Vemić, A. (2022). The most important fungi on wych elm (Ulmus glabra) trees in Montenegro. Agriculture and Forestry, 68 (3): 71-82. doi:10.17707/AgricultForest.68.3.05

DOI: 10.17707/AgricultForest.68.3.05

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THE MOST IMPORTANT FUNGI ON WYCH ELM (*ULMUS GLABRA*) TREES IN MONTENEGRO

SUMMARY

This paper contains results of investigation of the most important fungi found on wych elm (*Ulmus glabra*) trees in Montenegro. During the monitoring, 21 fungal species were found on wych elm (*Ulmus glabra*) in Montenegro. Fungus *Ophiostoma novo-ulmi* was the most frequent on young trees while species *Fomes fomentarius* and *Daldinia concentrica* were the most frequent on old trees. Fungi *Mycosphaerella ulmi* and *Stegophora ulmea* were the most common species on leaves. Majority of fungi in this study was for the first time recorded on wych elm (*Ulmus glabra*) in Montenegro. Obtained results will contribute to knowing about fungi associated with Dutch elm disease as well as fungi colonizing healthy trees left to spontaneous growth.

Keywords: *Ulmus glabra*, fungi, occurrence, distribution, trees decline

INTRODUCTION

Elm genus contains about 35 species of trees and shrubs (Cvjetićanin *et al*. 2016). In domestic forests, wych elm (*Ulmus glabra* Huds.) trees represent autochthonal species and often occur as admixed species in beech (*Fagus sylvatica*) forests (Tomić, 2004; Cvjetićanin *et al*., 2016).

Species from elm (*Ulmus* spp.) genus have great ecological importance that is even bigger because they are endangered across areal by Dutch elm disease. Rare localities like "Biogradska gora" National Park have forests with fully developed trees that avoided decline due to Dutch elm disease. These localities are from especially interest in discovering pathogenic fungi that colonize trees more tolerant to Dutch elm disease.

Early investigations of fungal species on genus *Ulmus* across the world were relatively rare. In his publication Saccardo (1898) described above 50 fungal species on genus *Ulmus* worldwide. Later, (Groove, 1935; Groove, 1937) recorded nearly 30 fungal taxa within this genus. On western *Ulmus* trees, Ellis and Ellis (1985) described above 30 fungal species while Phillips and Burdekin (1992) listed about 17 fungal species. In summary, early researches about fungal

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Recieved:30/04/2022 *Accepted:04/09/2022*

diversity on wych elm (*Ulmus glabra*) were rare, and besides Saccardo (1898) none of above literature sources precisely described the diversity of fungi on specific tree species from genus *Ulmus*. Species wych elm (*Ulmus glabra*) is from especial importance as widely distributed in Europe and one of the most endangered from Dutch elm disease. Saccardo (1898) reported 15 fungal species and Lanier *et al*. (1976) reported 5 fungal species on wych elm (*Ulmus glabra*) trees. Brasier (1981) reported 6 main fungi causing cancer disease in wych elm (*Ulmus glabra*) worldwide and 1 species causing tar spots on leaves. Also, some of these fungi, respectively species *Phomopsis oblonga* (Desm.) Trav. has been proved to be antagonistic against Dutch elm disease (Webber and Gibbs, 1984).

Recent investigations of the most important fungi on wych elm (*Ulmus glabra*) were still relatively rare. Typical plant pathology literature sources (Horst, 2013) listed the same fungal species as early researches. New researches were primarily focused on endophytic species and associated with gall-making insects (Kowalski, 2004). However, Medarević *et al*., (2011) found 10 fungal species on single wych elm (*Ulmus glabra*) tree in Goč nature reserve in Serbia. Also, new studies of fungi on wych elm (*Ulmus glabra*) trees were focused on finding of highly pathogenic species like *Botryodiplodia hypodermia* (Ellis & Everh.) Buisman outside their natural range (Bartnik *et al*., 2018). Recent investigations about fungal species on wych elm (Ulmus glabra) reported *Ophiostoma novo-ulmi* Brasier in "Biogradska gora" National Park as locality previously without Dutch elm disease (Vemić, 2022).

