The pine-wood nematode, *Bursaphelenchus xylophilus*, in Minnesota and Wisconsin: insect associates and transmission studies[1]

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The pine-wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer), was most commonly extracted from Cerambycidae emerging from nematode-infested pines in Minnesota and Wisconsin during 1981 and 1982. The greatest number of nematodes were extracted from *Monochamus scutellatus* (Say) and *Monochamus carolinensis* (Olivier). Low numbers of *B. xylophilus* were found in some buprestids but no nematodes were found in the curulionid and scolytid beetles examined. Two species of Cerambycidae, *Monochamus marmorator* (Kiby.) and *M. scutellatus* were associated with *B. xylophilus* from balsam fir in Minnesota. *Bursaphelenchus xylophilus* from insects associated with balsam fir were morphologically different from insects associated with pine. Dauer larvae of *B. xylophilus* were concentrated in the thoracic segments of *M. scutellatus* and *Monochamus mutator* (Lec.) examined. *Bursaphelenchus xylophilus* was transmitted to twigs during maturation feeding and to logs during oviposition by *M. carolinensis*, *M. mutator*, and *M. scutellatus*.

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Le *Bursaphelenchus xylophilus* (Steiner et Buhrer), un nématode du bois de pin fut communément extrait de cérambyx émergeant de pins infestés de nématodes au Minnesota et au Wisconsin durant les années 1981 et 1982. Le plus grand nombre de nématodes fut extrait des *Monochamus scutellatus* (Say) et *M. carolinensis* (Olivier). Un petit nombre de nématodes fut trouvé chez certains buprestes mais aucun ne fut trouvé chez les charançons et les scolytes examinés. Au Minnesota, le *M. marmorator* (Kiby.) et le *M. scutellatus*, provenant de bois de sapin étaient associés au *B. xylophilus*. La morphologie du nématode diffère selon qu'il est associé à des insectes venant du sapin ou du pin. Chez les spécimens de *M. scutellatus* et *M. mutator* (Lec.) examinés, on trouvé des masses de larves "dauer" (en latence) du nématode dans les segments thoraciques. Le transfert du *B. xylophilus* aux rameaux et aux billes se faisait, respectivement, durant l'alimentation de maturation et durant la ponte par les *M. carolinensis*, *M. mutator* et *M. scutellatus*.

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The pine-wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer), is the primary cause of a severe wilt disease of native pines (*Pinus thunberghii* Parl. and *Pinus densiflora* Sieb. et Zucc.) in Japan (Mamiya 1976; Mamiya and Enda 1972). In Japan, *B. xylophilus* is vectored primarily by cerambycid beetles (Coleoptera: Cerambycidae) (Mamiya and Enda 1972; Morimoto and Iwasaki 1972). The dauer larvae (transmission-stage larvae) of *B. xylophilus* enter the spiracles of the vectors before they emerge and are carried to young shoots of healthy trees on which the Cerambycidae undergo maturation feeding. Nematodes moult to the adult stage and enter the resin canals of host trees which exhibit rapid wilt symptoms and die within 3 months (Ishibashi and Kondo 1977; Mamiya 1976). Symptoms and development of pine-wilt disease in Japan have been discussed in detail in a number of review articles (Dropkin *et al.* 1981; Mamiya 1972, 1976; Wingfield *et al.* 1982*a*).

Bursaphelenchus xylophilus was first found in the United States in 1931 (Steiner and Buhrer 1934) at which time it was not recognized as a pathogen (Nickle *et al.* 1980). More recently, the nematode was found in this country in 1979 (Dropkin and Foudin 1979) and subsequently has been reported widespread throughout the United States on a wide range of conifer hosts, primarily *Pinus* spp. (Dropkin *et al.* 1981; Wingfield *et al.* 1982*a*). The association of *B. xylophilus* with dying trees in the United States has led to speculation that it may be a primary cause of mortality of native conifer species in this country (Dropkin *et al.* 1981).

