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FUNGI ASSOCIATED WITH THE PINE WOOD NEMATODE, *BURSAPHELENCHUS XYLOPHILUS*, AND CERAMBYCID BEETLES IN WISCONSIN

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The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) which is carried by cerambycid beetles (Coleoptera: Cerambycidae) causes a serious disease of native pines in Japan. Details of the life cycle of *B. xylophilus*, and the associated disease cycle have been presented in a number of recent reviews (Mamiya, **1976**, **1983**). The nematode was first found in the United States in 1931 (Steiner and Buhrer, **1934**; Nickle *et al.*, **1980**) and subsequently recognised as a pathogen (Dropkin and Foudin, **1979**).

Bursaphelenchus xylophilus is mycophagous (Kobayashi *et al.*, **1974**) and has apparently become adapted to feeding on living pine tissue. In Japan, some fungi associated with *B. xylophilus* in dying pines have been identified. In the United States, however, little attention has been given to the fungi associated with the nematode. The aim of this study was to identify fungi from the pupal chambers of cerambycid beetles in pine wood nematode infested trees, from the nematodes themselves and from cerambycid beetles emerging from the trees. The role of these fungi in the ecology of *B. xylophilus* is considered.

Twenty-year-old jack (*Pinus banksiana* Lamb) and red pines (*P. resinosa* Ait.), occurring at Black River Falls, Jackson Co., Wisconsin, were selected for study. Five trees of each species infested with *B. xylophilus* and cerambycid beetles were felled and cut into 60 cm bolts. Jack pine trees had died after being artificially girdled in a previous study (Wingfield, **1982**) and the red pines had died due to fire scorching and subsequent infestation of *Dendroctonus valens* (Le C.) (Wingfield, **1983**).

Pine bolts were transported to Minnesota where cerambycid beetle pupal chambers (64 from red and 61 from jack pine) were cut from the wood. Pupal chambers were placed on moistened filter paper in petri dishes and incubated at 25 C in sealed plastic buckets each containing an insecticide strip to suppress mite activity and kill nematodes in the wood.

After 2 wk, pupal chambers were examined for fungi using a dissection microscope. Conidia or ascospores of fungi sporulating on the wood surface were lifted from fruiting structures and plated on 2% Difco Malt Extract Agar (MEA) in Petri dishes. Petri dishes were incubated at 25 C for up to 4 wk. Fungi were then identified. Microscope preparations of these and other fungi in this study were deposited in the Herbarium of The National Collection of Fungi, Plant Protection Research Institute, Pretoria, South Africa (PREM).

Disks of wood 4 cm thick were removed from each tree sampled. Bark was removed and wood was chopped into 2 cm^3 blocks. Wood samples (60 g) from each tree were placed in Baermann funnels (Southey, **1970**) to extract nematodes. Nematodes were concentrated in a swing bucket centrifuge and placed on the surface of 1% water agar in the center of petri dishes. Petri dishes were incubated at 25 C in the dark for 2 wk and fungi sporulating on the agar surface were identified.

Red pine bolts remaining after pupal chambers and wood for nematode extractions were removed and placed in emergence traps previously used by Wingfield and Blanchette (**1983**). Traps were examined daily and cerambycid beetles collected.

Beetles were dissected into four parts: head with antennae, abdomen, thorax with legs, hind wings and elytra. Beetle thoraxes were plated separately from the remaining parts on water agar supplemented with 2% latic acid and 1% streptomycin sulphate. Dishes were incubated in the dark at 25 C for one week and fungi identified.

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TABLE I

FUNGI FROM CERAMBYCID PUPAL CHAMBERS IN *PINUS RESINOSA* AND *P. BANKSIANA*[a]

Fungi identified[b]
Arthrobotrys cladodes Drechsler var. *cladodes* (PREM 48209)
A. superba Corda (PREM 48208)
Ceratocystis allantospora Griffin (PREM 48206)
C. angusticollis Wright and Griffin (PREM 48203)
C. deltoideospora Olchow. and Reid (PREM 48202)
C. ips (Rumb) C. Moreau (PREM 48207)
C. minor (Hedgc.) Hunt (PREM 48205)
C. tenella Davidson (PREM 48201)
Ceratocystiopsis minima (Olchow. and Reid) Upadhyay (PREM 48204)
Chaetosphaeria sp.
Chloridium virescens var. *chlamydosporum* (van Beyma) W. Gams (PREM 48213)
Codinia sp.
Gliocladim roseum Bain
Phialophora verrucosa Medlar (PREM 48214)
Rhinocladiella elatior Mangenot (PREM 48211)
Spadicioides spp.

[a] Five *P. resinosa* and five *P. banksiana* trees sampled, with 64 and 61 pupal chambers from each species, respectively.

[b] PREM numbers are those of the National Collection of Fungi, Plant Protection Research Institute, Pretoria 0001, South Africa. Other than *C. ips* on *P. resinosa* and *C. virescens* on *P. banksiana* all fungi were found on both hosts.

The fungi in beetle pupal chambers of red and jack pine were similar (TABLE I). They included a number of *Ceratocystis* spp. *sensu lato* and *Ceratocystiopsis* sp. A mycoparasite, *Gliocladium roseum*, and a number of other fungi known to occur on wood were identified and isolated. In addition, two species of nematode-trapping fungi, *Arthrobotrys cladodes* var. *cladodes* and *A. superba* were found. Common contaminant fungi such as *Penicillium* spp. and *Trichoderma* spp. were present.

