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DISTRIBUTION AND NEW HOSTS OF THE PARASITIC FUNGUS Inonotus nidus-pici IN SERBIA

RASPROSTRANJENJE I NOVI DOMAĆINI PARAZITNE GLJIVE *Inonotus nidus-pici* U SRBIJI

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Abstract

Since 2013, symptoms indicative of *I. nidus-pici* infections were recorded in various stands in the central part of Serbia and several tree species from *Fagaceae* family were affected. After the detailed sampling, isolation and identification of the fungus using both classical and molecular methods, the current distribution and host range were determined. This fungus was recorded at 17 additional localities in the central part of Serbia, and *Q. cerris* was the most common and most affected host. Further, huge damages were occasionally recorded at *Q. petraea*, particularly when growing in mixed stands with *Q. cerris*. In addition, *Q. frainetto* and *Fagus sylvatica* were confirmed as new hosts of this pathogen in Serbia. Implications of these findings and potential control measures were discussed.

Key words: Inonotus canker, Quercus cerris, Q. petraea, oxidase reactions, white heart-rot

1. INTRODUCTION / UVOD

Inonotus sensu lato is a white rot basidiomycete genus causing heart rot on a large number of woody hosts. It belongs to *Hymenochaetaceae* within *Hymenochaetales* (Kirk et al., 2008) and most of the species are characterized by annual fruiting bodies, monomitic hyphal system, while the spores are varying in colour, going from colourless to coloured (Karadžić & Milenković, 2014; Wagner & Fischer, 2002). Based on the detailed morphological and molecular analyses, *Inonotus* s. I. the genus was split into five genera – Inonotus s.s., Inocutis, Inonotopsis, Mensularia, and Pseudoinonotus (Parmasto et al., 2014; Wagner & Fischer, 2001, 2002). Due to their ubiquitous distribution and wide range of hosts, they are considered the most important wood-decaying fungi together with some other species such as those from the Ganoderma, Fomes, Phellinus, Trametes, or Stereum genera (Bondarceva, 1998; Karadžić, 2010; Karadžić & Milenković, 2014; Karadžić et al., 2014, 2016, 2022; Kotlaba, 1984; Marinković & Šmit,



1965; Milenković et al., 2018; Overholts 1953; Ryvarden & Johansen, 1980; Ryvarden & Melo, 2014; Schwarze, 2007). Many of the species are parasitic causing significant damage to different hosts, while a large number are saprotrophic contributing to the decomposition of plant material. In the study of Karadžić and Milenković (2014), nine *Inonotus* s. I. species were recorded in the forests of Serbia and Montenegro, including five parasitic and four saprotrophic species. Among those five parasitic species, *Inonotus nidus-pici* Pilát was indicated as one of the most important (Karadžić & Milenković, 2014, 2015).

Inonotus nidus-pici is the cause of cankers and white heart-rot on a range of broadleaved hosts (Kotlaba, 1984), whereas in Serbian forests high infection rate was recorded in 2013 in the province of Vojvodina (Karadžić & Milenković, 2014). Namely, during the studies of the oak decline phenomenon in the natural forests managed by Vojvodinašume Public Enterprise (PE) (Karadžić et al., 2017), unusual symptoms were recorded on Turkey oak (*Quercus cerris* L.) and occasionally on pedunculate oak (*Quercus robur* L.) that have not been recorded in our forests before. Symptoms included typically opened, several-year-old cankers forming cavities with yellowish brown anamorphs on cankers darkening upon age, presence of resupinate fruiting bodies developed in cavities of trees, white heart-rot at the cross sections of infected trees, and after the detailed morphological analyses this fungus was determined as *I. niduspici* (Karadžić & Milenković, 2015). Interestingly, besides the negative effect on *Q. cerris* and other woody hosts, Garádi et al. (2021) and Papp et al. (2021) published some perspective medicinal properties of *I. nidus-pici*.

When studying the declines of various broadleaved hosts in natural ecosystems in the central part of Serbia, symptoms indicative for the infection of *I. nidus-pici* were recorded during the last few seasons. Besides *Q. cerris*, these symptoms were recorded on the other oak species as well as on the European beech (*Fagus sylvatica* L.) trees. Due to large damage caused by *I. nidus-pici* on broadleaved hosts, this study aimed to i) determine the distribution of this fungus in Serbia, ii) determine the host spectrum of this species, and iii) discuss the ecological and economical importance and provide guidelines for reducing the damages.

