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Distribution and reproduction of *Bursaphelenchus xylophilus* populations in wood and bark of western North American conifers

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Summary – The growth of populations of *Bursaphelenchus xylophilus* in wood and bark of *Abies grandis, Pinus contorta, Pseudotsuga menziesii, Tsuga heterophylla* and *Thuja plicata* was studied. Nematodes were inoculated into 6 cm diameter × 25 cm long stem segments of each tree species and incubated in plastic bags at room temperature (22 ± 4 °C). Nematode populations in the wood and bark were sampled separately at 4, 8 and 16 weeks after inoculation. Mean population densities in wood (pooled over sample dates) were 233, 13, 12, 2 and 0.03 nematodes/g dry wood for *P. contorta, P. menziesii, A. grandis, T. heterophylla* and *T. plicata,* respectively. Population densities in bark of the same species were 70, 267, 88, 113, and 21 nematodes/g dry bark, respectively. Nematode population growth was also studied in finely chopped autoclaved and non-autoclaved wood and bark of each tree species, and on filter papers impregnated with ethanol extracts of *P. contorta* and *P. menziesii* sapwood. Nematode population densities were greatest in *P. contorta* for both the autoclaved and non-autoclaved wood. After 6 weeks, population densities in non-autoclaved wood of *P. contorta, P. menziesii, A. grandis, T. heterophylla* and *T. plicata* was 137, 46, 5, 23 and 1 nematodes/g dry wood, respectively. Nematode populations grew more rapidly on filter papers impregnated with *P. contorta* extract than on filter papers impregnated with *P. menziesii* extract.

Résumé – ***Répartition et reproduction de populations de* Bursaphelenchus xylophilus *dans le bois et l'écorce des conifères de l'ouest de l'Amérique du Nord*** – La croissance de population de *Bursaphelenchus xylophilus* a été étudiée dans le bois et l'écorce d'*Abies grandis, Pinus contorta, Pseudotsuga menziesii, Tsuga heterophylla* et *Thuja plicata.* Le nématode a été inoculé dans des tronçons de tige (6 cm de diamètre et 25 cm de longueur) de chaque essence, puis mis en incubation dans des sacs de plastique, à la température ambiante (22 ± 4 °C). Les populations de nématodes ont été échantillonnées séparément dans le bois et l'écorce de chaque essence 4, 8 et 16 semaines après l'inoculation. Dans le bois, pour l'ensemble des dates d'échantillonnage, la densité moyenne était de 233, 13, 12, 2 et 0,03 nématodes par gramme de bois sec pour *P. contorta, P. menziesii, A. grandis, T. heterophylla* et *T. plicata* respectivement. Dans l'écorce, pour les mêmes essences, la densité était de 70, 267, 88, 113 et 21 nématodes par gramme d'écorce sèche. La croissance des populations de nématodes a été également étudiée dans le bois et l'écorce de chacune des essences finement hachés, stérilisés ou non à l'autoclave et sur du papier filtre imbibé d'un extrait à l'éthanol d'aubier de *P. contorta* et de *P. menziesii.* Que le bois soit stérilisé ou non, la plus forte densité de nématodes est observée chez *P. contorta.* Après 6 semaines, dans le bois non chauffé, la densité était de 137, 46, 5, 23 et 1 nématodes par gramme de bois sec pour *P. contorta, P. menziesii, A. grandis, T. heterophylla* et *T. plicata,* respectivement. Par ailleurs, les populations de nématodes croissaient plus rapidement dans le papier filtre imbibé d'extrait de *P. contorta* que dans celui imbibé d'extrait de *P. menziesii.*

Key words : Pine wilt disease, pine wood nematode, *Abies grandis, Pseudotsuga menziesii, Pinus contorta, Tsuga heterophylla, Thuja plicata.*

The pine wood nematode, *Bursaphelenchus xylophilus* colonizes dead and dying pines in Asia and North America (Robbins, 1982; Mamiya, 1984; Bergdahl, 1988; Bowers *et al.,* 1992). The nematode is vectored by wood boring beetles of the genus *Monochamus* (Cerambycidae), which undergo larval development and pupation in wood of *Pinus, Pseudotsuga, Picea* and *Abies* (Linit, 1988). Dauer juveniles of *B. xylophilus* climb onto young adult beetles immediately after eclosion and are transmitted to stressed or recently killed trees during beetle oviposition (Wingfield, 1983; Wingfield & Blanchette, 1983; Luzzi *et al.,* 1984; Edwards & Linit, 1992).

