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Entomogenous fungi associated with spruce bark beetle *Ips typographus* L. (Coleoptera, Scolytidae) in the Bohemian Forest

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Abstract
The incidence of entomogenous fungi directly associated with adults of the spruce bark beetle and with soils closely related to sites with presence of *Ips typographus* has been monitored within a period 1999–2000. Nine species of entomogenous fungi were discovered and 69 strains have been purified and deposited into the Collection of Entomogenous Fungi. *Verticillium lecanii* was the most frequent species associated with adults caught with pheromone traps, while *Beauveria bassiana*, *Paecilomyces farinosus* and *P. fumosoroseus* were among the most frequently collected fungi from spruce bark beetle adults in bark. Seven species of fungi were collected from soil samples after baited with great wax moth larvae with dominating status of *P. fumosoroseus*. Two species, *Paecilomyces penicillatus* and *Tolypocladium nubicola* were first time recorded from central Europe. *Beauveria bassiana* and *P. fumosoroseus* were the only species that have been detected in all systems, which were monitored.

Key words: *Ips typographus*, spruce bark beetle, entomogenous fungi, *Paecilomyces fumosoroseus*, *Beauveria bassiana*, Bohemian Forest

Introduction

The spruce bark beetle *Ips typographus* L. (Coleoptera: Scolytidae) is one of the most important pests of Norway spruce (*Picea abies*) in Central and Northern Europe. Increasing bark beetle populations are reported from many localities (Wegensteiner & Weiser 1996a), including large areas of Norway spruce forests at Šumava National Park, where bark beetle calamities are recorded periodically (Zatloukal 1998). In spite of this very little is known about its naturally associated antagonists, specially about pathogens limiting bark beetle populations under normal or outbreak conditions. Observations on the occurrence of entomogenous microorganisms in the adults of bark beetle revealed evidence of an entomopoxvirus in the cells of the midgut epithelium (Wegensteiner & Weiser 1995), of the microsporidia *Chytridiopsis typographi* Weiser (Microspora, Chytridiopsida) in the midgut cells and *Nosema typographi* Weiser (Microspora, Nosematidae) in the fat body, the connective tissues and in the gonads. Furthermore, *Malamoeba scolyti* Purrini (Rhizopoda, Amoebinae, Amoebidae) was found in

the midgut blind sacs and in the Malpighian tubules, and *Gregarina typographi* Fuchs (Sporozoa, Gregarinida, Gregarinidae) was observed in the midgut lumen (WEGENSTEINER & WEISER 1996a, WEGENSTEINER & WEISER 1996b). Regardless of varying frequency depending on locality and year, *Chytridiopsis typographi* seems to be the most dominant species, whereas *N. typographi* and *M. scolyti* were recorded in only a few specimens (WEGENSTEINER & al. 1996). In addition, two entomoparasitic nematodes, *Contortylenchus diplogaster* and *Cryptaphelenchus macrogaster*, were found in adults of *I. typographus* (WEGENSTEINER & WEISER 1996b, WEGENSTEINER & al. 1996).

In general, few records are available which associate entomogenous fungi with the adults of *I. typographus*. The entomogenous fungi *Beauveria bassiana* and *Paecilomyces farinosus* (Deuteromycotina, Hyphomycetes, Moniliales) were recorded as pathogens naturally associated with the adults of spruce bark beetle (VAUPEL & ZIMMERMANN 1996). In contrast, more than 700 species of fungi, mostly Hyphomycetes (Deuteromycotina) and Entomophthorales (Zygomycotina) from about 90 genera, are known as insect pathogens (CHARNLEY 1989). Genera that have been most often recorded as pathogenic to various species of scolytid beetles include *Beauveria*, *Metarhizium*, *Paecilomyces*, *Tolypocladium,* and *Verticillium* (DOBERSKI 1981a, 1981b, HOULE & al. 1987).

The aim of this study was to start introductory investigations on the occurrence of filamentous entomopathogenic fungi (Deuteromycotina, Hyphomycetes, Moniliales) directly associated with adults of *I. typographus* or in soils at sites closely related with presence of this pest in the Šumava National Park.