2. MATERIAL AND METHODS / MATERIJAL I METOD RADA

2.1 Study area and sampled hosts / Lokaliteti istraživanja i uzorkovani domaćini

The study was performed in natural broadleaved forests in Serbia (Table 1), and trees were of various ages and dimensions. All the hosts that showed symptoms indicative of *I. nidus-pici* infection were sampled, including *Q. cerris*, *Q. frainetto* Ten., *Q. petraea* (Matt.) Liebl., *Q. robur* and *F. sylvatica*. Localities where this fungus was previously recorded, as well as new distribution, are shown in Table 1.

2.2 Sampling, isolation, and morphological identification / Prikupljanje uzoraka, izolacija i morfološka identifikacija

Over 50 samples of decayed wood and fruiting bodies were randomly collected. Detailed photo documentation was taken in the field and fruitbodies found on the infected trees were collected. Determination was made by comparing all the morphological features with species description and various keys for the identification of European polypores (Černý, 1959, 1989; Ryvarden, 2005; Ryvarden & Melo, 2014), and following the methods of Nobles (1948, 1965) for the identification of pure cultures of wood-associated fungi. Collected samples of decay wood were taken from the transition zones between rot and healthy tissues and cut into smaller fragments, while surface sterilization was performed by immersion into 70% ethanol and short burning over an open flame. Sterilized fragments were plated onto Malt Extract Agar medium (MEA), prepared following the methods of Booth (1971) [18 g/L of malt extract (Merck, Germany) and 18 g/L agar (Torlak, Serbia)]. Plated samples were



Table 1. Studied localities, distribution, and hosts of the parasitic fungus *Inonotus nidus-pici* in Serbia. FE – forest estate; FA – forest administration; MU – management unit / Tabela 1. Istraživani lokaliteti, rasprostranjenje i domaćini parazitne gljive *Inonotus nidus-pici* u Srbiji. ŠG – šumsko gazdinstvo; ŠU – šumska uprava; GJ – gazdinska jedinica

No / Broj	Area / Područje	Locality / Lokalitet	Host / Domaćin	Year / Godina	Reference / Referenca
1	FE/ŠG Sombor	Kozara; Štrbac; Branjevina	Quercus cerris	2013	_
2	FE/ŠG Sremska Mitrovica, FA/ŠU Kupinovo	MU/GJ Kupinska Greda	Quercus robur	2013	
3	FE/ŠG Sremska Mitrovica, FA/ŠU Morović	MU/GJ Vinična-Žeravinac- Puk; Varadin Županja; Blata Malovanci; Smogva-Grabova Greda	Quercus robur	2013	Karadzić & Milenković, (2015)
4	FE/ŠG Novi Sad	Plavna- cerik; Ristovača	Quercus cerris, Q. robur	2013	
5	Fruška Gora NP	MU/GJ Ravne od. 5, 12a, 17a	Quercus cerris	2013	-
6	FE/ŠG Toplica, FA/ŠU Kuršumlija	MU/GJ Rankovica, od. 44b	Quercus cerris	2015	This study
7	FE/ŠG Toplica, FA/ŠU Kuršumlija	MU/GJ Rankovica, od. 56b	Quercus cerris	2016	This study
8	FE/ŠG Toplica, FA/ŠU Kuršumlija	MU/GJ Rankovica, od. 56b	Fagus sylvatica	2016	This study*
9	FE/ŠG Toplica, FA/ŠU Kuršumlija	MU/GJ Prolom, od. 69	Quercus cerris	2016	This study
10	FE/ŠG Toplica, FA/ŠU Blace	MU/GJ Veliki Jastrebac, od. 62	Quercus cerris	2016	This study
11	FE/ŠG Toplica, FA/ŠU Blace	MU/GJ Veliki Jastrebac, od. 63a	Quercus cerris	2016	This study
12	FE/ŠG Toplica, FA/ŠU Blace	MU/GJ Veliki Jastrebac, od. 63a	Quercus frainetto	2016	This study
13	FE/ŠG Toplica, ŠU Prokuplje	MU/GJ Mali Jastrebac, od. 23a	Quercus cerris	2016	This study
14	FE/ŠG Toplica, FA/ŠU Prokuplje	MU/GJ Mali Jastrebac, od. 38a	Quercus cerris	2016	This study
15	FE/ŠG Rasina, FA/ŠU Ražanj	MU/GJ Poslonske Planine od. 59c	Quercus cerris	2016	This study
16	FE/ŠG Golija Ivanjica, FA/ŠU Sjenica	MU/GJ Golija Javor, od. 17	Fagus sylvatica	2017	This study
17	FE/ŠG Beograd, FA/ŠU Lipovica	MU/GJ Lipovica	Quercus cerris	2017	This study
18	Beograd	Faculty of Forestry Arboretum	Quercus cerris	2017	This study
19	Novi Pazar	Locality Prćenova	Quercus cerris	2019	This study
20	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Lomnička reka, od. 19a	Quercus petraea	2021	This study
21	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Lomnička reka, od. 20c	Quercus cerris	2021	This study
22	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Lomnička reka, od. 20c	Quercus petraea	2021	This study
23	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Srndaljska reka, od. 119b	Quercus cerris	2022	This study

