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Survey of fungal species vectored by *Ips cembrae* to European larch trees in Raciborskie forests (Poland)

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The species composition of fungi associated with *Ips cembrae* was studied in the Raciborskie forests, Poland. The fungi were isolated from overwintered adults, larvae, new adults and from galleries at various stages of development. The results showed that there was a great diversity of fungi associated with this insect. We isolated 2877 cultures, including 61 species. The most important group of fungi were ophiostomatoid species. From these, *Ceratocystis laricicola*, *Ophiostoma brunneo-ciliatum* and *Graphium laricis* were commonly detected, whereas eight other species occurred less frequently or sporadically. *Ophiostoma ips* is documented here as a new associate of *I. cembrae*. *Ceratocystis laricicola* was shown to be the primary invader occurring most frequently at early stages of brood development, particularly in the sapwood. *Ophiostoma brunneo-ciliatum* and *G. laricis* were secondary invaders following *C. laricicola*. In the later stages of brood development other ophiostomatoid fungi appeared.

Key words: ophiostomatoid fungi, *Ips cembrae*, *Larix decidua*, fungi associated with bark beetles

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Druhové složení hub vázaných na kůrovce *Ips cembrae* bylo studováno v Raciborských lesích v Polsku. Houby byly izolovány z přezimujících brouků, larev, mladých brouků a z jejich chodbiček v různých stádiích vývoje. Výsledky ukázaly, že diverzita hub vázaných na tohoto brouka je velká. Izolovali jsme 2877 kmenů reprezentujících 61 druhů hub. Nejdůležitější skupinou byly ophiostomatální houby. Z nich byly nejčastěji nalezeny druhy *Ceratocystis laricicola*, *Ophiostoma brunneo-ciliatum* a *Graphium laricis*, zatímco dalších 8 druhů se vyskytovalo méně často až sporadicky. *Ophiostoma ips* je zde dokumentována jako nový průvodce kůrovce *I. cembrae*. Druh *Ceratocystis laricicola* se ukázal být primárním kolonizátorem, vyskytujícím se v ranných stádiích vývoje jeho potomstva, zejména v bělovém dřevě. *Ophiostoma brunneo-ciliatum* a *G. laricis* následovaly po *C. laricicola* jako sekundární kolonizátoři. V pozdějších stádiích vývoje potomstva se objevily další druhy ophiostomatálních hub.

INTRODUCTION

In Europe, the large larch bark beetle, *Ips cembrae* (Heer) (Coleoptera: *Scolytidae*) infests mainly *Larix decidua* Mill., but species of the genera *Pinus*, *Picea* and *Abies* may also be affected (Michalski and Mazur 1999). It is one of the most destructive insect pests of larch plantations and natural stands in Poland. The beetles breed in logs, wind-blown stems and dying trees, but at higher population densities also attack healthy trees. These insects have one or two generations per year. Adults from the first generation may fly in May, whereas the second generation initiates flying in July or August.

Phloem-feeding bark beetles are closely associated with various fungi that are transmitted in specialised structures or on the body surface. The most common mycelial fungi associated with bark beetles belong to the ascomycete genera *Ophiostoma* and *Ceratocystis*. Among these are species causing blue-stain in the sapwood of conifers and some of them can be pathogenic to the host plant, for example *Ophiostoma novo-ulmi* Brasier (Harrington 1993).

Several reports on the fungi associated with *Ips cembrae* have been published in Europe (Redfern et al. 1987, Kirisits et al. 2000, Kirisits 2001, Jacobs et al. 2003, Aghayeva et al. 2004, Kirisits 2004a, Kirisits 2004b) and Asia (Pashenova et al. 1995, Westhuizen et al. 1995, Peng et al. 1996, Yamaoka et al. 1998). No report has been published about mycobiota associated with *I. cembrae* in Poland. *Ceratocystis laricicola*, *Graphium laricis* and *Ophiostoma brunneo-ciliatum* were dominant fungal species in Europe. Among them, *C. laricicola* belonging to the *Ceratocystis polonica* (Siemaszko) C. Moreau species complex (Marin et al. 2005) is the most virulent species and probably plays an important role in tree death following attack by *I. cembrae* (Yamaoka et al. 1998, Kirisits 2001).

