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OCCURRENCE AND DISTRIBUTION OF *OPHIOSTOMA NOVO-ULMI* IN "BIOGRADSKA GORA" NATIONAL PARK

SUMMARY

During the monitoring of health condition in "Biogradska Gora" National Park a symptoms of Dutch elm disease were recorded. Analyses confirmed that fungus *Ophiostoma novo-ulmi* was present in symptomatic wych elm (Ulmus glabra) trees as causal agent of their wilting. This area is one of the rarest protected ecosystems of this type in the world and until now elm trees were considered to be free of Dutch elm disease. This is the first report of *Ophiostoma novo-ulmi* in "Biogradska Gora" National Park. Using genetically divergent forests as reservoirs for breathing programs against Dutch elm disease could be more difficult based on these results. Implementation of these results against Dutch elm disease on global and local scale was discussed.

Keywords: *Ulmus glabra*, *Ophiostoma novo-ulmi*, symptoms, Montenegro

INTRODUCTION

Forests of Biogradska Gora in Montenegro developed through natural processes, without anthropogenic disturbances and this kind of forests provide opportunity of studding close to nature management and environntal changes (Curovic *et al*., 2020; Nagel et al., 2013; Chivulescu *et al*., 2018). Area is characterized by great compositional and structural variability (Cagliero *et al*., 2021). Also, mixed forests in beech (*Fagus sylvatica* L.) dominated sites are incredibly rare and serve for observation of natural processes in temperate zone (Curovic *et al*., 2020). Changes in this kind of forests are still present and trees with lowest diameter have significantly shorter time to passage (Govedar *et al*., 2021).

Forest pathogens occur in all categories of world's forests and all tree species are susceptible to pathogens in some degree (Tainter and Baker, 1996; Sinclair and Lyon, 2005; Edmonds, 2013). There are multiple drivers influencing trees susceptibility to forest pathogens including invasion by alien pathogens, climate change, emergence of more virulent strains of pathogen, hybridization of fungal species, latent of cryptic pathogens, establishment of new associations

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between vectors and pathogens and introduction of new forest practices (Ghelardini *et al*., 2016).

The first step in disease management includes detection, survey and monitoring (Edmonds, 2013). New diseases in European forests were reported at an increasing rate over the last century (Stenlid *et al*., 2011). One of such diseases is Dutch elm disease characterized with rapid spread and multiple epidemics in the world (Brasier, 2000). Dutch elm disease is common name for disease caused by group of species from genus *Ophiostoma* (Kirisits, 2013). This disease caused significant loss in elm populations, primarily leading to decrease of their economic importance (Karadžić, 2010; Kirsits, 2013). Survival of elm species due to this pathogen isn't completely endangered because trees need to be in certain stage of development prior to infection (Brasier, 1996). However, there is also increased occurrence of pathogens in domestic forests including impacts of wood decay fungi (Radulović *et al*., 2020; Karadžić *et al*., 2020; Radulović *et al*., 2021) and unusual transmission from different host species (Vemić and Radulović, 2021). This holds great ecological pressure on elm trees in domestic forests. Considering still active epidemic of Dutch elm disease in Europe and reducing the number of vitally trees, keystones for protection are finding and production of genetically resistant trees (Santini *et al*., 2010). Resources of this material should be also searched in untouched forests.

During the monitoring of trees health condition in "Biogradska Gora" National Park in Montenegro a symptoms of Dutch elm diseases on wych elm (*Ulmus glabra* Huds.) were recorded. Laboratory analyses confirmed presence of pathogen *Ophiostoma novo-ulmi* Brasier in symptomatic trees. This is the first report of *Ophiostoma novo-ulmi* in Biogradska Gora (Montenegro) as area previously considered to be without Dutch elm disease. These results will contribute to knowledge about distribution of *Ophiostoma novo-ulmi* and ways of using untouched forests for genetically resistant material.

MATERIAL AND METHODS

Locality of research

Symptoms of Dutch elm disease were noticed in summer of 2017 in locality of National Park "Biogradska Gora" (42º53′39" N, 19º36′15" E) after detailed examination of entire area. Twigs from symptomatic trees were collected for laboratory analyses.

