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ASSEMBLAGES OF OPHIOSTOMATOID FUNGI VECTORED BY *Ips amitinus* (Coleoptera: Scolytinae) ON NORWAY SPRUCE DEPEND ON COLONIZATION TIME, POSITION ON THE HOST TREE AND DEVELOPMENT STAGE

VEKTORSKI ODNOS OFIOSTOMATOIDNIH GLJIVA I *Ips amitinus* (Coleoptera: Scolytinae) NA SMRECI OVISNO O VREMENU NASELJAVANJA, POLOŽAJU NA STABLU I FAZI RAZVOJA

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Summary

The small spruce bark beetle *Ips amitinus* is predominantly found in the spruce forests in mountainous areas of Central Europe. Its most important host trees are Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Under favourable weather and trophic conditions, this bark beetle can become dangerous, particularly for younger trees and plantations. The climate changes that we face today can be favourable to the species, which had not been economically important in the past but is currently causing forest damage. Information about the ecological/biological characteristics of *I. amitinus* in the literature is rare, especially for bark beetle–fungi associations; though bark beetle (Coleoptera: Scolytinae) species are known to be associated with variety of fungi. We investigated the factors affecting the associations of ophiostomatoid fungi with *I. amitinus* on Norway spruce. Material for this study was collected in the year 2010 near Dravograd, in north Slovenia, where Norway spruce trees were felled during the winter windthrow. Four hundred and forty-two samples (bark beetles and infested samples from wood discs, from two trees at 0.5 m, 6 m and 15 meters above the stump) were taken for ophiostomatoid fungi investigation. Isolation yielded a total of 625 isolates. Ophiostomatoid fungi were the most numerously represented group. Identified fungal isolates belonged to ten species. The most commonly found fungal associate was *Ophiostoma brunneo-ciliatum*, followed by *Grosmannia penicillata*, *Ophiostoma bicolor, Ceratocystiopsis minuta*, *Grosmannia piceiperda*, *Endoconidiophora polonica*, *Ophiostoma piceae*, *Ophiostoma fuscum*, *Grosmannia cucullata*, *Graphium fimbriisporum*. The association with *O. fuscum*, *G. cucullata* and *G. fimbriisporum* have not been demonstrated previously. The differences in distribution of fungi over different beetle life stages (adults, larvae, pupae) and infested wood were investigated.

KEY WORDS: small spruce bark beetle, vector, bark beetle life stages, associated fungi, forest protection, Slovenia, *Picea abies*

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INTRODUCTION
UVOD

The small spruce bark beetle *Ips amitinus* (Eichhoff, 1871) is taxonomically placed in Coleoptera, Curculionidae, Scolytinae. *I. amitinus* is predominantly found in the spruce forests in mountainous areas of Central Europe (Jurc & Bojović 2004). Principal hosts are *Picea* spp. and *Pinus* spp. (Cognato 2015), most frequently Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.). Occasionally, other pines (*P. cembra* L., *P. mugo* Turra), silver fir (*Abies alba* Mill.) and European larch (*Larix decidua* Mill.) are also attacked. This bark beetle often remains undetected because it is confused with other, more common bark beetle species with which it often co-occurs, such as *Ips typographus* L. (Knížek et al. 2001, Holuša et al. 2012, Jurc & Bojović 2004). *I. amitinus* is distinguished from other Eurasian *Ips* spp. by its straight antennal club sutures. The species is a secondary pest, primarily colonizing recently dead or weakened trees. Nevertheless, under favourable weather and trophic conditions it can become dangerous, particularly for younger trees and plantations (Jurc & Bojović 2004, Okland & Skarpaas 2008).

In Slovenian forests some species that had not been economically important in the past might now, due to climate changes, cause damages in forests (Jurc & Bojović 2004). Among them is *I. amitinus*. Data on the location of specimens in the collection of the Natural History Museum of Slovenia showed an *I. amitinus* location in Pohorje (coll. Peyer) and a location in Peca (coll. Pavlin), in addition to Košenjak (Jurc 2003, Jurc & Bojović 2004). A gradation of *I. amitinus* infestation appeared from 2002-2003 where there were stands of 70- to 80-year-old Norway spruce in the Alpine region of Slovenia, at an altitude of 1270-1500 m above sea level, where snow breakage, extreme drought and warm weather were recorded in the years prior to attack (Jurc & Bojović 2004). *I. amitinus* are still present at this location but at a low population level (Ribič 2007).

