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THE MOST IMPORTANT MYCOSES OF AUSTRIAN PINE (*PINUS NIGRA* ARNOLD.) IN THE AVALA AREA

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Abstract: Austrian pine is one of the species most often used for afforestation in our conditions. In the area of Avala, afforestation with Austrian and Scots pine began in 1899. The stands of Austrian pine located on the northern and northwestern foothills of the Avala Landscape of Outstanding Features are 115 years old, while the stands on the southern and southeastern side are about 65 years old. Two-year research in the area of Avala recorded the presence of 18 species of fungi on Austrian pine. Out of that number, 6 fungi were found on needles, 5 on needles and seeds, 2 on roots and stem bases, 1 on cones, 3 on trunks and branches and 1 species in vascular bundles. Of all the species, the greatest damage is caused by *Sphaeropsis sapinea* and species of the *Armillaria* genus.

Keywords: Austrian pine, mycoses, fungi, Avala

NAJVAŽNIJE MIKOZE CRNOG BORA (*PINUS NIGRA* ARNOLD.) NA PODRUČJU AVALE

Izvod: Crni bor je jedna od vrsta koja se u našim uslovima najčešće koristi za pošumljavanje. Na području Avale pošumljavanja crnim i belim borom počela su još 1899 godine. Sastojine crnog bora koje se nalaze na severnoj i severozapadnoj strani podnožja PIO Avala su starosti 115 godina, dok su sastojine koje se nalaze na južnoj i jugoistočnoj strani starosti oko 65 godina. Tokom dvogodišnjih istraživanja na području Avale na crnom boru je zabeleženo prisustvo 18 vrsta gljiva. Na četinama konstatovano je prisustvo 6 gljiva, na četinama i semenu 5, na korenu i pridanku 2, na šišaricama 1, deblu i granama 3 i u

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sprovodnim sudovima 1 vrsta. Od svih vrsta najveće štete pričinjavaju Sphaeropsis sapinea i vrste roda Armillaria.

Ključne reči: crni bor, mikoze, gljive, Avala

1. INTRODUCTION

Avala, the northernmost mountain in Šumadija, stands out in the wider area of Belgrade with its altitude of 506 meters. By the decision of the City of Belgrade of 2007, Avala was protected as a Landscape of Outstanding Features. It belongs to the Šumadija Mountain Range that represent a transitional zone between the Dinaric and Rhodope mountain ranges.

The area of the protected natural asset is characterized by the diversity and abundance of plant species. The recent flora of Avala consists of about 600 plant species from two classes, 86 families and 317 genera. A total of 6 forest and 4 meadow communities have been recognized and described on Mt. Avala. In the period from 1899 to 1906, Austrian and Scots pine plantations were established to replace clearings and thickets (Obratov-Petković, D., Đukić, M., 2000).

The family *Pinaceae* contains 11 genera, with 195 species of trees and infrequent shrubs. The *Pinaceae* family has the following three subfamilies: *Abietoideae*, *Pinoideae*, and *Laricioideae*. The *Pinoideae* subfamily includes only the *Pinus* genus, which is divided into 2 subgenera: *Pinus* and *Strobus*. Austrian pine belongs to the *Pinaceae* family, *Pinoideae* subfamily and *Pinus* genus and subgenus. Austrian pine is native to southern Europe, northwestern Africa, and western Asia. It belongs to the sub-Mediterranean floral element. In Serbia, it is found in the northwestern and central parts, and not so often in eastern Serbia. Earlier research identified several subspecies, but recent research has shown that there are only two subspecies, of which only ssp. *nigra* grows in Serbia (Cvjetičanin, R., et al., 2016).

In his research, Farjon, A. (2017) determines 5 subspecies of Austrian pine: *Pinus nigra* ssp. *nigra* (native to the east of Austria, north of Italy, Balkan Peninsula, Bulgaria, Romania, and the European portion of Turkey), *Pinus nigra* ssp. *laricio* (Corsica in France, southern Apennines and Sicily in Italy), *Pinus nigra* ssp. *pallasiana* (Kütahya Province in Turkey), *Pinus nigra* ssp. *dalmatica* (Croatia) and *Pinus nigra* ssp. *salzmannii* (southwestern Europe, Hérault and Pyrenees in France, Spain, the region of Diebel Djurdur in Algeria, and the Rif Mountain Range in the north of Morocco).