In Japan, *Monochamus alternatus* Hops. is the most important vector of *B. xylophilus* (Mamiya and Enda 1972; Morimoto and Iwasaki 1972). Preliminary investigations identifying insect associates of *B. xylophilus* in the United States have been reported from Missouri (Dropkin *et al.* 1981; Kondo *et al.* 1982; Linit *et al.* 1983) and Florida (Luzzi and Tarjan 1982). The following investigations were intended to identify the insect species most commonly carrying *B. xylophilus* in Minnesota and Wisconsin. The results of transmission

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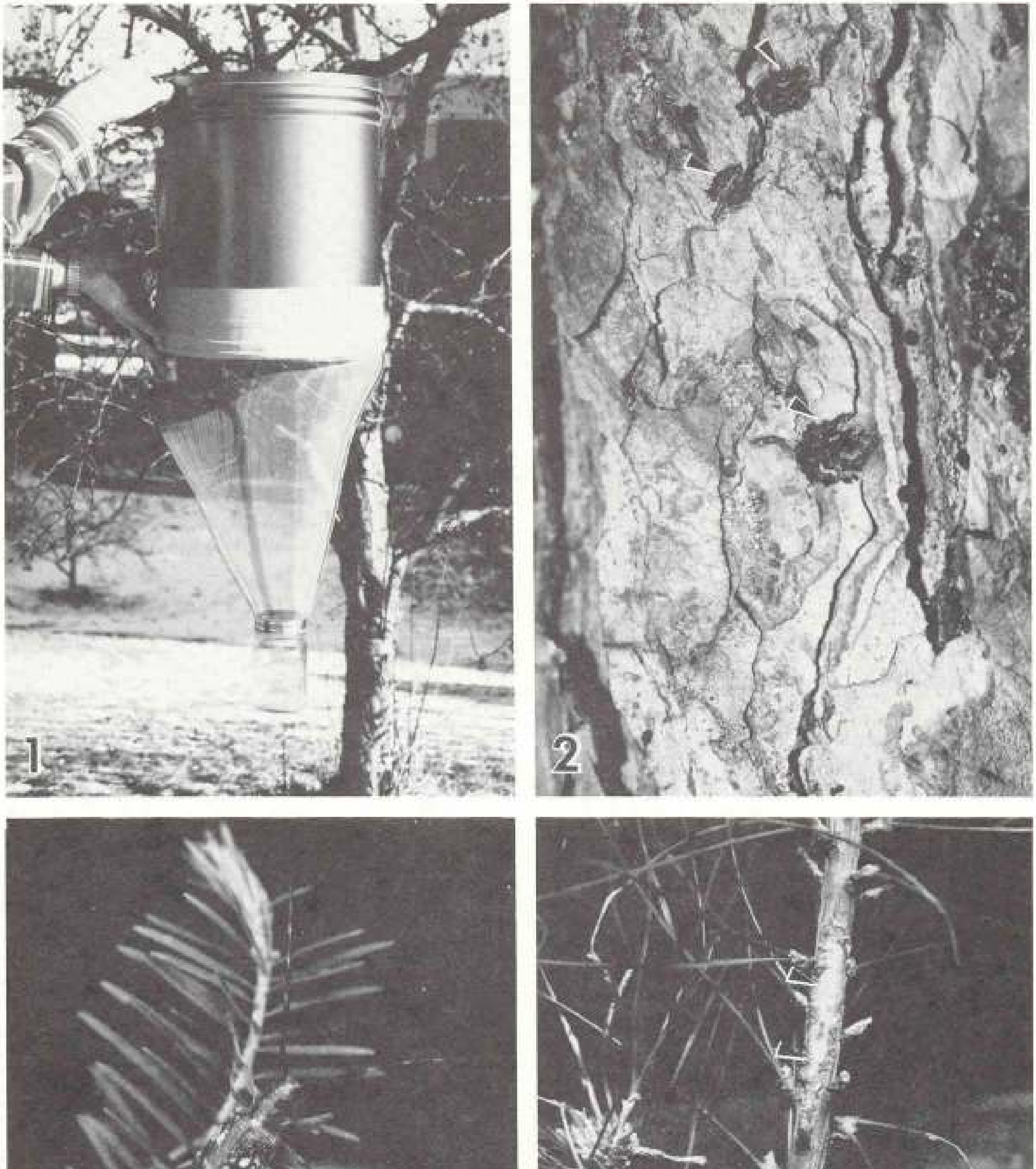

FIGS. 1–4. Emergence trap, oviposition damage, and maturation feeding by Cerambycidae. Fig. 1. Emergence trap for collecting Cerambycidae from pine infested with *B. xylophilus*. Fig. 2. Cerambycid oviposition niches (arrows) on red pine. Fig. 3. *Monochamus scutellatus* during maturation feeding on balsam fir seedling. Fig. 4. Maturation feeding damage (arrows) on red pine seedling.

