The effect of beetles on the dispersal stages of *Bursaphelenchus mucronatus* Mamiya & Enda (Nematoda: Aphelenchoididae) in wood chips of *Pinus sylvestris* L.

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The effect of the presence of beetle adults (*Monochamus galloprovincialis*, *Acanthocinus aedilis*, *Rhagium inquisitor* and *Hylobius abietis*) in Scots pine wood chips on the occurrence of the third (L_{III}) and the fourth (L_{IV}) dispersal stage of the nematode *Bursaphelenchus mucronatus* was studied. Possible infestation of the beetles with L_{IV} of the nematode while being in close contact with the pine chips was also explored. Adults of *M. galloprovincialis* and *A. aedilis* contributed to *B. mucronatus* L_{IV} formation in the chips. Callow adults of *M. galloprovincialis* were more efficient elicitors of moulting of L_{III} into L_{IV} than emerged beetles of the same species. Only *M. galloprovincialis* adults became infested with L_{IV} callow beetles containing more nematodes than emerged beetles.

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1. Introduction

The nematode, *Bursaphelenchus mucronatus* Mamiya & Enda 1979, a close relative of the pinewood nematode, *B. xylophilus* (Steiner & Buhrer 1934), that has been found to kill pine trees in Japan, China (Mamiya 1984, 1987) and the United States of America (Dropkin et al. 1981, Dropkin & Linit 1982, Kondo et al. 1982), is widely distributed in Japan (Mamiya & Enda 1979), China (Mamiya 1987) and Europe (De Guiran & Boulbria 1986, McNamara & Støen 1988, Magnusson & Schroeder 1989, Tomminen et al. 1989). Extensive pine mortality has not been associated with *B. mucronatus* but it appears to be of minor importance as a tree pathogen (Mamiya & Enda 1979, Tamura 1984). The reproduction and dispersal biology of the two species are more or less identical (Mamiya & Enda 1979). *B. mucronatus* and *B. xylophilus* are transmitted to new feeding grounds by beetle vectors, i.e. pine sawyers (*Monochamus* spp., Cerambycidae) serving as primary carriers in all regions where vector aspect has been studied (Mamiya & Enda 1972, Linit et al. 1983, Wingfield & Blanchette 1983, Carling 1984, Kinn 1986, Magnusson & Schroeder 1989, Tomminen 1990).

B. xylophilus and *B. mucronatus* are known to possess two alternative life cycles called propa-

gative and dispersal cycles (Ishibashi & Kondo 1977, Mamiya & Enda 1979, Wingfield et al. 1982). The former consists of an egg stage followed by four larval stages preceding adults of both sexes. The latter differs from the propagative cycle in having replaced the third and the fourth larval stage by stages designated as the third dispersal stage (L_{III}) and fourth dispersal stage (dauerlarva) (L_{IV}). Both of these dispersal stages are specialized stages; the former to surviving adverse living conditions such as drought, starvation and cold, thus being defined as a resting stage, and the latter to being transmitted to new feeding sources inside the tracheae of beetle vectors (Ishibashi & Kondo 1977, Kondo & Ishibashi 1978, Mamiya 1983).

The proportion of L_{III} in populations living in diseased or dead trees gradually increases with the deterioration of the trees (Mamiya 1983). In the winter and early spring the stage predominates in the populations in both species of *Bursaphelenchus* (Mamiya 1984, author unpubl.). The moulting of L_{III} in water into L_{IV} has been shown to be related to the developmental stages of the beetle vectors at the extraction time of the nematodes (Ishibashi & Kondo 1977). It has also been demonstrated that *B. xylophilus* L_{IV} formation can be attributed to certain variations in the incubation temperature under the circumstances where beetle vectors have not been available to the nematodes (Tomminen et al. 1988, Tomminen & Akar 1990). During the winter and spring in Japan, L_{III} aggregate around the pupal chambers of their vector beetle *Monochamus alternatus* Hope and later in spring, coinciding with the time of pupation of *M. alternatus*, L_{III} start moulting into L_{IV} (Mamiya 1983). Pine wood tissues and their extractives have been reported to possess qualities that promote moulting of *B. xylophilus* L_{IV} into adults (Mamiya 1983).

The main objective of this study was to determine the impact of beetle adult presence on the total population number and the occurrence of dispersal stages of *Bursaphelenchus mucronatus* in pine chips. Also examined was whether L_{IV}, if induced in the chips, would go into the beetles from the chips and if the age of the beetle adult would be of significance to the occurrence of the dispersal stages in chips as well as to their movement into the beetles.