In 1992, a fire destroyed 9060 ha of forest in the forest sections Rudy Raciborskie, Rudziniec and Kędzierzyn of the Raciborskie forests, Poland. After the fire, larch seedlings were planted at the reclaimed fire site. Dead trees were observed twelve years after the fire. Galleries of *Ips cembrae* were found on dead and declining larch trees.

In this study, species composition and occurrence frequency of fungi associated with *Ips cembrae* on European larch were investigated. The fungi were isolated from beetle bodies and galleries at various stages of the beetle life cycle.

MATERIAL AND METHODS

Sampling area

The study was conducted in the Rudy Raciborskie Forest District (SW Poland, Śląskie voivodeship, close to Racibórz, Bargłówka Forest Range, compartments 68g and 69b; 50° 12' 34" N, 18° 26' 57" E) in 2005. All materials were collected from 15-year old stands composed of European larch (*Larix decidua*) and Scots pine (*Pinus sylvestris* L.), where the larch was the dominant species. The diameters at breast height of the *L. decidua* trees ranged from 10 to 11 cm and the trees' average height was about 9 m.

Serious tree damage in these stands was mainly caused by *Ips cembrae* and moth *Coleophora laricella* (Hübn.). In order to determine the frequency and diversity of fungi associated with *I. cembrae*, trap trees infested by this insect were examined.

Insect and larvae collection

Overwintered adults. The beetles were collected in pheromone traps with commercially prepared CEMBRODOR® during their flight period (15–26 April). In total, 189 adults were collected and stored individually in sterile microtubes (1.5 ml) for later isolations.

Larvae and new adults. On 15 April, eight uninfested larch trees were felled. They were laid flat on the forest floor and left for colonisation by *Ips cembrae*. Four and six weeks after the main insect attack, 102 larvae and 44 new adults, respectively, were collected from insect galleries. Larvae and beetles were stored individually in sterile microtubes.

Sampling of gallery systems. Samples were taken from gallery systems found on felled trees two, four and eight weeks after the main attack. Samples taken two weeks after the main insect attack represented early stages of brood development, whereas in samples taken two weeks later larvae were present. In samples taken eight weeks after the main insect attack, young adults of *Ips cembrae* had already emerged from the galleries.

Culture procedures

Medium used for fungus isolations. All isolations were made on 2 % malt extract agar (2 % MEA; 20 g malt extract, 20 g agar, 1000 ml distilled water) supplemented with antibiotic tetracycline (200 mg per 1 litre of culture medium) to inhibit bacterial growth. Dishes were incubated at 20 °C for up to 30 days, in the dark, and checked daily for fungal growth. The fungal species were identified according to morphological and physiological characteristics using classical microbial techniques.

Isolation from gallery systems. Four 30-cm long sections with intact bark were cut from parts of the trunks infested by *Ips cembrae*. They were cut from the part of trunk located between 2 and 6 m from the base. In the laboratory the sections were cut into five discs, each of them six cm thick. The bark was separated from the wood under sterile conditions. Gallery fragments were disinfected with cotton wool saturated with 96 % ethanol, which covered the samples for 15 seconds, and dried with filter paper. Fungi were isolated from phloem in the female and larval galleries of *I. cembrae*, discoloured tissues surrounding the galleries and also from the outer sapwood layer, up to a depth of 20 mm. A surface layer of phloem was removed aseptically and 4 × 4 mm large fragments of phloem or sapwood were cut and placed on malt agar in Petri dishes. In total, 900 plant pieces collected from 75 gallery systems of *I. cembrae* were used in this study.

Isolation from insects. Within 12 hours of their collection, overwintered adults, larvae and new adults were placed in Petri dishes containing malt agar medium. Beetles and larvae, without surface sterilisation, were crushed using sterile tweezers.

