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Extended Abstract

Ophiostomatoid fungi associated with the Eastern Himalayan spruce bark beetle (*Ips schmutzenhoferi*) in Bhutan: Species assemblage and phytopathogenicity

Thomas Kirisits[1]*, Heino Konrad[1,2], Michael J. Wingfield[3] and Dal Bahadur Chhetri[4]

[1]Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna (BOKU), Hasenauerstraße 38, A-1190 Vienna, Austria.
[2]Present address: Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Department of Genetics, Unit of Ecological Genetics and Biodiversity, Hauptstraße 7, A-1140 Vienna, Austria.
[3]Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.
[4]Renewable Natural Resources (RNR) Research and Development Sub-centre, Darla, Department of Forest and Park Services, Ministry of Agriculture and Forests, Royal Government of Bhutan, Darla, Chhukha, Bhutan.

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A brief synthesis of recent studies on the ophiostomatoid fungi associated with the Eastern Himalayan spruce bark beetle, *Ips schmutzenhoferi* and on the pathogenicity of selected fungal associates of this insect to *Picea spinulosa* and *Pinus wallichiana* is presented. *I. schmutzenhoferi* is intimately associated with ophistomatoid fungi and eleven fungal associates belonging to the genera *Ceratocystis*, *Ceratocystiopsis*, *Grosmannia*, *Ophiostoma*, *Leptographium* and *Pesotum* were documented in a survey in Western Bhutan in 2001. In inoculation experiments with four ophiostomatoid fungi, conducted in 2005, *Leptographium* sp. 1, the most common fungal associate of *I. schmutzenhoferi*, displayed high levels of virulence to *P. spinulosa*. In contrast, *P. wallichiana* was highly resistant to inoculation with all four fungal species. The pathogenicity trials indicate that fungal associates of *I. schmutzenhoferi* and especially *Leptographium* sp. 1 prefer *P. spinulosa* over *P. wallichiana* as host, as is true of the insect itself.

Key words: *Ceratocystis bhutanensis*, *Ophiostoma sensu lato*, blue-stain fungi, fungal associates, insect-fungus symbiosis.

INTRODUCTION

The Eastern Himalayan spruce bark beetle, *Ips schmutzenhoferi* is a socio-economically important pest in temperate conifer forests at altitudes between about 2500 and 3400 m asl. in the Bhutan Himalayas (Kirisits et al., 2002, 2012). This bark beetle species preferentially infests Eastern Himalayan spruce, *Picea spinulosa*, but also Blue pine, *Pinus wallichiana* and occasionally logs of Eastern Himalayan larch, *Larix griffithiana*. *I. schmutzenhoferi* is an aggressive scolytine species that caused a destructive outbreak in Western and Central Bhutan in the 1980's.

Most conifer bark beetles live in association with ophiostomatoid fungi belonging to the ascomycete genera *Ophiostoma*, *Grosmannia*, *Ceratocystiopsis* and *Ceratocystis* and related anamorph genera such as *Leptographium* and *Pesotum*. They cause discoloration in the sapwood of conifers and are, therefore, commonly referred to as blue-stain fungi. Some fungal associates of conifer bark beetles are phytopathogenic and are able to kill their host trees.

Here, we briefly summarize recent studies on the ophiostomatoid fungi associated with *I. schmutzenhoferi* and on the pathogenicity of selected fungal associates of this insect to *P. spinulosa* and *P. wallichiana* (Kirisits et al., 2002, 2012; van Wyk et al., 2004; Konrad, 2006).

*Corresponding author. E-mail: thomas.kirisits@boku.ac.at.

Table 1. Ophiostomatoid fungi associated with *I. schmutzenhoferi* in Western Bhutan and their frequencies in relation to sources of isolation and isolation methods (directly from beetles collected from a *P. spinulosa* log, from the sapwood of a beetle-infested *P. spinulosa* tree and from insect galleries on *P. spinulosa* and *P. wallichiana*).

Fungal species	Sources of isolation / isolation methods		
	Beetles	**Sapwood**	**Beetle galleries**
Ceratocystiopsis cf. *minuta*	Not isolated	Not isolated	Common
Ceratocystis bhutanensis	Common	Not isolated	Not recorded
Grosmannia cf. *cucullata*	Common	Not isolated	Common
Leptographium sp. 1 (*Grosmannia* sp.)	Uncommon	Common	Dominant
Leptographium sp. 2	Rare	Rare	Rare
Ophiostoma cf. *ainoae*	Common	Common	Common
Ophiostoma floccosum (*Pesotum aureum*)	Rare	Not isolated	Not recorded
Ophiostoma quercus	Rare	Not isolated	Not recorded
Pesotum cf. *quercus*	Rare	Not isolated	Not recorded
Ophiostoma cf. *piceae*	Rare	Rare	Common
Ophiostoma setosum (*Pesotum cupulatum*)	Not isolated	Rare	Common