TABLE 1. Insects associated with *Bursaphelenchus xylophilus* infested pine and balsam fir from Minnesota and Wisconsin and the number of nematodes extracted from insects during 1981[a]

			No. of insects		No. of nematodes per insect	
Host and source	Insect family	Insect species	Examined	With *B. xylophilus*	Maximum	Average
Austrian pine						
Zimmerman, MN	Cerambycidae	*M. scutellatus*	21	20	65 039	12 415.4
		M. carolinensis	48	47	39 781	8094.3
		A. sexguttatus	15	14	43	8.9
		N. pusilus	2	0	0	—
		X. saggitatus	10	1	3	0.3
	Buprestidae	*Chrysobothris* sp.	7	2	7	1.6
	Curculionidae	*H. pales*	20	0	—	—
		P. picivorus[b]	20	0	—	—
		P. approximatus[b]	20	0	—	—
	Scolytidae	*I. pini*[b]	20	0	—	—
Austrian pine						
Durand, WI	Cerambycidae	*M. carolinensis*	2	1	9	4.5
Jack pine						
Black River Falls, WI	Cerambycidae	*M. carolinensis*	2	1	9	4.5
		M. mutator	3	3	25	15.3
Ponderosa pine						
LaCrosse, WI	Cerambycidae	*M. carolinensis*	2	2	11 662	5834.0
Red pine						
Spring Green, WI	Cerambycidae	*M. carolinensis*	52	52	49 875	10 515.6
		N. pusilus	1	1	14	14.0
White pine						
Independence, WI	Cerambycidae	*M. carolinensis*	9	7	920	138
Balsam fir						
Cloquet, MN	Cerambycidae	*M. scutellatus*	102	91	7963	543.1
		M. marmorator	10	9	8652	1686.4
	Buprestidae	*Chrysobothris* sp.	1	0	—	—

[a]Insects collected from the emergence traps containing *B. xylophilus* infested wood.
[b]Insects trapped associated with *B. xylophilus* infested trees in the field.

studies with *Monochamus carolinensis* (Olivier), *M. mutator* (Lec.), and *M. scutellatus* (Jay) are also discussed.

Materials and methods

Insect associates of B. xylophilus

Insects were collected from Austrian pine (*Pinus nigra* Arnold), jack pine (*Pinus banksiana* Lamb), ponderosa pine (*Pinus ponderosa* Laws.), red pine (*Pinus resinosa* Ait.), eastern white pine (*Pinus strobus* L.), and balsam fir (*Abies balsamea* (L.) Mill.) wood using emergence traps during the spring and summer of 1981 and from the wood of Austrian pine, jack pine, and red pine during the spring and summer of 1982. Wood bolts placed in emergence traps (Fig. 1) were collected from recently killed trees from various locations in Minnesota and Wisconsin (Table 1). Subsamples of wood (70 g fresh weight) from bolts were placed in Baermann funnels (Southey 1970) to ascertain whether *B. xylophilus* was present in the trees.

Wood bolts placed in emergence traps included branches (1.5−8.0 cm diameter) and sections from the main bole of trees (8−23 cm diameter). Insects emerging from red pine collected in Spring Green, Wisconsin were supplied by Dr. D. J. Hall, Forest Entomologist, Wisconsin Department of Natural Resources. In addition to wood samples from dead trees, bolts of Austrian pine cut from healthy, nematode-free trees and subsequently infested with cerambycid beetles and *B. xylophilus* as reported in a previous investigation (Wingfield 1982*a*) were also placed in emergence traps.

Insects emerging from caged wood were collected daily. Subsamples of Cerambycidae of unknown identity were retained and sent to Dr. D. C. L. Gosling, White Pigeon, Michigan, for identification. During 1981, insects collected were individually placed in 100 mL of water in a Waring blender and macerated for 1 min. Macerated insects were placed in Baermann funnels and nematodes were extracted after 24 h. Insects collected during 1982 were lightly macerated in 100 mL of water using a mortar and pestle and nematodes were extracted by placing the macerated insects in a double layer of tissue paper in plastic weighing boats. Another weighing boat was used as a cover to reduce evaporation.