MATERIAL AND METHODS

Insect baiting

Incidence of entomogenous fungi directly associated with the adults of spruce bark beetle and with soils closely related to sites with presence of *I. typographus* has been monitored during the years 1999 and 2000. The insect baiting systems described below constituted the primary sampling protocols of project:

a) The assay for entomogenous fungi associated with the adults caught within pheromon traps

Adults were removed from pheromone traps and stored in sterile plastic vials (8±1°C). Individual adults were positioned on the surface of 2% water agar in sterile Petri dishes (Fig. 1/1). Dishes were sealed with parafilm and were placed in an air-conditioned room (25±1°C, photoperiod 12/12 hr) to incubate for 5–7 days. When superficial growth of fungi was visible anywhere the integument the adult was processed further (see below). More than 800 adults collected from 26 different pheromone traps at 17 sites were evaluated.

b) The assay for entomogenous fungi associated with the adults collected from bark of spruce trees

Three bark samples (approx. 10×20 cm) were cut off from tree colonized with *I. typographus* and put into sterile plastic boxes with moist cotton in the lid of sterile Petri dishes. Containers were placed in the air-conditioned room described above for 5–7 days. After this incubation period, adults were removed from the bark, positioned on surface of 2% water agar in Petri dishes and processed identically to those collected in pheromone traps. A total of 56 trees on various sites across the Šumava National Park were evaluated (1999: 27 trees, 2000: 29 trees).

c) Presence of entomogenous fungi in soils

Soil samples were collected at sites closely related to *I. typographus* (directly under and close around colonized tree). Each sample consisted of 5–8 partial sub-samples collected at each site. Samples were stored in sterile plastic bags in a refrigerator (8±1°C) until processed in the laboratory. A modified "galleria bait method" (ZIMMERMANN 1986) was used for indirect isolation of entomogenous fungi. Synchronized populations of greater wax moth (*Galleria mellonella* L.) larvae were reared on Haydak's artificial diet (SKUHRAVÝ 1968). Homogenized soil samples were placed in sterile Petri dishes (40 ml of soil per Petri dish, 90 mm in diameter), moistened with sterile distilled water and the 3rd instar wax moth larvae (15 larvae of uniform size, 2 replicates per soil sample) were added and incubated for 14 days at 25±1°C. During the period of incubation, samples in containers were agitated gently and repositioned periodically to ensure that larvae remained exposed to the soil. All samples were evaluated on day 7, 10 and 14. When external growth of fungi was visible anywhere on the integument of the insect bait, cadavers were removed and placed into a new sterile Petri dish (Fig. 1/2) and incubated for another 3–5 days under conditions that favor growth and sporulation of most of entomogenous fungi (25°C, photoperiod 12/12 hrs). After 14 days of exposure, all remaining larvae that had no visible symptoms of mycosis, were removed from soil, immersed for 3 minutes into 1% solution of sodium hypochlorite for surface disinfection, then dipped into sterile distilled water for another 2–3 minutes and then placed on wet filter paper in sterile Petri dish for further incubation (5–7 days). If no external proliferation of fungi was observed on the cuticle of these larvae by the end of this incubation period, the soil sample was classified as having no entomogenous fungi present.

PURIFICATION AND IDENTIFICATION OF FUNGAL ISOLATES

Once, adequate growth of fungus was observed on cadavers (see Figs. 1/1–2) fungi were transferred to potato-dextrose agar plates (PDA, Difco) with a sterile needle. If necessary, subculturing was repeated until pure cultures were obtained. Purified isolates of fungi were identified after seven days of incubation at 25°C in the dark (KREISEL & SCHAUER 1987, FASSATIOVÁ 1979). When necessary, a slide mounting technique (GOETTEL & INGLIS 1997) or cultivation based on inoculation of small 2% agar blocks sandwiched and incubated between two sterile cover slips (HARRIS 1986) were used and fungi were determined using light microscope (BISSETT 1983, DOMSCH, DOMSCH & al. 1980, HUMBER 1997, SAMSON 1974).

LONG-TERM MAINTENANCE OF COLLECTED FUNGAL STRAINS

For long-term storage, purified cultures of all strains collected during this study were processed into alginate pellets (PEREIRA & ROBERTS 1991, EYAL & al. 1994). Pure cultures of each strain were grown on PDA plates first (Fig. 1/3), followed with production of biomass that was produced in submerged culture (PD broth, laboratory shaker, 300 rpm, 25°C, 3–5 days cultivation) and formulated by mixing with ground wheat bran (30%) and 1% aqueous sodium alginate (SIGMA). The mixture was stirred until the suspension was evenly dispersed. Adding drops of this mixture into coagulation bath of 0.25M aqueous $CaCl_2$ produce fresh pellets (Fig. 1/4). After 20 minutes in the $CaCl_2$ coagulation bath, fresh pellets were rinsed with sterile distilled water and air-dried. Dry pellets were placed into sterile plastic vials before storing them in a deep freezer (–23±1°C). Standard viability tests (EYAL & al. 1994) were

