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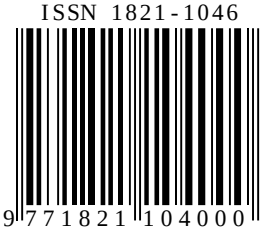

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Original scientific paper

ISOLATION OF RHIZOBACTERIA OF SESSILE OAK (*Quercus petraea* (Matt.) Liebl.) FROM THE FORESTS OF EASTERN SERBIA AND THEIR PRELIMINARY IDENTIFICATION

Sanja JOVANOVIĆ[1], Tanja BERIĆ[2], Aleksandar LUČIĆ[1], Olja STANOJEVIĆ[2], Vladan POPOVIĆ[1]

Abstract: *Rhizobacteria are a heterogeneous group of bacteria inhabiting the rhizosphere of plants, whose dynamics and diversity of metabolism and activity are conditioned primarily by root exudates. Functionally, these bacteria contribute to the improvement of plant performances, and are often members of the genera Bacillus and Pseudomonas. The rhizobacteria of forest species in Serbia have not been sufficiently studied. Sessile oak is one of the most important oak species in Serbia and one of the most produced species in forest nurseries. In this study, the rhizobacteria of sessile oak from natural populations of eastern Serbia are isolated and the representatives of genus Bacillus, i.e., Pseudomonas are identified preliminarily. Out of a total of 94 isolates, 35 were preliminarily identified as Bacillus spp., and out of 29 Gram-negatives, some of the isolates belong to genus Pseudomonas. Further research is necessary in order to identify the isolates more precisely and examine their impact on sessile oak.*

Key words: rhizobacteria, sessile oak, identification, *Bacillus*, *Pseudomonas*, eastern Serbia.

IZOLACIJA RIZOBAKTERIJA HRASTA KITNJAKA (*Quercus petraea* (Matt.) Liebl.) IZ ŠUMA ISTOČNE SRBIJE I NJIHOVA PRELIMINARNA IDENTIFIKACIJA

Izvod: *Rizobakterije predstavljaju heterogenu grupu bakterija koje naseljavaju rizosferu biljaka, čiji su i dinamika i diverzitet metablizma i aktivnosti uslovljeni pre svega*

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korenovim eksudatima. Funkcionalno, ove bakterije doprinose pospešivanju performansi biljke, i često su pripadnici rodova Bacillus i Pseudomonas. Rizobakterije šumskih vrsta u Srbiji nisu dovoljno istražene. Hrast kitnjak je jedna od najznačajnijih vrsta hrasta u Srbiji i najčešće proizvođenih u šumskim rasadnicima. U ovom radu izolovane su rizobakterije kitnjaka iz prirodnih populacija Istočne Srbije i preliminarno identifikovani predstavnici roda Bacillus, odnosno Pseudomonas. Od ukupno 94 izolata, 35 je preliminarno identifikovano kao Bacillus spp., a od 29 Gram negativnih, neki izolati pripadaju rodu Pseudomonas. Dalja istraživanja su neophodna radi preciznije identifikacije izolata i ispitivanja njihovog uticaja na hrast kitnjak.

Ključne reči: rizobakterije, hrast kitnjak, identifikacija, *Bacillus*, *Pseudomonas*, istočna Srbija

1. INTRODUCTION

Rhizobacteria are a heterogenous group of bacteria that inhabit plant rhizosphere. The availability of nutrients is the main reason of their dense population of this zone, where the abundance of bacteria is up to 100 times higher compared to surrounding land (Hajnal-Jafari et al., 2020; Vieira et al., 2020). The diversity and dynamics of metabolism and activity of rhizobacteria are conditioned by seasonality, availability of nutrients, type and physical and chemical characteristics of the soil, climate, plant genetics, as well as other biotic and abiotic factors. Plant species and its exudates secreted into the surrounding soil by means of its roots have a crucial role. Rhizodeposits of trees can contain up to 50% of carbon fixed by parent plant, and are incorporated into soil biomass (Baldrian, 2017). The changing environment affects the physiology of a plant, and in response to new circumstances, the plant changes the pattern of its exudates, spatially and temporally, realizing an effect on the associated microbial communities of the rhizosphere, in order to better adapt to new conditions. Plant Growth-Promoting Rhizobacteria (PGPR) stand out as a special functional group and they enhance plant growth. These bacteria are characterized by the ability of synthesis of phytohormones such as auxin, gibberellin, cytokinin, and inhibition of synthesis of ethylene, then dissolution of sparingly soluble forms of phosphates, mobilization of iron by production of siderophores, activation of induced systemic plant resistance, and various ways of exhibiting biocontrol activities (Vejan et al., 2016; Beneduzi et al., 2012). The beneficial effects of the above-mentioned mechanisms on the plant organism are reflected in the improvement of their performances – growth, fructification, resistance to biotic stressors, better adaptedness in relation to environmental conditions and survival in challenging habitats. It is considered that communication of PGPR with plants dates back to the arrival of first plants to land, when their co-evolution begins at the same time. (Lyu et al., 2021). In light of the fight against the pollution of the environment, climate changes and development of "green" technologies, PGPR have gained their place as a completely natural form of replacement of artificial fertilizers and pesticides, whose devastating effects on the environment have increasingly been examined (Backer et al., 2018), fully in line with the sustainability trends. Climate change problems are becoming more visible also in Serbia, so the need for their solution is more evident (Brašanac-

Bosanac, 2014). Biopreparations on the basis of individual bacterial strains or consortiums which are commercially widely available on the world market have also entered the course of legal regulations within the European Union (DuJardin, 2015). For that purpose, bacterial strains from the genera Bacillus and Pseudomonas are best characterized, and thus the most frequently used. They are characterized by great metabolic potential and the ability to degrade and synthesize an enormous number of compounds, adaptation to various habitat conditions, and a special feature of members of the genus Bacillus is the ability to form endospores in adverse environmental conditions, ensuring long-term survival of organisms. (Kloepper et al., 2004; Bakker et al., 2007; Kashyap et al., 2019; Dorjey et al., 2017). Ćirković-Mitrović (2014) examined the effect of BactoFil® on black walnut seedlings and confirmed its phytostimulating effect.

The rhizobacteria of forest species in Serbia are not thoroughly researched. Sessile oak (*Quercus petraea* (Matt.) Liebl.) is an autochthonous species of the Republic of Serbia and in the growing stock it is a second most valuable oak species with the share of 5.9% in the total volume (Banković et al., 2009). It builds 23 types of forests within the distribution area and is highly valued due to its technical value, wide application, as well as ecological and cultural significance. The need for sessile oak is great, as shown by the fact that it is one of two most commonly grown species in nursery production in Serbia. (Popović et al., 2019). Today, sessile oak forests are in unsatisfactory condition in terms of quality, health, age, poor vitality, thinned canopies, affected by the process of forest decline, which is further reflected in the frequency of fructification, amount and quality of seeds and natural regeneration (Krstić, 2016).

The aim of this study was to isolate sessile oak rhizosphere bacteria from its natural stands as well as the preliminary identification of isolates belonging to the genera Bacillus and Pseudomonas, as common soil microorganisms with the ability to promote plant growth. The obtained results can serve as a basis for further research of also the potential for manifestation of beneficial abilities on plant organism.

2. MATERIAL AND METHODS

In August 2020 soil samples were taken for the isolation of bacteria from the rhizosphere of natural sessile oak stands of the management units Ravna Reka I and Grabova Reka, within Majdanpek Forestry Administration. In each of the forest management units 6 healthy trees were randomly selected, at the base of which, at 50 cm from the tree, litterfall was removed and soil samples were taken at the depth of 0-30 cm, which were then deposited in sterile bags. The data on the precise location of the trees and altitude are shown in Tables 1 and 2.

Until the moment of processing, the samples were kept in a refrigerator at a temperature of 4ºC.

During processing, each individual soil sample was mixed, after which 1 g was taken and resuspended in 10 ml of distilled water. A 10^{-5} dilution was made and 1 ml was seeded in nutrient agar (Nutrient agar CM0003 Oxoid) substrate by pouring, in duplicate, after which the samples were incubated for 48 h at a temperature of 30ºC.

Table 1. *Data on location of trees from Ravna Reka 1 FMU*

Number of the tree	Coordinates	Altitude
Tree 1.	49 21 793 N 75 78 166 E	587 m
Tree 2.	49 22 048 N 75 78 248 E	624 m
Tree 3.	49 22 067 N 75 78 267 E	604 m
Tree 4.	49 22 363 N 75 78 211 E	628 m
Tree 5.	49 22 704 N 75 78 532 E	630 m
Tree 6.	49 22 959 N 75 78 423 E	644 m

Table 2. *Data on location of trees from Grabova Reka FMU*

Number of the tree	Coordinates	Altitude
Tree 1.	49 16 994 N 75 70 844 E	351 m
Tree 2.	49 16 950 N 75 70 923 E	368 m
Tree 3.	49 16 899 N 75 70 964 E	349 m
Tree 4.	49 16 768 N 75 71 017 E	325 m
Tree 5.	49 16 825 N 75 70 905 E	323 m
Tree 6.	49 16 950 N 75 70 680 E	285 m

After incubation, the grown colonies were purified until pure cultures were obtained. Thereafter, a preliminary identification of bacterial isolates was performed.

Gram staining reaction was determined for all isolates by the method of Suslow et al., 1982. The presence of threads indicates Gram-negative reaction, while their absence indicates Gram-positive reaction.

The catalase test indicates the presence of the enzyme catalase and it was performed on a microscope slide, as a reaction of a bacterial culture sample with 3% hydrogen peroxide (Schaad et al., 2001). All isolates were tested.

The bacterial isolates that showed a Gram-negative reaction were tested for the presence of cytochrome c oxidase in the respiratory chain, which preliminarily identifies aerobic bacteria. The oxidase test was performed by the method of Shields and Cathcart, 2010.

The bacteria that proved to be Gram-positive were tested for the possibility of endospore formation in adverse environmental conditions, which is a characteristic of bacteria of the genus *Bacillus*. Overnight cultures of individual isolates in the nutrient broth medium were made. Thereafter, 4 ml of each culture sample was incubated for 10 minutes at a temperature of 80ºC in a water bath (Memmert). After incubation, samples of each isolate were seeded on nutrient agar substrate in order to be tested for survival, and incubated for 48 h at a temperature of 30ºC.

The ability of Gram-negative isolates to synthesize green fluorescent pigment on KB nutrient medium with the addition of glycerol, which is a

characteristic of members of *Pseudomonas* bacteria genus, was also studied. The presence of this characteristic was observed under a UV lamp.

3. RESULTS AND DISCUSSION

The obtained results of isolation and preliminary characterization of sessile oak rhizobacteria from its natural stands in eastern Serbia are presented in Tables 3 and 4.

Table 3. *Preliminary characterization of bacterial isolates from sessile oak rhizosphere from the site of Grabova Reka FMU*

Bacterial isolate	Gram reaction	Oxidase test	Catalase test	Fluorescent pigment production	Growth at 80°C
GR1.1	+	/	+	/	-
GR2.1	-	+	+	-	/
GR2.2	-	-	+	+	/
GR2.3	+	/	+	/	+
GR2.4	-	+	-	+	/
GR2.5	-	-	+	-	/
GR2.6	+	/	-	/	+
GR2.7	-	-	+	+	/
GR2.8	+	/	+	/	+
GR2.9	-	-	+	+	/
GR2.10	-	+	-	+	/
GR3.1	+	/	+	/	-
GR3.2	-	-	+	-	/
GR3.3	+	/	+	/	+
GR3.4	+	/	+	/	+
GR3.5	+	/	+	/	+
GR3.6	+	/	+	/	+
GR3.7	+	/	+	/	+
GR3.8	+	/	+	/	+
GR3.9	-	-	+	-	/
GR4.1	-	-	+	-	/
GR4.2	+	/	+	/	+
GR5.1	+	/	+	/	+
GR5.2	+	/	+	/	-
GR6.1	-	+	-	+	/
GR6.2	+	/	+	/	+
GR6.3	-	-	+	-	/

Legend:
(/) The bacterial isolate has not been tested for the relevant characteristic.
(-) There is no appropriate enzyme or growth in the bacterial isolate, or its Gram reaction is negative.
(+) There is appropriate enzyme or growth in the bacterial isolate, or its Gram reaction is positive.

Table 4. *Preliminary characterization of bacterial isolates from sessile oak rhizosphere from the site of Ravna Reka 1 FMU*

Bacterial isolate	Gram reaction	Oxidase test	Catalase test	Fluorescent pigment production	Growth at 80°C
RR1.1	+	/	+	/	+
RR1.2	+	/	+	/	+
RR1.3	+	/	+	/	-
RR1.4	+	/	+	/	+
RR1.5	-	+	+	+	/
RR1.6	+	/	+	/	+
RR2.1	+	/	+	/	+
RR2.2	+	/	+	/	-
RR2.3	-	-	+	-	/
RR2.4	+	/	+	/	+
RR2.5	+	/	+	/	+
RR2.6	-	+	+	+	/
RR2.7	+	/	+	/	-
RR3.1	+	/	+	/	-
RR3.2	+	/	+	/	-
RR3.3	+	/	+	/	-
RR3.4	+	/	+	/	-
RR3.5	+	/	+	/	-
RR3.6	+	/	+	/	+
RR3.7	+	/	+	/	-
RR3.8	+	/	+	/	-
RR3.9	+	/	+	/	+
RR3.10	-	+	+	+	/
RR3.11	-	+	+	+	/
RR3.12	+	/	+	/	-
RR3.13	+	/	+	/	+
RR3.14	+	/	+	/	-
RR3.15	+	/	+	/	-
RR3.16	+	/	+	/	-
RR3.17	-	+	+	+	/
RR3.18	+	/	+	/	+
RR3.19	+	/	+	/	-
RR3.20	-	+	+	+	/
RR3.21	+	/	+	/	-
RR3.22	+	/	+	/	+
RR3.23	-	+	+	+	/
RR3.24	-	+	+	+	/
RR4.1	+	/	+	/	-
RR4.2	-	-	+	-	/
RR4.3	-	-	+	-	/
RR4.4	-	-	+	-	/
RR4.5	+	/	+	/	+
RR4.6	+	/	+	/	+
RR4.7	+	/	+	/	-
RR4.8	+	/	+	/	-
RR4.9	+	/	+	/	-
RR4.10	-	-	+	+	/
RR4.11	+	/	+	/	-
RR5.1	+	/	+	/	+
RR5.2	-	+	+	+	/
RR5.3	-	+	+	+	/
RR5.4	+	/	+	/	-

Bacterial isolate	Gram reaction	Oxidase test	Catalase test	Fluorescent pigment production	Growth at 80°C
RR5.5	+	/	+	/	+
RR5.6	+	/	+	/	+
RR5.7	+	/	+	/	-
RR6.1	+	/	+	/	+
RR6.2	+	/	+	/	+
RR6.3	+	/	+	/	+
RR6.4	+	/	+	/	+
RR6.5	+	/	+	/	+
RR6.6	+	/	+	/	+
RR6.7	-	+	+	+	/
RR6.8	+	/	+	/	-
RR6.9	+	/	+	/	-
RR6.10	-	+	+	+	/
RR6.11	+	/	+	/	-
RR6.12	+	/	+	/	-

Legend:
(/) The bacterial isolate has not been tested for the relevant characteristic.
(-) There is no appropriate enzyme or growth in the bacterial isolate, or its Gram reaction is negative.
(+) There is appropriate enzyme or growth in the bacterial isolate, or its Gram reaction is positive.

A total of 94 bacterial isolates were isolated from rhizosphere samples of 12 sessile oak trees from their natural stands of eastern Serbia. Out of that number, 67 originate from the site of Ravna Reka 1 FMU, while 27 isolates originate from the site of Grabova Reka FMU.

Out of the total number of isolated bacteria, 29 showed a Gram-negative reaction and 12 of them originate from Grabova Reka FMU. The remaining 65 isolates had a Gram-positive reaction, out of which 15 rhizosphere samples were from the site of Grabova Reka FMU.

All 29 Gram-negative isolates were tested for the presence of cytochrome c oxidase, 16 of which were oxidase positive and 19 bacteria had the ability to synthesize a yellowish green fluorescent pigment. The highest fluorescence was observed in isolates RR1.5, RR3.10, RR3.17, RR5.2 and RR5.3. Out of the total number of Gram-negative bacteria, 26 isolates proved to be catalase positive.

Gram-positive bacteria were tested for the presence of the enzyme catalase and out of the total number, 63 isolates synthesized this enzyme. Following the exposure to a temperature of 80°C, 35 bacterial isolates were viable and grew on nutrient agar.

After the performed tests, 34 of 94 isolates were Gram-positive, catalase positive and manifested growth after the exposure to a temperature of 80°C. Sixteen isolates had Gram-negative reaction with the production of fluorescent yellowish-green pigment, while 12 out of this number were oxidase positive.

The members of the genus *Bacillus* and *Pseudomonas* are very represented in nature. Changing environmental conditions require the development of adaptive strategies of organisms, and Bacillus endospores have proven to be one of the best solutions. These bacteria can especially be found in soils with different characteristics – acidic, alkaline or deserts, whereby the frequency of occurrence of some species shows significant correlations with altitude (Liu et al., 2019). Gagelidze et al. (2018) examined the presence of *Bacillus* and *Pseudomonas* in different soil types of Georgia and found that they occurred equally in black soil,

that *Bacillus* genus was more dominant in chernozem, alkaline soils and marshes, while *Pseudomonas* genus occurred more frequently in brown forest soils. Both sites sampled in this study are located on the type of dystric brown soil.

Due to their special complexity, forest soils are characterized by a particularly large diversity of bacteria. One study showed a decrease in the diversity of bacteria of the genus *Bacillus* from forest to eroded soils (Zhang et al., 2003), while in other no significant correlation was found (Liu et al., 2016). Dukunde et al. (2019) examined the influence of tree species on the structure of soil bacterial communities in temperate deciduous forests. They found that in beech and oak monocultures the soil pH is low with a high C:N ratio, and in these communities the abundance of oligotrophic compared to copiotrophic bacterial taxa is greater. Also, compared to mixed stands, monocultures showed a larger number of indicator species highly associated with each stand. While Grabova Reka FMU represents typical oak forests, in Ravna Reka 1 FMU there are mixed oak and beech forests.

No molecular o biochemical methods by which bacteria identification down to the level of species could be carried out were performed in this study. Based on the performed preliminary identification tests 35 potential members of the genus *Bacillus* were isolated. On the other hand, out of 29 Gram-negative isolates, some belong to the genus *Pseudomonas*.

The results confirm the diversity of forest soil bacteria, as well as the abundance of the genera *Pseudomonas* and *Bacillus* in the rhizosphere of sessile oak. 94 bacteria were isolated, however, since the research of the composition of bacterial communities was performed using a breeding method, we can state with certainty that the total diversity is much greater. The reason for relatively small number of Gram-negative bacteria, oxidase-positive isolates of potential pseudomonads can be the way of the bacteria seeding, i.e., pouring method, as a result of which some aerobic organisms could not grow. Further research is needed in order to perform more precise characterization of isolates. Thereafter, their relationships with sessile oak, the host plant would be studied.

4. CONCLUSIONS

Rhizobacteria are a large group of diverse microorganisms that inhabit the root zone of plants and in previous research conducted mainly on agricultural species, many isolates from the rhizosphere have shown potential in promoting plant growth and replacing artificial fertilizers, whose negative effects on the environment are increasingly being revealed. Sessile oak is one of the most important species in the flora of Serbia, and as such it is the subject of numerous studies.

In this study, 94 pure bacterial cultures were isolated from sessile oak rhizosphere, from its natural populations of eastern Serbia. Gram, catalase and oxidase tests were applied, as well as testing for fluorescent pigment synthesis, i.e., survival at 80 °C. While 35 isolates were preliminarily characterized as *Bacillus* spp., 29 Gram-negative isolates required additional research in order to confirm belonging to *Pseudomonas* genus. These results represent the first step in the study

of rhizosphere sessile oak communities and further studies are needed in order to determine the relationship with the parent plant.

