

First Reports of Silver Fir Blue Staining Ophiostomatoid Fungi Associated with *Pityokteines Spinidens*

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Abstract – *Nacrtak*

Pityokteines spinidens commonly occurs in Europe where it infests Silver fir and occasionally other conifers. Together with the two additional *Pityokteines* species (*P. curvidens* and *P. vorontzowi*) it has been reported as an important factor in Silver fir decline in some parts of Europe.

Bark beetles are known to be associated with diverse guilds of arthropods and microorganisms of which phoretic mites and ophiostomatoid fungi are among the best-known associates. Some ophiostomatoid fungi associated with bark beetles display high levels of virulence to their host trees and they are thus suspected to aid their bark beetle vectors in overcoming the defense mechanisms of living hosts. Increased local populations of *P. curvidens*, *P. spinidens* and *P. vorontzowi* have been recently noted in conifer forests in various parts of Croatia, resulting in high damage levels on Silver fir. This increase in the importance of *Pityokteines* species as forest pests of Silver fir in Croatia, prompted research on the biology of these bark beetles species and on their mite and fungal associates.

Preliminary reports of ophiostomatoid fungi associated with *P. spinidens* resulted from the inspections of bark beetle galleries in trees at the site Litorić (Gorski Kotar, Croatia) where severe case of Silver fir decline took place. Six ophiostomatoid fungi were isolated from the galleries of *P. spinidens*. These included *Ceratocystiopsis minuta*, *Ceratocystiopsis cf. alba*, *Graphium cf. fimbriisporum*, *Ophiostoma cf. cucullatum*, *Ophiostoma piceae sensu lato* and a *Pesotum* species. *Ophiostoma cf. cucullatum* was the most common species based on the occurrence of fungal structures in the insect galleries.

Keywords: *Abies alba*, *Scolytinae*, *Ceratocystiopsis*, *Graphium*, *Ophiostoma*, *Pesotum*

1. Introduction – *Uvod*

Silver fir (*Abies alba* Mill.) is vulnerable to an array of abiotic and biotic damaging factors, both inside and outside its natural range (Schwerdtfeger 1981, Altenkirch et al. 2002, Benz and Zuber 1997). Amongst insects, one important guild of Silver fir pests are phloem-feeding bark beetles (Coleoptera, Curculionidae, Scolytinae), particularly *Cryphalus piceae* Rtz. and *Pityokteines* species (Schwerdtfeger 1981, Altenkirch et al. 2002, Benz and Zuber 1997). *Pityokteines spinidens* Reitter commonly occurs in Europe and preferentially infests *A. alba*, but occasionally other conifers, too (Schwerdtfeger 1981, Altenkirch et al. 2002, Benz and Zuber 1997, Pfeffer 1995).

This bark beetle species usually attacks the lower trunk of Silver fir trees. *P. spinidens* and the two other *Pityokteines* species on *A. alba*, *P. curvidens* Germar and *P. vorontzowi* Jakobson normally infest stressed hosts, but can also attack healthy-looking, vigorous trees (Schwerdtfeger 1981, Benz and Zuber 1997). They have thus been suggested as contributing factors in Silver fir decline (Schwerdtfeger 1981, Benz and Zuber 1997), a still poorly understood syndrome in some parts of Europe that is explained to be caused by a variety of abiotic and biotic factors (Schwerdtfeger 1981, Altenkirch et al. 2002).

Bark beetles are known to be associated with diverse assemblages of microorganisms. Ophiostoma-

toid fungi are amongst the most intensively studied and probably most important fungal associates of bark beetles (Kirisits 2004, Kirschner 1998, Wingfield et al. 1993, Six 2003). They form a group of morphologically similar, but phylogenetically diverse ascomycetes, belonging to the genera *Ceratocystis*, *Ceratocystiopsis*, *Grosmannia* and *Ophiostoma* as well as related anamorph genera such as *Leptographium* and *Pesotum* (Kirisits 2004, Kirschner 1998, Wingfield et al. 1993, Six 2003, Zipfel et al. 2006). Many ophiostomatoid fungi cause intensive discoloration of the sapwood of bark beetle-infested trees, particularly conifers, and these species are therefore commonly referred to as blue-stain fungi (Kirisits 2004, Wingfield et al. 1993). Some ophiostomatoid species associated with bark beetles display high levels of virulence to their host trees and they are thus suspected to aid their bark beetle vectors in overcoming the defense mechanisms of living hosts (Kirisits 2004). However, the importance of ophiostomatoid fungi for bark beetles appears to be variable, and has been the source of controversial debates (Kirisits 2004, Six 2003).

