OPHIOSTOMATOID FUNGI ASSOCIATED WITH *IPS TYPOGRAPHUS* (L.) ON *PICEA ABIES* [(L.) H. KARST.] AND *PINUS SYLVESTRIS* L. IN NORTH-EASTERN POLAND

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ABSTRACT

This study dealt with the species distribution and frequency of ophiostomatoid fungi associated with the bark beetle *Ips typographus* on Norway spruce and Scots pine in north-eastern Poland. At all locations high spruce bark beetle damage has occurred in 2002-2003. Fungi were isolated from beetles and from brood systems of trees infested by the spruce bark beetle. The ophiostomatoid fungi were represented by 13 species. A similar spectrum of ophiostomatoid fungi as that recorded from *Picea abies* was associated with *I. typographus* on *Pinus sylvestris* trees. The most frequent ophiostomatoid species isolated from beetles, phloem and sapwood of Norway spruce were *O. bicolor* and *O. penicillatum*. The frequency of occurrence of ophiostomatoid fungi varied significantly among the examined locations. *O. bicolor* was the most frequently found species on Scots pine infested by *I. typographus*. The potential role of ophiostomatoid fungi in the epidemiology of *I. typographus* is discussed. Additionally, we also recorded how the ophiostomatoid fungi associated with spruce bark beetle could grow into phloem and sapwood of *Pinus sylvestris* trees.

KEY WORDS: *Ceratocystis polonica*, *Ips typographus*, *Ophiostoma piceaperdum*, ophiostomatoid fungi, *Picea abies*, *Pinus sylvestris*.

INTRODUCTION

The Eurasian bark beetle, *Ips typographus* L. (Coleoptera: Curculionidae, Scolytinae) is one of the important forest pests in the stands of *Picea abies*. It is a very aggressive species on *P. abies* trees, but other *Picea* species are occasionally colonized. When the populations of *I. typographus* increase to a high level, this insect can kill millions of spruce trees (Michalski 1998). During outbreaks of the spruce bark beetle, other tree genera like *Larix* Mill., *Pseudotsuga* Carričre, *Pinus* L. and *Abies* Mill. may also be infested (Michalski and Mazur 1999; Skuhravý 2002). A high aggressiveness of spruce bark beetle, *I. typographus* may be explained by possession of effective aggregation pheromones, a high tolerance to host tree resins and transmittion of phytopathogenic blue-stain fungi (Christiansen and Solheim 1990).

During gallery construction, the spruce bark beetle introduces various micro-organisms to the phloem and cambium of *P. abies* trees. There are yeasts, bacteria and fungi. The fungi which are most frequently associated with *I. typographus* are blue-stain fungi (Furniss et al. 1990). These fungi belong to the ascomycete genera *Ceratocystis* and *Ophiostoma* or to related anamorph genera (Wingfield et. al. 1993), and commonly are designated as "ophiostomatoid fungi". The ophiostomatoid fungi develop on the walls of larval galleries and adjacent sections of bark and sapwood, causing their discolouration by colonizing tracheids and living ray parenchyma cells. Together with associated ophiostomatoid fungi, *I. typographus* can overcome the resistance of healthy spruce trees (Christiansen 1985; Horntvedt et al. 1983). Among the transmitted blue-stain fungi, *C. polonica* is the most pathogenic species associated with the spruce bark beetle, *I. typographus*. It rapidly penetrates tracheids and causes disturbances in the water transport of living trees (Christiansen 1985; Horntvedt et al. 1983; Kirisits 1998; Kirisits and Offenthaler 2002; Krokene and Solheim 1998; Solheim 1988, 1991).

The first record of ophiostomatoid fungi associated with *I. typographus* in Poland originating from the Baltic areas of Norway spruce was made by Siemaszko (1939). He reported that *C. polonica*, *O. penicillatum*, *O. piceae*, *O. minutum* and *Graphium pycnocephalum* were associated with *I. typographus* in the Białowieża Forest. Fourteen ophiostomatoid species were found associated with spruce bark beetles in the mountain regions of Norway spruce in Poland (Jankowiak 2001, 2003, 2004a, b, 2005).

The main aim of this study was to determine the species composition of ophiostomatoid fungi associated with *I. typographus* in north-eastern Poland, particularly in a locally high population level of spruce bark beetles. This information could contribute to understanding the role of associated fungi in epidemiology of *I. typographus*. Additionally, we also recorded how the ophiostomatoid fungi associated with *I. typographus* could grow into phloem and sapwood of *Pinus sylvestris* trees.