Field-collected *Hylobius pales* (Herbst.), *Pachylobius picivorus* (Germ.), and *Pissodes approximatus* Hopkins, associated with *B. xylophilus* infested Austrian pine in Zimmerman, Minnesota, were obtained by placing freshly cut discs of white pine under dying trees (Ciesla and Franklin 1965; Thomas and Hertel 1969). In addition, *Chalcophora* spp. resting on *B. xylophilus* infested red pine in Black River Falls, Wisconsin were hand caught. These insects were macerated and examined for the presence of *B. xylophilus* in the same way as insects caught in emergence traps during the 1982 investigation.

Nematodes extracted from insects were concentrated in 8 mL of water, placed in graduated plastic culture dishes, and counted under a stereoscope. When nematode numbers were large, samples were diluted in water and two 8-mL subsamples were counted after thorough mixing. After counting, dauer larvae of nematodes extracted from the first 10 insects of any insect species collected were concentrated in a swing-bucket clinical centrifuge using 120-mL conical centrifuge tubes. These dauer larvae were then placed on the mycelium of *Botrytis cinerea* Pers.: Fr. growing on Difco potato-dextrose agar in 125-mL Erlenmeyer flasks. After 3 weeks, nematodes were extracted from cultures using Baermann funnels and adult male and female nematodes examined microscopically and confirmed to be those of *B. xylophilus*.

During 1982, 30 individuals of *Monochamus scutellatus* (Say) from Austrian pine wood and eight individuals of *Monochamus mutator* Lec. from red pine wood were divided into head (including antennae), thorax (including legs and wings), and abdominal segments. These parts were macerated separately using a mortar and pestle and nematodes were extracted in plastic weighing boats as described above.

Nematode transmission

Adult male and female *M. carolinensis* and *M. scutellatus* emerging from Austrian pine logs from Zimmerman, Minnesota and *M. mutator* emerging from red pine from Black River Falls, Wisconsin were placed in pairs within screen cages with white pine twigs in water and with a 12 cm long pine bolt (3−9 cm diameter) taken from healthy, nematode-free Austrian pine. Beetles were allowed to maturation feed on twigs and oviposit in the logs (Figs. 2−4). Logs and twigs were replaced at weekly intervals for up to 8 weeks. Every week, parts of the white pine twigs damaged by beetle maturation feeding and 1-cm^3 sections of bark and cambium surrounding all oviposition niches were removed and nematodes were extracted in plastic weighing boats. After 8 weeks, or when mortality of either beetle was observed, beetles were removed, macerated, and nematodes were extracted and counted.

Results

Insect associates of B. xylophilus

Insects collected from pine and balsam fir bolts during 1981 and 1982 and the number of nematodes extracted from these insects are presented in Tables 1 and 2. Dauer larvae extracted from Cerambycidae and Buprestidae were confirmed as those of *B. xylophilus* after moulting to the adult stage on *B. cinerea*. Nematodes extracted from *Ips grandicollis* (Eichh.), *Ips pini* (Say), *H. pales*, and *P. picivorus* were not *B. xylophilus*. Species of Cerambycidae were the most frequently collected insects from emergence traps and most commonly contained *B. xylophilus*. *Monochamus* spp. were the largest insects collected and contained the greatest number of nematodes. *Xylotrechus saggitatus*, and the smaller Cerambycidae such as *Amniscus sexguttatus* (Say) and *Neocanthocinus pusilus* (Kirby), contained few *B. xylophilus* (Tables 1 and 2). The maximum number of nematodes extracted from a single beetle during the two collection seasons was 65 039. *Monochamus carolinensis* from red pine in Wisconsin also contained relatively high numbers of *B. xylophilus* (Tables 1 and 2).

