Infestation of four coleopteran species by the pine wood nematode *Bursaphelenchus xylophilus* (Nematoda, Aphelenchoididae) living in wood chips

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Six coleopteran species were tested for their susceptibility to infestation by dauerlarval or propagative stages of *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934), living in wood chips: *Monochamus scutellatus* (Say), *Rhagium inquisitor* (L.), *Asemum striatum* (L.) (Cerambycidae), *Hylobius pales* (Herbst) (Curculionidae), *Dendroctonus valens* (LeConte) (Scolytidae) and *Pytho* sp. (Salpingidae). *M. scutellatus* and *R. inquisitor* very frequently became infested by dauerlarvae, though the numbers of larvae per beetle were low. *A. striatum* and *H. pales* also became infested by dauerlarvae, but the frequency of infestation and the numbers per beetle were considerably lower. In addition, one male of *H. pales* became infested by 20 individuals of propagative stages.

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1. Introduction

Under natural conditions, the pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970, which inhabits conifer wood, is known to be vectored mainly by *Monochamus* pine sawyers (Coleoptera: Cerambycidae) (Mamiya & Enda 1972, Kondo et al. 1982, Linit et al. 1983, Kobayashi et al. 1984). The nematodes enter a specialized life stage, called the fourth dispersal stage (dauerlarva), prior to crawling into the tracheids of the vectors (Mamiya 1984).

In 1984, the Finnish Plant Inspection Service discovered PWN in wood chips imported from North America (Rautapää 1986). This gave rise to concern that these nematodes might infest conifers native to Finland, and occasioned speculation about insects that could serve as vectors while the nematodes are living in wood chips.

The present study was an attempt to find potential vectors susceptible to infestation by the nematode while in close contact with contaminated wood chips.

2. Materials and methods

Experiment 1

Four different coleopteran species were chosen to be tested for their liability to become infested by

PWN dauerlarvae while in association with PWN-contaminated wood chips. The species were: the whitespotted sawyer, *Monochamus scutellatus* (Say), the ribbed pine borer, *Rhagium inquisitor* (L.), *Asemum striatum* (L.) (Cerambycidae) and the pales weevil, *Hylobius pales* (Herbst) (Curculionidae).

Two other coleopteran species, *Dendroctonus valens* LeConte (Scolytidae) and *Pytho* sp. (Salpingidae), were included in the experiment but the numbers tested were low.

All the beetle adults, except *M. scutellatus*, had recently been collected from the research forest of the University of Vermont in Jericho, VT. The sawyer adults were from a laboratory population, originating from Jericho, VT.

The wood chips used in the experiment were obtained from *Pinus strobus* L. (eastern white pine). The PWN strain used was a Vermont isolate from *P. strobus*.

Wood chips containing both dauerlarval and reproductive stages were used in the experiment. It has already been demonstrated that the dauerlarvae can be induced to form in wood chips without the presence of vectors when chips with nematodes in them are first incubated at low temperatures and then at an abruptly increased temperature (Tomminen et al. 1988). The dauerlarvae in this study had been obtained by this method.

The study was conducted at the Forest Pathology Laboratory of the University of Vermont.

A glass jar was filled with approximately 150 grams (fresh weight) of wood chips containing PWN, a known proportion of the population being in the dauerlarval stage. During the course of the experiment the sample of wood chips in the jar was twice replaced with new samples containing PWN with a known history of incubation. The incubation temperature varied between 20°C and 25°C.

All the beetles were kept in the jar for at least 2 days. The cerambycids had a tendency to remain on the surface of the chips and for this reason the jar was periodically agitated to force the beetles deeper inside the chips.

The beetles were then removed from the wood chip jar, dissected, and extracted using a modified version of the Baermann funnel technique. For each beetle placed in the jar, one individual from the same population was extracted as a control to ascertain that the beetles used in the experiment originated from a nematode-free population. The numbers of PWN dauerlarvae observed in each of the extractions were recorded.

Experiment 2

The purpose was to discover whether propagative or third dispersal stages of PWN would cling to the body or enter the tracheae of adult pales weevils under the following circumstances:

1) When adult weevils were kept in glass jars containing wood chips harbouring PWN of these life stages;
2) When juvenile weevils had been allowed to mature in wooden rearing boxes or in glass jars with white pine bark as breeding material and wood chips contaminated with these life stages of PWN.

The procedures were the same as in Experiment 1.

3. Results

Experiment 1

The population numbers and the proportions of PWN dauerlarvae used in three different wood chip samples are shown in Table 1. The results of the beetle extractions are shown in Table 2.

The individual of *M. scutellatus* with the maximum number of dauerlarvae (81) had been kept 5 days in the jar. It was observed under the dissecting microscope to discover whether the dauerlarvae were inside the tracheae or simply clinging to the body surface. The main metathoracic tracheae contained abundant nematodes, which were later identified as PWN dauerlarvae. PWN dauerlarvae were the only nematodes recovered from *M. scutellatus* and *R. inquisitor*.

