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Within-wood Spatial Dispersion of the Pinewood Nematode, *Bursaphelenchus xylophilus*[1]

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Abstract: Pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, spatial dispersion was determined in Scots pine, *Pinus sylvestris* L., bolts infested with the pine sawyer beetle, *Monochamus carolinensis* (Olivier) and in bolts without *M. carolinensis*. According to Taylor's power law and Green's index of dispersion, nematode dispersion was aggregated in both sets of bolts. The degree of aggregation did not differ significantly between beetle-infested and noninfested bolts, suggesting that the presence of *M. carolinensis* does not affect nematode dispersion within a bolt. Nematode population densities differed radially in bolts not infested with pine sawyers, but in a nonregular pattern. Moisture content of the bolts was correlated with population density of *B. xylophilus*, suggesting that nematode aggregates occur in areas of high moisture content.

Key words: Aphelenchoididae, Coleoptera, *M. carolinensis*, nematode, pine sawyer, pinewood nematode.

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, infests introduced and native conifers in North America. Its phoretic relationship with pine sawyer beetles, *Monochamus* spp., enables the nematode to be vectored from tree to tree (13). Nematodes adjacent to the beetle's pupal chamber molt to dauer juveniles and enter the respiratory system of the newly eclosed beetle (17). Although nematodes can be found throughout the respiratory system of the insect, they are concentrated within the tracheae arising from the metathoracic spiracles (13). The nematode is introduced into new host trees either through feeding (14,16) or oviposition wounds (5,16,25) made by pine sawyers.

The number of nematodes carried by an individual beetle can vary between populations and within populations of beetles, creating an aggregated dispersion of nematodes within a beetle population (13,23). *Monochamus carolinensis* (Olivier), the principal vector of *B. xylophilus* in the midwestern United States, can carry up to 79,000 nematodes per beetle upon emergence from naturally infested Scots pine trees (15). Similar results have been reported for *M. alternatus* Hope, the vector of the pinewood nematode in Japan (17). The number of nematodes carried by individual beetles is significant because it is correlated with the number of nematodes that enter a new host tree (5,14,23).

The purpose of this study was to determine if the spatial dispersion of *B. xylophilus* at the time of *M. carolinensis* pupation could explain the variation in the number of nematodes carried by individual beetles upon emergence from host trees. The objectives were to determine within-tree spatial dispersion of *B. xylophilus* in the presence and absence of *M. carolinensis* and to identify factors that may influence nematode dispersion.

MATERIALS AND METHODS

Seven apparently healthy, 30- to 35-year-old 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 these trees to ensure that each tree was free of *B. xylophilus*. Two bolts, each 40 cm in length, were removed from each tree. The ends of each bolt were dipped in hot paraffin to impede desiccation. Each bolt was inoculated, approximately 1 day after felling the trees, with an isolate of *Ophiostoma minus* (Hedgc.) H. & P. Sydow, a blue-

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stain fungus cultured on malt agar to enhance the growth of the nematode population (4). The fungus was introduced through two 1 cm diameter by 3 cm deep, holes: one hole at each end and on opposite sides of the bolt. One week later, 500 *B. xylophilus* of varying 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 were isolated from a Scots pine at the Thomas A. Baskett Wildlife Research and Education Center and cultured on *Botrytis cinerea* Pers. on potato dextrose agar (19). One bolt from each tree was randomly selected for oviposition by laboratory-reared *M. carolinensis* (12). Beetles were allowed to oviposit for approximately 3 days. The remaining bolt of each pair received no oviposition and served as a control. All bolts were kept in an insect rearing room at 27 C during beetle development, which ranged from 60 to 70 days. Paired bolts 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 14 bolts 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-thick disks. Three samples, 1.57 cm^3 each, were taken from each disk with a 1 cm diameter drill bit. The location of each sample was randomly selected from one of 12 circumferential points and then one of seven locations along a radial gradient from the outer edge of the disk to the pith. Nematodes were extracted using the modified Baermann funnel technique (19).

All statistical analyses were conducted using the Statistical Analysis System (18).

Spatial dispersion of pinewood nematode: Analysis of variance was used to determine if *B. xylophilus* population densities differed among disks within each bolt treatment. Taylor's power law (22) and Green's index of dispersion (8) were used to evaluate the within-tree dispersion of *B. xylophilus* in beetle-infested and noninfested pine bolts. Evaluation of nematode dispersal was conducted at the time of first beetle emergence from the beetle-infested bolt of each pair and at the same time in the associated control bolt. Dispersion was evaluated at this time because it marks the beginning of the association of the adult beetle with the dauer juvenile nematode (13).