Austrian pine is one of the species most often used for afforestation in our conditions. Its needles are dark green. It forms a wide crown and reaches large dimensions. Austrian pine prefers bright and warm exposures and is very resistant to adverse environmental conditions. It grows well on all soil types, even on extremely poor and shallow soils. Apart from being drought-prone and wind-resistant, it tolerates urban conditions.

Austrian pine in PA Avala is located in five compartments and 11 sections, on the northern and southern slopes. It builds pure or mixed stands. The stands of Austrian pine on the northern and northwestern foothills of Avala are 117 years old and the stands on the southern and southeastern side are about 67 years old.

2. MATERIAL AND METHOD

The research was conducted during 2018 and 2019 in the area of PA Avala in compartment 15 (section i), compartment 21 (sections a, b, c, d, e), compartment 23 (sections d, h), compartment 25 (sections k, n), and compartment 29 (section a).

The visual appearance of fruiting body was used in the identification of species. When it was not possible, the material with disease symptoms was transferred to the laboratory, where it was analyzed using standard phytopathological methods.

Tissues from which the material was isolated were first washed with running water for at least 30 minutes. Fungi were isolated from the freshly infected parts of lesion tissue which were cut into pieces smaller than 5 mm. Saprophytic species were isolated from older infected tissues. The pieces of excised tissue were surface-sterilized in sodium hypochlorite solution (2-6% active chlorine) for 2 minutes or in 70% ethanol for a few seconds and then washed in sterile distilled water. They were then transferred to appropriate nutritional media under aseptic conditions. Petri dishes were put into an incubator at appropriate temperatures, and the suitable time for species identification was determined through daily observation. Fungus cultures usually grew at temperatures between 21 and 25°C, but sometimes they were incubated at lower temperatures (below 15°C) or higher temperatures (above 34°C).

The formation of fruiting bodies was accelerated by changing external conditions (temperature, light regime), nutrition of fungi (replacement of nutrient-rich media with water-agar media), or long-term culture development. Identification was usually performed based on the culture characteristics, as well as morphological and physiological characteristics of the fungus using the following keys: Bondarceva, M. A. (1998), Dennis, R. W. G. (1978), Ellis, M., B., Ellis, J., B. (1985), Lanier, L., et. al. (1978), Minter, W.D. (1981) and Sutton B.C. (1980). The classification of fungi was performed according to the database available at www.indexfungorum.org/Names/Names.asp.

3. RESULTS AND DISCUSSION

The species of fungi identified on Austrian pine in the area of Avala are shown in Table 1.

Table 1. *Fungus species identified on Austrian pine in the area of Avala*

Latin name	Classification	Affected plant part	Significance
<i>Alternaria alternata</i> (Fr.) Kasser	Ascomycota, Pleosporaceae	Older needles, seed	+
<i>Armillaria</i> spp.	Basidiomycota, Physalacriaceae	Root and stem base	+++
<i>Auriscalpium vulgare</i> Gray	Basidiomycota, Auriscalpiaceae	Fallen cones	+
<i>Botrytis cinerea</i> Pers. ex Fr.	Ascomycota, Sclerotiniaceae	Needles, shoots, and seed	+
<i>Ceratocystis</i> spp.	Ascomycota, Ceratocystidaceae	Vascular bundles of stems	++

