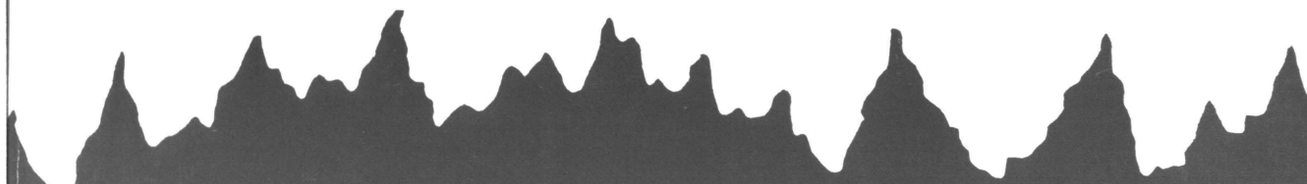
SILVA FENNICA

1990 · Vol. 24 N:o 3



SUOMEN METSÄTIETEELLINEN SEURA
SOCIETY OF FORESTRY IN FINLAND

SILVA FENNICA

A quarterly journal of forest science

PUBLISHER – JULKAISIJA

The Society of Forestry in Finland
Suomen Metsätieteellinen Seura r.y.

EDITORS – TOIMITUS

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Tilaukset ja tiedustelut pyydetään osoittamaan toimitukselle. Silva Fennican tilaushinta kotimaahan on 180 mk, ulkomaille 250 mk. Seuran jäsenille lehti jaetaan jäsenmaksua vastaan.

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Presence of *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoididae) fourth dispersal stages in selected conifer beetles in Finland

Jyrki Tomminen

TIIVISTELMÄ: BURSAPHELENCHUS MUCRONATUS-ANKEROISEN (NEMATODA: APHELENCHOIDIDAE) KESTOTOUKKIEN ESIINTYMINEN HAVUPUUKUORIAISISSA SUOMESSA

Tomminen, J. 1990. Presence of *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoididae) fourth dispersal stages in selected conifer beetles in Finland. Tiivistelmä: *Bursaphelenchus mucronatus* -ankeroisen (Nematoda: Aphelenchoididae) kestotoukkien esiintyminen havupuukuoriaisissa Suomessa. Silva Fennica 24(3): 273–278.

Fourth dispersal stages (dauerlarvae) of the nematode *Bursaphelenchus mucronatus* were surveyed in Finland during summers 1989 and 1990 examining field collected conifer beetle (Coleoptera) adults of the following species: *Monochamus sutor, M. galloprovincialis, Acanthocinus aedilis, Rhagium inquisitor, Asemum striatum, Spondylis buprestoides* and *Hylobius abietis*. All but the last one (Curculionidae) belong to genus Cerambycidae. The two *Monochamus* species were the only ones carrying *B. mucronatus* fourth dispersal stages, total numbers of nematode larvae per beetle being higher in *M. galloprovincialis*. The frequency of infestation was 24 % in *M. galloprovincialis* and 14 % in *M. sutor* but the difference was not statistically significant.

Tutkimuksessa selvitettiin *Bursaphelenchus mucronatus* -ankeroisen kestotoukkien esiintymistä suomalaisissa havupuukovakuoriaisissa (Coleoptera). Maastosta kerättiin seuraavien lajien aikuisia kesinä 1989 ja 1990: *Monochamus sutor, M. galloprovincialis, Acanthocinus aedilis, Rhagium inquisitor, Asemum striatum, Spondylis buprestoides* ja *Hylobius abietis*. Kaikki muut lajit paitsi vimeiseksi mainittu (Curculionidae) kuuluvat jäärien heimoon Cerambycidae. Ainoastaan *Monochamus* -suvun tukkijäärälajeista löytyi *B. mucronatus* -ankeroisen kestotoukkia. Kestotoukkamäärät kovakuoriaisaikuista kohti olivat korkeampia lajilla *M. galloprovincialis*. 24 % *M. galloprovincialis*-yksilöistä ja 14 % *M. sutor*-yksilöistä sisälsi kestotuokkia. Ero ei kuitenkaan ollut tilastollisesti merkittävä.

