Silva Fennica 1989, Vol. 23 N:o 4:271–277

Occurrence of the nematode *Bursaphelenchus mucronatus* Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland

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TIIVISTELMÄ: BURSAPHELENCHUS MUCRONATUS MAMIYA & ENDA 1979 (NEMATODA: APHELENCHOIDIDAE) ANKEROISEN ESIINTYMINEN SUOMESSA

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

A survey was conducted in Finland in 1988 to determine whether the pine wood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) or the closely related species *B. mucronatus* would occur in Finnish forests. Dead or dying standing trees and timber of two conifer species, *Pinus sylvestris* (Scots pine) and *Picea abies* (Norway spruce) were analyzed for the presence of these nematodes. *Monochamus* spp. pine sawyers (Coleoptera:Cerambycidae) were also collected and inspected for the presence of dispersal fourth juvenile stages (dauerlarvae) of the nematodes. The species *B. xylophilus* was not found, but *B. mucronatus* appeared to be widespread in the country. Individuals of this nematode were found both from Scots pine and Norway spruce. Adults of two *Monochamus* species were found, *M. galloprovincialis* and *M. sutor*. Only two of the examined beetles of the former species had dauerlarvae in or on their body.

Vuonna 1988 Suomen metsissä suoritettiin näytteiden keruu, jonka tarkoituksena oli selvittää esiintyykö mäntyankeroista, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) tai sen lähisukulaislajia, *B. mucronatus* maassamme. Näytteitä otettiin kuolleista tai kituvista männyn (*Pinus sylvestris*) ja kuusen (*Picea abies*) pystypuista sekä tukkipuutavarasta. Näytteiden oton yhteydessä kerättiin myös tukkijääräaikuisia (*Monochamus* spp.) (Coleoptera: Cerambycidae), joista etsittiin näiden ankeroisten kestotoukka-asteita. Lajia *B. xylophilus* ei tutkimuksessa löytynyt, mutta laji *B. mucronatus* osoittautui laajalti levinneeksi maassamme. Tämän lajin yksilöitä eristettiin sekä männyltä että kuuselta. *Monochamus*- tukkijääriä löytyi kahta eri lajia, *M. galloprovincialis* ja *M. sutor*. Ainoastaan kaksi tarkastetuista jääräaikuisista (edellinen laji) sisälsi ankeroisen kestotoukka-asteita.

Keywords: nematodes, pine, wood, surveys, Finland, *Bursaphelenchus xylophilus, B. mucronatus, Monochamus galloprovincialis, M. sutor, Pinus sylvestris, Picea abies.*
ODC 142+15

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Accepted September 20, 1989

1. Introduction

Pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Bührer 1934) Nickle 1970, has been involved in epidemics of pine wilt disease in Japan for many years (Mamiya 1984). The distribution of another species of the same genus, *B. mucronatus* Mamiya & Enda 1979 (BM), overlaps that of PWN to some extent (Mamiya & Enda 1979). However, the latter can be found from regions where PWN appears to be absent, i.e. areas with higher elevations. The biology of this species seems to be identical to that of pine wood nematode. BM has not been associated with tree mortalities. Both of these nematodes are spread by *Monochamus beetles (Cerambycidae) M. alternatus* Hops. being the most effective vector in Japan (Mamiya & Enda 1979, Kobayashi et al. 1984). The transmission of the dispersal fourth juvenile stages of these nematodes (dauerlarvae) occurs either during the maturation feeding of adult beetles or during the beetle's oviposition (Mamiya & Enda 1979, Wingfield & Blanchette 1983, Luzzi et al. 1984).

PWN has been killing some exotic pine species in the U.S.A. including *Pinus sylvestris L.*, but the native species appear to be more or less resistant (Dropkin & Foudin 1979, Nickle 1981, Malek & Appleby 1984). Several inoculation experiments conducted both in the field and in greenhouses have also shown PWN to be pathogenic to Scots pine (Bedker & Blanchette 1987, Linit & Tamura 1987).

PWN is known to occur also in Canada but has not been observed to cause pine wilt disease or related tree mortality. The nematode has only been found from trees which are dying or dead due to other reasons (Knowles et al. 1983, Pritam Singh, Forestry Canada, personal communication). There are several species of *Monochamus* in North America that have been found carrying the dauerlarvae of PWN (Dropkin et al. 1981, Linit et al. 1983, Wingfield & Blanchette 1983).