Dauer juveniles are also transmitted to healthy trees during maturation feeding by the beetles on young branches (Mamiya & Enda, 1972; Wingfield & Blanchette, 1983; Luzzi *et al.,* 1984; Linit, 1990). Under appropriate environmental conditions (Rutherford & Webster, 1987; Rutherford *et al.,* 1990) nematodes transmitted to susceptible pines via maturation feeding cause pine wilt disease. This disease has caused extensive mortality of *Pinus densiflora* and *P. thunbergii* in Japan (Mamiya, 1984, 1983 *b*), and sporadic mortality of *P. sylvestris* and *P. nigra* in the midwestern United States (Dropkin *et al.,* 1981; Rutherford & Webster, 1987; Bergdahl, 1988; Rutherford *et al.,* 1990).

Although *B. xylophilus* is normally not a pathogen of non-pine conifers, it may colonize them as a secondary invader. In North America, the nematode has been found in *Picea, Abies, Larix* and *Cedrus* that were killed by factors other than the nematode (Robbins, 1982; Bowers *et al.,* 1992). At present, it is uncertain whether *Pinus* spp. and non-pines are equally suitable for colonization by the nematode. Forge and Sutherland (1996) reported that population growth of *B. xylophilus* was greater in branch segments of *Pinus contorta* Dougl. than in *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga heterophylla* (Raf.) Sarg., *Abies grandis* (Dougl.) Lindl., and *Thuja plicata* Don. However, nematode distribution in the branch segments and factors responsible for the lower population growth in non-pine species were not evaluated.

The primary objective of this research was to compare growth of populations of *B. xylophilus* in wood and bark of the same five species. In order to determine if differences in nematode population growth in sapwood of *P. contorta* and *P. menziesii* were the result of chemicals in sapwood, we also compared population growth of the nematode on ethanol extracts from the wood of *P. contorta* and *P. menziesii.*

Materials and methods

Growth in stem segments

The main stems of two young trees (10 cm diameter at breast height, 15 to 25 years old) of *Pinus contorta, Pseudotsuga menziesii, Abies grandis, Tsuga heterophylla* and *Thuja plicata* were obtained from the Greater Victoria Watershed Forest near Victoria, British Columbia, Canada. Twelve 25-cm long segments were cut from each stem. Two 1-cm diameter holes were drilled radially to the center of each segment, and each segment was inoculated with the fungus *Ophiostoma piceae* (Munch) Syd. & P. Syd and nematodes as described previously (Forge & Sutherland, 1996).

Six segments from each tree were inoculated with 725 ± 50 individuals of a mucronate-tailed strain of *B. xylophilus* originally isolated from mixed-species wood chips at Clinton, British Columbia. The remaining six segments of each tree were inoculated with 608 ± 62 individuals of a round-tailed strain of the nematode originally isolated from a dead *Pinus banksiana* Lamb. at Smoky Lake, Alberta. The segments were arranged in a completely randomized design in a growth chamber at room temperature (22 ± 4 °C).

At 4, 8 and 16 weeks after inoculation, two replicate segments representing each combination of nematode isolate, replicate tree and tree species were randomly chosen for nematode sampling. Three adjacent 2-5-cm thick disks were cut from one end of each segment as described previously (Forge & Sutherland, 1996). The bark was separated from the wood of two disks at the cambium, and the bark and wood were chopped into 0.5 × 0.5 × 1.5 cm pieces and placed on separate Baermann funnels over two layers of laboratory tissue (Kimwipes, Kimberly-Clark, Mississauga, Ont.). The third disk was used for determing gravimetric moisture content of the segment. Nematodes emerging over 48 h were collected, placed in a covered counting slide and counted with a compound microscope at 40 to 200 × magnification. Dependent variables analyzed statistically were population densities in bark and wood (nematodes/dry mass of tissue), overall population density (nematodes from bark and wood/dry mass of bark and wood), and the percentage of nematodes inhabiting bark (nematodes from bark/nematodes from bark and wood). The effects of tree species, sample date and nematode strain on each dependent variable were analyzed using a nested three factor analysis of variance (GLM Procedure, SAS Inc., Cary, NC). Tree species was the main factor. The effect of each tree was nested within tree species and was used as the error term for testing the effect of tree species. Sample date and nematode strain were subfactors nested within each tree. Fisher's protected least significant differences (LSD) was used for comparing means.

