Pathogenicity of *Bursaphelenchus xylophilus* on three species of pine[1]

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Three species of 11-year-old pine trees were inoculated with *Bursaphelenchus xylophilus* in the field. Four branches in single whorls on red, Scots, and jack pine trees were wounded and inoculated with 10 000 nematodes each or with water extracts from *Botrytis cinerea* cultures. Prior to field inoculations, the pathogenicity of the nematode isolate was confirmed on seedlings in the greenhouse. Fourteen weeks after inoculation, 27 of 80 and 13 of 52 branches were dead or dying on Scots and jack pine trees, respectively. No symptoms were observed on red pine trees inoculated with *B. xylophilus* or on any controls. Branch death was attributed to the formation of girdling cankers resulting from inoculation. An average of 9.14, 10.39, and 0.02 nematodes were extracted per gram of wood from branch samples collected from Scots, jack, and red pine trees at 14 weeks, respectively, and at 58 weeks an average of 13.82, 1.01, and 0.05 nematodes per gram of wood sampled were recovered. Proportions of branch samples with nematodes declined from 14 to 58 weeks after inoculation. Although limited mortality of branches occurred, the pine wood nematode was not found to cause tree death following inoculation.

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Des pins rouges, des pins sylvestres et des pins gris âgés de 11 ans furent inoculés avec *Bursaphelenchus xylophilus*. Quatre branches par verticille furent blessées et inoculées avec 10 000 nématodes chacune ou avec des extraits à l'eau de cultures de *Botrytis cinerea*. Avant d'être inoculé au champs, le pouvoir pathogène du nématode avait été établi sur des semis en serre. Après 14 semaines, 27 des 80 branches de pin sylvestre et 13 des 52 branches de pin gris étaient mortes ou mourantes. Aucun symptôme ne fut observé sur les branches de pin rouge inoculées avec *B. xylophilus* ni sur les branches témoins. L'inoculation a provoqué des chancres qui auraient causé la mort des branches en les annelant. Le nombre moyen de nématodes par gramme de bois prélevé dans les branches était, respectivement, de 9,14, 10,39 et 0,02 après 14 semaines et de 13,82, 1,01 et 0,05 après 58 semaines pour les pins sylvestres, gris et rouges. La proportion de branches contenant des nématodes a diminué entre la 14e et la 58e semaine après l'inoculation. Même si quelques branches ont été tuées, les arbres inoculés avec le nématode du pin ne sont pas morts.

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Introduction

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, is considered to be the most serious pathogen of native *Pinus densiflora* Sieb. and Zucc. and *P. thunbergii* Parl., Japanese red and black pine, respectively, in Japan. In 1981, the disease associated with *B. xylophilus* resulted in the death of an estimated 10 million trees and a loss of 2 million cubic metres of wood (Mamiya 1983). *Bursaphelenchus xylophilus* was established as the primary cause of tree death in Japan when 13- to 25-year-old trees died within 3 months after inoculation with the nematode in the field (Kiyohara and Tokushige 1971). The nematode was found to be carried by cerambycid beetles and transmitted to pines during maturation feeding (Mamiya and Enda 1972; Morimoto and Iwasaka 1972). The biology of *B. xylophilus* and the etiology of the associated disease in Japan has been extensively investigated (Kobayashi *et al.* 1984; Mamiya 1983, 1984).

In the United States, *B. xylophilus* was first recognized as a pathogen in 1979 (Dropkin and Foudin). This report resulted in speculation regarding the potential threat of the nematode to pines in the United States and Canada. Subsequent investigations showed that *B. xylophilus* is widely distributed throughout North America, occurs on many conifer species, and is not associated with epidemic disease (Robbins 1982; Wingfield *et al.* 1984; Wingfield *et al.* 1982*b*). Herbarium specimens of *Aphelenchoides xylophilus* Steiner and Buhrer, first found in Louisiana in 1931, were recently rediscovered and found to be identical to *B. xylophilus* (Nickle *et al.* 1981). Wingfield *et al.* (1982*a*) found *B. xylophilus* associated with stressed trees and later showed that the nematode could be transmitted to dying trees during cerambycid beetle oviposition (Wingfield 1983; Wingfield and Blanchette 1983). The widespread distribution, absence of epidemic losses in native conifers, and presence of *B. xylophilus* for an extended period of time suggests endemicity of the nematode in North America (Wingfield *et al.* 1984; Wingfield *et al.* 1982*b*).