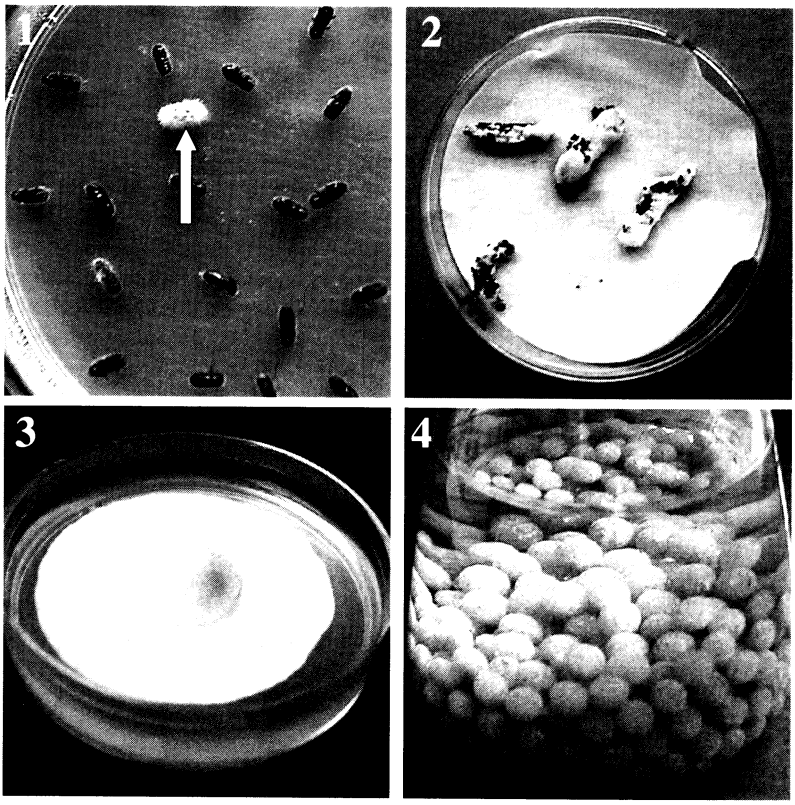

Fig. 1. – Demonstration of methodical aspects: 1 – fungus closely associated with adult of spruce bark beetle, 2 – external growth of fungi on cuticle of the *G. mellonella* larvae, 3 – stock culture of *Paecilomyces fumosoroseus* on PDA, 4 – new alginate pellets with incorporated biomass of collected strains of entomogenous fungi (photo by Z. Landa & P. Horňák, digital photography)

used prior to depositing pelletized strains into the Collection of Entomogenous Fungi (CEF, Department of Plant Production, Faculty of Agriculture, University of South Bohemia, České Budějovice). Twenty-five randomly chosen dry pellets were aseptically plated on a solid surface of 2% water agar in Petri dishes (square style grid – 5 rows each of 5 pellets) and incubated at 25°C for 3–5 days. The number of pellets covered with fungal mycelium and sporulation were determined before pellets were labeled as viable and deposited for long-term maintenance. All the strains reported on in this paper were deposited as separate sub-collection within the CEF.

Results

A total of 124 samples were collected at 65 sites in the Šumava National Park during the period from April 1999 to November 2000. In these samples, 69 strains of entomogenous fungi representing 9 species and 4 genera (anamorphs associated with Eurotiales and Hypocreales) of deuteromycetous fungi were discovered (Table 1, Figs. 2 & 3). In coherence with used insect baiting systems, the results of monitoring might be briefly summarized as follows.

More then 800 adults of spruce bark beetle were caught and processed that were collected

Table 1. – An overview of strains of the entomogenous fungi collected from various sources in the Šumava National Park in 1999–2000.

Species	Source			Total number
	Pheromone trap	Spruce bark	Soil	
Beauveria sp.	0	3	0	3
Beauveria bassiana (Bals.-Criv.) Vuill.	1	8	1	10
Paecilomyces sp.	0	3	0	3
Paecilomyces farinosus (Holm ex S.F. Gray) A.H.S. Brown et G. Smith	0	7	2	9
Paecilomyces fumosoroseus (Wize) A.H.S. Brown et G. Smith	1	6	10	17
Paecilomyces javanicus (Friederichs et Bally) A.H. S. Brown et G. Smith	0	0	3	3
Paecilomyces penicillatus (Höhnel) Samson*	0	1	0	1
Tolypocladium cylindrosporum W. Gams	0	0	1	1
Tolypocladium niveum (Rostr.) Bissett	0	0	6	6
Tolypocladium nubicola Bissett*	0	0	2	2
Verticillium lecanii (Zimm.) Viegas	13	1	0	14
Total amount of strains / source	15	29	25	69

* First time detected in the Czech Republic

Table 2. – Occurrence of the entomogenous fungi naturally associated with the adults of *Ips typographus* collected from pheromone traps in the Šumava National Park (year 2000).

