

Effect of *Monochamus carolinensis* on the Life History of the Pinewood Nematode, *Bursaphelenchus xylophilus*¹

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Abstract: The development of *Bursaphelenchus xylophilus* in pine wood infested with and free of *Monochamus carolinensis* was investigated. Formation of third-stage dispersal juveniles occurred in the presence and absence of pine sawyer beetles. The proportion of third-stage dispersal juveniles in the total nematode population was negatively correlated with moisture content of the wood. Formation of nematode dauer juveniles was dependent on the presence of the pine sawyer beetle. Dauer juveniles were present in 3 of 315 wood samples taken from non-beetle-infested Scots pine bolts and 81 of 311 samples taken from beetle-infested bolts. Nematode densities were greater in wood samples taken adjacent to insect larvae, pupae, and teneral adults compared with samples taken from areas void of insect activity. Nematodes recovered from beetle larvae, pupae, and teneral adults were mostly fourth-stage dauer juveniles, although some third-stage dispersal juveniles were also recovered. Dauer juvenile density was highest on teneral adult beetles.

Key words: *Bursaphelenchus xylophilus*, dauer juvenile, *Monochamus carolinensis*, nematode, pine sawyer, pinewood nematode.

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhner) Nickle, is a phytophagous and mycophagous nematode associated with stressed pine trees in North America. The nematode hatches as a second-stage juvenile and develops through two subsequent juvenile stages before becoming an adult (13). Under favorable conditions, such as adequate wood moisture, high nutritional reserves, and optimum temperatures, nematode development follows a reproductive pathway (15). When within-wood conditions deteriorate, the nematode switches to a dispersal pathway. The development of the nematode within stressed trees is closely associated with the development of beetles in the genus *Monochamus* (Coleoptera: Cerambycidae). *Monochamus* larvae form a pupal chamber in the xylem, and third-stage dispersal nematodes aggregate on the wall of the chamber (12). Third-stage dispersal ju-

veniles (J_{III}D) molt to fourth-stage dauer juveniles (J_{IV}D) and enter the respiratory system of the newly eclosed adult beetle (7). The dauer juvenile is important in the nematode life cycle because it is the stage carried by insect vectors to new host trees. The nematode is introduced into new host trees through feeding wounds (9,11) or oviposition wounds (3,20) made by *Monochamus* spp.

Monochamus carolinensis (Olivier) is the principal vector of *B. xylophilus* in the central United States (10). No data exist on the within-tree relationship between development of this beetle and that of the nematode. The objective of this study was to determine the influence of *M. carolinensis* presence on the formation and density of *B. xylophilus* dispersal juveniles.

MATERIALS AND METHODS

Seven apparently healthy Scots pine (*Pinus sylvestris* L.) trees were cut on the Thomas A. Baskett Wildlife Research and Education Center in Boone County, Missouri, during June 1990. Wood samples were taken from each tree to ensure that each was free of *B. xylophilus*. Two bolts, 40-cm long, were removed from each tree for a total of 14 bolts. The ends of each bolt were dipped in hot paraffin to impede desiccation. Approximately 24 hours after

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elling, each bolt was inoculated with an isolate of *Ophiostoma minus* ((Hdg.) H. & P. Sydow) on malt agar. Inoculations were made through two, 1-cm-diameter by 3-cm-deep drilled holes: one hole at each end and opposite side of the bolt. One week later, 500 *B. xylophilus* of various life stages suspended in 0.5 ml distilled water were inoculated into each of two similar holes: one opposite each of the fungal inoculation sites. The nematodes used in this study were obtained from a Scots pine at the Thomas A. Baskett Wildlife Research and Education Center and were cultured on *Botrytis cinerea* Pers. (17).