Frequency

Frequencies were computed using the following formula: F = (NF/NT) × 100, where F represents the frequency of occurrence (%) of each fungal species, NF represents the number of beetles, larvae or plant fragments, from which a particular fungus was isolated and NT represents the total number of a particular kind of sample from which fungi were attempted to isolate. Fungus frequencies were computed separately for phloem and sapwood fragments.

RESULTS

Fungus isolation from larvae and beetles

In this study, 646 fungus isolates were obtained from larvae and beetles. The most important group were ophiostomatoid fungi, represented by seven species: *Ceratocystis laricicola*, *Ceratocystiopsis minuta*, *Ophiostoma brunneo-ciliatum*, *O. piceae*, *Graphium laricis*, *Pesotum* sp. 1 and *Pesotum* sp. 2 (Tab. 1). *Ophiostoma brunneo-ciliatum* was the most commonly isolated fungus (isolated from 26.5 to 85.3 % of larvae and beetles). *Graphium laricis* was also isolated very often (45.5–75.5 %), except overwintered adult beetles (1.6 %). *Pesotum* sp. 1 was isolated at medium frequency only from larvae, whereas *Ophiostoma piceae* occurred most frequently on bodies of new adults (Tab. 1).

Generally, ophiostomatoid fungi were isolated from overwintered adults less frequently than from larvae and new adults. However, overwintered beetles were

more infested by other fungi. Among them, *Cladosporium cladosporioides*, *Penicillium* spp., *Mucor* spp., *Hormonema dematioides* and *Ceuthospora* cf. *pinastri* were most abundant. Fungi from the *Mucor* genus were also often isolated from new adults of *Ips cembrae* and *Penicillium* species from its larvae (Tab. 1).

Tab. 1. Fungi isolated from *Ips cembrae* adults and larvae.

Fungi	Frequencies of occcurrance (%), isolated from[1]		
	OA	L	NA
Ophiostomatoid fungi			
Ceratocystiopsis minuta (Siemaszko) H.P. Upadhyay & W.B. Kendr.		3.9	4.5
Ceratocystis laricicola Redfern & Minter	2.1	2.0	2.3
Graphium laricis K. Jacobs, Kirisits & M.J. Wingf.	1.6	75.5	45.5
Ophiostoma brunneo-ciliatum Math.-Käärik	26.5	85.3	75.0
Ophiostoma piceae (Münch) Syd. & P. Syd.		6.9	11.4
Pesotum sp.1		28.4	6.8
Pesotum sp. 2	1.1		
Other species			
Acremonium strictum W. Gams	0.6		
Acremonium of *Cordyceps militaris* L.			2.3
Alternaria alternata (Fr.) Keissl.	5.8		
Apiospora montagnei Sacc.	5.8		2.3
Aspergillus niger Tiegh.	0.6		
Botrytis cinerea Pers.	3.7		
Ceuthospora cf. *pinastri* (Fr.) Höhn.	7.4		
Chaetomium cochlioides Palliser	1.1		
Chaetomium globosum Kunze	2.6		2.3
Chaetomium sp.	1.6		
Cheiromycella microscopica (P. Karst.) Hughes	1.1		
Cladosporium cladosporioides (Fresen.) G.A. de Vries	18.0		
Clonostachys rosea f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams	2.6	2.9	2.3
Coniothyrium fuckelii Sacc.	1.1		
Curvularia oryzae Bugnic.	0.6		
Cylindrocarpon sp.	0.6		
Epicoccum nigrum Link	2.6		
Fusarium tricinctum (Corda) Sacc.	2.6		2.3
Hormonema dematioides Lagerb. & Melin	9.0		
Lecythophora hoffmannii (J.F.H. Beyma) W. Gams & McGinnis	2.6		
Mortierella sp.	7.4		
Mucor sp.	14.3		56.8
Paecilomyces farinosus (Holmsk.) A.H.S. Br. & G. Sm.	0.6	1.0	2.3

Penicillium spp.	13.8	28.4	2.3
Phialocephala sp.	2.1		
Phoma sp.	0.6		
Trichoderma sp.	4.2		
Ulocladium sp.	0.6		
Yeast	3.4	4.9	9.1
Unidentified			
Basidiomycetes (1 species)	0.7		
Other (3 species)	12.2		
Total isolates	302	244	100
Percentage of 'sterile' larvae and beetles	7.4	9.8	6.8
Number of investigated larvae and beetles	189	102	44