Tails of female *B. xylophilus* extracted from *Monochamus marmorator* (Kiby.) and *M. scutellatus*

TABLE 2. Insects associated with *Bursaphelenchus xylophilus* infested pine from Minnesota and Wisconsin and the number of nematodes extracted from insects during 1982[a]

Host and source	Insect family	Insect species	No. of insects: Examined	No. of insects: With *B. xylophilus*	No. of nematodes per insect: Maximum	No. of nematodes per insect: Average
Austrian pine						
Zimmerman, MN	Cerambycidae	*M. scutellatus*	8	7	22 570	4871
		M. carolinensis	49	39	10 872	4538
		A. sexguttatus	104	25	7793	236
		N. pusilus	22	6	101	8
		X. saggitatus	10	0	—	—
	Buprestidae	*Chrysobothris* sp.	1	1	4068	4068
Austrian pine[b]						
Zimmerman, MN	Cerambycidae	*M. scutellatus*	5	5	23 827	9149
		M. carolinensis	1	0	—	—
Red pine						
Black River Falls, WI	Cerambycidae	*M. mutator*	2	2	325	195
	Buprestidae	*Chalchophora* spp.[c]	29	0	—	—
	Scolytidae	*I. grandicollis*[c]	20	0	—	—

[a]Insects collected from emergence traps containing *B. xylophilus* infested wood.
[b]Wood bolts from previously cut, nematode-free trees previously described (Wingfield 1982*b*).
[c]Insects trapped associated with *B. xylophilus* infested trees in the field.

TABLE 3. Distribution of *Bursaphelenchus xylophilus* in three body sections (head, thorax, and abdomen) of *Monochamus scutellatus* and *Monochamus mutator*[a]

Insect species	Source	No. of insects		Mean *B. xylophilus*		
		Examined	With nematodes	Head	Thorax	Abdomen
M. scutellatus	Austrian pine Zimmerman, MN	30	26	69	2460	383
M. mutator	Red pine Black River Falls, WI	8	7	0	4615	96

[a]Insects collected from emergence traps containing *B. xylophilus* infested wood. Head section includes antennae, and thorax includes wings and legs.

TABLE 4. Summary of oviposition frequency and transmission of *B. xylophilus* by three *Monochamus* spp.

Comparison index	Description	*M. carolinensis*	*M. scutellatus*	*M. mutator*
a	Number of insect pairs	3	4	7
b	Pairs surviving 8 weeks	3	2	3
c	Pairs surviving 4–8 weeks	3	4	5
d	Total niches in 4–8 weeks	322	286	227
e	Total weekly tests (cages × weeks)	24	26	35
f	Niches per week (*d/e*)	13.4	11.0	6.5
g	Niches per week per pair (*f/c*)	4.47	2.75	1.3
h	Total niches per pair (*d/c*)	107.3	71.5	45.4
i	Number of pairs transmitting nematodes	3	3	4
j	Total nematodes transmitted	288	48	346
k	Nematodes transmitted per pair (*j/i*)	96	16	86.5
l	Total niches infested	29	7	14
m	% infested niches per pair (1/*n* × 100)	27	9.8	30.8
n	Beetles with nematodes at end of test	2	1	5
o	Beetles without nematodes at end of test	4	7	9
p	Mean survival length (weeks) for beetles in *n*	8	8	3.6
q	Total nematodes in surviving beetles	47	6	7469
r	Mean nematodes per surviving beetle (*q/n*)	23.5	6	1493.8

emerging from balsam fir logs were mucronate and unlike the more rounded tails of female *B. xylophilus* associated with insects from pine. The maximum number of nematodes recorded from a single *M. scutellatus* and *M. marmorator* from balsam fir were approximately the same (Table 1). These maxima were lower than those from any single insect from pine.

Bursaphelenchus xylophilus were present in the head, thoracic, and abdominal segments of *M. scutellatus* and *M. mutator* (Table 3). The greatest number of nematodes was always present in the thoracic region of the insects examined.

Nematode transmission

Only 3 of the 14 pairs of *Monochamus* spp. caged with white pine twigs transmitted *B. xylophilus* during maturation feeding over a period of 4 to 8 weeks. Transmission during maturation feeding by these beetles occurred four times with an average of 16.3 nematodes transmitted. These data are not tabulated. *Bursaphelenchus xylophilus* was also transmitted during oviposition. The total numbers of oviposition niches on pine bolts during the 4- to 8-week periods, numbers of nematodes in these niches, and data on survival of nematodes and beetles are summarized in Table 4.