continued / nastavak na sljedećoj stranici

No / Broj	Area / Područje	Locality / Lokalitet	Host / Domaćin	Year / Godina	Reference / Referenca
24	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Petinska reka, od. 18a	Fagus sylvatica	2022	This study
25	FE/ŠG Rasina, FA/ŠU Kruševac	MU/GJ Srndaljska reka, od. 101a	Fagus sylvatica	2022	This study
26	Vrnjačka Banja, Goč	Goč, jezero	Fagus sylvatica	2022	This study

continuation of Table 1 / nastavak Tabele 1

Note / Napomena. *Not confirmed by isolation, only cankers were recorded / *Nije potvrđena izolacijom, samo je rak zabilježen

incubated at approx. 18 °C in the dark, and after the first hypha appeared they were immediately transferred onto fresh MEA medium. Cultures are stored in the Culture Collection of the Faculty of Forestry in Belgrade.

Colony shape patterns were determined after two and six weeks of incubation at 20 °C in the dark on the MEA medium. Growth rates and cardinal temperatures were determined on the MEA and PDA (HiMedia, Mumbai, India) media following the methods of Karadžić et al. (2020), and pieces from actively growing colonies were transferred to the centre of 90 mm Petri dishes with medium (12 ml per Petri dish). Three replicates per isolate and temperature were prepared and incubated for 24 hours at 20 °C. In total, eight temperatures were selected for testing, including 10, 15, 20, 25, 27.5, 30, 32.5 and 35 °C, and plated replicates were left for incubation. After additional 24 hours of incubation, cross lines were made at the bottom of each Petri dish using the permanent marker, and growth was recorded every 24 hours.

The oxidation degree analyses were performed following the method of Davidson et al. (1938), and actively growing mycelial pieces were

transferred onto the MEA media poured into 90 mm Petri plates amended with gallic and tannic acids. Control plates contained pure MEA media, whereas the control fungus was *Trametes versicolor* (L. Fr.) Quél., taken from the Faculty of Forestry culture collection. The oxidation degree was determined after eight and 14 days of incubation at 20 °C in the dark (Karadžić & Milenković, 2015).

2.3 Molecular identification / Molekularna identifikacija

DNA was extracted from the representative isolate INP110a and PCR was performed using the ITS1 and ITS4 primers (White et al., 1990). DNA extraction, PCR reaction conditions and sequencing were performed following the methods described in Karadžić et al. (2020). Assembled sequences were aligned from two sequences obtained using both forward and reverse primer using the Bioedit (v 7.1.3). The sequence was compared with the NCBI database, with the option Nucleotide BLAST (Zhang et al., 2000). After the evaluation, a sequence of representative *I. nidus-pici* isolate was deposited to the NCBI nucleotide collection database.