MATERIAL AND METHODS

Study areas, collection of samples and fungal isolation

The investigations were conducted during 2002-2003 in four study plots located in 80-year-old stands of *Picea abies* growing in the Krynki Forest District (Sosnownik Forest Range, and Ostrów Forest Range), Supraśl Forest District (Borki Forest Range), Czarna Białostocka Forest District (Trzcianka Forest Range) and the Biebrza National Park (Werykle Protection Range). Additionally, we also took samples from *Pinus sylvestris* trees infested by *I. typographus* in the Hajnówka Forest District (Wilczy Jar Forest Range). In these stands, large numbers of trees were dying, mainly due to infestation by *I. typographus*. The population levels of the spruce bark beetle in study plots were high. In north-eastern Poland, in 2002, when climatic conditions were very favorable for bark beetles, *I. typographus* accomplished three generations (Łabędzki 2003). At all locations, high spruce bark beetle damage has occurred in 2002-2003, what was reflected in the amount of infested trees harvested (Table 1).

In order to determine the species spectrum and frequency of fungi associated with *I. typographus*, in each year trees infested by the first generation of this bark beetle species were chosen from May to June. On infested Norway spruce trees, development of *I. typographus* had reached the stage where young adults were present. The galleries taken from Scots pine trees in Hajnówka and Krynki were occupied by early stages of *I. typographus* larvae, with eggs and old beetles present. In contrast to these study plots, galleries taken from Czarna Białostocka were well developed with pupae and young beetles present.

A total of 28 Norway spruce and 5 Scots pine trees were analysed. From parts of the stem infested by *I. typographus*, 2-3 discs (approximately 20 cm thick) and chips (30 cm long) with intact bark were cut from each tree. In the laboratory, the bark was separated from the wood under sterile conditions, and gallery fragments from which isolations were intended were disinfected with 96% ethyl alcohol, using cotton wool. The disinfection lasted approximately 15 seconds and then gallery fragments were dried on filter paper. Isolation of fungi was made from phloem fragments taken from and around galleries of *I. typographus*, and also from the sapwood underneath insect galleries up to the depth of 10 mm into the stained sapwood. In the former case, fungi were isolated from pieces of phloem taken from maternal and larval galleries, as well as from discoloured areas around these galleries. After drying of samples, the surface layer of phloem tissues was removed with a sterile scalpel. Subsequently, fragments of phloem or sapwood, about 4×4 mm, were cut with a sterile scalpel or a chisel, and placed on a culture medium in 9 cm glass Petri dishes.

In May and June, beetles of *I. typographus* were collected from the galleries on infested Norway spruce and Scots pine trees. The beetles were disinfected in 96% ethyl alcohol for 15 seconds. After drying on a sterile blotting paper, the disinfected beetles were crushed on a microscopic slide and using a sterile scalpel were evenly spread over on the surface of medium. In total, the isolations were performed from 346 adults of *I. typographus*. All isolations were made on 2% malt extract agar (2% MEA; 20 g malt extract, 20 g agar, 1000 ml distilled water) supplemented with the tetracycline (200 mg per 1 litre of culture medium) to inhibit bacterial growth. Pure cultures of fungi were also grown on 2% MEA. The primary isolation plates were incubated at room temperature in the dark. Colonies of fungi growing from the phloem and sapwood fragments were compared on the basis of macro- and microscopic characteristics, and pure cultures were derived from representative colonies in order to identify the fungi. Cultures typical of each ophiostomatoid taxon have been deposited in the culture collection of the Laboratory of the Department of Forest Pathology, Hugo Kołłątaj University of Agriculture, Cracow, Poland. In 2002-2003, fungi were isolated from 1512 (Norway spruce) and 732 (Scots pine) phloem fragments taken from and around beetle galleries and from the sapwood (Tables 2, 3).

Data analyses

The frequency of occurrence of each fungal species was expressed as the percentage of the beetles, phloem and sapwood fragments, from which a given species was isolated in relation to the total number of the beetles or fragments from which isolations were made. Frequencies were computed using the following formula: $F = (NF/NT) \times 100$, where F represents the frequency of occurrence (%) of each fungal species, NF represents number of the beetles or fragments, from which a particular fungus was isolated and NT represents total number of the beetles or fragments from which fungal isolation was attempted.

TABLE 1. Volumne of infested timber [m^3] harvested in the investigated Forest ranges in 2001-2004.