[1] OA – overwintered adult; L – larvae; NA – new adults

Fungus isolation from *Ips cembrae* gallery systems

Fungi isolated from the phloem

We obtained 1157 fungus isolates, representing 33 taxa, from the phloem. Overall, 96.4 % of the 450 plant fragments taken from colonised trees contained fungi (Tab. 2). Ten species of ophiostomatoid fungi, *Ceratocystis laricicola*, *Ceratocystiopsis alba*, *C. minuta*, *Ophiostoma brunneo-ciliatum*, *O. ips*, *O. piceae*, *Graphium laricis*, *G. pycnocephalum*, *Ophiostoma* sp. and *Pesotum* sp. 1 were isolated from gallery systems. *Ophiostoma brunneo-ciliatum* was the most commonly isolated ophiostomatoid species. It was found in 74.0 % of all samples. *Graphium laricis* and *Ophiostoma piceae* were isolated from 21.6 and 15.8 % of all samples, respectively. Species not belonging to the ophiostomatoids were relatively rare. Among these fungi, *Clonostachys rosea* f. *rosea* and an unidentified Zygomycetes species were most abundant (Tab. 2).

The brood development phase of *Ips cembrae* had a relatively strong influence on the number of individual ophiostomatoid species in the phloem of beetle-infested larch trees (Tab. 2). These fungi were most often isolated two and especially six weeks after the main insect attack. Among the most common ophiostomatoid fungi, *Ceratocystis laricicola* and *Ophiostoma brunneo-ciliatum* were most strongly associated with the phloem at early stages of brood development, and *O. brunneo-ciliatum*, *Graphium laricis*, *Ceratocystiopsis minuta*, *C. alba* and *Ophiostoma ips* with the phloem taken from well-developed galleries. Eight weeks after the main insect attack the phloem was colonised mostly by *O. brunneo-ciliatum* and *Ophiostoma piceae*. Also non-ophiostomatoid species occurred commonly in this phase. Among them, *Clonostachys rosea* f. *rosea*, the unidentified Zygomycetes species, *Paecilomyces farinosus* and *Pezicula eucrita* were commonly isolated (Tab. 2).

Tab. 2. Fungi isolated from the phloem of *Larix decidua* after an attack of *Ips cembrae*.

Fungi	Frequencies of occurrence (%) 2, 4 and 8 weeks after attack			
	2	4	8	Total
Ophiostomatoid fungi				
Ceratocystiopsis alba (DeVay, R.W. Davidson & W.J. Moller) H.P. Upadhyay		18.7	0.7	6.5
Ceratocystiopsis minuta	0.7	36.0	9.3	15.3
Ceratocystis laricicola	46.7	20.7	16.7	28.0
Graphium laricis	22.7	39.3	2.7	21.6
Graphium pycnocephalum Grosm.	0.7	1.3	0.7	0.9
Ophiostoma brunneo-ciliatum	82.0	72.0	68.0	74.0
Ophiostoma ips (Rumbold) Nannf.	2.0	25.3		9.1
Ophiostoma piceae	4.0	4.0	39.3	15.8
Pesotum sp. 1		18.7		6.2
Ophiostoma sp.		2.0		0.7
Other species				
Acremonium strictum	3.3			1.1
Acremonium section *Nectroidae*		7.3		2.4
Alternaria alternata			1.3	0.4
Arthrobotrys superba Corda			6.0	2.0
Cladosporium cladosporioides			0.7	0.2
Clonostachys rosea f. *rosea*			62.7	20.9
Fusarium poae (Peck) Wollenw.			3.3	1.1
Fusarium tricinctum			6.0	2.0
Fusarium sporotrichioides Sherb.		0.7		0.2
Fusarium sp.			14.0	4.7
Geosmithia sp.	4.7	0.7		1.8
Geotrichum sp.			2.7	0.9
Mucor sp.	6.0		7.3	4.4
Paecilomyces farinosus		5.3	16.0	7.1
Penicillium spp. (2 species)	0.7	1.3	2.7	1.6
Pezicula eucrita P. Karst.			16.0	5.3
Rhinocladiella atrovirens Nannf.			12.0	4.0
Trichoderma sp.		4.0	10.7	4.9
Yeast	1.3			0.4
Unidentified				
Zygomycetes (1 species)			28.0	9.3
Others (2 species)	5.3			1.8
Total isolates	280	386	491	1157
Number of investigated galleries	25	25	25	75
Percentage of „sterile“ fragments	6.7	4.0		3.6
Number of investigated fragments	150	150	150	450