Concern about PWN in Finland arose after its detection in wood chips imported for the Finnish pulp industry in 1984 (Rautapää 1986).

Detailed information on the occurrence and the geographical distribution of PWN and BM was lacking and, hence in 1988 a large survey was conducted in Finland in order to determine whether PWN or its close relative BM would occur in Finnish conifer forests.

According to the literature there are three species of *Monochamus* occurring in Finland – *M. galloprovincialis* (Oliv.), *M. sutor* (L.) and *M. urussovi* (Fisch.) (=Rosenmülleri) (Saalas 1949). A second objective of the survey was then to investigate the presence of *Bursaphelenchus* spp. in Finnish pine sawyers.

The survey was funded by the Academy of Finland.

2. Materials and methods

Samples were cut both from dying and dead but still standing trees and cut timber of Scots pine and Norway spruce. Knowing that *Monochamus* pine sawyers would most likely serve as primary vectors for these nematodes the main emphasis was put on collecting wood samples from trees and timber infested with larval stages of these beetles.

Standing trees were felled and disks of 5–10 centimeters in thickness were cut out of the main stem at 1–3 m intervals. Similar disks were sawn out of timber containing larval galleries of pine sawyers. A total of 31 standing trees (all pines) and 44 roundwood timber (26 pines and 18 spruces) were sampled.

The disks were brought into the laboratory where a portion of them were extracted immediately using a modified Baermann funnel technique. However, the majority of the disks were first incubated at 25 C for a varied length of time prior to extraction to allow the nematode populations to build up to a detectable level. The wood collected at each sampling site was double-checked for the nematode after the first extraction round had been completed. All of the disks were cut in smaller pieces before the extraction.

During the survey an effort was made to collect adult pine sawyers for a nematode analysis. Between August 12 – September 5 a total of 80 adult *Monochamus* pine sawyers were collected (43 *M. galloprovincialis* and 37 *M. sutor*) when found feeding and ovipositing on fresh timber or windfallen trees. The numbers of individuals randomly chosen for nematode extraction were 30 and 25, respectively. Prior to the extraction the beetles were dissected. The extraction time for both the disks and the beetles was 48 hours.

The dauerlarvae found in the beetles were injected in a small amount of tap water into freshly cut healthy Scots pine bolts. The bolts were incubated at 25 °C for two weeks after which the bolts were sampled for nematode adults for identification.

Morphometric measurements were made on 53 females and 47 males of BM collected from Kuusankoski and Tuusula. These two sites were situated 130 km apart from each other in southern Finland. The specimens were killed by heat in a small amount of water on slides and the measurements were made immediately after the killing.

3. Results

The survey results appear in Figure 1 and Table 1. The positive findings of BM the number of sites being 19 (a total number of sites sampled 42), were almost invariably associated with *Monochamus* infested wood except for three cases where neither oviposition slits nor maturation feeding wounds of these beetles were detected. For one of the trees that came up negative with regard to pine sawyer beetles the nematodes were only extracted from the top portion of the stem. For the remaining two *Monochamus* negative trees the main extraction pattern was similar but there were also some nematodes recovered from the basal portion of the stem.

Table 1. The results of the finding of *Bursaphelenchus mucronatus* on the sample sites (Ps=*Pinus sylvestris;* Pa=*Picea abies;* T=timber; S=standing tree with wilted needles; W=windfall).

	Positive sample sites	Negative sample sites
Total number	19	23
Tree species	14Ps/7Pa	21Ps/2Pa
Condition of the tree	14T/6S/1W	13T/9S/1W
Signs of Monochamus	16	12