P. spinidens, *P. curvidens* and *P. vorontzowi* are well-known pests of Silver fir in Croatia. Since the beginning of 2000, damage levels by *Pityokteines* spp. on *A. alba* have been increasing in various parts of the country (Pernek 2005), prompting research on the biology of these bark beetles species and their natural enemies (Pernek 2005), as well as on their mite (Pernek et al. 2007) and fungal associates.

A. alba forms Dinaric beech-fir forests (generally called *Abieti-Fagetum dinaricum* Tregubov 1957) which inhabit Lika (with the dominant massifs of Velebit and Velika Kapela) and Gorski kotar (Vukelić and Baričević 2001). The stability of these forest ecosystems have recently turned out to be significantly influenced by the pest-fungal presence.

In Croatia, in forests managed by Hrvatske šume d.o.o. (Croatian forests Ltd.), Silver fir together with Norway spruce (*Picea abies* [L.] Karst.) is one of a few economically important tree species. According to Beuk et al. (2007) the annual allowable cut of Silver fir and Norway spruce is 11.1% (0.64 mil. m³). Quality of the Silver fir timber assortments from forests of fir and hardfern (*Blechno-Abietetum* Ht.) have been analysed by Šušnjar et al. (2005). According to the authors, among the diameter classes 37.5–82.5 cm, the quality classes A, B and C (according to European Committee for Standardization – CEN) have relative proportion of 80–90% of tree net volume. Qualitative classification of softwood round timber ENV 1927-1 allow no damages by insects or wood discoloration (blue stain) in quality classes A, B and C (HRN ENV 1927-1: 2000).

The insect-fungal association research is interesting and important from two points of view. First, they play an undoubtedly important role in the stability of forest ecosystems, and second, their presence in bark and wood influence significantly the timber quality, decreasing its economical value.

The research on fungal associates of *P. spinidens* and other *Pityokteines* species in Croatia revealed its importance upon the aforementioned. A preliminary study on a severe case of Silver fir decline in Litorić locality (Gorski Kotar) supports its necessity.

This preliminary research may also form the basis for further studies on the hitherto unknown interactions between *Pityokteines* spp. and their fungal associates.

2. Materials and Methods – Materijal i metode rada

The preliminary study of ophiostomatoid fungi associated with *P. spinidens* was conducted in April 2004. A stem section from a Silver fir tree, infested with *P. spinidens* was collected at Litorić (550 m a.s.l., 45°27'00" N and 15°04'06" E), located in Dinaric biogeographic region of Croatia (Fig. 1). Twenty gallery systems in the bark or wood were inspected for the occurrence of sexual and asexual stages of ophiostomatoid fungi. Fungi were isolated by transferring masses of ascospores obtained from perithecia and conidia taken from conidiophores and coremia to malt extract agar (MEA; 1000 ml tap water, 16 g agar agar, 20 g malt extract, 100 mg streptomycin sulphate) in plastic Petri dishes (6 cm diameter). Pure cultures were obtained by transferring ascospores, conidia or small pieces of mycelium to fresh MEA plates. Fungi were tentatively determined based on morphological characteristics of their sexual and asexual stages. Representative isolates of ophiostomatoid fungi associated with *P. spinidens* in Croatia are maintained in the culture collection of the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Austria. Selected strains were also deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

3. Results and Discussion – Rezultati rada i rasprava

In the gallery systems of the *P. spinidens* six fungi were found (Fig. 2). The preliminary study revealed the presence of *Ceratocystiopsis minuta* (Siemaszko)



Fig. 1 Research site Litorić - Salvage cut

Slika 1. Lokacija istraživanja Litorić - sanitarna sječa

H. P. Upadhyay & W. B. Kendrick, *Ceratocystiopsis* cf. *alba*, *Graphium* cf. *fimbriisporum*, *Ophiostoma* cf. *cucullatum*, *Ophiostoma piceae sensu lato* and a *Pesotum* species. *Ophiostoma* cf. *cucullatum*, which occurred both in its sexual and asexual stages was the most common species based on the occurrence of fungal structures in the insect galleries.