Tab. 3. Fungi isolated from the sapwood of *Larix decidua* after an attack of *Ips cembrae*.

Fungi	Frequencies of occurrence (%) 2, 4 and 8 weeks after attack			
	2	4	8	Total
Ophiostomatoid fungi				
Ceratocystiopsis alba		0.7	1.3	0.7
Ceratocystiopsis minuta	0.7	8.7	1.3	3.6
Ceratocystis laricicola	66.0	14.7	18.0	32.9
Graphium laricis	3.3	54.0	6.7	21.3
Graphium pycnocephalum			4.0	1.3
Ophiostoma brunneo-ciliatum	86.0	90.7	74.7	83.8
Ophiostoma ips		1.3		0.4
Ophiostoma piceae	5.3	0.7	40.7	15.6
Pesotum sp. 1		7.3		2.4
Ophiostoma sp.		0.7		0.2
Other species				
Acremonium section *Nectroidae*		4.0		1.3
Alternaria alternata			1.3	0.4
Arthrobotrys arthrobotryoides (Berl.) Lindau			1.3	0.4
Arthrobotrys superba			6.0	2.0
Clonostachys rosea f. *rosea*			81.3	27.1
Fusarium poae			2.0	0.7
Fusarium sporotrichioides		4.0		1.3
Fusarium tricinctum		4.0	4.0	2.7
Fusarium sp.			3.3	1.1
Hormonema dematioides		0.7		0.2
Mortierella sp.			0.7	0.2
Mucor sp.		2.0	25.3	9.1
Paecilomyces farinosus		2.7	23.3	8.7
Penicillium spp. (2 species)		9.3	7.3	5.6
Pezicula eucrita			2.7	0.9
Phialophora clavispora W. Gams			2.0	0.7
Rhinocladiella atrovirens			14.0	4.7
Trichoderma sp.			16.0	5.3
Unidentified				
Zygomycetes (1 species)			8.7	2.9
Other (2 species)	3.3			1.1
Total isolates	247	308	519	1074
Number of investigated galleries	25	25	25	75
Percentage of „sterile“ fragments	8.0	0.7		2.9
Number of investigated fragments	150	150	150	450

Fungi isolated from the sapwood

We obtained 1074 fungus isolates, representing 32 taxa, from the sapwood. Overall, 97.1 % of the 450 sapwood fragments taken from colonised trees contained fungi (Tab. 3). The same ophiostomatoid species were found in the sapwood as in the phloem of beetle-infested larch trees. *Ophiostoma brunneo-ciliatum* was the most common species in the sapwood of trees infested by *Ips cembrae*. It was isolated from 83.8 % of wood fragments. Two other ophiostomatoid fungi, *Graphium laricis* and *Ceratocystis laricicola* were isolated from 21.3 and 32.9 % of the fragments, respectively. Among non-ophiostomatoid fungi, *Clonostachys rosea* f. *rosea* and *Mucor* species were the most numerous ones (Tab. 3).

There were also similarities in the fungus species spectrum found in different stages of brood development. *Ophiostoma brunneo-ciliatum* was commonly isolated from the sapwood of all brood categories, whereas *Ceratocystis laricicola* occurred most frequently in the sapwood in early stages of brood development. *Graphium laricis* and *Ophiostoma piceae* were most numerous in the sapwood taken from well-developed and deserted galleries of *Ips cembrae*, respectively (Tab. 3).