3. RESULTS AND DISCUSSION / REZULTATI I DISKUSIJA

Morphological characters – size and shape of the collected fruiting bodies (Figures 1, 3 and 4), basidiospores, hymenial setae, and chlamydospores on the surface of anamorph are in the agreement with those of *I. nidus-pici* in the literature, parasitic fungus *I. nidus-pici* was therefore confirmed on various hosts in Serbia. All recorded characters of isolates were in congruence with findings of Karadžić & Milenković (2015), having the KEY PATTERN: 12122122421 obtained following Nobles (1948, 1965), and confirming the presence of *I. niduspici* from the collected samples. All obtained isolates were relatively slow growing on both



MEA and PDA media, with semi-aerial and white mycelium at the beginning that later changed colour from ochraceous to brownish (Figure 4d). The optimum temperature for growth on the MEA medium was at 30 °C averaging 2.47 ± 0.03 mm/day, whereas the optimum temperature on the PDA medium was at 25 °C averaging 1.24 ± 0.02 mm/day (Figure 2).



Figure 1. Inonotus nidus-pici symptoms on Quercus cerris trees: a-b- opened cankers with cavities and twoyear-old anamorph; c- one-year-old anamorph; d- basidiome (teleomorph) inside the log cavity; e- white heart-rot on the cross-section of the Q. cerris log / Slika 1. Simptomi Inonotus nidus-pici na stablima Quercus cerris: a-b- otvorene rak rane sa šupljinama i dvogodišnje plodnice (anamorph); c- jednogodišnja plodnica; d- plodonosno telo (teleomorph) unutar šupljine debla; e- bela trulež srčike na poprečnom preseku debla Q. cerris.

The different optimal temperature on the MEA and PDA media is not unusual, and this phenomenon was recorded in other fungi like in the case of *Neonectria punicea* (J.C. Schmidt: Fr.) Castlebury and Rossman (Karadžić et al., 2020).

In the reverse of older colonies, colour is also changed into brown in the middle of the colony after six weeks of incubation, whereas actively growing zones are ochraceous and whitish. In the older colonies, numerous brown, long



Figure 2. The growth rates of *I. nidus-pici* (INP) on MEA and PDA media at different temperatures (isolate INP110a), where *I. hispidus* (Bull.) Karst. The growth rate on the MEA medium (isolate SFB2016/15) was used for comparison / Slika 2. Porast *I. nidus-pici* (INP, izolat INP110a) na MEA i PDA podlogama na različitim temperaturama, gde je *I. hispidus* (Bull.) Karst. porast na MEA podlozi (izolat SFB2016/15) korišćen radi poređenja.

and spear-shaped setae were recorded, which corresponded with detailed descriptions showed in Pilát (1953), Černý (1959), Karadžić & Milenković (2015) or Bernicchia & Gorjón (2020). After the oxidation degree analyses, the isolates belonged to group 5 according to Davidson et al. (1938), indicating white-rot basidiomycetes.

The identity of *I. nidus-pici* was confirmed also by DNA sequencing. The obtained sequence was submitted to GenBank under the number: KX620772. To our best knowledge, this is the first molecular confirmation of *I. nidus-pici* in Serbia.

By 2013, little was known about the damages caused by *l. nidus-pici* in forests in Serbia and this fungus stayed unnoticed. However, significant damage was later recorded on *Q. cerris* trees (Figure 1), and this fungus can be considered one of the most important pathogens of this oak species. During the first screening of oak forests in Serbia, the distribution of this parasitic fungus was restricted to about five localities in the province of Vojvodina (Karadžić & Milenković, 2015). After the field monitoring in this study, *l. nidus-pici* was recorded at 17 additional localities across Serbia and known distribution is now substantially extended (Table 1).

The first record of *I. nidus-pici* in the central Serbia was in 2015 on *Q. cerris* trees in the oak forests in the management unit (MU) Rankovica near Kuršumlija (Table 1). Since that finding, the presence of this fungus was repeatedly recorded on Q. cerris trees in various areas (Table 1). A particularly serious situation was revealed in one stand in MU Rankovica (department 56b), where more than 50% of over-mature 140-yearold Q. cerris trees were infected showing multiple cankers with significant damage (Figure 1) and a tendency of further spreading. When felling the infected trees, cross-sections showed 70–80% of the surface being affected with white heartrot (Figure 1e), subsequently causing significant damage. A similar situation was recorded in other Q. cerris stands with a high percentage of infected trees.

