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The Most Important Parasitic and Saprophytic Fungi on Flowering Ash (*Fraxinus ornus*) in Parks of Serbia and Montenegro

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ABSTRACT

In order to fulfil the gap in domestic literature about biotic causes of flowering ash (*Fraxinus ornus*) decline in urban conditions, this paper presents the results of a five-year investigation of the most important parasitic and saprophytic fungi on this tree species in parks of Serbia and Montenegro. In total, 21 fungal taxa were recorded. Within recorded taxa, 2 taxa were found on leaves, 1 taxon was found on root, thin branches and bark, while 16 taxa were found on the trunk. On leaves *Ascochyta* spp. was recorded. The most significant fungi were *Armillaria mellea*, *Phellinus igniarius* aff. and *Inonotus hispidus*. Species *Meripilus giganteus* and *Schizophyllum commune* were the main successive fungi and were often found on substrate damaged by the most significant fungi or abiotic disorders. Species *Hymenoscyphus fraxineus* was recorded on single trees, as well as taxon *Neonectria* spp. The majority of recorded taxa, including invasive pathogen *Hymenoscyphus fraxineus* were for the first time found on flowering ash (*Fraxinus ornus*) in Serbia and Montenegro, especially in urban conditions of these countries. Better protection strategies of flowering ash (*Fraxinus ornus*) in this part of the region could be achieved based on these results.

Keywords: decline of park trees; manna ash; mycoses; distribution

INTRODUCTION

Genus ash (*Fraxinus*) contains about 60 species of broadleaved trees and shrubs and it is divided into 7 subsections (Cvjetićanin et al. 2016). Flowering ash (*Fraxinus ornus*) belongs to subsection *Ornus* and represents its typical species with flowers terminally or laterally in paniculate inflorescences on young twigs (Cvjetićanin et al. 2016). The species is autochthonous and a heliophyte; it belongs to sub-Mediterranean flora, grows in thermophilic oak forests, thermophilic bushes and occurs on dry, shallow soils (Cvjetićanin et al. 2016, Šeho et al. 2019). Also, flowering ash (*Fraxinus ornus*) is a bio-meliorative and decorative species (Jovanović 2007).

In domestic literature, there is almost no data about fungal causes of decline for this ecologically important tree species. Also, research that investigates mycoflora of this tree species is rare even in foreign literature (Farr and Rossman 2016). So far, new research about the occurrence of parasitic and saprophytic species on genus ash (*Fraxinus*) in this part of the region has mostly been directed to common ash (*Fraxinus excelsior* L.) (Vemić and Milenković 2018, Karadžić et al. 2019, Vemić 2020) and narrow-leaved ash (*Fraxinus angustifolia* Vahl) (Keča et al. 2017, Karadžić et al. 2019). Besides multiple benefits of flowering ash (*Fraxinus ornus*), there is a need for investigation of its mycoses because the other two autochthonous ash species in Europe are endangered by fungus *Hymenoscyphus fraxineus* (Kowalski) Baral, Queloz & Hosoya (Bakys et al. 2009, Gross et al. 2014), which causes greater ecological pressure to this tree species.

The main purpose of the research was to identify the most important parasitic and saprophytic species associated with the decline of flowering ash (*Fraxinus ornus*) trees in parks of Serbia and Montenegro. This way, knowledge about the decline of flowering ash (*Fraxinus ornus*) in urban areas is gained and can serve later as a basis for investigation of this tree species' mortality in natural

stands, especially due to climate change or intensive urbanization. Obtained results enable creating new or the improvement of the existing protection strategies for flowering ash (*Fraxinus ornus*) in this part of the region based on knowledge about fungal diversity colonizing this tree species.