<i>Cenangium acuum</i> Cooke & Peck	Ascomycota, <i>Helotiaceae</i>	Fallen cones	+
<i>Cladosporium herbarum</i> (Pers.) Link	Ascomycota, <i>Cladosporiaceae</i>	Fallen cones	+
<i>Cyclaneusma niveum</i> (Pers.) DiCosmo, Peredo & Minter.	Ascomycota, <i>Marthamycetaceae</i>	One-year and two-year old needles	++
<i>Epicoccum purpurascens</i> Ehrenb.	Ascomycota, <i>Didymellaceae</i>	Fallen cones i seme	+
<i>Fusarium moniliforme</i> J.Sheld.	Ascomycota, <i>Nectriaceae</i>	Needles and seed	+
<i>Heterobasidion annosum</i> (Fr.) Bref.	Basidiomycota, <i>Bondarzewiaceae</i>	Roots of uprooted trees	++
<i>Lophodermium pinastri</i> (Scharl.:Fr.) Chev.	Ascomycota, <i>Rhytismataceae</i>	Most often on fallen cones	++
<i>Lophodermium seditiosum</i> Minter, Staley & Millar	Ascomycota, <i>Rhytismataceae</i>	One-year and two-year needles	++
<i>Phellinus pini</i> (Thore:Fr.) Ames.	Basidiomycota, <i>Hymenochaetaceae</i>	Trunk of older trees	++
<i>Schizophyllum commune</i> Fr.	Basidiomycota, <i>Schizophyllaceae</i>	Trunk and branches	+
<i>Sphaeropsis sapinea</i> Dyko et Sutton,	Ascomycota, <i>Botryosphaeriaceae</i>	Young shoots and cones	+++
<i>Trichaptum abietinum</i> (Pers. ex J.F.Gmel.) Ryvar den	Basidiomycota, <i>Incertae sedis</i>	Trunk and branches	+
<i>Trichothecium roseum</i> Link.	<i>Incertae sedis</i>	Fallen cones and seed	+

+ - Fungi occur mainly as saprophytes or rarely as facultative parasites. They are not significant pathogens of Austrian pine.

+ + - Fungi can cause significant damage to Austrian pine, but due to the small-scale presence in this area, their significance is still small.

+ + + - Fungi cause significant damage. Multi-year infections can lead to the dieback of Austrian pine.

Two-year research in the area of Avala recorded the presence of 18 species of fungi on Austrian pine. Out of that number, 6 fungi were found on needles, 5 on needles and seeds, 2 on roots and stem bases, 1 on cones, 3 on trunks and branches, and 1 species in vascular bundles.

Of all the species, the most extensive damage is caused by *Sphaeropsis sapinea* and species of the *Armillaria* genus. The latter group includes the following species: *Ceratocystis spp.*, *Cyclaneusma niveum*, *Heterobasidion annosum*, *Lophodermium pinastri*, *Lophodermium seditiosum*, and *Phellinus pini*. The other 10 species have very little significance and most often occur as saprophytes.

Species of the *Armillaria* genus are the most common causes of dieback and decay in coniferous and broadleaved cultures of all ages. Coniferous cultures established on the sites of broadleaved species are particularly affected, especially if the sites have not been previously destumped. This fungus lives on stumps as a saprophyte, and then as a parasite attacks weakened coniferous trees, or develops as a weak parasite on broadleaved trees.

Exploring the species diversity of the *Armillaria* genus Keča et al. (2006) found the following five species in Serbia and Montenegro: *A. mellea*, *A. ostoyae*, *A.*

cepistipes, *A. gallica*, and *A. tabescens*. The most common species is *A. gallica* and *A. tabescens* the rarest.

S. sapinea colonizes young shoots and a severe attack usually leaves all shoots from the current growing season necrotic. A severe attack of this fungus was recorded in most compartments in the area of Avala. Perennial infections cause physiological weakness of trees, which leads to the dieback and eventually to the death of deformed trees.

Applying artificial inoculations of *Pinus nigra* seedlings, Milijašević, T. (2000) found that *S. sapinea* acts as a parasite. Uninjured shoots may be infected from April to August, although the symptoms of the disease are more difficult to notice in the summer months. This research indicates that the fungus can cause dieback of whole plants when it colonizes both the root collar and the root and forms fruiting bodies on them.