Besides *Q. cerris* and *Q. robur* which were listed as hosts of this fungus in Serbia (Karadžić & Milenković, 2015), three additional hosts from *Fagaceae* family were recorded in this study (Table 1). In 2016 *Q. frainetto* was recorded as a new host of *I. nidus-pici* in Serbia (Figure 4e). The habitat was a mixed oak forest in MU Veliki Jastrebac near Blace, and the infection rate was high on both *Q. frainetto* and *Q. cerris*. Another





Figure 3. *Inonotus nidus-pici* symptoms on *Quercus petraea* trees: a - opened canker and one- (arrow) and two-year-old anamorph; b - huge, opened cavity that spreads into the heartwood and the mass of released basidiospores; c – the initial development of canker and anamorph; d – cross-section through the *Q. petraea* log and white heart-rot / Slika 3. Simptomi *Inonotus nidus-pici* na stablima *Quercus petraea*: a- otvorene rak rane, jednogodišnje (strelica) i dvogodišnje plodnice; b- velika otvorena šupljina koja se širi u srčiku i masa oslobođenih bazidiospora; c- početak razvoja rak rane i plodnice; d- poprečni presek kroz deblo *Q. petraea* i bela trulež srčike.

oak *Q. petraea* was recorded as a host in MU Lomnička reka in 2020 (Table 1). In this stand, a dramatically high infection rate was recorded on approx. 80-year-old *Q. petraea*, causing significant damage to this important woody species (Figure 3). Finally, in 2019 *F. sylvatica* was confirmed as a host of *I. nidus-pici* (Figure 4a–c) near Sjenica, followed by two simultaneous findings on Goč and Jastrebac mountains (Figure 4a–c; Table 1). However, symptoms that resembled *I. nidus-pici* cankers without cavities with fruiting bodies were recorded on *Fagus sylvatica* earlier, but these records were not confirmed by isolation. Therefore, our findings in 2019 (and later in 2022) could be considered as the first ones on *F. sylvatica* in Serbia. This is probably not the final host range of *I. nidus-pici* in Serbia, considering that e.g. Kotlaba (1984)

published 17 different broadleaved hosts of this fungus in Czechoslovakia, including not only *Quercus* and *Fagus* but also e.g. *Acer* and *Fraxinus* as the respective host genera. Therefore, further monitoring of various stands and parks with potential woody hosts in Serbia is required.



Figure 4. *Inonotus nidus-pici* symptoms on *Fagus sylvatica* and *Quercus frainetto* trees: a-c- one- (arrows) and two-year-old anamorph on *F. sylvatica* stems; d - pure culture on the MEA medium after four weeks of incubation at 20°C in the dark; e - opened canker and anamorph on *Q. frainetto* stem / **Slika 4.** Simptomi *Inonotus nidus-pici* na stablima *Fagus sylvatica* i *Quercus frainetto*: a-c- jednogodišnje (strelica) i dvogodišnje plodnice na stablu *F. sylvatica*; d- čista kultura na podlozi MEA posle četiri nedelje inkubacije na 20 °C u tami; e- otvorene rak rane i plodnice na stablu *Q. frainetto*.



Inonotus nidus-pici mostly colonizes mature trees, rarely their branches with bigger diameters (Karadžić & Milenković, 2015), and considering a significant area with Q. cerris stands in Serbia (Banković et al., 2009), I. nidus-pici represents a high risk for their stability. A large percentage of Q. cerris stands in Serbia are of coppice origin due to extensive usage in the past, particularly after World War II, and the age of coppice stands is up to 60-70 years, whereas the age of seed-origin stands is going up to 150 years (Banković et al., 2009). Inoculum penetrates through wounds and scars from fallen branches, suggesting that older stands intensively utilized in the past now represent the ideal substrate for I. nidus-pici infection. These over-mature stands should be urgently regenerated to decrease risks of infection and damage by *I. nidus-pici*. Also, considering that Q. cerris is participating in various mixed stands (Banković et al., 2009), this host may serve as a huge inoculum reservoir for infection of surrounding trees. This situation was recorded in several mixed stands surveyed in this study when Q. cerris and other putative hosts occurred together (Table 1), suggesting that Q. cerris most likely was the primary source of inoculum.

