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Population dynamics of the pine wood nematode, *Bursaphelenchus xylophilus*, in excised branch segments of western North American conifers

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Summary – The population dynamics of two strains of the pine wood nematode, *Bursaphelenchus xylophilus,* were studied in excised branch segments of *Pinus contorta, Abies grandis, Pseudotsuga menziesii, Tsuga heterophylla* and *Thuja plicata* inoculated with the blue-stain fungus, *Ophiostoma piceae.* Nematodes were inoculated into small holes drilled into the center of the branch segments. Size and age-structure of the nematode populations were determined at regular intervals after inoculation. Nematode population growth occurred in branch segments of all tree species tested. However, population densities were significantly greater in *P. contorta* than in the other species. In one experiment, respective population densities in branch segments of *P. contorta, A. grandis, P. menziesii, T. heterophylla* and *T. plicata* increased from 2, 0.02, 0.1, 0.04 and 0.05 nematodes/g dry wood at 2 weeks after inoculation to 57, 15, 11, 13 and 6 nematodes/g dry wood after 16 weeks. The relative abundance of persistent third-stage juveniles (J3P) increased through time in all tree species and was greatest in *P. contorta* at most sample dates. The relative abundance of J3P was significantly greater in *P. contorta* than in the other species in one of two experiments only.

Résumé – ***Dynamique des populations de* Bursaphelenchus xylophilus *dans des branches excisées de conifères de l'ouest de l'Amérique du Nord*** – La dynamique des populations de deux souches de nématode des pins *(Bursaphelenchus xylophilus)* a été étudiée dans des segments de branche excisés d'*Abies grandis, Pinus contorta, Pseudotsuga menziesii, Tsuga heterophylla* et *Thuja plicata* dans lesquels on a inoculé le champignon responsable du bleuissement *(Ophiostoma piceae).* Des nématodes ont été inoculés avec le champignon dans de petits trous percés au centre des segments de branche. La taille et la struture par âge des populations de nématode ont été déterminées à intervalles de temps réguliers après l'inoculation. La population de nématode s'est développée dans les segments de branche de toutes les essences analysées. Toutefois, les densités de population étaient beaucoup plus importantes chez *P. contorta* que chez les autres essences. Dans une des expériences, les nombres respectifs de nématodes dans les segments de branche de *P. contorta, A. grandis, P. menziesii, T. heterophylla* et *T. plicata,* qui étaient de 2,0; 0,02; 0,1; 0,04 et 0,05 nématodes par gramme de bois sec 2 semaines après l'inoculation, sont passés à 57, 15, 11, 13 et 6 nématodes par gramme de bois sec après 16 semaines. L'abondance relative des juvéniles persistants du troisième stade (J3P) a augmenté avec le temps chez toutes les essences et elle était supérieure chez *P. contorta* à la plupart des dates d'échantillonnage. L'abondance relative des J3P était significativement plus importante chez *P. contorta* que chez les autres essences dans une des deux expériences seulement.

Key-words : *Bursaphelenchus xylophilus,* pine wilt disease, *Abies grandis, Pseudotsuga menziesii, Pinus contorta, Tsuga heterophylla, Thuja plicata.*

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, causes a vascular wilt disease of some species of *Pinus*. The nematode has caused extensive mortality of *Pinus thunbergii* and *Pinus densiflora* in Japan (Mamiya, 1984, 1983). The pine wood nematode is distributed across North America but has caused mortality only of *P. resinosa* in Maryland plantations (Harmon *et al.*, 1986), and *P. sylvestris* and *P. nigra* grown as ornamentals or in Christmas tree plantations in the midwestern United States (Rutherford *et al.*, 1990; Bergdahl, 1988; Rutherford & Webster, 1987; Dropkin *et al.*, 1981).

Bursaphelenchus xylophilus is vectored by wood boring beetles in the genus *Monochamus* (Linit, 1988; Mamiya, 1984). Immediately after being introduced into susceptible or recently killed pine trees, the nematodes feed on parenchyma and epithelial cells lining resin canals in the sapwood and cortex (Tamura & Mamiya, 1979; Mamiya & Kiyohara, 1972). At later stages of colonization the nematodes feed on fungi growing in the wood. *Bursaphelenchus xylophilus* feeds and reproduces on laboratory cultures of many different species of fungi isolated from wood (Fukushige, 1991*a, b;* Kobayashi *et al.*, 1974, 1975).