Years	Investigated Forest ranges				
	Werykle	Borki	Krynki*	Trzcianka	Wilczy Jar
2001	12.0	593.4	1590.8	297.6	550.1
2002	147.8	692.3	2482.2	638.9	1133.8
2003	91.8	1037.0	3157.1	728.9	1985.4
2004	–	263.8	1846.3	370.8	983.6

* total mean for forestries Sosnownik and Ostrów

TABLE 2. Frequency of occurrence (%) of ophiostomatoid fungi associated with *I. typographus* on Norway spruce from various niches collected at four locations.

Ophiostomatoid fungi	Krynki			Supraśl			Biebrzański NP.			Czarna Białostocka		
	B	P	S	B	P	S	B	P	S	B	P	S
Ceratocystis polonica (Siem.) C. Moreau	7	1	3	0	0	1	60	5	34	47	20	28
Ophiostoma ainoae H. Solheim	40	27	12	20	4	0	5	2	1	13	3	1
Ophiostoma bicolor Davids. & Wells	54	41	15	30	89	37	32	70	35	40	74	50
Ophiostoma flexuosum H. Solheim	0	0	0	5	0	0	0	0	0	0	0	0
Ophiostoma minutum Siem.	23	5	8	0	8	0	8	10	10	0	48	3
Ophiostoma penicillatum (Grosm.) Siem.	52	57	66	15	6	1	22	88	54	60	36	31
Ophiostoma piceae sensu lato	5	21	29	0	5	1	0	2	4	0	20	11
Ophiostoma piceaperdum (Rumb.) von Arx	5	4	4	0	0	0	3	0	0	0	1	0
Graphium fimbriisporum (Morelet) K. Jacobs. T. Kirisits & M.J. Wingf.	2	9	24	5	2	0	19	23	28	0	0	0
Graphium pycnocephalum Grosm.	0	1	0	0	7	1	0	0	0	0	4	2
Leptographium sp.	2	4	0	0	0	0	0	0	0	0	0	0
Pesotum sp.	2	1	1	4	1	0	0	0	0	13	3	0
Number of investigated fragments	100	504	294	40	180	174	37	60	120	30	90	90
Percentage of "sterile" fragments	4	5	15	45	4	66	8	0	0	7	3	18

B – beetles of *I. typographus*
P – phloem taken from and around galleries of *I. typographus*
S – sapwood underneath galleries of *I. typographus*

TABLE 3. Frequency of occurrence [%] of ophiostomatoid fungi associated with *I. typographus* on Scots pine from various niches collected at three locations.

Ophiostomatoid fungi	Hajnówka*			Krynki*		Czarna Białostocka**		
	B	P	S	P	S	B	P	S
Ceratocystis polonica	83	3	1	1	0	5	0	0
Ophiostoma ainoae	30	7	0	3	18	13	1	2
Ophiostoma bicolor	13	31	10	0	5	45	99	62
Ophiostoma ips	0	17	10	0	0	0	0	0
Ophiostoma minutum	8	9	6	1	0	15	0	0
Ophiostoma penicillatum	28	33	22	0	2	8	7	4
Ophiostoma piceae sensu lato	0	3	1	0	0	3	2	1
Ophiostoma piceaperdum	0	0	0	0	0	0	2	0
Graphium fimbriisporum	5	0	7	0	0	10	6	0
Graphium pycnocephalum	3	5	7	0	0	3	13	2
Leptographium sp.	0	0	0	0	0	18	0	0
Pesotum sp.	0	0	0	0	0	3	0	0
Number of investigated fragments	42	120	240	72	60	40	84	156
Percentage of "sterile" fragments	0	15	38	39	67	28	6	37

* samples with early stages of galleries development of *I. typographus*
** samples with late stages of galleries development of *I. typographus*
B – beetles of *I. typographus*
P – phloem taken from and around galleries of *I. typographus*
S – sapwood underneath galleries of *I. typographus*

RESULTS

Picea abies

In total, twelve species of the ophiostomatoid fungi were found on the four study plots. These included *C. polonica*, seven species of *Ophiostoma* sp., *G. fimbriisporum*, *G. pycnocephalum*, *Leptographium* sp. and *Pesotum* sp. (Table 2). Among the ophiostomatoid fungi, *O. penicillatum* and *O. bicolor* were most commonly isolated (Table 2). These fungi were most frequently isolated from the beetles, phloem and sapwood of spruce trees infested by *I. typographus*. The pathogenic species *C. polonica* was isolated in 0 to 60% of the samples. It was the only species occurring more frequently in the sapwood than in the phloem. Other common fungi, but not consistently occurring, were *O. ainoae*, *O. minutum*, *O. piceae*, and *G. fimbriisporum*. These species were found in most locations. Five other species (*O. piceaperdum*, *O. flexuosum*, *G. pycnocephalum*, *Leptographium* sp. and *Pesotum* sp.) were found, but at a very low frequency (Table 2).