Because *B. xylophilus* may be endemic to North America and the nematode can be transmitted to dying trees during vector oviposition, it is necessary to conduct inoculation studies to assess the pathogenicity of *B. xylophilus* in the United States. Most inoculation studies in the United States have been made on seedlings in greenhouses. Results from such inoculations have been variable and inconclusive and not always representative of results observed under natural conditions (Mamiya 1983). The studies presented here assess the pathogenicity of *B. xylophilus* in the greenhouse and field on native as well as introduced pine species.

Materials and methods

Study area

Treated trees were located in an 11-year-old plantation on the University of Minnesota Cloquet Forestry Center, Carlton Co., Minnesota (NE $^1/_4$, SW $^1/_4$, sec. 29, tp. 49 N, rge. 17 W). The study included 30 red pine (*P. resinosa* Ait.), 30 Scots pine (*P. sylvestris* L.), and 19

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jack pine (*P. banksiana* Lamb.) trees selected at random from one corner of the plantation.

Preparation of inoculum

The isolate of *B. xylophilus* for experimental use was obtained from one of its insect vectors, *Monochamus carolinensis* Olivier (Coleoptera: Cerambycidae), collected from a dead Austrian pine in Zimmerman, Minnesota, and established in monoxenic culture using methods previously described (Wingfield and Blanchette 1983). Nematodes for inoculation were grown on cultures of *Botrytis cinerea* Pers.:Fr. in 1000-mL Erlenmeyer flasks for 4 weeks in the dark at 25°C. Cultures of *B. cinerea* were grown on Difco potato dextrose agar for 2 weeks before the introduction of nematodes. Nematodes were extracted from cultures in sterile distilled water using pie pans (Kable and Mai 1968). They were collected 24 h later and concentrated by centrifugation. The concentration of nematodes was estimated through dilution and counting of aliquots and adjusted to 20 000 nematodes/mL. Water collected from pie pans after extraction of nematode-free cultures of *B. cinerea* was used as inoculum for the controls.

Prior to the field studies, the pathogenicity of the Austrian pine isolate of *B. xylophilus* was determined by inoculating 3-year-old Scots pine seedlings in the greenhouse. On 11 May 1983, 35 seedlings were inoculated with a water suspension of 5000 nematodes each and 20 seedlings were inoculated with the water obtained from extracting nematode-free cultures of *B. cinerea*. A wound, 2 cm long, that exposed the xylem was made by removing the bark on the main stem of the seedlings with a sterile scalpel. The inoculum was placed in contact with the wound surface, which was then covered with moist, sterile cotton and held in place with waterproof tape. The seedlings were maintained in the greenhouse and observed weekly for 2 months. Greenhouse temperatures ranged from 22 to 30°C during this time.

Inoculation technique

Twenty red and Scots pine trees as well as 13 jack pine trees were inoculated with 40 000 nematodes each on 2 June 1983. Wounds approximately 1 cm wide and 3 cm long, that exposed the xylem, were made on the upper surface of four branches on each tree. The four branches were located within a single branch whorl in the midcrown of each tree. Inoculum (0.5 mL) containing 10 000 nematodes was applied to the surface of each of the four wounds. The remaining trees (10 red pine, 10 Scots pine, 6 jack pine) were treated as controls, with 0.5 mL of water extracts from nematode-free cultures of *B. cinerea* applied to the wounds. All four wounds on each tree received the same treatment and the wrapped with waterproof tape.

Following treatment, trees were observed periodically for symptom development. On 12 September 1983, approximately 14 weeks after inoculation, 12 red pine (8 inoculated and 4 controls), 12 Scots pine (8 inoculated and 4 controls), and 10 jack pine (7 inoculated and 3 controls) trees were removed for examination. The remaining trees were harvested on 9 July 1984, approximately 58 weeks after inoculation. Upon removal of the trees, wounds were measured and samples taken for extraction of nematodes.