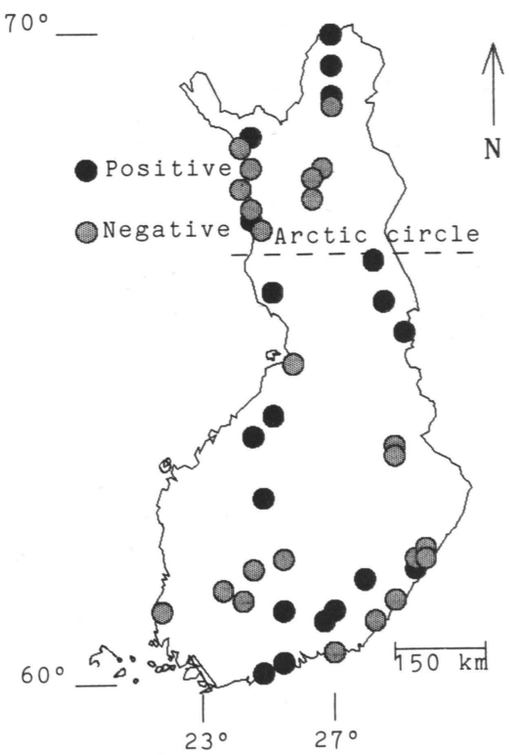

Fig. 1. The occurrence of the nematode *Bursaphelenchus mucronatus* in Finland.

The samples that yielded no nematodes were also relatively frequently infested with *Monochamus* juvenile stages. On as many as 12 out of 23 negative sampling sites the sampled wood was attacked by pine sawyer beetles either through feeding or ovipositioning. Occasionally these samples appeared to be heavily infested with *Monochamus* and yet they did not produce any nematodes of the species BM.

Since the incubation time preceding the extraction of the wood samples was highly variable the numbers of nematodes recovered from samples were not compared. However, they were recorded to obtain a general understanding of the numbers of nematodes inhabiting standing trees or timber in Finland. The highest number of BM (3.6 individuals/dry gram of wood) observed in the samples inspected immediately after the collection was for a sample collected from a recently died 17 m tall Scots pine in southern Finland (Tuusula). A suggested cause for the death of the tree was an infection of the root rot fungus *Heterobasidion annosum* (Fr.) Bref. The sample disk yielding this number of nematodes was cut at the height of 6 m along the stem. One of the authors had been collecting mating and ovipositing *M. galloprovincialis* adults from the same tree in 1986. At that time the tree still had green needles but the density of the crown was very sparse.

The overall highest number of BM (134 individuals/ dry gram of wood) detected was for a sample cut from a standing 17 m tall Scots pine in southern Finland (Lammi). However, the sample disk had been incubated at 25 °C for several weeks prior to the extraction. The tree was infested with the fungus *Endocronartium pini* (Pers.) Hiratsuka. There were no signs of *Monochamus* pine sawyers in this tree.

All of the pine sawyer adults were alive at the time of collection. The condition of each individual at the time of extraction was as follows: *M. galloprovincialis*: alive 8 (2 males and 6 females); recently died (7 males and 5 females); fungal growth covering the body (1 male and 3 females); *M. sutor*: alive (2 males and 4 females); recently died (11 males and 6 females); fungal growth covering the body (2 females).

Only two beetles appeared to have BM dauerlarvae in or on their body: one recently died *M. galloprovincialis* female (380 individuals) and one alive male of the same species (2 individuals). Thus, both the frequency as well as the total number of nematodes per beetle were very low.

The two southern populations of BM on which the measurements were made did not differ statistically significantly (BMDP t-test) in any of the variables under investigation and consequently the following data concerns of individuals of both populations.

Females (53):
L=0.77 mm (0.58-0.90); a=37.9 (26.9–51.1); b=9.7 (7.4–11.3); c=24.5 (16.9–30.9); V=71.9 (66.4–75.3); stylet=15.1 µm (11.3–17.3); mucro=4.3 µm (1.0–6.3).

Males (47):
L=0.74 mm (0.59–1.03); a=42.1 (24.8–56.0); b=9.6 (7.3–13.1); c=21.2 (16.7–29.4); stylet=14.8 µm (12.3–17.5); spicules=24.3 µm (17.5–30.0).

The shape of the bursa of the male was observed to be alike to the ones reported for BM by Mamiya and Enda 1979 and McNamara and Støen 1988, i.e. the bursa appeared to have two small projections on its terminal margin.

4. Discussion

BM has been reported in several European countries. The species of *Bursaphelenchus* reported from France 1978 (Scotto la Massese et al. 1978) has been shown to be much closer to BM than to PWN (DeGuiran & Boulbria 1986). This French population has turned out to possess some pathogenicity to local pine seedlings in greenhouse tests (DeGuiran & Boulbria 1985).

Yet another species of the same genus, morphologically identical to BM has been detected living in dead or dying deciduous trees, such as oak and cherry, in West Germany and Austria (Schauer-Blume 1987, Tomiczek 1988, Schauer-Blume & Sturhan 1989). The German population of this species has been identified as being of species *Bursaphelenchus fraudulentus* Rühm 1956 (Schauer-Blume & Sturhan 1989). This species originally contained in the genus *Aphelenchoides,* was described from *Prunus avium, Populus nigra* and *P. tremula* in West Germany in 1956 (Rühm 1956).