Keywords: *Bursaphelenchus mucronatus*, vectors, conifer beetles, Finland.
ODC 142+145.7

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Accepted October 18, 1990

1. Introduction

Several surveys have been conducted in recent years to locate the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970 (Nematoda: Aphelenchoididae) in coniferous forest in Europe (DeGuiran and Boulbria 1986, McNamara and Støen 1988, Tomiczek 1988, Magnusson and Schroeder 1989, Tomminen et al. 1989). *B. xylophilus* has not been detected in any of the surveys but another nematode species of the same genus, *B. mucronatus* Mamiya & Enda 1979, has been found to be widely distributed in European pine forests. In addition, it has also been found from Norway spruce in Finland (Tomminen et al. 1989).

Yet a third species, *B. fraudulentus* Rühm 1956 has been found from some broad-leaved trees (Rühm 1956, Schauer-Blume 1987, Schauer-Blume and Sturhan 1989). Neither of these nematode species has been associated with tree mortalities as a primary pathogen in Europe.

In Norway, Sweden and Finland *Monochamus* pine sawyers (Coleoptera: Cerambycidae) have been associated with the occurrence of *B. mucronatus* (McNamara and Støen 1988, Magnusson and Schroeder 1989, Tomminen et al. 1989). In Norway the nematode was found from cut logs containing *Monochamus* larval bore holes. In Sweden and Finland, fourth dispersal stages (dauerlarvae) were extracted from laboratory raised and from field collected pine sawyer adults, respectively.

The objective of this study was to increase the knowledge on the occurrence of *B. mucronatus* dauerlarvae in selected conifer beetle species in Finnish forests. At the same time it was attempted to verify the collecting results of the summer 1988 with regard to the absence of the pinewood nematode, *B. xylophilus,* in Finnish forests (Tomminen et al. 1989).

I wish to thank Miss Hannele Kettunen, Miss Pirjo Leppänen and Mr. Chris Olson for technical assistance in the field and in the laboratory, and Professors Erkki Annila and Matti Nuorteva for valuable comments on the manuscript. The study was funded by the University of Helsinki, the Academy of Finland and the Research Foundation of Finnish Natural Resources.

2. Materials and methods

Potential vector species were collected in the field during their swarming period in spring and summer 1989 and 1990 in Finland to study their frequency of infestation by *B. mucronatus* dauerlarvae (or *B. xylophilus* dauerlarvae). The species under investigation were: *Monochamus sutor* L., *M. galloprovincialis* Ol., *Acanthocinus aedilis* L., *Rhagium inquisitor* L., *Asemum striatum* L., *Spondylis buprestoides* L. and *Hylobius abietis* L.. All except the last species (Curculionidae) belong to family Cerambycidae.

The beetles were collected as adults in several locations throughout the country mainly from fresh pine and spruce logs where they had landed to mate and oviposit. Individuals of each species were also captured one by one whenever met during the search in the forests. The collection sites for *Monochamus* species are indicated in Figure 1. *Monochamus* pine sawyer adults were all collected in 1989. The main collection sites (1989 and 1990) for *A. aedilis, R. inquisitor* and *A. striatum* were as follows: Mäntsälä, 60°37'N, 25°17'E; Monninkylä, 60°29'N, 25°32'E; Savijoki, 60°40'N, 25°33'E; Vesivehmaa, 61°09'N, 25°42'E. The collection site for *S. buprestoides* was Tuohikotti (61°05'N, 27°03'E) (1989). Majority of the individuals of *H. abietis* were collected from sawdust piles during their peak swarming time early June 1989 in sawmills located in southern and central Finland (Vierumäki, 61°09'N, 25°52'E; Mäntyharju, 61°25'N, 26°53'E, Tuukkala, 61°43'N, 26°35'E).