Isolation frequencies of different ophiostomatoid fungi varied depending whether samples were taken from the immature beetles, phloem or the sapwood. The beetles were most commonly colonized by *O. bicolor*, *O. penicillatum*, *O. ainoae* and *C. polonica*. From the phloem taken from galleries of *I. typographus*, *O. penicillatum* and *O. bicolor* were most frequently isolated (Table 2). The sapwood underneath galleries of *I. typographus* was most frequently colonized by *O. penicillatum*, *O. bicolor* and *C. polonica* (Table 2).

The composition of ophiostomatoid fungi was qualitatively and quantitatively different at the various sites. The

virulent *C. polonica* was most frequently obtained from the beetles, phloem and sapwood of trees infested by *I. typographus* in the Biebrza National Park and the Czarna Białostocka Forest District (Table 2). In contrast, it was sporadically isolated from the beetles, phloem and sapwood of trees examined at Krynki and the Supraśl Forest Districts (Table 2). *O. penicillatum* was the most common fungus at all plots except one (in Supraśl), where *O. bicolor* was the most common species. *O. piceae* occurred with almost equal frequency on two study plots (in Krynki and Czarna Białostocka), whereas *O. minutum* was most frequently isolated from the phloem of trees infested by *I. typographus* in Czarna Białostocka and from the beetles in Krynki (Table 2).

Pinus sylvestris

A similar spectrum of ophiostomatoid fungi as that recorded from Norway spruce was associated with *I. typographus* on Scots pine. Most of the ophiostomatoid fungi found belonged to the genera *Ophiostoma* H. & P. Syd. (7 species), *Graphium* Corda (2 species), *Pesotum* Crane & Schoknecht (1 species), *Ceratocystis* Ellis & Halsted (1 species) and *Leptographium* Lagerberg & Melin (1 species). Apart from *O. flexuosum*, all of the ophiostomatoid fungi that occurred in the galleries of *I. typographus* on Norway spruce were also isolated from the phloem and sapwood of Scots pine. *O. ips* was recorded only in the galleries of *I. typographus* on Scots pine. The most frequent ophiostomatoid species in both phloem and sapwood was *O. bicolor.* Frequencies of occurrence of *O. bicolor* from immature beetles, phloem and sapwood ranged from 0 to 99% (Table 3). It occurred most frequently in the phloem and sapwood of trees which represented older developmental stages of *I. typographus* galleries (Czarna Białostocka). Other relatively common fungi were *O. ainoae* and *O. penicillatum.* The pathogenic species *C. polonica* was only occasionally isolated from pine samples. The frequency of other ophiostomatoid species was very low (Table 3).

The old beetles taken from the galleries of *I. typographus* in the Hajnówka Forest district were most frequently colonized by *C. polonica.* It was found also in the phloem and sapwood of Scots pine, but at a very low frequency (Table 3).

DISCUSSION

The assemblage of ophiostomatoid fungi associated with *I. typographus* in the present study was similar to the spectrum of fungi reported by Siemaszko (1939) in the Białowieża forest. He reported that *C. polonica*, *O. penicillatum*, *O. piceae*, *O. minutum* and *G. pycnocephalum* were associated with *I. typographus*. All the fungi displayed by Siemaszko were also found in this study. Apart from, 7 ophiostomatoid species recorded in this study had not been previously reported by that researcher. The newly reported species for the north-eastern Poland are: *O. ainoae*, *O. bicolor*, *O. flexuosum*, *O. piceaperdum*, *G. fimbriisporum*, *Graphium* sp. and *Leptographium* sp. All ophiostomatoid fungi found in this study were identified as associates of *I. typographus* in southern Poland (Jankowiak 2001, 2004a, b, 2005). The ophiostomatoid fungi in the present study have also been reported to be associated with *I. typographus* in other parts of Europe (Grubelnik 1998; Harding 1989; Kirisits 1996, 2001; Kirschner 1998, 2001; Kotýnková-Suchrová 1966; Krokene and Solheim 1996; Mathiesen-Käärik 1953; Salle et. al. 2003; Siemaszko 1939; Solheim 1986; Viiri 1997, 2002; Viiri and Lieutier 2004).