Extraction of nematodes

Treated branches were divided into distal and proximal portions, with respect to the main stem of the trees, at the center of the inoculation site. Two, 10-cm segments were collected from each branch for extraction of *B. xylophilus*. Each sample included either the distal or proximal half of the inoculation site. The fresh weight of the branch segments were recorded. Segments, including the bark, were then cut into approximately 1-cm^3 pieces. Nematodes were extracted from the branch samples using plastic weighing boats (8.1 × 8.1 cm), a modification on the pie pan technique for extraction of small samples. Square pieces of screen, 6.4 cm on a side, were placed in the weighing boats, creating a 5-mm gap between the bottom of the boat and the screen. Two Kimwipe tissues were placed over the screens and the wood samples were distributed evenly on top of the tissues. The edges of the tissues were folded back to cover the wood samples. Water was added to the boats until the samples were immersed. A second weighing boat was then inverted over the first as a cover to prevent evaporation. After 24 h the screen, tissues, and sample were removed from the boat and the water was collected.

In addition to the eight branch samples per tree, nematodes were extracted from a composite sample of wood from two 5 cm thick disks. The disks were removed from the main stem of the trees directly above and below the whorl of treated branches, debarked, and the xylem tissue cut into approximately 1-cm^3 pieces. Nematodes were extracted from a subsample of wood, approximately 70 g, in Baermann funnels (Southey 1970). The funnels were drained after 24 h and the water was collected.

Water samples collected from the weighing boats and Baermann funnels were incubated at 3°C for 24 h. Samples were concentrated by siphoning off the supernatant liquid and then stored at 3°C until nematode numbers were determined. Nematodes were counted directly and their numbers determined either by counting the entire sample or estimating the total number per sample by dilution.

Data analysis

The experiment was a factorial design with inoculum type and harvest date assigned to individual trees of the three pine species randomly. Individual trees were the experimental units. Variables of interest were percentage of branch circumference cankered, number of nematodes per gram of wood sampled, and the proportion of branches with nematodes. Average values for these variables were calculated by taking the mean of the four subsamples (i.e., individual branches) for each tree.

Percentage of branch circumference cankered, a measure of wound size, was calculated by dividing the width of the lesion or wound by circumference of the branch at the center of the inoculation site. Effects of inoculum type, harvest date, and species on wound size were examined by analysis of variance (Goodnight *et al.* 1982). Where differences among treatment means were detected, Bonferroni's method of multiple comparisons (Neter and Wasserman 1974) was used to identify significant differences.

Mann–Whitney and Kruskal–Wallis nonparametric statistical tests were used to examine the effects of inoculum type, harvest date, and tree species on the proportion of branches with nematodes per tree and the average number of nematodes extracted per branch (Conover 1980; Sall 1982). The average number of nematodes per branch was calculated by taking a weighted average of the number of nematodes per gram of wood for the proximal and distal samples based on sample weights. The average number of nematodes per branch for each tree was then calculated by summing the branch values and dividing by the number of branches. These nonparametric statistical tests were used to avoid assumptions of equal variances and normal distributions of the data.

The distribution of nematodes between the proximal and distal samples from inoculated trees was examined through the use of the Wilcoxson signed rank test (Delong 1982; Steel and Torrie 1980). The difference in the number of nematodes per gram of wood was calculated for each inoculated branch by subtracting the average number in the distal sample from that in the proximal sample. The average difference for each tree was then calculated by taking the sum of the differences for each branch and dividing it by the number of branches on the tree.

Results

Greenhouse inoculations

Twenty-two of the 35 Scots pine seedlings grown in the greenhouse were dead 8 weeks after inoculation with *B. xylophilus*. The proportion of dead seedlings that had been inoculated was significantly different ($P(\chi^2) < 0.01$) from the proportion for the controls, where 1 of 20 treated seedlings had died.

Field inoculation

Symptoms

Eight weeks after the field inoculations, chlorotic and dying branches were observed on several of the Scots and jack pine trees inoculated with *B. xylophilus* (Fig. 1). No symptoms were observed on any inoculated red pines or controls for all three

FIG. 1. Dead branch (arrow) on Scots pine 8 weeks after inoculation with *Bursaphelenchus xylophilus*.