MATERIALS AND METHODS

Field Methods

Field methods included the examination of terrain and taking samples for laboratory analyses. Terrain examination and taking samples was performed in the period of 2017-2022, 2-3 times a year in a range of 3 months. Terrain examination covered all larger cities in Serbia and Montenegro in search for flowering ash (*Fraxinus ornus*) trees. Special emphasis was put on the cities of Belgrade, Danilovgrad and Cetinje due to a significant number of flowering ash (*Fraxinus ornus*) trees. All trees with visible changes that can resemble mycosis symptoms or on whom visible fruit bodies of fungi were found were used as a sample for determination of fungi. Samples of symptomatic tissues including leaves, bark and branches were taken from symptomatic trees. Optionally, from these trees, fragments of wood or fruit bodies of macrofungi were collected. For taking samples from trees, a knife sterilized in 96% alcohol was used.

Laboratory Methods

Laboratory methods included determination of fungal species from the collected material. Conventional methods of identification based on morphological characteristics were used for the identification of fungi. Observation of fungal morphology was under enlargement of 400x using Am Scope B120 C E1 microscope. Preparation of temporary histological sections and isolation of mycelium from symptomatic tissues for microscopic analyses were conducted according to Muntanola-Cvetković (1990).

For identification of microfungi, descriptions by Ellis and Ellis (1985), Mel'nik (2000) and Kowalski (2006) were used. Some macrofungi were identified based on the morphology of carpophores according to Karadžić (2010). Microscopic identification of macrofungi was made using descriptions by Nobles (1948, 1965) and Stalpers (1978).

RESULTS

Diversity of recorded fungi on flowering ash (*Fraxinus ornus*) trees in Serbia and Montenegro is presented in Table 1.

On flowering ash (*Fraxinus ornus*) trees 21 taxa of parasitic and saprophytic fungi were recorded (Table 1). Whereby, 2 taxa were recorded on leaves, 1 taxon was recorded on root, thin branches and bark, while the remaining 16 taxa of fungi were recorded on the trunk. The most significant fungal taxa found on flowering ash (*Fraxinus ornus*) in parks of Serbia and Montenegro were *Armillaria mellea*, *Phellinus igniarius* aff. and *Inonotus hispidus* (Table 1, Figure 1, Figure 2). The other taxa that colonized the trunk represented successive species, mostly occurring on trees previously affected with the most significant fungi. The most significant was *Schizophyllum commune* and somewhat less significant was *Meripilus giganteus* of these successive species. Succession of fungal occurrence was also expressed on trees damaged by abiotic disorders. Table 2 shows the occurrence of successive fungi according to the condition of substrate.

All taxa of fungi were found in both investigated countries except the species *Hymenoscyphus fraxineus*, which was found only in Montenegro. *Hymenoscyphus fraxineus* was recorded only on one tree and it was isolated from necrotic leaf rachis (Figure 3). Also, necrosis was visible on one young green twig, but it was not isolated (Figure 3). Taxon *Neonectria* spp. caused more damage (Figure 3). Based on symptoms and laboratory analyses the fungus was identified up to the genus level.

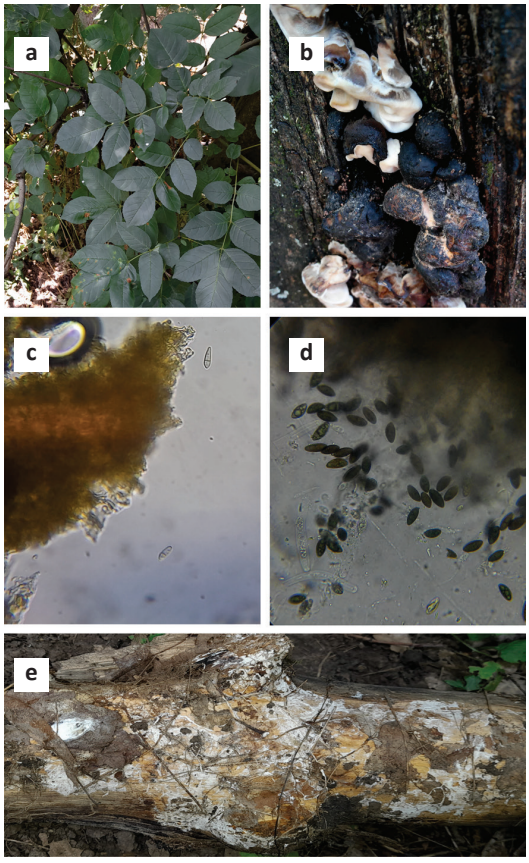

Figure 1. Fungi recorded on flowering ash (*Fraxinus ornus*): **(a)** *Ascochyta* spp. Symptoms; **(b)** *Bjerkandera adusta* and *Hypoxylon* spp. (old fruit body); **(c)** *Ascochyta* spp. part of pycnidium and conidia (pycnospores); **(d)** - *Hypoxylon* spp. ascospores; **(e)** *Armillaria mellea*.