Pathogens of wych elm (*Ulmus glabra*) in Montenegro are not much investigated. Knowledge about fungal species colonizing wych elm (*Ulmus glabra*) trees in this part of their areal can help in understanding patterns of decline before or after Dutch elm disease as main disease of elm trees. Also, knowledge about fungal pathogens of wych elm (*Ulmus glabra*) trees will help in screening trees that are more tolerant to Dutch elm disease and other pathogens at the same time for breeding programs. Another theoretical importance of these results is to study mycological complex on wych elm (*Ulmus glabra*) in this part of its areal.

Practical application of these results on global scale is to avoid production of plant material tolerant to Dutch elm disease but susceptible to other fungal pathogens. Also, on local scale practical application of obtained results is to prevent different fungal diseases in places where "disease escape" of *Ophiostoma novo-ulmi* is present or potentially produced seedlings tolerant to Dutch elm disease will be planted. Aims of this research were to investigate diversity of the most important fungi on wych elm and classify their role in decline progress through the number of first reports and distribution on different wych elm substrates in Montenegro. Tested null hypotheses were: a) Majority of recorded species aren't for the first time found on wych elm (*Ulmus glabra*) trees in Montenegro; b) Fungal species don't occur in succession on infected wych elm (*Ulmus glabra*) trees in Montenegro; c) There isn't difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in

Montenegro. In forward, results about investigating the pathogenic fungal complex of wych elm (*Ulmus glabra*) trees in Montenegrin forests were showed. Some saprophytic fungal species also were showed because their succession was associated with investigated parasitic fungi.

MATERIAL AND METHODS

Field researches were performed in wide range of different localities in Montenegro, including all managed forests and National Parks "Durmitor" and "Biogradska Gora". Field research included monitoring of health condition of wych elm (*Ulmus glabra*) trees whereby the occurrence of symptoms and fruit bodies of fungi were observed. Monitoring was performed during the period of 2017-2019 years, three times in each year. Trees in all stages of development were examined.

Samples from symptomatic trees were collected for laboratory identification of fungal species. Some fungal species, primarily macrofungi were identified directly in field based on description Karadžić (2010) and Hagara (2014).

Figure 1. A – Investigated localities in Montenegro (*Google Earth*), B – Isolated fungi on MEA media, ready for identification and preservation on agar slants

Laboratory identification of species was performed based on preparing temporary histological sections for microfungi or isolation and identification of pure cultures for macrofungi (Figure 1). Preparation of histological sections, isolation and identification of fungi was according Muntanola Cvetković (1990). Malt extract agar media (MEA, LAB M UK) was used for the isolation of fungi (Figure 1). Media was prepared according to manufacturer's protocol.

Identification of macrofungi through the characteristics of pure cultures was performed according to descriptions Nobles (1948; 1965) and Stalpers (1978). Identification of microfungi was performed through the characteristics of

microstructures based on descriptions Dennis (1978); Sutton (1980) and Ellis and Ellis (1985).

RESULTS AND DISCUSSION

Investigation revealed 21 fungal species on wych elm (*Ulmus glabra*) in Montenegro (Table 1).