Individuals of *A. striatum* occasionally contained varying numbers of stylet-bearing nematode species of the family Sphaerulariidae (W.R. Nickle USDA — personal communication). The highest numbers per beetle exceeded 4000.

In several cases *H. pales* contained an accumulation of unidentified rhabditid (Rhabditida) nematode dauerlarvae, the highest numbers per

Table 1. Data on the three wood chip samples used in Experiment 1. (–=not measured).

Sample:	1	2	3
Days in jar	34	3	33
Percent dauerlarvae: initial/final	37.5/28.1	3/–	73.7/17
Ind./g dry wood: initial/final	33/72	108/–	51/–

Table 2. Dauerlarval infestation of the beetles in Experiment 1.

Infested species:	*M. scu.*	*R. inq.*	*A. str.*	*H. pal.*
Days in jar	2–5	2–3	2–3	2–3
Beetles	8	17	18	33
Dauerlarvae: mean	21	6	0.7	0.9
max/min	81/6	21/0	3/0	2/0

beetle exceeding 5000. Most of these nematodes were recovered from underneath the elytra.

Neither *Dendroctonus valens* nor *Pytho* sp. (9 individuals per species) became infested by PWN dauerlarvae. Both these species contained other unidentified nematodes.

None of the control beetles of any species contained PWN dauerlarvae.

Experiment 2

A total of 249 adult pales weevils were tested for their susceptibility to contamination by life stages of PWN other than dauerlarvae. Sixty-two adults were kept in a glass jar with wood chips that contained propagative stages and in some cases, third dispersal stages of PWN. The weevils were held in the jar for periods varying from one day to four weeks. No PWN were extracted from the beetles.

Adult pales weevils were also placed in wooden rearing boxes or glass jars to breed on fresh white pine bark. After the new weevil generation had reached the final larval stages, approximately 1200 grams (fresh weight) of PWN-infested wood chips were placed in each box and in some cases mixed with the bark. Adult weevils were dissected and extracted soon after eclosion. It was not always possible to say whether a given adult belonged to the new generation or the parental population, but the majority of the extraction results concerned newly emerged weevils. Extraction was performed on 141 adults from the boxes and 46 from the jars. Periodic inspection of the chips and bark revealed large populations of PWN. During the course of the experiment the third dispersal stage gradually became dominant in the PWN populations. No dauerlarvae were detected in any of the sample extractions. PWN individuals were recovered from the bark, though in much lower numbers than from the chips. Only one beetle extraction revealed the presence of PWN. It concerned a male of a parental population placed in a glass jar. The weevil had been in the jar for 5 weeks in close association with wood chips. Twenty PWN individuals of both adult and propagative larval stages were recovered from the weevil. Whether these nematodes had been clinging to the surface of the weevil or had entered its tracheae was not ascertained.

4. Discussion

Monochamus scutellatus, *Asemum striatum* and *Hylobius pales* have been reported to carry PWN dauerlarvae under natural conditions (Dropkin et al. 1981, Linit et al. 1983). There is no previous evidence of the potential of *R. inquisitor* as a vector of PWN. *M. scutellatus* and *R. inquisitor* frequently became infested by PWN dauerlarvae under the present conditions (Experiment 1), though the numbers were not as high as the field observations for *Monochamus* spp. (Mamiya & Enda 1972, Kondo et al. 1982).

A. striatum and *H. pales* also became infested, but less frequently and in lower numbers than either of the cerambycids above. It seems probable that the few PWN observed in extractions of *H. pales* and *A. striatum* were not recovered from the tracheae but had been attached to the body surface at the time of extraction. Examination of

M. scutellatus revealed dauerlarvae aggregated in the main thoracic tracheae. Tracheal infestation of *R. inquisitor* was not confirmed, though it is reasonable to assume a similar association.

In one case in Experiment 2, a male *H. pales* became infested by 20 PWN (propagative stages) while in contact with wood chips containing PWN. This suggests that *H. pales* adults may pick up low numbers of different life stages of PWN from wood chips and transfer the nematodes to fresh conifer stumps or pine seedlings while breeding and feeding.

In conclusion, it can be hypothesized that if beetles susceptible to infestation by dauerlarvae or propagative stages of PWN become trapped in wood chip piles containing PWN, the nematodes may enter their tracheae or become attached to their body surface. As a result, these beetles could transport PWN from the wood chips to the living coniferous trees or fresh conifer material on which the beetles feed and breed.

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References

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.

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., 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. Missouri. 58 pp.

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.

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

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

Rautapää, J. 1986: Experiences with Bursaphelenchus xylophilus in Finland. — Bulletin OEPP/EPPO Bulletin 16:453–456.

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

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