OPHIOSTOMATOID FUNGI ASSOCIATED WITH THE SPRUCE BARK BEETLE (IPS TYPOGRAPHUS) NEW FOR POLAND: OCCURRENCE AND MORPHOLOGY

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Abstract

This is the first information on the occurrence of Ophiostoma ainoae, O. bicolor, O. cucullatum, O. flexuosum and O. piceaperdum in Poland. These fungi were associated with Ips typographus on Norway spruce. Various aspects of their morphology and frequency of occurrence are discussed. From among these species O. ainoae, O. bicolor and O. piceaperdum were the most frequent ones in larvae, beetles, and galleries of I. typographus.

Key words: ophiostomatoid fungi, Ophiostoma, Ips typographus, Picea abies

Introduction

The bark beetle (Ips typographus L. (Coleoptera: Scolytidae)) is one of the important forest pests on various species of spruce in Europe and Asia. It usually breeds in weakened trees and timber, but it may sometimes colonize healthy trees (Michalski and Mazur 1999).

The bark beetle is known to be associated with specific fungi. The adults of I. typographus may be the vectors of fungi from Ceratocystis sensu lato transmitting their perfect as well as imperfect stages (Paine et al. 1997). Most often propagules of these fungi are carried on the body surface of beetles (Solheim 1993 a) and in their digestive system (Furniss et al. 1990). Also mites associated with I. typographus play a very significant role in transfer of these fungi. As a rule these arachnids transfer several species of fungi, but only Ophiostoma bicolor Davids. et Wells was found relatively more frequently (Moser et al. 1989, 1997).

The life cycle of most of ophiostomatoid fungi is closely connected with insects as they develop on walls of larval galleries and adjacent sections of bark and sap-
wood, causing their discolouration. Many authors suspect that some fungi associated with *I. typographus* may possess pathogenic properties and may contribute to weakening and death of trees (Horntvedt et al. 1983, Christiansen and Solheim 1990, Krokene and Solheim 1997).

Over the last few years the diversity of ophiostomatoid fungi associated with *I. typographus* in Europe and Asia was investigated by Solheim (1986) in Norway, Harding (1989) in Denmark, Yamaoka et al. (1997) in Japan, Kirisits et al. (1998) in Austria, and Kirschner and Oberwinkler (1999) in Germany. This study showed, that the bark beetle is associated with more than 16 species from the genus *Ceratocystis sensu lato*, mainly species of *Ophiostoma*. The first record of ophiostomatoid fungi associated with *I. typographus* in Poland was made by Siemaszko (1939). In his study he recognized and described five species of ophiostomatoid fungi: *Ceratocystis polonica* (Siem.) C. Moreau, *Ophiostoma penicillatum* (Grosm.) Siem., *O. piceae* (Münch) H. et P. Syd., *O. minutum* Siem., and *Graphium pycnocephalum* Grosm.

The objectives of this study were to obtain a comprehensive view of the ophiostomatoid fungi associated with *I. typographus*, hitherto unknown in Poland, and to compare morphological features and ecological relations of *O. ainoae* H. Solheim, *O. bicolor*, *O. cucullatum* H. Solheim, *O. flexuosum* H. Solheim and *O. piceaperdum* (Rumb.) von Arx occurring in Poland with data reported from other countries in Eurasia.

**Materials and methods**

**Study areas and fungal isolations**

Investigations were carried out during 1998–2001 in three experimental plots in Norway spruce stands situated in the Ustroń Forest District (Holcyna Forest Range, compartment 90b), Gorce National Park (Łopuszna Forest Range, compartment 75b) and Krynica Experimental Forest (Kopciowa Forest Range, compartment 3d).