This study revealed that mature *Q. cerris* stands in Serbia are often affected by the parasitic

fungus I. nidus-pici. However, other oak species like Q. frainetto, Q. petraea and Q. robur were also recorded as hosts, posing high ecological and economical risks in different ecosystems in Serbia. Particularly high risks are present in Quercetum frainetto-cerris Rudski zonal forests (Tomić & Rakonjac, 2013) as ecologically very important in Serbia (and other countries of the Balkan Peninsula) since both major hosts seemed to be susceptible to I. nidus-pici infections. To decrease the level of damage and source of inoculum, it is recommended to remove all trees with symptoms from the affected stands. In some cases, the rotation should be shortened (rotation concerning forest pathology) considering the influence of drought stress and the fact that older and mature stands are the most susceptible to infections. In management planning, advantage should be given to mixed stands rather than to pure stands, shorter rotation of most susceptible hosts, and during tending and thinning any mechanical damage should be decreased to a minimum. Nevertheless, the careful monitoring of mature Q. cerris and other potentially susceptible hosts is urgently required to promptly react in the cases of new infections and I. nidus-pici spreading.

4. CONCLUSIONS / ZAKLJUČCI

- Inonotus nidus-pici is a white rot basidiomycete that causes significant heartrot damage on various hardwood hosts;
- it was found in 22 different locations in Serbia;
- previously known hosts in Serbia were *Quercus cerris* and *Q. robur*;
- three new hosts were recorded in this study: *Q. frainetto, Q. petraea,* and *Fagus sylvatica;*
- *Quercus cerris* is the most frequent host and significant damage caused by white heart rot was recorded in its stands;

- besides *Q. cerris*, substantial damage was also recorded on *Q. petraea* trees, posing high risks to this valuable species;
- stand age is discussed as a potential cause of higher recording frequency during the last decade;
- avoiding mechanical damage during tending and thinning was suggested as a short-term measure to decrease the risks of future infections;
- flexible management planning and rotation concerning forest pathology were suggested as one of the long terms measures.

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References / Literatura

- Banković, S., Medarević, M., Pantić, D., & Petrović, N. (2009). The National Forest Inventory of the Republic of Serbia-The growing stock of the Republic of Serbia. Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia-Forest Directorate, Belgrade.
- Bondartseva, M. A. (1998). *Opredelitel' gribov Rossii. Poryadok Afilloforov'iye. Vyp. 2* [Определитель грибов России. Порядок Афиллофоровьие. Вып. 2]. Nauka, Leningrad.
- Bernicchia, A., & Gorjón, S. P. (2020). Polypores of the Mediterranean Region. Romar SRI.
- Booth, C. (1971). *Methods in microbiology Vol. 4*. Academic Press, London.
- Davidson, R. W., Campbell, W. A., & Blaisdell, J. D. (1938). Differentiation of wood-decaying fungi by their reaction on gallic or tannic acid medium. *Journal of Agricultural Research*, 57(9), 683–695.
- Garádi, Z., Dékány, M., Móricz, Á.M., Gaál, A., Papp, V., Béni, S., & Ványolós, A. (2021). Antimicrobial, Antioxidant and Antiproliferative Secondary Metabolites from *Inonotus nidus-pici*. *Molecules*, 26, 2–9.
- Karadžić, D., Milenković, I., & Radulović, Z. (2022). Česte lignikolne gljive u šumama Srbije i njihova lekovita svojstva. Univerzitet u Beogradu, Šumarski fakultet.
- Karadžić, D., Milenković, I., Milanović, S., Golubović Ćurguz, V., & Tomović, Z. (2017). Najznačajnije parazitske i saprofitske gljive u hrastovim šumama na području JP "Vojvodinašume". JP Vojvodinašume, Petrovaradin.
- Karadžić, D. (2010). *Šumska fitopatologija*. Univerzitet u Beogradu, Šumarski fakultet.
- Karadžić, D., & Milenković, I. (2014). The most common Inonotus species in the forests of Serbia and Montenegro. *Šumarstvo, 66*(3–4), 1–18.
- Karadžić, D., & Milenković I. (2015). Contribution to the knowledge of parasitic fungus *Inonotus nidus*-

pici Pilat the agent of canker of broadleaved forest trees. *Šumarstvo, 67*(1–2), 15–29.