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ISOLATION OF RHIZOBACTERIA OF SESSILE OAK (*Quercus petraea* (Matt.) Liebl.) FROM THE FORESTS OF EASTERN SERBIA AND THEIR PRELIMINARY IDENTIFICATION

Sanja JOVANOVIĆ, Tanja BERIĆ, Aleksandar LUČIĆ, Olja STANOJEVIĆ, Vladan POPOVIĆ

Summary

Rhizobacteria are a heterogeneous group of bacteria inhabiting the rhizosphere of plants, whose dynamics and diversity of metabolism and activity are conditioned primarily by root exudates. Functionally, these bacteria contribute to the improvement of plant performances, and are often members of the genera *Bacillus* and *Pseudomonas.* The beneficial effects are reflected in the improvement of their performances – growth, fructification, resistance to biotic stressors, better adaptedness in relation to environmental conditions and survival in challenging habitats. In light of the fight against the pollution of the environment, climate changes and development of "green" technologies, PGPR have gained their place as a completely natural form of replacement of artificial fertilizers and pesticides, whose devastating effects on the environment have increasingly been examined, fully in line with the sustainability trends.

The rhizobacteria of forest species in Serbia have not been sufficiently studied. Sessile oak is one of the most important oak species in Serbia and one of the most produced species in forest nurseries. At present, sessile oak forests are in unsatisfactory condition in terms of quality, health, age, poor vitality, thinned canopies, affected by the process of forest decline, which is further reflected in the frequency of fructification, amount and quality of seeds and natural regeneration.

The aim of this study was to isolate sessile oak rhizosphere bacteria from its natural stands and preliminary identification of isolates belonging to the genera *Bacillus* and *Pseudomonas*, as common soil microorganisms with the ability to promote plant growth.

Subsequent to performed Gram, catalase and oxidase reactions and examination of growth at 80°C and production of fluorescent pigment the rhizobacteria of sessile oak from natural populations of eastern Serbia were isolated and members of the genera *Bacillus,* i.e., *Pseudomonas* were preliminarily identified. Out of a total of 94 isolated bacteria, 35 potentially belong to the genus *Bacillus* and out of 29 Gram-negatives, some of the isolates belong to the genus *Pseudomonas.* Further research is necessary in order to identify the isolates more precisely and examine their impact on sessile oak.

IZOLACIJA RIZOBAKTERIJA HRASTA KITNJAKA (*Quercus petraea* (Matt.) Liebl.) IZ ŠUMA ISTOČNE SRBIJE I NJIHOVA PRELIMINARNA IDENTIFIKACIJA

Sanja JOVANOVIĆ, Tanja BERIĆ, Aleksandar LUČIĆ, Olja STANOJEVIĆ, Vladan POPOVIĆ

Sažetak

Rizobakterije predstavljaju heterogenu grupu bakterija koje naseljavaju rizosferu biljaka, čiji su i dinamika i diverzitet metablizma i aktivnosti uslovljeni pre svega korenovim eksudatima. Funkcionalno, ove bakterije doprinose pospešivanju performansi biljke, i često su pripadnici rodova *Bacillus* i *Pseudomonas*. Blagodetni efekti ogledaju se u

poboljšavanju njihovih performansi – rasta, plodonošenja, otpornosti na biotičke stresore, boljoj prilagođenosti na uslove sredine i opstanku na izazovnim staništima. U svetlu borbe sa zagađenošću životne sredine, klimatskim promenama i razvoju „zelenih'' tehnologija, PGPR su dobile svoje mesto kao potpuno prirodan vid zamene veštačkih đubriva i pesticida, čiji se devastirajući efekti po okolinu sve više razmatraju, potpuno u skladu sa tendencijama održivosti.

Rizobakterije šumskih vrsta u Srbiji nisu dovoljno istražene. Hrast kitnjak je jedna od najznačajnijih vrsta hrasta u Srbiji i najčešće proizvođenih u šumskim rasadnicima. Šume kitnjaka su danas nezadovoljavajućeg stanja u pogledu kvaliteta, zdravstvenog stanja, starosti, slabe vitalnosti, razređenih krošnji, zahvaćeni procesom sušenja, što se dalje odražava na učestalost plodonošenja, količinu i kvalitet semena i prirodnu obnovu.

Cilj ovog rada je bio izolacija bakterija rizosfere hrasta kitnjaka iz njegovih prirodnih sastojina i preliminarna identifikacija izolata koji pripadaju rodovima *Bacillus* i *Pseudomonas*, kao čestim zemljišnim mikroorganizmima sa sposobnostima promocije rasta biljaka.

Nakon izvedene Gram, katalaza, oksidaza reakcije, zatim ispitivanja rasta na 80°C i proizvodnju fluorescentnog pigmenta, izolovane su rizobakterije kitnjaka iz prirodnih populacija Istočne Srbije i preliminarno identifikovani predstavnici roda *Bacillus*, odnosno *Pseudomonas*. Od ukupno 94 izolovane bakterije, 35 potencijalno pripada rodu *Bacillus*, a od 29 Gram negativnih, neki izolati pripadaju rodu *Pseudomonas*. Dalja istraživanja su neophodna radi preciznije identifikacije izolata i ispitivanja njihovog uticaja na hrast kitnjak.

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THE REMOVAL OF PATHOGENIC MICROORGANISMS IN A BIOLOGICAL SYSTEM WITH FLOATING ISLANDS

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Abstract: *Pathogenic microorganisms in water pose a great risk to human health. Therefore, it is necessary to find an efficient, environmentally friendly, and economically acceptable solution for their removal from polluted and wastewater. This paper presents the efficiency of a biological system with floating islands in the removal of pathogenic microorganisms from the water of a polluted urban river. The modified floating treatment wetland consisted of a collection tank, 4 calls with floating islands and 1 cell with algae, which enabled additional water polishing. The results of the research showed that the biological system constructed on the bank of this river had a high efficiency in reducing the number of various groups of pathogenic microorganisms. Within the cells with the floating islands, 100% efficiency in the removal of coliform bacteria of faecal origin was achieved, and the reduction of pathogens was continued within the cell with algae. The realised efficiency of removal of total coliform bacteria was 100% in all cells, except in the cell 4 with decorative aquatic macrophytes, in which the efficiency was 97%. The number of intestinal enterococci was reduced in the range of 92 to 97% in cells with plants, and up to 98% in the cells with algae. The floating islands and algae also enabled a high reduction in the number of aerobic heterotrophs and facultative oligotrophs. In addition, the ratio of these microorganisms had a value above 1 during the entire period of water treatment, which indicated that natural processes of self-purification of polluted water ran smoothly in*

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the floating treatment wetland. Due to the reduction of pathogenic microorganisms, water that belonged to class V, i.e., III, after the discharge from the biological system, had the characteristics of water with excellent ecological status (class I).

Key words: rhizofiltration, plants, algae, polluted water, urban river.

UKLANJANJE PATOGENIH MIKROORGANIZAMA U BIOLOŠKOM SISTEMU SA PLUTAJUĆIM OSTRVIMA

Izvod: *Patogeni mikroorganizmi u vodama predstavljaju veliki rizik po ljudsko zdravlje. Zbog toga je neophodno naći efikasno, ekološki pogodno i ekonomski prihvatljivo rešenje za njihovo uklanjanje iz zagađenih i otpadnih voda. Ovaj rad prikazuje efikasnost biološkog sistema sa plutajućim ostrvima u uklanjanju patogenih mikroorganizama iz vode zagađene urbane reke. Modifikovani biološki sistem se sastojao od sabirnog rezervoara, 4 bazena sa plutajućim ostrvima i 1 bazena sa algama, koje su omogućile dodatno poliranje vode. Rezultati istraživanja su pokazali da je biološki sistem konstruisan na obali ove reke imao visoku efikasnost u redukciji brojnosti različitih grupa patogenih mikroorganizama. U okviru bazena sa plutajućim ostrvima ostvarena je 100% efikasnost u uklanjanju koliformnih bakterija fekalnog porekla, a u okviru bazena za algama redukcija patogena je nastavljena. U svim bazenima ostvarena je efikasnost uklanjanja ukupnih koliformnih bakterija od 100%, osim u četvrtom bazenu sa dekorativnim akvatičnim makrofitama u kome je efikasnost bila 97%. Broj crevnih enterokoka je redukovan u granicama 92-97% u bazenima sa biljkama, a u bazenu sa algama do 98%. Plutajuća ostrva i alge su omogućili i visoko smanjenje brojnosti aerobnih heterotrofa i fakultativnih oligotrofa. Pored toga, odnos ovih mikroorganizama je imao vrednost iznad 1 tokom celog perioda prečišćavanja, što je ukazalo da su se u sistemu neometano odvijali prirodni procesi samoprečišćavanja zagađene vode. Zahvaljujući redukciji patogenih mikroorganizama voda koja je pripadala V odnosno III klasi je po izlasku iz biološkog sistema imala karakteristike vode sa odličnim ekološkim statusom (I klasa).*

Ključne reči: rizofiltracija, biljke, alge, zagađena voda, urbana reka.

1. INTRODUCTION

The most common risk to human health associated with water comes from the presence of microorganisms that cause various diseases (Jasper *et al.*, 2013). Surface waters contain a variety of microorganisms, including bacteria, fungi, protozoa, and algae, some of which can produce toxins, transmit, or cause diseases. Moreover, various intestinal pathogens can be found in water, such as *Salmonella* sp., *Shigella* sp., *Escherichia coli* and others. (Chapman and Kimstach, 1996). Sewage, agricultural runoff, and rainwater as well as household wastewater which are often mixed with each other and discharged into rivers without prior treatment pose a big problem. Such waters carry with them various pathogens and pose a great risk. A typical municipal sewage can contain from 10 to 100 million coliform bacteria in 100 ml and 1 to 50 million *Escherichia coli* or faecal streptococci in 100 ml (Chapman and Kimstach, 1996). A serious problem arises also when it is impossible to completely remove pathogenic microorganisms by conventional water treatment, which leads to their potential appearance in drinking water.

Various biological systems, such as constructed wetlands or floating islands, have the ability to remove various pollutants from polluted waters. (Čule *et al.*, 2021; Čule *et al.*, 2017; Kadlec and Wallace, 2008; Sharma *et al.*, 2021; Vymazal, 2007). The removal occurs by imitation of natural processes, without the use of chemicals and additional energy, and due to the symbiotic relationship between the basic components of biological systems such as plants, algae, small invertebrates, zooplankton, microorganisms, substrate, and water. (Brašanac-Bosanac *et al.*, 2020; Chen *et al.*, 2017; Davis, 1995; Prasad and de Oliveira Freitas, 2003; Yeh *et al.*, 2015). In recent years, great interest has been shown in the use of these nature-based solutions for the removal of pathogenic microorganisms from water (Vymazal, 2011). Although there are different opinions about their effectiveness in reducing pathogens (Ghermandi *et al.*, 2007; Watson *et al.*, 1989) it is evident that they can be used independently, within hybrid systems or as a complement to conventional treatment (Jasper *et al.*, 2013). Floating islands, which are used for the treatment of polluted waters, consist of mash platforms, substrate, and terrestrial and aquatic plants with associated beneficial microorganisms (Benvenuti *et al.*, 2018; Chance *et al.*, 2019; Sharma *et al.*, 2021). They can be placed within existing reservoirs, basins or directly on a river or a lake.

Topčiderka river has been used for years as a collector of wastewater, rainwater, agricultural and urban runoff, and industrial wastewater. Therefore, throughout the year its water is within the limits of class V based on most chemical, physical chemical and microbiological parameters for water quality assessment. (Čule *et al.*, 2017). The sources of pollution of this urban river are numerous, and its high pollution also endangers the integrity of the water of the Čukarički Rukavac armlet located near the most important recreational centre in Belgrade. To examine the possibility of using biological systems for the treatment of a polluted river, a modified floating treatment wetland (FTW) was constructed on its bank. This paper presents the achieved efficiency of an environmentally friendly and cost-effective solution for the removal of pathogenic microorganisms from water.

2. MATERIAL AND METHODS

The biological system consisted of a collection tank (5.0 m^3) and four cells with floating islands (area 3.0 m^2 and volume 3.0 m^3, each) (Figure 1). To provide additional polishing of water the fifth cell with algae was added (area 3.0 m^2 and volume 1.5 m^3). The collection tank and cells were placed on levelled soil and connected with plastic pipes (Čule *et al.*, 2017). A water meter was placed on each supply branch of cells 1-4, which enabled precise control of the amount of water coming from cells with floating islands to cell 5. In this way, equal amounts of water from cells 1-4 were poured into the cell with algae. Owing to the inlet and outlet construction of the collection tank and cells, the water moved gravitationally through the system (Čule *et al.*, 2017). Since the coverage of the cells with floating islands was 100%, anaerobic conditions were expected in them. To introduce a larger amount of dissolved oxygen into the FTW, the inlet into the cell 5 was placed on its upper edge, i.e., 50 cm above the ground (Figure 2). In this way, but

also due to larger free water surface, an additional reduction of pollutants is enabled, for the removal of which an aerobic environment is necessary. In each of the four cells there were 3 floating islands (1.0 m x 1.0 m). The buoyant platform of the floating islands was made of light thermoplastic mass, with handrails and circular openings at the bottom (diameters 8.4 cm and 5.0 cm). Stone wool was used as a substrate. The platform construction has enabled floating of the islands on the water and maintaining of above-ground biomass above its surface, as well as the growth of roots and rhizomes in the water. Non-invasive plants and plants suitable for rhizofiltration (Blaylock and Huang, 2000; Čule *et al.*, 2021; Čule *et al.*, 2016; Dushenkov *et al.*, 1995; Kumar *et al.*, 1995; Salt *et al.*, 1995) were used to form the vegetation of floating islands. Total of 25 (cells 1-3) or 30 (cell 4) seedlings were planted on each island. *Phragmites australis* (Cav.) Trin. ex Steud, was planted within cell 1, *Canna indica* L. in cell 2, while in cell 3 there was a mix of *P. australis* and *C. indica* at the ratio of 12:13. On each island in cell 4 there were also mixed plantations created by planting *Iris pseudacorus* L. (8 seedlings), *Iris sibirica* 'Perry's Blue' (5 seedlings), *Alisma plantago - aquatica* L. (5 seedlings), *Lythrum salicaria* L. (5 seedlings) and *Menyanthes trifoliata* L. (6 seedlings) (Figure 1). Algae were introduced in into cell 5 directly from the river. A monoculture of macroscopic algae from the genus *Cladophora* sp. was formed within this cell (Figure 2).

Figure 1. *Cells with floating islands (cell 1 – on the left; cells 2-4 – on the right)*

Figure 2. *Cell 5 with algae and construction of water inlet*

The FTW start-up period lasted a month and a half, after which the monitoring of its efficiency in the removal of pollutants began. The treatment cycle began by bringing water from the river into the collection tank by means of a pump and further distribution of water, by gravitational flow, simultaneously to cells 1-4. Hydraulic retention time (HRT) in cells was 6 days. After this period the water

flowed due to gravity into cell 5, where it also remained for 6 days. The water treatment was then completed, and the treated water was discharged into the river.

To examine the quality of polluted and purified water on the basis of sanitary and microbiological parameters, water sampling was performed according to the standard method SRPS EN ISO 19458: 2009 (Water quality - Sampling for microbiological analyses). Polluted water samples were taken at the beginning of the cycle at the FTW inlet. After 6 days, water was sampled in cells 1-4, and after the next 6 days in cell 5, too. The water sample was 1 litre of composite sample taken from 5 places in each cell (each corner and centre). Water samples were brought to the laboratory in refrigeration devices and stored until the moment of analyses according to the prescribed protocol.

Sanitary and microbiological analyses included determination of the number of total coliform bacteria in 100 ml (TCB), the number of coliform bacteria of faecal origin in 100 ml (CBoFO), the number of intestinal enterococci in 100 ml (IE), the number of aerobic heterotrophs in 1 ml (H) and facultative oligotrophs in 1 ml (FO), as well as their ratio. The number of total coliform bacteria and the number of coliform bacteria of faecal origin was determined in accordance with the standard method SRPS EN ISO 9308-2:2015 (Water quality - Enumeration of *Escherichia coli* and coliform bacteria — Part 2: Most probable number method.), while the number of intestinal enterococci was determined based on the instructions for use of rapid tests made by Idexx (IDEXX Water Enterolert-E and IDEXX Water Enterolert-DW, AFNOR IDX 33/04-02/15). The number of aerobic heterotrophs, the number of facultative oligotrophs and their ratio was determined based on Petrović *et al.* (1998).

3. RESULTS

The results of microbiological analysis of polluted and purified water from sanitary and ecological aspect (Tables 1 and 2) have shown that biological system was extremely effective in removing various categories of microorganisms, which impair water quality.

The number of coliform bacteria of faecal origin (CBoFO) in inflow amounted to 1,209,800.0 in 100 ml (Table 1.). The number of these pathogenic microorganisms was reduced after only six days of treatment in cells with plants (Table 2). The lowest value of CBoFO has been recorded in cell 2 where the species *C. indica* reduced the number of bacteria to 166.4 in 100 ml (Table 1) with 100% removal efficiency (Table 2). Compared to other cells, the reduction of number of CBoFO was the least in cell 4 with decorative macrophytes, but removal efficiency was also 100% (Table 2) in relation to the initial content of these pathogenic microorganisms in the inflow. At the end of treatment, CBoFOs were below the detection limit (Table 1) so that the efficiency of CBoFOs removal was 100% (Table 2).

The number of total coliform bacteria (TCB) was equal to the number of CBoFO in the inflow (Table 1). The reduction of TCB was the highest in cell 1 with the species *P. australis* where 1,299.7 coliform bacteria was recorded in 100 ml (Table 1), and the lowest in cell 4 with decorative macrophytes with the

removal efficiency of 97% (Table 2). At the end of the treatment, after the discharge of water from cell 5, the removal efficiency of TCB was 100% (Table 2).

The number of intestinal enterococci (IE) in the inflow amounted to 2,419.8 in 100 ml (Table 1). The removal of IE has been significant already after first 6 days of treatment in cells with plants, so that the number of IE ranged within the limits from 71.7 to 196.8 in 100 ml (Table 1). The lowest number of IE was recorded in cell 2 with the species *C. indica* with the removal efficiency of 97% (Table 2). In cell 1 with the species *P. australis* the removal of IE was the least compared to other cells and it amounted to 92% (Table 2). Until the end of the treatment the number of IE was reduced to the value of 46.4. Therefore, the removal efficiency for IE in the modified FTW was 98% (Table 2).

Table 1. *The number of coliform bacteria of faecal origin, total coliform bacteria, and intestinal enterococci in polluted and purified water*

Parameter	Sampling location					
	Inflow	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
CBoFO	1,209,800.0	224.7	166.4	435.2	3,150.0	<1.0
TCB	1,209,800.0	1,299.7	1,413.6	1,986.3	34,850.0	2.0
IE	2,419.8	196.8	71.7	157.6	166.4	46.4

Each value represents the value of a composite sample taken from 5 spots in a cell. CBoFO – the number of coliform bacteria of faecal origin in 100 ml, TCB – the number of total coliform bacteria in 100 ml, IE – the number of intestinal enterococci in 100 ml. Cell 1 – *Phragmites australis* (Cav.) Trin. ex Steud., Cell 2 - *Canna indica* L., Cell 3 - *P. australis* and *C. indica*, Cell 4 - *Iris pseudacorus* L., *Iris sibirica* 'Perry's Blue', *Alisma plantago - aquatica* L., *Lythrum salicaria* L. and *Menyanthes trifoliata* L., Cell 5 - algae (*Cladophora* sp.).

Table 2. *The efficiency of removal of pathogenic microorganisms from polluted water (%)*

Parameter	Sampling location				
	Cell 1	Cell 2	Cell 3	Cell 4	Outflow of FTW
CBoFO	100	100	100	100	100
TCB	100	100	100	97	100
IE	92	97	93	93	98

CBoFO –coliform bacteria of faecal origin, TCB –total coliform bacteria, IE - intestinal enterococci. Cell 1 - *Phragmites australis* (Cav.) Trin. ex Steud., Cell 2 - *Canna indica* L., Cell 3 - *P. australis* i *C. indica*, Cell 4 - *Iris pseudacorus* L., *Iris sibirica* 'Perry's Blue', *Alisma plantago - aquatica* L., *Lythrum salicaria* L. and *Menyanthes trifoliata* L., Outflow of FTW – outflow of cell 5.

The number of aerobic heterotrophs in the inflow amounted to 77,000.00 in 1 ml (Table 3). The reduction of number of aerobic heterotrophs (H) was also significant after only 6 days of the treatment in cells with plants and it ranged within the limits from 3,182.00 to 16,364.00 in 1 ml (Table 3). The lowest number of H was recorded in cell 1 with the species *P. australis*, and the highest in cell 2 with the species *C. indica* (Table 3). At the end of the treatment in the water of cell 5 the number of H amounted to 1,591.00 in 1 ml (Table 3).