2. Material and methods

2.1. Establishment of nematode populations in pine chips

The pine chips in the study were produced from young healthy *Pinus sylvestris* L. trees (trees had been felled in late May and early June) growing in Tuusula, in southern Finland. The chips were stored in a freezer at –20°C and thawn out shortly before they were used.

The nematode populations in the chips were established by inoculating 200 g (fresh weight) of chips in sealable plastic bags with approximately 200 nematodes each. Two different geographic isolates of *B. mucronatus* were used in inoculations: Isolate I (Muddusjärvi, Finland, 69°02′N, 27°03′E) and Isolate II (Enontekiö, Finland, 68°24′N, 23°36′E). Both isolates were originally extracted from *Monochamus* infested round pine logs and since they were both of northern origin they were thought to respond similarly enough to the study treatments, thus making it unnecessary to treat them separately. During the course of the experiment fungi, including *Graphium* sp., established growth on the chips. However, a thorough fungal determination was not conducted.

The pine chips used in the study were divided into 5 treatments according to their history of incubation following the nematode inoculations (Chip incubations):

I incubation at room temperature (20–25°C) for 5 months;
II incubation at room temperature for 6 months;
III incubation at room temperature for 12 months;
IV incubation at room temperature for 5 months, moved to refrigerator at 3°C for 20 days followed by incubation at room temperature for 7 days;
V Same as IV with the exception of the incubation at room temperature following the cold treatment in the refrigerator lasting 22–27 days.

Treatments I–III will be cited as *room treatment* and IV–V as *cold treatment* later in the text.

The reason for the classification above was the variation in the timing of the availability of given beetle adults. In some cases during the

statistical analysis of the study data some chip classes were combined for analytical purposes due to the lack of statistical differences in the parameter values under comparison. Where such combinations were performed this will be distinctly stated in the results section.

2.2. Beetles

Four different species of conifer beetles were included in the study, all having a common and widespread occurrence in Finland: *Monochamus galloprovincialis* (Olivier), *Acanthocinus aedilis* (L.), *Rhagium inquisitor* (L.) and *Hylobius abietis* L. The first three species are members of the family Cerambycidae, *M. galloprovincialis* and *A. aedilis* of the subfamily Lamiinae and *R. inquisitor* of the subfamily Lepturinae, while the last one belongs to the family Curculionidae. *M. galloprovincialis* pine sawyers were reared to adults in the laboratory in Scots pine bolts. A portion of these beetles was removed from their pupal chambers prior to their emergence and correspondingly classified as *callow* adults characterized by their soft cuticle and its light brown colour. In contrast, already emerged pine sawyer adults were grouped as *emerged* adults possessing a hardened dark-coloured cuticle (the post-emergence age for these beetles was not determined).

All the other beetle species were field-collected from several locations in southern Finland during the peak swarming season of each species. The main collection sites were: Tuusula 60°21′N, 25°04′E; Mäntsälä, 60°37′N, 25°17′E; Haarajoki 60°31′N, 25°11′E and Savijoki, 60°40′N, 25°33′E. Sexes were treated separately in *M. galloprovincialis* and *A. aedilis*.

2.3. Beetle-nematode association

Beetles were placed in small cylindrical plastic jars (diameter of the bottom and the top = 53 mm, height = 90 mm, volume = 198 cm^3) one beetle per jar with small amount of pine chips from one of the five chip incubations containing nematodes of the species *B. mucronatus*. The jars were sealed with plastic caps to prevent the beetles from escaping. The jars were periodically agitated to force the beetles into close contact with the chips. A control jar with chips of the corresponding treatment was established for each beetle jar. For each beetle used in the study one beetle of the same species originating from the same source was extracted as a control to determine the initial level of *B. mucronatus* L_{IV} infestation in the beetles.

The beetle jars and their respective control jars were kept at room temperature (21–24°C) for a varying number of hours classified as follows (Jar incubations):

1. Less than 101 h
2. 101–200 hours
3. 201–300 hours and
4. exceeding 300 h.

Subsequently, the beetles were removed from the jars, at the latest on the first day they were found dead, and cut into 3–4 pieces to facilitate the nematode departure from the tracheae or other parts of the internal structures during the extraction. For extraction purposes the pieces of the beetles wrapped in single-layer paper towel tissue were submerged in tap water (initial temperature roughly 30°C, extraction at room temperature) in test tubes for 48 hours followed by disposal of the beetle remains and examination of the tube water for nematodes. L_{IV} of *B. mucronatus* were counted and a portion was cultured either on *Botrytis cinerea* Pers. or Scots pine chips to obtain adults for identification.