- Karadžić, D., Milenković, I., Golubović Ćurguz, V., & Obradović, S. (2018). The most important parasitic fungi causing cankers, galls and witches' brooms on forest trees in Serbia. *Šumarstvo*, 70(3–4), 25–52.
- Karadžić, D., Milenković, I., & Radulović, Z. (2016). Contribution to the knowledge of parasitic and saprophytic fungi on Persian walnut (*Juglans regia* L.) trees in Serbia. *Šumarstvo, 68*(3–4), 87–103.
- Karadžić, D., Radulović, Z., & Milenković, I. (2014). Ganoderma species in the forests of Serbia and Montenegro. Sumarstvo, 66(1–2), 1–19.
- Karadžić, D., Stanivuković, Z., Milanović, S., Sikora, K., Radulović, Z., Račko, V., Kardošová, M., Durkovič, J., & Milenković, I. (2020). Development of *Neonectria punicea* Pathogenic Symptoms in Juvenile *Fraxinus excelsior* Trees. *Frontiers in Plant Science*, *11*, 592260. https://doi. org/10.3389/fpls.2020.592260
- Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). *Dictionary of the FUNGI* (10th ed.). CABI International, Wallingford, Oxon, UK.
- Kotlaba, F. (1984). Zeměpisné rozšíření a ekologie chorošů (Polyporales s.l.) v Československu. Academia, Praha.
- Marinković, P., & Šmit, S. (1965). *Gljive razarači bukovog drveta u šumama i na stovarištima u Srbiji*. Institut za šumarstvo i drvnu industriju.
- Milenković, I., Tomšovský, M., Karadžić, D., & Veselinović, M. (2018). Decline of Paulownia tomentosa caused by Trametes hirsuta in Serbia. Forest Pathology, 48(4), e12438. https://doi. org/10.1111/efp.12438
- Nobles, K. M. (1948). Studies in Forest Pathology: VI. Identification of cultures of Wood-rooting fungi. *Canadian Journal of Research, 26c*(3), 281–431.

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- Nobles, K. M. (1965). Identification of cultures of wood-inhabiting Hymenomycetales. *Canadian Journal of Botany*, *43*, 1097–1139.
- Overholts, L. O. (1953). *The Polyporaceae of the United States, Alaska and Canada*. University of Michigan Press, Ann Arbor.
- Parmasto, E., Saar, I., Larsson, E., & Rummo, S. (2014). Phylogenetic taxonomy of *Hymenochaete* and related genera (Hymenochaetales). *Mycological Progress*, 13, 55–64
- Pegler, D. N. (1964). A survey of the Genus Inonotus (Polyporaceae). Transactions of the British Mycological Society, 47(2), 175–195.
- Pilát, A. (1953). Hymenomycetes novi vel minus cogniti Cehoslovakiae: II. Acta Musei Nationalis Pragae, 9B(2), 1–109.
- Ryvarden, L. (2005). *The genus Inonotus, a synopsis*. Fungiflora, Oslo.
- Ryvarden, L., & Johansen, I. (1980). A preliminary polypore flora of East Africa. Fungiflora, Oslo.
- Ryvarden, L., & Melo, I. (2014). *Poroid fungi of Europe*. Fungiflora, Oslo.
- Schwarze, F. W. M. R. (2007). Wood decay under the microscope. *Fungal Biology Reviews*, 21(4), 133–170.
- Tomić, Z., & Rakonjac, LJ. (2013). *Šumske fitocenoze Srbije*. Institut za šumarstvo Beograd.

- Wagner, T., & Fischer, M. (2001). Natural groups and a revised system for the European poroid *Hymenochaetales* (Basidiomycota) supported by nLSU rDNA sequence data. *Mycological Research*, 105(7), 773–782.
- Wagner, T., & Fischer, M. (2002). Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l. and phylogenetic relationships of allied genera. *Mycologia*, 94(6), 998–1016.
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR protocols: a guide to methods and applications (pp. 315–322). Academic Press, San Diego, California, USA.
- Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1/2), 203–214.
- Černy, A. (1959). *Inonotus nidus-pici* Pilát rezavec datli, velmi škodlivý parazit některých listatých dřevin v ČSR. *Sbornik VŠZ v Brné, 28*, 65–87.
- Černy, A. (1989). *Parazitické Drevokazné houby*. Vydalo ministerstvo lesniho a vodniho hospodárstvi a drevozpracujiciho prumyslu ČSR ve Statnim zemedelskem nakladatelstvi v Praze.