I. amitinus, like other bark beetles, are vectors of various fungi. The fungal spore attaches to the bark beetles bodies' and are dispersed to new host plants (Harrington 1993). Known fungal associates of the *I. amitinus* beetle are several genera from ascomycetes, mostly known as ophiostomatoid fungi. Ophiostomatoid fungi cause considerable economic losses in the forestry and timber production due to intensive discolorations or sap staining and vascular wilt diseases (Gibbs 1993, Harrington 1993). However, information regarding the basic ecological characteristics of *I. amitinus* is scarce (Holuša et al. 2012). The association of *I. amitinus* with ophiostomatoid fungi is equally poorly researched (Grosmann 1931, Kirisits et al. 2000).

Because environmental changes can influence bark beetle distribution as well as the distribution of its associated fungi, research on different bark beetle-fungi associations is essential (Linnakoski et al. 2010, Rice et al. 2008). Thus,the aim of this study was to obtain a better understanding of the association between the ophiostomatoid fungi assemblage connected with *I. amitinus* in Norway spruce. Therefore, we investigated 1) species composition of fungi associated with *I. amitinus* and we define their pathogenicity for the host tree, 2) the association between fungi and the *I. amitinus* development stage, 3) the influence of colonization time and 4) the influence of colonization position within the Norway spruce.

MATERIALS AND METHODS
MATERIJALI I METODE

Sample collection – *Uzimanje uzoraka*

Material for this study was collected in Dravograd, in north Slovenia (Koroška, 46°38′45″N 15°2′10″ E, altitude 1270 m a.s.l.) where Norway spruce trees were felled during the winter 2010 windthrow. They were naturally infested by the bark beetle *I. amitinus* in spring 2010. 204 beetles, 40 larvae, and 40 pupae were collected and placed individually in sterile tubes. Wood discs were cut from two trees at 0.5 m, 6 m and 15 meters above the stump (6 wood discs in total) and transported to the laboratory. Material was collected at the beginning of June 2010, immediately after the first colonization of bark beetles, and one month later in July 2010, when the first beetle generation had already built their galleries and the young adults were not emerging from the wood yet.

Fungal isolation – *Izolacija gljiva*

Fungi were isolated from sapwood of steam discs following a methodology similar to Solheim (1992a, 1992b) and Kirisits (2010). All the laboratory work was done in the laboratory of Forestry Institute of Slovenia in the Department of Forest Protection. Stem discs were split longitudinally in the laboratory one day after they were cut in the field. Three circular sections from each disc were taken, 18 total sections, and 158 wood chips in total were placed into a growing medium of 2% malt extract agar (MEA; 2% Bacto™ Malt Extract, 1.5% Difco™ Agar Technical; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Pieces of wood were taken from under female galleries at 2 mm, 5 mm and at every subsequent 5 mm into the sapwood to a depth of 35 mm using sterile technique used by Haberkern et al. (2002), Solheim (1992a; 1992b) and Kirisits (2009). Petri dishes were incubated in the dark at 22°C to 25°C till the mycelia started to produce.

Insect-associated fungi were gathered from the beetles. Each adult beetle (as well as each pupa and larva) was placed directly on to the MEA plates and smashed. Petri dishes were stored in the dark at 22°C to 25°C till the mycelia started to produce. They were inspected every day for fungal

Tab. 1 Ophiostomatoid fungi identified on I. amitinus (adults, larvae, pupae) and wood, with frequencies
Tab. 1 Identificirane vrste gljiva na I. amitinus (adulti, larve, kukuljice) i drvu, s frekvencijama