The pine chips in the beetle jars and the control jars were extracted upon the removal of the beetles. The extraction procedure was as follows: the jar was filled with tap water and subsequently stored at room temperature for 48 hours. Upon the completion of the extraction time the chips were removed and placed in a heating oven at 95°C for 3–4 days for assessment of the dry weight of wood. The nematodes in the jars were counted and a portion was individually picked (50–60, rarely less, depending on the availability of the nematodes in a given sample) for determining the percentage of the total nematode population in the following life stages:

1. third dispersal stage (L_{III}) and
2. fourth dispersal stage (L_{IV}) (dauerlarva).

L_{III} is characterized by its comparatively large size, relatively thick cuticle, and a dense accu-

mulation of granules in the intestinal cells (Mamiya & Enda 1979, Kondo & Ishibashi 1978). In respect of the Finnish *B. mucronatus* the tip of its tail, regardless of the future sex, is strongly mucronated, being identical to the female tail (author unpubl.). L_{IV} lacks any visible internal structures in the anterior part of its body including stylet and oesophagus and it has a clearly dome-shaped head and strongly mucronated tail (Mamiya & Enda 1979, Kondo & Ishibashi 1978, author unpubl.).

2.4. Statistical analyses

Analysis of variance and Brown-Forsythe or Welch (variances not equal) analysis of variance were used to determine the statistically significant differences ($P < 0.05$) among the data that were normally distributed. In some cases transformations were applied to the data to obtain or improve the required normality (log transformations on numerical data and arcsine transformations on proportional data). When the requirements for the normality were not gained in spite of the transformation endeavours nonparametric analysis of variance (Kruskal-Wallis) was used. The pairwise comparisons following analysis of variance were executed using the *t*-test with pooled or separate variances. The time that beetles were kept in the jars was used as a rational variable in test Spearman rank correlation analysis to determine correlations between the time and the population parameters of interest.

3. Results

3.1. *Monochamus galloprovincialis*

M. galloprovincialis were collected from the different chip treatments as follows:

I (2 callow and 9 emerged beetles),
II (1 callow),
III (3 callow and 1 emerged),
IV (no beetles) and V (5 emerged).

According to the analysis of variance the chips in different room treatments (I, II, III) did not differ in the parameter values under comparison. Thus, all the room temperature cases were combined.

The sex of the beetle did not have any significance in variations in numbers of L_{IV} in the beetles, total population number or the proportions and numbers of dispersal stages of *B. mucronatus* in the pine chips. Beetles were divided between different incubation time classes (jar incubations) in the following way: less than 101 hours (2 callow and 4 emerged beetles), 101–200 hours (3 callow and 5 emerged) and 201–300 hours (1 callow and 6 emerged). Incubation time, however, did not contribute to variation in population parameters under study, thereby offering a chance to combine the data over the incubation hours.

The total population number of *B. mucronatus* in the chips was not affected by the presence of either callow or emerged adult beetles (Table 1). Similarly, the number of L_{III} was not influenced

Table 1. Occurrence of *Bursaphelenchus mucronatus* in Scots pine wood chips (ind. per gram dry wood, mean and range) and beetle adults following the exposure of the chips to the presence of adult beetles. TOT = total population number; L_{III} = third dispersal stage; L_{IV} = fourth dispersal stage; % = percentage of a given developmental stage in the population. — * statistically significant at 5% level when compared to the corresponding control group (chips without beetle presence). Room treatment chips were constantly incubated at room temperature while cold treatment chips experienced a few days exposure to 3°C.

Beetle species	TOT	$L_{IIIchips}$	$L_{III\%chips}$	$L_{IVchips}$	$L_{IV\%chips}$	$L_{IVbeetle}$	*n*
M. galloprovincialis							
Cold treatment chips							
Emerged	41 (9–129)	22 (2–77)	43 (26–60)	3 (0–8)*	8 (3–14)*	–	5
Control	64 (4–208)	32 (3–160)	43 (15–77)	0.3 (0–2)	0.4 (0–2)	–	16
Room treatment chips							
Callow	124 (48–273)	39 (15–116)	33 (14–46)*	57 (3–110)*	40 (6–64)*	186 (0–985)	6
Emerged	103 (43–300)	51 (22–167)	48 (31–80)	14 (0–103)	11 (0–54)*	5 (0–40)	10
A. aedilis	86 (18–169)	50 (7–112)	52 (31–81)	6 (0–27)	6 (0–18)	–	21
R. inquisitor	49* (9–140)	22 (4–77)	38 (17–55)	1 (0–5)	5 (0–32)	–	11
H. abietis	127 (35–240)	76 (29–166)	67 (34–91)	3 (0–8)	2 (0–6)	–	7
Control, all beetles	94 (4–352)	55 (3–338)	52 (15–96)	2 (0–21)	2 (0–19)	–	57