In our conditions, *C. niveum* has two infection periods. The first period is from late April to late August, and the second from mid-October to early December. Besides this species, *C. minor*, which occurs on Scots pine, is another species common in our country. Some authors consider species of this genus primary parasites, while some cite them as weak parasites or saprophytes. By artificial inoculations of two-year-old Scots pine seedlings, Karadžić, D. (1980) found that the infection is realized through stomata and then spreads in the mesophyll. The first symptoms are noticed after three months, and the apothecia begin to form four months after the inoculation. According to this research, *C. minor* acts as a parasite under controlled conditions.

According to Lazarev, V. (1980, 2004), the biology of *Lophodermium* species is characterized by 4 phases of development: the phase of infection, the phase of latency, the phase of transition to the saprophytic nutrition, and the phase of reproduction. The phase of infection is limited to the period of a mass release of ascospores, and penetration is possible through the cuticle or the stoma. The latency phase lasts 20-30 days and sometimes may be skipped. The phase of transition to the saprophytic nutrition is characterized by the formation of lush mycelium that spreads along the needle axis. It also spreads in the radial direction by filling resin ducts. Chlorotic spots become dark red, and the formation of pycnidia begins. At the end of this phase, the entire needle is necrotic and turns dark red. In the last phase, apothecia are formed. When mature, they open with a longitudinal crack and release ascospores. Transverse black or brown lines are also formed at this stage. Studying the degree of aggressiveness of *Lophodermium* species, Lazarev, V. (1981, 2004) found *L. pinastri* on older primary needles of seedlings in forest nurseries and stands, older secondary needles (2 and 3 years), and secondary needles attached to dead branches. *L. seditiosum* was found on younger primary needles of seedlings in forest nurseries, secondary needles of various ages, and most often on needles younger than one year in forest nurseries, cultures, and stands.

In the research of parasitic and saprophytic fungi in Austrian pine cultures in Serbia, Karadžić, D., Milijašević, T. (2008) found 41 species of fungi on Austrian pine, 45 species on Scots pine, while 24 species were found on both Austrian pine and Scots pine. *Mycosphaerella pini*, *Sphaeropsis sapinea*, *Cenangium feruginosum*, *Gremmeniella abietina*, and *Armillaria spp* cause the most extensive damage and dieback of Austrian pine trees.

In the study of the pathogenic mycoflora of *Pinus* species, Karadžić, D., (1987) states that *Scirrhia pini*, *Sphaeropsis sapinea*, *Cenangium feruginosum*, and *Gremmeniella abietina* pose a practical problem in the establishment and maintenance of Austrian pine cultures, and perennial infections can lead to the dieback of trees. *Heterobasidion annosum* is also stated as a fungus that poses a practical problem in the establishment and maintenance of Austrian pine cultures and causes dieback.

H. annosum occurs less frequently on Austrian pine. In the area of Avala, it was observed on the lateral veins of one fallen tree in compartment 23 (section d). Another two species that can cause great damage to Austrian pine (*Phellinus pini* and *Ceratocystis spp.*) were found on individual (felled) trees, but have had no economic significance so far. *P. pini* occurs on old pine trees and considering Austrian pine, it attacks only trees over 100 years of age. The resistance of younger pines is explained by their greater ability to secrete resin. The resin quickly seals the injured places on the bark and thus prevents infections because the penetration of mycelium into the tree is prevented. The decaying process in the vertical direction progresses about 15 cm per year. The lower and central parts are most affected.

4. CONCLUSIONS

Based on two years of research, the following conclusions can be drawn:

- In the area of Avala, Austrian pine recorded the presence of 18 species of fungi. Out of that number, 6 fungi were found on needles, 5 on needles and seeds, 2 on roots and stem bases, 1 on cones, 3 on trunks, and branches and 1 species in vascular bundles
- Of all species, *Sphaeropsis sapinea* and species of the *Armillaria* genus cause the greatest damage. A severe attack of *S. sapinea* was recorded in most compartments in the area of Avala. Young needles of almost all shoots were infected. Species of the *Armillaria* genus were found on most decaying and dead Austrian pine trees. Austrian pine trees with common infections of *S. sapinea*, *C. niveum* and *L. seditiosum* are particularly endangered. These trees have a significantly decreased photosynthetic rate.
- Of other species, the presence of *Phellinus pini* should be noted. This species causes centre rot of old pine trees, with the lower and central part of the tree being affected most. In the area of Avala, it was found on several felled trees but has had no economic significance so far. However, with the increasing age of trees, greater damage can be expected.
- *H. annosum* was recorded in the area of Avala on the lateral veins of one fallen tree in compartment 23 (section d). This species poses practical problems in the establishment and maintenance of Scots pine cultures but it is not of great significance for Austrian pine.

- Species that occur on cones and seeds (*A. alternata*, *B. cinerea*, *E. purpurascens*, *F. moniliforme*, and *T. roseum*) can cause significant damage only if the cones and seeds are improperly collected and transported, or improperly stored.
- Other species develop mainly as saprophytes or rarely as facultative parasites. They are not significant as pathogens of Austrian pine.

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Rezime

Istraživanja najvažnijih mikoza crnog bora na području Avale vršena su tokom 2018. i 2019. godine u 5 odeljenja i 11 odseka. Konstatovano je prisustvo 18 vrsta gljiva. Na četinama je konstatovano prisustvo 6 gljiva, na četinama i semenu 5, na korenu i pridanku 2, na šišaricama 1, deblu i granama 3 i u sprovodnim sudovima 1 vrsta.

Od svih vrsta najveće štete pričinjavaju *Sphaeropsis sapinea* i vrste roda *Armillaria*. Jak napad *S. sapinea* zabeležen je u većini odeljenja na području Avale. Inficirane su mlade četinama skoro svim izbojcima. Vrste roda *Armillaria* konstatovane su na većini stabala u fazi sušenja, kao i na već osušenim stablima crnog bora. Na nekim stablima su konstatovane zajedničke infekcije gljivama *S. sapinea*, *C. niveum* i *L. seditiosum*. Ova stabla imaju znatno redukovan fotosintetski potencijal. *H. annosum* predstavlja praktičan problem u podizanju i održavanju kultura belog bora i nema većeg značaja za crni bor.

Od ostalih vrsta treba ukazati na prisustvo gljive *Phellinus pini*. Ova vrsta za sada nema ekonomski značaj. Međutim, sa starošću stabala mogu se očekivati i veće štete. Vrste koje se javljaju na šišaricama i semenu mogu izazvati značajnije štete samo ako se šišarice i seme nepravilno sakupljaju i transportuju, ili nepravilno skladište. Ostale vrste se razvijaju uglavnom kao saprofiti ili retko kao fakultativni paraziti. Nisu značajni kao uzročnici bolesti na crnom boru.

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Summary

The research of the most important Austrian pine mycoses in the area of Avala was performed during 2018 and 2019 in 5 compartments and 11 sections. The presence of 18 species of fungi was determined. Out of that number, 6 fungi were found on needles, 5 on needles and seeds, 2 on roots and stem bases, 1 on cones, 3 on trunks and branches and 1 species in vascular bundles.

Of all the species, the greatest damage is caused by *Sphaeropsis sapinea* and species of the genus *Armillaria*. A severe attack of *S. sapinea* was recorded in most compartments in

the area of Avala. Young needles of almost all shoots were infected. Species of the *Armillaria* genus were found on most trees in the decaying phase, as well as on dead Austrian pine trees. Joint infections of *S. sapinea*, *C. niveum*, and *L. seditiosum* were found on some trees. These trees have a significantly decreased photosynthetic rate. *H. annosum* poses practical problems in the establishment and maintenance of Scots pine cultures but it is not of great significance for Austrian pine.

Other species whose presence should be noted include *Phellinus pini*. This species has had no economic significance so far. However, with the age of trees, greater damage can be expected. Species that occur on cones and seeds can cause significant damage only if the cones and seeds are improperly collected and transported, or improperly stored. Other species develop mainly as saprophytes or rarely as facultative parasites. They are not significant as pathogens of Austrian pine.