During the early stages of colonization in pine trees, *B. xylophilus* populations undergo several generations of a propagative life-cycle which consists of eggs, second-, third- and fourth-stage juveniles, and mature males and

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females (Mamiya, 1975). At two to three months after infection the juveniles begin developing into specialized persistent third-stage juveniles (J3P) rather than maturing into reproductive adults (Fukushige & Futai, 1987; Mamiya, 1984, 1983). The J3P were previously referred to as dispersal third-stage juveniles (Mamiya, 1975, 1983, 1984; Thong & Webster, 1991). However, because the J3P are adapted for long-term survival and are not directly involved in dispersal (Mamiya, 1984; Kondo & Ishibashi, 1978; Ishibashi & Kondo, 1977; Thong & Webster, 1991) they will here be referred to as persistent third-stage juveniles. The factors triggering J3P formation have not been clearly identified.

In spring the J3P aggregate around pupal chambers of *Monochamus* (Linit, 1988; Mamiya, 1984). As the *Monochamus* adults emerge from puparia the J3 molt to dispersal fourth-stage juveniles which move into the spiracles of young adult beetles (Mamiya, 1984; Linit, 1988). The dispersal fourth-stage juveniles (J4D) are transmitted to healthy trees during maturation feeding of the beetles on young shoots (Mamiya & Enda, 1972; Wingfield & Blanchette, 1983; Luzzi *et al.*, 1984; Linit, 1990). The J4 are also transmitted, during beetle oviposition, to trees stressed or recently killed by the nematode or other factors (Wingfield, 1983; Wingfield & Blanchette, 1983; Luzzi *et al.*, 1984; Edwards & Linit, 1992). Transmission via oviposition on trees stressed or killed by other factors allows populations of the nematode to persist in areas where it does not act as a primary pathogen.

Monochamus beetles engage in maturation feeding and oviposition on conifers other than pines, especially *Pseudotsuga, Abies* and *Picea* (Linit, 1988). *Picea excelsa (= P. abies)* and *Cedrus deodara* have been killed by *B. xylophilus* under extreme inoculum pressure and environmental conditions in Japan (Mamiya, 1983). The nematode has not been documented causing a wilt disease of any non-pine conifers under fields conditions in North America, but it has been found in wood samples from trees of *Picea, Abies, Larix* and *Cedrus* killed by other factors (Robbins, 1982; Bowers *et al.*, 1992).

Little is known of the comparative suitability of non-pine conifers for colonization by populations of *B. xylophilus,* or how the life-history of the nematode may differ in the wood of non-pine tree species. The objective of this research was to compare, via inoculation into excised branch segments colonized by fungi, the population dynamics of *B. xylophilus* in several conifers indigenous to western North America : *Pinus contorta* Dougl., *Pseudotsuga menziesii* (Mirb.) Franco, *Abies grandis* (Dougl.) Lindl., *Tsuga heterophylla* (Raf.) Sarg., and *Thuja plicata* Donn.

Materials and methods

General protocol

All branch segments were cut from trees in the Greater Victoria Watershed near Victoria, British Columbia, Canada. For each of the two experiments, two branches, at least 8 cm diameter at the base, were cut from three adjacent mature (70 ± 10 years old) trees of each species. The branches were cut into 25 cm long segments. Eight segments with diameters of 5 to 8 cm were selected from each tree. Two 1-cm diameter holes, for nematode inoculation, were drilled radially to the centre of each branch segment. The centre of each hole was located 8 cm from the end of the segment, leaving approximately 8 cm separating each hole. The cut ends of all segments, including the nematode inoculation holes, were dipped into a conidial suspension of *Ophiostoma piceae* (Munch) Syd. & P. Syd. prepared by rinsing the surface of two 4 week-old cultures of the fungus (growing on 1.5 % malt agar) into 1l of distilled water, resulting in a cloudy suspension; the number of spores/ml was not quantified. After inoculation with *O. piceae* the segments were incubated 5 days at 22 ± 4 °C in sealed polyethylene bags before they were inoculated with nematodes.

Two strains of the nematode were used in the experiments. The British Columbia strain was isolated from wood chips at Clinton, British Columbia. The Alberta strain was isolated from a dead *Pinus banksiana* Lamb. at Smoky Lake, Alberta. The nematodes were reared on mats of a nonsporulating culture of *Botrytis cinerea* Pers. : Fr. grown on Difco potato dextrose agar amended with 10 % glycerol. The nematodes were harvested by rinsing the lid and agar surface of a plate, and cleaned by allowing them to twice migrate through six layers of laboratory tissue (Kimwipes, Kimberly-Clark, Mississauga, Ont.) on a Baermann funnel.