Clonostachys rosea f. *rosea*, *Mucor* species and *Paecilomyces farinosus* were also commonly isolated from sapwood at final stages of brood development of *Ips cembrae* (Tab. 3).

DISCUSSION

In this study, ophiostomatoid fungi were the most common fungus associates of *Ips cembrae*, represented by eleven species. These fungi were isolated from the beetles, larvae as well as from gallery systems. The high frequency of *Ophiostoma brunneo-ciliatum*, *Ceratocystis laricicola*, *Ceratocystiopsis minuta* and *Graphium laricis* suggests a close association of these species with *I. cembrae*. This is the first report on the occurrence of *C. laricicola*, *O. brunneo-ciliatum* and *G. laricis* from Poland.

A similar spectrum of ophiostomatoid fungi was associated with *Ips cembrae* in other parts of its distribution range in Europe (Redfern et al. 1987, Kirisits 2001, Kirisits et al. 2000, Kirisits 2004a, Kirisits 2004b) and Asia (Pashenova et al. 1995, Yamaoka et al. 1998). However, *Ophiostoma bicolor* R. W. Davidson & D. E. Wells, *O. fusiforme* Aghayeva & M. J. Wingf., *O. lunatum* Aghayeva & M. J. Wingf. (Aghayeva et al. 2004, Kirisits 2004a) and *O. laricis* Van der Westh., Yamaoka & M. J. Wingf. (Yamaoka et al. 1998) were not recorded in this study.

Among ophiostomatoid species, *Ophiostoma ips* was found in association with *Ips cembrae* for the first time. This species had been described before as an associate of *Ips acuminatus* (Gyll.), *I. sexdentatus* Börner and *Orthotomicus proximus* Eichh. on *Pinus sylvestris* (Kirisits 2004a).

Among ophiostomatoid fungi, *Ophiostoma brunneo-ciliatum* was commonly found in galleries and on beetle bodies of *Ips cembrae*. This species occurred in all niches with frequencies ranging from 26.5 to 85.0 %. These results suggest that *O. brunneo-ciliatum* is the most common fungus associated with *I. cembrae* in Poland. The species' frequency of occurrence did not vary significantly among different brood development stages of *I. cembrae*. In this study, *O. brunneo-ciliatum* was the most common species two, four and eight weeks after insect attack. This fungus is weakly pathogenic to Scots pine (Lieutier et al. 1989) and larch (Yamaoka et al. 1998).

In the present study, an important element of the mycobiota of *Ips cembrae* was *Ceratocystis laricicola*. The frequency of this fungus declined with time. These changes were particularly clear in the sapwood infested by *I. cembrae*. In later stages of brood development *C. laricicola* was less numerous than in the early stages. It seems that *C. laricicola*, like *Ceratocystis polonica* on *Picea abies* (L.) H. Karst., has the ability to colonise rapidly the sapwood of larch trees infested by *I. cembrae*. The results of inoculation studies (Redfern et al. 1987, Yamaoka et al. 1998, Kirisits 2001) indicate that this fungus is the only fungus associated with *I. cembrae* capable of killing larch trees.

Graphium laricis was a third most numerous species isolated from the phloem and sapwood of *Ips cembrae* galleries. In isolation from the sapwood of trees, *G. laricis* occurred sporadically in early stages of brood development and was more frequently found at later ones. This indicates that this fungus rather followed *Ceratocystis laricicola* and *Ophiostoma brunneo-ciliatum* in penetrating the sapwood of larch trees attacked by *I. cembrae*.

Ceratocystiopsis minuta and *C. alba* were isolated in highest frequencies from older gallery systems. They might play a similar role as *Graphium laricis* in the succession after an attack by *Ips cembrae*. These species were more often isolated from the phloem than from sapwood. These results confirm the findings of Jankowiak (2005), who found *C. minuta* more frequently in the phloem than in the sapwood of spruce trees attacked by *Ips typographus*. In Poland, *C. minuta* is known to be associated with Norway spruce infested by *I. typographus* (Siemaszko 1939, Jankowiak 2005, Jankowiak and Hilszczański 2005) and with Scots pine infested by *Tomicus piniperda* L. (Jankowiak 2006a), whereas *C. alba* was found in gallery systems of *Hylurgops palliatus* (Gyll.) on Scots pines (Jankowiak 2006b).