Laboratory methods

Fungus was isolated using standard procedures described in Brasier (1981). After the surface sterilization of elm wood, a small pieces of dark xylem tissues were placed on selective media. Fresh grown pieces of mycelium were transferred on 3% MEA to obtain pure cultures. Storage of cultures was according recommendations described in Brasier (1981). Fresh 10 days old cultures were used for morphological and molecular identification.

Fungus was identified on both morphological and molecular criteria. Morphological identification was performed based on the characteristics of pure

cultures and microstructures according to data described in Brasier (1981) and Brasier (1991). Examination of microstructures was performed using microscope AM Scope B120 C E1. Molecular identification was performed with Phire Plant Direct PCR Kit using ITS1/ITS4 combination of primers. Complete PCR procedure was performed according to manufacturer's recommendations.

RESULTS AND DISCUSSION

Recorded symptoms

Symptoms recorded in investigated area included wilting of trees and premature leaves shedding (Figure 1 A-B). Bark damages caused by the smaller European elm bark beetle (*Scolytus multistriatus* March.) provided additional evidence about presence of Dutch elm disease (Figure 1 C).

All symptoms were visible in summer period and based on their degree infection probably started some time earlier (Figure 1). Smaller twigs were equally infested as larger ones and fungus was more easily isolated from these twigs (Figure 1).

Figure 1. Symptoms of Dutch elm disease on wych elm (*Ulmus glabra*) A-B wilting of trees, C – damages of the smaller European elm bark beetle *Scolytus multistriatus*

Also, symptoms were recorded in less protected stands and between less protected stands and old-growth forest (Figure 1). Distant parts of old-growth forests were still without any symptoms of Dutch elm disease.

Identification of fungus

Species isolated from sampled tissues was identified as *Ophiostoma novo-ulmi* (Figure 2). Colonies were white, concentric with presence of aerial mycelium in central part (Figure 2 - A).

Conidia were from *Sporotrix* stage and abundant, single celled, holoblastic, ellipsoid to ovoid (Figure 2 - B). Also, some conidia aggregated in spore drops (Figure 2 B). Anamorph with synemates – *Pesotim* (*Graphium*) was absent in obtained cultures.

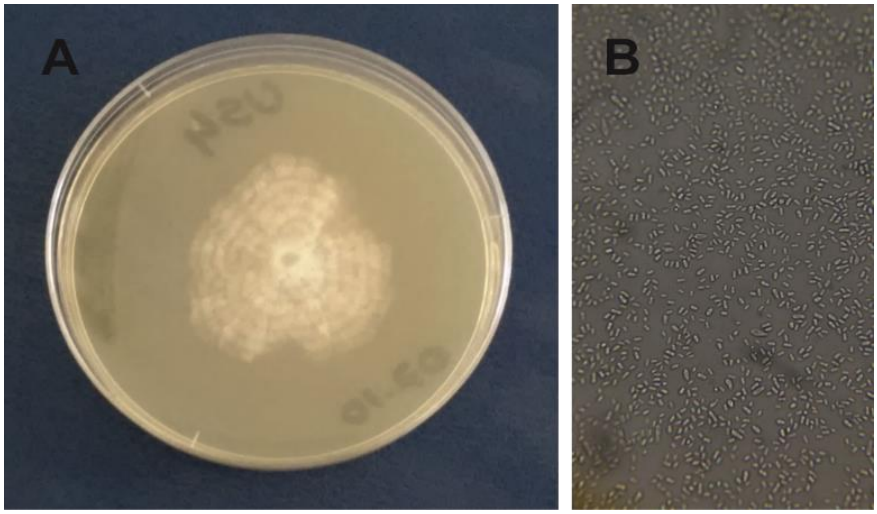

Figure 2. *Ophiostoma novo-ulmi:* A – characteristics of culture, B – conidial spores

Prior to this study "Biogradska Gora" National Park was considered to be free of Dutch elm disease. This is the first report of Dutch elm disease caused by *Ophiostoma novo-ulmi* in "Biogradska Gora" National Park. Results will have practical application in evaluating the potential of old-growth forests as source of genetically divergent trees that can be used in breeding programs for resistance to Dutch elm disease. Also, another practical application is for improving protection strategies of elm trees in "Biogradska Gora" National Park.