The fungal associates of the beetle *I. typographus* occurred with varying frequency in different environmental conditions and investigations (Grubelnik 1998; Harding 1989; Jankowiak 2005; Kirisits 1996, 2001; Kirschner 2001; Siemaszko 1939; Solheim 1986; Viiri 1997; Viiri and Lieutier 2004). In the present study, *C. polonica* was not regularly isolated, whereas *O. bicolor* and O. *penicillatum* were the most commonly and consistently occurring species. These two last species were also the most frequently isolated from phloem and sapwood of spruce trees in southern Poland (Jankowiak 2005). In another Polish study (Siemaszko 1939), *O. penicillatum*, *C. polonica* and *G. pycnocephalum* have been found as common associates of *I. typographus* in the Białowieża forest.

The quantitative differences are most obvious for the most virulent fungal associate of *I. typographus*, *C. polonica*. There are also significant differences in the frequency of occurrence of *O. piceaperdum* (Kirisits 2004). It seems that various factors may explain the variation of the composition of the ophiostomatoid fungi of *I. typographus*. According to Kirisits (2001, 2004), the isolation procedures, differences in sampling and timing of isolations can greatly affect the frequencies of occurrence of ophiostomatoid fungi. In this study, however, the composition of ophiostomatoid fungi differed significantly between locations.

According to Solheim (1992a, b) and Jankowiak (2005), a temporal succession of fungi into phloem and sapwood could be responsible for the varying frequencies of ophiostomatoid fungi on Norway spruce trees. *C. polonica* is the primary invader, occurring most frequently in the sapwood of *P. abies* trees during the early stages of brood development of *I. typographus* (Jankowiak 2005; Solheim 1992a, b). In contrast to *C. polonica*, *O. ainoae*, *O. minuta* and *O. piceae* are secondary and tertiary invaders into the phloem and the sapwood of spruce trees and mainly colonise dying and dead tissues (Jankowiak 2005; Solheim 1992a, b). In this study, *C. polonica* was also isolated more frequently from the sapwood than from the phloem of spruce trees. However, these results also clearly showed that *C. polonica* was not a dominant fungus in the sapwood of trees attacked by *I. typographus*. *O. bicolor* occurred in the sapwood of trees more frequently than *C. polonica*. *O. bicolor* abundantly accompanies *I. typographus* also in southern Poland, where it already colonizes trees at early stages of development of *I. typographus* galleries (Jankowiak 2005). In this study, the fact that the galleries were collected from dying Norway spruces could greatly affect the frequency of occurrence of *C. polonica*, which could have been replaced by other fungi (e. g. *O. bicolor*) in dying tissues.

Qualitative and quantitative differences in the composition of fungal associates of *I. typographus* may also be related to the population dynamics of *I. typographus*. Harding (1989) and Solheim (1993a) suggested that *C. polonica* may occur at low frequencies during endemic periods of *I. typographus*, when beetles colonize weakened and dead trees. At this time, it may be replaced by other species. During the epidemic phase, the frequency of *C. polonica* may increase, when vigorous trees are attacked. There is no direct evidence for this hypothesis. In Norway, the frequency

of *C. polonica* has been low during endemic periods, whereas the frequency has been higher during the epidemic phase (Solheim 1992a, 1993a). In contrast, in Denmark (Harding 1989) and Austria (Grubelnik 1998; Kirisits et. al. 2000; Kirisits 2001), no differences were found between beetle population levels. In a French study, in a local abundant population of spruce bark beetle, *C. polonica* occurred at moderately high frequencies (Viiri and Lieutier 2004). The isolation results from this study also do not support Harding's and Solheim's hypothesis. This study has shown that *C. polonica* on five study sites, where the population levels of the spruce bark beetle were high, occurred sporadically (in Krynki and Supraśl) or at moderately high frequencies (in Czarna Białostocka and Biebrza National Park). Additionally, *C. polonica* also was the most abundant species isolated from the beetles infesting Norway spruce and Scots pine in Hajnówka. Our results indicates that *C. polonica* become replaced by other species, especially by *O. bicolor* and *O. penicillatum*.