In total 123 Norway spruce trees infested by *I. typographus* were felled during the period from June to August, and then analysed for presence of fungi associated with this bark beetle. From the infested trunk section of each tree six–eight discs were cut, and their bark was separated from wood under sterile conditions. Selected gallery fragments were disinfected with 96% ethyl alcohol. Fungi were isolated from: a) fragments of *I. typographus* galleries in periderm, phloem, and outer layers of sapwood to the depth of 5 mm, b) fragments of discoloured and undiscoloured sapwood underneath galleries of *I. typographus* to the depth of 50 mm. After drying of samples a surface layer of tissues was removed with a sterile scalpel and fragments, 4 × 4 mm in size, were cut and transferred to a medium. All isolations and cultures of fungi were performed on 2% malt extract agar (MEA) with an addition of antibiotic – tetracycline (200 mg/l of MEA). Incubation of fungi
took place at room temperature in the dark. Colonies of fungi were compared on
the basis of macro- and microscopic characteristics, and from representative colo-

nies pure cultures were developed in order to identify the fungi. Transferring small
pieces of mycelium made the pure cultures. In total, the fungi were isolated from
13 630 fragments of galleries and adjacent sapwood. In the first case 842 galleries
of *I. typographus* were considered.

In June and July larvae and beetles of *I. typographus* were collected from its gal-

leries on infested trap trees. The larvae, before isolation of fungi, were bathed in
sterile water for 30 s, or disinfected in 96% ethyl alcohol for 30 s. The beetles were
bathed in sterile water for 30 s, or disinfected in 96% ethyl alcohol for 15 and 30 s.
After drying on a sterile blotting paper the disinfected larvae and beetles were

crushed on a microscopic slide and using a sterile scalpel were evenly spread over
the surface of medium. In total, the isolations were performed from 789 adults
and 500 larvae of *I. typographus*.

Frequencies were computed using the following formula: \( F = \frac{(N_F/N_T) \times 100}{Yamaoka et al. 1997} \), where \( F \) represents the frequency of occurrence of each fun-
gal species, \( N_F \) represents number of larvae, adults, fragments of galleries and sap-
wood underneath the galleries from which a particular fungus was isolated, and
\( N_T \) represents total number of larvae, adults, fragments of galleries and sapwood
underneath the galleries from which isolations were made.

**Morphological investigations**

The morphological description of fungi was based on: a) cultures grown on the

medium, b) teleomorphs and anamorphs of fungi found in galleries of *I. typographus* developed under natural conditions. In the case of cultures the isolates

for further investigations were obtained by the following method: the conidia or
ascospores formed in cultures or under natural conditions were evenly spread over
the medium, and then the fragments of the developed mycelium were transferred
onto fresh medium. When the mycelium appeared, a disc, 7 mm in diameter, was
cut and placed in a central position of Petri dishes with the medium. Investigations

were carried out on 25 isolates from larvae, beetles and galleries of *I. typographus*.
The measurement of a culture diameter was carried out on isolates in two repeti-
tions after four days since the inoculum was taken. Fungi were grown at 22°C in
the dark. During this study the teleomorphs and anamorphs were measured and
the mycelium was checked after 10–21 days since the beginning of incubation. Pro-
duced teleomorphs and anamorphs were taken from each isolate at random. The
mean values of individual morphological characteristics were computed on the ba-
sis of 30–50 measurements.
Results

*Ophiostoma ainoae* was one of the most commonly isolated taxa in this study. From among the specimens collected in galleries of this spruce bark beetle, 39.6% of larvae and 35.2% adults were colonized. This fungus colonized exactly 27% of galleries. 6.4% of sapwood fragments from underneath galleries of *I. typographus* were also colonized (Table 1). *Ophiostoma ainoae* produced perithecia both on the surface of the 2% MEA medium and in the galleries of *I. typographus*. The synanamorphs were *Peso* and *Hylorhinocladia* type (Table 2, Photos 1–9).