	1st collection (52[1])		2nd collection (152[1])		Beetle Total (204[1])		Wood (158[1])		Larva (40[1])		Pupa (40[1])		Total (442[1])	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Ophiostoma bicolor	13	25,0	25	16,4	38,0	18,6	20	12,7	8	20	8	20	74	16.7
Ophiostoma brunneo-ciliatum	10	19,2	61	40,1	71,0	34,8	20	12,7	10	25	32	80	133	30.1
Ophiostoma fuscum	1	1,9	8	5,3	9,0	4,4	2	1,3	6	15	0	0	17	3.8
Ophiostoma piceae	8	15,4	11	7,2	19,0	9,3	2	1,3	0	0	2	5	23	5.2
Grosmannia cucullata	4	7,7	4	2,6	8,0	3,9	0	0,0	0	0	2	5	10	2.3
Grosmannia penicillata	6	11,5	32	21,1	38,0	18,6	36	22,8	6	15	14	35	94	21.3
Grosmannia piceiperda	17	32,7	10	6,6	27,0	13,2	4	2,5	6	15	2	5	39	8.8
Endoconidiophora polonica	2	3,8	8	5,3	10,0	4,9	16	10,1	0	0	0	0	26	5.9
Ceratocystiopsis minuta	4	7,7	39	25,7	43,0	21,1	6	3,8	10	25	14	35	73	16.5
Graphium fimbriisporum	3	5,8	2	1,3	5,0	2,5	0	0,0	0	0	0	0	5	1.1
Total	68		200		268		106		46		74		494	
Number of ophiostomatoid fungi	10		10		10		8		6		7		10	
Yeast	13	25.0	30	19.7	43	21.1	5	3.2	20	50.0	15	37.5	83	18.8
Penicillium spp.	7	13.5	3	2.0	10	4.9	32	20.3	2	5.0	4	10.0	48	10.9
Total	88		233		321		143		68		93		625	

N Number of strains of the respective fungal species obtained from the different samples
% frequency of fungus per sample (N fungi/N samples)*100
[1] Number of samples per beetle life stage (bark beetles, larvae, pupae) or wood chip

growth. Where necessary, cultures were purified by transferring small pieces of mycelium onto new 2 % MEA plates. We added autoclaved pieces of conifer wood (spruce) to the medium and placed cultures under UV light, to stimulate ascocarp production.

Samples were carefully examined under the lens of an Olympus SZX 12 and further under an Olympus BX51 microscope. Fungi were identified based on their anamorph and teleomorph structures (Upadhyay 1981, Grylls & Seifert 1993, Wingfield et al. 1993, Jacobs & Wingfield 2001, Linnakoski et al. 2010). They were compared with fungi collected in previous research (Repe et al. 2013). Reference strains of all of the isolated ophiostomatoid fungi associated with *I. amitinus* in Slovenia were deposited in the culture collection of the Laboratory for Forest Protection (ZLVG) at the Slovenian Forestry Institute, Ljubljana, Slovenia.

DATA ANALYSIS
ANALIZA PODATAKA

The difference in number of fungi species observed at wood and adult bark beetle sampling sites between the months was compared with a Mann-Whitney test. Because the bark beetles' early development stages were mainly found to occur on the surface, samples from 2 mm into the wood were taken for further analyses. The differences in fungi species per substrate for the depth and height were compared with a Friedmann test. The height (for the comparison between depths) and depth (for the comparison between heights) were taken as the block (Quinn & Keough 2002). When the results of this test were significant, it was followed by the Steel test (Steel 1959). The differences between the development stages were compared using a Kruskal-Wallis test followed by a Dwass-Steel test.

The differences in species assemblages between the two development stages and the adult bark beetle in the different months were analysed with Permanova (Anderson 2001) using the library »vegan« (Oksanen et al. 2011) in R statistics software (R Development Core Team 2011). When multiple groups were compared pairwise, the P-value was adjusted with the Holm adjustment (Holm 1979). Additionally, for every ophiostomatoid fungi species, the differences between months for the adults and the differences between development stages over both months were tested. First, a Chi square test was performed, followed by the Ryan test (Ryan 1960).