C. minuta is a common, widespread and unspecific fungal associate of conifer bark beetle species. It is, for example associated with many European bark beetles on spruce, pine and larch (Kirisits 2004, Kirschner 1998). *Ceratocystiopsis* cf. *alba* may be identical with *Ceratocystiopsis alba* (DeVay, R. W. Davidson & W. J. Moller) H. P. Upadhyay, a fungus that is unrelated to *Ceratocystiopsis* and *Ophiostoma* (Hausner et al. 1993). Several attempts to isolate *Ceratocystiopsis* cf. *alba* on MEA from ascospores were unsuccessful in the present study as well as in other investigations on the fungal associates of conifer bark beetles (T. Kirisits, unpublished). This phenomenon

was also reported by Kirschner (Kirschner 1998) for *C. alba*. These observations indicate that *Ceratocystiopsis* cf. *alba* and *C. alba* cannot grow on standard artificial media. They may therefore be mycoparasites of other ophiostomatoid species or other fungi occurring in bark beetle galleries. *C. alba* and *Ceratocystiopsis* cf. *alba* have previously been reported as fungal associates of several conifer bark beetle species in Europe, including *Dryocoetes autographus* Rtzb., *Hylurgops palliatus* Gyll., *Ips amitinus* Eichh., *Ips cembrae* Heer and *I. typographus* L. (Kirisits 2004, Kirschner 1998, Kirisits – unpublished).

Graphium cf. *fimbriisporum* resembles *Graphium fimbriisporum* (Morelet) K. Jacobs, T. Kirisits & M. J. Wingfield and *Graphium pseudormiticum* M. Mouton & M. J. Wingfield, two species that are morphologically very similar to each other but can be distinguished based on molecular markers and ecological differences (Jacobs et al. 2003). *G. fimbriisporum* is associated with various bark beetle species on Norway

spruce (*Picea abies* [L.] Karst) in Europe, while *G. pseudormiticum* is an associate of pine bark beetles in Europe and South Africa (Kirisits 2004, Jacobs et al. 2003, Mouton et al. 1993). *Ophiostoma* cf. *cucullatum*, the most common fungal associate of *P. spinidens* in this study, resembles *Ophiostoma cucullatum* (Solheim), which is a rare associate of various bark beetles on *Picea* spp. in Europe and Asia (Kirisits 2004, Kirschner 1998). Further investigations are needed to clarify whether the fungus on *A. alba* is identical with *O. cucullatum* or it is a new fungal species.

Isolates of *Ophiostoma piceae sensu lato* may either belong to *Ophiostoma piceae* (Münch) H. & P. Sydow (= *Ophiostoma piceae sensu stricto*) or *Ophiostoma quercus* (Georgévitch) Nannf. or both species are associated with *P. spinidens*. *O. piceae* and *O. quercus* are reproductively isolated sibling species that can hardly be distinguished based on morphological characteristics (Brasier and Kirk 1993, Hamschlagler et al. 1994, Harrington et al. 2001). However, we think that isolates associated with *P. spinidens* on *A. alba* very likely belong to *O. piceae sensu stricto*, as this is the species that commonly occurs on conifers, whereas

O. quercus in Europe has been commonly found on hardwoods, but rarely on conifers (Brasier and Kirk 1993). *O. piceae* is a very common, unspecific fungal associate of numerous bark beetle species in Europe, North America and Asia (e. g. Kirisits 2004, Kirschner 1998). Identification of the *Pesotum* species isolated from the galleries of *P. spinidens* in absence of a sexual stage was not possible, as there is presently no monographic treatment of this group of anamorphic fungi.