Table 1. Fungi recorded on flowering ash (*Fraxinus ornus*) in Serbia and Montenegro.

Taxon of fungus	Part of tree	Recorded morphological characteristics
Ascochyta spp.	Leaves	Pycnidia 120 µm, epiphyllous, light brown Conidia hyaline, septate, ellipsoid, ends round, 6-10 x 2-3 µm
Armillaria mellea (Vahl. ex Fr.) Kummer	Root	Pileus 2-11.5 cm, stipe 5-1.5 cm, anulus 3-9 mm, hymenophore lamelloid Basidia 30 x 6.5 µm, cystidia 15 x 8 µm, spores hyaline 7 x 6.5 µm, Marginal and aerial hyphae 1-4 µm
Bjerkandera adusta (Willd.) P. Karst.	Trunk	Basidiocarps 3-4 cm, grey, hymenophore poroid Basidia 9-13 x 2-3 µm, Cystidia absent, Spores hyaline 4-5 x 3 µm, Marginal hyphae 2-5 µm, Aerial hyphae 2-5 µm
Botrytis cinerea Pers.	Little twigs	Conidiophores hyaline, spherically, up to 2 mm Conidia in clusters, hyaline, round 8-14 x 7-9 µm
Exidia spp.	Trunk	Basidiocarps 5-50 cm, black, soft, resupinate, hymenophore poroid Basidia septate, 16 x 9 µm, Cystidia absent, Spores hyaline 14 x 5 µm
Fomes fomentarius (L.) Fr.	Trunk	Basidiocarps 10-50 cm, grey, zonate, hard, hymenophore poroid Basidia 20-30 x 10 µm, Cystidia absent, Spores hyaline 19 x 6-7 µm, Marginal hyphae 1.5-5 µm, Aerial hyphae 2-3 µm
Ganoderma applanatum (Pers.) Pat.	Trunk	Basidiocarps 20-40 cm, dark brown, upper surface dull, hard, hymenophore poroid, dark zones between layers Basidia 11-15 x 6-8 µm, Cystidia absent, Basidiospores 7-9 x 5-6 µm, brown, Marginal hyphae 2-9 µm, Aerial hyphae 1-2 µm
Hymenoscyphus fraxineus Baral, Queloz, Hosoya	Leaves	Apothecia were not recorded. Phialides dark, up to 24 µm, phialoconidia 3-4 x 2-2.5 µm, first forming conidium was clavate 7 x 2.5 µm
Hypoxylon spp.	Trunk	Stromata grey, black when old, hemispherical. Ascospores dark brown, 15 x 7 µm
Ischnoderma spp.	Trunk	Basidiocarps 12-15 cm, fleshy, concentric, brown and black Basidia 12-18 x 6 µm, Cistidia absent, Spores hyaline 6 x 2 µm, Marginal and aerial hyphae 2-5 µm
Inonotus hispidus (Bull.) Karst.	Trunk	Basidiocarps 10-16 cm, hirsute, reddish-orange to reddish-black, hymenophore poroid, yellowish-brown Basidia 20-27 x 9-11 µm, Cistidia absent, Setae 24 x 8 µm, Spores brown 8-10 x 6-8 µm, Marginal hyphae 1-9 µm
Meripilus giganteus (Pers.) P.Karst	Trunk	Basidiocarps 6-30 cm, circular, young fleshy and hard when old, grey to ochraceous Basidia 25-40 x 7-8 µm, Cistidia 20-40 x 6-8 µm, Spores hyaline 6-7 x 5-6 µm, Marginal and aerial hyphae 2.5-7 µm
Neonectria spp.	Bark	Stromata red, perithecia 300 µm, spores hyaline, septate 9-18 x 4-7 µm
Omphalotus olearius (DC.) Singer	Trunk	Basidiocarps flashy, yellow to orange, pileus 5-10 cm, stipe 4-10 x 1-3 cm, anulus absent, Spores hyaline 5-7 x 5-7 µm
Polyporus squamosus (Huds.) Fr.	Trunk	Basidiocarps 10-30 cm, laterally stipitate, reniform and circular, azonate, flaky, hymenophore poroid, white Basidia 40-70 x 9-12 µm, Cystidioles 20-35 x 6-8 µm, Spores hyaline 14-17 x 5-6 µm, Marginal hyphae 2-6 µm, Aerial hyphae 3-6 µm
Pleurotus spp.	Trunk	Basidiocarps 9-10 cm, pileus brown-grey, soft, hymenophore lamelloid Basidia 50 x 8 µm, Cystidia absent, Spores hyaline 10 x 4 µm
Phellinus igniarius aff.	Trunk	Basidiocarps 8-20 cm, sessile, grey, hard, margin concolorous, hymenophore porid, pale brown Basidia 9-13 x 6-7 µm, Cistidia absent, Setae 14-17 x 4-6 µm, Spores hyaline 5-6.5 x 4-6 µm, Marginal hyphae 2-6 µm, Aerial hyphae 1-3 µm
Schizophyllum commune Fr.	Trunk	Basidiocarps 3-5 cm, semi-sessile, shelly, curved rim, grey, hirsute, hymenophore lamelloid, brown Basidia 40-55 x 7-10 µm, Cistidia absent, Spores hyaline 6-7 x 2 µm, Marginal hyphae 2-4 µm, Aerial hyphae 1.5-6 µm
Stereum hirsutum (Willd.) Pers.	Trunk	Basidiocarps resupinate or semi-resupinate, zonate, ash grey, slightly hirsute, hymenophore poroid, yellow Basidia 30-45 x 3.5-4.5 µm, Cistidia absent, Spores hyaline 6 x 2.5 µm
Trametes hirsuta (Wulf.) Pil.	Trunk	Basidiocarps 4-7 cm, effused, hirsute, white or light grey, zonate, hymenophore poroid, white or grey Basidia 15-22 x 5-7 µm, Cystidioles 12-18 x 3-5 µm, Spores hyaline 6-9 x 2-2.5 µm, Marginal hyphae 2-4 µm, Aerial hyphae 3-9 µm
Trametes versicolor (L.) Lloyd	Trunk	Basidiocarps 4-7 cm, sessile, in clusters, concentric, motley, hymenophore poroid, pores angular Basidia 15-20 x 5-6, Cistidia absent, Spores hyaline 5-6 x 2 µm, Marginal hyphae 3-4 µm, Aerial hyphae 2-3 µm