Table 1. Fungi found on wych elm in Montenegro

Fungal species	Type of disease	Colonized part of tree
***Armillaria mellea* (Vahl. Ex Fr.) P. Kummer**	Root rot	Root
***Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn ex Ryvarden**	Heart rot	Trunk
***Bjerkandera adusta* (Willd.) P. Karst.**	Sap rot	Trunk
***Daldinia concentrica* (Bolton) Ces. & De Not.**	Sap rot	Trunk
***Hypoxylon multiforme* (Fr.) Fr.**	Sap rot	Trunk, Branches
***Fomes fomentarius* (L.) Fr.**	Heart rot	Trunk
***Fomitopsis pinicola* (Sw.) P. Karst.**	Heart rot	Trunk
***Ganoderma applanatum* (Pers.) Pat.**	Heart rot	Trunk
***Hypoxylon rubiginosum* (Pers.) Fr.**	Sap rot	Trunk
***Irpex lacteus* (Fr.) Fr.**	Sap rot	Trunk
***Ischnoderma resinosum* (Schrad.) P. Karst.**	Sap rot	Trunk
***Ophiostoma novo-ulmi* Brasier**	Wilt	Trunk, Branches, Root
***Phylloporia ribis* (Schumach.) Ryvarden**	Heart rot	Trunk
***Polyporus squamosus* (Huds.) Fr.**	Heart rot	Trunk
***Mycosphaerella ulmi* Klebahn**	Leaf spot	Leaves
***Pleurotus cornucopie* (Paulet) Rolland**	-	Trunk, Fallen branches
***Stegophora ulmea* (Schwein.) P. Syd. & Syd.**	Leaf spot	Leaves
***Stereum hirsutum* (Willd.) Pers.**	Sap rot	Trunk, Fallen branches
Trametes gibbosa (Pers.) Fr.	Heart rot	Trunk
***Xylaria polymorpha* (Pers.) Grev.**	Sap rot	Trunk
***Schizophyllum commune* Fr.**	Sap rot	Trunk, Stumps

On wych elm (*Ulmus glabra*) trees, 21 fungal species was found, where by 1 fungal species was found on rot, 2 fungal species were found on leaves, 18 species were found on trunk and 3 species were found simultaneously on trunk and fallen branches (Table 1). The most common species were *Armillaria mellea*, *Daldinia concentrica*, *Fomes fomentarius*, *Ganoderma applanatum*, *Polyporus squamosus*, *Mycosphaerella ulmi*, *Ophiostoma novo-ulmi*, *Pleurotus cornucopie* and *Stegophora ulmea*. Species *Ophiostoma novo-ulmi* was the most common, distributed over entire areal of wych elm (*Ulmus glabra*) in Montenegro. The most important species are *Ophiostoma novo-ulmi* and heart rot fungi. Besides *Ophiostoma novo-ulmi* sa the most important pathogen, heart rot fungi were major pathogens on older trees that avoided Dutch elm disease (Table 1).

Some representative examples of found fungal species on wych elm's (*Ulmus glabra*) trunk in Montenegro are showed in Figure 2, Figure 3, Figure 4 and Figure 5.

Figure 2. Some fungi found on wych elm: A – *Armillaria mellea*; B – *Daldinia concentrica*; C – *Bjerkandera adusta*

Figure 3. Some fungi found on wych elm: A – *Hypoxylon multiforme*; B – *Fomes fomentarius*; C – *Fomitopsis pinicola*

Figure 4. Some fungi found on wych elm: A – *Ganoderma applanatum*; B – *Hypoxylon rubiginosum* and *Irpex lacteus*; C – *Ischnoderma resinosum*; D – *Phylloporia ribis*; E – *Polyporus squamosus*; F – *Trametes gibbosa*

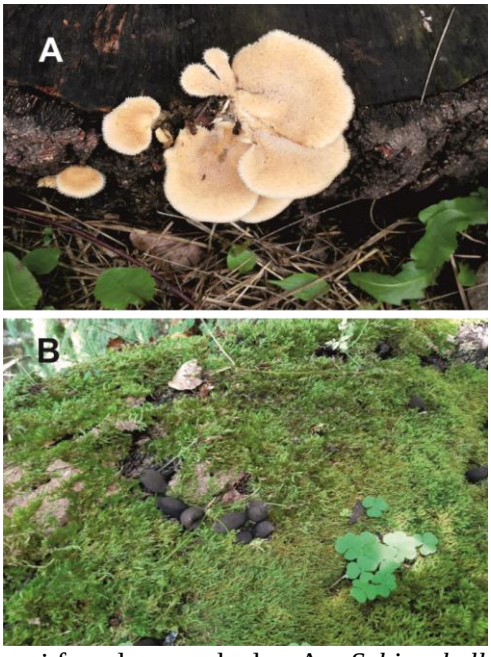

Figure 5. Some fungi found on wych elm: A – *Schizophyllum commune*; B – *Xylaria polymorpha*

On the other side, fungi occurring on leaves causing spots or occurring on entire habitus of trees are showed in Figure 6. All species were found on the trees of different categories of development.