This study showed that *Ophiostoma piceae* is a relatively important associate of *Ips cembrae* in Poland. However, it was isolated mainly from phloem and sapwood

in final stages of brood development. This indicates that *O. piceae* is a tertiary phloem and sapwood invader of larch trees infested by *I. cembrae*. This result agrees with reports on fungal succession on spruce trees attacked by *Ips typographus* (Harding 1989, Solheim 1992a, Solheim 1992b, Jankowiak 2005) but is in contrast to the findings by Yamaguchi (1995) and Peng et al. (1996), who suggested that *O. piceae* is an important and virulent associate of *Ips cembrae* in Japan.

This study indicated that temporal succession of fungi in the phloem and sapwood was responsible for the changes in ophiostomatoid fungi frequencies in various *Ips cembrae* brood development stages on larch trees. *Ceratocystis laricicola* as the primary invader occurred frequently at early stages of brood development, particularly in the sapwood. *Ophiostoma brunneo-ciliatum* and *Graphium laricis* as secondary invaders followed *Ceratocystis laricicola*. In the later stages of brood development other fungi species appeared. In contrast to our results, Japanese studies (Yamaguchi 1995, Peng et al. 1996) showed that *Ophiostoma piceae* was the principal blue-stain fungus infecting beetle-attacked larch trees. However, the opinions about fungal invasion in sapwood of trees attacked by *I. cembrae* in Japan were very divided, because Yamaoka et al. (1998) considered *C. laricicola* the most important fungal associate of this insect in Japan. Interestingly, the pattern of fungal invasion in phloem and sapwood observed in the present study was similar to that found on *Picea abies* infested by *Ips typographus* (Solheim 1992a, Solheim 1992b, Jankowiak 2005). These authors reported that fungi carried by *I. typographus* invaded Norway spruce sapwood in a successional pattern with the most pathogenic *Ceratocystis polonica* first, followed by other beetle-transmitted *Ophiostoma* and *Graphium* species.

All the ophiostomatoid species occurred more abundantly on new adults than on overwintered beetles caught with a trap. This phenomenon is well known in the relationship between bark beetles and fungi. Similar relations were found between *Tomicus piniperda* and *Leptographium wingfieldii* M. Morelet (Gibbs and Inman 1991), and *Ips typographus* and *Ceratocystis polonica* (Jankowiak 2004). Masuya et al. (1998) supposed that fungal spores on beetle bodies were probably exposed to extreme environmental fluctuations during beetle flight. Therefore, overwintered adults usually carry fewer fungi than emerging beetles.

Non-ophiostomatoid fungi were also frequently carried by *Ips cembrae* beetles. Our studies showed further that overwintered adults were more contaminated with non-ophiostomatoid fungi. The presence of these fungi could be the result of contact of *I. cembrae* individuals with soil during overwintering. Species not belonging to the ophiostomatoid fungi were also found in galleries of *Larix decidua* infested by *I. cembrae*. *Clonostachys rosea* f. *rosea*, the dominant non-ophiostomatoid species in this study, was also reported from bark beetles galleries in Poland (Bałazy 1962) and Germany (Kirschner 2001). According to Kirschner (2001) this fungus is a mycoparasitic and mycophilic fungus. Other fun-

gal species found belong to insect-pathogenic fungi (*Paecilomyces farinosus*), phytopathogenic fungi (*Alternaria alternata*, *Cylindrocarpon* sp. and *Fusarium* spp.), wood-colonising fungi (*Rhinocladiella atrovirens*, *Phialophora clavispora*) and other ecological groups. Another important group of fungi isolated from gallery systems of *I. cembrae* were endophytes such as *Epicoccum nigrum*, *Pezicula eucrita* and *Coniothyrium fuckelii*, which were frequently isolated from symptomless and uncolonised larch trees (Kowalski and Kehr 1992).

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