Growth in chopped wood and bark

Two experiments were conducted, one to assess nematode growth in non-autoclaved wood and bark, and the other to assess nematode growth in autoclaved and non-autoclaved wood and bark. The British Columbia strain of the nematode was used in both experiments. Bark was peeled from freshly cut stem segments of each tree species used in the previous experiment, and chopped into 0.5 × 0.5 cm pieces. The wood of each species was chopped into 1 × 1 × 1 cm pieces, frozen at − 20 °C, and chopped into fine splinters using a large capacity Waring blender.

For the first experiment, approximately 10 g (dry mass) of bark of each species was placed into six separate glass Petri dishes (10 cm diameter). An additional eight Petri dishes were filled with 10 g (dry mass) finely chopped wood of each tree species. All dishes were inoculated with 203 ± 20 nematodes in 0.125 ml of sterile water and 1 ml of a suspension of *O. piceae* spores. The spore suspension was prepared by rinsing the surface of two 4 week-old cultures (1.5 % malt agar) into 500 ml of sterile distilled water.

For the second experiment, approximately 7 g (dry mass) bark of each species was placed into six separate Petri dishes. An additional sixteen Petri dishes were filled with 10 g (dry mass) finely chopped wood of each species. Three dishes of bark and eight dishes of wood of each species were autoclaved (1.02 bar at 121 °C) for 30 min before inoculation. The remaining dishes of wood and bark of each species were not autoclaved. All dishes were inoculated with 89 ± 7 nematodes in

0.025 ml sterile water and a 0.5 cm^2 plug of a non-sporulating culture of *Botrytis cinerea* (Münch) Pers : Fr. The dishes from both experiments were sealed with Parafilm and arranged in a completely randomized experimental design and incubated in darkness at room temperature.

After 6 weeks nematodes were extracted from each Petri dish by placing the contents on a Baermann funnel for 24 h. Nematodes emerging from each sample were counted as described above and expressed as the number of nematodes per g of dry plant tissue. Data for bark and wood were analyzed separately. The effects of tree species (first experiment) or tree species and autoclaving (second experiment) on nematode population density were analyzed using a completely randomized analysis of variance model. The data were log transformed prior to analysis to reduce mean-correlated variance.

Growth on wood extracts

Sapwood of *P. contorta* and *P. menziesii* was chopped into 1 × 1 ×1 cm chips. 500 ml of chips of each species were covered with ethanol in a 1 l beaker and incubated at room temperature for 48 h. The extract was decanted and filtered through filter paper (Whatman No 1), yielding approximately 300 ml of extract for each species.

Three Whatman No 1 filter papers were placed in each of 48 glass Petri dishes and autoclaved. For each tree species, 24 dishes were prepared by applying a 10-ml aliquot of the extract to the filter papers in each dish. All dishes were left uncovered in a flow chamber until the ethanol evaporated (approximately 4 h). Spores from two different 4 week-old cultures of *O. piceae* grown on 1.5 % malt agar were suspended in : *i)* 500 ml of sterile water and *ii)* 500 ml of sterile 2 % malt extract. For each extract, twelve dishes were moistened with 5 ml of the water-spore suspension and the remaining dishes were moistened with 5 ml of the malt extract-spore suspension. All dishes were subsequently inoculated with 120 ± 10 nematodes in 0.025 ml of sterile distilled water.

At 1, 2, 4 and 6 weeks after inoculation, nematodes were extracted from three dishes representing each combination of extract and added nutrients. The filter paper in each dish was cut into 1 cm^2 pieces and the contents of each dish rinsed into a Baermann funnel. Nematodes emerging over 24 h were counted as described previously. The experiment was repeated once using the same procedures. Data from the two runs of the experiment were treated as blocks in the analyses.

Data were analyzed using a three factor randomized complete block analysis of variance. The main-effects factors were extract (*P. contorta vs P. menziesii*), nutrient amendment (water *vs* malt extract), and sample time. The data were log-transformed prior to analysis to reduce mean-correlated variance.