O. piceaperdum has also been suggested to play a special role in the population dynamics of *I. typographus* (Harding 1989; Solheim 1993a; Viiri and Lieutier 2004). Harding (1989) reported that *O. piceaperdum* may be able to overcome the resistance of even vigorous Norway spruce, whereas Kirisits (1996, 1998) suggested that it is only able to kill considerable portions of the phloem. Recently, Jankowiak (2005) have studied the mycobiota in southern Poland and have recorded that *O. piceaperdum* may occur frequently in the sapwood of Norway spruce during the early stages of *I. typographus* brood development. In this study, *O. piceaperdum* was not recorded at all or occurred very rarely. Our results do not support the idea that *O. piceaperdum* plays a special role in the population dynamics of spruce bark beetle in north-eastern Poland.

We cannot explain differences in the frequency of *C. polonica* between the study plots and *O. piceaperdum* which occurred at very low frequencies in all the locations examined. The occurrence of *C. polonica* and *O. piceaperdum* in the various sites within the distribution range of *I. typographus* probably depends on different factors. This difference may be caused by the different environmental conditions in examined stand of *P. abies* (e.g. stand density). Some authors have suggested that the genetic variation and patogenicity levels of different strains of *C. polonica* and *O. piceaperdum* may have a strong influence on the frequency of fungi isolated from the galleries of *I. typographus* (Kirisits and Angelberger 1999; Krokene and Solheim 2001; Viiri and Lieutier 2004).

The mycobiota of *I. typographus* on Norway spruce proved to be very similar to that of *I. typographus* on Scots pine. Among ophiostomatoid fungi, *O. ips* was never reported in association with *I. typographus*. It was associated with *I. sexdentatus* (Börn.), *I. acuminatus* (Gyll.), *Orthomicus laricis* (Fabr.) and *Tomicus piniperda* (L.), which commonly infest Scots pine (Kirisits 2004; Kirschner 2001).

In the present study, *C. polonica* was isolated from a high percentage of the beetles, whereas it was found only in a few samples of phloem and sapwood of Scots pine growing on the Hajnówka Forest district study plot. *C. polonica* also was only sporadically present in samples of phloem and sapwood at the remaining localities. This results showed that the pathogenic *C. polonica* is considerably less able to colonize the phloem and sapwood of Scots pine than tissues of Norway spruce trees. The same observation was made by Christiansen, Solheim (1990) who reported that this fungus was nonpathogenic in young Scots pine. The fact that *C. polonica* only sporadically colonized phloem and sapwood of Scots pine may be caused by the low tolerance of the fungus to resins, structure of the water conducting vessels or some other factors. It seems that *C. polonica* is more specialized to colonize the sapwood of Norway spruce than sapwood of Scots pine. This fungus is consistently associated exclusively with the phloem-feeding bark beetles on Norway spruce, mainly with *I. typographus*, *I. duplicatus* Sahlb. and *I. amitinus* Eichn. (Kirisits 2001, 2004; Krokene and Solheim 1996). *O. bicolor*, in contrast, seems to be better adapted to colonize phloem and sapwood of Scots pine. In the present study, it was the most common species occurring in the phloem and sapwood of Scots pine at all localities. *O. bicolor* is much less pathogenic to Norway spruce than *C. polonica* (Harding 1989; Horntvedt et al. 1983; Kirisits 1998; Solheim 1988), but experimental inoculations with *O. bicolor* on live Scots pine have not been conducted yet. The fact that *O. bicolor* grew farther into the phloem and sapwood of pine than other fungi may indicate that this fungus is more pathogenic to Scots pine than other fungi. *O. bicolor* is associated with the phloem-feeding bark beetles on Norway spruce, Scots pine and European larch in Europe (Kirisits 2004; Kirschner 2001). This indicates that *O. bicolor* is more than *C. polonica* non-vector-specific fungus and occurs on various conifer trees.

This study showed that ophiostomatoid fungi are the most frequent species transmitted by *I. typographus* in north-eastern Poland. The *C. polonica* and *O. piceaperdum* were isolated relatively rarely, therefore a particular role of these fungi in epidemiology *I. typographus* was not confirmed. These results rather suggested that *O. bicolor* may play a special role in the population dynamics of *I. typographus*. It seems that pathogenic *C. polonica* is unable to invade live phloem and sapwood of Scots pine infested by *I. typographus*. Our observations showed that the fungus best adapted to colonize phloem and sapwood of Scots pine attacked by spruce bark beetles is *O. bicolor*. To further test this fact, more comprehensive studies of the ophiostomatoid fungi of *I. typographus* colonizing Scots pine are needed.

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