Laboratory-reared *M. carolinensis* (8) were allowed to oviposit on one randomly selected bolt from each tree for approximately 3 days. The remaining bolt of each pair received no oviposition and served as a control. All bolts were held in an insect rearing room at 27 C during the period of beetle development, which ranged from 60 to 70 days. Bolts constituting a pair were removed from storage upon emergence of the first beetle from the infested bolt. A 5-cm thick disk was removed from each end of the bolt and discarded to avoid an edge effect caused by desiccation. The remaining portion of the bolt was cut with a band saw into fifteen 2-cm-wide disks. Three 1.57-cm³ randomly located samples were removed from each disk with a 1-cm-d drill bit and electric drill. Thus, 45 samples were taken from each bolt, for a total of 315 samples from each treatment. Four samples from beetle-infested bolts were eliminated from the analysis due to experimental error resulting in a final sample size of 311. All samples were assayed for nematodes with the modified Baermann funnel technique (17). The number of third-stage dispersal juveniles, fourth-stage dauer juveniles, and all other *B. xylophilus* life stages was determined for each sample. The wet weight of each wood sample was determined before nematode extraction. After extraction, each sample was dried at 125 C for approximately 48 hours to determine dry weight. The mois-

ture content of each sample was calculated. All statistical analyses described below were conducted using the Statistical Analysis System (16).

Effect of moisture content on B. xylophilus life stages: Spearman's rank correlation was used to determine whether the proportion of third-stage dispersal juveniles in the total nematode population was correlated with moisture content of the wood samples. A nested analysis of variance was used to partition the variance associated with moisture content among treatments, bolts, disks, and samples. Analysis of variance was used to test for differences in moisture content between treatments.

Effect of Monochamus on B. xylophilus population density: Chi-square analysis was used to determine whether the ratio of third-stage dispersal juveniles to fourth-stage dauer juveniles, each expressed as a percentage of the total number of dispersal nematodes ($J_{III D}$ and $J_{IV D}$), was independent of the presence or absence of *M. carolinensis* in the bolt. One-way analyses of variance were run on log transformed [$\ln(x + 1)$] data to determine if mean densities of third-stage dispersal juveniles and fourth-stage dauer juveniles differed between bolt treatments. A nested analysis of variance was used to partition the variation associated with total nematode density (all life stages) among treatments, bolts, disks, and samples. Analysis of variance was used to test for differences in total nematode densities between the two treatments.

B. xylophilus association with Monochamus life stages: Additional wood samples were taken adjacent to *M. carolinensis* galleries in the beetle-infested bolts. Wood forming the walls of the gallery surrounding developing insects was removed by inserting a drill bit into the gallery. Each sample was classified as being adjacent to a mature larva, pupa, or teneral adult, dependent on the life stage of the insect removed from the gallery at the time of sampling. Wood samples from beetle-infested bolts described previously ($n = 311$) were

used as nonadjacent control samples. Samples taken adjacent to insect activity were irregular in volume because of variation in the diameter of insect galleries; thus, the number of nematodes in the adjacent and nonadjacent samples was expressed as the number of nematodes per gram of dry wood. The number of third-stage dispersal juveniles, fourth-stage dauer juveniles, and total number of *B. xylophilus* was determined for each sample. Analysis of variance and least square means were used to determine whether proximity of insect activity influenced total nematode density in the wood; *t*-tests were used to compare mean density of third-stage dispersal juveniles and fourth-stage dauer juveniles within each of the wood sample types. All data were log-transformed to minimize variation in nematode densities.

Monochamus carolinensis mature larvae, pupae, and teneral adults ($n = 48$) were removed from galleries. Each individual was macerated, and nematodes were collected with the modified Baermann technique. The number of third-stage dispersal juveniles, fourth-stage dauer juveniles, and the total number of *B. xylophilus* collected from each was determined. Analysis of variance was performed on log-transformed data to determine whether the density of *B. xylophilus* life stages differed among the life stages of the beetle.

RESULTS

Effect of moisture content on B. xylophilus life stages: A significant negative correlation was found between the proportion of third-stage dispersal juveniles in the total nematode population and moisture content of the wood samples ($r = -0.31$; $P = 0.0001$). Moisture content ranged from 11 to 69% in individual wood samples. The proportion of third-stage dispersal juveniles in the total nematode population ranged from 0.01 to 0.99, with a mean of 0.5 (± 0.2).

Mean percentage moisture content was higher in the control bolts (41.8% \pm

12.8%) than in the beetle-infested bolts (34.2% \pm 9.1%); however, this difference was not significant ($F = 1.98$; $df = 1,12$; $P = 0.185$). Seventy-four percent of the variation in moisture content was attributable to treatment effects. The remaining variation was associated with differences among the samples.