Fungi sporulating on water agar to which *B. xylophilus* had been added included two nematode trapping fungi (*A. cladodes* var. *cladodes* and *A. superba*) which were also present in pupal chambers. These fungi were found on nematodes from one jack and three red pine. In addition, a single nematode from red pine was found colonised by an endoparasitic *Harposporium* sp. This fungus was not isolated and the species could not be identified.

Three species of cerambycid beetles were collected from red pine bolts. These include six *M. scutellatus*, 148 *M. carolinensis* and 14 *Neocanthocinus pusilus* (Kirby). Other than probable contaminant fungi (*Aspergillus* spp., *Alternaria* sp., *Aureobasidium* sp., *Cladosporium* sp., *Helminthosporium* sp., *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp.) a nematode parasite, *Arthobotrys cladodes* var. *cladodes*, an insect pathogen, *Beauvaria bassiana* (Bals.) Vuill., a mycoparasite, *Gliocladium roseum* and *Ceratocystis minor* were isolated from these insects.

Fungi have previously been isolated from pine wood nematode infested trees in Japan (Kobayashi *et al.*, **1975**; Mamiya and Tamura, **1976**; Yoneda *et al.*, **1980**) and in the United States (McGawley *et al.*, **1980**). In Japan, fungi have also been isolated from *B. xylophilus*. This is, however, the first report of fungi isolated from cerambycid beetles, the wood associated with the beetles before emergence and from nematodes extracted from the same wood. Most fungi previously found associated with pine wood nematode infested wood were common saprophytes and probably not specifically associated with *B. xylophilus*.

An objective of this study was to look at fungi closely associated with *B. xylophilus* and its vectors. The most commonly recorded fungi and those of particular interest here were *Ceratocystis* spp. (*sensu lato*) known to be associated with insects (Upadhyay, **1981**), particularly bark beetles (Coleoptera: Scolytidae). *Ceratocystis* spp. were most commonly found in the pupal chambers of cerambycid beetles. *Ceratocystis* spp. produce spores in slimy masses that adhere to insect surfaces or are carried in specialised gland cells (mycangia) of the insects (Whitney, **1982**). *Ceratocystis* spp. sporulating in pupal chambers of cerambycid beetles are also likely to adhere to the surface of these insects when they emerge. However, unlike bark beetles, adult cerambycid beetles do not penetrate bark of dead pines during infestation. The only contact these insects have with pines is when the male beetle chews an oviposition niche in the bark and, by means of an ovipositor, places an egg under the bark surface. This limited contact is unlikely to be sufficient to transmit *Ceratocystis* spp. Fungi associated with cerambycid beetles probably rely on some means other than adult beetles to return them to beetle galleries.

Isolations from beetles yielded few of the fungi found in pupal chambers. This supports the view that the insects themselves might not be responsible for completing the life cycle of the fungi

found in their pupal chambers. Cerambycid beetles, like bark beetles, exist together with many microorganisms on their bodies (Whitney, **1982**). Some of these microorganisms, such as nematodes and mites, are mobile and might be important in transferring fungi from one cerambycid gallery to the next.

Further research is necessary to establish the role of mites in the transmission of fungi found on *B. xylophilus* and its vectors. In this study, mites are likely to have left cerambycid beetles before isolation. This could explain the absence from the beetles of fungi found in pupal chambers. Mites phoretic on cerambycid beetles are known to feed on fungi (Moser and Roton, **1971**) including *Ceratocystis* spp. and can disseminate fungal spores (Moser *et al.*, **1974**). Mites are also important in the dissemination and fertilisation of *Ceratocystis* spp. associated with bark beetles (Brasier, **1978**; Bridges and Moser, **1983**).

All *Ceratocystis* spp. recorded here are known in North America (Upadyhay, **1981**). Only *C. allantospora* has been associated with cerambycid beetles (Griffin, **1968**). *Ceratocystis angusticollis* has not previously been associated with insects (Olchowecki and Reid, **1974**) whereas all other *Ceratocystis* spp. have been associated with scolytid bark beetles (Upadhyay, **1981**; Griffin, **1968**; Olchowecki and Reid, **1974**). *Bursaphelenchus xylophilus* is a mycophagus nematode and feeds on fungi in dead trees. Some of the fungi recorded here, such as *Ceratocystis* spp. which are adapted for insect transmission, could provide an important food source for the nematodes in dead wood.

Two species of nematode-trapping fungi, *Arthrobotrys superba* and *A. cladodes* var. *cladodes* were found in beetle pupal chambers and on *B. xylophilus*. Nematode trapping fungi have been found associated with *B. xylophilus* in Japan (Mamiya and Tamura, **1976**; Yoneda *et al.*, **1980**; Tamura, **1980**). This is, however, the first record of nematode-trapping fungi associated with *B. xylophilus* in the United States.

Predators and parasites of *B. xylophilus* and its vectors including nematode-trapping fungi, could be significant in maintaining ecological homeostasis (Pimental, **1963**). It is probable that *B. xylophilus* was introduced into Japan (Mamiya, **1983**). The introduction of natural enemies such as parasitic fungi into Japan might be useful in reducing nematode populations there (Saiki *et al.*, **1984**). Further research to evaluate the role of fungi associated with the pine wood nematode and its vectors should therefore be encouraged. An understanding of the ecology of these fungi and other associates of *B. xylophilus* should improve our understanding of the disease associated with the nematode.

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Key Words: *Bursaphelenchus xylophilus, Ceratocystis* spp., *Ophiostoma* spp., *Arthrobotrys* spp., insect associated fungi, nematode parasitic fungi.

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