*Ophiostoma bicolor* was the fungus most frequently isolated from sapwood underneath galleries of *I. typographus* with a frequency of occurrence of 10.5%. It was especially common in larvae and beetles (36.8% and 32.8, respectively). Over 30% of galleries of *I. typographus* were also colonized by this species (Table 1). This fungus produced perithecia both on the surface of the 2% MEA medium and in the galleries of *I. typographus*. The anamorph was *Hylorhinocladia* type (Table 2, Photos 10–13).

*Ophiostoma cucullatum* was not found in larvae. From the other niches examined, it occurred in lower frequencies. This species was most often isolated from beetles collected in galleries (3.5%). It occasionally colonized the galleries and sapwood underneath galleries of *I. typographus* (1.0 and 0.2%, respectively) (Table 1). *Ophiostoma cucullatum* produced perithecia only on the surface of the 2% MEA medium. The anamorph was *Peso* type (Table 2, Photos 14–19).

*Ophiostoma flexuosum* was most often isolated from beetles collected in galleries (7.3%) similarly as the previous species. 2.6% of larvae were also colonized by this fungus. In the galleries and sapwood underneath galleries of *I. typographus* this species occurred less frequently, ranging from 1.1 to 0.2% (Table 1). This fungus produced perithecia only on the surface of the 2% MEA medium. The anamorph was *Sporothrix* type (Table 2, Photos 20–23).

*Ophiostoma piceaperdum* was isolated from 12.5–20.0% of beetles and larvae collected in galleries of *I. typographus*. Frequencies of occurrence from the galleries

| Table 1 |
|-----------------|-----------------|-----------------|-----------------|
| **Fungus**      | **Larvae**      | **Beetles**     | **Galleries**   |
| *Ophiostoma ainoae* | 39.6            | 35.2            | 27.0            | 6.4            |
| *Ophiostoma bicolor* | 36.8            | 32.8            | 30.8            | 10.5           |
| *Ophiostoma cucullatum* | –               | 3.5             | 1.0             | 0.2            |
| *Ophiostoma flexuosum* | 2.6             | 7.3             | 1.1             | 0.2            |
| *Ophiostoma piceaperdum* | 20.0           | 12.5            | 23.3            | 5.1            |
| Number of examined larvae, beetles, fragments of galleries and fragments of sapwood | 500 | 789 | 10 100 | 3 530 |

*Total mean for all methods of disinfection.
Characteristics of anamorphs, teleomorphs and cultures of *Ophiostoma ainoae*, *O. bicolor*, *O. cucullatum*, *O. flexuosum* and *O. piceaperdum* (µm)