The branch segments were inoculated with nematodes by placing 0.5 ml of a suspension of the nematodes into each of the two holes and plugging the holes with cotton; inoculum densities and life-stage distributions are reported in descriptions of the individual experiments. The segments were returned to polyethylene bags and arranged in a completely randomized design on a laboratory bench at room temperature.

At designated times after inoculation, replicate branch segments were arbitrarily chosen for estimating nematode population densities. The surface of each segment was washed vigorously with a wire brush and water. Three adjacent 2.5 cm thick disks were cut from each segment; the centre disk contained one of the inoculation sites and the other two disks were taken from each side of the centre disk. The two side disks from each segment were chopped into 0.5 × 0.5 × 2 cm chips and placed in a 10 cm diameter Baermann funnel over two layers of laboratory tissue for nematode extraction for 48 h.

The first 30 (first experiment) or 50 (second experiment) nematodes observed in each sample were classified according to the following life-stages : second- and third- stage juveniles (J2 + J3), fourth-stage juveniles (J4), persistent third-stage juveniles (J3P), adult fe-

males, and adult males. J4 were distinguished from second- and third-stage juveniles by the presence of genital primordia visible with the light microscope at 400 × magnification. J3P were distinguished from propagative juveniles by their large size, lack of sexual differentiation, and densely packed lipids (Mamiya, 1975; Kondo & Ishibashi, 1978).

The centre disk from each segment was used for determining gravimetric moisture content of the wood. Dry mass was determined after drying the segments at 100 °C for 48 h and moisture contents were expressed as the percentage of dry wood mass. The remaining pieces of each branch segment were incubated at room temperature in polyethylene bags for an additional 72 h. After incubation the freshly cut surfaces were observed with a stereomicroscope for the presence of fungal hyphae and synnemata or perithecia of *Ophiostoma*. The extent of hyphal growth on the cut surfaces was rated using a 0 to 4 scale (0 = no hyphae; 1 = trace of hyphae present; 2 = hyphae observed on up to 30 % of surface area; 3 = hyphae on 30 to 70 % of surface area; 4 = hyphae on 70 to 100 % of surface).

Experiment 1

Branch segments used in the first experiment were cut July 28, 1992. *P. contorta, A. grandis, P. menziesii* and *T. heterophylla* were evaluated in this experiment. All segments were inoculated with 474 ± 28 individuals of the British Columbia strain. The life-stage distribution of the population was 13 : 2 : 1 : 2 for J2 + J3 : J4 : female : male, respectively. Nematode populations were sampled each week after inoculation for 8 weeks. At each sample date three branch segments of each tree species, representing each of the three replicate trees of each species, were arbitrarily selected and nematode populations sampled.

Experiment 2

Branch segments used in the second experiment were cut November 24, 1992. *P. contorta, A. grandis, P. menziesii, T. heterophylla* and *T. plicata* were evaluated in this experiment. Four segments from each of the fifteen trees (three replicate trees × five species) were inoculated with 804 ± 59 individuals of the British Columbia strain, with a life-stage distribution of 12 : 1 : 2 : 2 : 2 for J2 + J3 : J4 : J3P : females : males, respectively. The other four segments from each of the fifteen trees were inoculated with 798 ± 74 individuals of the Alberta strain, with a life-stage distribution of 50 : 1 : 6 : 11 : 3 for J2 + J3 : J4 : J3P : females : males, respectively. At 2, 4, 8 and 16 weeks after the branches were inoculated, three replicate segments (representing the three replicate trees) of each combination of nematode isolate and tree species were arbitrarily selected and nematode populations sampled.

Data analyses

The nematode population density for each segment was expressed as the number of nematodes per gram of dry wood. The relative abundance of J3P was calculated as [(number of J3P/number of nematodes classified to life-stage) × 100]. When fewer than ten nematodes were present in a sample, the relative abundance of J3P was not calculated and was entered into analyses as missing data.