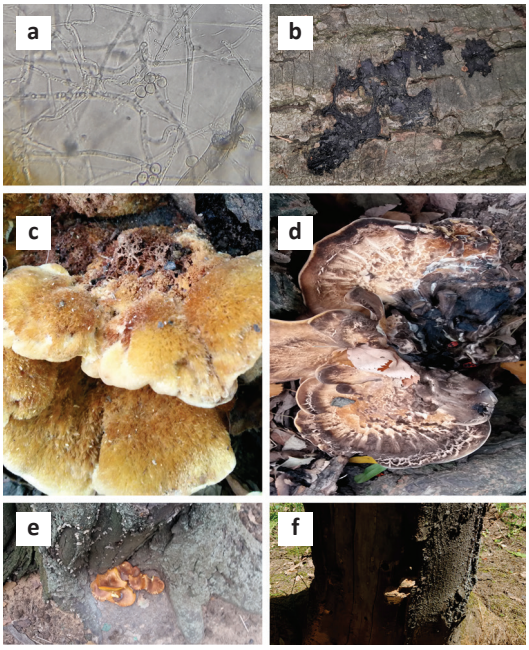

Figure 2. Fungi recorded on flowering ash (*Fraxinus ornus*): **(a)** *Botrytis cinerea*; **(b)** *Exidia* spp.; **(c)** *Inonotus hispidus*; **(d)** *Meripilus giganteus*; **(e)** *Omphalotus olearius* and *Schizophyllum commune*; **(f)** *Phellinus igniarius* aff.