<table>
<thead>
<tr>
<th></th>
<th><em>Ophiostoma ainoae</em></th>
<th><em>Ophiostoma bicolor</em></th>
<th><em>Ophiostoma cucullatum</em></th>
<th><em>Ophiostoma flexuosum</em></th>
<th><em>Ophiostoma piceaperdum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perithecial base</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MEA</td>
<td>90.2–209.1 (134.0) × 94.3–205.0 (135.8)</td>
<td>137.7–243.0 (203.6) × 129.6–259.2 (206.8)</td>
<td>72.9–137.7 (96.1) × 72.9–137.7 (100.2)</td>
<td>82.0–164.0 (112.9) × 82.0–151.7 (107.1)</td>
<td>178.2–243.0 (202.3) × 178.2–267.3 (216.5)</td>
</tr>
<tr>
<td>Natural substrate</td>
<td>97.2–194.4 (146.9) × 97.2–194.4 (138.5)</td>
<td>129.6–324.0 (246.4) × 137.7–364.5 (249.7)</td>
<td>– –</td>
<td>72.9–137.7 (100.2) × 72.9–137.7 (100.2)</td>
<td>121.5–299.7 (189.9) × 129.6–291.6 (179.6)</td>
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<tr>
<td><strong>Length of neck</strong></td>
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<tr>
<td>MEA</td>
<td>500.0–1048.0 (711.7)</td>
<td>648.0–1247.4 (921.5)</td>
<td>291.6–567.0 (427.4) with ostiolar hyphae</td>
<td>229.6–500.2 (384.8)</td>
<td>226.8–947.7 (532.7)</td>
</tr>
<tr>
<td>Natural substrate</td>
<td>388.8–1020.3 (593.2)</td>
<td>534.6–1498.5 (902.9)</td>
<td>– –</td>
<td>– –</td>
<td>226.8–907.2 (989.3)</td>
</tr>
<tr>
<td><strong>Width of neck at the base</strong></td>
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<tr>
<td>MEA</td>
<td>20.0–44.0 (28.3)</td>
<td>56.7–97.2 (69.7)</td>
<td>24.3–40.5 (30.0)</td>
<td>16.4–41.1 (25.1)</td>
<td>40.5–97.2 (53.1)</td>
</tr>
<tr>
<td>Natural substrate</td>
<td>24.0–44.0 (28.3)</td>
<td>40.5–129.6 (71.0)</td>
<td>– –</td>
<td>– –</td>
<td>32.4–56.7 (41.9)</td>
</tr>
<tr>
<td><strong>Width of neck at the tip</strong></td>
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<tr>
<td>MEA</td>
<td>12.0–30.0 (14.3)</td>
<td>24.3–48.6 (33.1)</td>
<td>16.2–24.3 (20.8)</td>
<td>8.2–32.8 (12.3)</td>
<td>12.1–40.5 (20.5)</td>
</tr>
<tr>
<td>Natural substrate</td>
<td>16.2–20.2 (16.3)</td>
<td>20.2–40.5 (30.2)</td>
<td>– –</td>
<td>– –</td>
<td>16.2–44.5 (30.1)</td>
</tr>
<tr>
<td><strong>Length of ostiolar hyphae</strong></td>
<td></td>
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<tr>
<td>MEA</td>
<td>16.0–81.0 (36.1)</td>
<td>– lack</td>
<td>56.7–162.0 (109.6)</td>
<td>24.6–86.1 (41.1)</td>
<td>lack</td>
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<tr>
<td><strong>Ascospores</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Size including sheaths</td>
<td>4.0–5.5 (4.8) × 2.0–2.75 (2.4)</td>
<td>4.75–7.5 (5.6) × 2.5–4.5 (3.0)</td>
<td>5.0–6.25 (5.3) × 2.5–5.5 (3.2)</td>
<td>3.5–5.25 (4.7) × 1.25–2.5 (1.6)</td>
<td>5.5–7.75 (7.4) × 3.0–5.5 (4.9)</td>
</tr>
<tr>
<td>Shape</td>
<td>cylindrical in face and side view</td>
<td>rectangular in face and side view</td>
<td>ellipsoidal in face and reniform in side view</td>
<td>allantoid in face and baccilliform in side view</td>
<td>ellipsoidal in face and hat-shaped in side view with brims</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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</tr>
<tr>
<td><strong>Type of anamorph</strong></td>
<td>Pesotum sp. and Hyalorhinocladiella sp.</td>
<td>Hyalorhinocladiella sp.</td>
<td>Pesotum sp.</td>
<td>Sporothrix sp.</td>
<td>Leptographium piceaperdum</td>
</tr>
<tr>
<td><strong>Synnematal length on MEA for</strong></td>
<td><strong>Pesotum:</strong> 7.5–35.0 (19.7)</td>
<td>151.0–418.2 (239.2)</td>
<td>4.0–44.0 (24.2)</td>
<td>60.2–324.0 (161.5)</td>
<td></td>
</tr>
<tr>
<td><strong>natural substrate</strong></td>
<td><strong>for Pesotum:</strong> 180.0–860 (390.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Conidia size</strong></td>
<td><strong>for Pesotum:</strong> 3.0–9.5 (5.7) × 1.25–4.0 (2.7)</td>
<td>3.5–8.0 (5.4) × 2.25–3.0 (2.6)</td>
<td>2.5–8.0 (5.6) × 1.25–3.75 (2.4)</td>
<td>3.25–7.0 (4.7) × 2.25–4.0 (2.7)</td>
<td></td>
</tr>
<tr>
<td><strong>shape</strong></td>
<td><strong>for Pesotum:</strong> cylindrical, ellipsoidal and clavate</td>
<td>ellipsoidal and clavate</td>
<td>cylindrical and tear-shaped</td>
<td>clavate</td>
<td>ellipsoidal and truncate</td>
</tr>
<tr>
<td><strong>Cultures on MEA after four days: structure and colour, grow in 22°C</strong></td>
<td>often immersed, aerial mycelium in white aggregation; hyaline to brown, 16–22 mm</td>
<td>immersed, hyaline-light yellow to light brown, 28–35 mm</td>
<td>immersed, aerial mycelium woolly, flocose; pale yellow, 12–26 mm</td>
<td>immersed, aerial mycelium flocose; dark brown to black, 13–15 mm</td>
<td>immersed, aerial mycelium olive-green, very miserly; brown to black, 37–48 mm</td>
</tr>
</tbody>
</table>