The data on nematode population densities and relative abundance of J3P were each analyzed using a nested analysis of variance (GLM procedure, SAS Inc., Cary, NC). Tree species was the main factor in each experiment and the replicate trees of each species were designated as a nested factor. Time (first experiment), or nematode isolate and time (second experiment) were designated as subfactors nested within each of the replicate trees of each species. Nematode population densities were *log (x + 1)* transformed before analyses to minimize mean-correlated variance. Fisher's protected least significant differences (LSD) were computed for testing differences between means.

The relationships between population density and relative abundance of J3P were studied using linear regression and nonlinear regression (REG and NLIN procedures, SAS Inc.). For the nonlinear regression analyses, attempts were made to fit an asymptotic model of the form $y = c\,(1-e^{-bx})$ to the data. Individual branch segments were treated as separate experimental units for regression analyses. Regressions were computed for each tree species in each experiment, for each experiment with data for the different tree species combined, and for each tree species with data from the two experiments combined. *Log*-transformed and non-transformed data were analyzed.

Data on percentage moisture and fungal colonization were analyzed using the same analysis of variance models used to analyze population densities and J3P relative abundance.

Results

Experiment 1

Nematode population densities were affected by tree species and time ($P = 0.02$ and $P = 0.0001$, respectively). Population densities increased through time in all tree species (Fig. 1). The overall mean population densities (averaged over time) were 24.2, 4.8, 4.9, and 1.4 nematodes/g dry tissue for *P. contorta, A. grandis, T. heterophylla,* and *P. menziesii,* respectively; the overall mean population density for *P. contorta* was greater than population densities in the other species ($P \leq 0.05$). Population densities in *P. contorta* were not greater than in the other species at any individual sample times.

J3P formed in all tree species (Fig. 1). The pattern of increase in J3P relative abundance varied among tree species ($P = 0.02$ for the species × time interaction

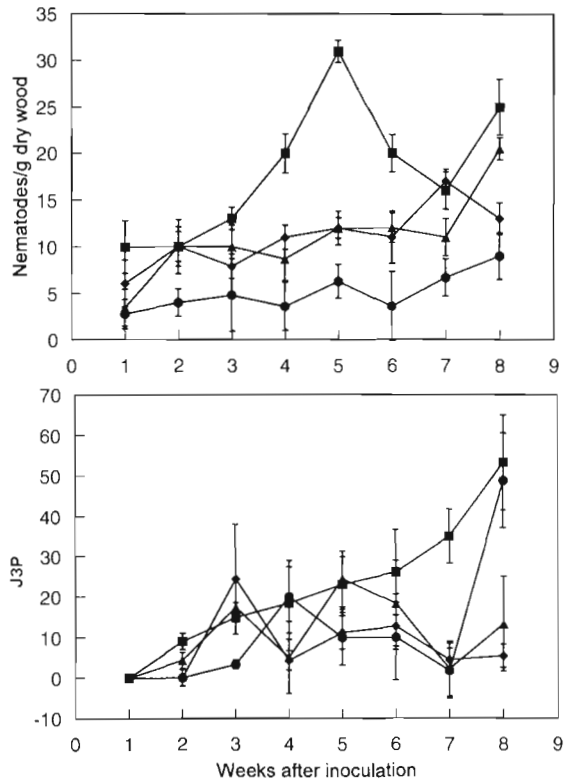

Fig. 1. *Experiment 1. Effect of time on mean (n = 3) nematode population densities (top) and persistent third-stage juveniles (J3P) percentage (bottom) of* Bursaphelenchus xylophilus *in branch segments of* Pinus contorta (■), Pseudotsuga menziesii (●), Abies grandis (▲) *and* Tsuga heterophylla (◆).

term). The mean relative abundance of J3P fluctuated erratically over the 8 weeks in *A. grandis, T. heterophylla* and *P. menziesii*. In *P. contorta* the mean relative abundance of J3P increased monotonically from 0 at the 1-week sample time to 59 % on the 8th week. The overall mean relative abundance of J3P (averaged over the 8 weeks) was greater in *P. contorta* than in the other species ($P \leq 0.05$). The relative abundance of J3P in *P. contorta* was greater ($P \leq 0.05$) than in all other species only at the 7-week sample time. At the 8-week sample time J3P relative abundance was greater ($P \leq 0.05$) in *P. contorta* and *P. menziesii* than in the other species, but the mean for *P. menziesii* at this date was based on only two replicates.