In 1985 the Finnish Plant Quarantine Service, National Board of Agriculture found BM from timber imported to Finland from Siberia, Soviet Union (Annikki Lahtinen, personal communication). Norway, however, was the first country among the Nordic countries to find BM in logs cut out from forest grown Scots pine (McNamara & Støen 1988). In 1988 BM was also reported in Sweden in Scots pine (Magnusson & Schroeder 1989).

This survey was a second attempt to locate PWN or BM in Finnish forests. In 1984 the surroundings of two harbors and three papermills were examined for the presence of either PWN or BM but neither of these nematodes were recovered in any of the samples extracted (Hokkanen 1985, Rautapää 1986).

According to the results the species *B. mucronatus* appears to be widely distributed in Finnish conifer forests. It seems to occur both in spruce and pine although the observations are more frequent in pine. All the other observations in the literature on the occurrence of BM are from pines but according to our results Norway spruce serves also as a host of BM. It has to be noted, though, that Wingfield et al. 1983 reported of a balsam fir *(Abies balsamea* L. (Mill) isolate of PWN the females of which had a similar, although shorter in length, mucro as BM. In Canada this "M" form of PWN has also been found from black spruce (*Picea mariana* (Mill) B.S.P.) (Forest insect... 1985).

According to the results one has to focus on collecting samples from trees or logs infested with *Monochamus* beetles in order to ensure positive findings of this nematode. In the three cases where BM was detected without being associated with signs of Monochamus, one cannot exclude the possibility of the presence of the beetles having been overlooked by the observers. An obvious lack of pine sawyer infested wood among the samples collected during the earlier survey very likely accounted for the failure to detect this nematode in 1984 (Annikki Lahtinen, Finnish Plant Quarantine Service, National Board of Agriculture, personal communication).

A general pattern of the population sizes inside standing trees and timber seems to suggest very low levels. When reared at constant high temperatures in the laboratory the populations increase several-fold.

From the standpoint of the species status of the Finnish population of BM it appears to be morphologically close to all of the BM populations reported from other parts of the world. When comparing the measurements of the Finnish population to the ones from Japan there are differences in size individuals of the Finnish population being smaller (Mamiya & Enda 1979). This divergence, however, could be due to the differences in the methods used for preparing the specimens for measuring. In our study the nematodes were not fixed but instead the individuals were simply killed with heat followed by an immediate measuring. The crucial characters (the presence of the female mucronate tail and its length, the shape of the caudal alae of males as well as the spicule), however, are similar to those of the Japanese BM as well as to the ones of the Norwegian population of BM (McNamara & Støen 1988). Also the characters of the German *Bursaphelenchus* species recovered from deciduous trees (Schauer-Blume & Sturhan 1989) appear to be in accordance with our observations for Finnish BM.

The frequency with which the pine sawyers were carrying the dauerlarvae was observed to be very low. Also the numbers of nematodes per beetle was minimal. The average numbers of the dauerlarvae of PWN observed in Japan and in the U.S.A. are 15 000 and 19 000 per individual *Monochamus* adult, respectively (Mamiya & Enda 1972, Linit et al. 1983). However it should be noted that these average numbers may vary depending upon the local conditions numbers being high in areas with large populations of

nematodes. This could also relate to Finnish populations.

Magnusson and Shroeder (1989) reported high maximum numbers of dauerlarvae recovered from individuals of two *Monochamus* species (*M. galloprovincialis, M. sutor*). However, their results do not represent a real field situation since the beetles were reared to adults in laboratory conditions. These artificial conditions might have been conducive to successful reproduction of the nematode resulting in high nematode density in the logs where the juvenile stages of the beetles were developing. The number of dauerlarvae very apparently is in direct proportion to the size of the nematode population as a whole. Yet, Magnusson's and Schroeder's observation gives further credit to the hypothesis *Monochamus* species serving as primary vectors for BM also in northern Europe.

PWN does not seem to occur in Finland. The survey will be replicated in the summer 1989 further to clarify the distribution pattern of BM as well as the frequency with which the possible vectors are infested with the dauerlarvae of BM.

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