RESULTS
REZULTATI

Fungal isolation and identification – *Izolacija gljiva i identifikacija*

Ophiostomatoid fungi were the most numerously represented group in this research (Tab. 1). Identified fungal isolates belonged to ten species: *Endoconidiophora polonica* (Siemaszko) Z.W. de Beer, T.A. Duong & M.J. Wingf., (Syn.: *Ceratocystis polonica* (Siemaszko) C. Moreau, Revue Mycol., Paris 17 (Suppl. Colon. no. 1): 22 (1952), Index Fungorum, http://www.speciesfungorum.org/Names/SynSpecies.asp?RecordID=810316), *Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhyay & W.B. Kendr., *Ophiostoma*

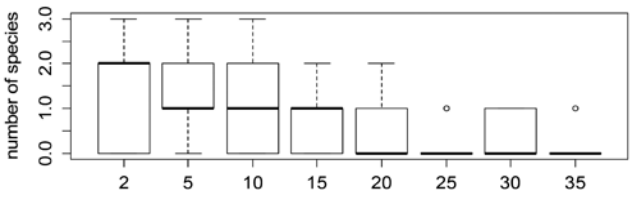

Fig. 1 The presence of ophiostomatoid fungi species in wood at different depths in mm (X axis) in the tree.
Slika 1 Prisutnost vrsta ofiostomatoidnih gljiva na uzorcima iz različitih dubina drva mm(X-os).

bicolor R.W. Davidson & D.E. Wells, *Ophiostoma brunneo-ciliatum* Math.-Käärik, *Ophiostoma piceae* (Münch) Syd. & P. Syd., *Ophiostoma fuscum* Linnak., Z.W. de Beer & M.J. Wingf., *Graphium fimbriisporum* (M. Morelet) K. Jacobs, *Grosmannia cucullata* (H. Solheim) Zipfel, Z.W. de Beer & M.J. Wingf., Kirisits & M.J. Wingf., *Grosmannia penicillata* (Grosmann) Goid., and *Grosmannia piceiperda* (Rumbold) Goid. In addition to species from the ophiostomatoid group, we found also *Penicillium* spp. and yeasts.

Fungi isolated from wood – *Gljive izolirane iz drva*

During the first collection in June 2010, logs had just been colonized by the bark beetles. At that time, the wood was not yet been blue-stained, and 'predictably', no ophiostomatoid fungi were found (t=96, P<0.001). During the second collection period, blue staining was present in the sapwood. The average discoloration depth was 19.3 mm.

The following eight ophiostomatoid fungi, including their imperfect states, were collected from wood: *E. polonica*, *C. minuta*, *O. bicolor*, *O. brunneo-ciliatum*, *O. fuscum, O. piceae*, *G. piceiperda*, *G. penicillata*, as well as *Penicillium* spp. and yeasts.

The species *G. penicillata* was present in 44% of sapwood circle sections and was obtained from 25% of sapwood pieces, representing the most commonly found fungi. It was followed by *O. bicolor* and *O. brunneo-ciliatum* species that were found in almost 14% of all sapwood pieces. The species *O. brunneo-ciliatum* was present in 77% of all circle sections and *O. bicolor* was found in 56%. The species *E. polonica* was found on 33% of sapwood circle sections and 11% of all sapwood pieces.

With increase in depth, the number of fungi species decreased. Only the species *G. penicillata* and *E. polonica* penetrated deeper than 15 mm into the sapwood, and we observed differences in ophiostomatoid fungi species richness over the depth of penetration (χ^2=21.03, df=7, p<0.01) (Fig. 1).

More fungal species were observed in the higher parts of the tree (6 and 15 meters) compared to lower part of the tree (0.5 m) (χ^2=14.22, df=2, p<0.01). There were very few fungi detected at the lowest sampling height, namely just *O. bicolor* and *O. brunneo-ciliatum.*

Fungi isolated from bark beetles – *Gljive izolirane iz potkornjaka*

On adult beetles, 10 ophiostomatoid fungal species were found, in contrast to pupae and larvae where 7 and 6 ophiostomatoid fungal species, respectively, were found (Table 1). We did not find differences in ophiostomatoid fungi species richness between the first and second collection times on bark beetles (U = 3694, P = 0.47). However, we did find differences in ophiostomatoid fungi assemblages on bark beetles (F=10.8, p<0.001, R^2=0.076) between the sampling periods.