by the presence of an adult beetle, whereas the presence of the latter significantly contributed to an increase in the number of L_{IV} in the chips. The proportion of L_{III} in the chips was not affected by the presence of emerged beetles but in the presence of callow beetles the percentage decreased (room treatment chips). With callow beetles the proportion of L_{IV} was conclusively higher in chips with the beetle than in control chips. The same situation could be seen with emerged beetles in both chip incubation treatments, although the difference was not as pronounced.

Adults that had prematurely been removed from their pupal chambers (callow beetles) contained more L_{IV} than old mature beetles that had emerged on their own (Table 1) (Kruskal-Wallis test statistic = 4.80, P = 0.05*). Similarly, the percentage and number of L_{IV} in the chips were higher in jars with callow beetles (Table 1) (P = 0.01** and 0.05*, respectively).

The frequency of beetle infestation with L_{IV} upon the removal of the beetles from the jars was 44% (n = 16, callow and emerged beetles combined) in the room treatment of the chips, whereas in the cold treatment of the chips none of the beetles became contaminated (n = 5, emerged beetles) (Table 1). None of the control beetles were infested (n = 32), confirming that beetles used in the study were initially free of *B. mucronatus*.

3.2. *Acanthocinus aedilis*

All the chips used originated from room treatment I. Sex did not cause variation in the variables under investigation, thereby allowing combinations of data to be made. The incubation time did correlate to some extent with certain variables such as the number of L_{III} per dry gram of wood (r = 0.51*) and the percentage of L_{III} (r = 0.51*). None of the beetles in the jars became infested with L_{IV} (Table 1). Similarly, all the control beetles were free of *B. mucronatus* L_{IV} (n = 21). However, some of the beetles did contain other unidentified nematode species.

Since the incubation time affected at least some of the variables, a separate one-way analysis of variance was performed on the data inside the different incubation time groups. In the first group (less than 101 hours) the beetle presence in the chip jars did not have any impact on the total population number or the numbers and proportions of dispersal stages in the chips (n = 5). A similar situation prevailed in the second group (101–200 hours) (n = 7). However, in the third group (201–300 hours) (n = 9) both the number and the percentage of L_{IV} was affected by the presence of the beetle. The number of L_{IV} (per dry gram of wood) in 'beetle chips' (mean = 12, range = 0–27) exceeded the one in chips without the beetle (mean = 2, range = 0–14) (P = 0.05*). Likewise, the proportion of L_{IV} was higher in 'beetle chips' (mean = 9.4%, range = 0–18) than in the chips without the beetle (mean = 2%, range = 0–7.5) (P = 0.05*).

3.3. *Rhagium inquisitor*

Only chips from the room treatment were used and the only incubation time class represented was 101–200 hours. There were more nematodes in control chips than in the 'beetle chips' (Table 1). None of the beetles in the jars became infested with L_{IV}. Similarly, all the control beetles were free of nematodes (n = 11). The presence of beetle adults in the chips did not have a clear impact on the nematode L_{IV} formation (Table 1).

3.4. *Hylobius abietis*

The chips used all originated from the room treatment and the incubation time regarding all the beetles exceeded 300 hours. The presence of the beetles did not have any impact on any of the variables under examination (Table 1). All the control beetles were free of *B. mucronatus* L_{IV} (n = 7) but they contained other unidentified nematode species.

4. Discussion

The only treatment where the total population number was affected by the presence of the adult beetles was with the species *Rhagium inquisitor*. Nematode mortality occurred in the chips when the beetles were retained among the chips. The reason for the decline in the population number remained unknown.

