Hitchhikers with Invasive *Tetropium fuscum* (Fabr.) (Coleoptera:Cerambycidae) in Atlantic Canada

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Abstract

Tetropium fuscum (Fabr.) (Coleoptera:Cerambycidae) is native to Europe and apart from the Halifax, Nova Scotia area, is unknown elsewhere in North America (Smith & Hurley, 2000). *Tetropium fuscum* poses no primary threat to *Picea abies* Karst. in Europe and is regarded there as a secondary insect that usually attacks severely weakened or recently dead trees. An infestation of *Tetropium fuscum* was discovered in 1998 in red spruce trees (*Picea rubens* Sarg.) in the Halifax Regional Municipality in Nova Scotia, Atlantic Canada (Smith and Hurley, 2000). The trees had very little resistance and repeated attacks by the beetles ultimately led to the death of the trees. Several species of ophiostomatoid fungi were found in association with the *T. fuscum* infestation.

Very little is known about the fungal associates of *Tetropium* spp (Mathiesen 1951; Mathiesen-Käärik 1953; Kotýnková-Sychrová, 1966; Solheim, 1996). Mathiesen (1951) described *O. tetropii* associated with *Tetropium* sp. on spruce in Europe and later (Mathiesen-Käärik, 1953) listed a few more species isolated from old larval galleries of the beetle. *Ophiostoma tetropii* has been reported from *Picea abies* in Finland (Virri, 1997), Norway (Solheim, 1986) and Czechoslovakia (Kotýnková-Sychrová, 1966).

The aim of this study was to characterize the ophiostomatoid fungi associated with *T. fuscum* in Atlantic Canada. We also compared the associates of *T. fuscum* with ophiostomatoid fungi associated with *Tetropium* species in Europe. *Ophiostoma* species associated with the indigenous, transcontinental species (*T. cinnamopterum* Kirby), which occupies the same niche in North America, were also compared to those associated with *T. fuscum* occurring in Canada.

Ophiostoma species were isolated from the stained sapwood adjacent to the beetle galleries of *Tetropium fuscum* from wood bolts collected in Halifax, Nova Scotia. Additionally, ascospores from perithecia and conidia from sporulating anamorphic structures in the pupal chambers of *T. fuscum* were aseptically transferred to 2% MEA and 2% CSMEA. Isolations were also made by allowing living *T. fuscum* adults to move around on 2% CSMEA. The resulting colonies were purified by transferring to fresh 2% MEA and incubated at 20°C and 25°C. Similar isolations were also made from ascospores and conidia obtained from sexual and asexual stages occurring in the galleries of the native *T. cinnamopterum* on red spruce and white spruce in the Point Pleasant Park area. Isolated strains were grown on 2% MEA and Oatmeal agar (OA). All measurements and microscopic observations were performed on fungal structures produced on 2% MEA and OA.

DNA extractions from pure cultures grown on commercial potato dextrose agar (PDA), were made using the UltraClean™ Microbial DNA kit (MO BIO, USA). Amplifications of the ITS1-5.8S-ITS2 (internal transcribed spacer regions) were done using standard protocols for PCR reactions using Ready-to-go (RTG) PCR-beads (Amersham Pharmacia Biotech) (Primers: NS7 and ITS4) (White *et al.,* 1990). PCR products were purified using the UltraClean™ PCR-cleanup kit (BIO/CAN Scientific) and sequenced using the Big-dye terminator cycle sequencing pre-mix kit (Applied Biosystems) on an ABI PRISM 310 automatic sequencer (Perkin Elmer Applied Biosystems) (Primers: NS7, ITS2, ITS3 and ITS4) (White *et al.,* 1990). Sequence data were edited using Sequencher (Gene Codes Corporation, Michigan, USA) and a computer-generated alignment (Wisconsin Package Version 10.1, Genetics Computer Group (GCG), Madison, Wisconson; Canadian Bioinformatics Resource, http://www.cbr.nrc.ca/) was manually adjusted in PAUP* v.4.0b8 (Phylogenetic Analysis Using Parsimony) (Swofford 1999). Phylogenetic relationships were inferred using a heuristic search in PAUP* v.4.0b8. Confidence levels were estimated using Bootstrap analysis (1000 replicates).