Location	Site	Date[1]	Number of adults		Isolated fungus[2]
			Total	Infected	
České Žleby	Medvědice	April 28	95	12	*V. lecanii*
České Žleby	Medvědice	April 28	99	19	*V. lecanii*
České Žleby	Medvědice	April 28	71	8	*V. lecanii*
Strážný	Kunžvart	May 11	110	19	*V. lecanii* *B. bassiana*
Železná Ruda	Plesná	May 11	14	1	*V. lecanii*
Modrava	Sokol	May 30	13	2	*V. lecanii*
Rejštějn	Buzošná	June 2	39	2	*V. lecanii*
Srní	Tmavý potok	June 2	38	2	*V. lecanii*
Rejštejn	Pěnivý potok	September 13	4	1	*V. lecanii*
Srní	Tmavý potok	September 13	4	1	*V. lecanii*
Srní	Tmavý potok	September 13	2	1	*V. lecanii*
Modrava	Novohradské močály	September 13	65	5	*V. lecanii*
Modrava	Novohradské močály	September 27	21	4	*V. lecanii*
Modrava	Novohradské močály	September 27	55	1	*P. fumosoroseus*

[1] Date when adults of spruce bark beetle were collected from pheromone traps
[2] Pure cultures of strains deposited in culture collection of entomogenous fungi of the University of South Bohemia

Table 3. – The entomogenous fungi naturally associated with the adults of *Ips typographus* collected from infested spruce trees in the Šumava National Park (year 1999).

Location	Source	Date[1]	Isolated fungus	Isolate[2]
Modrava	wind-damaged area, decayed tree	June 10	*Paecilomyces farinosus*	Pfa 1
Modrava	forest, broken tree	June 10	*P. fumosoroseus*	Pfr 2
Modrava	clearing, dead tree	June 10	*Beauveria bassiana*	Bba 3
Stožec	forest, decayed tree	June 10	*P. fumosoroseus*	Pfr 4
Modrava	wind-damaged area, decayed tree	June 3	*P. farinosus*	Pfa 5
Modrava	forest, trap-tree	May 6	*B. bassiana*	Bba 6
Strážný	decayed tree	June 29	*Beauveria* sp.	Bsp. 7
Modrava	decayed tree	June 29	*P. farinosus*	Pfa 8
Kvilda	trap-tree	June 29	*Verticillium lecanii*	Vle 9
Kvilda	decayed tree	June 29	*P. fumosoroseus*	Pfr 10
Kvilda	trap-tree	June 29	*B. bassiana*	Bba 11
Kvilda	decayed tree	June 29	*B. bassiana*	Bba 12
Modrava	tree trap	July 22	*B. bassiana*	Bba 13
Modrava	wind-damaged area, decayed tree	June 3	*Beauveria* sp.	Bsp.14
Modrava	trap-tree	June 29	*P. farinosus*	Pfa 15
Modrava	trap-tree	June 29	*P. fumosoroseus*	Pfr 16
Kvilda	decayed tree	September 15	*Paecilomyces* sp.	Psp. 17
Stožec	chop down tree	September 30	*Beauveria* sp.	Bsp.18

[1] Date when bark samples with adults of *I. typographus* were collected at particular site
[2] Pure cultures of strains deposited in culture collection of entomogenous fungi of the University of South Bohemia

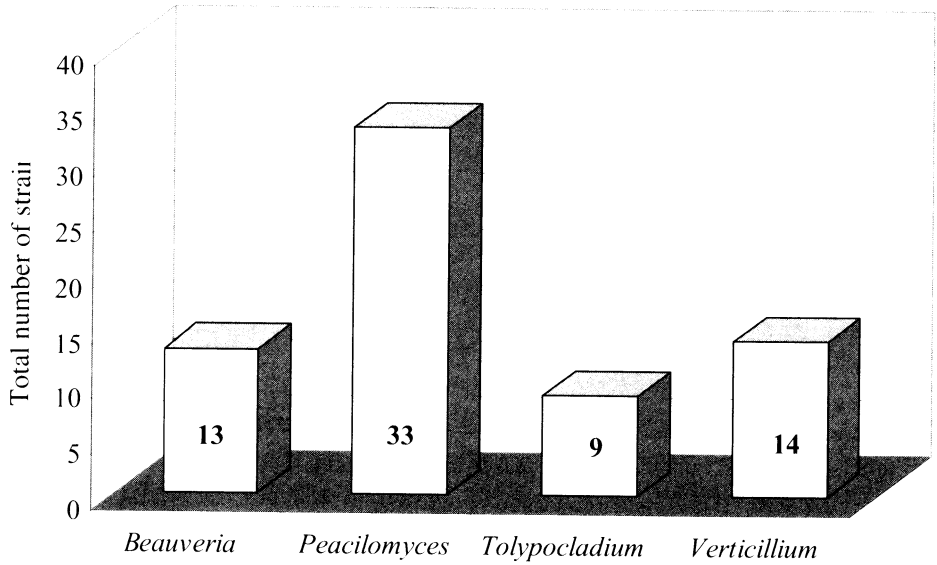

Fig. 2. – An overview of genera structure of the entomogenous fungi associated with the spruce bark beetle *I. typographus* in the Šumava National Park.