*Sizes of perithecial are given as vertical × horizontal.*
Photos 1–9. *Ophiostoma ainoae*: 1 – perithecium on the wood, 2 – *Pesotum* state anamorph on the malt agar medium, 3 – conidia, 4 – ascospores, 5 – perithecium on the malt agar medium, 6 – top of neck, 7 – *Pesotum* state anamorph on the wood, 8 – *Hyalorhinocladiella* state anamorph, 9 – culture on the malt agar medium after 14 days (photo by T. Kowalski)
and sapwood underneath galleries of *I. typographus* ranged from 23.3 to 5.1% (Table 1). *Ophiostoma piceaperdum* produced perithecia both on the surface of the 2% MEA medium and in the galleries of *I. typographus*. The anamorph was *Leptographium piceaperdum* K. Jacobs et M.J. Wingfield (Table 2, Phots 24–28).

Photos 10–13. *Ophiostoma bicolor*: 10 – peritheciun on the periderm, 11 – peritheciun on the malt agar medium, 12 – ascospores, 13 – culture on the malt agar medium after 14 days (photo by T. Kowalski)
Discussion

*Ophiostoma ainoae* was reported from Austria, Denmark, Norway, Sweden, and Japan in association with *I. typographus* (Solheim 1986, Harding 1989, Yamaoka et al. 1997, Kirisits et al. 1998). In the present study this species belonged to fungi often

Photos 20–23. *Ophiostoma flexuosum*: 20 – peritheciun on the malt agar medium, 21 – ascospores, 22 – *Sporothrix* state anamorph, 23 – culture on the malt agar medium after 14 days (photo by T. Kowalski)
associated with *I. typographus*. Most frequently *O. ainoae* colonized larvae and adults collected in galleries of this bark beetle. Also in other countries *O. ainoae* was frequently isolated from beetles collected in galleries of *I. typographus* (Solheim 1992a, Kirisits et al. 1998). It was most numerously isolated in Japan where it colonized over 80% of beetles of *I. typographus* f. *japonicus* Niijima (Yamaoka et al. 1997). The galleries of *I. typographus* investigated during this study were relatively abundantly colonized by *O. ainoae*. Results of other authors indicate a great variability in the degree of colonization of galleries of *I. typographus* by this fungus. In Norway *O. ainoae* colonized 0–22.0% of gallery fragments (Solheim 1993 b), in Denmark and Sweden only 6.0–8.0% (Harding 1989), while in Japan 51.2–61.5% (Yamaoka et al. 1997).

Ophiostoma ainoae belonged to fungi, which from the layer of wood adjacent to galleries of I. typographus was relatively rarely isolated. This indicates that O. ainoae is a species little expansive, and it is not adapted to colonize the deeper sapwood layers. The same was observed by Solheim (1992 b) and by Harding (1989).