The most common species was *O. brunneo-ciliatum,* it was followed by the species *C. minuta*, *O. bicolor* and *G. piceiperda* (Table 1). Fungi isolated from pupae and larvae were also dominated by the species *O. brunneo-ciliatum*. The species *E. polonica* was not isolated from either the pupae or the larvae (Table 1).

Comparisons of ophiostomatoid fungi isolated from different I. amitinus life stages and wood – *Usporedbe vrsta ofiostomatoidnih gljiva izoliranih iz različitih razvojnih faza I. amitinus i drveta*

Isolation of ophiostomatoid fungi was most successful from adult beetles. We did find differences between ophiostomatoid fungi assemblages (Fig. 2) on bark beetles compared with wood (χ^2=2.42, df=3, p<0.01).

There was a slight difference in ophiostomatoid fungi species richness between the beetle life stages and wood. In wood, nine ophiostomatoid fungal species were found; whereas 8 were found on pupae and larvae and 11 fungal species were found on adult beetles (χ^2=9.87, df=3, p<0.05).

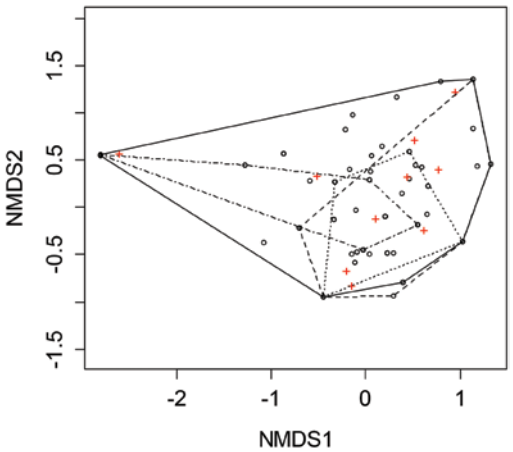

Fig. 2 Differences in ophiostomatoid fungi assemblages in wood and on bark beetles in different developmental stages using an NMDS plot (non-metric multidimensional scaling). Continuous line = adult, dashed line = larvae, dotted line = pupae, dot-dash line = wood.
Slika 2 Razlike u skupinama ofiostomatoidnih gljiva na drvetu i potkornjacima u različitim razvojnim stadijima upotrebom NMDS ploha (non-metric multidimensional scaling). Neprekinuta linija = odrasli kukci, isprekidana linija = ličinke, točkasta linija = kukuljice, linija-crtica linije = drvo.

DISCUSSION
RASPRAVA

Which ophiostomatoid fungi are vectored by I. amitinus? – *Kojim ofiostomatoidnim gljivama je I. amitinus vektor?*

This study showed that *I. amitinus* is involved in ophiostomatoid fungi dispersal. Despite the fact that our research studies were carried out on rather small sample we found ten different ophiostomatoid fungi as well as yeast and *Penicillium* spp. The results were based on samples collected from one location in one breeding season of *I. amitinus*. Fungal species found can be compared with other investigations of the bark beetle *I. amitinus* in Europe (Grosmann 1931, Kirisits et al. 1998). Our findings concurred with an investigation by Kirisits et al. (1998) that was made on *Norway spruce* trees. Additionally, we found associations that were not previously recorded, namely those with species *G. cucullata*, *G. fimbriisporum* and *O. fuscum*.