The number of facultative oligotrophs (FO) in the inflow amounted to 94,000.00 in 1 ml (Table 3). After 6 days of treatment in cell with plants the number of FO was reduced, and it ranged from 13,182.00 to 35,909.00 in 1 ml (Table 3). The lowest number of FO was recorded in cell 3 in which species *P.*

australis and *C. indica* were planted, and the highest was in the cell 4 with decorative macrophytes (Table 3). The number of FO in the water of cell 5 at the end of the treatment amounted to 23,318.00 in 1 ml (Table 3).
The ratio of aerobic heterotrophs and facultative oligotrophs (FO/H) both in polluted and purified water was above 1. The value of this parameter in the inflowing water amounted to 1.22 while in cells it ranged from 1.28 to 14.66. The lowest value of FO/H was recorded in cell 2 with the species *C. indica*, and the highest in cell 5 with algae.

Table 3. *The number of aerobic heterotrophs and facultative oligotrophs and their ratio in polluted and purified water*

Parameter	Sampling location					
	Cell	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
H	77,000.00	3,182.00	16,364.00	5,909.00	11,818.00	1,591.00
FO	94,000.00	26,667.00	20,952.00	13,182.00	35,909.00	23,318.00
FO/H	1.22	8.38	1.28	2.23	3.04	14.66

Each value represents a value of a composite sample taken from 5 spots in a cell. H – the number of aerobic heterotrophs in 1 ml, FO – the number of facultative oligotrophs in 1 ml, FO/H – the ratio of aerobic heterotrophs and facultative oligotrophs, Cell 1 – *Phragmites australis* (Cav.) Trin. ex Steud., Cell 2 - *Canna indica* L., Cell 3 - *P. australis* and *C. indica*, Cell 4 - *Iris pseudacorus* L., *Iris sibirica* 'Perry's Blue', *Alisma plantago - aquatica* L., *Lythrum salicaria* L. and *Menyanthes trifoliata* L., Cell 5 - algae (*Cladophora* sp.).

4. DISCUSSION

Polluted and wastewater contain various pathogens, such as parasites, viruses, and bacteria, which can cause serious diseases in humans and animals (hepatitis A, typhus, cholera, dysentery, etc.). Coliform bacteria (TCB and CBoFO) and intestinal enterococci (IE) are most used as indicators of water contamination by pathogens (National Risk Management Research Laboratory, 2000). The mechanism of pathogen removal itself is not clear enough, but it is assumed that they are removed from water based on physical and biological processes (Stottmeister *et al.*, 2003). In the inflow of polluted water, pathogenic microorganisms are bound to solid particles in the water or are present in it in the form of suspensions (National Risk Management Research Laboratory, 2000). Those that are bound to solid particles are removed from the water through the processes of filtration, sedimentation, adsorption, and aggregation (Ottová *et al.*, 1997). Some of the pathogens, which are free in water, compete with other microorganisms and usually do not survive (National Risk Management Research Laboratory, 2000), because only the microorganisms that can make symbiotic relationships with the root system can survive in the rhizosphere. Within cell 5 with algae, where a larger surface area of free water appeared, pathogen elimination occurred also due to UV radiation (Ottová *et al.*, 1997). The species *P. australis* and *A. plantago* - aquatica are mentioned in the literature as very efficient plants, which, due to root exudates, provide stimulating effects for the growth of colonies of beneficial microorganisms, and inhibition of further pathogen development in polluted water (Stottmeister *et al.*, 2003). Watson *et al.* (1989) state that various engineered biological systems for purification of polluted water have an

exceptional ability to reduce the number of pathogenic microorganisms in water, whereby their efficiency ranges around 90% for coliform bacteria and about 80% for intestinal enterococci.

The results of assessing the efficiency of the modified FTW in this study confirm the general efficiency of biological systems for water treatment, noting that higher efficiency was achieved in modified FTW than the data stated in the literature. Within the cells with floating islands, 100% efficiency in removal of CBoFO was achieved, and pathogen reduction was continued within the cell with algae. In all cells, the efficiency of TCB removal was 100%, except in the cell 4 with decorative aquatic macrophytes, in which the efficiency was 97%. The number of IE was reduced in the range from 92 to 97% in cells with plants, and up to 98% in the cell with algae. The good efficiency of the modified FTW in the removal of various pathogens can be indicated by comparing the number of CBoFO, TCB and IE, as parameters for assessing the ecological status of water (The Official Gazette of the Republic of Serbia, 2011), in inflow and outflow of modified FTW. Based on the number of CBoFO and the number of TCB, the inflow belonged to class V, and based on the number IE to class III (The Official Gazette of the Republic of Serbia, 2012). After the passage of water through the biological system and a significant reduction in the number of pathogenic microorganisms, the outflow of FTW was classified in class I (water with excellent ecological status) (The Official Gazette of the Republic of Serbia, 2012).

Aerobic heterotrophs (H) are indicators of water quality from the aspect of its organic pollution. The high value of these microorganisms indicates waters, which are highly loaded with organic substances that are subject to microbiological decomposition. On the other hand, facultative oligotrophs (FO) are indicators of water less loaded with organic matter. Based on the results of this study the inflow of the FTW belonged to class III, i.e., water with moderate ecological status in terms of number of H (The Official Gazette of the Republic of Serbia, 2012). After the passage through the biological system, based on the reduced number of H, the water had the characteristics of water with good ecological status (The Official Gazette of the Republic of Serbia, 2012). In this way, a shift for one water quality class has been achieved. Based on the ratio of the number of facultative oligotrophs and aerobic heterotrophs (FO / H), the possibility of water self-purification is determined. As can be seen in the tables presented, the value of FO/H was above 1 during the entire water treatment, which indicates a satisfactory possibility of self-purification. In addition, water in which the value of FO/H is above 10, which was the case with the water of outflow of the biological system, belong to the I class of waters (The Official Gazette of the Republic of Serbia, 2011).

5. CONCLUSION

Various types of biological systems, which enable the removal of various types of pollutants from water, are gaining more and more importance not only in scientific research but also in practice. This green technology enables achieving the ecological optimum with the preservation and sustainability of natural resources. Since it does not require high investments, it enables quantitative and qualitative economic, social, and other effects in a long term, so it is also economically

acceptable. On the other hand, secondary and tertiary processes in conventional water treatment plants require a heavy use of technology, energy and various chemicals that additionally pollute the environment, while they can fail in complete removal of pathogens. In this paper, it is shown that the modified FTW has a high efficiency in removing pathogens from the water of a polluted urban river. The results clearly show that the overall achieved efficiency of the biological system in the removal of coliform bacteria of faecal origin and total coliform bacteria is 100%, and for intestinal enterococci 98%. Also, floating islands and algae enabled a high reduction in the number of aerobic heterotrophs and facultative oligotrophs. In addition, the ratio of these microorganisms had a value above 1 during the entire water treatment, which indicated that the natural processes of self-purification of polluted water ran smoothly in the FTW. Due to the reduction of pathogenic microorganisms, water that belonged to class V, i.e., III, after the discharge from the biological system, had the characteristics of water with excellent ecological status (class I). Surface waters belonging to this class, based on limit values of pathogenic microorganisms, provide conditions for the functioning of ecosystems, life and protection of fish, and can be used for drinking water supply with prior treatment by filtration and disinfection, bathing and recreation, irrigation and industrial use.

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THE REMOVAL OF PATHOGENIC MICROORGANISMS IN A BIOLOGICAL SYSTEM WITH FLOATING ISLANDS

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Summary

The most common risk to human health associated with water comes from the presence of microorganisms that cause various diseases. Sewage, agricultural runoff and rainwater as well as household wastewater which are often mixed with each other and discharged into rivers without prior treatment pose a big problem. Such waters carry with them various pathogens and pose a great risk. Various biological systems can remove various pathogenic microorganisms from polluted and wastewater. Removal occurs by imitation of natural processes, without the use of chemicals and additional energy, and due to the symbiotic relationship between the basic components of biological systems such as plants, algae, small invertebrates, zooplankton, microorganisms, substrate, and water. The Topčiderka river has been used for years as a collector of wastewater, rainwater, agricultural, urban and industrial wastewater. Therefore, throughout the year its water is within the limits of class V of waters based on most chemical, physical chemical and microbiological parameters for water quality assessment. To assess the possibility of using biological systems for the treatment of a polluted river, a modified floating treatment wetland (FTW) was constructed on its bank. This paper presents the achieved efficiency of an environmentally friendly and cost-effective solution for the removal of pathogenic microorganisms from water. The biological system consisted of a collection tank, four cells with floating islands and one cell with algae which enabled additional polishing. In each of the four cells there were 3 floating islands. The species Phragmites *australis* (Cav.) Trin. ex Steud, *Canna indica* L., *Iris pseudacorus* L., *Iris sibirica* 'Perry's Blue', *Alisma plantago - aquatica* L., *Lythrum salicaria* L. and *Menyanthes trifoliata* L. were used for forming of island vegetation. The algae were introduced into cell 5 directly from the river. The FTW star-up period lasted a month and a half, after which the monitoring of its efficiency in the removal of pathogenic microorganisms began. The results of this research show that total realised efficiency of the modified FTW in the removal of coliform bacteria of faecal origin and total coliform bacteria is 100%, and 98% for intestinal enterococci. In addition, the floating islands and algae enabled a high reduction in the number of aerobic heterotrophs and facultative oligotrophs. Also, the ratio of these microorganisms had a value above 1 during the entire water treatment, which indicated that the natural processes of self-purification of polluted water ran smoothly in the system. Due to the reduction of pathogenic microorganisms, water that belonged to class V, i.e., class III, after the discharge from the biological system, had the characteristics of water with excellent ecological status (class I).

UKLANJANJE PATOGENIH MIKROORGANIZAMA U BIOLOŠKOM SISTEMU SA PLUTAJUĆIM OSTRVIMA

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Rezime

Najčešći rizik po ljudsko zdravlje povezan sa vodom, potiče od prisustva mikroorganizama, koji izazivaju različite bolesti. Veliki problem predstavljaju kanalizacione, poljoprivredne i kišne otpadne vode, kako i otpadne vode iz domaćinstva, koje se često međusobno mešaju i bez prethodnog tretmana ispuštaju u reke. Ovakve vode sa sobom nose različite patogene i predstavljaju veliki rizik. Različiti biološki sistemi imaju mogućnost da uklanjaju različite patogene mikroorganizme iz zagađenih i otpadnih voda. Uklanjanje se dešava imitacijom prirodnih procesa, bez upotrebe hemikalija i dodatne energije, a zahvaljujući simbiotičkoj vezi između osnovnih komponenti bioloških sistema kao što su biljke, alge, mali beskičmenjaci, zooplankton, mikroorganizmi, supstrat i voda. Topčiderska reka se već godinama koristi kao kolektor otpadnih, kišnih, poljoprivrednih, gradskih i industrijskih voda. Zbog toga se tokom cele godine nalazi u granicama V klase voda na osnovu većine hemijskih, fizičko-hemijskih i mikrobioloških parametara za ocenu kvaliteta voda. Kako bi se ispitala mogućnost korišćenja bioloških sistema za tretman zagađene reke, na njenoj obali je konstruisan modifikovani sistem sa plutajućim ostrvima. Ovaj rad prikazuje postignutu efikasnost ekološki pogodnog i ekonomski isplativog rešenja za uklanjanje patogenih mikroorganizama iz vode. Biološki sistem se sastojao od sabirnog tanka, četiri bazena sa plutajućim ostrvima i jednog bazena sa algama, koji je omogućavao dodatno poliranje. U svakom od četiri bazena su se nalazila po 3 plutajuća ostrva. Za formiranje vegetacije ostrva korišćene su vrste *Phragmites australis* (Cav.) Trin. ex Steud, *Canna indica* L., *Iris pseudacorus* L., *Iris sibirica* 'Perry's Blue', *Alisma plantago - aquatica* L., *Lythrum salicaria* L. i *Menyanthes trifoliata* L. Alge su u bazen 5 unesene direktno iz reke. Period uhodavanja biološkog sistema je trajao mesec i po dana, nakon čega je započet monitoring njegove efikasnosti u uklanjanju patogenih mikroorganizama. Rezultati istraživanja pokazuju da je ukupna ostvarena efikasnost biološkog sistema u uklanjanju koliformnih bakterija fekalnog porekla i ukupnih koliformnih bakterija 100%, a crevnih enterokoka 98%. Takođe, plutajuća ostrva i alge su omogućili visoko smanjenje brojnosti aerobnih heterotrofa i fakultativnih oligotrofa. Pored toga, odnos ovih mikroorganizama je imao vrednost iznad 1 tokom celog perioda prečišćavanja, što je ukazalo da su se u sistemu neometano odvijajali prirodni procesi samoprečišćavanja zagađene vode. Zahvaljujući redukciji patogenih mikroorganizama voda koja je pripadala V odnosno III klasi je po izlasku iz biološkog sistema imala karakteristike vode sa odličnim ekološkim statusom (I klasa).

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VARIABILITY OF HUNGARIAN OAK (*Quercus frainetto* Ten.) FROM THE TERRITORY OF LIPOVICA ACCORDING TO MORPHOLOGICAL TRAITS OF SEEDLINGS

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Abstract: *Natural populations of Hungarian oak in Serbia are found near the northwestern boundary of the range of distribution of this significant forest species. The survival of forest border provenances is endangered due to climate change, small population size, low species competitiveness, etc. Therefore, ancillary measures of population regeneration support are recommended along with broadening of their genetic diversity. Although the knowledge of the levels and structure of genetic diversity of populations is a prerequisite for successful conservation and use, research of this topic regarding Hungarian oak is rare. The analyses of morphological traits of one-year-old seedlings of 40 half-sib families are carried out in order to gain insight into the variability of Hungarian oak population in Lipovica.*

The intrapopulation variability was determined based on two measured morphological parameters and one derived ratio. The obtained results show there is a significant variability of morphological traits of seedlings on the level of half-sib families and they indicate a high phenotypic variability of the researched traits. The results of the analysis of variance show statistically significant differences between the researched half-sib families for all observed traits.

The researched gene pool of Hungarian oak is characterized by a satisfactory degree of genetic variability and represents a good starting point for the process of further breeding. In order to confirm the results of this research, i.e., to determine more precisely genetic structure of the population, it is necessary to perform analyses of various phenotypic traits in specially designed field plantations as well as the analysis of adequate DNA markers.

Key words: seedlings, variability, gene pool, population.

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VARIJABILNOST SLADUNA (*Quercus frainetto* Ten.) SA PODRUČJA LIPOVICE PREMA MORFOLOŠKIM SVOJSTVIMA SADNICA

Izvod: *Prirodne populacije sladuna u Srbiji nalaze se blizu severozapadne granice areala ove značajne šumske vrste. Opstanak rubnih provenijencija ugrožen je usled promene klime, male veličine populacija, niske kompetitivnosti vrste i dr. Zbog toga se preporučuju pomoćne mere potpomaganja obnove populacija uz proširivanje njihove genske raznolikosti. Premda je poznavanje nivoa i strukture genetske raznolikosti populacija preduslov uspešne konzervacije i korišćenja, istraživanja ove tematike na sladunu su retka. U cilju upoznavanju varijabilnosti populacije sladuna u Lipovici izvršene su analize morfoloških svojstava jednogodišnjih sadnica 40 linija polusrodnika.*

Na osnovu dva merena morfološka parametara i jednog izvedenog odnosa utvrđena je unutarpopulaciona varijabilnost. Dobijeni rezultati su pokazali postojanje značajne varijabilnosti morfoloških svojstava sadnica na nivou linija polusrodnika i ukazuju na visoku fenotipsku varijabilnost istraživanih svojstava. Rezultati analize varijanse pokazuju statistički značajne razlike između istraživanih linija polusrodnika za sva posmatrana svojstva.

Istraživani genofond sladuna odlikuje se zadovoljavajućim stepenom genetičke varijabilnosti i predstavlja dobru polaznu osnovu za proces daljeg oplemenjivanja. Radi potvrde rezultata ovog istraživanja odnosno preciznije determinacije genetske strukture populacije, neophodno je sprovesti analiza raznovrsnih fenotipskih svojstava u posebno dizajniranim poljskim zasadima kao i analiza prikladnih DNA markera.

Ključne reči: sadnice, varijabilnost, genofond, populacija.

1. INTRODUCTION

Hungarian oak (*Quercus frainetto* Ten.) is an oak species growing in thermophilic deciduous forest in southeastern Europe. Palaeoecological proofs suggest that Hungarian oak was present in Peloponnese more than 6000 years ago (Jahns, 1993). Previous studies of genetic diversity of oak forests have shown that *Q. frainetto* belongs to *Dascia* section, it is genetically close to *Q. pubescens* and clearly differentiated from *Q. robur* and *Q. petraea* (Finkeldey, 2001; Petit et al., 2002; Belletti et al., 2005; Curtu et al., 2007; Fortini et al., 2015). It grows in a wide vegetation belt rich in woody species, mostly in habitats with humid continental climatic conditions, frequent summer droughts, highest amount of precipitation in spring, wide temperature range, low winter temperatures (Horvat et al., 1974). As one of the edificators, Hungarian oak in habitats in Serbia occurs together with Turkey oak in the climatogenic community of Hungarian and Turkey oak *Quercetum frainetto-cerridis* Rudski 1949. A number of sub-associations representing various ecological variants of Hungarian oak and Turkey oak have been described (Stajić et al., 2008) and several geographic variants within the broadly understood forest of Hungarian oak and Turkey oak (Tomić and Rakonjac, 2013). It is in contact with populated places and agricultural land, so it is under constant pressure, which leads to a gradual reduction of the area of these forests. Due to the anthropogenic influence in the last century, the areas under Hungarian oak forests have been significantly reduced, and that has had a very negative effect

on its genetic diversity. Natural regeneration of Hungarian oak is very difficult, it is mainly regenerated vegetatively, which additionally affects the reduction of genetic diversity. In addition, in competition with Turkey oak as biologically stronger species, the regeneration of Hungarian oak is insufficient, so it is necessary to fill in the gaps, reestablish its participation in establishing stands or replace it by the reproductive material originated from selected seed sources.

Genetic improvement programs include individual selection of plus trees from the most valuable populations and testing of their offspring in comparative trials at different sites (Wright, 1976). The long-term survival of species is closely related to their genetic diversity (Gapare, 2014). Under the influence of changed environmental conditions, biotic pathogens and damage, the survival and evolution of species depend on the levels of genetic diversity (Reed and Frankham, 2003). Research dealing with genetic diversity and identifying populations with high genetic variability can help reduce the risk of biodiversity loss (Souto et al., 2015). The increase in the adaptability of the species through genetic improvement could greatly increase the ecological, economic and social values of Hungarian oak as well as improve the conservation of this species in its natural habitat (Cetera et al., 2018). In order to create a basis for the preservation of Hungarian oak gene pool and the directed management of available resources, detailed studies of the level, patterns and causes of genetic diversity and structure are necessary.

The aim of the research in this paper was to determine the intrapopulation variability of Hungarian oak according to the morphological traits of one-year-old seedlings. The obtained results can be used for preliminary insight in genetic variability of the researched population, improvement of production of nursery stock and continuation of research on genetic diversity.

2. MATERIAL AND METHODS

For the research in this paper acorns were collected from the harvest of 2019, originating from Lipovica Forest Management Unit, of Belgrade Forest Enterprise. Based on phenotypic traits and abundance of yields 40 plus trees evenly distributed on the area of FMU were selected, whereby the minimal distance between the trees was at least 50 m in order to avoid relatedness. Seeds were collected from trees in order to grow half-sib families where mother is known, but not the other parent, according to the method of genetic analysis of trees (Isajev and Mančić, 2001). Three kilograms of ocularly healthy and undamaged acorns were collected per each tree, regardless of their dimensions. After collection the acorns were dried at 35% of humidity and stored at a temperature of 3-5 ºC. The acorns were sawn in April 2020 separately per plus trees in the nursery of the Institute of Forestry in Belgrade. In uniform environmental conditions, seedlings were produced in a bed measuring 1x40 m. The distance between the rows in a bed is 15 cm and rows are parallel with the shorter side of the bed.