TABLE 1. Number of symptomatic branches on three pine species by treatment, 14 weeks after inoculation with *Bursaphelenchus xylophilus*

Species and treatment	No. of branches			% dead or dying
	Inoculated	Healthy	Dead or dying	
Scots pine				
Inoculated	80	53	27	33.8
Control	40	40	0	0
Jack pine				
Inoculated	52	39	13	26.9
Control	24	24	0	0
Red pine				
Inoculated	79[a]	79	0	0
Control	40	40	0	0

[a]One branch was broken during inoculation and therefore excluded.

species. Fourteen weeks after treatment, 27 and 13 branches were observed to be dead or dying on inoculated Scots and jack pine, respectively (Table 1). On symptomatic branches the portions of branches beyond the inoculation sites were dead. Because inoculation sites were located in the internodal regions between lateral branches, symptomatic branches usually died back to the next set of lateral branches toward the main stem (Fig. 2).

Proportions of branches girdled or closed

Differences in wound response between inoculated and control treatments for all three pine species were observed.

FIG. 2. Dead Scots pine branch 8 weeks after inoculation with 10 000 *Bursaphelenchus xylophilus*. Branch is dead from the first set of lateral branches below the inoculation site (arrow).

Callus was usually observed around the original wound on control branches but not on branches inoculated with *B. xylophilus* (Figs. 3*a*–3*d*). Fifteen of 32 and 11 of 44 inoculated Scots pine branches were girdled at the inoculation site 14 and 58 weeks after treatment for an average proportion per tree of 0.468 and 0.299, respectively (Table 2). No wounds on inoculated Scots pine branches were closed by callus at 14 or 58 weeks. In contrast, 11 of 16 and 20 of 23 Scots pine branches treated as controls had wounds completely closed by callus at 14 and 58 weeks and no branches were girdled. Similar results were observed for jack pine, but branches on red pine trees inoculated with *B. xylophilus* were never completely girdled (Table 2). Fourteen of 180 branches were broken at the inoculation site on trees harvested 58 weeks after treatment, probably as a result of heavy snow cover during the winter. These branches were treated as missing and average values per tree were calculated based on the remaining branches.

Percentage of branch cankered

Significant main effects ($P < 0.05$) among species, harvest date, and inoculum type on percentage of branch circumference cankered were detected with analysis of variance. The species-inoculum type interaction was also significant ($P < 0.05$). This significant interaction appeared to be the result of a change in ranking in the average percentage of branch circumference cankered for Scots pine. Inoculated Scots pine had the largest average percentage of branch circumference cankered of the three species but also had the smallest average for the control wounds of the three species as well (Table 3). All other interaction terms were statistically nonsignificant ($P > 0.05$). Because an interaction between species and inoculum type was detected, separate analyses were done to examine the effects of inoculum type and harvest date by species as well as to examine the effects of species and harvest date by inoculum type.

Significant effects of inoculum type and harvest date on percentage of branch circumference cankered for each of the three species were detected with analysis of variance. The interactions of inoculum type and harvest date were not significant for any of the three species. Pooling the effects of harvest date, the average percentage of branch circumference cankered for wounds inoculated with *B. xylophilus* were significantly larger than wounds treated as controls for all three species in separate analyses (Table 3).