This species was for the first time isolated from galleries of I. typographus in Norway and described from a conidial stage Pesotum by Solheim (1986). Taxonomic characteristics of a teleomorphic stage produced on a medium differ to a considerable degree from morphological characteristics presented for this species by Solheim (1986), who however examined this species under different growth conditions (2% malt, 1.5% agar). The bases of perithecium were considerably larger, and its neck longer while at the base and tip it was narrower. Similar differences were noticed by Yamaoka et al. (1997). In the present study it was also found that dimensions of ascospores were larger than reported by Solheim (1986), and it was observed that there were certain differences in size of perithecium depending on a substrate. Perithecia developed on the medium had smaller perithecial bases and longer necks than perithecia developed on the wood. Ophiostoma ainoae produced synanamorphs Pesotum and Hyalorhinocladiella. The latter one was not mentioned in the description of Solheim (1986), but it was reported by Yamaoka et al. (1997).


Ophiostoma bicolor was one of the most commonly isolated from larvae and beetles taxa in this study. In Japan this species was isolated a little more frequently, i.e. from almost 45% of beetles of I. typographus f. japonicus, as compared with Poland (Yamaoka et al. 1997), while isolations carried out by Furniss et al. (1990) in Norway showed over twice as frequent colonization of beetles by O. bicolor than in the present study. Also a relatively numerous occurrence of O. bicolor in larvae and beetles was confirmed by Moser et al. (1997), who found that this species was the most frequent fungus occurring on bodies of mites associated with I. typographus f. japonicus.

Ophiostoma bicolor in this study was the fungus most frequently isolated from sapwood underneath galleries of I. typographus. A comparison of results obtained by many authors in Europe and Asia showed that the occurrence of O. bicolor in galleries of I. typographus is quite strongly diversified (Harding 1989, Solheim 1993 b, Krokene and Solheim 1996, Yamaoka et al. 1997). In Japan O. bicolor was isolated from 11.4% of fragments (Yamaoka et al. 1997), while in Norway Solheim (1993 b), and Krokene and Solheim (1996) found O. bicolor in 22.0–100.0% of gallery fragments in artificially colonized spruce trunks with beetles of I. typographus. In Denmark this species was isolated from 20.0–80.0% of I. typographus gallery fragments (Harding 1989). In the present study it was found that in wood layers, adjacent to galleries of I. typographus, O. bicolor was the most frequently occurring species among the fungi under discussion. On the basis of results of this study it
may be concluded that *O. bicolor* may colonize wood in its deeper layers. Also according to Harding (1989) *O. bicolor* was, beside *C. polonica*, the most frequently isolated species from the depth of 3 cm.

This species was for the first time isolated from *Picea engelmannii* Parry and described by Davidson and Wells (Davidson 1955). Also the descriptions of the species were presented by Kotýnková-Sychrová (1966), Griffin (1966) and Upadhyay (1981). The dimensions of perithecia and ascospores, as well as dimensions of synnemata and conidiospores produced on a medium, were within limits of the diagnostic descriptions published for this species by Kotýnková-Sychrová (1966) and Upadhyay (1981). This study revealed differences in size of perithecial bases depending on substrate. The bases of perithecia produced on the medium had considerably smaller dimensions than bases of perithecia developed on the wood.

*Ophiostoma cucullatum* sporadically accompanied *I. typographus* in southern Poland. It was isolated considerably more frequently from beetles than from galleries. It is interesting that it was not found in larvae, which often were present in galleries in a direct neighbourhood of beetles. The results of this study suggest that *O. cucullatum* is quite strongly associated with beetles of *I. typographus*. Also Yamaoka et al. (1997) in Japan found this fungus only in beetles collected in galleries of *I. typographus* f. *japonicus*. During the present study *O. cucullatum* sporadically colonized galleries of *I. typographus* and adjacent wood. Also in Austria (Kirisits et al. 1998), Norway (Solheim 1986) and Denmark (Harding 1989) this species was sporadically isolated from galleries of *I. typographus*.