At the end of the first growing season, root collar diameter and the height of the seedlings were measured on a random sample of 50 seedlings per plus tree and based on the measured values the vigor index according to Roller 1977 was calculated. Root collar diameter was measured by vernier caliper with the accuracy of 0.1 mm, and the height of the seedlings with a ruler, with accuracy of 0.5 mm.

Morphological traits of the seedlings were described by means of descriptive statistical indicators: arithmetic mean (x), standard deviation (SD), coefficient of variability (CV %). For the purpose of determining intrapopulation variability the analysis of variance (ANOVA) was used. The analyzed variability factor was a tree. All the above-mentioned statistical analyses were performed using the statistical program STATISTICA 7.0 (StatSoft Inc. 2004).

3. RESULTS AND DISCUSSION

Table 1 shows the parameters of descriptive statistics for the researched properties of seedlings.

The mean value of the root collar diameter of the studied half-sib families amounts to 3.7 mm and ranges from 2.6 mm (half-sib family 40) to 5.2 mm (half-sib family 12) with a standard deviation from 0.4 mm to 1.5 mm. According to the parameter root collar diameter the most homogenous is a half-sib family 5 (13.4%), and the most heterogenous half-sib families are 23 and 27 (36.1%).

The mean value of seedling height based on the studied half-sib families amounts to 11.4 cm and ranges from 6.6 cm (half-sib family 40) to 18.5 cm (half-sib family 1) with a standard deviation ranging from 1.0 to 7.2. According to the seedling height parameter the most homogenous is a half-sib family 7 (11.8%), and the most heterogenous half-sib family is 36 (57.5%).

The mean value of the vigor index of the seedlings amounts to 3.1 and ranges from 2.1 (half-sib families 6 and 10) to 4.1 (half-sib family 4) with a standard deviation ranging from 0.3 to 1.8. According to the parameter vigor index of seedlings the most homogenous is a half-sib family 3 (10.6%), and the most heterogenous is a half-sib family 4 (45.4%).

The most variable trait on the level of the studied half-sib families is a seedling height (41.3%), while root collar diameter is the least variable trait (29.1%).

Table 1. *Descriptive statistics for the measured morphological traits of seedlings*

Tree	d (mm)			h (mm)			h/d		
	M	SD	CV	M	SD	CV	M	SD	CV
1	4.7	0.8	16.5	18.5	6.5	35.4	3.9	1.0	26.8
2	4.9	0.9	18.4	15.8	4.4	27.7	3.3	1.0	30.7
3	4.1	0.6	15.9	13.4	2.3	17.5	3.3	0.3	10.6
4	3.2	0.6	17.7	12.4	4.7	37.7	4.1	1.8	45.4
5	3.0	0.4	13.4	8.4	1.7	20.4	2.9	0.7	23.0
6	4.0	1.4	34.8	8.3	2.4	28.8	2.1	0.4	18.0
7	2.8	0.6	22.3	8.4	1.0	11.8	3.2	0.9	29.0
8	3.6	0.9	24.4	13.7	4.7	34.2	3.8	1.1	29.4
9	4.1	0.8	20.3	12.5	2.7	21.7	3.1	0.7	21.2
10	3.6	1.0	28.0	7.5	2.5	33.6	2.1	0.4	19.8
11	3.1	0.7	23.7	10.3	2.2	21.7	3.4	0.6	18.4
12	5.2	1.5	29.9	17.4	5.7	33.0	3.4	0.7	21.0
13	4.1	1.0	24.9	11.6	2.7	23.3	3.0	1.0	34.7
14	3.9	1.2	29.8	9.4	3.3	35.5	2.5	0.7	29.3
15	3.4	1.0	29.1	10.2	3.3	32.5	3.1	0.6	20.9
16	4.4	1.4	31.8	13.8	6.0	44.0	3.2	0.9	29.3
17	3.8	1.0	27.0	10.9	2.6	24.4	3.0	0.7	21.9

18	**3.5**	**1.1**	**31.9**	**10.8**	**3.1**	**28.6**	**3.1**	**0.6**	**17.8**
19	3.5	0.8	23.7	13.4	5.6	42.3	3.7	0.9	24.5
20	3.7	0.8	20.2	11.7	2.4	20.2	3.2	0.6	18.5
21	3.9	0.8	19.4	11.0	3.3	30.2	2.9	0.8	28.2
22	3.6	0.8	22.3	12.5	5.3	42.2	3.5	1.0	28.6
23	4.0	1.4	36.1	11.9	2.0	16.7	3.2	0.9	28.3
24	3.6	0.7	18.7	9.1	2.5	27.9	2.6	0.6	23.4
25	4.3	0.7	16.9	15.7	7.1	45.3	3.6	1.2	33.8
26	3.1	0.6	20.4	8.1	2.2	27.8	2.6	0.6	24.5
27	3.7	1.3	36.1	8.7	3.5	40.1	2.4	0.5	21.2
28	3.0	0.5	15.3	7.7	2.0	26.3	2.6	0.7	27.8
29	4.0	0.8	19.4	12.4	5.4	43.4	3.0	1.0	32.2
30	3.4	1.2	34.6	11.2	3.0	26.6	3.5	0.6	17.6
31	3.8	0.8	22.1	11.5	5.1	44.3	3.0	1.2	38.1
32	4.0	1.3	32.4	13.1	7.2	54.9	3.2	0.9	27.8
33	4.1	1.2	29.8	14.7	4.9	33.4	3.7	1.3	34.2
34	2.9	0.8	29.4	8.8	1.9	21.8	3.2	0.7	22.0
35	3.7	1.3	35.6	11.3	4.0	35.0	3.2	1.0	32.2
36	3.3	0.8	25.6	8.9	5.1	57.5	2.6	1.0	38.0
37	3.8	1.1	29.4	11.7	5.0	43.0	3.0	0.7	23.3
38	4.1	1.0	24.9	14.7	4.6	31.2	3.7	0.9	23.8
39	3.5	1.0	28.0	10.3	3.6	34.8	3.0	0.6	20.5
40	2.6	0.8	32.1	6.6	1.4	21.1	2.8	0.8	28.8
Mean	3.7	1.1	29.1	11.4	4.7	41.3	3.1	0.9	30.2

Legend: d-root collar diameter; h- seedling height; h/d- height and root collar diameter ratio

Table 2. *Analysis of variance for the measured morphological traits of seedlings*

Parameter	SS Effect	df Effect	MS Effect	F	p
d	119.077	39	3.053	3.192	0.0000
h	2968.76	39	76.12	4.624	0.0000
h/d	83.162	39	2.132	2.842	0.0000

Based on the obtained results of the analysis of variance (ANOVA) it can be concluded that statistically significant differences have been determined between the studied half-sib families for all analyzed morphological traits of seedlings. (Table 2).

The obtained results indicate there is a significant level of phenotypic variability on the level of half-sib families, taking in consideration the researched morphological traits of seedlings. The researched population of Hungarian oak is characterized by a satisfactory degree of genetic variability so it can be used in breeding processes and it is a potential source of a reproductive material of a good quality. Statistically significant differences determined between individual parent trees (genotypes) in the analyses of morphological traits of acorns have also indicated a high level of intrapopulation genetic variability (Popović et al. 2020). For the needs of afforestation and auxiliary measures of natural regeneration of Hungarian oak, acorns are used and seedlings are produced in a generative way, which is why the properties and quality of reproductive material are among the key factors for achieving success in these ventures.

The knowledge on genetic diversity and population structure is the basis for preserving and improving the gene pool of a species. So far, modest research has been done to determine the genetic diversity of Hungarian oak populations in Serbia, so there is a real need for that. The analyses of the amount and distribution

of genetic variability within and among populations can be used to determine genetic diversity and establish programs for the conservation of genetic resources (Dumolin-Lapegue et al., 1997). The distribution of genetic diversity within and among populations is a function of gene flow rates among populations, and the level of gene flow depends on the spatial distribution of habitats, size and degree of isolation of populations, pollen and seed movements between populations (Bruschi et al. 2003). Great genetic diversity of populations is very important for survival and stability, but in addition, adequate ecological management is necessary for their preservation and in situ conservation (Bruschi et al. 2003). The adaptability of forest tree populations to climate change is largely determined by individual levels of relatedness (Lloret and García, 2016). Uncontrolled use of resources and felling of trees, which result in a decrease in population size, increase the risk of loss of genetic resources and long-term survival of populations (Gilpin and Soule, 1986). In small populations, reduced genetic diversity may be the result of genetic drift, and in subsequent generations, loss of phenomena heterozygosity and inbreeding may lead to reduced condition in these otherwise stable populations (Bruschi et al., 2003). Genetically improved stands and populations with drought tolerance will be highly valued in the future (Apostol et al., 2020).

Based on the performed research, it can be concluded that in the researched population of Hungarian oak there is a high level of variability of the researched morphological traits of seedlings. The obtained results are the basis for the continuation of research that needs to be conducted in order to provide guidelines and recommendations for the conservation and directed use of the genetic resources of Hungarian oak in this population and in the territory of Serbia. For more complete understanding of variability of Hungarian oak in part of its natural range of distribution the research should be continued and broadened using molecular markers and analyses of various phenotypic traits in specially designed experimental plantations.

4. CONCLUSIONS

The obtained results provide a preliminary assessment of the genetic variability of the researched plus trees and contribution to the knowledge of the analyzed properties of seedlings and represent a good starting point for future research in species breeding.

On the level of the researched half-sib families the following mean values were obtained: root collar diameter amounts to 3.7 mm (ranging from 2.6 to 5.2 mm), the height of seedlings amounts to 11.4 cm (ranging from 6.6 to 18.5 cm), and vigor index of seedlings amounts to 3.1 (ranging from 2.1 to 4.1).

The occurrence of statistically significant differences for the analyzed morphological traits of seedlings on the level of half-sib families clearly indicates there is a genetic differentiation and high level of intrapopulation variability.

The determined variability of morphological characteristics of one-year-old seedlings can serve as an indicator of further development of seedlings of selected half-sib families and for improvement of production of Hungarian oak reproductive material of good quality.

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VARIABILITY OF HUNGARIAN OAK (*Quercus frainetto* Ten.) FROM THE TERRITORY OF LIPOVICA ACCORDING TO MORPHOLOGICAL TRAITS OF SEEDLINGS

Vladan POPOVIĆ, Aleksandar LUČIĆ, Ljubinko RAKONJAC, Sanja JOVANOVIĆ, Ivica LAZAREVIĆ

Summary

Hungarian oak (*Quercus frainetto* Ten.) is an oak species growing in thermophilus deciduous forests in southeastern Europe. Palaeoecological proofs suggest that Hungarian oak was present in Peloponnese more than 6000 years ago. Natural regeneration of Hungarian oak is very difficult, it is mainly regenerated vegetatively, which additionally affects the reduction of genetic diversity. In addition, in competition with Turkey oak as biologically stronger species, the regeneration of Hungarian oak is insufficient, so it is necessary to fill in the gaps, reestablish its participation in establishing stands or replace it by the reproductive material originated from selected seed sources.

The aim of the research in this paper was to determine the intrapopulation variability of Hungarian oak according to the morphological traits of one-year-old seedlings.

For the research in this paper acorns from the harvest of 2019 were used, originating from Lipovica Forest Management Unit, of Belgrade Forest Enterprise, Belgrade. Based on phenotypic characteristics and abundance of yields 40 plus trees evenly distributed on the area of FMU were selected. In the nursery of the Institute of Forestry in Belgrade, in uniform environmental conditions, seedlings were produced separately per plus trees. At the end of the first growing season, root collar diameter and the height of the seedlings were measured on a random sample of 50 seedlings per plus tree and based on the measured values the vigor index was calculated.

Based on the researched half-sib families, the following mean values were obtained: root collar diameter amounted to 3.7 mm (ranging from 2.6 to 5.2 mm), height of seedlings amounted to 11.4 cm (ranging from 6.6 to 18.5 cm), vigor index of seedlings amounted to 3.1 (ranging from 2.1 to 4.1).

The results of the analysis of variance (ANOVA) showed that the researched half-sib families differ significantly for all analyzed morphological traits of seedlings, which indicates a high level of intrapopulation genetic variability.

The obtained results contribute to the knowledge on the analyzed traits of seedlings, preliminary assessment of genetic variability of the researched plus trees and represent a good starting point for future research on species breeding, and also can serve for improvement of production of Hungarian oak reproductive material of good quality.

VARIJABILNOST SLADUNA (*Quercus frainetto* Ten.) SA PODRUČJA LIPOVICE PREMA MORFOLOŠKIM SVOJSTVIMA SADNICA

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Rezime

Sladun (*Quercus frainetto* Ten.) je vrsta hrasta koja raste u termofilnim listopadnim šumama u jugoistočnoj Evropi. Paleoekološki dokazi sugerišu da je sladun bio prisutan na Peloponezu pre više od 6000 godina. Prirodno obnavljanje sladuna je veoma otežano, uglavnom se obnavlja vegetativnim putem, što dodatno utiče na smanjenje genetičkog diverziteta. Osim toga u konkurenciji cera kao biološki jače vrste, obnavljanje sladuna je nedovoljno, pa je potrebno popunjavanje praznina, ponovno uspostavljanje učešća u izgradnji sastojina ili zamene reproduktivnim materijalom koji potiče iz odabranih semenskih izvora.

Cilj istraživanja u ovom radu bio je da se utvrdi unutarpopulaciona varijabilnost sladuna prema morfološkim svojstvima jednogodišnjih sadnica.

Za istraživanja u ovom radu upotrebljen je žir iz uroda 2019. godine, poreklom iz GJ „Lipovica“, ŠG „Beograd“ Beograd. Na osnovu fenotipskih karakteristika i obilnosti uroda izdvojeno je 40 materinskih stabala ravnomerno raspoređenih po površini gazdinske jedinice. U rasadniku Instituta za šumarstvo u Beogradu, u ujednačenim uslovima sredine proizvedene su sadnice odvojeno po materinskim stablima. Na kraju prvog vegetacionog perioda na slučajnom uzorku od 50 sadnica po materinskom stablu izmereni su prečnik u korenovom vratu, visina sadnica i izračunat je koeficijent jedrine.

Na nivou istraživanih linija polusrodnika dobijene su sledeće prosečne vrednosti: prečnik u korenovom vratu iznosi 3,7 mm (od 2,6 do 5,2 mm), visina sadnica iznosi 11,4 cm (od 6,6 do 18,5 cm), koeficijent jedrine sadnica iznosi 3,1 (od 2,1 do 4,1).

Rezultati analize varijanse (ANOVA) pokazuju da se istraživane linije polusrodnika statistički značajno razlikuju za sva analizirana morfološka svojstva sadnice što ukazuje na visok nivo unutarpopulacone genetske raznolikosti.

Dobijeni rezultati daju doprinos poznavanju analiziranih svojstava sadnica, preliminarnu procenu genetičke varijabilnosti proučavanih materinskih stabala i predstavljaju dobru polaznu osnovu za buduća istaražiavanja u oplemenjivanju vrste, a mogu poslužiti i za unapređenje proizvodnje kvalitetnog repromaterijala sladuna.

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MORPHOLOGICAL VARIABILITY OF WILD SERVICE TREE (*Sorbus torminalis* (L.) Crantz) FRUIT AND SEEDS FROM THE AREA OF KOŠUTNJAK

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Abstract: *Wild service tree (Sorbus torminalis (L.) Crantz) is an autochthonous deciduous species that have highly valuated timber. It often occurs individually in oak and beech forests, but it is necessary to monitor the intensity of regeneration and presence in these forests. The fruits of this species are edible and medicinal, usually contain 2-4 seeds, which are characterized by dormancy. The fruits and seeds collected from trees from the area of Košutnjak (Belgrade, Serbia) were used in this study. This study aimed to determine the variability of fruits and seeds in the population. Length and width of fruit and also determined length, width, and thickness of the seed were measured. The dimensions of fruits and seeds from this population were by results reported in previous studies. The obtained results showed clear differences between trees in morphological attributes of fruits and seeds (OneWay ANOVA, p<0,05). The average fruit contained more than two seeds and a maximal number of seeds per one fruit was 5. At one tree were recorded a high number of empty fruits. The obtained results indicate a high degree of genetic variability in this population, but also indicate the need for testing with other morphological and molecular markers.*

Key words fruits and seeds, variability, morphological markers.

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VARIJABILNOST MORFOLOŠKIH KARAKTERISTIKA PLODOVA I SEMENA BREKINJE (*Sorbus torminalis* (L.) Crantz) SA PODRUČJA KOŠUTNJAKA

Izvod: *Brekinja (Sorbus torminalis* (L.) Crantz*) je autohtona vrsta lišćara koja ima veoma cenjeno i vredno drvo. Često se stablimično javlja u hrastovim i bukovim šumama, ali neophodno je pratiti intenzitet njenog obnavljanja i zastupljenost u ovim šumama. Plodovi ove vrste su jestivi i lekoviti, sadrže obično 2-4 semena koja karakteriše dormantnost. U radu su korišćeni plodovi i semena brekinje sakupljeni sa stabala koja rastu na području Košunjaka (Beograd, Srbija). Cilj ovog istraživanja je da se utvrdi varijabilnost plodova i semena brekinje u ovoj populaciji. Plodovima je izmerena dužina i širina i određen je broj semena koji se nalazi u plodu, a semenu su mereni dužina, širina i debljina. Dimenzije plodova i semena iz ove populacije nalaze se u opsegu vrednosti koje su prijavljene u ranijim istraživanjima. Dobijeni rezultati su pokazali da postoje jasne razlike između stabala u krupnoći plodova i semena (OneWay ANOVA, p<0,05). Broj semena u plodu maksimalno je iznosio 5, a u proseku se u plodu nalazilo više od dva semena. Kod jednog stabla je zabeležen veliki broj plodova bez semena. Dobijeni rezultati ukazuju na visok stepen genetičke varijabilnosti u ovoj populaciji, ali i ukazuju na potrebu za testiranjem drugim morfološkim i molekularnim markerima.*

Ključne reči: brekinja, plodovi i seme, varijabilnost, morfološki markeri.

1. INTRODUCTION

Wild service tree (*Sorbus torminalis* (L.) Crantz) is a diploid (2n=34), monoecious, heliophytic, fast-growing species, widespread in southern, western, and central Europe, northwestern Africa, and southeast Asia (Welk et al., 2016), with an altitude from 100 m.a.s.l. (Romania) to 2200 m.a.s.l. (Turkey). Current occurrence centers of this species are located in France and the Balkan region (Nicolescu et al., 2009). The limiting factor in the vertical distribution of this species is the amount of heat in the summer (Welk et al., 2016). In stands, it occurs individually (in the beech and pine forests) and in groups (in the oak forests) with an occurrence of 0.1 to 30 individuals/ha (Demesure-Musch, Oddou-Muratorio, 2004). This tree is widely indigenous in Serbia, represented as an accompanying species in the oak and beech forests up to 1500 m a.s.l. (Cvjetićanin and Perović, 2000). In terms of endangerment, it is categorized as a species "at-risk" (Banković et al., 2009), due to habitat fragmentation, possible disturbance of the genetic structure of populations, difficult natural regeneration, and poor competitiveness of the species (Demesure-Musch, Oddou-Muratorio, 2004).

Forest ecosystems are exposed to a constant process of fragmentation due to the implementation of other forms of land use (Tyrväinen, Miettinen, 2000). This type of anthropopresure is especially present in urban forest ecosystems (Raskovic, 2015), such as Kosutnjak. Forest areas in cities have a special value since they are one of the last refuges of flora and fauna and as such represent important ecosystems in terms of preserving diversity and integrity of nature with optimal land use to improve environmental quality (Milovanović et al., 2010). As one of the species of forest fruit trees, wild service tree represents an important

ecosystem component from the aspect of biodiversity, nutrition of ornithofauna, and landscape appearance of the Košutnjak forest.

Wild service tree blooms during late spring and forms fruits that ripen in the fall of the same year. The flowers are hermaphroditic, and pollination is entomophilic. The fruits are apparent, ellipsoid (pear-shaped), brown with light spots (Cvjetićanin and Perović, 2000). According to Aldasoro et al. (1998), the fruit has a multilayered epidermis, which is a morphological characteristic that is unknown in other *Sorbus* species. The seed is located inside the fleshy fruit, has a pronounced dormancy of the embryo, and, as with other species of the genus *Sorbus*, characterized by the absence of endosperm (Regent, 1980). The fruits are edible, and their medical properties are well known. They are common food for birds, especially thrushes (*Turdus sp.*), which play a significant role in spreading seeds over long distances and thus gene exchange between populations (Demesure et al., 2000).

