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The early stages of fungal succession in *Pinus sylvestris* phloem and sapwood infested by *Tomicus piniperda*

Abstract: Fungal invasion of Scots pine phloem and sapwood was investigated during a period of 15 weeks following attack by the pine shoot beetle, *Tomicus piniperda* (L.). The study was conducted in Mielec-Mościska, where the pine trees were heavily damaged by shot-feeding of *T. piniperda*. In order to determine the species richness and occurrence frequency of fungi associated with *T. piniperda* in temporal succession, living and trap trees infested by *T. piniperda* were used. Results revealed great diversity of fungi associated with *T. piniperda*, including 3758 cultures and 57 fungi species. The most important groups of fungi were the blue-stain fungi and molds, including mainly *Penicillium*, *Trichoderma* and *Mucor* genera. Among ophiostomatoid fungi, *Ophiostoma minus* and *O. piceae* were the dominant species. Occasionally isolated species were *Leptographium lundbergii*, *L. procerum*, *L. wingfieldii*, *Graphium pycnocephalum* and *Graphium* sp. 'W'. Molds and pathogenic *O. minus* were the first invaders of both phloem and sapwood, however molds were more frequently isolated from phloem and sapwood at a depth of 5 mm. *Ophiostoma piceae* and *L. lundbergii* followed *O. minus* in the sapwood invasion. These species were successively replaced by *L. wingfieldii*, *L. procerum* and *Graphium* species in the later stages of fungal invasion in pine sapwood.

Additional key words: blue-stain fungi, molds, ophiostomatoid fungi.

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Introduction

Blue-stain fungi are considered as primary colonizers of the freshly cut logs. These fungi utilize easily usable substrates such as simple sugars and starch stored in the ray parenchyma cells. Later, the wood is colonized by decay fungi which degrade all the major wood components (cellulose, hemicelluloses and lignin) and saprotrophic species (Seifert 1993).

Phloeophagous bark beetles introduce various microorganisms to the phloem and cambium of trees. Most of them belong to the yeasts and ophiostomatoid fungi. Many species of ophiostomatoid fungi (including *Ceratocystis*, *Ceratocystiopsis* and *Ophiosto-ma*) cause sap-stain in the phloem and sapwood of trees infested by bark beetles. Only the most virulent of them are able to colonize fresh sapwood after insect's attack. Less virulent species follow the primary invaders (Kirisits 2004).

The larger pine shoot beetle, (*Tomicus piniperda* (L.); *Coleoptera: Scolytidae*) is native to Europe, North Africa and Asia. It is one of the main insect pests of *P. sylvestris* L. in Poland. It usually reproduces in recently fallen or dead trees but can also attack stressed living trees. The most serious damage has been found in pine stands growing nearby sawmills and wood yards, where *T. piniperda* may contribute to tree death. The pine shoot beetle attacks both the trunks and shoots

of pines, but the destruction of shoots by adult maturation feeding is the most serious (Michalski and Mazur 1999).

Tomicus piniperda is scolytid with a relatively loose relationship with ophiostomatoid fungi. In Europe, this insect carries numerous species of *Ophiostoma* and their anamorphs, however none of these occurs with high frequency in different populations of the scolytid (Mathiesen 1950, Rennerfelt 1950, Mathiesen-Käärik 1953, Morelet 1988, Lieutier et al. 1989, Piou and Lieutier 1989, Gibbs and Inman 1991, Solheim and Långström 1991, Wingfield and Gibbs 1991, Jacobs and Wingfield 2001). These reports also showed that *Ophiostoma minus* (Hedgc.) Syd. & P. Syd. and *Leptographium wingfieldii* M. Morelet were consistently associated with *T. piniperda* in Europe.

In Poland, Siemaszko (1939) found *O. minus*, *O. piceae* (Münch) Syd. & P. Syd. and *O. piliferum* (Fr.) Syd. & P. Syd. associated with *T. piniperda* in pine stands near Warsaw. Jankowiak (2006a) reported that *T. piniperda* may carried twelve species of ophiostomatoid fungi, mostly *O. minus* and a *Leptographium* spp.

The present study describes the fungal colonization of Scots pine tissues during 15 weeks followed by a successful attack of *T. piniperda*.

Material and methods

The study was conducted in Mielec-Mościska (50°194'25''N, 21°29'39''E) in 2005. All materials were collected from the 40–50-year old stand, where *P. sylvestris* was a dominant species. The stand was located beside the timber store and trees were heavily damaged by shot-feeding of *T. piniperda*. In order to determine the frequency and diversity of fungi associated with *T. piniperda* feeding in temporal succession, the living and trap trees infested by *T. piniperda* were examined.