Effect of Monochamus on B. xylophilus population density: The mean number of *B. xylophilus* (all life stages) did not differ between treatments ($F = 0.65$; $df = 1,12$; $P = 0.436$). Seventy-eight percent of the variation associated with total nematode density was attributable to differences among samples; most of the remainder was due to differences among bolts.

The ratio of third-stage dispersal juveniles to fourth-stage dauer juvenile nematodes recovered from wood samples was dependent on bolt treatment according to chi-square analysis ($\chi^2 = 66.37$; $df = 1$; $P = 0.0001$). The mean number of third-stage dispersal juveniles did not differ between treatments ($F = 2.17$; $df = 1,624$; $P = 0.141$) (Table 1). Fourth-stage dauer juvenile density was higher in beetle-infested bolts than in the noninfested bolts ($F = 3.91$; $df = 1,624$; $P = 0.048$). Eighty-one of 311 wood samples from beetle-infested bolts contained dauer juveniles. In comparison, only three of 315 wood samples taken from nonbeetle-infested bolts contained dauer juveniles. Two samples came from the same bolt and contained 1 and 2 dauer juveniles, respectively. The third sample, taken from a different bolt, contained 20.

B. xylophilus association with Monochamus life stages: *B. xylophilus* density (all life stages) differed significantly among wood samples taken adjacent to galleries containing beetle larvae, pupae, teneral adults, and samples not adjacent to insect activity ($F = 9.97$; $df = 3,353$; $P = 0.0001$). Nematode densities in wood samples adjacent to beetle larvae, pupae, and teneral adults were each significantly higher than those in the nonadjacent samples (Table 2). Wood samples taken adja-

TABLE 1. Mean number of *Bursaphelenchus xylophilus* third-stage dispersal juveniles and fourth-stage dauer juveniles in wood samples taken from Scots pine bolts infested with and not infested with *Monochamus carolinensis*.

Bolt treatment	No. samples		Mean†	SD
	Total	With nematodes		
		All nematode life stages		
Beetle-infested	311	276	59.59 a	248.65
Uninfested	315	279	52.25 a	98.99
		Third-stage dispersal juveniles		
Beetle-infested	311	241	21.48 a	79.00
Uninfested	315	268	29.93 a	63.83
		Fourth-stage dauer juveniles		
Beetle-infested	311	81	21.61 a	193.32
Uninfested	315	3	0.07 b	1.13

† Means within a nematode life-stage followed by the same letter are not significantly different according to analysis of variance ($P \leq 0.05$).

cent to beetle larvae had the lowest mean nematode density, followed by samples adjacent to teneral adults and pupae; however, these differences were not significant.

There was no difference in the density of third-stage dispersal juveniles versus fourth-stage dauer juveniles in the wood adjacent to galleries containing *M. carolinensis* larvae or pupae (Table 3). Dauer juvenile density was higher in the wood surrounding galleries containing teneral adults. Densities were similar in wood samples from areas nonadjacent to insect activity but differed significantly when analysis was conducted on the log-transformed data.

Total nematode density differed among beetle life stages ($F = 28.98$; $df = 2,45$; $P = 0.0001$) (Table 4). The number of nematodes recovered was lowest on beetle larvae and highest on teneral adults. Individual larvae carried between 0 and 350

nematodes. Pupal densities ranged from 0 to 3,375 nematodes. The range for teneral adults was 200 to 30,800 nematodes per individual. Few third-stage dispersal juveniles were recovered. More than 98% of all *B. xylophilus* recovered from the beetles were dauer juveniles.

DISCUSSION

The mechanisms that mediate the switch in *B. xylophilus* development from the reproductive to the dispersal pathway are poorly understood. Ishibashi and Kondo (4) noted a correlation between total *B. xylophilus* population density and an increase in the proportion of third-stage dispersals in laboratory fungal cultures. They suggested that this stage is the survival stage under conditions of starvation. Mamiya reported that the proportion of third-stage dispersal juveniles in diseased trees gradually increased as the deterioration of the

TABLE 2. Mean number of *Bursaphelenchus xylophilus* (all life stages) per gram of wood in Scots pine wood samples taken adjacent to and away from *Monochamus carolinensis* activity.