The moisture content of all species dropped between the first and second weeks and did not change thereafter (Table 1). The moisture contents did not differ significantly among tree species. Perithecia, synnemata, or both, of *Ophiostoma* were observed on the cut surfaces of segments of all tree species. The rating of mycelial growth observed on cut surfaces was affected by an interaction between species and time ($P = 0.002$). Mean

Table 1. *Percent moisture of branch segments of* Pinus contorta *(Pc),* Pseudotsuga menziesii *(Pm), Abies grandis (Ag),* Tsuga heterophylla *(Th) and* Thuja plicata *(Tp).*

Week	*Pc*	*Pm*	*Ag*	*Th*	*Tp*
Experiment 1					
1	114	109	115	109	n.d.
2	76	78	84	75	n.d.
3	84	65	79	85	n.d.
4	85	81	92	89	n.d.
5	82	69	92	86	n.d.
6	104	80	86	94	n.d.
7	82	68	69	65	n.d.
mean	89	79	88	86	
LSD ($P \leq 0.05$) = 19					
Experiment 2					
2	97	89	88	77	90
4	81	90	81	81	95
8	95	91	84	83	88
16	92	88	84	76	90
LSD ($P \leq 0.05$) = 17					

n.d. = not determined.

Table 2. *Mean rating of hyphal growth on cut surfaces of branch segments of* Pinus contorta *(Pc),* Pseudotsuga menziesii *(Pm),* Abies grandis *(Ag),* Tsuga heterophylla *(Th) and* Thuja plicata *(Tp) in experiments 1 (n = 3) and 2 (n = 6).*

Week	*Pc*	*Pm*	*Ag*	*Th*	*Tp*
Experiment 1					
1	0.0	0.0	0.0	0.0	n.d.
2	2.0	0.3	1.0	1.0	n.d.
3	1.0	0.5	1.7	1.0	n.d.
4	n.d.	n.d.	n.d.	n.d.	n.d.
5	3.3	0.3	2.3	1.3	n.d.
6	3.7	1.0	4.0	1.0	n.d.
7	3.7	2.3	3.3	2.0	n.d.
mean	2.3	0.7	2.1	1.1	
LSD ($P \leq 0.05$) = 0.99					
Experiment 2					
2	2.0	0.5	1.8	0.8	0.0
4	3.0	1.3	2.0	0.2	0.0
LSD ($P \leq 0.05$) = 0.87					

See " materials and methods " for rating scale.
n.d. = not determined.

hyphal growth on the cut surfaces of *P. contorta* and *A. grandis* was greater than on the other species (Table 2).

Experiment 2

Tree species and time had significant main-factor effects on nematode population densities ($P = 0.0016$ and

$P = 0.0001$, respectively). The population densities increased through time in all tree species. Overall mean population densities (averaged over time) were greater in *P. contorta* than in the other species ($P \leq 0.05$). Nematode population densities were greater ($P \leq 0.05$) in *P. contorta* than in all other species at all individual sample times except *P. menziesii* at the 4- and 16-week sample times. Because the effect of nematode isolate was not significant, data for the two strains were pooled for presentation (Fig. 2).

Only one sample of *T. plicata,* taken at the 16-week sample time, yielded enough nematodes to calculate J3P relative abundance. Consequently, data for *T. plicata* were omitted from the analysis of J3P relative abundance. The relative abundance of J3P increased through time in all species (Fig. 2). There was an interaction ($P = 0.013$) between the effects of tree species and nematode strain; at the 16-week sample date, J3P of the British Columbia isolate were present at greater relative abundance in *P. contorta, T. heterophylla* and *A. grandis,* and J3P of the Alberta strain were present at greater relative abundance in *P. menziesii.*