Further investigations, including especially DNA sequence comparisons are needed to clarify the taxonomic placement of most of the ophiostomatoid fungi identified as associates of *P. spinidens* in our study. In addition, more intensive surveys, based on material from other areas and using isolations directly from *Pityokteines* beetles and their immature stages as well as from the phloem and sapwood of infested trees, will likely lead to the identification of additional ophiostomatoid fungi associated with *Pityokteines* spp. None of the six fungi reported here as associates of *P. spinidens* appear to be virulent pathogens to their host trees in our preliminary study. This is also sup-

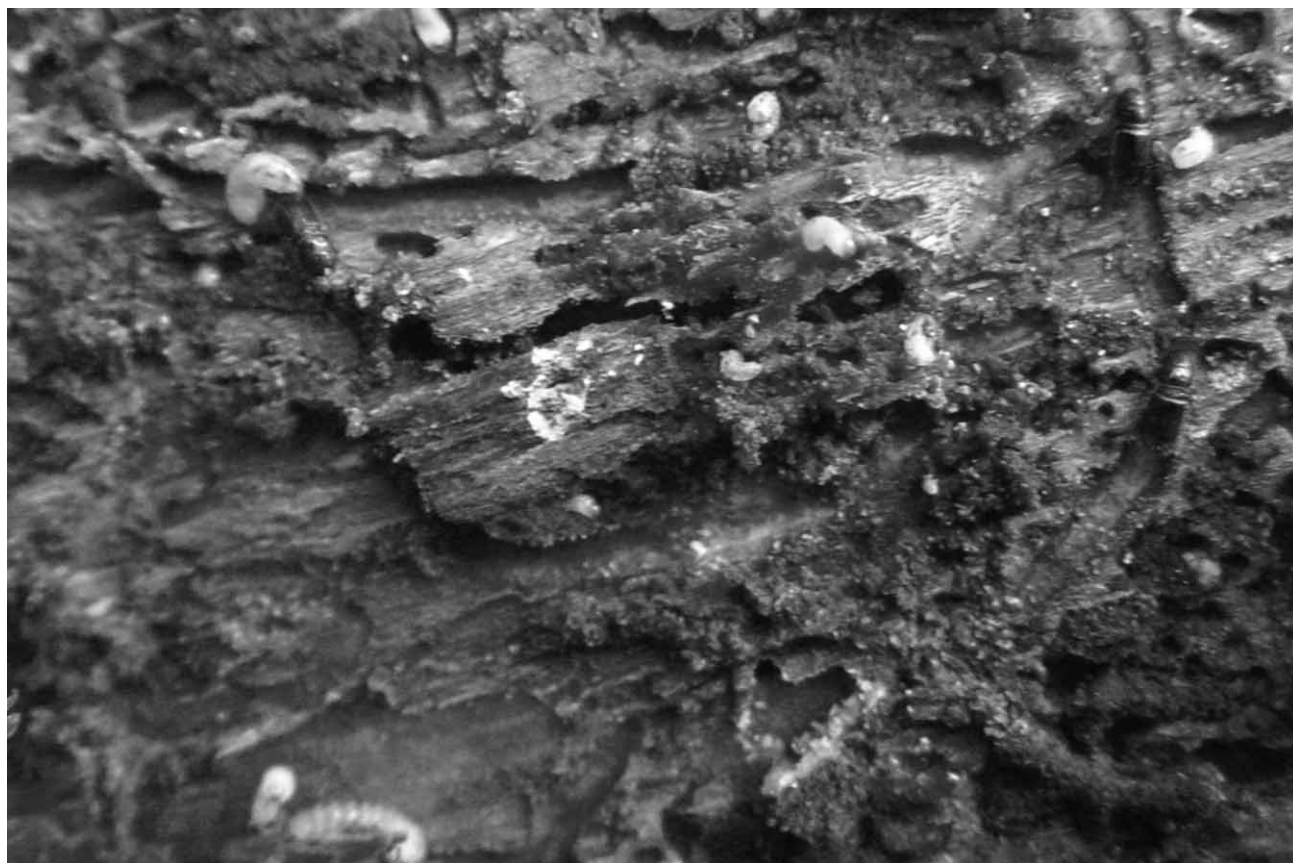


Fig. 2 Sampled gallery systems of *P. spinidens* with larvae, adult bark beetles and a blue stain revealed in some galleries