For the trap trees, 18 uninfested Scots pine trees were felled in mid March. They, were laid flat on the forest floor with intact crowns and left for colonization by *T. piniperda*. Insects main attack started on April the 1st. Samples from these trees were taken 2, 4, 6, 8, 10 and 15 weeks after the main attack. The living trees infested by *T. piniperda* were felled 2, 4, 6, 8, 10 and 15 weeks after the main attack. In total 12 living trees were analysed.

Four 30 cm long sections with intact bark were cut from parts of the trunk infested by *T. piniperda*. They were cut from a part of trunk located between 2 and 6 m away from the base. In the laboratory the sections were cut into the 6 cm thick discs. The bark was separated from the wood under sterile conditions, and gallery fragments were disinfected with cotton wool saturated with 96% ethanol, which covered samples for 15 sec. It was followed by drying with filter paper.

Fungi were isolated from phloem taken from the female and larval galleries of *T. piniperda* and their discoloured surroundings, and also from the sapwood lying up to 35 mm beneath galleries along a radii. From an individual radius four samples were taken: from depth of 0.5 mm, 10 mm, 20 mm and 35 mm. The radii were selected to correspond with the position of galleries with successful brood establishment. Development of visible blue-stain in sapwood of Scots pine after attack by *T. piniperda* was also noted. A surface layer of phloem was removed aseptically and 4 × 4 mm fragments of phloem or sapwood were cut, and placed on 2% malt extract agar (MEA: 20 g malt extract, 20 g agar, 1000 ml distilled water, 200 mg tetracycline) in 9 cm plastic Petri dishes.

Fungi were incubated at room temperature in the dark. Fungi were identified on the basis of their morphology. Altogether, 3600 fragments were taken from 600 galleries examined (Table 1).

Frequency was defined as the percentage of isolates of individual species in relation to the total number of isolates. For a different sapwood depth, frequency was expressed as the percentage of sapwood fragments, from which a given species was isolated in relation to the total number of fragments from which isolations were made.

Simpson diversity index (D) was used to compare the fungal diversity (Simpson 1949). The formula for Simpson index is:

$$D = 1 - \sum_{i=1}^{i=s} P_i^2$$

where P_i is the probability of sampling species *i*, *i* is the frequency of species *i* / total frequency for all species, *S* is the species richness (number of species per sample). D ranges from zero to one and denotes the probability that two randomly selected individuals in a community belong to different species. A value close to zero suggests that dominant species may exist in a population, and conversely, a value close to one indicates species equitability, that is, the species are evenly distributed. Dominance or subordinance in fungal communities was determined with Camargo's index (Camargo 1993). A dominant species is present if $P_i > 1/S$, where S represents number of species per sample; the number of competing species in a community.

Results

Fungi isolated from the phloem infested by *T. piniperda*

A total of 2145 fungal isolates, representing 57 species, were obtained from Scots pines phloem infested with *T. piniperda*. Fungi were isolated from 91.3% of 1200 fragments (Table 1). The most fre-

Table 1. Fungi isolated from the phloem of *Pinus sylvestris* trees after an attack by *Tomicus piniperda*