The mean and variance of the number of *B. xylophilus* per sample ($n = 45$) was calculated for each of the seven bolts in both treatments. Data were log transformed ($\log_{10}(x + 1)$) to meet the assumption of normality. Taylor's power law is defined as $s^2 = am^b$, where s^2 = population variance, a = y intercept of population density, m = population mean, and b = slope of the population density. The t statistic was used to test the hypothesis that each slope did not deviate significantly from a value of 1 (22). An F statistic was used to compare slopes between treatments. A slope significantly greater than 1 represents an aggregated dispersion; a slope significantly less than 1 represents a uniform dispersion. A slope value not significantly different from 1 represents a random dispersion.

Green's index of dispersion, defined as $[(s^2/m) - 1]/(n - 1)$, where s^2 = population variance, m = population mean, and n = number of individuals in each bolt, was used to verify the results of Taylor's power law. This index is independent of changes in population density. The mean number of nematodes per sample ($n = 45$) and its associated variance was calculated for each bolt. Data were transformed as above. A Green's index value of 0 represents a random dispersion. A significant positive departure from 0 indicates an aggregated dispersion, whereas a negative departure indicates a uniform dispersion. A t statistic was used to test for significance of index values for each of the treatments (8). An additional t statistic was used to compare index values between treatments.

Effect of radial location and moisture content on pinewood nematode: Wood samples were classed according to their radial location within a bolt to evaluate the population density of nematodes from the edge of a bolt to the pith. Analysis of variance was conducted on $\log_{10}$-transformed data to determine if mean pinewood nematode densities differed among radial locations within treatments. Comparison of density patterns in beetle-infested and noninfested bolts was conducted to determine the influence of beetle presence on radial population densities of the pinewood nematode.

The wet weight of each wood sample was determined, then each sample was dried at 125 C for 48 hours and weighed. The moisture content of each wood sample was determined as follows:

$$\text{moisture content (\%)} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100$$

to investigate the relationship between moisture content and nematode density. Spearman's rank correlation procedure was used to investigate the relationship between percentage of moisture content and *B. xylophilus* density. Wood samples from both treatments were pooled for this analysis.

RESULTS

Spatial dispersion of Bursaphelenchus xylophilus: B. xylophilus population densities did not differ among disks within logs with beetles ($F = 1.15$; df = 14, 296; $P = 0.31$) or without beetles ($F = 1.38$; df = 14, 300; $P = 0.16$), indicating that the nematode population had dispersed from the inoculation sites and colonized the entire log. *B. xylophilus* populations were aggregated in beetle-infested and noninfested bolts according to Taylor's power law and Green's index of dispersion. The calculated slope values for both the beetle-infested and noninfested bolts differed from 1 according to Taylor's power law (Table 1). The slope value of beetle-infested bolts was higher than that of noninfested bolts; however, the significance of the differences was minimal ($F = 4.80$; df = 1, 11; $P = 0.05$). Results using Taylor's power law were supported by Green's index of dispersion (Table 2). There were no differences in the calculated index values between treatments ($t = -0.4862$; df = 12; $P = 0.64$).

TABLE 1. Calculated *y*-intercept (a) and slope (b) values for the relationship between the mean and variance of *Bursaphelenchus xylophilus* population densities in beetle-infested and noninfested Scots pine blots.

Treatment	a	b	*n*	r^2	*t*	$P > t$
Beetle-infested	−1.30	2.77	7	0.86	5.49	0.017
Noninfested	0.81	1.98	7	0.92	7.82	0.005

Effects of radial location and moisture content on pinewood nematode: The mean number of *B. xylophilus* per sample differed among radial distances in bolts without beetles ($F = 2.72$; df = 6, 308; $P = 0.01$) but not in beetle-infested bolts ($F = 2.13$; df = 6, 304; $P = 0.0502$) (Table 3). Differences in radial nematode population densities in the noninfested bolts followed no pattern relative to radial location, and high variances made interpretation of the mean separation procedure difficult. Mean (±S.D.) *B. xylophilus* population densities in beetle-infested bolts ranged from 21 (±39) nematodes at 4 cm to 147 (±513) nematodes at 2 cm. Mean densities in noninfested bolts ranged from 26 (±27) nematodes at 7 cm to 79 (±129) nematodes at 6 cm. The range of values for both treatments was similar, with the exception of the 2-cm value in the beetle-infested bolts, which coincided with the location of beetle larval activity and may reflect aggregation of dauer juvenile nematodes not present in bolts without beetles.

TABLE 2. Green's index of dispersion values for *Monochamus carolinensis*-infested and noninfested Scots pine bolts.

Treatment	Green's index value†	*n*	*t*	$P > t$
Beetle-infested	0.05* a	7	3.23	0.02
Noninfested	0.21* a	7	3.53	0.01

† Green index values followed by an asterisk differed significantly from 0 according to a *t* test; values followed by the same letter did not differ significantly according to a *t* test ($P \leq 0.05$).