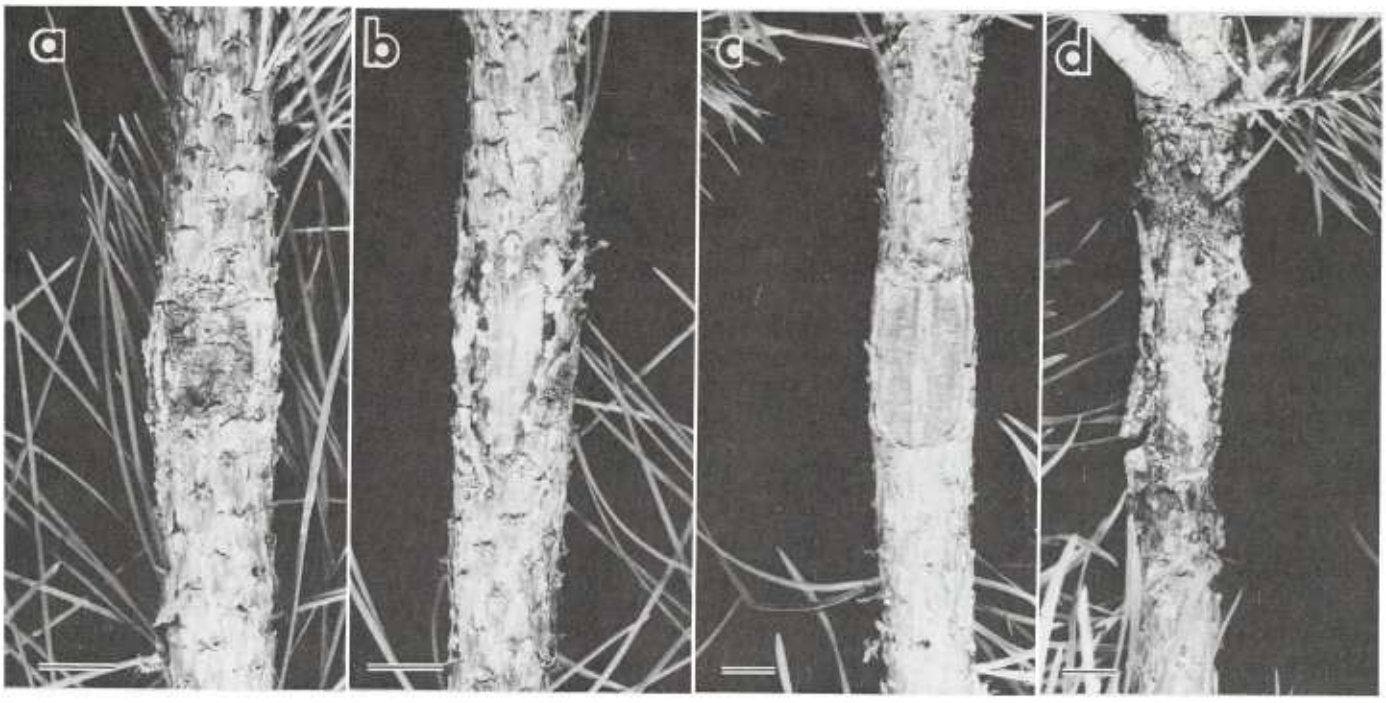

FIG. 3. Wound response of red pine (*a* and *b*) and Scots pine (*c* and *d*) 58 weeks after treatment. Wounds treated as controls (*a* and *c*) are completely closed by callus tissue. Note the excessive resin and inhibition of callus tissue around the wounds inoculated with *Bursaphelenchus xylophilus* (*b* and *d*).

TABLE 2. Average proportion of branches per tree girdled at the inoculation site or wounds closed by callus tissue 14 and 58 weeks after treatment for three pine species

Species and treatment	No. of trees sampled		Proportion of branches							
			Girdled				Closed			
	14 weeks	58 weeks	14 weeks		58 weeks		14 weeks		58 weeks	
Scots pine										
Inoculated	8	12	0.468	(0.057)[a]	0.299	(0.080)	0	(0)	0	(0)
Control	4	6	0	(0)	0	(0)	0.688	(0.187)	0.861	(0.063)
Jack pine										
Inoculated	7	6	0.321	(0.141)	0.055	(0.055)	0	(0)	0.042	(0.042)
Control	3	3	0	(0)	0.083	(0.083)	0.333	(0.333)	0.250	(0.250)
Red pine										
Inoculated	8	12	0	(0)	0	(0)	0	(0)	0	(0)
Control	4	6	0	(0)	0	(0)	0.438	(0.258)	0.736	(0.066)

[a]Values in parentheses are standard errors of the means.

Significant effects of species and harvest date on percentage of branch circumference cankered were detected for trees inoculated with nematodes. There was no significant interaction of species and harvest date for inoculated trees. At 14 weeks after treatment, the average percentage of branch circumference cankered for inoculated Scots, jack, and red pines were 79.4, 78.2, and 43.0, respectively (Table 4). The average percentage of branch circumference cankered of both Scots and jack pines were significantly different from red pine but were not significantly different from one another. At 58 weeks after treatment, only inoculated Scots pine wounds were significantly larger than inoculated red pine wounds. Inoculated jack pine wounds were not significantly different from either Scots or red pines at 58 weeks. Only for inoculated jack pine was a significant reduction in average percentage of branch circumference cankered detected from 14 to 58 weeks. No significant effects of species and harvest date were detected with analysis of variance for the control treatments, so no further analyses were done.

TABLE 3. Average percentage of branch circumference cankered for three species of pine by treatment

Species	Average % cankered		
	Inoculated	Control	LSD
Scots pine	69.0*a*	4.6*b*	13.7
Jack pine	61.9*a*	17.1*b*	17.9
Red pine	36.0*a*	6.5*b*	8.8

NOTE: Means within a row followed by the same letter were not significantly different ($P = 0.05$); LSD, least significant difference.