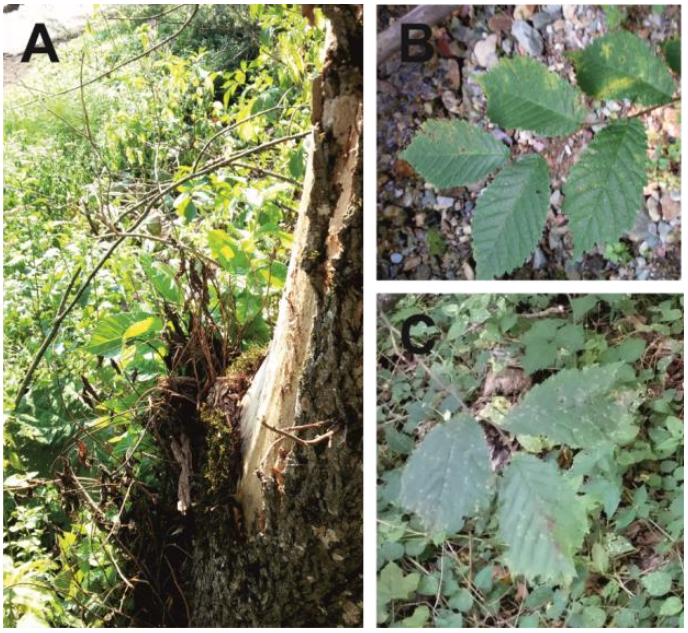

Figure 6. Some fungi found on wych elm: A – *Ophiostoma novo-ulmi*; B – *Mycosphaerella ulmi*; *Stegophora ulmea*

From the investigated fungal species, 12 species were found for the first time on wych elm in Montenegro (Table 2).

Table 2. Fungi found for the first time on wych elm in Montenegro

Fungal species	Type of disease	Colonized part of tree
***Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn ex Ryvarden**	Heart rot	Trunk
***Bjerkandera adusta* (Willd.) P. Karst.**	Sap rot	Trunk
***Daldinia concentrica* (Bolton) Ces. & De Not.**	Sap rot	Trunk
***Hypoxylon multiforme* (Fr.) Fr.**	Sap rot	Trunk, Branches
***Hypoxylon rubiginosum* (Pers.) Fr.**	Sap rot	Trunk
***Irpex lacteus* (Fr.) Fr.**	Sap rot	Trunk
***Ischnoderma resinosum* (Schrad.) P. Karst.**	Sap rot	Trunk
***Phylloporia ribis* (Schumach.) Ryvarden**	Heart rot	Trunk
***Mycosphaerella ulmi* Klebahn**	Leaf spot	Leaves
***Stegophora ulmea* (Schwein.) P. Syd. & Syd.**	Leaf spot	Leaves
Trametes gibbosa (Pers.) Fr.	Heart rot	Trunk
***Xylaria polymorpha* (Pers.) Grev.**	Sap rot	Trunk

Recorded fungi on wych elm (*Ulmus glabra*) revealed high presence of different species. This way, the first null hypothesis was rejected and alternative hypothesis that majority of recorded species are for the first time found on wych elm (*Ulmus glabra*) trees in Montenegro was accepted. Mycological complex of this tree species was significantly revealed in this part of wych elm (*Ulmus glabra*) areal.