Even if *I. amitinus* are causing more damages now then they have in the past (Jurc & Bojović 2004, Ribič 2007, Okland & Skarpaas 2008), the assemblages of ophiostomatoid fungi in this investigation showed that the associated species are not highly pathogenic, referring to the pathogenicity researches that were made with fungi species found also during this research (Repe et al. 2013, Kirisits and Offenthaler 2002, Kirisits 1998, Jankowiak and Kolarík 2010). The association of fungi was dominated by the species *O. brunneo-ciliatum* (Tab. 1). *C. minuta* was very commonly observed as well and is associated with a broad range of bark beetles as well as different host trees (Kirisits 2004). According to the pathogenicity researches, the most virulent fungal associate of bark beetles on Norway spruce is *E. polonica* (Repe et al. 2013 Kirisits and Offenthaler 2002, Kirisits 1998, Jankowiak and Kolarík 2010), found also during this research. However *E. polonica* was one of the least presented species in association with *I. amitinus* (Table 1), noting the fact that the sampling was undertaken at only one location and that the abundance of *E. polonica* could be different at other locations. Our observation of *E. polonica* was not as common as reported by Kirisits *et al.* (2000). It represented 5.9% of the associated fungi in this investigation and was found on 10.1% of all wood chips and 4.9% of bark beetles. It was not isolated not from pupae nor from larvae. Revising *E. polonica* abundance in prior investigations of other bark beetles on *P. abies* trees in Europe, the frequency of occurrence has differed considerably. It has rarely appeared as the dominant species (Solheim 1986, Kirisits 2010, Krokene & Solheim 1996) and was often not found at all or was found in moderate quantities (Jankowiak et al. 2009, Sallé et al. 2005, Giordano et al. 2013). It has been speculated that the composition of fungal associates may differ during different phases of population dynamics (Harding 1989, Solheim 1992a, Solheim 1992b, Kirisits 2004, Kirisits 2010) or over different localities in Europe (Kirisits 2004).

Wood colonization by *I. amitinus* – *Kolonizacija drva od* I. amitinus

At the beginning of bark beetle colonization, fungi were not yet present in the sapwood. Fungi penetrated into the wood slowly; an occurrence which was also presented in the research of ophiostomatoid fungi penetration in *P. abies* trees performed by Solheim (1992b). At the second collection time, after the bark beetles had been established in the tree, ophiostomatoid fungi were present. *I. amitinus* thus effectively transmitted the ophiostomatoid fungi. The most abundant species, namely, *G. penicillata*, was present in just 25% of wood chips out of the 158 samples taken from 18 circular sections. The complex of fungi found in wood was very similar to those found on the different beetle life stages (Table 1). The difference was that there were fewer fungi found on wood and the composition of fungi on wood differed from this on bark beetles.

At 0.5 m above the stump, few fungi were present, though there was not a strong beetle colonization either. It is known that *I. amitinus* prefer to colonize parts of trees with smaller dimensions (Jurc & Bojović 2004, Ribič 2007, Okland & Skarpaas 2008), consequently, beetles are more present higher up on the trunk of adult trees. Witrylak (2008) established that the most abundant *I. amitinus* colonization was where the habitat is more suitable for bark beetle development that is in the middle part of the stem, where the bark was 2-3 mm thick. In our research, accordingly, it was shown that the number of isolated fungi increased with the sample height. Just 9.5% of fungi was found on lowest sampling height (0.5 meters), 42.5% of fungi was found at sampling height of 6 meters and 48% of fungi on sampling height of 15 meters.

Comparison of the fungi isolated from wood and different beetle life stages – *Usporedba gljiva izoliranih iz drva i različitih razvojnih faza potkornjaka*

During this research, ophiostomatoid fungi were isolated from all bark beetle life stages (adult bark beetles, larvae, pupae) and wood. On adult bark beetles, the majority of ophiostomatoid fungi were found, and the smallest number was found on larvae. Considering the quantity of collected samples, the most ophiostomatoid fungi were found on pupae. This can be explained by the simple fact that feeding larvae penetrate deeper into the wood and can bore their way in front of fungi penetration. When the specimens start to pupate, thick layers of conidiophores and ascoscarps develop around them in the pupal chambers. After young specimens transform from pupae, they become inoculated with conidia and ascospores (Kirisits 2004).

The smallest number of fungi were found on wood, because fungi penetrated into the wood slowly. Possibly more fungi or different fungal composition could be found if the research had continued for a few more months. The average discoloration depth (19.3 mm) was comparable to research in Poland (Kirisits 2010) which showed discoloration depth of 18.5 mm, or Norway (Solheim 1991), which showed a discoloration depth of 19.9 mm seven weeks after colonization. The speed of colonization depended on the percentage of humidity in the wood and consequently depends on the quantity of oxygen and temperature. The first fungus that colonized the tree was *E. polonica*, followed by *O. bicolor*, *G. penicillata*, and *O. ainoae* (Solheim 1992b). *E. polonica* tolerated low levels of oxygen and also grew very quickly, which was a good combination for colonization of trees (Solheim 1992b).