For the most part, neither the proportion nor the number of the third dispersal stage (L_{III}) was noted to be influenced by the presence of the beetle adults in the chips. This may have been at least partly due to a certain degree of inaccuracy associated with telling the L_{III} apart from the third propagative stage (L_3) using light microscopy of 400–1000 magnification. L_3 can also harbour high densities of granules in their intestinal cells under certain conditions (author unpubl.). However, in the presence of callow *Monochamus galloprovincialis* the proportion of L_{III} clearly decreased which may be indicative of the population preparing itself to be carried away to new feeding grounds inside their beetle vectors. This dynamic shift should coincide with a simultaneous increase in the proportion of L_{IV}. This, indeed, was clearly the case in the present study.

It has previously been shown that a culturing of *Bursaphelenchus xylophilus* in pine chips at low temperatures (3°C or 12°C) (cold treatment) followed by an extended exposure of the chips to high temperatures (27–30°C) will bring about formation of the fourth dispersal stage (L_{IV}) in chips (Tomminen et al. 1988, Tomminen & Akar 1990, Tomminen et al. 1991). It has also been suggested that the presence of the vector beetle is imperative in stimulating L_{IV} production in *B. xylophilus* populations (Ishibashi & Kondo 1977). In Ishibashi's and Kondo's study L_{III} moulted into L_{IV} in water incubated at 20°C when the nematodes were extracted from pupal chambers of *Monochamus alternatus* immediately following the removal of the pupae and adults of the beetles from the chambers. Ishibashi & Kondo proposed the insect hormones to play a vital role as a stimulus for L_{IV} generation.

In the present study it was clearly demonstrated that the presence of adults of *Monochamus galloprovincialis* and *Acanthocinus aedilis* in Scots pine chips containing a nematode *Bursaphelenchus mucronatus* increases both the number and the proportion of L_{IV} in the nematode populations. In the case of *A. aedilis* the event seemed to become more conspicuous over time as the only statistically significant result concerned beetles that had been kept in the pine chip jars longer than 200 hours. Beetle species of both genera, *Monochamus* (Mamiya & Enda 1972, Linit et al. 1983, Kobayashi et al. 1984, Luzzi et al. 1984) and *Acanthocinus* (Kobayashi et al. 1984), the former being the most prominent and primary vector genus of *B. xylophilus* in Japan (Mamiya & Enda 1972, Kobayashi et al. 1984) and the United States of America (Kondo et al. 1982, Linit et al. 1983) and of *B. mucronatus* in Finland (Tomminen et al. 1989, Tomminen 1990), have been found to carry L_{IV} of *B. xylophilus* in the field.

In the present study callow adults of *M. galloprovincialis* that were prematurely extracted from their pupal chambers in Scots pine logs appeared to be more efficient in stimulating the increase in proportion and number of L_{IV} of *B. mucronatus* in chips than older beetles that had been allowed to emerge from the logs on their own. Based on the information obtained in the present study it cannot be determined what kinds of factors may have been responsible for the differences in the L_{IV} formation in the chips between the two beetle age classes. If the insect hormones emitted by beetle adults govern the generation of L_{IV} as suggested by Ishibashi & Kondo (1977) then the result of this study indicates the production of given types of hormones of importance being more prolific in callow beetles. Since the larval stages of *Monochamus* do not stimulate *B. xylophilus* L_{III} to moult into L_{IV} (Ishibashi & Kondo 1977) the significance of CO_2 exhaled by different developmental stages of the beetles appears to be of minor importance.

The nematodes in the present study that were referred to as having experienced the cold treatment had been incubated at room temperature no longer than 5 months prior to the cold treatment. Consequently, the nematode populations may still have lived in an environment capable of sustaining nematode reproduction, rendering it unattractive for the propagative second stages (L_2) to moult in massive numbers into L_{III}. This was further supported by the tendency of the total population number to be higher in wood chips that had continuously been incubated at room temperature as compared to the chips with the cold treatment in their history of incubation. Apparently the cold treatment halted the potential reproduction which continued in the chips maintained constantly at room temperature. At the low temperature of the cold treatment (3°C) all the nematode activity is very likely reduced to a

minimum (Mamiya 1975). Since the previous studies have demonstrated cold treatment alone without the beetle presence affecting *B. xylophilus* populations by increasing the number and proportion of L_{IV}, both of these factors (beetle presence and cold treatment) seem to be involved in the process.

Although a similar increasing trend of the proportion of L_{IV} was seen in the chips with adults of *Rhagium inquisitor*, the effect was not as apparent as with the two other cerambycid beetles in the study. However, the presence of *Hylobius abietis* in the nematode-infested chips did not precipitate any variation in the proportions and numbers of the dispersal stages of *B. mucronatus*. As the significance of these species as vectors of *B. mucronatus* seem to be of minor importance (Tomminen 1990) the result is not unexpected.