This species was for the first time isolated from beetles of *I. typographus* in Norway and described from the conidial stage *Phialographium* by Solheim (1986). In comparison with the taxonomic characteristics of perithecia given by this author (under different growth conditions), the isolates investigated in the present study produced perithecia having slightly larger dimensions of a perithecial basis and longer ostiolar hyphae.

*Ophiostoma flexuosum* belonged to species rarely associated with *I. typographus*. Also in other European countries it was only sporadically isolated from larvae, beetles and galleries of *I. typographus* (Solheim 1986, Harding 1989).

This species was for the first time isolated from galleries of *I. typographus* in Norway and described from the conidial stage *Sporothrix* by Solheim (1986). In comparison with the morphological characteristics of perithecia reported by this author (under different growth conditions) the isolates investigated during the present study produced perithecia having considerably shorter necks and longer ostiolar hyphae.

*Ophiostoma piceaperdum* inhabits various species of trees from genera *Pinus*, *Picea* and *Pseudotsuga*, and is associated with insects from genera *Dryocoetes*, *Hylurgops*, *Pityogenes*, *Polygraphus*, as well as with *I. typographus* (Griffin 1966, Kotýnková-Sychrová 1966, Olchowecki and Reid 1974, Harrington and Cobb 1988, Perry 1991, Jacobs et al. 1998, 2000) and *I. duplicatus* (Krokene and Solheim 1996). *Ophiostoma piceaperdum* was isolated from galleries of *I. typographus* in North America (Wright and Cain 1961, Griffin 1966, Olchowecki and Reid 1974, Jacobs et al. 2000), Asia (Yamaoka et al. 1997) and also in various countries of Europe, in-
cluding Norway (Solheim 1986), Denmark (Harding 1989), Austria (Kirisits et al. 1998) and former Czechoslovakia (Kotýnková-Sychrová 1966). In Australia this species was isolated from wood of Douglas fir imported from northwestern regions of North America, and it may create a threat to plantations of conifers in that continent (Mireku and Simpson 2002).

*Ophiostoma piceaperdum* was one of the most common fungi isolated in this study. It was a fungus frequently associated with larvae and beetles of *I. typographus*. Similar colonization of beetles was reported from Japan (Yamaoka et al. 1997). The galleries of *I. typographus* were colonized by *O. piceaperdum* to a similar degree as its larvae and beetles. The results of many authors in Europe and Asia indicate that the frequency of colonization of *I. typographus* galleries by *O. piceaperdum* may vary (Harding 1989, Solheim 1986, 1992 a, Yamaoka et al. 1997). In Japan *O. piceaperdum* was isolated from 21.2–26.9% of tissue fragments taken from egg and larval galleries of *I. typographus f. japonicus* (Yamaoka et al. 1997). Solheim (1986, 1993 b) reported in Norway a sporadic occurrence of this fungus in galleries of *I. typographus*, but in the case of artificial colonization of Norway spruce trees with beetles of *I. typographus* the occurrence of *O. piceaperdum* was frequent (Solheim 1992 a). In Denmark and Sweden Harding (1989) isolated *O. piceaperdum* from about 25–40% of the investigated fragments. *Ophiostoma piceaperdum* belonged to the species least frequently isolated from stained and unstained wood adjacent to galleries of *I. typographus*. This can indicate a lack of active penetration of wood adjoining to galleries of this bark beetle. A similar observation was reported by Harding (1989) and by Solheim (1992 b).