Kirisits (2004) anticipated that the host tree had a more important influence on assemblages of fungi than the species of bark beetle investigated. Bark beetles *I. typographus* and *I. amitinus* have very similar niches, so we can assume that the fungi associated with these two beetles were similar. The complex of fungi associated with *I. typographus* was more abundant than that associated with *I. amitinus* (Kirisits 2004, Repe et. al 2013); however, all fungi associated with *I. amitinus* could also be found in association with *I. typographus*. Further investigations, with different methods and different sampling plots may also find other fungi that have not yet been found in association with *I. amitinus*.

Penicillium spp. and yeast were found as well. Yeasts were found at quite high frequencies in association with *I. amitinus* bark beetle and its developmental life stages but not in connection with wood. Some investigations have already suggested (Grosmann 1931, Six 2003) that yeast might be an important bark beetles associate. In our research yeast was not as common on wood, which was in accordance with previous research in Norway (Solheim1992b). Contrary to yeast, *Penicillium* spp. were found to be more abundantly associated with wood.

CONCLUSIONS
ZAKLJUČCI

Our research of ophiostomatoid fungi associated with *I. amitinus* yielded ten ophiostomatoid fungal taxa. Fungi that were found on bark beetles and its earlier life stages were found on wood after the beetles' colonization. *I. amitinus* is a good ophiostomatoid fungi vector and it does inoculate fungi into Norway spruce trees. Like other bark beetles, it lives in association with yeast as well. Fungi assemblage connected with *I. amitinus* in Norway spruce depend on colonization time, position on the host tree and development stage.

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Sažetak

Mali osmerozubi smrekov pisar *Ips amitinus* najčešće naseljava smreku u montanskima područjima Središnje Europe. Najvažniji domaćin je obična smreka (*Picea abies*) i bijeli bor (*Pinus sylvestris*). U ugodnim vremenskim i trofičnim uvjetima, potkornjak postaje opasan, posebno za mlađa stabla u plantažama. Klimatske promjene, s kojima se suočavamo danas, mogu biti povoljne za vrste koje nisu bile ekonomski važne u prošlosti, a u zadnje vrijeme počinju pričinjavati štete u šumama. Informacije o ekološkim/biološkim obilježjima *I. amitinus* su u literaturi rijetke, posebice za asocijacije potkornjaka i gljiva; iako je poznato da su vrste potkornjaka (Coleoptera: *Scolytinae*) povezane s različitim gljivama. Istraživali smo čimbenike koji utječu na asocijacije ofiostomatoidnih gljiva s *I. amitinus* na običnoj smreki. Materijal za studiju bio je prikupljen 2010. godine u blizini Dravograda, na sjeveru Slovenije, gdje je u zimskim vjetrovima bila porušena obična smreka. Za izolacije ofiostomatoidnih gljiva prikupili smo 442 uzorka (kukci i zaraženo drvo - uzorci iz drvenih diskova, s dva stabla na 0,5 m, 6 m i 15 metara iznad panja). Uzeto je ukupno 625 izolata. Ofiostomatoidne gljive su bile najbrojnije zastupljene skupine. Identificirali smo deset vrsta gljiva. Najčešća je bila *Ophiostoma brunneociliatum*, slijedile su *Grosmannia penicillata*, *Ophiostoma bicolor*, *Ceratocystiopsis minuta*, *Grosmannia piceiperda*, *Endoconidiophora polonica*, *Ophiostoma piceae*, *Ophiostoma fuscum*, *Grosmannia cucullata*, *Graphium fimbriisporum*. Povezanost *I. amitinus* s *O. fuscum*, *G. cucullata* i *G. fimbriisporum* bila je prvi put potvrđena. Istraživali smo razlike u pojavljivanju pojedinih vrsta gljiva u različitim stadijima života potkornjaka (adulti, ličinke, kukuljice) i zaraženih uzoraka drva.

KLJUČNE RIJEČI: mali osmerozubi smrekov pisar, vektor, razvojni stadij potkornjaka, asocijacije gljiva, zaštita šuma, Slovenija, *Picea abies*