Taxon	Number of isolates (% frequency) in the 2, 4, 6, 8, 10 and 15 week after attack						
	2	4	6	8	10	15	Total
Ophiostomatoid fungi							
Graphium pseudormiticum M. Mouton & M.J. Wingf.					4(0.8)	1(0.2)	5(0.2)
Graphium pycnocephalum Grosm.				10(2.7)	45(9.5)	25(5.2)	80(3.7)
Graphium sp. "W"	1(0.5)			17(4.6)	9(1.9)	6(1.3)	33(1.5)
Leptographium lundbergii Legerb. & Melin		1(0.3)	2(0.8)	13(3.5)			16(0.7)
Leptographium procerum (W. B. Kendr.) M.J. Wingf.				6(1.6)	7(1.5)		13(0.6)
Leptographium wingfieldii M. Morelet			1(0.4)		2(0.4)	11(2.3)	14(0.7)
Ophiostoma minus (Hedgc.) Syd. & P. Syd	31(15.4)	24(6.7)	9(3.4)	64(17.3)	108(22.9)*	33(6.9)	269(12.5)
Ophiostoma piceae (Mûnch) Syd. & P. Syd	1(0.5)	4(1.1)	29(10.9)	64(17.3)	107(22.7)*	109(22.9)*	314(14.6)
Pesotum sp.				2(0.5)		12(2.5)	14(0.7)
Other							
Acremonium sp.		6(1.7)	1(0.4)			9(1.9)	16(0.7)
Altenaria alternata (Fr.) Keissl.		2(0.6)			2(0.4)		4(0.2)
Arthrinium sp.					2(0.4)		2(0.1)
Aspergillus niger Tiegh.	7(3.5)	1(0.3)				1(0.2)	9(0.4)
Botrytis cinerea Pers.						5(1.0)	5(0.2)
Chloridium sp.			1(0.4)			1(0.2)	2(0.1)
Cladosporium cladosporoides (Fresen.) G.A. de Vries		1(0.3)	1(0.4)			1(0.2)	3(0.1)
Cytospora pinastri Fr.				2(0.5)	1(0.2)	1(0.2)	5(0.2)
Dipodascus aggregatus Francke-Grosm.		2(0.6)	2(0.8)	10(2.7)	52(11.0)	14(2.9)	80(3.7)
Epicoccum nigrum Link	2(1)	1(0.3)		1(0.3)	1(0.2)		5(0.2)
Epithyrium resinae (Sacc. & Berl.) Sacc.		2(0.6)					2(0.1)
Exophiala sp.					2(0.4)	4(0.8)	6(0.3)
Fusarium sp.				1(0.3)	3(0.6)		4(0.2)
Geosmithia sp.				5(1.4)	3(0.6)	8(1.7)	16(0.7)
Geotrichum candidum Link	3(1.5)						3(0.1)
Gliocladium catenulatum J.C. Gilman & E.V. Abbot				1(0.3)		1(0.2)	2(0.1)
Harposporium sp.		2(0.6)					2(0.1)
Heterobasidion annosum (Fr.) Bref.						5(1.0)	5(0.2)
Hormonema dematioides Lagerb. & Melin	12(6.0)	24(6.7)	8(3.0)	9(2.4)	1(0.2)		54(2.5)
Lecythophora hoffmannii (van Beyma) W. Gams & McGinnis		8(2.2)	3(1.1)	1(0.3)	4(0.8)	2(0.4)	18(0.8)
Leptodontidium beauverioides de Hoog	1(0.5)						1(0)
Mortierella ramanniana var. *ramanniana* (A. Mõller) Linnem.	1(0.5)	2(0.6)	17(6.4)	9(2.4)	5(1.1)	5(1.0)	39(1.8)
Mucor spp.		13(3.6)	7(2.6)	33(8.9)	21(4.4)	22(4.6)	96(4.5)
Myrothecium cf. indicum P. Rama Rao		12(3.3)	1(0.4)				13(0.6)
Oidiodendron tenuissimum (Peck) S. Hughes	4(2.0)	9(2.5)		2(0.5)			15(0.7)
Paecilomyces variotii Bainier						2(0.4)	2(0.1)
Penicillium spp.	57(28.4)*	147(40.8)*	81(30.5)*	59(16.0)	39(8.3)	17(3.6)	400(18.6)
Pezicula eucrita P. Karst.	3(1.5)	10(2.8)	2(0.8)				15(0.7)
Phialocephala cf. dimorphospora Kendrick		5(1.4)		2(0.5)	1(0.2)		8(0.4)
Phialophora bubaki (Laxa) Schol-Schwarz			1(0.4)			4(0.8)	5(0.2)
Phialophora clavispora W. Gams						5(1.0)	5(0.2)
Phialophora sp.						3(0.6)	3(0.1)
Phoma sp.	3(1.5)	2(0.6)					5(0.2)
Rhinocladiella atrovirens Nannf.	4(2.0)	7(1.9)	6(2.3)	4(1.1)		3(0.6)	24(1.1)
Rhizoctonia sp.		5(1.4)		1(0.3)			6(0.3)

Taxon	Number of isolates (% frequency) in the 2, 4, 6, 8, 10 and 15 week after attack						
	2	4	6	8	10	15	Total
Sepedonium chrysospermum (Bull.) Fr.		3(0.8)	1(0.4)				4(0.2)
Sporothrix sp.					5(1.1)	6(1.3)	11(0.5)
Trichoderma spp.	67(33.3)*	32(8.9)	86(32.3)*	50(13.6)	48(10.2)	152(31.9)*	435(20.3)
Trimmatostroma sp.				1(0.3)			1(0)
Verticillium falcatum (Petch) W. Gams						1(0.2)	1(0)
Yeasts		22(6.1)					22(1)
Unidenified							
Basidomycetes (two species)						8(1.7)	8(0.4)
Other (five species)	4(2)	13(3.6)	7(2.6)	2(0.5)			26(1.2)
Number of isolates	201	360	266	369	472	477	2145
Number of species	16	28	20	25	23	30	57
Simpson index	0.7779	0.8057	0.7824	0.8811	0.8548	0.8319	0.8817
Percentage of "sterile" fragments	13(6.5)	8(4)	9(4.5)	5(2.5)	1(0.5)	6(3)	52(8.7)
Number of investigated fragments	200	200	200	200	200	200	1200
Number of galleries	100	100	100	100	100	100	600

*Dominant species

quent group included blue-stain fungi and *Penicillium*, *Trichoderma* and *Mucor* species. A group of nine ophiostomatoid fungi was isolated from the phloem. The most common species among them were *O. piceae* (14.6%) and *O. minus* (12.5%) (Table 1).