Table 4. – The entomogenous fungi naturally associated with the adults of *Ips typographus* collected from spruce trees in the Šumava National Park (year 2000).

Location	Site	Date [1]	Source [2]	Number of adults	
				Total	Infected
Borová Lada	Buková slať	May 2	4	6	6
Borová Lada	Buková slať	May 3	4	17	3
Strážný	Kužvart	May 3	4	10	3
České Žleby	Medvědice	May 3	3	12	4
Plešný	Trojmezná	May 16	2	13	2
Plešný	Trojmezná	May 16	2	32	2
Železná Ruda	Plesná	May 16	5	10	1
Borová Lada	Buková slať	May 16	1	20	3
Modrava	Novohuťské močály	May 30	1	20	1
Borová Lada	Buková slať	May 30	1	15	1
Borová Lada	Buková slať	May 30	3	30	2
Rejštejn	Pěnivý potok	May 30	1	21	2
Modrava	Sokol	May 30	1	4	1
Rejštejn	Pěnivý potok	May 30	1	21	6
Modrava	Novohuťské močály	September 27	2	6	1
Modrava	Novohuťské močály	September 27	4	4	1

[1] Date when bark samples with the adults of *I. typographus* were collected at particular site
[2] Bark samples collected from: 1 – trap tree, 2 – newly infested tree, 3 – broken tree at wind-damaged area, 4 – decayed tree, and 5 – chop down tree before bark off

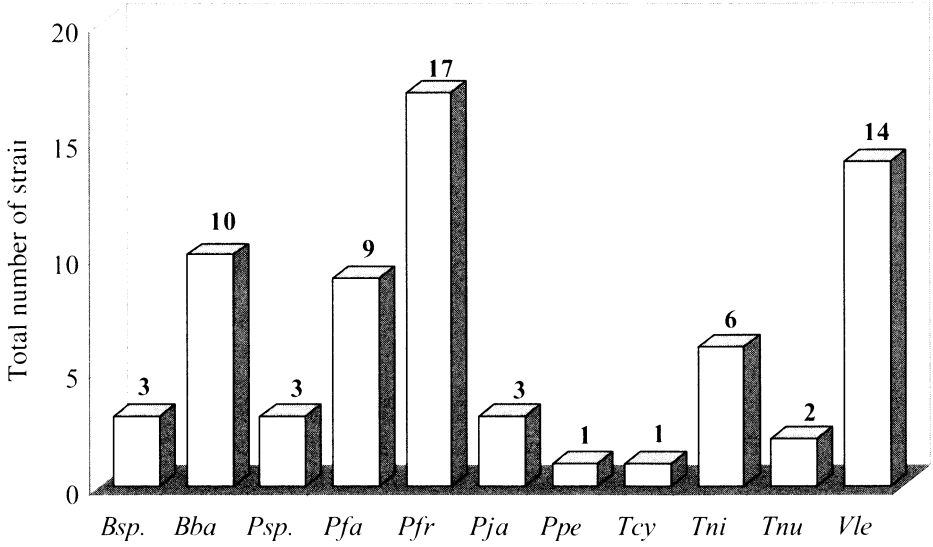

Fig. 3. – An overview of species structure of the entomogenous fungi associated with the spruce bark beetle *I. typographus* in the Šumava National Park.

in 26 pheromone traps at 19 sites during the period from March–September 2000. Entomogenous fungi were discovered on adults collected in 14 traps (53.8%). *Verticillium lecanii* was the dominant species isolated from adults collected in 13 pheromone traps with only single strains of *Beauveria bassiana* and *Paecilomyces fumosoroseus* being isolated for a total of 15 strains collected overall (Table 2).

The occurrence of entomogenous fungi within a population of *I. typographus* adults collected from spruce bark was monitored during this two-year study. More than 150 spruce bark samples from a total of 56 trees were collected from various sources during the period from June 1999 to September 2000. Entomogenous fungi were discovered in 18 of the 27 trees (66.7%) that were evaluated in 1999. After being processed in the laboratory, 18 strains of entomogenous fungi were isolated from the surface of infected beetles: *Beauveria bassiana* (5 strains), *Paecilomyces farinosus* (4 strains) *P. fumosoroseus* (4 strains) and *V. lecanii*

Table 5. – The entomogenous fungi isolated from soil samples using "galleria bait method" at various sites of the Šumava National Park (period September – November 2000).

