

Temporal Pattern of Pinewood Nematode Exit from the Insect Vector *Monochamus carolinensis*¹

MARC J. LINIT²

Abstract: Laboratory-reared *Monochamus carolinensis* (Olivier) were used to study the temporal pattern of pinewood nematode dauer larval exit from this beetle vector. Exit rates of dauer larvae were determined by comparing the mean number of dauer larvae carried by adult beetles 0, 7, 14, or 21 days after emergence from the log in which they developed. Density of dauer larvae was highest in beetles on the day of their emergence and dropped slowly through the subsequent age classes. The rate of nematode exit was low during the first week (4.5%) and higher during weeks 2 (20.5%) and 3 (13.1%). A total of 38.1% of the initial dauer larvae exited the beetles during the 3-week observation period.

Key words: *Bursaphelenchus xylophilus*, insect vector, pine wilt disease, pinewood nematode, *Monochamus carolinensis*.

Beetles in the genus *Monochamus* are the principal vectors of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle, 1970, throughout the known distribution of the nematode (8). The nematode is the causal agent of pine wilt disease, as well as the reason for an international embargo of coniferous wood from the United States and Canada (1). Wood-boring beetles provide the only known means of transport for pinewood nematodes from infected to non-infected host trees and logs. The association between the nematode and insect vector is thus an essential component in the transmission of pinewood nematodes through both primary and secondary pathways (8,18).

Dauer larvae of *B. xylophilus* inhabit the respiratory tracheae of adult stages of *Monochamus* spp. The majority of dauer larvae inhabit the tracheae arising from the meta-thoracic spiracle. The number of dauer larvae carried by individual *M. carolinensis* (Olivier) adults is quite variable and ranges from 0 to > 100,000 (2,9,10,17). The process of nematode exit from *M. alternatus* Hope, the principal vector of pinewood nematode in Japan, has been studied by several investigators because of the importance of the nematode-associated pine wilt

disease in that country (11). Dauer larvae are reported to exit the beetle through the spiracles, move toward the tip of the beetle's abdomen, travel down the setae on the terminal abdominal sclerites, and drop off (6,12).

The number of dauer larvae carried by adult *M. alternatus* decreases with time following emergence of the adult beetle from the tree in which it developed (6,12). Enda (3) reported that 25% of all dauer larvae exit the tracheal system of *M. alternatus* within 10 days of beetle emergence from the tree in which it developed, 87% by day 20, and 94% by day 30. In a study by Nakane (13) exit began immediately upon beetle emergence but is slow during week 1, maximized during week 2, and reduced beyond that time. Hosoda and Kobayashi (5) reported the earliest dauer larval exit is 3-5 days after beetle emergence, with the majority of nematode exit occurring after day 10. In another study (4), dauer larval exit reached its maximum during week 3, declined through week 5, and was low through week 10. In a recent study (15), the majority of dauer larvae exited 10-40 days after *M. alternatus* emergence. Some Japanese workers (6) conclude, however, that the rate of dauer larval exit is so discontinuous that generalization may not be realistic.

Similar studies have not been conducted on North American vectors of the pinewood nematode. The objective of this study was to monitor the exit rate of pinewood

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² Department of Entomology, University of Missouri-Columbia, Columbia, MO 65211.

nematode dauer larvae from adult *M. carolinensis*.

MATERIALS AND METHODS

Monochamus carolinensis adults were reared in Scots pine (*Pinus sylvestris* L.) logs under laboratory conditions. The procedure described by Linit (7) was modified in order to obtain adult beetles infested with pinewood nematode dauer larvae. Each log was inoculated with ca. 50,000 pinewood nematodes (all life stages) before being subjected to beetle oviposition. Two holes (1.27 cm d) were drilled ca. 4 cm into the xylem tissue at opposite ends of the log. The nematodes (ca. 25,000), suspended in 2 ml sterile, distilled water, were pipetted into each hole which was then plugged with styrofoam and sealed with petroleum jelly. The logs were held at 30 C and 70% relative humidity through the completion of beetle development and emergence. Newly emerged beetles were collected daily and placed in wooden boxes with water, pine foliage, and pine logs on which they could mate and oviposit for 0, 7, 14, or 21 days. Thus, each beetle belonged to one of four beetle age classes. At the appropriate time, individual beetles were dissected and the number of dauer larvae carried by each beetle was determined by the Baermann technique (14).

Beetles were reared in five batches of logs that differed only in the time of year they were cut: July, August, November (2 batches) 1983, and February 1984. Previous rearing experience has shown that beetles reared in logs cut during the winter months tend to carry fewer dauer larvae than beetles reared in logs cut in summer. Beetles reared in each batch of logs were assigned to all of the four age classes. The experiment was analyzed as a randomized complete block with log batch being the block effect.

Evaluation of the temporal pattern of nematode exit from adult *M. carolinensis* was made by comparison of the mean number of nematodes carried by beetles in each age class. A chi-square test of independence was used to compare the frequency

of beetles carrying no nematodes within each age class. Analysis of variance was used to test for differences in mean density of dauer larvae among age classes of beetles. Dauer larval densities were transformed ($\log + 1$) to stabilize within-age class variances. Nematode exit was partitioned among beetle age classes and expressed as a percentage of initial density of dauer larvae. During the study, 221 beetles were reared and dissected.

RESULTS AND DISCUSSION

Nematodes were recovered from beetles in all age classes; however, a large range of nematode densities was observed among individual beetles within each age class. All age classes contained beetles in which no nematodes were found. The maximum number of nematodes recovered from individual beetles exceeded 70,000 for each age class. The proportion of beetles within an age class that carried no nematodes was independent of age class ($\chi^2 = 3.15$; $df = 3$; $P = 0.368$), suggesting that total nematode evacuation of beetles did not occur in the later age classes.

Significant differences were found in the mean number of nematodes among log batches ($F = 3.68$; $df = 4, 12$; $P = 0.035$) but not among age classes ($F = 0.97$; $df = 3, 12$; $P = 0.437$). There was no interaction among these variables. Mean density of dauer larvae was highest on the day of beetle emergence and dropped slowly through the subsequent age classes (Table 1). The rate of mean nematode exit was low during the first week (4.5%), increased greatly during week 2 (20.5%), and declined somewhat during week 3 (13.1%). A total of 38.1% of the initial dauer larvae exited the beetles during the 3-week observation period.

Exit of *B. xylophilus* dauer larvae from beetle vectors in the genus *Monochamus* is an important step in the process of transmission of the pinewood nematode to new host trees. This is the first report of exit rates of pinewood nematode dauer larvae from any North American vector. The exit pattern was similar to that reported by

TABLE 1. Dauer larvae collected from adult *Monochamus carolinensis* in four age classes.

Age class (days post- emergence)	No. of adult beetles examined	Dauer larvae (mean no.)	Nematodes exited† (no.)	Exit‡ (%)
0	70	21,151	959	4.5
7	45	20,192	4,328	20.5
14	54	15,864	2,768	13.1
21	52	13,096		
Total	221		8,055	38.1

† The between-week difference in mean number of nematodes.

‡ The number of nematodes exited expressed as a percentage of the mean number of nematodes in age class 0.

many Japanese investigators for *M. alternatus*.

Walsh and Linit (16) reported that *M. carolinensis* oviposition begins about 5–7 days after adult beetle emergence and continues through the life of the female. Pinewood nematode dauer larval exit from *Monochamus* spp. is low before initiation of oviposition activity, suggesting that nematode transmission through oviposition wounds is the predominant pathway to new host trees and logs.

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