Adults of *M. galloprovincialis* were the only beetles that became infested with *B. mucronatus* L_{IV} in this study. The average number of L_{IV} was higher in callow adults than in emerged beetles. Under natural conditions, beetle adults of *Monochamus* linger inside their pupal chambers for several days prior to their emergence (Saalas 1923, 1949, Pershing & Linit 1986, Linit 1988). Similarly, Pershing & Linit (1986) have observed that immediately after eclosion of *Monochamus carolinensis* (Olivier) reared on an artificial diet in the laboratory the adult's integument is soft and light in colour. According to Pershing & Linit, adult activity is not initiated until after several days during which the exoskeleton sclerotizes. The sclerotization may play a role in making it less attractive (chemical effect) or more difficult (structural effect) for the nematodes to enter the beetle's tracheae. However, the higher numbers of L_{IV} in the callow beetles may simply have been a direct result of the higher than average density of L_{IV} in the chips with callow beetles. Thus, the age of the beetle per se may not be so crucial in determining the prospective infestation level of the beetles but rather it might affect the initial density of L_{IV} in the chips and in so doing indirectly contribute to high numbers of L_{IV} in the beetle's tracheae. There are still other factors that may play a role, such as the type of insect hormone emitted by a given beetle (chemical determinant), the amount of CO_2 exhaled by the beetle (chemical determinant), and the anatomical structure of the spiracles or tracheae of the beetle (structural determinant). It has been demonstrated that CO_2 attracts the *B. xylophilus* L_{IV} to move into the tracheae of *M. alternatus* (Miyazaki et al. 1978a,b, Ikeda et al. 1981) and some unsaturated fatty acids isolated from the beetles, their excretions and the pupal cell wall seem to cause *B. xylophilus* aggregation to the pupal cell of the sawyer (Miyazaki et al. 1977a, b, Ikeda et al. 1981). It has also been shown that homogenates of, and lipid extracts from, 4th instar larvae of *Monochamus carolinensis* possessed qualities of an attractive nature to *B. xylophilus* from Scots pine (Bolla et al. 1989). Carbon dioxide has been shown to aid in the host finding of the infective stage juveniles of *Neoaplectana carpocapsae* Weiser (Gaugler et al. 1980). Furthermore, the dauer larvae of *N. carpocapsae* have been found to exhibit strong attraction of a chemical nature to the larvae or faeces of several insects (Schmidt & All 1978, 1979). In a study by Tomminen & Akar (1990) emerged *Monochamus scutellatus* (Say) pine sawyer adults became infested with L_{IV} of *B. xylophilus* when the beetles were kept in glass jars with nematode-infested pine chips (mean = 21, $n = 8$). In the same study *Rhagium inquisitor*, *Asemum striatum* L. (Cerambycidae) and *Hylobius pales* (Herbst) also became infested with L_{IV} of *B. xylophilus* but the averages were very low. Linit et al. (1983) found L_{IV} of *B. xylophilus* from field-collected *H. pales*, the highest numbers per beetle not exceeding 300. Their observation provides sound evidence that under certain circumstances this curculionid is also capable of obtaining L_{IV} in its tracheae.

Since the origin and incubation history of the pine chips used in the study were highly variable between the beetle species no definite comparisons could be made between the species. At any rate, the results do indicate *M. galloprovincialis* as being of the greatest significance among the beetle species tested in the regulation of *B. mucronatus* dispersal stages in the chips. The fact that *A. aedilis* also had an impact on the proportion L_{IV} in the chips does indicate the species' potential as a carrier of *B. mucronatus*, regardless of the negative result in respect of the contamination of the beetle itself. Callow adults were not tested and had they been included in the study the result may have been different.

The results of the present study invariably support the idea of the pine sawyer genus *Monochamus* being the most important beetle vector group for *B. mucronatus* in Finland. The role of the other beetles in the study as vectors for the nematode seems to be of minor importance. It should be remembered, however, that all the other beetles tested were mature and the inclusion of callow adults may have enhanced the chance of obtaining higher densities of L_{IV}. The observation of field-collected *H. pales* adults being infested with L_{IV} of *B. xylophilus* in the United States (Linit et al. 1983) may well be explained by the fact that those beetles almost without a doubt had come into contact with the nematodes inside their pupal chambers prior to their emergence. Thus, the curculionids were still callow soft-bodied adults at the time of the encounter. Even if *A. aedilis* and *R. inquisitor* do not act as noteworthy vectors for B. mucronatus in the field, these cerambycids do exhibit qualities making them potential candidates in spreading the nematode from contaminated pine chips closer to the oviposition points of the beetles.