There were 201, 360, 266, 369, 472 and 477 isolates represented by 16, 28, 20, 25, 23 and 30 species 2, 4, 6, 8, 10 and 15 weeks after the main beetles attack. Two weeks after main beetles attack the phloem was colonized mainly by *Trichoderma* and *Penicillium* species. *Ophiostoma minus* was also common (Table 1).

Two weeks later the frequency of *O. minus* and *Trichoderma* spp. was almost three times and four times lower, respectively. In contrast, the frequency of *Penicillia* increased. There were two other ophiostomatoid fungi found: *L. lundbergii* Lagerb. & Melin and *O. piceae*, though these species were only occasionally isolated (Table 1).

Six weeks after *T. piniperda* attack the phloem was colonized mostly by *Penicillium* and *Trichoderma* species. The frequency of *O. minus* was 3.4%. In contrast, the frequency of *O. piceae* increased (10.9%) (Table 1). *Leptographium wingfieldii* which had not been present in the samples taken two weeks earlier occurred sporadically (Table 1).

Eight weeks after main insect attack, the group commonly occurring were ophiostomatoid species. The sapwood was colonized mainly by *O. minus* (17.3%) and *O. piceae* (17.3%). In contrast, the frequency of *Penicillia* and *Trichoderma* spp. was lower (Table 1).

Ophiostoma minus and *L. lundbergii* reached the highest frequency (22.9% and 9.5%, respectively) ten weeks after insect attack. Frequency of *O. piceae* also increased (22.7%). In contrast, the frequency of *Penicillia* decreased (8.3%) (Table 1).

Fifteen weeks after attack the phloem was colonized mostly by *O. piceae* and *Trichoderma* species. Their frequencies were 22.9% and 31.9%, respectively. Frequency of *O. minus* decreased about three times, compared to earlier records. *Penicillia* reached the lowest frequency (3.6%) (Table 1).

The highest number of species was found fifteen weeks after main attack of *T. piniperda*. Species richness and diversity were the lowest in the second week after insects attack. Only two ophiostomatoid species, *O. minus* and *O. piceae*, were present at all phases of fungal succession. At early stages, the dominant fungi were *Penicillium* spp. and *Trichoderma* spp., and in the later and final stages *O. minus*, *O. piceae* and *Trichoderma* spp. (Table 1).

Fungi isolated from Scots pine sapwood infested by *T. piniperda*

A total of 1613 fungal isolates, representing 50 species, were obtained from Scots pines sapwood infested with *T. piniperda*. Fungi were isolated from 54.4% of 2400 fragments (Table 2). The most frequent group included blue-stain fungi, *Penicillium* and *Trichoderma* species. In the group of nine ophiostomatoid fungi isolated from the sapwood the most common species were *O. minus* (37.4%) and *O. piceae* (12,2%) (Table 2).

There were 173, 134, 87, 254, 477 and 488 isolates represented by 15, 20, 17, 20, 19 and 25 species 2, 4, 6, 8, 10 and 15 weeks after main beetles attack. Two weeks after main beetle attack the sapwood was colonized mainly by *Penicillium* spp. and *O. minus* (Table 2). The latter usually occurred at the depth of 35 mm, where *Penicillia* and and *Trichoderma* spp. were also common (Fig. 1a). *Ophiostoma piceae* and *Graphium* sp. 'W' were detected only in 1.2% of samples (Table 2).

Table 2. Fungi isolated from the sapwood of *Pinus sylvestris* trees after an attack by *Tomicus piniperda*