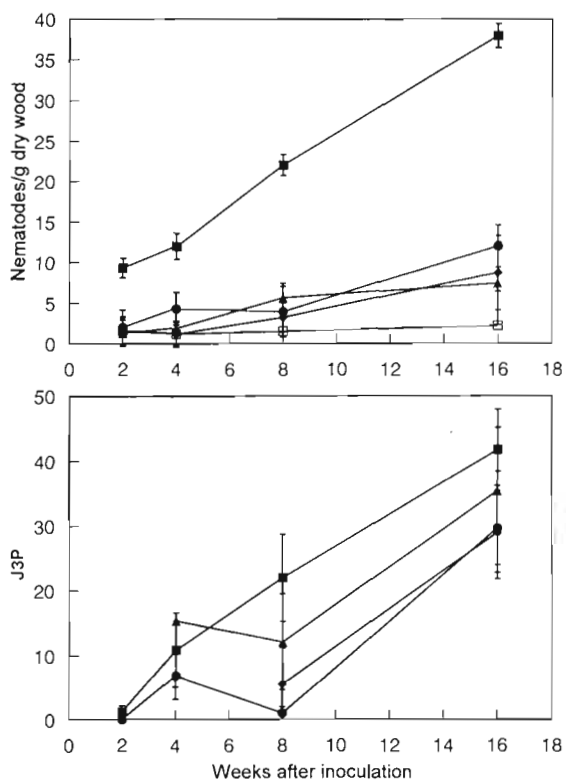

Fig. 2. *Experiment II. Effect of time on mean (n = 6) population densities (top) and persistent third-stage juveniles (J3P) percentage (bottom) of* Bursaphelenchus xylophilus *in branch segments of* Pinus contorta (■), Pseudotsuga menziesii (●), Abies grandis (▲), Tsuga heterophylla (◆) *and* Thuja plicata (□).

Wood moisture contents did not differ among tree species and were similar to wood moisture contents in the first experiment (Table 1). Data on fungal growth on cut surfaces of the branch segments was recorded only at the 2- and 4-week sample times (Table 2). The data were similar to the first experiment with the exception that no perithecia or synnemata of *Ophiostoma* were observed on cut surfaces of *T. plicata.*

Relationships between population density and J3P relative abundance

The relative abundance of J3P was not correlated with population densities for any tree species in either experiment (Fig. 3). For regressions computed using data combined from the two experiments, R^2 values for *P. contorta, P. menziesii, T. heterophylla* and *A. grandis* were 0.14, 0.22, 0.14 and 0.06, respectively. *R*- squared values for experiments 1 and 2, computed with data from all tree species combined, were 0.17 and 0.31, respectively. Plots of residuals from the nonlinear regressions indicated that the asymptotic model did not fit the data adequately.

Discussion

Both strains of *B. xylophilus,* inoculated into fungus-colonized branch segments, reproduced in all tree species tested. Nematode population densities were greater in branch segments of *P. contorta* than in the segments of other species. We did not observe any systematic differ-

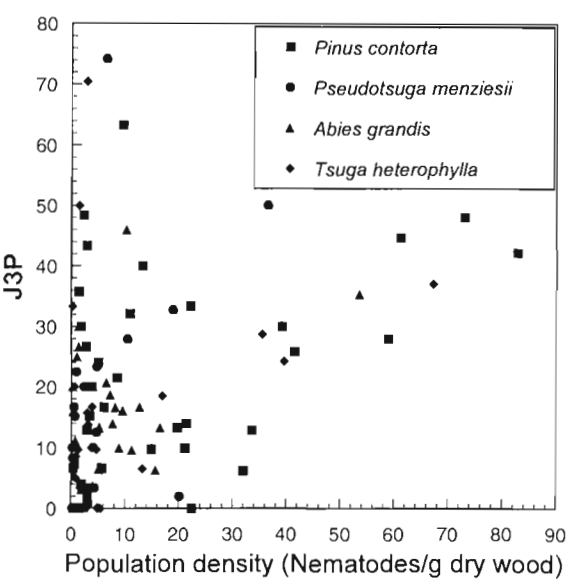

Fig. 3. *Relationships between population densities and persistent third-stage juveniles (J3P) relative abundance for* Bursaphelenchus xylophilus *populations in branch segments of* Pinus contorta, Pseudotsuga menziesii, Abies grandis *and* Tsuga heterophylla. *Data are combined from two different experiments.*

ences in the ability of the two strains to reproduce in branch segments of the different conifers.

Our primary objective was to compare the dynamics of *B. xylophilus* populations in the wood of different tree species, given that conditions were optimal for nematode colonization. Under natural conditions, freshly killed trees would be open to colonization by numerous different fungi. The blue-stain fungi, *Ophiostoma* spp. and *Ceratocystis* spp., are carried by bark beetles and are usually among the first fungi colonizing freshly cut wood. In order to mimic natural conditions the segments were inoculated with *O. picea* to ensure that a least one species of blue-stain fungus had an opportunity to colonize the wood and we did not attempt to control the growth of additional fungi in the branch segments.