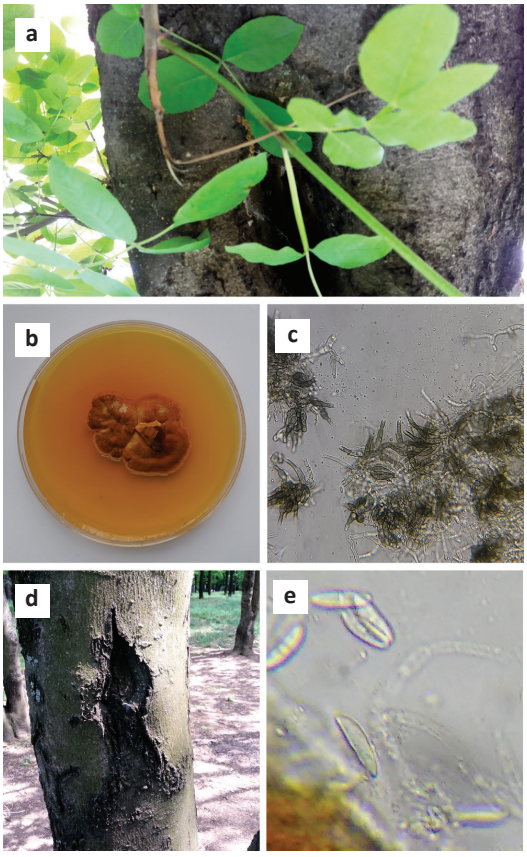

Figure 3. Fungi recorded on flowering ash (*Fraxinus ornus*): **(a)** *Hymenoscyphus fraxineus* symptoms; **(b)** *Hymenoscyphus fraxineus* culture; **(c)** *Hymenoscyphus fraxineus* phialides and phialoconidia; **(d)** *Neonectria* spp. symptoms; **(e)** *Neonectria* spp. ascospores (mature and immature).

Table 2. Occurrence of successive fungi according to the condition of substrate.

Damaging cause	Condition of substrate	Recorded fungal taxon
Snow	Breakage	*Exidia* spp.
		Pleurotus spp.
Wind		*Schizophyllum commune*
		Stereum hirsutum
Unknown	Stumps	*Ischnoderma* spp.
		Meripilus giganteus
		Omphalotus olearius

DISCUSSION

This research showed in detail the diversity of parasitic and saprophytic fungi on flowering ash (*Fraxinus ornus*) trees in urban conditions of Serbia and Montenegro. The majority of found taxa were recorded for the first time on flowering ash (*Fraxinus ornus*) in this part of the region. Further molecular analyses are needed to closely identify and confirm all recorded fungal taxa in this study, particularly *Ascomycota* and *Basidiomycota* fungi within certain species complexes or those fungi that were impossible to identify to the species level based on morphological characteristics due to old, dry and damaged fruit bodies.

Problems regarding the decline of trees species from genus *Fraxinus* in Europe culminated through the occurrence of fungus *Hymenoscyphus fraxineus*. Flowering

ash (*Fraxinus ornus*) can also be affected with this fungus (Kirisits and Schwanda 2015), although damaging consequences are much less frequent because the fungus is limited to leaves and surrounding tissues (Kirisits 2017). Confirmed presence of fungus *Hymenoscyphus fraxineus* on flowering ash (*Fraxinus ornus*) in this part of the region has great importance for understanding the distribution and ecology of this pathogen.