Taxon	Number of isolates (% frequency) in the 2, 4, 6, 8, 10 and 15 week after attack						
	2	4	6	8	10	15	Total
Ophiostomatoid fungi							
Graphium pseudormiticum					5(1.0)		5(0.3)
Graphium pycnocephalum				3(1.2)	24(5.0)	25(5.1)	52(3.2)
Graphium sp. "W"	2(1.2)			12(4.7)	7(1.5)	3(0.6)	24(1.5)
Leptographium lundbergii		2(1.5)	1(1.1)	20(7.9)			23(1.4)
Leptographium procerum				6(2.4)	5(1.0)	6(1.2)	17(1.1)
Leptographium wingfieldii					4(0.8)	28(5.7)	32(2)
Ophiostoma minus	61(35.3)*	10(7.5)	7(8.0)	103(40.6)*	278(58.3)*	145(29.7)*	604(37.4)
Ophiostoma piceae	2(1.2)	2(1.5)	20(23.0)	47(18.5)	84(17.6)	41(8.4)	196(12.2)
Pesotum sp.						5(1.0)	5(0.3)
Other							
Acremonium sp.	1(0.6)	1(0.7)				4(0.8)	6(0.4)
Aspergillus niger	2(1.2)						2(0.1)
Cheatomium sp.					1(0.2)		1(0.1)
Chloridium sp.						1(0.2)	1(0.1)
Cladosporium cladosporoides		1(0.7)					1(0.1)
Cytospora pinastri			1(1.1)				1(0.1)
Dipodascus aggregatus		1(0.7)	1(1.1)	7(2.8)	14(2.9)		23(1.4)
Epicoccum nigrum			1(1.1)	2(0.8)			3(0.2)
Epithyrium resinae			2(2.3)				2(0.1)
Exophiala sp.						3(0.6)	3(0.2)
Fusarium sp.					2(0.4)		2(0.1)
Geosmithia sp.				4(1.6)	4(0.8)	3(0.6)	11(0.7)
Geotrichum candidum	1(0.6)						1(0.1)
Gliocladium catenulatum						1(0.2)	1(0.1)
Heterobasidion annosum						9(1.8)	9(0.6)
Hormonema dematioides	8(4.6)	12(9.0)	3(3.4)	4(1.6)	2(0.4)		29(1.8)
Lecythophora hoffmannii		5(3.7)	2(2.3)	3(1.2)	6(1.3)	2(0.4)	18(1.1)
Mortierella ramanniana var. *ramanniana*	1(0.6)	1(0.7)		1(0.4)	1(0.2)	1(0.2)	5(0.3)
Mucor spp.			2(2.3)	4(1.6)	5(1.0)	5(1.0)	16(1)
Myrothecium cf. indicum		2(1.5)					2(0.1)
Oidiodendron tenuissimum		2(1.5)		2(0.8)			4(0.2)
Paecilomyces variotii				1(0.4)			1(0.1)
Penicillium spp.	64(37.0)*	65(48.5)*	16(18.4)	14(5.5)	5(1.0)	3(0.6)	167(10.4)
Pezicula eucrita	1(0.6)	2(1.5)	1(1.1)				4(0.2)
Phialocephala cf. dimorphospora		1(0.7)					1(0.1)
Phialophora clavispora						2(0.4)	2(0.1)
Phialophora sp.						5(1.0)	5(0.3)
Rhinocladiella atrovirens	3(1.7)	2(1.5)		5(2.0)		2(0.4)	12(0.7)
Rhizoctonia sp.			1(1.1)			2(0.4)	3(0.2)
Sepedonium chrysospermum		1(0.7)	1(1.1)	2(0.8)			4(0.2)
Sporothrix sp.					7(1.5)	3(0.6)	10(0.6)
Thysanophora penicillioides (Roum.) W.B. Kendr.	1(0.6)						1(0.1)
Trichoderma spp.	21(12.1)	2(1.5)	22(25.3)*	9(3.5)	21(4.4)	180(36.9)*	255(15.8)
Yeasts		19(14.2)					19(1.2)

Taxon	Number of isolates (% frequency) in the 2, 4, 6, 8, 10 and 15 week after attack						
	2	4	6	8	10	15	Total
Unidentifited							
Basidomycetes (two species)			2(2.3)			7(1.4)	9(0.6)
Other (five species)	2(1.2)	3(2.2)	4(4.6)	5(2.0)	2(0.4)	2(0.4)	18(1.1)
Number of isolates	173	134	87	254	477	488	1613
Number of species	15	20	17	20	19	25	50
Simpson index	0.7206	0.7272	0.8366	0.7853	0.6228	0.7613	0.8064
Percentage of "sterile" fragments	206(51.5)	293(73.3)	252(63)	15338.3)	43(10.8)	52(13)	1095(45.6)
Number of analysed fragments	400	400	400	400	400	400	2400
Number of analysed galleries	100	100	100	100	100	100	600

*Dominant species

Two weeks later the frequency of *O. minus* was six times lower. In contrast, the frequency of *Penicillia* increased. They occurred mostly at depth of 5–15 mm. In deeper layers of sapwood the occurrence frequency of *Penicillia* was lower. Frequency of *O. piceae* did not changed, and *L. lundbergii* replaced *Graphium* sp. 'W' (Fig. 1b, Table 2).

Six weeks after attack the sapwood was colonized mostly by *O. piceae*, *Penicillium* and *Trichoderma* species (Table 2). Fungi occurred usually at the depth of 5 mm. Deeper their frequencies were very low (Fig. 1c).

Eight weeks after attack, the group commonly occurring were ophiostomatoid species. *Graphium pycnocephalum*, *Graphium* sp. 'W' and *L. procerum* (W.B. Kendr.) M.J. Wingf. had not been present in the samples taken two weeks earlier. *Ophiostoma minus* colonized mostly the sapwood underneath galleries and occurred usually at the depth of 5–15 mm (Fig. 1d, Table 2).

Ophiostoma minus reached the highest frequency (58.3%) ten weeks after insects attack. *Ophiostoma piceae* frequency decreased slightly at that time (Table 2). There were five other ophiostomatoid species detected including *G. pycnocephalum* Grosm., *Graphium* sp. 'W', *L. procerum*, *G. pseudormiticum* M. Mouton & M.J. Wingf. and *L. wingfieldii*, though the last 3 species were relatively less numerous. *Dipodascus aggregatus* Francke-Grosm., *Penicillia* and *Trichoderma* species were more frequent (Table 2). *Ophiostoma minus* occurred frequently in all layers of sapwood. This fungus occurred in 66% of samples taken from a depth of 35 mm, while *O. piceae* occurred only in 10% of samples from this layer (Fig. 1 e).

