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Notes on mycobiota associated with *Ips typographus* from the Šumava Mts. (Czech Republic)

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In 1999 and 2000, stem samples of Norway spruce (*Picea abies*) infested by bark beetle (*Ips typographus*) from one spruce stand locality affected by massive infestation by *Ips typographus* in the central part of the Šumava mountains were taken. The mycobiota of 20 adults was studied. Eighteen species of microscopic fungi were recorded. Yeasts and ophiostomatoid fungi were detected most frequently.

Key words: *Picea abies*, ophiostomatoid fungi, bark beetles

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V letech 1999 a 2000 byly vždy na jedné vybrané lokalitě postižené kůrovcovou kalamitou ve střední části Šumavy odebrány vzorky kmenů smrku (*Picea abies*) napadených lýkožroutem smrkovým (*Ips typographus*). Z odebraných vzorků smrku bylo odchyceno celkem 20 dospělých brouků a zjišťovány mikroskopické houby, které přenášejí. Celkem bylo nalezeno 18 druhů mikroskopických hub, mezi nimiž dominovaly kvasinky a ophiostomatální houby.

INTRODUCTION

Fungi and insects are important factors in trees pests in different parts of the world. Mycological and phytopathological research in the last 20 years has been focused on understanding the importance and role of fungi – bark beetle associations in trees dieback. The role of fungi associated with bark beetles as potential pathogens of vascular tissues is investigated (Krokene and Solheim 1996, Yamaoka et al. 1997, Kirschner 2001).

In this field, spruce bark beetle (*Ips typographus)* is frequently studied. The mycobiota associated with this beetle species was investigated in Europe (e.g. Solheim 1986, 1993; Krokene and Solheim 1996; Kirisits 1998) and Japan (Yamaoka et al. 1997). Fungi, especially ophiostomatoid, associated with spruce

bark beetles are known to be pathogenic to various species of trees (e.g. Krokene and Solheim 1996, Kirisits 1998).

So far, the mycobiota of eight species of bark beetles (*Hylurgops palliates*, *Ips typographus*, *Myelophilus piniperda*, *Pityogenes chalcographus*, *Scolytus intricatus*, *Tomicus minor*, *T. piniperda*, *Xyloterus lineatus*), including *Ips typographus*, has been investigated in the Czech Republic (Novotný and Šrůtka 2004). Fungi associated with bark beetles, including *I. typographus*, from Central Bohemia were investigated by Kotýnková-Sychrová (1966). She studied a very limited number of adult bark beetles and did not pay attention to the frequency of occurrence of these fungi. Phytopathological aspects of fungi associated with *Ips typographus*, especially *Ceratocystis polonicum* and *Ophiostoma bicolor*, were investigated by Jankovský and Mrkva (1997) and Jankovský et al. (1998, 2003).

The aim of the present study is to contribute to the knowledge of mycobiota of *Ips typographus* which causes mass dieback of Norway spruce in the Šumava Mts. (southwest part of the Czech Republic).

MATERIALS AND METHODS

Samples of spruce stems (*Picea abies*) including galleries and adults of *Ips typographus* were taken from spruce stands from two localities in the central part of the Šumava Mts., South Bohemia, Czech Republic. One was near the village of Modrava (48°58'44"N 13°28'42"E) and the second one was on the slope of Mt. Luzný (48°58'47"N, 13°28'54"E). The samples from the first study site were taken in August 1999 and samples from the second study site were taken in June 2000. The spruce stands were affected by mass infestation by *Ips typographus*. Both localities belong to the study sites of the Institute of Landscape Ecology, Academy of Sciences of the Czech Republic.

Tab. 1. Overview of studied samples.

Sample No.	Date	Locality	Samples
1	2 Aug 1999	Modrava	10 adults beetles from 1 stem
2	21 June 2000	Mt. Luzný	10 adults beetles from 1 stem

Mature beetles were excised from the bark and separately washed in 5 ml of sterile water with Tween 80 for 3 minutes in an ultrasonic cleaner. Washed beetles, 1 ml of the suspension and detritus from galleries were separately inoculated on Petri dishes with 2 % malt extract agar. After 10–14 days of incubation at room temperature the fungi were isolated and identified.