Results

Growth in stem segments

Nematode population densities in wood and in wood plus bark were significantly greater in segments of *P. contorta* than in the other tree species ($P \leq 0.05$; Table 1). Nematode population densities in bark did not differ significantly among tree species. The overall percentage of nematodes inhabiting bark was significantly lower for *P. contorta* than the other species ($P \leq 0.05$). Population densities in wood were also affected by an interaction between the factors of tree species and sample date ($P \leq 0.02$); peak population densities in wood occurred at 8 weeks for *P. contorta* and *T. plicata,* and at 16 weeks for the other species.

There were no consistent differences between the population densities of the two nematode strains in wood or wood plus bark. Nematode strain had a marginally significant effect on population densities in bark ($P = 0.06$; Table 2). The overall mean (pooled over tree species) population density in bark was greater for the mucronate-tailed British Columbia strain than for the round-tailed Alberta strain. Although the Alberta strain was present at greater population densities than the British Columbia strain in bark of *P. menziesii,* there was no significant interaction between nematode strain and tree species.

The moisture content of *P. contorta* segments was consistently less than the other species (Table 3).

Growth in chopped wood and bark

In the first experiment nematode populations were significantly greater in the chopped wood of *P. contorta* than in chopped wood of the other species except *P. menziesii* ($P \leq 0.05$; table 4). There were no significant

Table 1. *Population densities (nematodes/g dry tissue) of* Bursaphelenchus xylophilus, *and percentage of the sampled population occupying bark, in stem segments of* Pinus contorta, Pseudotsuga menziesii, Abies grandis, Tsuga heterophylla *and* Thuja plicata.

	Wood	Bark	Wood + bark *	% in bark **
P. contorta	233 *a*	70 *a*	131 *a*	19 *a*
P. menziesii	13 *b*	267 *a*	62 *b*	74 *b*
A. grandis	12 *b*	88 *a*	20 *b*	70 *b*
T. heterophylla	2 *b*	113 *a*	22 *b*	87 *b*
T. plicata	0.03 *b*	21 *a*	2 *b*	95 *b*

* Nematodes recovered from bark + wood/dry mass of bark + wood; ** Nematodes recovered from bark/nematodes recovered from bark + wood. Means (n = 24) within a column followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$); each value is the mean of eight replicates segments sampled at 4, 8 and 16 weeks after inoculation.

Table 2. *Population densities (nematodes/g dry tissue) of the British Columbia and Alberta strains of* Bursaphelenchus xylophilus *in the bark of stem segments of* Pinus contorta Pseudotsuga menziesii, Abies grandis, Tsuga heterophylla *and* Thuja plicata.

	British Columbia	Alberta
P. contorta	142	21
P. menziesii	151	336
A. grandis	119	84
T. heterophylla	244	6
T. plicata	49	0.2
Pooled mean *	141	89

* Pooled means are significantly different ($P \leq 0.05$). Mean of twelve replicates.

Table 3. *Percent moisture of stem segments of* Pinus contorta, Pseudotsuga menziesii, Abies grandis, Tsuga heterophylla *and* Thuja plicata.

	Weeks after inoculation		
	4	*8*	*16*
P. contorta	98 (14)	89 (15)	84 (8)
P. menziesii	116 (6)	113 (6)	118 (6)
A. grandis	111 (11)	115 (14)	118 (17)
T. heterophylla	168 (42)	147 (20)	158 (15)
T. plicata	143 (33)	154 (25)	127 (13)

Values in parentheses are standard deviations of the means (n = 8).

differences among nematode populations in bark of the various tree species. In the second experiment tree species and autoclaving affected populations in chopped wood ($P \leq 0.001$ for main effects of both factors; Table 4). Autoclaving the wood before inoculation resulted in significantly greater ($P \leq 0.05$) nematode populations for all tree species. For both autoclaved and non-autoclaved wood, populations were significantly greater in *P. contorta* than in the other species ($P \leq 0.05$). Neither autoclaving nor tree species had a significant effect on nematode populations in bark. In both experiments hyphae were observed growing between fragments of wood of all species except *T. plicata*.