Slika 2. Uzorkovani hodnici *P. spinidens* s ličinkama, odraslim kukcima i plavilom drva vidljivim u pojedinim hodnicima

ported by the lack of intensive blue-stain observed in the phloem and sapwood of the stem section obtained from the attacked Silver fir tree in Litorić.

4. Conclusion – *Zaključak*

This preliminary study revealed the necessity of further research on bark beetle-fungi associations in Silver fir forest ecosystems in Croatia. Clarifying the fungal species presence in gallery systems of bark beetles and investigating their »collaborating« influence to the trees' health are of a significant importance. The results of future research can be implemented in the pest-disease control measurements supporting the stability of these forest ecosystems, and also reducing the damage in timber quality classes A, B and C as the consequence of bark beetle-fungi presence.

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Sažetak

Prvi nalaz ofiostomatoidnih gljiva, uzročnika plavila drva obične jele u asocijaciji s *Pityokteines spinidens*

Obična jela (*Abies alba* Mill.) osjetljiva je na utjecaj mnogih abiotskih i biotskih čimbenika i u svom prirodnom arealu i izvan njega (Schwerdtfeger 1981, Altenkirch i dr. 2002, Benz i Zuber 1997). Među kukcima važna su skupina štetnika na običnoj jeli potkornjaci (Coleoptera, Curculionidae, Scolytinae), posebice *Cryphalus piceae* Rtz. i vrste iz roda *Pityokteines* (Schwerdtfeger 1981, Altenkirch i dr. 2002, Benz i Zuber 1997). *Pityokteines spinidens* Reitter uobičajeno pridolazi u Europi i primarno naseljava običnu jelu, a povremeno dolazi i na drugim četinjačama (Schwerdtfeger 1981, Altenkirch i dr. 2002, Benz i Zuber 1997, Pfeffer 1995). Ova vrsta potkornjaka obično napada niže dijelove debla obične jele. *P. spinidens* i druge dvije vrste iz ovoga roda, *P. curvidens* Germar et *P. vorontzowi* Jakobson uobičajeno naseljavaju stresna stabla, međutim mogu napasti i izgledom zdrava, vitalna jelova stabla (Schwerdtfeger 1981, Benz i Zuber 1997). S obzirom na navedeno oni se smatraju čimbenicima koji pridonose fenomenu sušenja obične jele (Schwerdtfeger 1981, Benz i Zuber 1997), još uvijek slabo razumljivom sindromu u nekim europskim zemljama koji se objašnjava posljedicom utjecaja različitih abiotskih i biotskih čimbenika (Schwerdtfeger 1981, Altenkirch i dr. 2002).

Poznato je da potkornjaci mogu biti udruženi s različitim mikroorganizmima. Ofiostomatoidne su gljive veoma mnogo istraživane te vjerojatno i u najvažnijim gljivičnim vezama s potkornjacima (Kirisits 2004, Kirschner 1998, Wingfield i dr. 1993, Six 2003). One razvijaju skupinu morfološki sličnih, ali filogenetski različitih askomiceta, koje pripadaju rodovima *Ceratocystis*, *Ceratocystiopsis*, *Grosmannia* i *Ophiostoma*, te njihovim anamorfnim rodovima, kao što su *Leptographium* i *Pesotum* (Kirisits 2004, Kirschner 1998, Wingfield i dr. 1993, Six 2003, Zipfel i dr. 2006). Mnoge ofiostomatoidne gljive uzrokuju intenzivnu diskoloraciju bijeli drveća napadnutoga potkornjacima, posebice četinjača, i poznate su pod zajedničkim imenom uzročnici »plavila drva« (Kirisits 2004, Wingfield i dr. 1993). Pojedine ofiostomatoidne gljive u asocijaciji s potkornjacima pokazuju izrazitu virulentnost i stoga se opravdano pretpostavlja da potpomažu svojim vektorima u svladavanju obrambenih mehanizama svojih životnih domaćina (Kirisits 2004). Međutim, važnost ofiostomatoidnih gljiva za potkornjake, odnosno njihova uloga varira i kao takva je uzrok kontroverznih rasprava (Kirisits 2004, Six 2003).