Species of ophiostomatoid fungi sporulated in the tunnels of *T. fuscum* and *T. cinnamopterum.* Three species of ophiostomatoid fungi were associated with *P. rubens* infested by *T. fuscum* in Atlantic Canada: *Ophiostoma tetropii, Ophiostoma piceae* and *Pesotum fragrans.* One strain of *O. floccosum* was isolated from a girdled *P. rubens* tree in Point Pleasant Park. No evidence of *T. fuscum* infestation was observed in this tree. Three strains of unidentified *Pesotum* spp. were also isolated from *P. rubens* attached by *T. fuscum.*

Ophiostoma tetropium was abundant in *P. rubens* attacked by *T. fuscum.* This species is homothallic and produces perithecia prolifically in culture, even after several subcultures. In culture *Ophiostoma tetropii* produces a *Hyalorhinocladiella* anamorph as well as a *Leptographium*-like anamorph with a short stipe. The strains isolated from in the Halifax area corresponds morphologically and phylogenetically to a strain from Europe, recognized as *O. tetropii* (CBS 428.94). This strain also has a *Hyalorhinocladiella* as well as *Leptographium* –like anamorph. Mathiesen (1951) described *O. tetropii* from Norway spruce (*Picea abies*) infested by *Tetropium* species in Europe. Unfortunately, no type material was designated and a single living strain of this species was deposited at CBS (CBS140.51=MUCL9530). The strain in CBS, however, no longer corresponds to the original description of *O. tetropii* (Mathiesen, 1951). The *"Scopularia"*-like (=*Leptographium*) state reported by Mathiesen (1951) was not observed and a distinct *Sporothrix* state characterized by obvious denticles on the apices of the conidiogenous cells was observed in culture. In addition, the ITS sequence of this strain does not correspond to the strain from Europe that has been identified as *O. tetropii.* Analysis of the data, group CBS140.51 together with other species of *Ophiostoma,* such as *O. stenoceras., O. narcissi* and *O. rostrocoronatum,* all characterized by white colonies and *Sporothrix* anamorphs with distinct denticles (Upadhyay, 1981).

Based on the evidence, we no longer consider the ex-type strain of *O. tetropii* (CBS140.51=MUCL9530) as authentic for this species and propose that the European strain of *O. tetropii* (CBS428.94) be considered representative for *O. tetropii.* In the absence of a designated type specimen, we propose that BPI 595681, deposited by Hunt as *O. tetropii,* be designated as the epitype for *O. tetropii,* while the illustration accompanying the original description serves as the lectotype. Griffin (1968) reported *O. tetropii* from *Picea mariana* in Ontario. This report could not be confirmed as the herbarium material (MFB7394) was deteriorated and overgrown with *Aspergillus* sp.

Ophiostoma tetropii *does not appear to be pathogenic in North America and is not known to be major economic importance in Europe. In the near future, we intend to initiate inoculation studies on red spruce with fungal associates of* T. fuscum *in Canada as part of a comprehensive risk assessment.*

Ophiostoma piceae was frequently isolated from both *Picea rubens* and *P. glauca*, including from trees attacked by either *T. fuscum* and the native *T. cinnamopterum.* The presence of this species comes as no surprise as it is commonly found throughout North America (Uzunovic *et al.,* 1999; Harrington *et al.,* 2001) and is considered a generalist not associated with a specific niche. The strains from Atlantic Canada were morphologically similar to descriptions in literature of this species (Upadhyay, 1981; Harrington *et al.,* 2001). Strains identified as *O. piceae,* based on their morphology, grouped together in analyses of ITS sequences with other strains of *O. piceae,* including the ex-type strain of *O. piceae* (CBS 108.21).

Pesotum fragrans was isolated a few times during the study. Mathiesen-Käärik (1953) described this species from galleries of *Ips sexdentatus* in *Pinus sylvestris* logs. This fungus is widespread and has been reported from USA, New Zealand and Australia (Harrington *et al.,* 2001). *Pesotum fragrans* does not appear to be exclusively associated with *Tetropium fuscum* or red spruce. It was also isolated from Balsam fir, ambrosia beetles and the native *Tetropium cinnamopterum.* On OA, a bright yellow pigment is produced, that appears to characteristic of this species. ITS sequences of the strains from Halifax are identical to the strain (CBS 279.54) that is considered to be authentic for this species (Harrington *et al.,* 2001). Although *P. fragrans* has not been reported from Canada before, it appears to have been present in Canada for a considerable period of time. Two of the strains (NLC 348, NLC 349) used were isolated from the sapwood of dead balsam fir trees, attacked by *Trypodendron lineatum* (Oliv.) in Newfoundland, Canada in 1982. We also examined a dried specimen from Quebec, Canada, collected in 1986. This suggests that *P. fragrans* is not a new introduction to Canada associated with *T. fuscum.*

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