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References

Bolla, J. A., Bramble, J. & Bolla, R. I. 1989: Attraction of Bursaphelenchus xylophilus pathotype MPSy-1, to Monochamus carolinensis larvae. — Jap. J. Nematol. 19(12):32–37.

Carling, D. E. 1984: Some insect associates of the pinewood nematode in eastern Virginia. — Can. J. Forest Res. 14:826–829.

De Guiran, G. & Boulbria, A. 1986: Le nèmatode des pins. Caractèristiques de la souche française et risque d'introduction et d'extension de Bursaphelenchus xylophilus en Europe. — Bulletin OEPP/EPPO Bulletin 16:445–452.

Dropkin, V. H., Foudin, A., Kondo, E., Linit, M., Smith, M. & Robbins, K. 1981: Pinewood nematode: A threat to U.S. forests? — Plant Disease 65(12):1022–1027.

Dropkin, V. H. & Linit, M. 1982: Pine wilt — a disease you should know. — J. Arboriculture 8(1):1–6.

Gaugler, R., LeBeck, L., Nakagaki, B. & Boush, G. M. 1980: Orientation of the entomogenous nematode Neoaplectana carpocapsae to carbon dioxide. — Environ. Entomol. 9:649–652.

Ikeda, T., Miyazaki, M., Oda, K., Yamane, A. & Enda, N. 1981: The chemical ecology of Monochamus alternatus on the relationship with pine wood nematodes and host trees. — XVII IUFRO World Congress, Japan. Division 2, Proceedings.

Ishibashi, N. & Kondo, E. 1977: Occurrence and survival of the dispersal forms of pine wood nematode, Bursaphelenchus lignicolus Mamiya and Kiyohara. — Appl. Entomol. Zool. 12(4):293–302.

Kinn, D. N. 1986: Incidence of pinewood nematode dauerlarvae and phoretic mites associated with long-horned beetles in central Louisiana. — Can. J. Forest Res. 17:187–190.

Kobayashi, F., Yamane, A. & Ikeda, T. 1984: The Japanese pine sawyer beetles as the vector of pine wilt disease. — Ann. Rev. Entomol. 29:115–135.

Kondo, E. & Ishibashi, N. 1978: Ultrastructural differences between the propagative and dispersal forms in pine wood nematode, Bursaphelenchus lignicolus, with reference to the survival. — Appl. Entomol. Zool. 13(1):1–11.

Kondo, E., Foudin, A. S., Linit, M. J., Smith, M. T., Bolla, R., Winter, R. E. K. & Dropkin, V. H. 1982: Pine wilt disease: nematological, entomological, and biochemical investigation. — Agric. Exp. Stn., Univ. Mo. 58 pp.

Linit, M. J. 1988: Nematode-vector relationships in the pine wilt disease system. — J. Nematol. 20(2):227–235.

Linit, M. J., Kondo, E. & Smith M. T. 1983: Insects associated with the pinewood nematode, Bursaphelenchus xylophilus (Nematoda: Aphelenchoididae), in Missouri. — Environ. Entomol. 12:467–470.

Luzzi, M. A., Wilkinson, R. C. & Tarjan, A. C. 1984: Transmission of the pine wood nematode Bursaphelenchus xylophilus, to slash pine trees and log bolts by a cerambycid beetle, Monochamus titillator, in Florida. — J. Nematol. 16(1):37–40.

Magnusson, C. & Schroeder, M. 1989: First record of a Bursaphelenchus-species (Nematoda) from Monochamus beetles in Scandinavia. — Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz 63:53–54.

Mamiya, Y. 1975: The life history of the pine wood nematode, Bursaphelenchus liqnicolus. (In Japanes with English summary) — Jap. J. Nematol. 5:16–25.

— 1983: Pathology of the pine wilt disease caused by Bursaphelenchus xylophilus. — Ann. Rev. Phytopathol. 21:201–220.

— 1984: The pine wood nematode. — In: Nickle, W. R. (ed.), Plant and insect nematodes: 589–626. Marcel Dekker Inc. New York.