Fifteen weeks after attack the sapwood was colonized mostly by *O. minus* and *Trichoderma* species. Their frequencies were 29.7 and 36.9%, respectively. Number of *O. piceae* isolates decreased more than twice in comparison to earlier records. In contrary, *L. wingfieldii* increased its share to 5.7% (Table 2).

The highest number of species was found fifteen weeks after main attack by *T. piniperda*. Species richness and diversity were lowest in the second week af-

ter attack. Only two ophiostomatoid species, *O. minus* and *O. piceae*, were present at all phases of fungal succession. At early stages, the dominant fungi were *Penicillium* spp. and *O. minus* and in the later and final stages *O. minus* and *Trichoderma* spp. (Table 2).

Development of blue-stain had started very slowly and has increased very rapidly since the 8th week after attack (Fig. 2).

Discussion

The pattern of fungal invasion in sapwood of trees attacked by bark beetle was described by Solheim (1992a and 1992b). He reported that fungi carried by *Ips typographus* (L.) invaded the Norway spruce sapwood in a successional pattern with the most pathogenic *Ceratocystis polonica* (Siemaszko) C. Moreau first, followed by other beetle-transmitted *Ophiostoma* and *Graphium* species. It seems that a pattern of fungal colonization of pines after pine shoot beetle attack, may be different. In contrast to the *I. typographus*, our studies showed that, the association of *T. piniperda* with ophiostomatoid fungi is not very strong. Some populations of *T. piniperda* may be more strongly connected with *Penicillia* and yeasts than with ophiostomatoid fungi (Jankowiak 2006a, Kirisits 2001).

The community of fungi associated with *T. piniperda* in the present study was similar to those associated with the insect in other parts of Europe (Mathiesen-Käärik 1953, Lieutier et al. 1989, Gibbs and Inman 1991, Solheim and Långström 1991, Wingfield and Gibbs 1991). The study showed that among ophiostomatoid species, *O. minus* together with *Penicillia* and *Trichoderma* species were the first colonizers of pine trees, particularly of a sapwood. *Penicillium* and *Trichoderma* species were more frequent than *O. minus*. Presence of *Penicillia* and *Trichoderma* species may be due to the contact of *T. piniperda* individuals with a soil during overwintering in bark at a tree base. These species were also found in the sapwood of uninfested *P. sylvestris* trees two weeks after felling (Tarociński and Stolarski 1972, Chaverri and Samuels 2003).
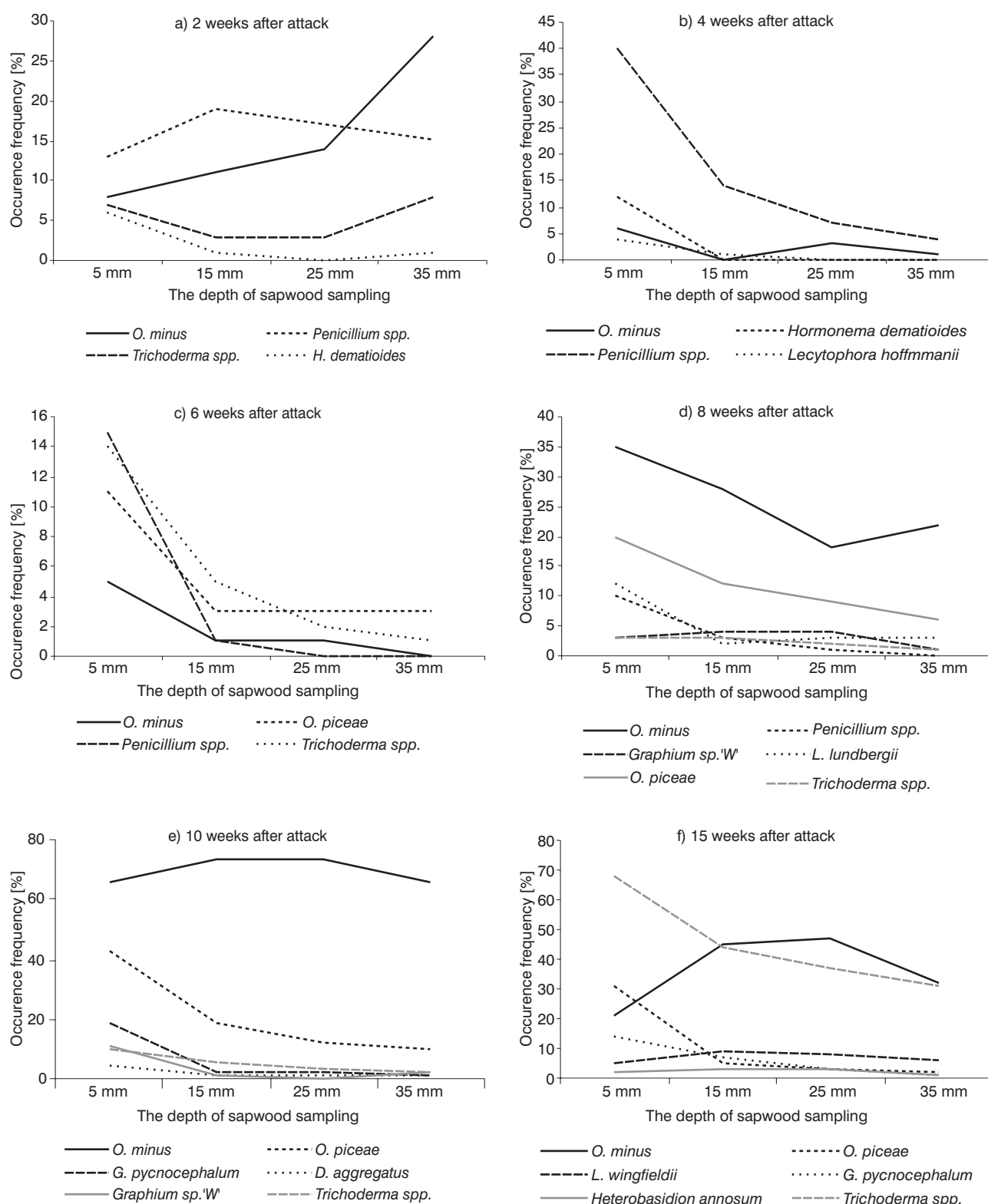
Fig. 1. Occcurence frequency of the most commonly appearing fungi in sapwood of Scots pine infested by *T. piniperda*