Treatment	No. wood samples	Mean†	SD
Larval gallery	20	183.10 a	447.83
Pupal gallery	15	534.23 a	1074.29
Teneral adult gallery	11	350.65 a	497.11
Nonadjacent	311	60.15 b	242.57

† Means followed by the same letter did not differ significantly according to a test of least significant differences ($P \leq 0.05$). Statistical analyses were conducted on log-transformed data; raw means are reported.

TABLE 3. Mean number of *Bursaphelenchus xylophilus* third-stage dispersal juveniles and fourth-stage dauer juveniles per gram of wood in Scots pine wood samples taken adjacent to and away from *Monochamus carolinensis* activity.

<i>B. xylophilus</i> life stage	No. wood samples	Mean†	SD	<i>P</i> > <i>t</i>
	Beetle larval galleries			
Third-stage dispersal juveniles	20	102.24	223.23	0.143
Fourth-stage dauer juveniles	20	73.44	212.36	
	Beetle pupal galleries			
Third-stage dispersal juveniles	15	83.57	107.72	0.891
Fourth-stage dauer juveniles	15	412.62	1022.70	
	Teneral adult galleries			
Third-stage dispersal juveniles	11	7.69	13.12	0.034
Fourth-stage dauer juveniles	11	302.61	481.03	
	Nonadjacent to beetle galleries			
Third-stage dispersal juveniles	311	21.48	79.00	0.001
Fourth-stage dauer juveniles	311	21.61	193.32	

† Statistical analyses were conducted on log-transformed data; raw means are reported.

trees became advanced (14) and suggested that depletion of food reserves, low temperature, or moisture content of the wood could be important (15). Low temperature was not a factor in the present study, as all bolts were held at 27 C for the duration of the experiment. As moisture content of wood samples in the present study decreased, the proportion of third-stage dispersal juveniles in the total nematode population increased. Similar relationships have been described by Tomminen et al. (19) in pine chips and Suzuki and Kiyohara (18) in pine seedlings. We cannot determine from the present study whether

formation of third-stage dispersal juveniles was influenced directly by decreasing moisture content in the wood or indirectly by a secondary process. For example, food quantity (fungal growth) may be related to moisture content of the wood and could influence third-stage dispersal juvenile formation. The present study indicates that the presence of insects is not necessary for third-stage dispersal juvenile formation.

Total *B. xylophilus* population density was not affected by the presence or absence of *M. carolinensis* in experimental bolts. The density of third-stage dispersal juveniles was likewise not affected. Formation of *B. xylophilus* dauer juveniles has been thought to occur exclusively in the presence of an insect vector (15). Tomminen et al. (19) recently reported dauer juvenile formation in insect-free wood chips subjected to various incubation temperatures, but the number of dauer juveniles never exceeded 5% of the total population. Kiyohara and Bolla (5) reported dauer juveniles to compose 8 to 18% of the total nematode population on cultures of *B. cinerea* and glycerol. In the present study, *M. carolinensis* had a dramatic effect on the formation of dauer juveniles in pine bolts. A total of only 23 dauer juveniles was collected from 315 wood samples taken from beetle-free bolts—less than 1% of the total *B. xylophilus* population density of those

TABLE 4. Mean number of *Bursaphelenchus xylophilus* recovered from *Monochamus carolinensis* life stages.

Beetle life stage	No. samples	Mean†	SD
All nematode life stages			
Larva	22	30.86 a	81.57
Pupa	14	1676.57 b	4452.90
Teneral adult	12	6915.25 c	9304.47
Third-stage dispersal juveniles			
Larva	22	2.18 a	6.46
Pupa	14	31.93 a	73.03
Teneral adult	12	33.50 a	71.66
Fourth-stage dauer juveniles			
Larva	22	25.32 a	68.60
Pupa	14	1619.50 a	4308.20
Teneral adult	12	6858.42 b	9327.31

† Means within a nematode life stage followed by the same letter did not differ significantly according to a Student-Newman-Keuls test ($P \leq 0.05$). Statistical analyses were conducted on log-transformed data; raw means are reported.

samples. Dauer juvenile density was significantly higher in wood samples from beetle-infested bolts. The dauer juvenile stage is transported by insect vectors (9); thus, formation of this stage is essential if successful invasion of a new host tree is to occur. In a previous study (10), four species of wood-boring cerambycids were recovered upon emergence from pines naturally infested by *B. xylophilus*. *Monochamus carolinensis* adults carried a mean of 19,152 *B. xylophilus*; the other cerambycids averaged fewer than 300. Similar results were reported by Mamiya (15) and Wingfield and Blanchette (21). This suggests that a *Monochamus*-specific compound, produced during beetle development, initiates dauer juvenile formation or is attractive to dauer juveniles within the wood of infested pines.