Given all the above factors, it is necessary to pay attention to its presence and monitor the potential and intensity of natural regeneration of this species, especially in urban forest complexes that are exposed to intense anthropogenic pressure. In this paper, we investigated the morphological characteristics of fruits and seeds from the population in the area of Košutnjak in Belgrade. The conducted research will give a clearer picture of the genetic diversity and morphological characteristics of fruits and seeds in this area.

2. MATERIAL AND METHOD OF WORK

The fruits were collected in the period 20 to 25 September 2020. from 5 wild service trees growing at Košutnjak area. The presence of 25 adult trees was recorded in this area (Picture 1). Collecting of seeds was possible only from 5 trees, which in the field were marked with the numbers BR10, BR11, BR13, BR14, and BR15 (Table 1).

Košutnjak forests are administratively located in two management units: MU "Košutnjak", which is managed by PE "Srbijašume" and MU "Šuma Košutnjak - Trim staza", which is managed by the Institute for sport and sports medicine of the Republic of Serbia. The total area of these management units is 346.74 ha (305.97 ha and 40.77 ha, respectively). Trees selected as test trees in this study grow in the area of MU "Šuma Košutnjak - Trim staza".

The fruits were collected by picking from the branches of the tree. The collected fruits (Picture 2) were stored in a plastic bag in the refrigerator for 2 weeks. From each of the 5 trees, 40 fruits were selected and length and width were measured, as well as the number of seeds in each fruit. The seeds were extracted from fruits and used for seed length, seed thickness, and seed width measurements (Figure 3). All measurements were performed using a digital nonius, with an accuracy of 0.1 mm. The obtained data were processed in Statistica 7.0 software. Mean values of measured characteristics, also standard deviation, and minimum and maximum values were calculated for observed morphological characteristics of fruits and seeds. Differences between trees were tested using one-factor analysis of variance (OneWay ANOVA, $p < 0.05$), and grouping was performed by Tukey

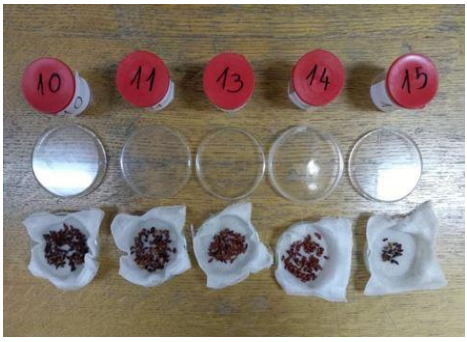
HSD post-hoc test. The performed dendrogram cluster analysis was used to observe the morphological closeness between the tested trees.

Figure 1. *Map of trees on Košutnjak*

Table 1. *Marks, coordinates, dimensions and yield abundance for recorded trees*

Mark on the map	Geographical longitude	Geographical latitude	Tree diameter (cm)	Tree height (m)
B10	44753083	20436712	25.5	9.0
B11	44753680	20436723	29.3	11.0
B13	44768890	20427299	36.0	8.5
B14	44768747	20427206	34.5	9.0
B15	44767881	20427940	31.2	12.0

Picture 2. *Sampled fruits*



Picture 3. *Extracted seeds from sampled fruits*

3. RESULTS

3.1. Fruits morphological characteristics

According to the morphological characteristics of the fruits (Table 2), tree BR13 had the highest mean values of fruit length (13.33 mm), and maximum fruit length (17.3 mm). The fruits from tree BR14 were the widest according to the average values of fruit width (10.66 mm). The lowest mean value of fruit length was recorded for fruits from tree BR11 (11.29 mm), while the lowest mean value of fruit width was in fruits from tree BR15 (7.80 mm). On average, the largest number of seeds in the fruit were observed in fruits originating from tree BR10 (2.46) and BR14 (2.51), and at least in tree number BR15 (1.14). A maximum of 5 seeds per fruit was extracted from fruits originating from trees BR10 and BR14. Fruits without seeds were recorded in all trees. The highest number of fruits without seeds was recorded in tree number BR15.

Table 2. *Mean (standard deviation), maximum and minimum values of the observed characteristics of fruits*

Tree N=200	Fruit length (mm)		Fruith width (mm)		Number of seeds in fruit	
	Mean value* (sd)	min-max	Mean value* (sd)	min-max	Mean value* (sd)	min-max
BR10	14.36^{c} (1.20)	11.60-16.30	8.75^{a} (0.99)	6.90-10.50	2.46^{a} (1.31)	0.00-5.00
BR11	11.29^{a} (0.89)	8.80-12.60	8.91^{a} (0.97)	7.10-11.40	2.05^{a} (1.06)	0.00-4.00
BR13	13.33^{b} (1.35)	10.60-17.30	10.04^{b} (1.21)	7.00-11.70	2.03^{a} (1.28)	0.00-4.00
BR14	12.98^{b} (0.93)	10.00-14.40	10.66^{b} (1.04)	7.30-11.90	2.51^{a} (1.37)	0.00-5.00
BR15	11.91^{a} (1.04)	9.30-14.20	7.80^{c} (0.77)	7.00-10.10	1.14^{b} (0.67)	0.00-3.00

**Tukey HSD post-hoc test, $p<0.05$*

3.2. Seeds morphological characteristics

From 200 fruits (5 trees × 40 fruits) were extracted a total of 343 seeds (Figure 4). The mean value of seed length is highest in tree BR10 (7.26 mm) and lowest in tree BR11 (5.40 mm). The average thickness is highest at seeds from tree BR13 (2.39 mm), and the smallest in tree BR10 (1.94 mm) and BR15 (1.94 mm). The highest mean value of seed width was recorded in seeds from tree BR14 (3.83 mm), and the smallest at tree BR15 (2.71 mm). The longest seed is recorded in tree BR10 (5.90-8.60 mm), and the shortest in tree BR11 (4.10-6.90 mm). The thickest seeds were recorded at trees BR13 (1.20-3.40 mm) and BR14 (1.40-3.30 mm), and the thinnest at tree BR15 (1.00-2.70 mm). The largest variations in thickness were observed in tree BR11 (0.50-4.50 mm). The widest seeds were recorded at tree BR13 (2.80-4.80 mm), and at the opposite is tree number BR15 which has the narrowest seed (2.10-3.90 mm).

Table 3. *Mean (standard deviation), maximum and minimum values of the observed characteristics of seeds*

Tree N=343	Seed length (mm)		Seed thickness (mm)		Seed width (mm)	
	Mean value* (sd)	min-max	Mean value* (sd)	min-max	Mean value* (sd)	min-max
BR10	7.26^{c} (0.53)	5.90-8.60	1.94^{b} (0.37)	0.90-2.80	3.42^{b} (0.37)	2.00-4.30
BR11	5.40^{b} (0.58)	4.10-6.90	2.27^{ac} (0.67)	0.50-4.50	3.60^{ab} (0.51)	2.40-5.10
BR13	6.56^{a} (0.65)	5.40-7.90	2.39^{a} (0.42)	1.20-3.40	3.75^{a} (0.45)	2.80-4.80
BR14	6.22^{a} (0.46)	5.10-7.20	2.31^{a} (0.36)	1.40-3.30	3.83^{a} (0.43)	2.80-4.60
BR15	6.11^{a} (0.56)	4.80-7.00	1.94^{bc} (0.47)	1.00-2.70	2.71^{c} (0.42)	2.10-3.90

** Tukey HSD post-hoc test, p <0.05*

3.3. Differences between trees in the observed morphological characteristics of fruits and seeds

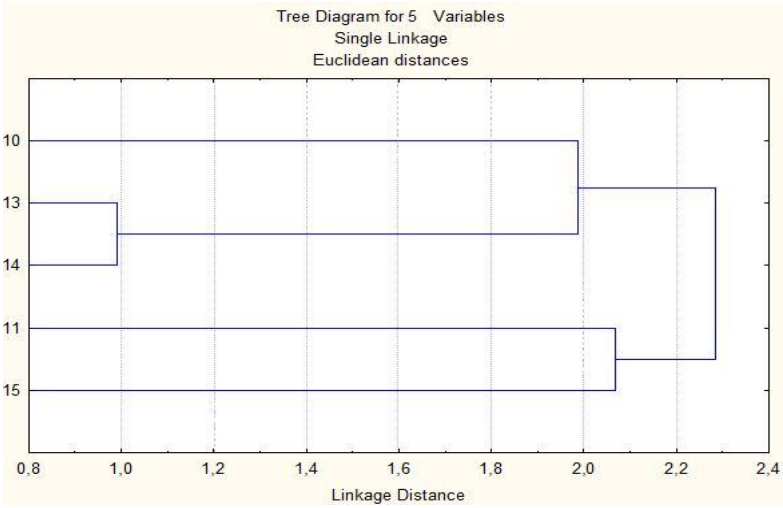
Analysis of variance for observed characteristics of the fruits (length and width of fruit and number of seeds in fruits) and seeds (length, thickness and width of seed) shows significant differences between the trees (Table 4). Fruits and seeds originating from one tree were separated in relation to other trees, and all trees differ significantly in the observed morphological characteristics of fruits and seeds.

Grouping according to the Tukey post-hoc test showed a large number of groups for each of the observed morphological characteristics of fruits and seeds (Table 2 and Table 3).

Table 4. *Analysis of variance for the observed characteristics of fruits and seeds (OneWay ANOVA, p <0.05)*

Parameter	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Fruit length	225.33	4	56.33	234.79	195	1.20	46.7858	0.0000
Fruit width	150.47	4	37.62	196.77	195	1.01	37.2786	0.0000
Number of seeds in fruit	73.83	4	18.46	264.93	195	1.36	13.5858	0.0000
Seed length	159.89	4	39.97	102.19	338	0.30	132.2051	0.0000
Seed thickness	13.04	4	3.26	75.32	338	0.22	14.6343	0.0000
Seed width	22.79	4	5.70	65.02	338	0.19	29.6226	0.0000

The dendrogram of cluster analysis (Graph 1) indicates the separation two groups of trees according to the observed morphological characteristics of fruits and seeds. In one group there are tree BR13 and BR14 which are grouped at a relatively short distance. Tree number BR10 is later connected with this group. Trees number BR11 and BR15 are grouped at a relatively large distance forming a separate group.



Graph 1. *Dendrogram of cluster analysis*

4. DISCUSSION

McAllister (2005), considers that the length of fruit can be very important and that it is more stable than the width, which varies depending on the availability of water and the number of viable seeds. In this study was found that there is equal variation, both in fruit length and fruit width. However, Bednorz (2006) states that the width of the fruit is more stable than the length, despite large variations in the number of seeds. Most often, in the examined populations, the fruits contained two seeds, but also fruit without seeds, and even fruits with six seeds each. The average number of seeds was 2-4 per fruit in our research, but a large number of fruits without seeds were recorded in fruits from tree 15. In several cases, 5 seeds per fruit were recorded (for seeds from trees 10 and 14). There are usually 2-4 seeds in one fruit (McAllister, 2005), which is confirmed in this research. Orsanić et al. (2009), recorded slightly larger fruits in three Croatian populations, but at the same time, the average number of seeds in yield from these populations was lower than in our study, and in all three populations it had a mean value below 2 seeds per fruit.

The measured values of seed length, thickness, and width in this study are in the range reported by Bednorz (2006), for seeds originating from 13 populations of this species in Poland. Research conducted in Bosnia and Herzegovina also shows a similar range of variation in the length and width of seeds (Hodzic et al., 2016).

Based on all observed morphological characteristics of yield and seeds, we can conclude that the most similar trees are 13 and 14, which are located close to each other in the field (Graph 1). However, we cannot conclude that the similarities and differences between the trees have a direct connection with the spatial arrangement of trees because these trees are most similar to tree 10, which is also at a greater distance from them compared to tested trees 11 and 15. A high degree of intrapopulation variability in populations in Europe has been observed using

different molecular markers, and an overview of these studies is given in note (Hodzic et al., 2016). The results of our study indicate high intrapopulation variability.

5. CONCLUSION

Fruit dimensions are not necessarily an indicator of the number of seeds per fruit, but according to the average values, the largest fruits also contained the largest number of seeds on average. Morphological characteristics of fruits and seeds from trees growing in the area of Košutnjak showed a high degree of variability. All observed traits are in the range of values reported in previous studies. Differences between trees indicate a high level of genetic diversity that should be examined on a larger sample and using other morphological and molecular markers.

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MORPHOLOGICAL VARIABILITY OF WILD SERVICE TREE (*Sorbus torminalis* (L.) Crantz) FRUIT AND SEEDS FROM THE AREA OF KOŠUTNJAK

Slavko PAVLOVIĆ, Ivona KERKEZ JANKOVIĆ, Jovana DEVETAKOVIĆ, Mirjana ŠIJAČIĆ-NIKOLIĆ

Summary

Wild service tree (*Sorbus torminalis* (L.) Crantz), (2n = 34), is heliophytic, fast-growing species, widespread in southern, western, and central Europe, northwestern Africa, and southeast Asia from 100-2200 m.a.s.l. In stands, it occurs individually (in the beech and pine forests) and in groups (in the oak forests). This tree is widely indigenous in Serbia, categorized as a species "at-risk", and represented as an accompanying species in the oak and beech forests up to 1500 m a.s.l. As one of the species of forest fruit trees, wild service tree represents an important ecosystem component, especially in urban forests ecosystems, which are under the high rate of antropopressure.

In spring this species forms hermaphroditic flowers which are entomophilic pollinated and ellipsoid (pear-shaped), brown with light spots fruits will form and be ripen in the fall of the same year. The fruit has a multilayered epidermis and seeds have a pronounced dormancy of the embryo and, it is characterized by the absence of endosperm.

Due to habitat fragmentation, possible disturbance of the genetic structure of populations and difficult natural regeneration, and poor competitiveness of the species it is necessary to pay attention to its presence and monitor the potential and intensity of natural regeneration of this species, especially in urban forest complexes. In this paper, we investigated the morphological characteristics of fruits and seeds from the population in the area of Košutnjak in Belgrade.

The fruits were collected in September 2020. from 5 wild service trees growing at Košutnjak area. Trees were selected as test trees in this study grow in the area of MU "Šuma Košutnjak - Trim staza" (40.77 ha). The fruits were collected by picking from the branches of the tree. From each of the 5 trees, 40 fruits were selected. The number of seeds in each fruit, length and width of fruit, seed length, seed thickness, and seed width were measured using a digital nonius, with an accuracy of 0.1 mm. Mean values of measured characteristics, standard deviation, minimum and maximum values were calculated in Statistica 7.0 software. Differences between trees were tested using OneWay ANOVA ($p < 0.05$), a grouping of trees was performed by Tukey HSD post-hoc test, also dendrogram cluster analysis was used to observe the morphological closeness between the tested trees.

From 200 fruits (5 trees x 40 fruits) were extracted a total of 343 seeds. Fruits without seeds were recorded in all trees. Analysis of variance for observed characteristics of the fruits and seeds showed significant differences.

Grouping according to the Tukey post-hoc test showed a large number of groups. The dendrogram of cluster analysis indicates the separation of two groups.

Morphological characteristics of fruits and seeds from trees growing in the area of Košutnjak showed a high degree of variability. All observed traits are in the range of values reported in previous studies. Differences between trees indicate a high level of genetic diversity that should be examined on a larger sample and using other morphological and molecular markers.

VARIJABILNOST MORFOLOŠKIH KARAKTERISTIKA PLODOVA I SEMENA TEST STABALA BREKINJE (*Sorbus torminalis* (L.) Crantz) SA PODRUČJA KOŠUTNJAKA

Slavko PAVLOVIĆ, Ivona KERKEZ JANKOVIĆ, Jovana DEVETAKOVIĆ, Mirjana ŠIJAČIĆ-NIKOLIĆ

Rezime

Brekinja (*Sorbus torminalis* (L.) Crantz), (2n = 34), je heliofitna, brzorastuća vrsta rasprostranjena u južnoj, zapadnoj i centralnoj Evropi, severozapadnoj Africi i jugoistočnoj Aziji od 100-2200 m n.v. Javlja se stablimično (u pojasu šumam bukve i borova) i grupimično (u pojasu hrastovih šuma). Autohtona je vrtsa u Srbiji, kategorisana kao "vrsta pod rizikom", zastupqljna kao prateća vrsta u hrastovim i bukovim šumama do 1500 m n.v. Kao jedna od šumskih voćkarica, predstavlja važnu komponentu šumskih ekosistema, posebno u urbanim šumskim kompleksima koji su izloženi visokoj stopi antropopritiska.

U proleće ova vrsta formira hermafroditne cvetove koji se oprašuju entomofilno, dok se plodovi eloipsoidnog (kruškolikog) oblika smeđe boje sa svetlim pegama formiraju i sazrevaju u jesen iste godine. Plodovi imaju višeslojni epidermis, a semenke imaju izraženu dormantnost embriona, kao i karakteristično odsustvo endosperma.

Usled fragmentacije staništa, mogućeg poremećaja genetske strukture populacija i otežane prirodne obnove i slabe konkurentnosti vrste, potrebno je obratiti pažnju na prisustvo ove vrste i pratiti potencijal i intenzitet prirodne obnove, posebno u urbanim šumskim kompleksima. U ovom radu istražene su morfološke karakteristike plodova i semena iz populacije sa područja Košutnjaka u Beogradu.

Plodovi su sakupljeni u septembru 2020. godine sa 5 samoniklih stabala. Selektovane individue nalaze se na području GJ. „Šuma Košutnjak - Trim staza" (40,77 ha). Plodovi su sakupljani branjem sa grana. Sa svakog od 5 stabala odabrano je po 40 plodova. Broj semena u svakom plodu, dužina i širina ploda, dužina semena, debljina i širina semena izmereni su pomoću digitalnog nonijusa sa tačnošću od 0,1 mm. Srednje vrednosti izmerenih karakteristika, standardne devijacije, minimalne i maksimalne vrednosti izračunate su u softveru Statistica 7.0. Razlike između stabala su testirane pomoću jednofaktorijalne analize varijanse (OneWay ANOVA, $p < 0,05$), grupisanje stabala je izvršeno Tukei HSD post-hoc testom, takođe je korišćena denrogramska klaster analiza za posmatranje morfološke bliskosti između testiranih stabala.

Iz 200 plodova (5 stabala × 40 plodova) izvađeno je ukupno 343 semena. Plodovi bez semena zabeleženi su na svim stablima. Analiza varijanse za uočene karakteristike plodova i semena pokazala je značajne razlike. Grupisanje prema Tukey post-hoc testu pokazalo je veliki broj grupa. Dendrogram klaster analize ukazuje na razdvajanje dve grupe.

Morfološke karakteristike plodova i semena drveća koje raste na području Košutnjaka pokazale su visok stepen varijabilnosti. Sve uočene osobine su u opsegu vrednosti zabeleženih u prethodnim studijama. Razlike između drveća ukazuju na visok nivo genetske raznolikosti koji bi trebalo ispitati na većem uzorku i koristeći druge morfološke i molekularne markere.

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Original scientific paper

FIR AND NORWAY SPRUCE STANDS FROM THE PLANNING ASPECT IN THE AREA OF ĐEREKARSKI OMAR FOREST MANAGEMENT UNIT IN SOUTHWESTERN SERBIA

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Abstract: *The results of the research of fir and Norway spruce mixed stands in the area of Đerekarski Omar Forest management unit situated in southwestern Serbia are presented in this paper. The stands which are the subject of this research belong to a complex of frigophilic coniferous forest types. The forests in the Đerekarski Omar Forest management unit are managed in accordance with the planning documents starting from 1961 when the first planning of these forests was performed. The results of this paper were based on the measurement of stationary sample plots established in homogeneous parts of the stands. In all sample plots fir is a dominant tree species. The values of volume and volume increment in studied stands are significantly above the average at the level of Serbia for fir and Norway spruce stands. The health condition of the stands is good, whereby a positive circumstance is the process of natural regeneration of these stands both on locations where there is a sparse canopy and in closed-canopy stands. On the other hand, number of trees, mean tree diameter and qualitative stand structure indicate the need for a detailed analysis of the planned works.*

Key words: fir and Norway spruce forests, southwestern Serbia, forest management plans.

