to our knowledge has not been isolated from coniferous wood. Notwithstanding this fact, both nematode isolates grew well on this fungus as has been observed in previous studies (DOZONO and YOSHIDA 1974; KONDO et al. 1982). *Diplodia pinea* is a common, weakly pathogenic fungus usually found associated with die-back and tip blight of pines (PUNITHALINGHAM and WATERSON 1970). Although it occurs on pine and not balsam fir, this fungus is not likely to provide a food source for *B. xylophilus* in nature, since it is not commonly isolated from the wood of dead trees. The slow development of *B. xylophilus* on this fungus may support this view.

Ceratocystis ips is transmitted by various Scolytid bark beetles (UPHADYAY 1981) and is the fungus most commonly isolated from blue stained timber in the North Central United States (Authors unpublished; HIMELICK 1982). Recent research has shown than *B. xylo-*

philus is transmitted to cut timber and dying trees (WINGFIELD 1982 a) during oviposition (WINGFIELD and BLANCHETTE 1983). Once transmitted to cut timber or dying trees, the nematode is thought to perpetuate itself on fungi such as *C. ips* which transmitted to timber close to the same time that Cerambycid oviposition occurs (WINGFIELD 1982 b). *C. ips* does not, however, occur in balsam fir which is less extensively colonized by blue stain fungi (Authors unpublished). The association of *B. xylophilus* with *C. ips* in dead pines in Minnesota and the lack of this association on balsam fir may explain why *B. xylophilus* from pine feeds readily on *C. ips* in culture while the balsam fir isolate of *B. xylophilus* does not reproduce on this fungus.

Differences between isolates of *B. xylophilus* from balsam fir and pine are based on experiments using single isolates of the nematodes. All adult female nematodes extracted from balsam fir in this study had mucronate tails which suggests that the isolate used in laboratory and greenhouse experiments is representative of a population of *B. xylophilus* present in balsam fir. The presence of other biological variants of *B. xylophilus* in conifers including balsam fir is likely. This view is supported by reports of isolates of *B. xylophilus* of varying pathogenicity from pines in Japan (J. W. RIFFLE, Forest Sciences Laboratory, University of Nebraska, personal communication). The possible existence of more pathogenic races of the nematode present in different parts of the world and the significance of this fact in quarantine measures deserves further attention.

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Summary

Bursaphelenchus xylophilus was found on balsam fir which were dying primarily of root disease and insect infestation. Adult female nematodes from balsam fir had mucronate tails similar to those in *B. mucronatus*. Nematodes from balsam fir did not mate with *B. mucronatus* but did mate with *B. xylophilus* from pines. The nematode from balsam fir is considered a morphological variant of *B. xylophilus* regardless of its similarity with *B. mucronatus*. *B. xylophilus* from balsam fir killed only balsam fir seedlings while a pine isolate of the nematode killed only pine seedlings in greenhouse inoculations. Both nematode isolates reproduced well on cultures of *Botrytis cinerea* but only the pine isolate of *B. xylophilus* reproduced on *Ceratocystis ips*, a fungus common on pines but which does not occur in balsam fir.

Résumé

Comparaison du nématode habitant le bois de pin, Bursaphelenchus xylophilus, selon qu'il est extrait du pin ou du sapin baumier

Le nématode du bois de pin, *Bursaphelenchus xylophilus* a été découvert sur sapin baumier au Minnesota et dans le Wisconsin, mais apparemment il ne constituait pas la cause primaire de la mortalité des arbres. A la différence du *B. xylophilus* issu des pins, les femelles adultes provenant du sapin baumier ont des appendices mucronés semblables à ceux de *B. mucronatus*. Toutefois, *B. xylophilus* provenant du sapin baumier peut être croisé avec *B. xylophilus* venant du pin et pas avec *B. mucronatus*. Les individus isolés sur sapin baumier sont pathogènes pour des semis de sapins baumiers élevés en serre et ne tuent pas les semis de pin sylvestre et de Pinus resinosa. L'inverse est vrai de *B. xylophilus* isolé de pins dans le Minnesota qui tue les semis de pins et pas ceux de sapin baumier. Les isolats de *B. xylophilus* issus de pins et de sapin baumier se reproduisent tous deux bien sur des cultures de *Botrytis cinerea*, mais l'isolat issu de sapin baumier se reproduit très faiblement sur des cultures de *Ceratocystis ips*.

Zusammenfassung

Ein Vergleich des Kiefernholz bewohnenden Nematoden Bursaphelenchus xylophilus aus Kiefer und Balsamtanne

Bursaphelenchus xylophilus wurde in Balsamtannen gefunden, bei denen primär Wurzelerkrankungen und Insektenbefall zum Absterben führten. Erwachsene weibliche Nematoden aus Balsamtannen hatten stachelspitzige Hinterenden, ähnlich denen von *B. mucronatus*. Nematoden aus Balsamtannen paarten sich nicht mit *B. mucronatus*, wohl aber mit *B. xylophilus* aus Kiefern. Die Nematoden von der Balsamtanne werden trotz ihrer Ähnlichkeit mit *B. mucronatus* als morphologische Variante von *B. xylophilus* angesehen.

B. xylophilus von Balsamtannen brachte nur Sämlinge dieser Baumart zum Absterben, während Nematoden von Kiefern bei Inokulationen im Gewächshaus nur Kiefernsämlinge abtöteten. Beide Nematodenisolierungen vermehrten sich gut auf Kulturen von *Botrytis cinerea*. Auf *Ceratocystis ips*, einem häufig an Kiefern, nicht aber an Balsamtannen vorkommenden Pilz, vermehrte sich nur das *B. xylophilus*-Isolat aus Kiefern.

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