Location – site	Source[1]	Infected larvae[2]			Isolated fungus
		Day 7	Day 10	Day 14	
Rejštejn – Zhůřská Slať	1	–	–	+	*Tolypocladium niveum*
Modrava – Novohradský močál	2	–	–	+	*T. niveum*
Plešný – Trojmezná	2	–	–	+	*Paecilomyces farinosus*
Plešný – Trojmezná	2	–	–	+	*T. niveum*
Plešný – Trojmezná	2	–	–	+	*P. javanicus*
Plešný – Trojmezná	2	–	–	+	*P. fumosoroseus*
Plešný – Trojmezná	2	–	–	+	*P. fumosoroseus*
Plešný – Kaliště	3	–	–	+	*P. javanicus*
Plešný – Kaliště	4	–	–	+	*P. javanicus*
Plešný – Kaliště	4	–	–	+	*P. fumosoroseus*
Plešný – Smrčina	4	–	–	+	*P. fumosoroseus*
Plešný – Smrčina	4	–	–	+	*P. fumosoroseus*
Plešný – Smrčina	4	–	–	+	*P. fumosoroseus*
České Žleby – Medvědice	4	–	–	+	*T. nubicola*
Strážný – Častá	3	–	+	–	*T. niveum*
Modrava – Modravská stráň	3	–	–	+	*T. nubicola*
Modrava – Modravská stráň	3	–	–	+	*P. fumosoroseus*
Modrava – Modravská stráň	3	–	–	+	*P. fumosoroseus*
Strážný – Kunžvart	2	–	+	+	*T. niveum*
Strážný – Kunžvart	2	–	+	+	*T. cylindrosporum*
Strážný – Kunžvart	2	–	–	+	*P. fumosoroseus*
Modrava – Sokol	3	–	–	+	*P. fumosoroseus*
Modrava – Sokol	2	–	–	+	*T. niveum*
Kvilda – Nad Vltavskou cestou	4	–	–	+	*Beauveria bassiana*
Kvilda – Nad Vltavskou cestou	4	–	–	+	*P. farinosus*

[1] Soil samples were taken: 1 – under healthy spruce tree, 2 – under spruce tree newly infested with the bark beetle, 3 – under dead spruce tree, and 4 – under chop down and bark-off spruce tree

[2] Superficial growth of mycelium recorded (+) or not recorded (–) on surface of the integument of *G. mellonella* larvae

(1 strain). Four additional strains were collected but were not determined to the species level (*Beauveria* sp. – 3, *Paecilomyces* sp. – 1) (Table 3).

Similarly, the entomogenous fungi were discovered on the *I. typographus* adults in bark samples in 16 of the 29 trees (55.2%) that were collected during the period from May to September 2000 (Table 4). After being processed in the laboratory, 11 strains of entomogenous fungi were isolated from the surface of infected beetles: *Beauveria bassiana* (3 strains), *P. farinosus* (3 strains), *P. fumosoroseus* (2 strains), *Paecilomyces penicillatus* (Höhnel) Samson (1 strain) and two *Paecilomyces* sp. that have not been determined to species level yet.

Finally, 42 soil samples were collected and processed in the laboratory using the "galleria bait method". A total of 25 entomogenous strains of fungi from 7 different species were re-isolated from cadavers of *G. mellonella* larvae: *P. fumosoroseus* (10 strains), *P. farinosus* (2 strains), *P. javanicus* (3 strains), *Tolypocladium niveum* (6 strains), *T. nubicola* (2 strains), *T. cylindrosporum* (1 strain), and *B. bassiana* (1strain) (Table 5). Eight other isolates were collected, but have not been determined to the species level yet. Discovery of *Paecilomyces penicillatus* (1 strain) and *Tolypocladium nubicola* (2 strains) represent the first records of these species in the Czech Republic.

A separate sub-collection, which consists of the 69 strains of various entomogenous fungi, isolated during this project was established within the Collection of Entomogenous Fungi at USB. Each item represents pure biomass of the strain of the species, which was formulated as dry alginate pellets (approximately 300–400 pellets per strain). When assessed using an *in vitro* assay, all pellets were fully viable (growth of mycelium and sporulation) and all items, which were deposited in CEF are available for further study.

Discussion

Scolytid bark beetles that colonize living conifers are well known as frequently associated with specific fungi that are carried in specialized structures on the body surface called mycangia. Mycangia are cuticular structures that function to harbor fungal spores and mycelia of specific blue-stain fungi. These fungi are usually introduced into the tree during the attack process. The continuing association suggests that there is mutual benefit to the fitness of both beetles and fungi (Paine & al. 1997). The spruce tree pathogenic fungus *Ceratocystis polonica* and other blue-stain fungi (e.g. *C. coerulescens, C. minor, Ambrosiella* sp., *Ophiostoma bicolor,* and *Ophiostoma penicillatum*) were isolated with high frequency from the inoculations with *I. typographus* (Krokene & Solheim 1996, Paine & al. 1997). The hypothesis was confirmed (Krokene & Solheim 1997, Krokene & Solheim 1998) that aggressive bark beetle species (including *I. typographus*) vector virulent fungi that may help them to kill trees, but also some non-aggressive bark beetles might vector pathogenic fungi.