Although *B. xylophilus* may undergo limited population growth under axenic conditions in complex media (Bolla & Jordan, 1982), there is no evidence that it feeds on any substrates in wood other than living cells of either plant or fungal origin. *B. xylophilus* feeds on many species of fungi in addition to parenchyma cells and epithelial cells lining resin canals (Kobayashi *et al.*, 1974, 1975; Fukushige, 1991*a,b*). Because parenchyma cells may stay viable for months after a tree or branch has been cut, nematodes in the branch segments could have been feeding on tree cells or fungal hyphae. As a result, we can only speculate on whether the observed differences among tree species in suitability for nematode colonization are the result of differences in *i)* physicochemical characteristics of the wood environment, *ii)* the abundance or nutritional quality of parenchyma and epithelial cells, or *iii)* differences in the availability of palatable fungal hyphae.

We observed that nematode population densities were generally correlated with the observed level of hyphal growth on freshly cut surfaces of branch segments : *P. contorta* > *A. grandis* > *T. heterophylla* > *P. menziesii*. However, caution must be used when interpreting this relationship. Although rapid colonization of cut surfaces may reflect the inherent suitability of sapwood of different tree species for rapid fungal growth, it may not reflect the availability of palatable hyphae for nematodes in the sapwood. Other factors that may be responsible for the observed differences in population growth are discussed elsewhere (Forge & Sutherland, 1996).

The formation of persistent third-stage juveniles (J3P) is important for the survival and dispersal of *B. xylophilus* populations in nature. Under field conditions the J3P reach peak relative abundance in autumn and winter (Mamiya, 1983, 1984; Fukushige & Futai, 1987) and appear to be physiologically adapted for surviving low temperatures, desiccation and long periods without feeding (Kondo & Ishibashi, 1978; Ishibashi & Kondo, 1977). The formation of J3P is also a necessary developmental step preceding the formation of dispersal fourth-stage juveniles which are carried by *Monochamus* beetles.

The relative abundance of J3P increased through time in all species, and was usually greater in *P. contorta* than in the other species. Date-to-date fluctuations in J3P relative abundance also appeared to be less variable in *P. contorta* than in the other species. It is not clear if the lower relative abundance of J3P in non-pines is the result of chemical cues arising directly from the wood environment, or from lower population densities. Mamiya (1990) reported that J3P formation in laboratory cultures was stimulated by fatty acids characteristic of *Pinus* spp. However, the greater J3P relative abundance was correlated with greater population densities. The accumulation of pheromones at high population densities trigger formation of dauer larvae in *Caenorhabditis elegans* (Riddle *et al.*, 1987) and a similar process could regulate formation of J3P of *B. xylophilus*. Giblin and Kaya (1984) also found that media amendments which increased population densities of *Bursaphelenchus seani* increased the relative abundance of J3P.

If J3P formation is induced by high population densities, then population densities and J3P relative abundance in wood should be correlated. Mamiya (1983, 1984) observed that the relative abundance of J3P increased rapidly after population densities increased in infected pines in Japan. Similarly, mean population densities and relative abundances both increased through time in our research. However, when the data were considered on a segment by segment basis, there was no correlation between population density and J3P relative abundance. We often observed high J3P relative abundance in samples with low population densities. Our interpretation of the relationship between population density and J3P relative abundance is based on the assumption of an even nematode distribution within the 1 cm thick × 8 cm diameter disks that were sampled. There could have been pockets of high nematode population density and J3P relative abundance in disks with low overall population density.

The relatively low population densities developing in non-pines should result in low rates of acquisition by *Monochamus* beetles. The probability of emerging *Monochamus* beetles acquiring nematodes, and the mean number of nematodes per beetle, are both directly related to moisture and the density of nematode populations in wood (Togashi, 1989).

Although our data show that *B. xylophilus* can reproduce in fungus-colonized branch segments of selected non-pine species from western North America, it is only rarely found in these species. For instance, out of 3706 samples from dead and dying conifers in British Columbia, only one sample of *P. menziesii* contained *B. xylophilus* (Bowers *et al.*, 1992); no other non-pine species in British Columbia have been reported with the nematode. *Monochamus* beetles are often found in western North America but oviposit most frequently on species of *Pinus* (Bowers *et al.*, 1992). In addition to low rates of vector visitation, low frequencies of acquisition of nema-

todes by *Monochamus* beetles, resulting from low nematode population densities in wood, probably contribute to the rarity of populations of *B. xylophilus* in forests dominated by non-pine conifers.

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