Considering that fungus *Hymenoscyphus fraxineus* causes the decline of leaves on flowering ash (*Fraxinus ornus*) (Kirisits 2017), being familiar with the diversity of other fungi occurring on them has significant importance in defining decline progress. Spots on the leaves of flowering ash (*Fraxinus ornus*) in this part of the region were possibly caused by *Ascochyta* spp. The presence of *Venturia fraxini* Aderh. which also has two-celled spores, but which are bigger and light brown instead of hyaline spores of *Ascochyta* spp. (Ellis and Ellis 1985), has not been confirmed for now. Also, fruit bodies of *Venturia fraxini* are perithecia (pseudothecia), unlike pycnidia of *Ascochyta* spp., and only the anamorph of this fungus colonizes living leaves (Ellis and Ellis 1985). Fungus *Venturia orni* Ibrahim, Schlegel & Sieber morphologically similar to *Venturia fraxini* (Ibrahim et al. 2016) also was not found. This points to further investigation of fungal diversity in natural stands in order to confirm the presence of these species, especially using molecular methods. It is assumed that air pollution in urban areas influenced the absence or markedly reduced presence of these species considering susceptibility of leaf pathogens to the external environment (Kowalski 2013).

Bark necrosis and further damages as a consequence of their development have great significance in urban conditions in reducing ornamental value of trees (Tello et al. 2005). This research showed the presence of *Neonectria* spp. on the bark of flowering ash (*Fraxinus ornus*) trees. Difficulties in sampling tissues due to tree cutting and age of necrosis excluded more detailed analyses. Previous research demonstrated that species *Neonectria punicea* (J.C.) Schmidt Castl. & Rossman caused bark necrosis on common ash (*Fraxinus excelsior*) in cases where bark had been previously damaged (Karadžić et al. 2020). Since bark damages in urban conditions are frequent due to different causes, it is assumed that this species was also present on flowering ash (*Fraxinus ornus*) trees in parks of Serbia and Montenegro.

Decay of trees also has great importance in urban conditions (Tello et al. 2005). However, due to their different bioecological characteristics in urban conditions, lignicolous fungi that cause heart rot and have hard fruit bodies are less frequently distributed (Vasaitis 2013). This theory was confirmed by this research, but also, at some level, there were exceptions from this rule. This can be explained due to the proximity of typical forest ecosystems near the investigated park trees.

Finally, pathogenicity tests are recommended for certain *Ascomycota* fungi recorded in this study, primarily *Ascochyta* spp. and *Neonectria* spp. to evaluate their role in flowering ash (*Fraxinus ornus*) trees' decline in urban conditions.

The obtained results enable adequate protection strategies in parks with flowering ash (*Fraxinus ornus*) trees in this part of the region. Identification of the most important fungal species associated with diseases of trees potentiate taking such protection measures that will decrease or eliminate their presence.

CONCLUSIONS

This study identified 21 taxa of parasitic and saprophytic fungi on flowering ash (*Fraxinus ornus*) trees in parks of Serbia and Montenegro. Results and conclusions that follow from them can be presented as following:

- On flowering ash (Fraxinus ornus) trees 21 taxa of parasitic and saprophytic fungi were recorded. On leaves 2 taxa were found, 1 taxon was found on the root, thin branches and bark, while 16 taxa were found on the trunk. The majority of taxa were found for the first time on flowering ash (Fraxinus ornus) trees in Serbia and Montenegro, especially in urban areas.
- Invasive pathogen *Hymenoscyphus fraxineus* was for the first time recorded on flowering ash (*Fraxinus ornus*) in Montenegro. This represents one of the southernmost findings of this fungus and has great significance in studying ecology of this fungus. It is considered that *Hymenoscyphus fraxineus* is also present on flowering ash (*Fraxinus ornus*) in Serbia due to more favorable ecological conditions for this fungus.
- The most significant species were *Armillaria mellea*, *Phellinus igniarius* aff. and *Inonotus hispidus*. The other taxa occurred less frequently or successively, causing smaller damages to trees.
- Recommended protection strategies are focused on regulating a mixture of tree species in parks and local forest stands as well as lowering damages of trees because many fungal species found in this research also colonize other tree hosts.

Author Contributions

Author AV designed the research, performed field investigations, laboratory analyses and wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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