Compared with association of spruce bark beetle with tree pathogenic blue-stain fungi, very little is known about the association of scolytid beetles with other groups of fungi, including entomopathogenic species. First, entomogenous fungus *Beauveria bassiana* has been recorded on *Scolytus multistriatus* (Doane 1959) and *S. scolytus* (Doberski 1981a). Also, *Cordyceps militaris, Hirsutella thompsonii, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces farinosus, Paecilomyces fumosoroseus,* and *Verticillium lecanii* were found as pathogenic to *Scolytus scolytus* (Doberski 1981a, 1981b) or *S. multistriatus* (Houle & al. 1987). However, *B. bassiana* only has been recorded on spruce bark beetle and tested in combination with pheromone traps for pathogenicity and the possibility to auto-disseminate and induce epizootics within a population by contaminated adults of *I. typographus* (Vaupel & Zimmermann 1996).

In contrast to sporadic records, five species of entomogenous fungi (*B. bassiana, P. farino-*

sus, *P. fumosoroseus*, *P. penicillatus*, and *V. lecanii*) with many separate isolates were collected directly from adults of spruce bark beetle and another three species of entomogenous fungi (*Tolypocladium cylindrosporum*, *T. niveum*, and *T. nubicola*) were collected from the soil samples. However, *B. bassiana* and *P. fumosoroseus* were the only species collected from all monitored systems (adults from pheromone traps or bark and soil samples).

Three species of entomogenous fungi (*B. bassiana*, *P. fumosoroseus*, and *V. lecanii*) were found to be auto-disseminated along with the *I. typographus* adults that were caught by pheromone traps, but *V. lecanii* was the only species, which was detected regularly. Fourteen strains of *V. lecanii* have been collected and formulated into alginate pellets for long-term storage. The evaluation of viability and virulence based on standard set of *in vitro* and *in vivo* assays has been conducted with the strain Vle-9, including RAPD fingerprinting. Preliminary results indicate that Vle-9 had excellent viability and was virulent to a number of different insect hosts (LANDA & al. in press). In general, *V. lecanii* is already well known and used for routine biological control of many insect pests on various greenhouse crops. Several bio-insecticides based on *V. lecanii* are already registered (e.g. Mycotal®, Vertalec®) (WRIGHT & CARRUTHERS 1999). Similarly, a biopreparation containing the fungus *P. fumosoroseus* (strain PFR 97-Apopka) is registered in the USA (PFR™ 20%WDG) and the EU (PreFeRal®) and used in routine biological control program against various greenhouse pests (e.g. whiteflies, aphids, thrips and coccids), as well as some important insect pests of field crops (OSBORNE & LANDA 1992). Myco-insecticide containing fungus *B. bassiana* (Boverol®) was already used to induce epizootics in populations of *I. typographus* using pheromone traps to disseminate spores by contaminated adults (VAUPEL & ZIMMERMANN 1996).

Five species of fungi (*B. bassiana*, *P. farinosus*, *P. fumosoroseus*, *P. penicillatus*, and *V. lecanii*) were isolated from adults of *I. typographus* after beetles were collected from bark. *B. bassiana* (with 8 strains), *P. farinosus* (7), and *P. fumosoroseus* (6) were the most dominat species among those collected. Furthermore, a single strain of *Paecilomyces penicillatus* was discovered, which is the first record of this species on scolytid beetles in general. Both *B. bassiana* and *P. fumosoroseus* performed like typical entomogenous fungi, which penetrate host cuticle, develops inside the host and completes its developmental cycle with external growth and sporulation on the dead host (Figs. 4/1–9). Direct association of diverse species of the entomogenous fungi with the adults in bark indicates mechanisms that might be utilized to control this pest using the endemic natural enemies. Again, preliminary experiments were conducted, and fungal infections of spruce bark beetle adults were induced with endemic strains of *B. bassiana*, *V. lecanii*, and *P. fumosoroseus* after application of suspensions of conidia to the surface of trap-trees. Otherwise the adults of spruce bark beetles were infected later in bark, after being contaminated with spores, which were applied on surface of three trap logs (LANDA & al. – unpubl.).