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SASTOJINE JELE I SMRČE SA PLANSKOG ASPEKTA NA PODRUČJU GAZDINSKE JEDINICE „ĐEREKARSKI OMAR“ U JUGOZAPADNOJ SRBIJI

Izvod: *U radu su predstavljeni rezultati istraživanja mešovitih sastojina jele i smrče na području gazdinske jedinice „Đerekarski Omar“ koja se nalazi u jugozapadnoj Srbiji. Sastojine koje su predmet ovog istraživanja pripadaju kompleksu frigorifilnih četinarskih tipova šuma. Šumama u gazdinskoj jedinici „Đerekarski Omar“ gazduje se u skladu sa planskim dokumentima počevši od 1961. godine, kada je izvršeno prvo uređivanje ovih šuma. Rezultati ovog rada nastali su na osnovu premera stacioniranih oglednih polja postavljenih u homogenim delovima sastojina. Na svim oglednim poljima jela je dominantna vrsta drveta. Vrednosti zapremine i zapreminskog prirasta u istraživanim sastojinama su znatno iznad prosečnih na nivou Srbije za sastojine jele i smrče. Zdravstveno stanje sastojina je dobro, pri čemu pozitivnu okolnost predstavlja proces prirodne obnove ovih sastojina kako na mestima gde je došlo do otvaranja sklopa tako i pod potpunim sklopom sastojine. S druge strane, broj stabala, srednji prečnik stabala i kvalitativna struktura stastojine ukazuju na potrebu detaljne analize planiranih radova.*

Ključne reči: šume jele i smrče, jugozapadna Srbija, planovi gazdovanja šumama.

1. INTRODUCTION

Serbia is considered a country with a medium forest coverage. Of its total area, 29.1% (Vojvodina 7.1%, Central Serbia 37.6%) is under forests. Compared to the global aspect, forest coverage of Serbia is close to the global forest coverage which amounts to 30%, and significantly lower than the European which reaches 46% (Banković, S. et al., 2009).

An important indicator of forest coverage is related to the qualitative structure of forests, whereby their origin is one of the attributes which most closely determine forest coverage in a qualitative sense. Compared to the total vegetation coverage in the growing stock of Serbia coppice forests are dominant with 64.7%, natural stands of high origin cover 27.5%, and artificially established stands (with plantations) 7.8%. The average volume in forests of Serbia amounts to 161 m^3/ha, whereby in high forests it is 254 m^3/ha, in coppice forests 124 m^3/ha and in artificially established forests (plantations) it is 136 m^3/ha (Banković, S. et al., 2009a).

The growing stock of the Republic of Serbia is dominated by beech which participates in the total volume with 40.5%, and in volume increment with 30.6%, followed by Turkey oak with 13.0% share in volume and 11.4% in volume increment and Sessile oak with 5.9% in volume and 6.1% in volume increment. Norway spruce is the most wide-spread coniferous species and its share in volume and volume increment is 5.2%, and 6.7% respectively. The share of Austrian pine and Scots pine in total volume and volume increment is 4.5% and 9.8%, respectively, while fir’s share in volume and volume increment is 2.3% and 2.2% (Banković, S. et al., 2009a).

Fir forests cover relatively small area of 25,600 ha, 65.6% of which is state-owned. In addition, stands of natural origin dominate with a share of 95.3%, and cultivated stands are present on 4.7% of the area (Banković, S. et al., 2009a)

In Serbia, fir is spread on high mountain massifs and the most important sites are in the west, on mountains Tara, Zlatibor (Murtenica), Zlatar, Čemerno, Golija, Mokra Gora, Prokletije, Šar Planina, Kopaonik and Goč, Veliki Jastrebac, and in the east on Stara Planina. The northernmost site is on Rtanj and Malinik.

Within the basic ceno-ecological coordinate system fir occurs in two forest complexes (Jovanović, B., Jović, N. 1981., Jović, N. et al., 1991):

- Complex (belt) of mesophilic beech and mixed beech and conifer types of forests;
- Complex (belt) of frigophilic coniferous forest types.

The researched stands are located in the complex of frigophilic coniferous forest types within Đerekarski Omar Forest management unit (FMU) which belongs to Gornjeibarsko forest area. Norway spruce and fir forests (*Piceo-Abietetum*) on brown podzolic soils within state-owned forests in Gornjeibarsko forest area are spread on an area of 1,749.6 ha.

Considering the above, the objective of this paper is an analysis of the condition of fir and spruce stands and natural regeneration process in these stands with particular reference to forest management plans for these forests.

2. OBJECT OF THE RESEARCH, MATERIAL AND METHODS

Đerekarski Omar Forest management unit is located between 20˚06′30" and 20˚11′30" of east longitude and 42º55′30" and 43˚01′00" of north latitude. Pešter plateau stands out in this mountainous area and for small part of its length it borders with this forest management unit. The FMU belongs to Dinaric mountain massif. The characteristic of these mountains are mostly limestone ridges which are torn and diverge. The FMU is located in Đerekarska depression which is situated on the sides of such two ridges that start from Krstača top. The lowest elevation within the FMU and the highest point of the FMU are at 1,160 m and 1,689 m above mean sea level, respectively. The main characteristic of the relief in the FMU is brokenness of the terrain, large abundance of hills with depressions and steep sides, so it is normal that different exposures are present, although northern and southern and partly eastern exposures are predominant.

The researched stands belong to the group of ecological units (ecological types) – Norway spruce and fir forests (*Abieti - Piceetum abietis*, Mišić et Popović, 1978) on acidic brown and brown podzolic soils and they are situated on the sites which are regularly managed in accordance with the Forest Management Plan. The basic function of these forests is production.

For the purpose of presenting the climate of the subject area the data have been collected from meteorological station in Sjenica which is the closest to the sample plots, for the reference period from 1981 to 2010. In terms of regional climate, this area belongs to humid continental climate of slightly altered type. The mean annual temperature in the analysed period is 6.7°C with the maximum

measured temperature being 36.2°C and the minimum -35.6°C. The mean annual precipitation is 749.5 mm, while mean annual relative humidity is 77%.

Within the Đerekarski Omar FMU, six sample plots were established with the average area of 0.25 ha. When setting up sample plots, care was taken to meet the conditions of habitat and stand homogeneity. Sample plots 1, 2 and 6 are located in the compartment 31, section A. Sample plots 4 and 5 are situated in the compartment 23, section A. Sample plot 3 is located in the compartment 22, section B. Sample plots are square in shape. The diameters and heights of all trees above taxation limit were measured on all sample plots and bore cores were extracted from five trees in each diameter size class in order to determine volume increment. Also, on all sample plots the abundance of young growth was determined by placing four square surfaces 1x1 m, at 10 m from the tops of the corners at an angle of 45° (Figure 1).

In addition, for each tree on the sample plot a silvicultural class was determined. Quality is determined using grades from 1 to 8 whereby grades 1, 2 and 3 were assigned to trees in the first biological position (dominant trees). Grade 1 is given to the best quality trees, straight, full-boled, with a properly developed canopy. Grade 2 was given to lower quality trees that are located in the first biological position and grade 3 was given to curved trees, trees with improperly developed canopy and trees with damages that are located in the first biological position. Grades 4, 5 and 6 are assigned to codominant trees according to the same criteria as for trees in the first biological position based on the quality of the trunk and the canopy. Grades 7 and 8 are assigned to the trees of the understorey, whereby 7 is assigned to healthy and vital trees and 8 to the trees that are not vital but damaged or dead.

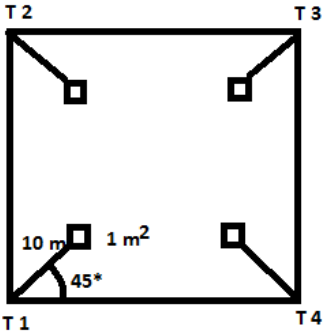

Figure 1. *Schematic representation of a sample plot*

Figure 2. *The researched stand on SP 1 (Source: Martać N.)*

The volume tables method was used for calculation of volume, whereby for fir two-way table for fir on Goč was used (Banković, S. et al., 1990), while for Norway spruce two-way table for Norway spruce on Kopaonik was used (Banković, S. et al., 2003). The current volume increment was determined according to the diameter increment method, by applying Meyer's differential method. Different functions were tested for modelling the height curve, and the selection of the final model was made on the basis of statistical parameters of regression and correlation analysis, as well as on the basis of the degree of

coincidence of equalized and empirical data. Data processing was performed using Microsoft Excel and Stat graphics.

3. RESULTS OF THE RESEARCH AND DISCUSSION

Habitat, as well as stand conditions with different intensity affect the development of trees in the stand and exactly those different development processes of certain trees in a stand lead to differentiation of trees in terms of diameter, height and other structural elements, which creates a specific internal structure of stands – stand structure.

For the purpose of creation of quality forest management plans at the stand level it is necessary to have a reliable information on forest condition which also means performing permanent monitoring of forest condition. Furthermore, considering that one of the strategic goals of forest management in Serbia is the increase of stand mixedness in order to provide greater forest stability, the knowledge on the structural development of these stands becomes even more significant.

Table 1. *Overview of taxation indicators*

Sample plot	Number of trees N/ha			Basal area G (m^2)			Volume V (m^3)			Volume increment Iv (m^3)		
	Fir	Norway spruce	Total	Fir	Norway spruce	Total	Fir	Norway spruce	Total	Fir	Norway spruce	Total
SP 1	872	45	917	54.1	3.8	57.9	732.1	55.7	787.8	9.4	0.9	10.4
SP 2	819	33	852	53.9	4.3	58.2	735.6	61.8	797.4	8.7	0.9	9.6
SP 3	864	18	882	39.2	1.3	40.5	518.6	83.6	602.2	8.9	0.9	9.8
SP 4	631	99	730	42.5	3.8	46.3	594.4	82.6	677.0	11.9	1.9	13.8
SP 5	731	87	818	43.8	5.8	49.6	601.1	80.17	681.27	11.3	1.5	13.0
SP 6	486	37	523	34.8	3.8	38.6	480.3	55.0	535.3	11.7	1.1	12.8
$\bar{x}$	**733.8**	**53.2**	**787.0**	**44.7**	**3.8**	**48.5**	**610.3**	**69.8**	**680.2**	**10.3**	**1.2**	**11.6**
$S_{\bar{x}}$	**62.0**	**13.2**	**58.9**	**3.2**	**0.6**	**3.4**	**43.3**	**5.6**	**41.8**	**0.6**	**0.2**	**0.8**
cv (%)	**20.7**	**60.8**	**18.3**	**17.5**	**38.1**	**17.3**	**17.4**	**19.7**	**15.1**	**14.3**	**34.6**	**15.9**

3.1. Number of trees and diameter structure

In the researched stands on sample plots the total number of trees pre ha above the taxation limit (10 cm) ranges from 523 (SP 6) to 917 (SP 1) (Chart 1).

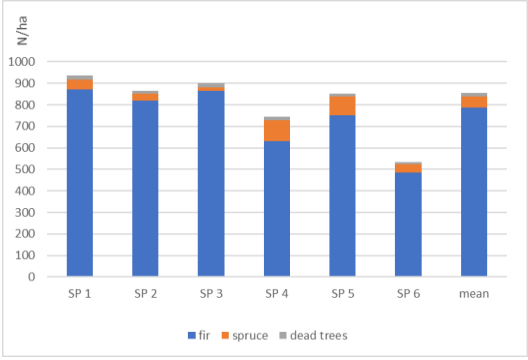

Chart 1. *Number of trees in the researched stands*

Fir is a dominant tree species in all sample plots and according to mean values number of trees per hectare amounts to 787. The share of fir in the total number of trees is 93%, while remaining 7% belongs to Norway spruce. The average number of dead trees per hectare is 23. Observing the obtained results and the results of previous research carried out on Mt.Tara (Stamenković et. al., 1990), Kopaonik (Šljukić et. al., 2017) and Zlatar (Popović., 2017) it is evident that the number of trees per sample plot as well as the average number is within the range of number of trees on other sites in Serbia where these forests occur.

Diameter structures of fir and Norway spruce in the researched stands are presented in Chart 2.

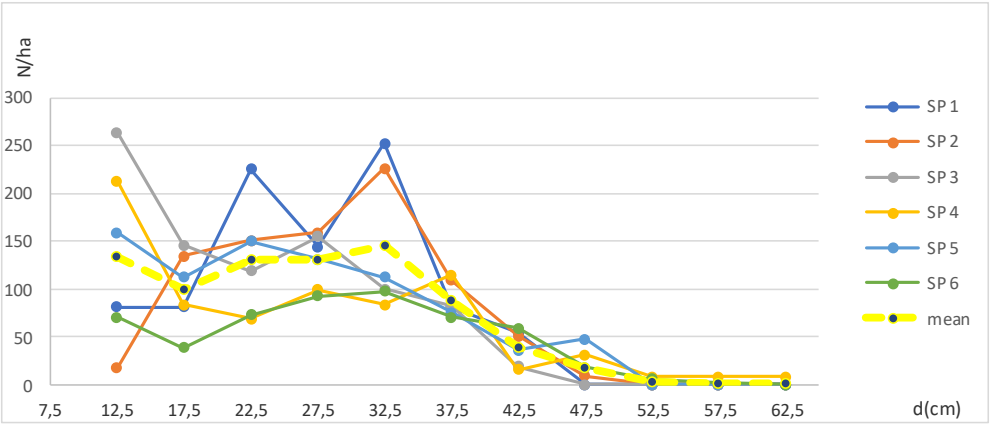

Chart 2. *The structure of the researched stands*

In structural terms, these forests are characterized by a pronounced diversity of structural forms, from a structure close to even-aged stands (SP 2), through a structure close to selection forests (SP 3) to the structure similar to all-aged stands (SP 1). The shape of summary lines of tree distribution in all cases is conditioned by fir as a dominant species. If the line representing the average for all sample plots is observed, we come to the conclusion that the structure of the researched stands corresponds the most to the structure of even-aged stands, whereby thin and trees of medium thickness are dominant with a minimal presence of trees of large dimensions. The results of this research show that the researched stands are similar to the researched stands on other sites in Serbia. On the basis of a research carried out in Norway spruce and fir forest on Kopaonik, Šljukić et al. (2017), state those are very diverse stands in terms of structural forms, from a structure close to even-aged stands, two-storied, to typical multi-storied all-aged stands.

The values of stand mean diameter and mid-diameter of dominant fir and Norway spruce trees in researched stands are presented in Table 2.

Table 2. *Stand mean diameters (dg) and mid-diameters of dominant fir and Norway spruce trees (dg max) in the researched stands*

Sample plot	Type of tree	d_g (cm)	$d_{g\ max}$ (cm)
SP 1	Fir	28.1	37.6
	Norway spruce	26.2	34.2
SP 2	Fir	28.9	38.2
	Norway spruce	24.7	37.1
SP 3	Fir	24.1	42.5
	Norway spruce	24.0	35.8
SP 4	Fir	29.3	37.4
	Norway spruce	27.8	34.4
SP 5	Fir	27.6	36.5
	Norway spruce	24.2	32.8
SP 6	Fir	30.4	41.1
	Norway spruce	28.7	37.6

Stand mean diameter (d_g) and mid-diameter of dominant trees ($d_{g\ max}$) are calculation categories which are under dominant influence of stand conditions that arise as a consequence of silvicultural treatment. Stand mean diameter of fir (d_g) ranges from 24.0 cm (SP 3) to 30.4 cm (SP 6), while mid-diameter of dominant trees ($d_{g\ max}$) ranges from 37.4 cm (SP 4) up to 42.5 cm (SP 3). On the other hand, Norway spruce as an accessory tree species reaches lower diameter values of both stand mean diameter and mid-diameter of dominant trees. The above confirms the statement that there is a small number of trees of the largest dimensions in the researched stands, as well as that the trees of medium and small dimensions are dominant there.

3.2. Tree Height

Height curves of the researched stands are presented in Chart 3.

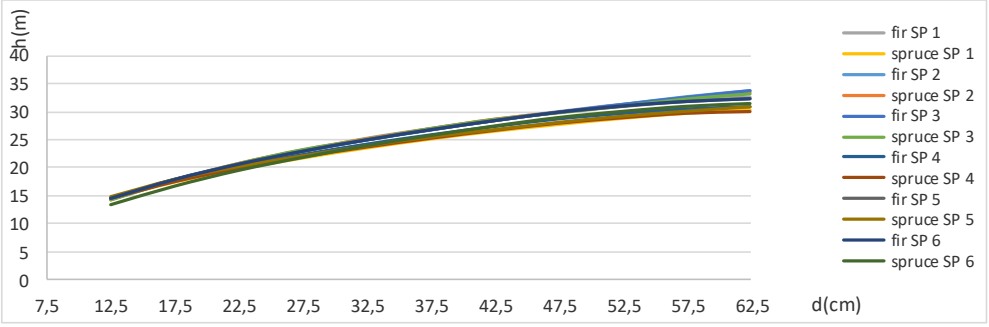

Chart 3. *Height curves of the researched stands*

Height curves confirm the statement that when establishing sample plots great attention was paid to achieving that the conditions in which sample plots were established were homogenous. Therefore, the variation of heights per sample plots is small, and differences are more pronounced in Norway spruce.

The values of mean stand height and mean height of the dominant trees of fir and Norway spruce in the researched stands are presented in Table 3.

Table 3. *Mean stand height (hg) and mean height of the dominant trees (hg max) of fir and Norway spruce in the researched stands*

Sample plot	Tree species	h_g (m)	$h_{g\,max}$ (m)
SP 1	Fir	23.2	26.8
	Norway spruce	22.9	26.6
SP 2	Fir	23.5	27.1
	Norway spruce	22.8	26.4
SP 3	Fir	20.2	29.9
	Norway spruce	20.1	27.9
SP 4	Fir	23.7	26.8
	Norway spruce	23.4	25.9
SP 5	Fir	23.1	26.4
	Norway spruce	22.6	26.2
SP 6	Fir	24.1	28.1
	Norway spruce	24.8	29.4

There is no pronounced variability of mean stand heights of fir *(h_g)*. The highest variability is on SP 4 where it amounts to 23.7 m, while the lowest variability amounting to 20.2 m is on SP 3. The largest mean height of the dominant trees *($h_{g\,max}$)* is on SP 3 amounting to 29.9 m. For Norway spruce, the variability of mean stand heights is somewhat more pronounced, whereby the difference between the highest on SP 6 and the lowest on SP 4 amounts to 4.7 m. Observing mean stand heights and mean heights of the dominant trees it can be concluded that fir is dominant in relation to Norway spruce in all sample plots, except for SP 6. Also, it can be stated that the heights in the researched stands approximate the heights in stands on other sites (Popović, 2017; Šljukić, 2017).

3.3. Basal Area

Basal area of the researched stands is presented on Chart 4.

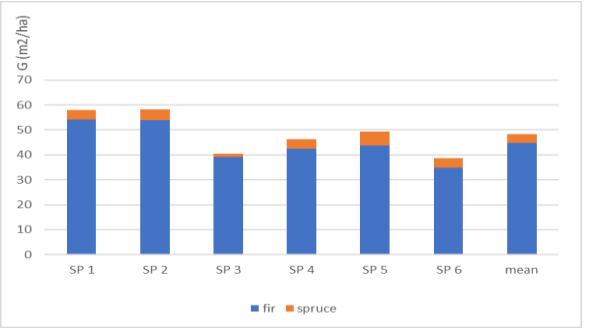

Chart 4. *Basal area of the researched stands*

Mean basal area in the researched stands amounts to 48.5 m^2/ha which is less than in the stands on Mt.Tara (Stamenković et al. 1990). Also, the obtained basal area is significantly smaller than the mean basal area in the stands on Kopaonik which amounts to 58.4 m^2/ha (Šljukić, 2017), as well as in the area of Kneževo (Govedar et al. 2008).

3.4. Volume and Volume Increment

The volume of the researched stands is presented in Chart 5.

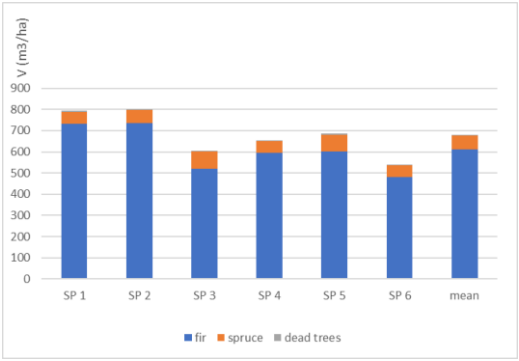

Chart 5. *Volume in the researched stands*

The mean volume of these forests amounts to 680.1 m^3/ha, with a mixture ratio of 0.9:0.1 in favour of fir, whereby the share of dead trees of both species amounts to 1.8% in total. Observing each sample plot individually, the lowest productivity is recorded in the stand on SP 6 and the highest in the stand on SP 2. Volume and volume increment of these stands indicate they are highly productive. A high value of volume (exceeding 400 m^3/ha) in preserved mixed stands of fir and Norway spruce is determined in the area of Ljubišnja in Montenegro (Stojanović et al., 2000), in the areas of Potok and Drinići in the Republic of Srpska ranging from 504 m^3/ha up to 716 m^3/ha (Govedar, 2005), in the area of Kneževo 726.9 m^3/ha (Govedar et al., 2008), in the area of Mt.Tara (Stamenković et al. 1990; Medarević, 2005), and in the area of Kopaonik 776.9 m^3/ha (Šljukić et al., 2017).