Penicillia usually grow only on a surface of wood and utilize available simple carbon compounds (Zabel and Morrell 1992, Seifert 1993). However, in our study, *Penicillium* and *Trichoderma* species were detected also in deeper layers of sapwood, particularly at

the early stages of colonization. The highest occurrence frequency of *O. minus* in deeper layers of sapwood was recorded two weeks after insects attack. It was connected with an absence of a visible blue-stain. It seems that fungal spores could have been distrib-
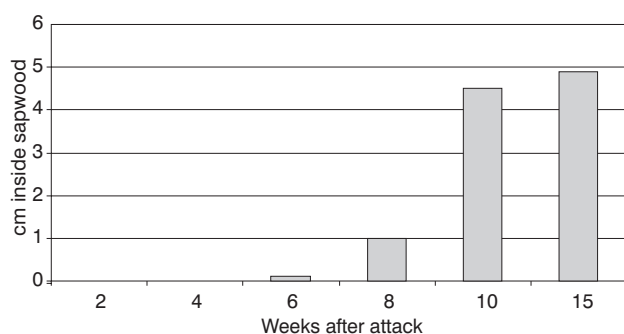
Fig. 2. Development of visible blue-stain in sapwood
of Scots pine after attack by *Tomicus piniperda*

uted by sap flowing to deeper layers of sapwood. Development of visible blue-stain started very slowly. The more rapid colonization by blue-stain fungi took place six weeks after insects attack, and in this phase the blue-stain became visible. It seems that an explosive development of blue-stain fungi in the sapwood may be caused by changes in sap flow intensity. Wang (1983), Yamaoka et al. (1990) and Kirisits and Offenthaler (2002) report that sap flow density decreases distinctly within first weeks after natural or artificial infection of conifers by blue-stain fungi. Münch (1907) observed, that penetration of the fresh sapwood by *O. minus* was very slow. It cannot be excluded that a slow growth of majority of blue-stain fungi in the initial stages of colonization is an effect of antagonistic activity of *Penicillia* and *Trichoderma*, since they can produce volatile and non-volatile metabolites limiting the growth of other community components (Wells and Bell 1979, Kwaśna 1987).

The results showed that *O. minus* was the first serious colonizer of both, phloem and sapwood. The fungus had persisted during the initial stages of colonization and became the dominant within 8–15 weeks after *T. piniperda* attack. *Ophiostoma minus* is commonly associated with *T. piniperda* (Kirisits 2004). The big differences in its frequency in various stages suggest a loose relation with *T. piniperda* (Mathiesen 1950, Mathiesen-Käärik 1953, Siemaszko 1939, Lieutier et al. 1989, Gibbs and Inman 1991, Solheim and Långström 1991, Masuya et al. 1998, Masuya et al. 1999, Jankowiak 2006a). In this study, *Ophiostoma minus* was a dominant fungus in the sapwood of pines. This indicates that *O. minus* is more specialized than other ophiostomatoid fungi to colonize the sapwood of Scots pine trees infested by *T. piniperda*. The ability of *O. minus* to relatively rapid colonization of the sapwood of trees was reflected by the high level of virulence of this species to pine (Lieutier et al. 1989, Solheim and Långström 1991, Långström et al. 1993, Solheim et al. 1993 and Solheim et al. 2001) and by its ability to tolerate low concentrations of oxygen (Solheim et al. 2001).