Ophiostomatoid fungi were identified using following literature: Upadhyay (1981), Solheim (1986), Grylls and Seifert (1993) and Yamaoka et al. (1997).

RESULTS

In the present study, 18 fungal species were detected in Norway spruce samples infested with *Ips typographus*. Yeasts and ophiostomatoid fungi dominated the fungal community, but other filamentous fungi were recorded, too. Five fungal species (yeast sp. 1, *Graphium* cf. *fimbriisporum*, *Ophiostoma piceaperdum*, a sterile dark mycelium and *Ophiostoma bicolor*) were recorded in association with at least 40 % of beetles. Small differences were observed in the occurrence of fungi on bodies of beetles, in the suspension and in galleries. More fungal species were found in galleries than in the suspension or beetle bodies. All dominant species, excluding *Ophiostoma bicolor*, were found in the suspension, beetle bodies and galleries (Table 2).

In 1999, 13 species were isolated from beetles from Modrava. Five dominant species (yeast sp. 1, *Graphium* cf. *fimbriisporum*, *Ophiostoma bicolor*, *Ophiostoma minutum* and *Ophiostoma piceaperdum*) were observed in association with at least 40 % of beetles.

In 2000, 11 fungal species were detected in association with beetles from the locality Mt. Luzný. The fungal community was dominated by three species (yeast sp. 1, *Ophiostoma piceaperdum* and a sterile dark mycelium) which occurred in association with at least 40 % of beetles.

DISCUSSION

Many of the fungal species, especially ophiostomatoid fungi, isolated in the present study, are well known for *Ips typographus*, i.e. *Ceratocystis polonica*, *Ophiostoma bicolor*, *O. piceaperdum* (formerly *O. europhioides*), *O. minutum* (formerly *Ceratocystiopsis minuta*), *O. piceae*, *Graphium* spp. and *Leptographium* spp. (e.g. Solheim 1986; Kirschner 1994, 2001; Krokene and Solheim 1996; Yamaoka et al. 1997).

Kotýnková-Sychrová (1966) investigated fungi associated with *Ips typographus* in the Czech Republic. She isolated seven species of ophiostomatoid fungi (*Ophiostoma bicolor*, *O. minutum*, *O. penicillatum*, *O. piceae*, *O. piceaperdum*, *O. serpens* and *Graphium pycnocephalum*), but she did not pay attention to the frequency of occurrence of these fungi. In the present study we recorded 10 species of ophiostomatoid fungi and their frequency of occurrence. *Ophiostoma bicolor*, *O. minutum*, *O. piceae* and *O. piceaperdum* were found in the present study and by Kotýnková-Sychrová (1966).

Tab. 2. Fungi recovered in the mycobiota study of *Ips typographus* from the Šumava Mts. (T – total occurrence, B – body of adult, G – gallery, S – suspension, % – percentage of beetles colonised by fungi).

Species of fungi	Modrava 1999 (10 beetles)			Luzný 2000 (10 beetles)			Both localities		
	B%	S%	G%	T%	B%	S%	G%	T%	T%
basidiomycete sp. 1					10	20		20	10
Ceratocystis polonica (Siemaszko) C. Moreau			10	10	10	10	10	20	15
Cladosporium herbarum (Pers.: Fr.) Link			10	10					5
Graphium cf. *fimbriisporum* (Morelet) K. Jacobs, T. Kirisits et M. J. Wingf.	40	30	60	90	30	10	20	30	60
Graphium sp. 2							10	10	5
Leptographium sp. 1	10		30	30					15
Leptographium sp. 2					20	10	20	20	10
Mucor racemosus Fresen.			20	20					10
Ophiostoma bicolor Davidson et Wells	60	70	20	80					40
Ophiostoma piceaperdum (Rumbold) Arx	30	30	30	40	40	30	60	70	55
Ophiostoma minutum Siemaszko	20	40	40	60					30
Ophiostoma piceae (Münch) H. et P. Sydow						10		10	5
Ophiostoma sp. 1			10	10					5
Phoma sp.	10			10					5
sterile dark mycelium	10		30	30	20	60	20	60	45
sterile light mycelium	10		30	30		10	0	10	20
yeast sp. 1	90	100	50	100	90	90	80	90	95
yeast sp. 2							10	10	5
Number of species	9	5	12	13	7	9	8	11	18