All the beetles (Table 1) were dissected in the laboratory, wrapped in tissue paper and placed in small vials filled with water for extraction of the nematodes. Extraction time was 24 hours after which the dissected beetles were removed from the vials and discarded. The water extraction was examined with a light microscope for possible nematodes. If nematodes resembling *B. mucronatus* and *B. xylophilus* dauerlarvae (cannot be told apart by appearance) were encountered they were counted and a portion of them was injected on either *Botrytis cinerea* petri dishes or on *Pinus sylvestris* wood chips. After an incubation at 23–28°C the cultures were examined for adult nematodes to confirm the identification.

Kruskal-Wallis one-way analysis of variance was used to determine statistical differences in the numbers of *B. mucronatus* dauerlarvae in different beetle species and also to test differences in geographic distribution of dauerlarval infestation of *Monochamus* pine sawyer adults. To compare the frequency of dauerlarval infestation of the two *Monochamus* species the Yates corrected chisquare test of independence was used.

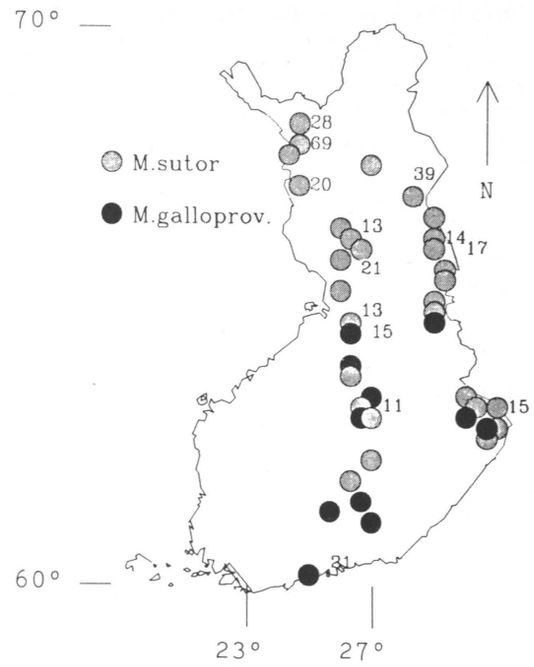

Fig. 1. Collection sites of *Monochamus sutor* and *M. galloprovincialis* in 1989 in Finland. Individual numbers exceeding 10 per collection site are shown.

3. Results

B. mucronatus fourth dispersal stages were only found in *Monochamus* pine sawyers (Table 1). Dauerlarvae of the pinewood nematode, *B. xylophilus,* were not found in any of the beetles examined. From the table it can be calculated that the frequency of infestation of *B. mucronatus* dauerlarvae was 14 % for *M. sutor* and 24 % for *M. galloprovincialis*. The difference in the frequency of infestation was not statistically significant (Yates corrected chisquare = 3.19, Df = 1).

The total numbers of dauerlarvae per beetle were significantly higher in *M. galloprovincialis* at 0.05 % significance level (Kruskal-Wallis one-way analysis of variance). The maximum numbers of *B. mucronatus* dauerlarvae per beetle for *M. sutor* and *M. galloprovincialis* were 1325 and 1500, respectively.

Figure 1 shows the collection sites of the two *Monochamus* pine sawyers with individual numbers of beetles indicated when exceeding 10 per collection site. There was no statistical difference in the geographic distribution of dauerlarval infestation of either one of the *Monochamus* species.

None of the other nematode species found from the beetles (Table 1) were identified. However, a great majority were stylet-bearing nematodes except the ones that were recovered from *H. abietis* which almost without an exception consisted of a rhabditid species (Rhabditida).

Table 1. Occurrence of *Bursaphelenchus mucronatus* fourth dispersal stages in selected conifer beetles in Finland. (For collecting data refer to Materials and methods).