Volume increment in the researched stands ranges from 9.6 m^3/ha up to 13.8 m^3/ha, and it's mean value is 11.5 m^3/ha, whereby fir participates with 89%, and spruce with 11%. If diameter structure obtained on sample plots and increment are taken in consideration, with some caution it can be concluded that in terms of production structural all-agedness is more suitable to the current situation, presuming that it is a consequence of more favourable conditions of lateral light.

3.5. New Growth

Growth and development of new growth of various tree species depends on its microhabitat conditions, age, height and position (Krstić, et al., 1997) and its height increment depends on the tree species, edaphic, climatic and other factors (Bunuševac, 1951).

The average number of new growth individuals of fir in the researched stands amounts to 39,375, while the average number of new growth individuals of Norway spruce is 9,600, which is lower number of new growth individuals compared to even-aged stands in the area of Kneževo (Govedar, et al., 2008). New growth occurs equally both under the closed canopy and in places where canopy is sparse, considering that in the areas with sparse canopy its development is more intensive due to an increased amount of light. The age of the new growth is different and there is a new growth aged from 1 to over 10 years which is suppressed and dormant due to lack of light. When conducting the research individual seedlings aged up to 1 year were not taken into account due to a high risk of their decay, but it should be noted that that they are abundant due to a good seed yield of both tree species in the previous year.

Figure 3. *New growth development in the area with a sparse canopy (Source: Martać N.)*

3.6. Silvicultural Class

The trees of the 7th silvicultural class are the most abundant within the researched stands, followed by the 5th and the 4th classes which represent the trees from the second biological position. The fourth most abundant trees are the highest quality trees and in the fifth place are the trees of somewhat lower quality and are located in the first biological position. The 1st and the 2nd class represented by the trees from the first biological position are approximately equally represented with the total of about 250 trees per ha. Dead trees are also present in all three biological positions and their number in the researched stands is on average 23 trees per ha. Taking into account the above, it can be concluded that a sufficient number of the highest quality trees per hectare is present, but it is necessary to implement cultivation measures in the form of thinning which would provide more space for development of crowns of the highest quality trees and thus have effect on increase of tree diameters and stand quality.

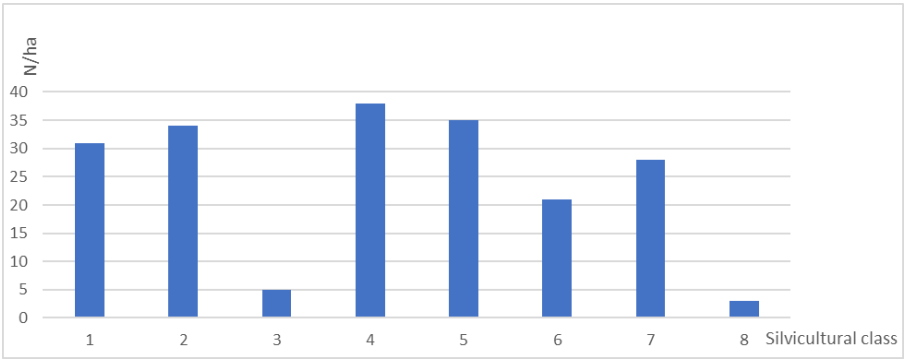

Chart 6. *Overview of stand quality*

4. CONCLUSIONS

Based on the obtained results on the structural characteristics of fir and Norway spruce forests within the researched area, the following can be stated:

- The volume of the researched stands per ha exceeds significantly the mean volume for Gornjeibarsko forest area and amounts to 262 m^3/ha.
- The volume increment of the researched stands also exceeds the mean for Gornjeibarsko forest area.
- The determined volume and volume increment of the researched stands are higher compared to the means of fir and Norway spruce forests in the territory of Serbia amounting to 301 m^3/ha and 8.35 m^3/ha, respectively.

According to the applicable Forest management plan all researched stands belong to the same forest management class and in all stands group shelterwood felling is planned. The above indicates that in all stands group-shelterwood system of management with long regeneration period is applied, which is typical for all-aged stands. Thereat, in all researched stands group-shelterwood felling is planned. Considering the above and the research results which clearly indicate that in terms of structure some of the researched stands are even-aged, (aged 85 at breast height), and that there is a number of suppressed trees (4^{th} and 5^{th} silvicultural class) in the stands that prevent normal crown development of the highest quality trees and thus affect their qualitative and quantitative characteristics, it should be noted that more attention should be paid to delineation of stands and creation of adequate Forest management plans in the form of planning of thinning and shelterwood felling with short regeneration period.

Furthermore, it is necessary to emphasize that the part of Gornjeibarsko forest area that has been included in this research is a special refuge habitat for fir regardless of whether pure or mixed stands are in question. Compared to other sites of mixed coniferous and broadleaved forests and coniferous forests in Serbia, fir (pure or mixed with Norway spruce) occurs here also in the structural form typical of even-aged forests.

Despite the increasingly intense effect of climatic extremes on forests, compared to some other sites in Serbia, these stands have a satisfactory health and negative biotic effects are present rarely and only in individual cases.

What completes the overall picture in a positive sense is an unhindered spontaneous forest renewal also under closed canopy, which clearly indicates the priority of natural regeneration in these forests. By "moderate and deliberate" intensity felling, in relation to the present condition of forests, a principle of sustainable production and yield will be ensured in a long-term, while supporting the permanence and evenness of water aquifers since this area is an active source of potable water for the nearby village.

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FIR AND NORWAY SPRUCE STANDS FROM THE PLANNING ASPECT IN THE AREA OF ĐEREKARSKI OMAR FOREST MANAGEMENT UNIT IN SOUTHWESTERN SERBIA

Nikola MARTAĆ, Branko KANJEVAC, Vlado ČOKEŠA, Natalija MOMIROVIĆ, Branka PAVLOVIĆ, Danilo FURTULA

Summary

The condition of fir and Norway spruce mixed stands in the area of Đerekarski Omar Forest management unit in southwestern Serbia are researched from the planning aspect in this paper.

The researched stands belong to the group of ecological units (ecological types) – Norway spruce and fir forests (Abieti - Piceetum abietis, Mišić et Popović, 1978) on acidic brown and brown podzolic soils. The basic function of these forests is production.

The total number of trees in the researched stands ranges from 523 to 917 trees per ha, whereby fir is dominantly represented in all stands. Mean volume of these forests amounts to 680.1 m3/ha with a mixture ratio of 0.9:0.1 in favour of fir. Volume increment in the researched stands ranges from 9.6 m3/ha up to 13.8 m3/ha, and the mean value amounts to 11.5 m3/ha, whereby the shares of fir and Norway spruce are 89% and 11%, respectively. The volume and volume increment of the researched stands are significantly higher compared to the mean values of the same elements within Gornjeibarsko forest area, as well as compared to the Serbian average.

The average number of new growth individuals of fir in the researched stands amounts to 39,375, while the average number of new growth individuals of Norway spruce is 9,600. New growth occurs equally both under the closed canopy and in places where canopy is sparse, considering that in the areas with sparse canopy its development is more intensive due to an increased amount of light.

The part of Gornjeibarsko forest area that has been included in this research is a special refuge habitat for fir regardless of whether pure or mixed stands are in question. Despite the increasingly intense effect of climatic extremes on forests, compared to some other sites in Serbia, these stands have a satisfactory health and negative biotic effects are present rarely and only in individual cases. In addition, an unhindered spontaneous forest renewal represents a positive circumstance, which clearly indicates the priority of natural regeneration in these forests. By “moderate and deliberate” intensity felling, in relation to the present condition of forests, a principle of sustainable production and yield will be ensured in a long-term, while supporting the permanence and evenness of water aquifers since this area is an active source of potable water for the nearby village.

SASTOJINE JELE I SMRČE SA PLANSKOG ASPEKTA NA PODRUČJU GAZDINSKE JEDINICE „ĐEREKARSKI OMAR“ U JUGOZAPADNOJ SRBIJI

Nikola MARTAĆ, Branko KANJEVAC, Vlado ČOKEŠA, Natalija MOMIROVIĆ, Branka PAVLOVIĆ, Danilo FURTULA

Rezime

U radu je sa planskog aspekta proučavano stanje mešovitih sastojina jele i smrče na području gazdinske jedinice „Đerekarski Omar“ u jugozapadnoj Srbiji.

Istraživane sastojine pripadaju grupi ekoloških jedinica (ekoloških tipova) - šume smrče i jele (*Abieti - Piceetum abietis*, Mišić et Popović 1978) na kiselim smeđim i smeđim podzolastim zemljištima. Osnovna funkcija ovih šuma je proizvodna.

Ukupan broj stabala u istraživanim sastojinama se kreće od 523 do 917 stabala po ha, pri čemu je jela dominantno zastupljena u svim sastojinama. Prosečna zapremina ovih šuma iznosi 680,1 m^3/ha, sa razmerom smese 0,9:0,1 u korist jele. Zapreminski prirast u istraživanim sastojinama kreće se od 9,6 m^3/ha do 13,8 m^3/ha, a prosečna vrednost iznosi 11,5 m^3/ha, pri čemu jela učestvuje sa 89%, a smrča sa 11%. Zapremina i zapreminski prirast istraživanih sastojina su značajno veći u odnosu na prosečne vrednosti istih elemenata u okviru Gornjeibarskog šumskog područja, kao i u odnosu na prosek Srbije.

Prosečna brojnost podmlatka jele u istraživanim sastojinama iznosi 39 375, dok je prosečna brojnost podmlatka smrče 9 600 jedinki. Podmladak se podjednako javlja kako pod potpunim sklopom tako i na mestima gde je došlo do prekida sklopa, s tim da se na mestima prekida sklopa on intenzivnije razvija usled povišene količine svetlosti.

Deo Gornjeibarskog šumskog područja koji je bio obuhvaćen ovim istraživanjem predstavlja poseban stanišni refugijum za jelu, bilo da se radi o čistim ili mešovitim sastojinama. I pored sve intenzivnijeg uticaja klimatskih ekstrema na šume, ove sastojine u odnosu na neke druge lokalitete u Srbiji imaju zadovoljavajuće zdravstveno stanje i samo je pojedinačno i retko prisustvo negativnih biotičkih uticaja. Osim toga, pozitivnu okolnost predstavlja nesmetano spontano podmlađivanje, što jasno ukazuje na prioritet prirodnog obnavljanja u ovim šumama. Sečom „umerenog i odmerenog“ intenziteta u odnosu na sadašnje stanje šuma obezbediće se dugoročno princip trajnosti proizvodnje i prinosa, uz to podržavajući stalnost i ravnomernost izdani vode s obzirom da je ovo područje aktivno vodoizvorište pitke vode za obližnje naseljeno mesto.

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DETERMINATION OF LEAF AREA INDEX (LAI) AT LEVEL II SAMPLE PLOTS ACCORDING ICP MANUAL

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Abstract: *The paper describes the methodology for determining LAI according to the ICP forest methodology, where hemispherical photographs were taken on a network of fixed points placed on the surfaces of three Sample plots Level II. Hemispherical photographs were processed by the Hemisfer software package. The data obtained by image processing were entered into the ICP Forests database. The obtained LAI values represent the response to the state of vegetation under the influence of different ecological conditions as well as anthropogenic influences, and will be the part of future annual monitoring at Sample plots of the Level II points*.

Key words ICP Forests, leaf area index, method Hemispherical photography, Hemisfer software.

ODREĐIVANJE INDEKSA LISNE POVRŠINE (LAI) NA BIOINDIKACIJSKIM TAČKAMA NIVOA II PREMA ICP PRIRUČNIKU

Izvod: *U radu je opisana metodologija za određivanje indeksa lisne površine prema ICP Forest metodologiji, po kojoj su snimljene hemisferne fotografije na mreži fiksnih tačaka postavljenih na površinama tri Bioindikacijske tačke Nivo-a II. Hemisferne*

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fotografije su obrađene programskim paketom Hemisfer softer. Podaci dobijeni obradom slika uneti su u bazu podataka ICP Forests. Dobijene LAI vrednosti pružaju odgovor o stanju vegetacije pod uticajem različitih ekoloških uslova, kao i antropogenih uticaja i biće deo budućeg godišnjeg monitoringa na Bioindikacijskim tačkama Nivoa II.

Ključne reči: ICP Forests, indeks lisne površine, metod hemisfernih fotografija, Hemisfer softver.

1. INTRODUCTION

The leaf area index (LAI) represents the ratio of leaf area and soil surface (Pince, 1993) and represents one of the most important characteristics of trees and forest stands (Fleck et al., 2020). LAI can be used to assess or predict the health status and invasion of pests (Pokorny and Stojnić, 2012; Wang et al., 2018) or plant productivity and biomass production potential (Pokorny and Stojnić, 2012; Scurlock et al., 1999), for monitoring the growth and changes of vegetation (Zhili et al., 2013), to drive the ecological system, crop growth and forest growth models (Myneni et al., 2002; Sonnentag et al., 2007).

Tree canopy is an extremely important parameter that interacts with global changes in the environment, and a reliable and objective assessment of leaf area index (LAI) is necessary for a better understanding of this relationship (Sidabras and Augustaitis, 2015).

LAI (measured in m^2/m^2) is defined as half the total leaf area of the forest canopy divided by the ground area below the canopy (Chen and Black, 1991).

Numerous direct, semi-direct and indirect methods have been developed to estimate the leaf area index (LAI), as well as subjective assessment methods (Jonckheere et al., 2004, Fleck et al., 2020).

For ICP Forests is very important that model applications know the maximum LAI that is reached during the vegetation period, since the annual development of leaves may be derived from this value (Fleck et al., 2020). The all methods that are applied in ICP Forest plots focus on measuring the maximum LAI (LAI_{max}) and had to prove that it is able to extract this quantity.

Several direct and indirect methods are used within ICP Forestry. Litterfall measurements and biomass harvesting are direct measurements. Even if direct measurement methods provide the most reliable LAI estimate, they are less commonly used because they are usually more laborious than other methods. Direct methods serve as a standard for validation of indirect and remote methods (Fleck et al., 2020).

Hemispherical photography, Plant Canopy Analyzer, SunScan Ceptometer and Airborne LiDAR are indirect measurements that are applied in ICP Forest plots. The essence of indirect methods is to quantify the penetration of light through the canopy and based on that to calculate amount of leaf area that allows the relationship between light above and below the canopy. The biggest limitation of these methods is the measurement at very low rates of light penetration. The leaf area index over 6 causes penetration rates of about 5% which poses a great challenge for optical instruments (Gower et al., 1999).

ICP Forests Programme (International Cooperative Programme on Forest Condition Monitoring) has been continuously performed in the Republic of Serbia since 2003 (Rakonjac et al., 2020). Institute of Forestry has been proclaimed as the National Focal Center of the Republic of Serbia by the Directorate of Forests – Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia.

The main subject of ICP Forests is the monitoring of anthropogenic (primarily air pollution) and abiotic factors on the condition and development of Europe's forest ecosystems (Gagić-Serdar et al., 2019). ICP Forests Programme has been performed on sample plots Level I and Level II through monitoring vitality and health condition. Level I include monitoring, observation and analyses of forest condition on sample plots in 16 x 16 km grid. Level II includes the intensive monitoring and deal with a greater number of input parameters of permanent observation plots (Stefanović et al., 2017). As part of the ICP Forests program the leaf area index will be monitored starting in 2021, on the Sample plots of Level II.

The application of LAI methods on a yearly basis is envisaged only within the framework of intensive monitoring of Level II, in order to obtain reliable information on the significant interannual variation of the leaf area shown by the forest canopy.

2. MATERIAL AND METHODS

An indirect method Hemispherical photography (fish-eye photography), was used to determine the LAI at the Sample plots of Level II in Serbia.

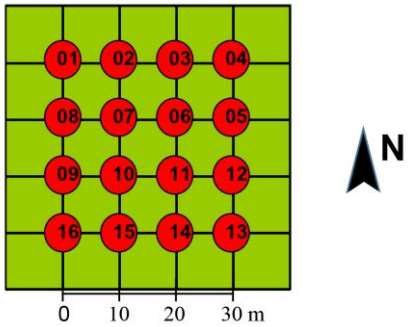
Hemispherical photography (fish-eye photography) involves shooting photographs, registration, classification and calculation

The photographs were taken by looking upwards, with a wide-angle lens (180 °) quantifying the potential solar radiation at the point of observation. The photographs obtained in this way in the highest resolution contain the most comprehensive information in the corner. The photographs were taken in the early morning hours (conditions without direct light), avoiding the effect of reflection. The exposure was set automatically. During the photography, the zenith and azimuth (orientation) were determined. In order for the camera to be positioned at the top, towards the zenith, a self-leveling carrier was used, and a spirit level with three axes of leveling was attached to the camera itself. Photographs should be taken in the summer and winter. Summer photographs are taken during maximum foliation (from mid-July to mid-August), and winter after the leaves fall (before the buds burst in spring).

The classification of photographs is done automatically, determining which pixels of the image represent the visible and which the invisible part of the sky. An algorithm was used for the calculation, which is used to calculate the index of solar radiation and LAI.

During 2021, on the Sample plots of Level II at the following localities: Kopaonik, Crni vrh and Mokra Gora, a permanent grid for taking hemispherical photographs was set, which are used to obtain the leaf area index (LAI). A grid of points at a distance of 10 meters has been set up, within 50 m x 50 m, and photographs were taken from the inner 16 points. Such an arrangement of points is

placed on a 30 m x 30 m grid, which covers an area of 0.25 ha. The illustration is shown at the Picture 1.



Picture 1. *Measurement design*

Positions for obtaining photographs were moved when they were close to or on an obstacle (tree, construction), by 5 times the diameter of the obstacle itself. All obstacles that were above the lens (branches, leaves) were removed. Positions for taking photographs are permanently marked on the field, with pegs and plastic tape.

To avoid ground vegetation, the camera is mounted on legs with a lens at a height of 1.3 m from the ground. The position of the camera is oriented so that the upper edge of the picture represents the north.

A Nikon D5000 camera and a Nikkor 10.5 mm full-frame (DX) wide-angle fisheye lens were used to take the photographs. A three-level spirit level is attached to the camera. All pictures were taken with delayed release (set to 10 seconds) to prevent camera shake or any movement of the camera.

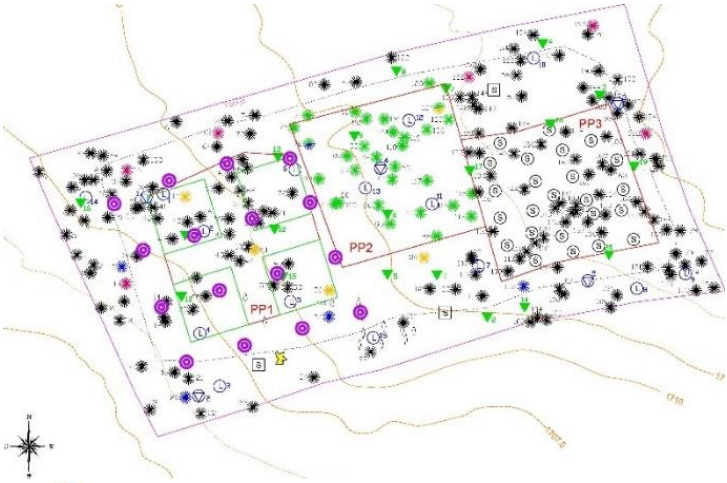
Picture 2. *Shooting hemispherical photography*

Taken photographs were analysed in Hemisfer software available from the web site: http://www.wsl.ch/dienstleistungen/software/hemisfer. At first automatic threshold determination were done and then LAI analysing. Because of very dense canopy the threshold for picture were done according to Ridler and Calvard (1978) method

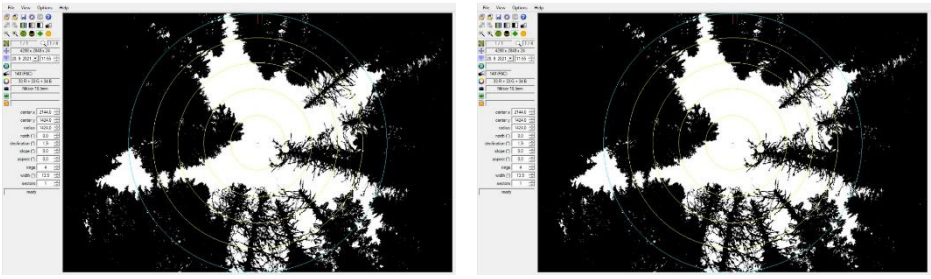
3. RESULTS AND DISCUSSION

The hemispherical photographs were analysed in 4 rings of 13.5°.