Dutch elm disease is caused by three closely related species from genus *Ophiostoma* including *Ophiostoma ulmi* (Buisman) Nannf., *Ophiostoma novo-ulmi* Braiser and *Ophiostoma himal-ulmi* Braiser & Mehrotra (Kirisits, 2013). From these species *Ophiostoma ulmi* was responsible for the first outbreak of disease in the Europe and North America on the beginning of 20th century (Gibbs, 1978; Brasier, 2000) and later in the middle of 20th century was replaced by *Ophiostoma novo-ulmi* (Brasier, 1991; Brasier, 2000). On the other side *Ophiostoma himal-ulmi* occurs endemically on Himalyan elm (*Ulmus wallichiana* (Planch.)) and currently there isn't reported its significant pathogenicity (Kirisits, 2013).

Species *Ophiostoma novo-ulmi* is considered to contain two subspecies whereby susp. novo-ulmi caused epidemic in Europe and subsp. americana caused epidemic in North America (Brasier, 1979; Brasier, 1991). From especially concerns is that hybridization between these subspecies exists and hybrid showed very rapid emergence and that further complex hybrids are now

expanding (Brasier and Kirk, 2010). At this point more molecular analyses are required to evaluate routes of introduction in this area and characterization of present strain.

Fungus *Ophiostoma novo-ulmi* is still actively invasive and isolates decade after the outbreak showed similar characteristics as isolated early after the outbreak (Brasier and Webber, 2019). Anyway, clonal researches against DED are now more promising, despite that wych elm (*Ulmus glabra*) was more susceptible than the field elm (*Ulmus minor* Miller) (Solla *et al*., 2005). However, investigation of less genetically divergent *Ophiostoma novo-ulmi* populations showed that wych elm (*Ulmus glabra*) was less infested than the field elm (*Ulmus minor*) (Łakomy *et al.*, 2016). All this points to further researches about genetic background of booth pathogen and host in order to create proper strategies for preservation of this species.

National Park "Biogradska Gora" was well known for a long time as natural reserve where Dutch elm disease is relatively little or non-present. Previous studies (Karadžić, 1996; Karadžić *et al*., 1999) didn't confirm presence of *Ophiostoma* species in this area pointing to conclusions that wych elm (*Ulmus glabra*) could be more resistant or "disease escape" is more likely in this kind of ecosystem. However, old-growth forests are much more resistant and tolerant to diseases in comparison to the other categories of forests (Lazarev, 2001). This means that sources of tolerant trees could still be found in these forests including Biogradska Gora especially because healthy trees were still found in this area. Based on these results tolerant trees and "disease escape" were more likely in distant parts of old-growth forest. Further protection of this National Park against DED should be in eliminating symptomatic trees in less protected area as well as adopting new strategies in regulating rules for the passage of visitors in more protected area – old-growth forest. This will reduce accidental transfer of inoculum or disturbing ecological stability of these forests.

CONCLUSIONS

Performed research leaded to next conclusions:

- ✓Fungus *Ophiostoma novo-ulmi* was isolated from wych elm (*Ulmus glabra*) trees in "Biogradska Gora" National Park.
- ✓All wych elm (*Ulmus glabra*) trees from whom *Ophiostoma novo-ulmi* was isolated had typical symptoms of Dutch elm disease without showing any form of tolerance.
- ✓All reported cases of *Ophiostoma novo-ulmi* in this study were in less protected zone and beginning of the old-growth forest located around lake "Biogradsko jezero".
- ✓Elm trees deep in old-growth forest didn't have any symptoms of Dutch elm disease at the moment this study was performed.
- ✓This is the first report of Dutch elm disease in "Biogradska Gora" National Park.

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