Growth on wood extracts

Nematode populations increased through week 4 in all combinations of wood extract and nutrient amendment (Table 5). Wood extract, nutrient amendment and sample date all had significant main-factor effects ($P \leq 0.01$) on nematode populations. Nematode populations were greater in plates amended with malt extract than non-amended plates. For the amended plates, populations sampled at 6 weeks and pooled over sample dates were greater in plates made with *P. contorta* extract than in those with *P. menziesii* extract.

Table 4. *Mean population densities (nematodes/g dry tissue) of* Bursaphelenchus xylophilus *in autoclaved and non-autoclaved chopped wood and bark of* Pinus contorta, Pseudotsuga menziesii, Abies grandis, Tsuga heterophylla *and* Thuja plicata, *and inoculated with* Ophiostoma piceae *(experiment 1) or* Botrytis cinerea *(experiment 2).*

	Bark		Wood	
	A *	*NA* **	*A* *	*NA* **
Experiment 1				
P. contorta	n.d.	4326 *a*	n.d.	10152 *a*
P. menziesii	n.d.	5314 *a*	n.d.	6651 *ab*
A. grandis	n.d.	8353 *a*	n.d.	3039 *bc*
T. heterophylla	n.d.	4485 *a*	n.d.	3673 *bc*
T. plicata	n.d.	3254 *a*	n.d.	1931 *c*
Experiment 2				
P. contorta	2077 *a*	3214 *a*	813 *a*	137 *a*
P. menziesii	1923 *a*	1627 *a*	155 *b*	46 *b*
A. grandis	2244 *a*	1020 *a*	88 *b*	5 *c*
T. heterophylla	1384 *a*	526 *a*	167 *b*	23 *b*
T. plicata	5395 *a*	1792 *a*	51 *c*	1 *c*

* A = Autoclaved. ** NA = Not autoclaved. n.d. = not determinated. Experiments 1 and 2 inoculated with 203 ± 20 and 89 ± 7 nematodes per Petri dish, respectively.
Means (bark : n = 6; wood : n = 8) within a column and the same experiment followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Discussion

The wood of non-pines studied appears to be inferior to the wood of *P. contorta* as a habitat for *B. xylophilus*. Population densities of the nematode were significantly lower in wood of stem segments of non-pine species than in *P. contorta*. In contrast, population densities of the nematode in bark of stem segments were similar for all species studied.

The Alberta and British Columbia strains used in the second experiment have round and mucronate tails, respectively. Because tail shape is the only visible characteristic distinguishing *B. xylophilus* from the non-pathogenic *B. mucronatus* in Asia and Europe, there has been some interest in determining if mucronate-tailed and round-tailed strains of *B. xylophilus* from North America are biologically distinct (Wingfield *et al.*, 1983; Panesar & Sutherland, 1989; Riga *et al.*, 1991; Sutherland *et al.*, 1991). In the present study, the British Columbia

Table 5. *Population densities (nematodes/Petri dish) of* Bursaphelenchus xylophilus *growing on filter papers impregnated with ethanol extracts of the wood of* Pinus contorta *or* Pseudotsuga menziesii, *moistened with water or 2% malt extract, and inoculated with* Ophiostoma piceae.

	Weeks after inoculation				
	1	*2*	*4*	*6*	*Pooled mean*
MALT EXTRACT					
P. contorta	688 *a*	3355 *a*	4570 *a*	9837 *a*	4613 *a*
P. menziesii	901 *a*	2028 *a*	2207 *a*	2408 *b*	1886 *b*
WATER					
P. contorta	324 *b*	703 *b*	819 *b*	769 *c*	654 *c*
P. menziesii	309 *b*	409 *b*	925 *b*	511 *c*	539 *c*

Means (n = 6) within a column followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

strain was usually present at greater population densities in bark (but not wood) than the Alberta strain. However, the biological significance of these data is questionable since we did not find any consistent difference between the two strains in previous research (Forge & Sutherland, 1996).

The water content of *P. contorta* stem segments was significantly less than the other tree species. However, in a previous study (Forge & Sutherland, 1996) we observed greater population growth in *P. contorta* than in the other tree species despite similar moisture contents.

The differences in population growth of *B. xylophilus* in wood of these conifers may reflect differences in physical structure. In pines the nematode primarily inhabits resin canals (Mamiya & Kiyohara, 1972; Mamiya, 1983 *b,* 1984), but of the genera considered in this study only *Pinus* and *Pseudotsuga* possess resin canals (Jane, 1970). Furthermore, the resin canals of *Pseudotsuga* are smaller and less numerous than in *Pinus* (Jane, 1970).