Numbers of nematodes

The average numbers of nematodes extracted from branches

TABLE 4. Average percentage of branch circumference cankered at the inoculation site for three pine species at 14 and 58 weeks after treatment

Species and harvest date	Treatment: Inoculated No. of trees sampled	Inoculated Average % cankered	Inoculated Standard error	Control No. of trees sampled	Control Average % cankered	Control Standard error
Scots pine						
14 weeks	8	79.4*a*	(4.1)	4	10.0	(7.4)
58 weeks	12	62.1*ab*	(6.8)	6	0.9	(0.5)
Jack pine						
14 weeks	7	78.2*a*	(5.3)	3	17.1	(9.1)
58 weeks	6	42.9*bc*	(7.7)	3	17.1	(12.4)
Red pine						
14 weeks	8	43.0*bc*	(4.2)	4	10.9	(7.1)
58 weeks	11	30.9*c*	(3.6)	6	3.6	(0.8)

NOTE: Means within a column followed by the same letter were not significantly different (experimentwise error rate $P < 0.05$).

TABLE 5. Average number of nematodes extracted per gram of wood sampled at 14 and 58 weeks after treatment for Scots, jack, and red pine branches

Species treatment	14 weeks No. of trees sampled	14 weeks Average	14 weeks Standard error	58 weeks No. of trees sampled	58 weeks Average	58 weeks Standard error
Scots pine						
Inoculated	8	9.14	(3.81)	11	13.82	(12.93)
Control	4	0.00*	(0)	6	0.00*	(0)
Jack pine						
Inoculated	7	10.39	(6.14)	6	1.01	(0.90)
Control	3	0.00*	(0)	3	0.00 NS	(0)
Red pine						
Inoculated	8	0.02	(0.01)	11	0.05	(0.05)
Control	4	0.00 NS	(0)	6	0.00 NS	(0)

NOTE: *, a significant difference between the mean response of inoculated and control treatments within species for each harvest date (Mann–Whitney test, $P(T) < 0.05$); NS, a nonsignificant difference between treatment means (Mann–Whitney test, $P(T) > 0.05$).

for the three pine species are presented in Table 5. On average, the greatest numbers of nematodes were recovered from Scots pine, with an average number per gram of wood sampled of 9.14 after 14 weeks and 13.82 at 58 weeks. Very few nematodes were recovered from red pine. At 14 and 58 weeks the average numbers of nematodes recovered from jack pine branches were 10.39 and 1.01, respectively. No nematodes were recovered from any of the control samples extracted at 14 weeks. Two nematodes, one from each of a single jack and red pine sample, were recovered from two control samples extracted at 58 weeks. Significant differences between the average numbers of nematodes extracted for inoculated and control treatments were observed for Scots pine trees at 14 and 58 weeks after inoculation and for jack pine at 14 weeks (Table 5). The treatment means were not significantly different for jack pine at 58 weeks or for red pine trees at both 14 and 58 weeks after inoculation. Significant differences among the average number of nematodes extracted for the three species sampled at 14 weeks after inoculation with *B. xylophilus* were observed. Differences among the average numbers of nematodes extracted for the three pine species were not significant for control branches sampled at 14 and 58 weeks or inoculated branches sampled at 58 weeks. No nematodes were recovered from the composite samples taken from the main stems of the three species sampled at 14 and 58 weeks for either treatment.

The average difference in the number of nematodes per gram of wood for the proximal and distal samples was −6.0, which was significantly less than zero ($P < 0.01$). This indicates that there were greater numbers of nematodes per gram of wood in the distal samples of inoculated trees.

Proportion of branches with nematodes

The largest proportions of branches per tree with nematodes were found 14 weeks after inoculation (Table 6). The average proportion of branches with *B. xylophilus* at 14 weeks for inoculated Scots pine was 0.969, for jack pine 0.893, and red pine 0.156. No branches treated as controls contained *B. xylophilus* at 14 weeks.