Species	Number of beetles (checked/infested)	*B. mucronatus* dauerlarvae in infested beetles (mean/st.dev.)	Other nematodes
Monochamus sutor	336/47	116/242	–
M. galloprovincialis	63/15	169/392	–
Acanthocinus aedilis	135/–		+
Rhagium inquisitor	83/–		+
Asemum striatum	31/–		+
Spondylis buprestoides	12/–		+
Hylobius abietis	195/–		+

4. Discussion

Monochamus pine sawyers serve as primary vectors for *B. xylophilus* in Japan (Mamiya and Kiyohara 1972, Kobayashi et al. 1984) and the United States of America (Linit et al. 1983). The average numbers of *B. xylophilus* dauerlarvae per vector beetle have ranged from 15 000 to 19 000, respectively. Beetle species of other genera from Cerambycidae and also from other families (Buprestidae, Curculionidae) have also been found to carry *B. xylophilus* dauerlarvae, though the numbers per beetle have been considerably lower (Linit et al. 1983, Kobayashi et al. 1984). In the United States such species as *Hylobius pales* and *Pissodes approximatus* (Curculionidae) and *Asemum striatum* and *Arhopalus rusticus* (Cerambycidae) have been found carrying *B. xylophilus* the density of the dauerlarvae per beetle varying between 10–298 (Linit et al. 1983).

In the present study *Monochamus* pine sawyers proved to be the primary carriers of *B. mucronatus* dauerlarvae in Finland. In fact, they were the only species with infestations among the beetle special studied. *B. xylophilus* dauerlarvae were not encountered. In Japan, where *B. mucronatus* partly coexists with *B. xylophilus, Monochamus* pine sawyers act as vectors for *B. mucronatus*, too (Mamiya and Enda 1979). However, although none of the other beetles turned positive with regard to *B. mucronatus* dauerlarval infestation it does not exclude the possibility of detecting positive cases when sample size is increased. In a study by Tomminen and Akar (1990) it has been shown that *Rhagium inquisitor* and *Asemum striatum* adults become infested by *B. xylophilus* dauerlarvae when kept in close contact with *B. xylophilus* contaminated wood chips.

The fact that all beetles species without infestation of *B. mucronatus* dauerlarvae did carry other nematode species (Table 1) the reverse situation being true with the two *Monochamus* species may suggest competition between different nematode species in inhabiting phoretically beetle adults. However, further studies would be necessary to verify such a relationship. It is well-known that individual bark beetles can be infected with more than one genus or species of parasitic nematodes (Massey 1974).

The average densities of dauerlarvae per beetle were not nearly as high as the densities of *B. xylophilus* dauerlarvae in their primary vectors in Japan and the United States. *M. galloprovincialis* appeared to carry higher numbers of dauerlarvae in average than *M. sutor*. The former species is strictly a pine species whereas the latter reproduces more frequently on spruce (Saalas 1923, 1949, author unpublished). In the light of this the higher numbers in *M. galloprovincialis* may reflect the nematode's preference in host species Scots pine being more attractive than Norway spruce. On the other hand, since the distribution of these two pine sawyer species differs, *M. sutor* being distinctly more common in eastern and northern parts of the country as compared to more southerly distribution of *M. galloprovincialis* (Figure 1), there may be other factors involved such climatic factors, for instance.

In Sweden in Magnusson's and Schroeder's study (1989) the encountered *B. mucronatus* dauerlarva numbers per beetle were high the maximums being 13 000 and 29 400 for *M. sutor* and *M. galloprovincialis*, respectively. Magnusson's and Schroeder's beetles were not field collected adults, however, but adults that emerged in laboratory from field collected pine logs. The conditions during the storage in the laboratory must not have been identical to field conditions but more likely had been conducive to nematode reproduction affecting the eventual numbers of dauerlarvae in the logs. Schroeder and Magnusson (1989) have demonstrated *M. sutor*'s capability to serve as a vector for *B. mucronatus*. In their laboratory studies *B. mucronatus* was transferred to small pieces of fresh Scots pine and Norway spruce branches during pine sawyer adults maturation feeding and to Scots pine and Norway spruce logs during beetles oviposition.

The present survey results confirm the results of the summer 1988 survey in Finland (Tomminen et al. 1989) with regard to the occurrence of the two *Bursaphelenchus* species in Finnish conifer forests. *B. mucronatus* is widely distributed in both pine and spruce stands and is very likely vectored by two *Monochamus* species, *M. sutor* and *M. galloprovincialis* in Finland whereas *B. xylophilus* does seem to be absent in Finland.

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