The pattern of fungal succession in Scots pine phloem and sapwood infested by *T. piniperda* was dif-

ferent than in other countries. Gibbs and Inman (1991) recorded mostly pathogenic *L. wingfieldii* on the windthrown pines. The fungus was isolated less frequently from new galleries than old ones. This suggested that propagules of *L. wingfieldii* had been introduced only by a few colonizing adults and then grew rapidly along the medullary rays and tracheids and established itself in some of the initially uninfected gallery systems. Our results may only partly support the results of Gibbs and Inman (1991), because *L. wingfieldii* occurred more frequently in the later than initial stages of fungal colonization. The results of this study were confirmed by Jankowiak (2006a) observations that in Poland *L. wingfieldii* is very weakly associated with *T. piniperda* and it colonizes pine tissues in later stages of fungal succession after this beetle attack.

Leptographium procerum and *L. lundbergii* were rare in the *T. piniperda* galleries. Unlike *L. wingfieldii*, *L. lundbergii* had already colonized the wood in 4th week after insect attack and then followed *O. minus*. *Leptographium lundbergii* and *L. procerum* in pine wood colonized by *T. piniperda* were recorded also by Mathiesen-Käärik (1953), Gibbs and Inman (1991) and Jankowiak (2006a).

Ophiostoma piceae followed *O. minus* as a secondary invader of phloem and sapwood, at first less frequently, but with increasing frequency in time. *Ophiostoma piceae* was recorded in Sweden (Solheim and Långström 1991), Poland (Siemaszko 1939), England (Gibbs and Inman 1991) and Austria (Kirisits 2001), but not in France (Lieutier et al. 1989). Jankowiak (2006a) showed that *O. piceae* was an important associate of *T. piniperda*. This fungus is known to be a common associate of phloeophagous bark beetles in North America and Eurasia (Kirschner 2001, Kirisits 2004). Considering the time of its invasion *O. piceae* seems to be a secondary invader, what does not agree with data from Norway (Solheim 1992a, 1992b), where it was the third invader of Norway spruce sapwood infested by *I. typographus*. The results of inoculation studies indicate that *O. piceae* is weakly pathogenic (Nevil and Alexander 1992, Krokene and Solheim 1998, Yamoka et al. 2000, Jankowiak 2006a). Our study suggests also that *O. piceae* may play more important role in colonizing phloem than sapwood. These results confirm the findings of Jankowiak (2005), who observed that *O. piceae* was better adapted to the colonization of phloem than sapwood of Norway spruce.

Graphium species were the third group of invaders in phloem and sapwood. Among them *G. pycnocephalum* and *Graphium* sp. 'W' were the most abundant. Together with *Leptographium* species they may 'replace' *O. minus* and *O. piceae* in later stages of succesion. It was confirmed in the studies of Solheim (1992a and 1992b), who found *Graphium* to be ter-

tiary and quaternary invader of Norway spruce sapwood. *Graphium pycnocephalum* is commonly associated with phleophagous bark beetles, e.g. *Pityogenes chalcographus* (L.), *Ips typographus, I. acuminatus* (Gyll.), *Hylurgops ligniperda* (Fabr.) and *T. piniperda* (Kirisits 2004). *Graphium* sp.'W' is used here in the broad sense (Seifert and Okada 1993), while its taxonomic position is being investigated. The species seems to be closely connected to *H. palliatus* (Gyll.) than to *T. piniperda* (Jankowiak 2006b).

This study indicates that *Hormonema dematioides* Lagerb. & Melin may also play important role in colonization of pine tissues in early stages of fungal succession. This species is known sap-stain fungus (Hermanides-Nijhof 1977) and was often isolated from overwintered adults of *T. piniperda* in Poland (Jankowiak, unpublished). *Hormonema dematioides* was reported as a common fungal associate of *T. piniperda* in France (Lieutier et al. 1989), Sweden (Solheim and Långström 1991), Poland (Siemaszko 1939) and Japan (Masuya et al. 1998). Endophytes, such as *Epicoccum nigrum* Link, *Lecythophora hoffmannii* (J.F.H. Beyma) W. Gams & McGinnis, *Pezicula eucrita* P. Karst., *Phialocephala* cf. *dimorphospora* Kendrick and *Ceutospora pinastri* Fr. were also numerous and possibly important group of fungi isolated from gallery systems of *T. piniperda*. These fungi species are known from symptomless Scots pine wood (Kowalski and Kehr 1996, Kowalski and Stańczykiewicz 2000).

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