— 1987: Origin of the pine wood nematode and its distribution outside the United States. — In: Wingfield, M. J. (ed.), Pathogenicity of the pine wood nematode:

59–65. APS Press, The American Phytopathological Society.

Mamiya, Y. & Enda, N. 1972: Transmission of Bursaphelenchus lignicolus (Nematoda: Aphelenchoididae) by Monochamus alternatus (Coleoptera: Cerambycidae). — Nematologica 18:159–162.

— 1979: Bursaphelenchus mucronatus n. sp. (Nematoda: Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. — Nematologica 25:353–361.

McNamara, D. G. & Støen, M. 1988: A survey for Bursaphelenchus spp. in pine forests in Norway. — Bulletin OEPP/EPPO Bulletin 18:353–356.

Miyazaki, M., Oda, Ka. & Yamaguchi, A. 1977a: Behavior of Bursaphelenchus lignicolus toward unsaturated fatty acids. — J. Jap. Wood Res. Soc. 23:255–261.

— 1977b: Deposit of fatty acids in the wall of pupal chamber made by Monochamus alternatus. — J. Jap. Wood Res. Soc. 23:307–311.

Miyazaki, M., Yamaguchi, A. & Oda, K. 1978a: Behavior of Bursaphelenchus liqnicolus in response to carbon dioxide. — J. Jap. Wood Res. Soc. 60:203–208.

— 1978b: Behavior of Bursaphelenchus liqnicolus in response to carbon dioxide released by respiration of Monochamus alternatus pupae. — J. Jap. Forest Soc. 60:249–254.

Pershing, J. C. & Linit, M. J. 1986: Biology of Monochamus carolinensis (Coleoptera: Cerambycidae) on Scotch pine in Missouri. — J. Kansas Entomol. Soc. 59(4):706–711.

Saalas, U. 1923: Die Fichtenkäfer Finnlands. Studien über die Entwicklungsstadien, Lebenweise und geographische Verbreitung der an Picea excelsa Link. lebenden Coleopteran nebst einer Larvenbestimmungstabelle. — Ann. Acad. Sci. Fennicae, A 22. 746 p.

— 1949: Suomen metsähyönteiset. — Werner Söderström Osakeyhtiö, Porvoo. Helsinki. 719 pp.

Schmidt, J. & All, J. N. 1978: Chemical attraction of Neoaplectana carpocapsae (Nematoda: Steinernematidae) to insect larvae. — Environ. Entomol. 7:605–607.

— 1979: Attraction of Neoaplectana carpocapsae (Nematoda: Steinernematidae) to common excretory products of insects. — Environ. Entomol. 8:55–61.

Tamura, H. 1984: Distribution of Bursaphelenchus xylophilus and B. mucronatus and tissue reaction in the inoculated branches of pine trees. — Abstracts of papers. First Int. Congr. Nematology. Guelph, Ontario, Canada, August 5–10.

Tomminen, J. 1990: Presence of Bursaphelenchus mucronatus (Nematoda: Aphelenchoididae) fourth dispersal stages in selected conifer beetles in Finland. — Silva Fennica 24(3):273–278.

Tomminen, J. & Akar, H. 1990: Infestation of four coleopteran species by the pinewood nematode Bursaphelenchus xylophilus (Nematoda, Aphelenchoididae) living in wood chips. — Entomol. Fennica 1:171–174.

Tomminen, J., Halik, S. & Bergdahl, D. R. 1988: Dauerlarvae of Bursaphelenchus xylophilus formed in wood chips of Pinus strobus. — E.S.N. XIXth Int. Nematology Symp. Uppsala, Sweden, 7–13 August 1988. Abstracts. Nematologica 1988, 34:298.

— 1991: Effects of temperature and incubation on occurrence of different life stages of Bursaphelenchus xylophilus in wood chips. — J. Nematology 23(4):477–484.

Tomminen, J., Nuorteva, M., Pulkkinen M. & Väkevä, J. 1989: Occurrence of the nematode Bursaphelenchus mucronatus Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland. — Silva Fennica 23(4):271–277.

Wingfield, M. J., Blanchette, R. A., Nicholls, T. H. & Robbins, K. 1982: The pine wood nematode: a comparison of the situation in the United States and Japan. — Can. J. Forest Res. 12:71–75.

Wingfield, M. J. & Blanchette, R. A. 1983: The pine-wood nematode, Bursaphelenchus xylophilus, in Minnesota and Wisconsin: insect associates and transmission studies. — Can. J. Forest Res. 13:1068–1076.

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