Monochamus carolinensis transmitted the greatest number of nematodes per pair of insects (Table 4, index *k*) apparently as a result of frequent oviposition (index *h*) and a moderately large percentage of niches with nematodes (index *m*). All three pairs survived the 8-week test period (index *p*). *Monochamus mutator* transmitted an average of 86.5 nematodes per pair (index *k*). *Monochamus mutator* made only 1.3 oviposition niches per week per pair, compared with 4.47 by *M. carolinensis* (index *g*), thereby reducing its overall transmission effectiveness. *Monochamus scutellatus* was intermediate in oviposition activity (Table 4, indices g and *h*), but transmission was minimal by three of the four pairs (index *m*). This resulted in the smallest rate of transmission among the three species of insect.

Although these data are few and the range of infestations per niche is great for all three insect species, they do suggest that these species differ in effectiveness as vectors of *B. xylophilus*. Such differences may be due to survival rate of the vectors, oviposition frequency, and the numbers of nematodes present in the beetles initially. Some beetles still contained nematodes after feeding for 8 weeks (Table 4, indices *g* and *h*). However, beetles that had fed for shorter time periods contained considerably greater numbers of nematodes (index *r*).

Discussion

Insects in the family Cerambycidae are most commonly associated with *B. xylophilus* in Minnesota and Wisconsin. This observation is consistent with reports from other regions of the United States (Linit *et al.* 1983) and Japan (Mamiya and Enda 1972; Morimoto and Iwasaki 1972). Although *B. xylophilus* was reported associated only with Cerambycidae in Japan, the nematode has been reported associated with *H. pales* and *P. approximatus* (Coleoptera: Curculionidae) in Missouri (Linit *et al.* 1983). *Bursaphelenchus xylophilus* was not extracted from these species or the related *P. picivorus* in this study. Although certain species of weevils such as *H. pales* undergo maturation feeding (Baker 1972), and would have the opportunity to transmit *B. xylophilus*, they apparently seldom carry the nematode (Linit *et al.* 1983; Mamiya and Enda 1972) and probably do not serve as common vectors of *B. xylophilus*. Of the Buprestidae examined in this study, only three insects contained *B. xylophilus*. Buprestidae have been reported as associates of *B. xylophilus* in the United States (Linit *et al.* 1983), but we are not aware of a report of the nematode carried by Buprestidae in Japan.

This is the first report of *M. marmorator*, *M. mutator*, *N. pusilus*, and *X. saggitatus* carrying *B. xylophilus*. The absence of *B. xylophilus* from *I. grandicollis* is consistent with previous reports that *B. xylophilus* is not carried by Scolytidae (Mamiya and Enda 1972; Dropkin *et al.* 1981).

Monochamus alternatus is the primary vector of *B. xylophilus* in Japan and a single beetle can carry up to 175 000 dauer larvae of the nematode (Mamiya and Enda 1972). *Monochamus scutellatus* and *M. carolinensis* from various pine species in this study were found to carry the highest number of nematodes. These maxima were, however, considerably lower than those reported from *M. alternatus* in Japan. The maximum number of nematodes and the mean number per insect for *M. scutellatus* and *M. carolinensis* in this study are similar to those from *M. carolinensis* in Missouri (Linit *et al.* 1983). The low numbers of nematodes extracted from these species in 1982 may have been related to the small numbers of nematodes in the wood from which beetles were collected. *Bursaphelenchus xylophilus* feeds on fungi (Dozono and Yoshida 1974; Kobayashi *et al.* 1974; Kiyohara 1976; Kiyohara and Tokushige 1971) and probably survives on fungi in dead wood after tree death. Various factors, including the amount of blue stain caused by certain fungi in dead pines, could influence the number of nematodes in logs and consequently the numbers of dauer larvae in beetles emerging from these logs. The third *Monochamus* sp., *M. mutator*, trapped from pine in this investigation, carried fewer nematodes than did *M. scutellatus* and *M. carolinensis*. The number of beetles of this species trapped were small. However, the number of nematodes extracted from this species (comparing nematode numbers in various body parts, Table 3) was comparable to those extracted from *M. scutellatus*. For this reason, we would expect that *M. mutator* would be an equally good vector of *B. xylophilus* as *M. carolinensis* and *M. scutellatus* in Minnesota and Wisconsin.