P. spinidens, *P. curvidens* i *P. vorontzowi* poznati su štetnici obične jele u Hrvatskoj. Od početka 2000. godine štete nastale od spomenutih vrsta iz roda *Pityokteines* na jeli povećavale su se u različitim područjima, što je potaknulo istraživanje biologije ovih potkornjaka i njihovih prirodnih neprijatelja (Pernek 2005, Pernek i dr. 2007) te njihovih asocijacija s gljivama.

Obična je jela sastavni dio dinarskih bukovo-jelovih šuma (*Abieti-Fagetum dinaricum* Tregubov 1957) koje nastanjuju Liku (s dominantnim masivima Velebita i Velike Kapele) te Gorski kotar (Vukelić i Baričević 2001). Stabilnost ovih šumskih ekosustava nedavno je prilično narušena prisutnošću potkornjaka i gljiva, odnosno njihovih zajednica.

U Hrvatskoj, u šumama kojima gospodare Hrvatske šume d.o.o., obična jela zajedno s običnom smrekom (*Picea abies* /L./ Karst.) nalazi se među nekoliko ekonomski značajnih vrsta drveća. Prema Beuku i dr. (2007) godišnji etat obične jele i smreke iznosi 0,64 mil. m³, odnosno 11,1 % ukupnoga godišnjega etata po vrstama drveća kojima gospodare Hrvatske šume d.o.o. Analizirajući kakvoću sortimenata obične jele dobivenih iz šuma jele s rebračom (*Blechno-Abietetum* Ht. 1950), Šušnjar i dr. (2005) utvrdili su sljedeće. Unutar debljinskih razreda 37,5 – 82,5 cm razredi kakvoće A, B i C (prema Europskomu standardu – CEN) nalaze se s relativnom zastupljenošću 80 – 90 % neto obujma stabla. Prema normi HRN ENV 1927-1: 2000, Oblo drvo četinjača – Razvrstavanje po kakvoći, 1. dio: Smreke i jele, oštećenja od kukaca i diskoloracija drva (uključujući plavilo drva) nisu dopuštena upravo u spomenutim razredima kakvoće A, B i C.

Ovim preliminarnim istraživanjem utvrđene su ove ofiostomatoidne gljive u hodnicima *P. spinidens*: *Ceratocystiopsis minuta*, *Ceratocystiopsis cf. alba*, *Graphium cf. fimbriisporum*, *Ophiostoma cf. cucullatum*, *Ophiostoma piceae* sensu lato i *Pesotum sp.* Vrsta *Ophiostoma cf. cucullatum* najproširenija je u hodnicima potkornjaka.

Istraživanja asocijacija potkornjaci-gljive zanimljiva su i važna iz dvaju osnovnih razloga. Prvo, takve asocijacije imaju nedvojbeno važnu ulogu u stabilnosti šumskih ekosustava, i drugo, njihova prisutnost u kori i drvu značajno utječe na kakvoću trupaca, umanjujući njihovu ekonomsku vrijednost.

*Ovo preliminarno istraživanje gljivičnih uzročnika s *P. spinidens* i drugim vrstama potkornjaka iz roda *Pityokteines* u Hrvatskoj, a u vezi s ekstremnim sušenjem obične jele na Litoriću (Gorski kotar), dalo je prve rezultate koji će poslužiti kao smjernice u pronalaženju veza i interakcija između vrsta iz roda *Pityokteines* i njihovih gljivičnih asocijacija, te njihov pojedinačan i zajednički utjecaj na zdravstveno stanje obične jele.*

Ključne riječi: *Abies alba, Scolytinae, Ceratocystiopsis, Graphium, Ophiostoma, Pesotum*

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