Bursaphelenchus xylophilus densities in wood adjacent to *M. carolinensis* larvae, pupae, and teneral adults were all greater than densities in nonadjacent wood samples. Mamiya (12) documented an increase in nematode density around sites of *M. alternatus* larval activity and a subsequent increase in sites adjacent to pupal activity. Bolla et al. (1) and Miyazaki et al. (22) have demonstrated *B. xylophilus* attraction to lipid extracts of fourth instar and adult *Monochamus*, respectively. Kobayashi et al. (6) reported that fungi within the genera *Ophiostoma* and *Verticicladiella* were found in the larval galleries and pupal chambers of developing *M. alternatus*. Dauer juveniles climb the long perithecial neck of blue stain fungi located within the pupal chamber of *M. alternatus* before moving to newly formed adult beetles (15). These fungal concentrations could provide a food source that supports high nematode densities and facilitates movement of dauer juveniles onto their insect vectors.

Ishibashi and Kondo (4) reported that 87% of the *B. xylophilus* collected from *M. alternatus* larval galleries were third-stage dispersal juveniles. This proportion decreased through pupal development to only 1.2% of the population in adult beetle galleries. In contrast, dauer juvenile pop-

ulation percentage increased from 11.5 to 98.8%. Similar patterns were found in the present study. Third-stage dispersal density decreased from 55.8% of the total *B. xylophilus* population in wood surrounding *M. carolinensis* larval galleries to 2.2% of the population surrounding galleries with teneral adults. The dauer juvenile component increased from 40.1% to 86.3%.

Mamiya (15) recovered dauer juveniles from teneral adults but not larvae or pupae of *M. alternatus* extracted from *B. xylophilus*-infested wood. We recovered both dispersal stages of the nematode from *M. carolinensis* larvae, pupae, and teneral adults, although the numbers recovered from larvae were very low. While a few third-stage dispersals were recovered from macerated beetles, over 98% of all nematode recovered from the beetles were dauer juveniles.

Dauer juvenile movement onto immature stages of the beetle is nonproductive because the exoskeleton (including the tracheal system, which is an invagination of the exoskeleton) is shed during each beetle molt (2). Therefore, only dauer juveniles that move onto beetles after adult eclosion are assured of transport as the beetle emerges from the tree in which it developed. Twelve of 14 pupae examined in this study were surrounded by wood containing dauer juveniles. Of these, eight carried dauer juveniles; five carried fewer than 120, but two individuals carried more than 7,000 and 14,000, respectively. Such high numbers suggest that dauer juvenile movement onto pupae is not random. The fate of these nematodes during beetle eclosion is not known.

The within-wood development of *B. xylophilus* was affected by abiotic and biotic factors. We feel that decay processes initiated by the death of the host tree regulate the association between the nematode and the beetle it depends on for transport to new hosts. Adult *Monochamus* beetles are attracted to dying pines for mating and oviposition. With the death of the tree, the moisture content of the phloem and xylem tissues begins to decrease. This decrease

regulates the timing of beetle and nematode development and acts to synchronize their association. Beetles must oviposit while the phloem is moist and suitable as a food resource for their progeny. *B. xylophilus* introduced through beetle feeding (9,11) or oviposition wounds (3) reproduce while the moisture content of the xylem is suitable and then switch to the dispersal pathway as the moisture content drops. The decrease in moisture content coincides with the development of the beetle, so that third-stage dispersal juveniles are present during beetle pupal formation and adult eclosion. We believe that a chemical compound associated with *Monochamus* pupation or adult eclosion mediates *B. xylophilus* dauer juvenile formation. Other species of subcortical insects (10) may not produce this compound, thus limiting their association with *B. xylophilus*.

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