This paper represents the first report of insects associated with *B. xylophilus* from a nonpine host. Balsam fir wood sampled in Minnesota has yielded low numbers (less than 10 adults per 60 g fresh weight of wood) of *B. xylophilus* in comparison with the number of this nematode commonly extracted from pines (M. J. Wingfield and R. A. Blanchette, unpublished). This may explain the fact that nematode numbers extracted from *M. scutellatus* from balsam fir were lower than those extracted from pine. The lower numbers of *B. xylophilus* in balsam fir is thought to be related to the limited occurrence of blue-stain fungi in fir as compared with pine. In addition, balsam fir does not have resin canals in which *B. xylophilus* are reported to develop and move in pine (Mamiya 1972, 1976).

The mucronate tails of female *B. xylophilus* from insects emerging from balsam fir wood were similar to those observed on adult females extracted from balsam-fir wood in Minnesota. An isolate of these nematodes was pathogenic on balsam fir seedlings but not on pine seedlings and was less adapted to feed on certain fungi common on pine than *B. xylophilus* from pine (Wingfield *et al.* 1982). Balsam fir is the exclusive host of *M. marmorator* (Baker 1972) whereas *M. scutellatus* is found on many conifer species including *Larix* spp., *Picea* spp., *Abies* spp., and *Pinus* spp. (Wilson 1962). The strain of *B. xylophilus* from balsam fir may therefore not be restricted to balsam fir by means of a host-specific vector.

The presence of *B. xylophilus* in beetles emerging from bolts used in a previous study to show that the nematode is transmitted to cut timber (Wingfield 1982*a*) proves that it can survive in cut timber for a season. The nematodes apparently feed on stain fungi and emerge from these bolts with cerambycid beetles. By this means, cut timber would serve as a reservoir for the pine-wood nematode as well as the associated cerambycid vectors. This illustrates a life cycle for the pine-wood nematode in the absence of apparently susceptible host species (Wingfield 1982*b*). Forestry practices reducing the amount of cut timber and dying trees to reduce bark-beetle populations should also serve to reduce populations of Cerambycidae and *B. xylophilus*.

Wingfield (1982*a*) suggested that vectors transmit *B. xylophilus* during oviposition on the logs and stressed trees. The means of transmission, however, was not known. Preliminary transmission studies presented here confirm that *B. xylophilus* is commonly transmitted during vector oviposition and are consistent with similar results from Florida (Luzzi and Tarjan 1982). Transmission of *B. xylophilus* was more common during oviposition than during maturation feeding. That some beetles contained nematodes at the end of the 8-week period suggests that transmission of *B. xylophilus* by its vectors may be inefficient. However, once nematodes are established on a log or in a susceptible tree, they are able to reproduce extremely fast (Kiyohara and Suzuki 1978; Mamiya 1972, 1976) and their numbers would increase rapidly.

The number of nematodes in beetles would undoubtedly affect the success of transmission during oviposition and maturation feeding. Although this investigation has shown that most *M. carolinensis* and *M. scutellatus* emerging from Austrian pine and *M. mutator* emerging from red pine are infested with a relatively large number of nematodes, it is impossible to know whether beetles used in transmission studies are carrying nematodes or how many nematodes they might contain. For this reason, additional transmission studies with larger numbers of beetles are required to better understand the patterns of transmission of *B. xylophilus*. In addition, transmission studies using seedlings and trees in the forest are required to ascertain whether *B. xylophilus* can be transmitted and kill seedlings as well as older trees. Preliminary studies of this nature have been reported from other parts of the United States (Linit *et al*. 1983; Luzzi and Tarjan 1982).

Cerambycidae are attracted to, and oviposit in dead and dying trees and cut timber (Baker 1972; Ikeda *et al*. 1980; Ikeda *et al*. 1981). Transmission of *B. xylophilus* at this stage in the life cycle of the vector will result in the pine-wood nematode being present in conifers dying as a result of any cause. This may explain the association of *B. xylophilus* with trees in Minnesota, Iowa, and Wisconsin which were stressed by various pathogens and insects and in which *B. xylophilus* appeared to be a secondary component of the disease complex (Wingfield *et al*. 1982*b*). Careful observations ensuring the absence of all biotic and abiotic factors capable of stressing trees are, however, necessary to ascertain that *B. xylophilus* was not transmitted to stressed trees during vector oviposition and that the nematode was the primary cause of tree death.

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