Data from our experiments with chopped wood indicate that chemical composition also influences population growth of *B. xylophilus.* If physical structure is the main factor influencing suitability for colonization by *B. xylophilus,* then differences between tree species should be reduced in finely chopped wood. Our data are not consistent with the hypothesis that wood structure restricts nematode colonization of non-pines; we found that population growth was significantly greater in the chopped wood of *P. contorta* than in chopped wood of the other species. Supporting observations are provided by the effect of autoclaving, which would be more likely to alter the chemical environment of wood than its physical structure. Autoclaving resulted in a substantial increase in suitability for nematode population growth for all tree species.

The chemical composition of wood could affect *B. xylophilus* populations directly, or indirectly by influencing fungal species composition or the abundance of palatable hyphae. *B. xylophilus* feeds and reproduces on laboratory cultures of many different species of fungi isolated from wood, including *B. cinerea* and *Ophiostoma* spp. (Kobayashi *et al.,* 1974, 1975; Fukushige, 1991 *a, b*). We found that nematode population growth differed among tree species when fungal species composition was controlled. In both experiments with chopped wood, one utilizing *O. piceae* only and one utilizing *B. cinerea* only, we found that nematode population growth was greatest in the wood of *P. contorta.* We speculate that the abundance of palatable hyphae differed between tree species, but it is difficult to define and accurately quantify the amount of palatable hyphae in wood. Fungal growth on chopped *T. plicata* appeared less than on the other species. However, differences between *P. contorta* and the other species, especially *P. menziesii,* were not as apparent. After six weeks, hyphae were observed growing between fragments of wood of all species except *T. plicata,* suggesting that the fungi were not completely consumed by the nematodes and population growth may not have been limited by the abundance of food at that time.

Population growth of *B. xylophilus* was greater in the presence of ethanol extracts of *P. contorta* than in extracts of *P. menziesii.* These observations may have resulted from differences in wood extracts acting directly on the nematodes, rather than differences in the abundance of palatable hyphae. Fungal growth on plates made with *P. menziesii* extract appeared to be equal to or greater than growth on plates with extracts of *P. contorta.* Had differences in nematode population growth been due to differences in nutrient content of the wood extracts and resulting fungal growth, then these differences should have been masked by amending the plates with malt extract. In contrast, differences in nematode population growth between plates made with *P. contorta* and *P. menziesii* extracts were greater in the presence of malt extract.

Previous research has shown that compounds present in *Pinus* spp. have direct positive effects on the life-history and population growth of *B. xylophilus.* Mamiya (1990) demonstrated that population growth of this nematode in agar media was increased by the presence of fatty acids characteristic of *Pinus* spp. Similarly, Hinode *et al.* (1987) found that the pine monoterpene *B*-myrcene increased population growth and the rate of molting from dauer juveniles to adults. Compounds diffusing into agar from wood blocks of *Pinus* spp. and *Larix leptolepsis* also induced molting of dauer juveniles to adults at a greater rate than compounds diffusing out of the wood of other conifers (Mamiya, 1983 *a*).

Our data indicate that the suitability of non-pine conifers as habitats for *B. xylophilus* may depend on whether the abundant nematodes in bark can move into the pupal chambers of *Monochamus* located in sapwood. *Bursaphelenchus xylophilus* is attracted over short distances by carbon dioxide or fatty acids given off by *Monochamus* pupae (Miyazaki *et al., in* Mamiya, 1984). However, the limits of chemoattraction in logs have not been precisely defined. Warren and Linit (1992) found that nematode aggregation of populations of the nematode in logs of *P. sylvestris* was not affected by the presence of *Monochamus*.

Concern over the possibility of *B. xylophilus* becoming established in Europe has led to an embargo against softwood lumber from North America. Due to vector preference for *Pinus* spp. (Linit, 1988; Bowers *et al.*, 1992) and the limited population growth of *B. xylophilus* in wood of non-pine conifer species, it appears unlikely that non-pine conifer species would be infested with significant populations of *B. xylophilus*. The research presented here indicates that ensuring lumber of non-pine conifers is free of bark should further reduce the likelihood of it containing significant populations of *B. xylophilus*.

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