Sap rot and heart rot fungi were detected. Sap rot and heart rot fungi have different survival strategies, sap rot fungi use "ruderal strategy", primarily are focused on fresh, injured substrate (Vasaitis, 2013). Rot characterized by investigated fungi was easily recognized through presence of their fruit bodies in advanced stages of decay (Figures 2-5). From Table 1 it can be seen that majority of heart rot fungi on wych elm (*Ulmus glabra*) follow Haddow-Etheridge concept (Vasaitis, 2013). This concept represents type of fungal heart rot development that is triggered by naturally aging of trees or other natural factors influencing tree vitality. Fungi *Fomes fomentarius*, *Fomitopsis pinocola*, *Polyporus squamosus* and *Ganoderma applanatum* colonize wide range of broadleaved hosts (Karadžić *et al*., 2016; Vemić and Milenković, 2018; Radulović *et al*., 2020; Karadžić *et al*., 2020). Trees that are in early stages or avoided Dutche elm disease had fungal infection associated either with their injury or aging based on these results. Heart rot fungi associated with Haddow-Etheridge concept are mostly host specified (Vasaitis, 2013). However, majority of found heart rot fungi, primarily species *Ganoderma applanatum*, *Fomes fomentarius*, *Fomitopsis pinocola* and *Polyporus squamosus* can be found on wide ranges of trees hosts (Hagara, 2014). This makes protection strategies difficult but more concrete in the same time due to knowledge about identified fungal species.

Also, symptoms on leaves were specific for fungi *Mycosphaerella ulmi* and *Stegophora ulmea* (Figure 6 B, C) and also served for identification in addition to their morphological characteristics. Symptoms of *Mycosphaerella ulmi* were typical red and brown spots on leaves, often mixed with chlorosis and discoloration of leaves (Figure 6 B). Symptoms of *Stegophora ulmea* were typical black tar spots, pale at the beginning and tyrning black with mature (Figure 6 C). Spots were typical for this species and identification is often possible just based on symptoms. In scientific literature it is unclear if this species is synonym for *Systrema ulmi* (Schleicher). In this research we consider this to be the same species based on symptoms occurring and morphological characteristics of fungi.

Symptoms from *Ophiostoma novo-ulmi* were characteristics for this species (Figure 6 A). One of the first symptoms was wilting of thin branches, which soon affected entire tree (Figure 6 A). Formal opinion is that wych elm (*Ulmus glabra*) is more infested by Dutch elm disease than the field elm based on early researches (Townsend, 1971; Brasier, 1977) as well as recent research about clonal resistance (Solla *et al*., 2005). However, experiences from foreign researches also showed that in some cases the field elm (*Ulmus minor*) trees can be more endangered by epidemic (Łakomy *et al*., 2016). During this research heavy wilting was recorded on both wych elm (*Ulmus glabra*) and the field elm

(*Ulmus minor* Mill.). In this point it was hard to evaluate which species declines faster in these ecological conditions because it is also impacted by different subtypes of *Ophiostoma novo-ulmi* present in this part of areal.

Recorded fungal species in this study were found on trees damaged due to abiotic factors, wilting caused by *Ophiostoma novo-ulmi* and decay caused by *Ganoderma applanatum*, *Fomes fomentarius*, *Fomitopsis pinocola* and *Polyporus squamosus*. Interestingly, some typical secondary colonizers *Auricularia auricula-judae* (Bull. ex St-Amans) Wettst and *Tremella mesenterica* Retz. ex Hook were not found in this investigation. These species are often found on declining broadleaf trees species in domestic forests (Radulović *et al*., 2022). These results rejected the second null hypotheses and accepted alternative hypothesis that fungi occur in succession on infected wych elm (*Ulmus glabra*) trees in Montenegro. Results revealed that trunk is the most endangered part of trees with majority of found fungi (Table 1, Table 2). These findings rejected the third null hypothesis that there isn't difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in Montenegro. Alternative hypothesis that there is difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in Montenegro was accepted.