TABLE 6. Average proportion of branches per tree with *Bursaphelenchus xylophilus* for three species of pine sampled 14 and 58 weeks after treatment

Species and treatment	14 weeks			58 weeks		
	No. of trees sampled	Branches with nematodes	Average proportion per tree	No. of trees sampled	Branches with nematodes	Average proportion per tree
Scots pine						
Inoculated	8	31/32	0.969 (0.031)	12	10/42	0.242 (0.071)
Control	4	0/16	0 (0)	6	0/23	0 (0)*
Jack pine						
Inoculated	7	25/28	0.893 (0.051)	6	6/23	0.278 (0.109)
Control	3	0/12	0 (0)*	3	1/11	0.111 (0.083) NS
Red pine						
Inoculated	8	5/32	0.156 (0.066)	12	3/42	0.068 (0.049)
Control	4	0/16	0 (0) NS	6	1/24	0.042 (0.042) NS

NOTE: Values in parentheses are standard errors of the means; *, a significant difference between the mean response of inoculated and control treatments within species for each harvest date (Mann–Whitney test, $P(T) < 0.05$); NS, a nonsignificant difference between treatment distributions (Mann–Whitney, $P(T) > 0.05$).

Significant differences between the average proportions of branches per tree with nematodes for inoculated and control treatments were observed for Scots and jack pines sampled at 14 weeks as well as Scots pines sampled at 58 weeks (Table 6). There were also significant differences among the average proportions of branches with nematodes for the three pine species inoculated with *B. xylophilus* sampled at 14 weeks. Reductions in the proportions of Scots and jack pine branches with *B. xylophilus* between 14 and 58 weeks after treatment were significant.

Discussion

The ability of *B. xylophilus* to kill pine seedlings in the greenhouse has been well documented (Dropkin *et al.* 1981; Dwinell 1985; Kondo *et al.* 1982; Myers 1982). Results presented here document canker development but not tree death associated with *B. xylophilus* inoculations of forest trees and suggest that the ability of the nematode to kill seedlings should not be extrapolated to large, field-grown trees. These results support previous field inoculation studies where trees under normal conditions did not die following inoculation with *B. xylophilus* (Wingfield *et al.* 1986).

Inoculation with *B. xylophilus* resulted in the inhibition of wound closure for all three species. Scots and jack pine appeared to be equally affected and were more sensitive to *B. xylophilus* than red pine. On Scots and jack pine trees, wounds inoculated with *B. xylophilus* often enlarged to girdle branches. This resulted in branch death distal to the inoculation site.

Cankers associated with *B. xylophilus* inoculation have not been previously reported. In other inoculations of forest trees (Kiyohara and Tokushige 1971; Wingfield *et al.* 1986) wounds were made using drill bits or increment borers and nematodes were introduced primarily into the xylem. In our study only the phloem was removed to expose the xylem without injuring it greatly. Inoculation wounds of this type, made without injury to the xylem, are similar to maturation feeding wounds produced by cerambycid beetles, the vectors of *B. xylophilus* (Kondo *et al.* 1982; Wingfield and Blanchette 1983).

The number of nematodes inoculated into the wounds was probably much larger than would be transmitted during feeding by the beetles (Kobayashi *et al.* 1984; Wingfield *et al.* 1984). This may explain why girdling cankers have not been previously associated with cerambycid feeding. In Japan, nematodes have been shown to move away from the site of inoculation rapidly in susceptible trees such as *P. thunbergii* and *P. densiflora* (Mamiya 1983). In this study, nematodes appeared to remain restricted to the site of inoculation and were not found in the main stems of the inoculated trees. This restriction of movement and development of girdling cankers in Scots and jack pines could represent a degree of susceptibility greater than that observed for red pine but less than that observed in Japan.

Drought stress can result in increased susceptibility of Japanese pines to infestation of *B. xylophilus* and enhance disease development (Kiyohara and Suzuki 1978; Suzuki 1984). The lack of tree death observed in this study might be attributed to the absence of a stress significant enough to enhance disease development.

In these studies, *B. xylophilus* was found to be moderately pathogenic on the native North American and exotic species of pines tested. Inoculations of the pine wood nematode did not result in tree death. Results do, however, confirm those from previous studies (Wingfield *et al.* 1986) and report for the first time cankers associated with inoculation of *B. xylophilus*. The development of cankers and the restriction of nematode movement might be the result of a resistance host response. Based on canker development and nematode numbers, red pine may be considered nearly immune to infection by *B. xylophilus*, while Scots pine and jack pine appear to be more susceptible.

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