TABLE 3. The mean number of *Bursaphelenchus xylophilus* at each of seven radial locations from beetle-infested and noninfested Scots pine bolts.

Distance (cm)†	No. wood samples	Mean no. nematodes‡	S.D.
	Beetle-infested		
1	46	46.4 a	114.2
2	59	146.8 a	513.3
3	48	22.0 a	32.7
4	44	21.0 a	38.6
5	41	39.8 a	105.7
6	44	60.5 a	209.5
7	30	49.0 a	91.6
	Noninfested		
1	53	49.2 ab	83.7
2	49	47.9 a	45.1
3	40	51.2 ab	100.7
4	41	56.0 b	156.8
5	55	50.4 ab	88.9
6	45	78.9 ab	128.8
7	32	25.9 b	27.0

† The distance of samples from the edge of a bolt to the center.

‡ Means with the same letter within the same treatment did not differ significantly according to a Student-Newman-Keuls test. Mean separations based on $\log_{10}$-transformed means; raw means are reported above.

Moisture contents of individual samples ranged from 11% to 69%, with a mean of 38% (±12%). A weak, but significant, correlation was found between moisture content and the total number of nematodes ($r = 0.18$; $P > r = 0.0001$).

DISCUSSION

Within-tree dispersion of *B. xylophilus* has been reported to be random (10) and aggregated (20). Both studies, however, determined nematode dispersion in pine seedlings soon after nematode inoculation. In the present study, dispersion was determined to be aggregated at the time of beetle eclosion, approximately 60 to 70 days postinoculation.

Aggregation is characteristic of other plant-parasitic nematodes, including *Ditylenchus* spp. and *Aphelenchoides* spp. (3,24). Aggregation is also frequently observed in entomopathogenic nematodes such as *Steinernema carpocapsae* Weiser (9) and mycophagous nematodes such as *Ditylenchus* spp. (2). The within-wood aggregative behavior of *B. xylophilus* may influence the number of nematodes carried by individual beetles. Edwards and Linit (6) documented that female *M. carolinensis* randomly search the surface of a log before egg deposition. The potential nematode load of a developing beetle may be a function of random egg laying and subsequent larval and pupal development. Beetle larvae and pupae that develop near a nematode aggregate may emerge carrying a large number of nematodes, whereas beetles that develop away from a nematode aggregate are likely to carry a low number. Beetles that carry a high number of nematodes transmit a greater number of nematodes into a new host tree through either feeding (14,23) or oviposition wounds (5) than beetles with few nematodes. Success of *B. xylophilus* in invading a new host tree may depend on insects developing near nematode aggregates.

Aggregation of *B. xylophilus* populations within a tree may be a function of several factors. Within-tree aggregation of the nematode may reflect the location of food sources within a tree. Although *B. xylophilus* is phytophagous and mycophagous, developing nematode populations at the time of beetle emergence probably rely on fungi as a food source (17). A predominant fungus found in deteriorating pine tree is *O. minus*, which is introduced into trees by bark beetles. Growth of the fungus tends to occur longitudinally, rather than radially and tangentially, because of reduced mechanical resistance (7). Radial spread is second fastest because most pits, the openings in the primary cell walls of trees, are oriented radially. Tangential growth has the lowest rate of spread due to low density of pits (7). Fungal growth, therefore, has a concentrated dispersion within a tree. If nematode populations reflect blue stain fungus dispersion, it is reasonable to assume that higher nematode population densities would occur on the periphery of a bolt where high fungal densities are found. Nematodes were not concentrated near the periphery of the bolts; thus it is unlikely that *O. minus* alone regulates the within-wood dispersion of the nematode. Furthermore, *B. xylophilus* is known to feed on other genera of fungi found in dying pines (11).

If within-wood nematode movement followed a path of least resistance, nematode population densities would be highest on the periphery of a bolt due to the large longitudinal resin canals within a tree. Nematode numbers, however, did not follow a radial gradient, suggesting that plant anatomy does not affect nematode dispersion at the time of beetle emergence.

A second factor that may influence nematode dispersion is within-tree moisture conditions. The moisture content of wood samples was highly variable and was weakly correlated with nematode density. The pattern of nematode dispersion, therefore, may be influenced by the distribution of moisture within a bolt.

The effect of moisture content on *B. xylophilus* population dynamics has been documented in the United States and Japan. Tamura (21), working with a Japanese strain of the nematode in seedlings, reported nematode population densities increased as moisture content increased. Dwinell (4), reported that *B. xylophilus* densities in pine chips declined as the percentage of moisture in the chips declined. Bergdahl and Halik (1) found nematode numbers were highest in pine chips with a moisture content of 38%. Fewer nematodes, however, were found in wood chips with higher moisture contents. Nematode populations that are near areas of high moisture content may be able to increase their densities by remaining in the reproductive pathway of development. *Bursaphelenchus xylophilus* populations in areas of low moisture follow a dispersal pathway (17) and may form aggregates. Aggregation may reduce exposure to desiccation and adverse temperatures (9).