OPHIOSTOMATOID FUNGI ASSOCIATED WITH *I. SCHMUTZENHOFERI*

In July 2001, we conducted a survey of ophiostomatoid fungi associated with *I. schmutzenhoferi* in Western Bhutan (Kirisits et al., 2002, 2012; van Wyk et al., 2004; Konrad, 2006). Specimens of *I. schmutzenhoferi*, excavated from their galleries or collected from pheromone traps, stem discs and stem sections from *I. schmutzenhoferi*-infested *P. spinulosa* and *P. wallichiana* trees and logs as well as bark and wood samples containing bark beetle breeding galleries were collected. These collections were made in forests and at wood depots at various localities in the districts of Thimphu and Wangdue Phodrang. Fungi were isolated on malt extract agar and cycloheximide-malt extract agar. Isolations were made directly from the insects, from the sapwood of a beetle-infested *P. spinulosa* tree and from ascospores and conidia taken from perithecia and asexual fungal structures in and around breeding galleries of the insects.

Eleven fungal species were found to be associated with *I. schmutzenhoferi* (Table 1). These included *Ceratocystiopsis* cf. *minuta*, *Ceratocystis bhutanensis* (van Wyk et al., 2004), *Grosmannia* cf. *cucullata*, *Leptographium* sp. 1 (associated with a *Grosmannia* teleomorph), *Leptographium* sp. 2, *Ophiostoma* cf. *ainoae* and five taxa in the *Ophiostoma piceae* species complex (Kirisits et al., 2002, 2012; Konrad, 2006). The latter comprised *Ophiostoma floccosum* (*Pesotum aureum*), *Ophiostoma quercus*, *Ophiostoma* cf. *piceae*, *Ophiostoma setosum* (*Pesotum cupulatum*) and *Pesotum* cf. *quercus* (Konrad, 2006; Kirisits et al., 2012). The assemblages and frequencies of ophiostomatoid fungi varied greatly in the niches that were examined and depended on the isolation methods used (Table 1). Overall, *Leptographium* sp. 1 appeared to be the dominant fungal associate of *I. schmutzenhoferi* and *O.* cf. *ainoae* was also common.

PATHOGENICITY OF FUNGAL ASSOCIATES TO *P. SPINULOSA* AND *P. WALLICHIANA*

In spring 2005, two isolates each of *C. bhutanensis*, *Leptographium* sp. 1 (*Grosmannia* sp.), *O.* cf. *ainoae* and *O.* cf. *piceae* were included in a pathogenicity trial on *P. spinulosa* and *P. wallichiana* in the district of Bumthang in Central Bhutan (Kirisits et al., 2012). Each of 15 pole-sized *P. spinulosa* and *P. wallichiana* trees (diameter at breast height between 20 and 27 cm) were wound-inoculated twice with each of the isolates. Sterile malt extract agar was used as control treatment. About seven weeks after inoculation, the outer bark was removed around the inoculation sites and the lengths of the necrotic lesions in the phloem were measured. All four fungi caused only small necrotic lesions in the phloem of *P. wallichiana* trees, with average lesion lengths ranging from 17.2 to 28.2 mm. *Ceratocystis bhutanensis*, *O.* cf. *ainoae* and O. cf. *piceae* also caused relatively small lesions on *P. spinulosa* (range of average lesions lengths: 29.6 to 34.4 mm). In contrast, *Lepographium* sp. 1 incited very long phloem lesions on *P. spinulosa* (Figure 1), averaging 223.2 and 296.3 mm for the two isolates. In a mass inoculation experiment on small trees (diameter at breast height between 10 and 12 cm), also conducted in 2005, *C. bhutanensis* displayed a very low level of virulence to *P. wallichiana* and a moderate level of virulence to *P. spinulosa* (Kirisits et al., 2012). Thus, this fungus appears to have a very limited or no ability to kill its host trees, even when inoculated at high dosages.

CONCLUSIONS

Our studies in Bhutan (Kirisits et al., 2002, 2012; van Wyk et al., 2004; Konrad, 2006) have shown that *I. schmutzenhoferi* is intimately associated with ophio-

Figure 1. A. Necrotic lesion in the phloem (right) and on the corresponding sapwood surface (left) of a *P. spinulosa* tree caused by *Leptographium* sp. 1. in the pathogenicity trial, B. The longest phloem lesion incited by *Leptographium* sp. 1 on *P. spinulosa*, measuring 69.5 cm.

stomatoid fungi. The most common fungal associate of this bark beetle species, *Leptographium* sp. 1 displayed high levels of virulence to *P. spinulosa*. In contrast, *P. wallichiana* appears to be highly resistant to the fungal associates of *I. schmutzenhoferi*. Moreover, the pathogenicity trials suggest that fungal associates of *I. schmutzenhoferi* and especially *Leptographium* sp. 1 prefer *P. spinulosa* over *P. wallichiana* as host, as is true of the insect itself. Our studies on *I. schmutzenhoferi* (Kirisits et al., 2002, 2012; van Wyk et al., 2004; Konrad 2006) are the first to consider the association of ophiostomatoid fungi with a conifer bark beetle in the Himalayan region. They also contribute to knowledge regarding the occurrence, taxonomy, ecology and pathogenicity of these organisms in a largely unexplored part of Asia.

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