This species was for the first time described by Rumbold in 1936 as *Ceratostomella piceaperda* Rumbold (Jacobs et al. 2000). In the 1960s Wright and Cain (1961) isolated a similar species from spruce and pine, differing from the previous one by the shape of ascospores and described it as *Ceratocystis europhioides* Wright et Cain. Upadhyay (1981) considered *C. europhioides* as a synonym of *O. piceaperdum*, while Solheim (1986) made a clear distinction between these two species. In the last publication concerning this problem Jacobs et al. (2000) support the opinion of Upadhyay (1981) and accept *O. europhioides* and *C. pseudoeurophioides* Olchoweczki et Reid as synonyms of *O. piceaperdum*. The morphological characteristics of *O. piceaperdum* observed during this study are very close to the diagnostic characteristics given by Wright and Cain (1961), as well as Jacobs et al. (1998). The differences in size of perithecia depending on a substrate were observed. The perithecia produced on the medium had larger perithecial bases and shorter necks than perithecia produced on the wood.

**Summary**

The investigations concerned certain aspects of morphology and frequency of occurrence of *Ophiostoma ainoae, O. bicolor, O. cucullatum, O. flexuosum* and *O. piceaperdum* in Poland. The isolates of fungi were made from larvae, beetles, and
galleries of *Ips typographus* on Norway spruce trees growing in the Gorce National Park, Krynica Experimental Forest and Ustroń Forest District. The morphological characteristics were based on cultures grown on MEA at 22°C, as well as on teleomorphs and anamorphs found in galleries of *I. typographus* developed in natural conditions. Also the occurrence frequency of fungi in larvae, beetles, galleries of *I. typographus*, and wood adjacent to these galleries was determined. It was found that other authors basically within limits of the diagnostic characteristics reported the taxonomic characteristics of these fungi. The greatest differences between some morphological characteristics were mainly connected with a kind of a substrate on which fructifications of fungi were grown. From among the species investigated during this study the following were the most frequently occurring in larvae, beetles, and galleries of *I. typographus*: *O. ainoae*, *O. bicolor* and *O. piceaperdum*. The fungi *O. flexuosum* and *O. cucullatum* considerably more often inhabited larvae and beetles, while in galleries they occurred sporadically. *Ophiostoma ainoae* was the most frequently occurring species on the larvae and beetles, and *O. bicolor* most frequently colonized the galleries and adjoining wood.

**Streszczenie**

NOWE DLA POLSKI GRZYBY OFIOSTOMATOIDALNE TOWARZYSZĄCE KORKNIKOWI DRUKARZOWI (*IPS TYPOGRAPHUS*): WYSTĘPOWANIE I MORFOLOGIA

Badania koncentrowały się na niektórych aspektach morfologicznych oraz na wykazaniu częstości występowania Ophiostoma ainoae, O. bicolor, O. cucullatum, O. flexuosum i O. piceaperdum w Polsce. Izolaty grzybów wyodrębniono z larw, imagines oraz żerowisk *Ips typographus* na świerkach rosnących na terenie Gorczańskiego Parku Narodowego, Leśnego Zakładu Doświadczalnego w Krynicy i Nadleśnictwa Ustroń. Morfologię badano z wykorzystaniem kultur hodowanych na pożywce MEA w temperaturze 22°C oraz stadiów teleomorficznych i anamorficznych grzybów występujących w żerowiskach kornika drukarza z „natury”. Określono także częstości występowania grzybów w larwach, imagines, żerowiskach oraz drewnie przylegającym do żerowisk *I. typographus*. Stwierdzono, że cechy taksonomiczne badanych grzybów zasadniczo mieściły się w granicach cech diagnostycznych podanych dla tych gatunków przez innych autorów. Największe różnice pomiędzy niektórymi cechami morfologicznymi były związane z rodzajem substratu, na którym wzrastały owocniki grzybów. Wśród badanych grzybów najczęściej występowały w larwach, imagines i żerowiskach *I. typographus*: *O. ainoae*, *O. bicolor* i *O. piceaperdum*. Grzyby *O. flexuosum* i *O. cucullatum* znacznie częściej zasiedlały larwy i imagines, a w żerowiskach owada występowały sporadycznie. Spośród badanych grzybów *O. ainoae* najczęściej zasiedlał larwy i imagines, a żerowiska oraz przylegające do nich drewno – *O. bicolor*.
Literature


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