At the two study sites, significant differences in the composition of the mycobiota, specifically in the occurrence of dominant species, were find, but some frequently occurring fungi (*Ophiostoma piceaperdum* and *Graphium* cf. *fimbriisporum*) were recorded at both localities. The differences in mycobiota composition are probably caused by origin or by time of sampling of beetles. The investigated beetles were sampled on one spruce stem each year. The investigated beetles were born in one maternal gallery or the fungi grew through the plant tissue (wood and bark) and colonised more beetle galleries.

The differences in mycobiota composition were compared with results by Solheim (1992) and studies by Kirschner (2001), too. In the present study, yeast sp. 1, *Graphium* cf. *fimbriisporum*, *Ophiostoma piceaperdum*, a sterile dark mycelium and *Ophiostoma bicolor* were isolated most frequently. *Ceratocystis polonica*, *Graphium* sp., *Ophiostoma penicilliatum* and *O. bicolor* were dominant species in the study by Solheim (1992); and Kirschner (2001) recorded most frequently *Ophiostoma minutum* (as *Ceratocystiopsis minutum*), *O. piceae* and *O. piceaperdum* (as *O. europhioides*), *O. japonicum* and *O. bicolor*. The men-

tioned fungal species are associated with *Ips typographus* and frequencies of their occurrence differ from case to case.

According to Solheim´s (1992) classification of fungal succession in spruce infested by *Ips typographus*, most recorded fungi (almost all species of ophiostomatoid fungi) in the present study belong to secondary or tertiary invaders. Only *Ceratocystis polonica* belongs to primary invaders. Quaternary invaders were recorded in very low frequency (e.g. *Mucor racemosus*, *Cladosporium herbarum*, *Phoma* sp.), but some of these fungi (*Cladosporium herbarum*, *Phoma* sp.) are known as saprophytes or endophytes of conifers, including spruce (Petrini and Fisher 1988, Butin and Kowalski 1990).

The method of direct isolation (beetles are taken from their galleries and directly placed on agar medium) is most frequently used to study the mycobiota of bark beetles (Kirschner 1994, 2001; Krokene and Solheim 1996; Solheim 1986; Yamaoka et al. 1997). In the present study, the mycobiota of *Ips typographus* was investigated by using an ultrasonic cleaner. Kubátová et al. (2002) employed the same method during a study of fungi associated with *Scolytus intricatus* as in the present study. We did not know if the method of using an ultrasonic cleaner was appropriate to study fungi associated with bark beetles, because Kubátová et al. (2002) recorded ophiostomatoid fungi in low frequencies. Our results are similar to observations by Kirschner (1994, 2001), Solheim (1992) and Yamaoka et al. (1997), who used the method of direct isolation and therefore the method based on using an ultrasonic cleaner is applicable for the study of the mycobiota of bark beetles.

An attention was paid to the occurrence of *Ceratocystis polonica*, because this species is known to be potentially pathogenic or pathogenic to spruce (Kirisitis 1998). In the present study, this species was detected in low frequency each year.

In 2000, a so far unidentified basidiomycete (sp. 1) (Table 2) was isolated. It was morphologically similar to *Dacryobolus ipidophyllus*, which is known from beetle galleries (Fassatiová 1954), but the full description of this species was not available to the authors of the present paper. Some other basidiomycete species (e.g. *Amylostereum* spp.) are known for their symbiosis with insects (Slippers et al. 2002) and the observed unidentified basidiomycete could be symbiotic with *Ips typographus*.

Yeast sp. 1 occurred more frequently in the suspension and on insect bodies than in galleries. Intestines of beetles could be probably its ecological niche and therefore this species occurs less frequently in galleries.

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