The dispersion of *B. xylophilus* at the time of beetle emergence was aggregated. Aggregation did not depend on the presence or absence of the pine sawyer but may be related to other factors, such as variable moisture content within a tree. Areas of adequate moisture content may enable nematode populations to continue reproduction, allowing large numbers to be achieved.

LITERATURE CITED

1. Bergdahl, D. R., and S. Halik. 1990. Development of *Bursaphelenchus xylophilus* populations in wood chips with different moisture contents. Journal of Nematology 22:113–118.

2. Cairns, E. J. 1953. Relationship of the environmental moisture conditions of the mushroom-spawn nematode, *Ditylenchus* spp., to its control by heat. Mushroom Science II. Gembloux.

3. Croll, N. A. 1970. The behavior of nematodes. New York: St. Martin's.

4. Dwinell, L. D. 1986. Ecology of the pinewood nematode in southern pine chip piles. USDA-Forest Service, Southeastern Forest Experiment Station Research Paper SE-258.

5. Edwards, O. R., and M. J. Linit. 1992. Transmission of *Bursaphelenchus xylophilus* through oviposition wounds of *Monochamus carolinensis* (Coleoptera: Cerambycidae). Journal of Nematology 24:133–139.

6. Edwards, O. R., and M. J. Linit. 1991. Oviposition behavior of *Monochamus carolinensis* (Coleoptera: Cerambycidae) infested with the pinewood nematode. Annals of the Entomological Society of America 84:319–323.

7. Fares, Y., J. D. Goeschl, and P. J. H. Sharpe. 1980. Dynamics of bark beetle-fungus symbiosis I. Pine tree anatomy and fungus growth pattern. Technical Bulletin No. 1630. Asheville NC: U.S. Department of Agriculture, Forest Service.

8. Green, R. H. 1966. Measurement of nonrandomness in spatial distributions. Researches on Population Ecology 8:1–7.

9. Ishibashi, N., and E. Kondo. 1990. Behavior of infective juveniles. p. 140. *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

10. Kiyohara, T., and K. Suzuki. 1978. Nematode population growth and disease development in the pine wilt disease. European Journal of Forest Pathology 8:285–292.

11. Kobayashi, T., K. Sazaki, and Y. Mamiya. 1974. Fungi associated with *Bursaphelenchus lignicolus,* the pine wood nematode. Journal of the Japanese Forestry Society 56:136–145.

12. Linit, M. J. 1985. Continuous laboratory culture of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with notes on larval development. Annals of the Entomological Society of America 78:212–213.

13. Linit, M. J. 1988. Nematode–vector relationships in the pine wilt disease system. Journal of Nematology 20:227–235.

14. Linit, M. J. 1990. Transmission of pinewood nematode through feeding wounds of *Monochamus carolinensis* (Coleoptera: Cerambycidae). Journal of Nematology 22:231–236.

15. Linit, M. J., E. Kondo, and M. T. Smith. 1983. Insects associated with the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoid-

idae), in Missouri. Environmental Entomology 12: 467–470.

16. Luzzi, M. A., R. C. Wilkinson, and A. C. Tarjan. 1984. Transmission of the pinewood nematode, *Bursaphelenchus xylophilus,* to slash pine trees and log bolts by a cerambycid beetle, *Monochamus titillator,* in Florida. Journal of Nematology 16:37–40.

17. Mamiya, Y. 1984. The pine wood nematode. Pp. 589–626 *in* W. R. Nickle, ed. Plant and insect nematodes. New York: Marcel Dekker.

18. SAS Institute. 1985. SAS user's guide: Statistics, 5th ed. Cary, NC: SAS Institute.

19. Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries, and Food Reference Book 402. London: Her Majesty's Stationery Office.

20. Tamura, H. 1976. Distribution of *Bursaphelenchus lignicolus* in the dead pine trees. Japanese Journal of Nematology 6:60–66.

21. Tamura, H. 1986. Occurrence of the dispersal third stage juveniles of the pinewood nematode in pine logs. Transactions of the Annual Meeting of the Kansai Branch of the Japanese Forestry Society 37: 201–203.

22. Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189:732–735.

23. Togashi, K. 1985. Transmission curves of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) from its vector, *Monochamus alternatus* (Coleoptera: Cerambycidae), to pine trees with reference to population performance. Applied Entomology and Zoology 20:246–251.

24. Wallace, H. R. 1963. The biology of plant parasitic nematodes. New York: St. Martin's.

25. Wingfield, M. J. 1983. Transmission of pine wood nematode to cut timber and girdled trees. Plant Disease 67:35–37.