Seven species of entomogenous fungi were identified from soils collected at sites related with occurrence of spruce bark beetle after baiting with great wax larvae. Among these, *P. fumosoroseus* was the most dominant species (10 strains), followed by *Tolypocladium niveum* (6). The abundance of *P. fumosoroseus*, together with its presence in all other monitored systems indicates the importance and potential of this entomogenous fungus. All other species collected from soil samples were detected sporadically, however, some records are unique, because it was the first time when certain species were detected in the Czech Republic (*Paecilomyces penicillatus* – 1 strains, *Tolypocladium nubicola* – 2 strains). Furthermore, when compared with pheromone traps and bark, soil was the only monitored system, where species of the genus *Tolypocladium* were detected. All three discovered species, *T. nubicola*, *T. cylindrosporum*, and *T. niveum* are known as larval pathogens of mosquitoes (WEISER 1991, WEISER & MAŤHA 1988a, WEISER & MAŤHA 1988b). However, those fungi were collected after

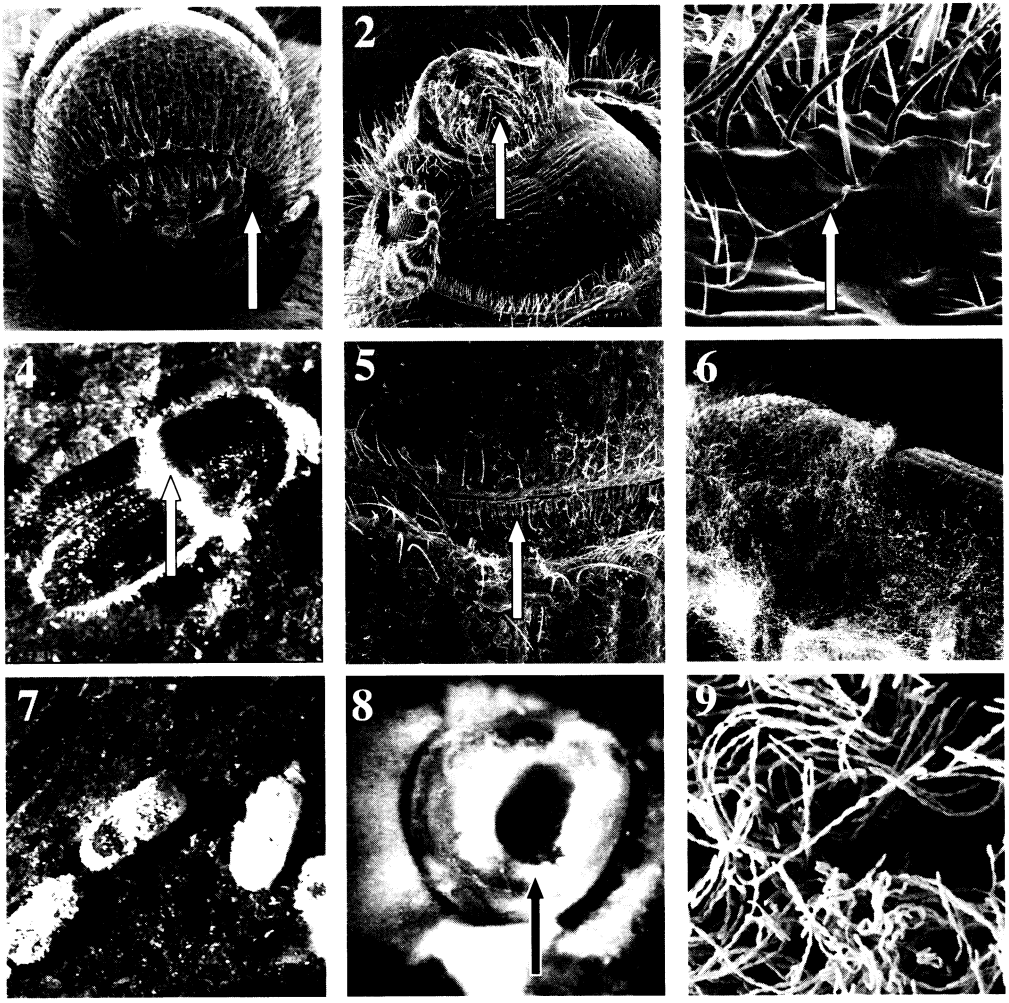

Fig. 4. – Development of entomogenous fungus *Paecilomyces fumosoroseus* on adult of bark beetle *Ips typographus*: first symptoms of fungal infection are usually detectable at the anterior of prothorax (1) and around mouth parts (2), infection proceeds with occurrence of separate hyphae (3) followed by creation of superficial mycelial net on the integument of infected host (4-5, posterior of prothorax) and finished when dense mycelium overgrow host cuticle (6-7). Dead host is mummified (8 – haemocoel filled with mass of mycelia) and cycle is completed when newly born conidia are created on an aerial mycelium (9) (photo by Z. Landa & P. Horňák, SEM and digital photography)

baiting the soil with the alternative host and there is not evidence yet, that these fungi might induce infection of any developmental stage of spruce bark beetle.

The results of this introductory survey of the entomogenous fungi directly or indirectly associated with the spruce bark beetle indicate that those microorganisms might play an important role in limiting populations of this pest under normal or outbreak conditions. Furthermore, this study might be considered as a contribution to the overall biodiversity of the Šumava National Park, the unique area of the Bohemian Forest.

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