Sažetak

Gljive iz roda Inonotus uzrokuju belu trulež drvenastih domaćina i obuhvataju parazitske i saprotrofske predstavnike. Jedan od tipičnih parazitskih predstavnika je gljiva I. nidus-pici Pilát, uzročnik otvorenih rak-rana i bele truleži srčike lišćara. Glavni domaćini ove parazitske gljive su hrastovi, prvenstveno hrast cer (Quercus cerris L.), ali i vrste iz roda Acer, Fraxinus i drugih rodova. Prethodnih decenija nisu zabeležene veće štete uzrokovane ovom gljivom i ona je bila neprimetna u našim šumama. Međutim, tokom 2013. godine zabeležene su velike štete i širenje ove parazitske gljive na hrastu ceru, a u pojedinačnim slučajevima i lužnjaku (Q. robur L.) na pet lokaliteta u šumama kojima upravlja šumsko gazdinsvo "Vojvodinašume" i Nacionalni park "Fruška Gora" (Karadžić & Milenković 2015). Od 2013. godine zabeležene su značajne štete na području uže Srbije i stabla različitih drvenastih domaćina su pokazivala simptome infekcije ovom parazitskom gljivom. Detaljnim terenskim istraživanjima i monitoringom velikog broja lišarskih sastojina, prikupljeni su uzorci sa simptomatičnih stabala zahvaćenih procesima truleži koji su podvrgnuti testovima izolacije u laboratoriji. Dobijeni izolati su morfološki i molekularno analizirani i kasnije podvrgnuti testovima na reakcije oksidaze sa dodatkom galne i taninske kiseline radi određivanja tipa truleži. Zajedno sa morfologijom polnih i bespolnih plodonosnih tela na inficiranim stablima, izvršena je konačna identifikacija. Izolati su pokazivali spori rast na MEA i PDA hranljivim podlogama, u početku su bili svetli, a pri dužoj inkubaciji u mraku menjali su boju u narandžastu, smeđu, a zatim u tamno-smeđu. Posle sprovedenih reakcija oksidaze izolati su pripadali grupi 5 prema Davidson et al. (1938), ukazujući na belu trulež iz razdela Basidiomycota. Na površini plodonosnih tela, kao i u čistim kulturama bio je prisutan veliki broj

kopljastih seta i na osnovu poznatih ključeva ova gljiva je identifikovana kao *I. nidus-pici*. Dobijeni rezultati su potvrđeni molekularnim analizama i sekvenciranjem ITS regiona. Parazitska gljiva *I. nidus-pici* je zabeležena na 17 novih lokaliteta u Srbiji, a pored cera i lužnjaka, nađena je na hrastu kitnjaku (*Q. petraea* (Matt.) Liebl.), hrastu sladunu (*Q. frainetto* Ten.) i bukvi (*Fagus sylvatica* L.). Na ceru su zabeležene značajne ekonomske štete usled truleži srčike najvrednijeg dela debla, a sličan obim šteta je zabeležen i na hrastu kitnjaku. Nalaz na bukvi predstavlja velike ekonomske i ekološke rizike, uzimajući u obzir zastupljenost i važnost ove vrste u šumama Srbije. Nalaz na hrastu sladunu predstavlja velike ekološke rizike, jer ova vrsta gradi važne zonalne šumske zajednice sa hrastom cerom. Izbegavanje mehaničkih oštećenja i uklanjanje stabala sa simptomima su preporučeni kao kratkoročne mere, dok je skraćenje i uvođenje "patološke ophodnje" preporučeno kao dugoročna mera za smanjenje šteta od parazitske gljive *I. nidus-pici*.

Ključne reči: bela trulež srčike, Inonotus rak, Quercus cerris, Q. petraea, reakcije oksidaze