Highly pathogenic species *Botryodiplodia hypodermia* was not found in this investigation. This fungus causes elm dieback and is potential factor endangering elms in Europe (Bartnik *et al*., 2022). Possible explanation is because *Botryodiplodia hypodermia* is much less prevalent in Europe than in North America (Bartnik *et al*., 2018). Also, forest stands and climate characteristics in Montenegro are somewhat different than in other geographically distant parts of Europe. However, it is possible that species was present and unsuccessful isolations were due to decrease of mycelium activity in tissues. Also, cancer symptoms that remind on this species were not detected during the investigation.

Montenegro is one of the rarest countries that have fully grown wych elm (*Ulmus glabra*) trees, left to spontaneous development without Dutch elm disease, enabling researches about other fungi associated with trees decline. Production of trees material that is more tolerant to Dutch elm disease and also doesn't have special susceptibility to other pathogens is more possible based on these results. Practically, on global scale, production of trees material that is more tolerant to mechanical damages that favorize development of sap rot fungi *Bjerkandera adusta*, *Daldinia concentrica*, *Hypoxylon multiforme*, *Hypoxylon rubiginosum*, *Irpex lacteus*, *Ischnoderma resinosum*, *Stereum hirsutum*, *Xylaria polymorpha* and *Schizophyllum commune* should be done. The usage of proveniences with thicker bark and more dense wood should be more intensive for breeding programs. In modern forestry genetic engineering is important factor in disease management (Edmonds, 2013). Also, wych elm (*Ulmus glabra*) as the other admixed species in beech forests requires different strategies and programs in order to achieve diversity of these forests (Schulze *et al*., 2016).

In domestic forests, integral protection is the most applicable protection method. Between everything, this means measures against Dutch elm disease should also be focused on other fungi. Because majority of found species are less host specified this means that protection strategies should be focused on other trees species occurring with wych elm (*Ulmus glabra*), primarily beech (*Fagus sylvatica* L.). Operations like thinning and pruning should be more intense in domestic beech forests to avoid transmission of inoculum. Also, the other forest operations should be performed more precisely to avoid damaging of trees and making entry points for sap rot fungi.

CONCLUSIONS

Prior to this research there were no detailed studies about fungal pathogens on wych elm (*Ulmus glabra*) in Montenegro. Based on performed research all conclusions can be pointed on next way:

On wych elm (*Ulmus glabra*) there were found 21 fungal species. Based on part of tree, 1 species was found on rot and base of tree, 2 species were found on leaves, 18 species were found on trunk and 3 species were found simultaneously on trunk, branches or root.

Majority of species occurs in succession. Often necrotic spots on leaves caused by *Mycosphaerella ulmi* and *Stegophora ulmi* (*Systrema ulmi*) leads to physiological stress in trees allowing *Armillaria mellea* and other rot fungi to develop active infections and cause decline of trees that avoided infection of *Ophiostoma novo-ulmi*.

Species *Mycosphaerella ulmi* and *Stegophora ulmi* (*Systrema ulmi*) were also found solitarily on leaves on youngest seedlings. Mortality of young wych elm (*Ulmus glabra*) specimens were recorded in some cases. In other cases, young wych elm (*Ulmus glabra*) continued development but it is still unclear about further health condition on this these trees.

Sap and heart rot fungi were the most diverse group of fungi colonizing wych elm (*Ulmus glabra*). Trees that had some form of resistance or "disease escape" from Dutch elm disease were severe damaged by these pathogens.

Species *Ophiostoma novo-ulmi* was still the most important pathogen of wych elm (*Ulmus glabra*) causing the wilting of trees across entire country.

Majority of fungal species, 12 species were found for the first time on wych elm (*Ulmus glabra*) in Montenegro. These results will contribute to knowledge about mycoflora of wych elm (*Ulmus glabra*) in Montenegro.

ACKNOWLEDGEMENT

Ministry of Education, Science and Technological Development, Republic of Serbia finances scientific work of University of Belgrade, Faculty of Forestry in 2022 year based on Contract about realization number: 451-03-9/2022-14/200169.

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