Kopaonik sample plot

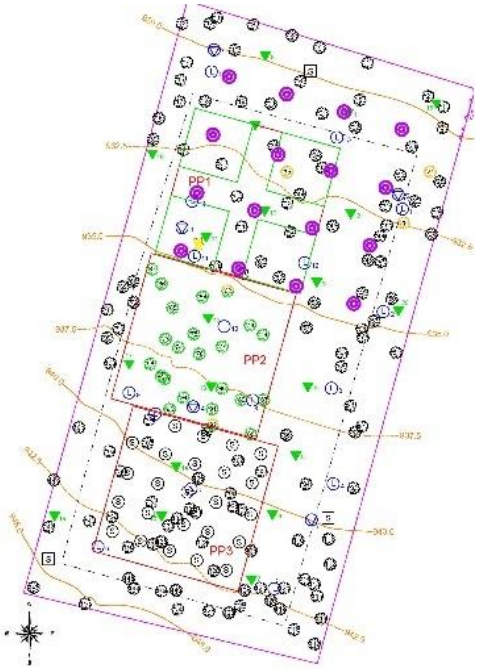


Picture 3. *Shooting position for hemispherical photographs for LAI on Kopaonik Sample plot*

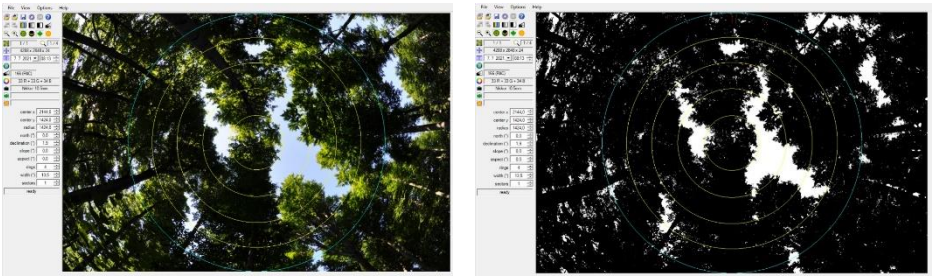


Picture 4. *Left: Image analysis 67000400012809211155512O in Hemisfer software*; *Right: The threshold for picture 67000400012809211155512O according to Ridler and Calvard (1978)*

Crni vrh sample plot

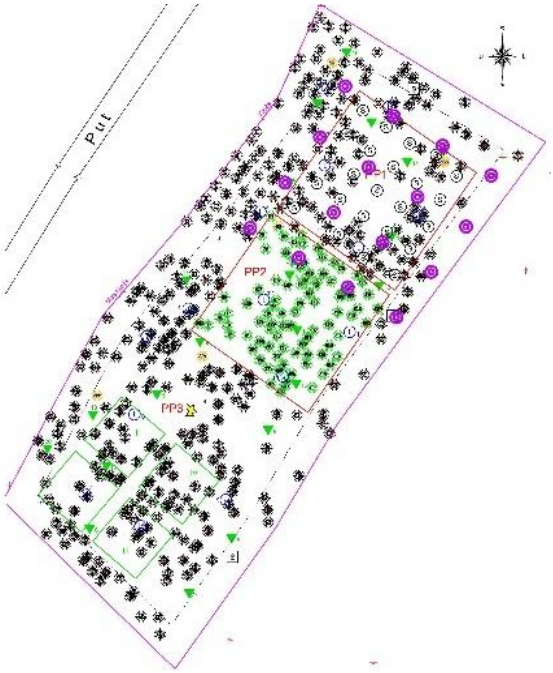


Picture 5. *Shooting position for hemispherical photographs for LAI on Crni vrh Sample plot*

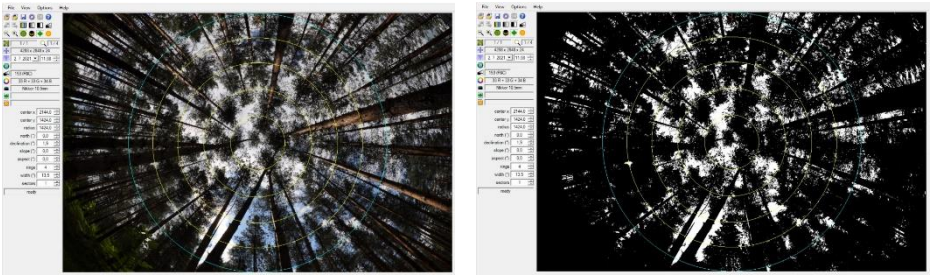


Picture 6. *Left: Image analysis 67000400010707210811335 1 in Hemisfer software*; *Right: The threshold for picture 67000400010707210811335 1 according to Ridler and Calvard (1978)*

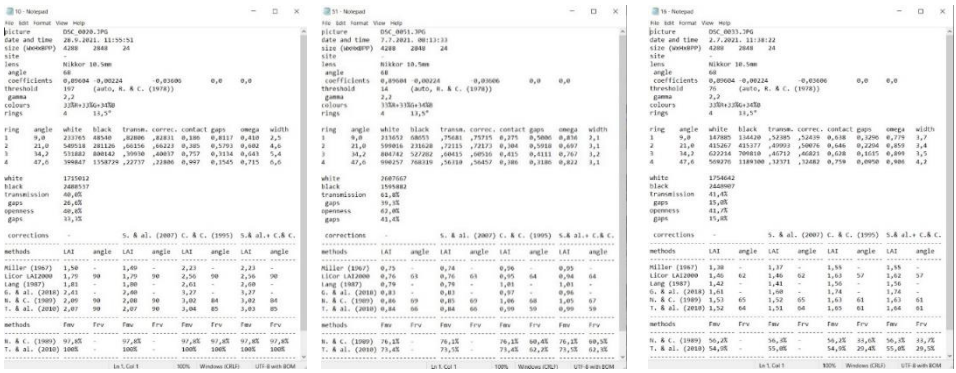
Mokra Gora sample plot



Picture 7. *Shooting position for hemispherical photographs for LAI on Mokra Gora Sample plot*



Picture 8. *Left: Image analysis 67000500010207211138223 3 in Hemisfer software; Right: The threshold for picture 67000500010207211138223 3 according to Ridler and Calvard (1978)*



Picture 9. *Example of LAI results for 67000400012809211155512O (left), 67000400010707210811335 1 (middle), 67000500010207211138223 3 (right) files*

Picture 9. represent example of a results file for LAI, as obtained with the Hemisfer software. It contains general information about the picture (name, size), camera and lens. In addition, there is information about the angle, slope, threshold, gamma, channels (red, green and blue channels), rings, sectors (12 azimutal sectors, grouped by 3) and leaf angle (leaf angle limited between 35°-70°).

They are also shown results per ring (angle - average zenith angle in the ring, ignored, white, black, transmission - total gap fraction within the defined rings, transmission corrected after Schleppi et al. (2007), contact number based on the corrected transmission, gaps - proportion of large gaps estimated according to Chen and Cihlar (1995), omega - value of canopy clumping after Chen and Cihlar (1995) and width - foliar element width calculated for Chen and Cihlar (1995)), per ring and sector (azimuth, ignored, white, black, transmission and contact) and total (white - pixels (sky), black - pixels (canopy), ignored - pixels ignored, transmission - light transmission in 2D (azimutal equidistant projection of the hemisphere), openness - canopy openness in 3D, also called sky view factor (solid angles of the hemisphere) and gaps - of which large gaps).

The software outputs the LAI results according to Miller (1967), LiCor LAI2000, Lang (1987), Gonsamo et al. (2018), Norman and Campbell (1989) and Thimonier et al. (2010) with corrections: slope and non-linearity correction (Schleppi et al., 2007), clumping correction (Walter and Torquebiau, 2000), clumping correction (Lang and Xiang, 1986), clumping correction (Chen and Cihlar, 1995), robust regression (Gonsamo et al., 2018), ellipsoidal method (Norman and Campbell, 1989) and weighted ellipsoidal method (Thimonier et al., 2010). In addition, values of Fmv = transmission (total gap fraction) in vertical projection and Frv = fraction of large gaps in vertical projection are displayed by Norman and Campbell (1989) and Thimonier et al. (2010) method.

In Table 1. the basic data on the sample plots were shown and the LAI results recalculated in the Hemisfere software by the method of Thimonier et al., 2010, Non-linearity correction (Schleppi et al., 2007) and Canopy clumping (Chen and Cihlar, 1995).

The leaf area index is expressed in the LAI_{max} column, since the data refer to summer photographs when the plants are in maximum foliation, so the value of LAI is the maximum value of the index during one calendar year.

LAI_{max} values in summer measurement in Kopaonik Sample plot range from 0.3 to 6.58, in Crni vrh Sample plot range from 0.24 to 4.74 and in Mokra Gora Sample plot range from 0.56 to 4.21.

According to the data expressed in Table 1. the highest value of LAI is at point 8 on Sample plot Kopaonik 6.58, and the GAP fraction point is 2.43%. While the lowest value of LAI is 0.24 at point 7 on Sample plot Crni vrh, and the GAP is 72.70%. The highest mean value of LAI 2.55 is Sample plot on Kopaonik, and the lowest on Crni Vrh Sample plot 1.94 (mean value of LAI Mokra Gora Sample plot is 2.08).

Tabela 1. *Minimum and maximum values of LAI of individual images on Sample plots of the Level II in the vegetation period mean value*

Sample plot	Number of shots	Mean value LAI	Mean value GAP %	Minimum value LAI	GAP %	Maximum value LAI	GAP %
Kopaonik	16	2.55	37.04	0.30	58.40	6.58	2.43
Crni vrh	16	2.08	31.02	0.24	72.40	4.74	2.43
Mokra Gora	16	1.94	35.00	0.56	34.00	4.21	3.63

Leaf area index in response to various environmental impacts as well as the age of individuals in the tree layer have been subject of research for many authors (Balandier et al., 2006, Kantor et al., 2009, Bequet et al., 2012, Pokorny and Stojnić, 2012) so the data collected in this way from the permanent points on Sample plots Level II will be a useful for further research.

4. CONCLUSIONS

The results of the analysis of hemispherical photographs of the first survey on Sample plots of the Level II experimental fields indicate that the difference in the leaf area index exists in relation to the dominant woody vegetation. In coniferous species (Kopaonik and Mokra Gora), the average leaf area index with 16 measured hemispherical photographs is higher than in the area where the deciduous species (Crni vrh) is dominant. Also, the minimum value of the leaf surface index at certain points from which the photographs were taken is the lowest on the experimental surface Crni vrh, where the dominant species is deciduous. Bearing in mind that this is the first recording on Sample plots of the Level II, it can be stated that there are differences, but that only a long series of results obtained by recording in the coming period will allow correlating the values of leaf area index and density and age of vegetation of dominant species in the tree layer, as well as changes in climatic and other environmental factors that are part of the intensive monitoring of the condition at Sample plots of the Level II.

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DETERMINATION OF LEAF AREA INDEX (LAI) AT LEVEL II SAMPLE PLOTS ACCORDING ICP MANUAL

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Summary

When assessing the potential for photosynthetic activity and thus indirectly the vitality of vegetation in the studied area, one of the factors that is analysed is the leaf area index (LAI). LAI represents the total leaf area per unit of analysed area (m^2/m^2). As it is almost impossible to do this directly in the forest floor by collecting the assimilation organs and measuring them, in these cases this is done by various indirect methods. One of them is the analysis of hemispheric photographs with suitable software packages. The paper presents an analysis of hemispherical photographs made according to the ICP forestry methodology with the Hemispfer software package calculated by LAI. The obtained data were entered into the ICP Forests database with all relevant data on the quality and conditions that were when the photographs were taken. The results of the calculated LAI values will be used in further research on the impact of various ecological and anthropogenic factors on vegetation when a sufficient number of replications are performed during the summer and winter photographs in the following years.

ODREĐIVANJE INDEKSA LISNE POVRŠINE (LAI) NA BIOINDIKACIJSKIM TAČKAMA NIVOA II PREMA ICP PRIRUČNIKU

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Rezime

Kada se ocenjuje potencijal za fotosintetičku aktivnost, a time posredno i vitalnost vegetacije na istraživanom prostoru, jedan od faktora koji se uzima u obzir je indeks lisne povrsine (LAI - leaf area index). LAI predstavlja ukupnu površinu listova po jedinici analizirane površine (m^2/m^2). Kako je u šumskom sklopu gotovo nemoguće u spratu drveća to obaviti direktno sakupljanjem asimilacionih organa i njihovim premerom, to se u ovim slučajevima radi različitim indirektnim metodama. Jedna od njih je analiza hemisfernih fotografija pogodnim softverskim paketima. U radu je prikazana analiza hemisfernih fotografija načinjenih prema metodologiji ICP forestry sa softverskim paketom Hemisfer koji izračunava LAI. Dobijeni podaci su uneti u ICP Forests bazu podataka sa svim relevantnim podacima o kvalitetu i uslovima koji su bili kada su fotografije snimane. Rezultati izračunatih vrednosti LAI će se koristiti u daljim istraživanjima uticaja različitih ekoloških i antropogenih faktora na vegetaciju kada se izvrši dovoljan broj ponavljanja tokom letnjih i zimskih fotografisanja u narednim godinama.

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MOST COMMON SPECIES OF DEFOLIATING INSECTS OF BROADLEAVED FORESTS: ICP LEVEL I MONITORING IN 2021

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Abstract: *In order to make conclusions related to the selection of the most appropriate improvement measures, it is necessary to define the condition of forests through monitoring their health state. ICP for Forests Monitoring deals with human factors (mainly air pollution) and biotic factors that impair vitality and hinder the development of forest ecosystems in Europe and Serbia. Since broadleaved forests experience stronger attacks of plant diseases and pests, they have more severe defoliation. Beech is the most common broadleaved species at the ICP sample plots in Serbia. It is followed by oak species – sessile oak, Turkey oak and Hungarian oak. The largest number of insect species develop on the assimilation organs of forest trees. The paper presents the most common species of defoliators detected at SP in Serbia in 2021.*

Key words: pests, monitoring, defoliators, crown condition

NAJZASTUPLJENIJE VRSTE INSEKATA DEFOLIJATORA LIŠĆARSKIH ŠUMA U OKVIRU PRAĆENJA NA BIT NIVO-A I U 2021. GODINI

Apstrakt: *Uvid u stanje šuma radi donošenja zaključaka o neophodnim merama unapređenja, moguće je utvrditi praćenjem zdravstvenog stanja šuma. Predmet ICP-a za praćenje šuma su antropogeni (uglavnom zagađenje vazduha) i biotički faktori štetni po vitalnost i razvoj šumskih ekosistema u Evropi, kao i u Srbiji. Lišćarske šume, zbog jačeg napada biljnih bolesti i štetočina, imaju jače izraženu defolijaciju. Od lišćarskih vrsta, najzastupljenije vrste na bioindikacijskim tačkama Nivo-a 1 u Srbiji je bukva, zatim*

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hrastovi: cer, sladun i kitnjak. Najveći broj insekata razvija se na asimilacionim organima šumskog drveća. U radu će biti prikazane najčešće vrste defolijatora koje su detektovane na BIT u Srbiji u 2021. godini.

Ključne reči: štetočine, monitoring, defolijatori, stanje kruna.

1. INTRODUCTION

Periodic variations in results emphasise the importance of continuous monitoring of defoliation and additional assessments of different environmental data that can contribute to a better understanding of what is detected each year. Having assessed the condition of samples (drying and chlorosis), the next step is to carry out integrated system monitoring by determining soil changes, analysing leaves, assessing deposition and other similar studies (Popović et al., 1995).

Continuous forest condition monitoring at reconstructed Level I sample plots has been conducted according to the Manual of the ICP programme for forest condition monitoring since 2003. The Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia – Forest Administration has entrusted the coordination and management of the ICP for Forests Programme to the Institute of Forestry as the National Focal Center for Forest Condition Monitoring of the Republic of Serbia. Our country has been actively participating in this programme every year since 2003 and reported on the results to the Forest Administration and the ICP Forest Centre. All data collected are entered into a coded database at the end of the year (UNECE, 2010).

Forest ecosystems are exposed to the opposing forces of the debatable discourse of climate change or global warming. Global warming can positively affect plant growth (Saxe et al., 2001; Pretzsch et al., 2014) by prolonging the tree growing season. On the other hand, it can increase the pressure of herbivorous insects on forest ecosystems. Mild winters increase the survival rate of overwintering insects (Pureswaran et al., 2018), and warm climates encourage insect development and increase the number of generational cycles per year.

The defoliator is a collective name for the insects that reduce the assimilation surface. In the years of outbreaks, they cause massive damage that can drastically affect the growth, increment and yield of trees, or change their phenotypic characteristics, such as the tree's natural shape (Mihajlović, 2008).

2. METHOD AND CRITERIA

Faced with the growing concern about the state of forests in Europe in the early 1980s, the UN/ECE established the International Cooperative Program for the Assessment and Monitoring of the Effects of Air Pollution on Forests (ICP Forests) under the Convention on Long-range Transboundary Air Pollution (CLRTAP) in 1985.

In 1986, the European Union adopted a plan to protect forests from air pollution, and the legal basis for co-financing in EU member states was provided by EEC Regulation No.3528 / 86. (UNECE, 2010).

Since then, ICP Forests and the EU have worked closely together to monitor forests. The programme now involves 46 countries (states), including all EU members, Canada and the United States.

Forest condition assessments use standardised methods that form a significant basis for the exchange of expert knowledge (ICP Forests: Manual on methods and criteria for harmonised sampling, assessment, monitoring and analysis of the effects of air pollution on forests).

The methodology was developed as an important platform and is mandatory for all participants in the Programme. The results and recommendations provide a scientific basis for decision-making in the field of air quality control, forestry development policy and strategy, and environmental protection in general.

Monitoring activities, in this way, aim at the resolutions of the Ministerial Conferences on the Protection of Forests in Europe and provide information on three key indicators of sustainable forest management.

Since 2003, 130 observation plots have been installed in the territory of the Republic of Serbia, on a systematic grid of 16 x 16 km (103 sample plots), and since 2004 in a grid of 4 x 4 km too. Monitoring of forest condition at Level I observation plots primarily refers to the observation and assessment of defoliation (mandatory) and discolouration (optional) of tree crowns.

Furthermore, data on soil gathered under the programme are expected to contribute to carbon accumulation estimates, as the EU's input for the implementation of the Kyoto Protocol mechanisms under the UN Climate Change Convention (UNFCCC). Regular observations of Level I plots on the entire territory of Serbia during the growing season (June-September) in 2021, resulted in the assessment of tree defoliation and vitality. The assessment included defoliation and discolouration, as well as monitoring of the damage caused by biotic and abiotic agents. Each individual tree within the observation plot was assessed and any visible damage and change detected were recorded. The research was conducted on all 2,928 trees of Level I observation plots, 359 of which were conifers and 2,569 broadleaves. This shows that broadleaved trees, as well as damage inflicted to them, including the damage caused by insects, have significantly contributed to the complete picture of the state of Serbia's forests in 2021.

All injuries were recorded by cause and classified by type. This paper uses data from the field forms – logbooks that are filled in the field by researchers from the Institute of Forestry.

Undetermined damage causes or the insects themselves (larvae, imago) were later determined in the laboratory of the Institute of Forestry, Belgrade, by the method of cultivation or using identification keys.

Defoliation classes are shown in tables and assessed visually in the field (Table 1).

Table 1. *Defoliation classes by UN/ECE[2] and EU[3] classification*

Defoliation class	Defoliation degree	Loss of foliage (%)
0	None	0-10
1	Slight	10-25
2	Moderate	25-60
3	Severe	60-100
4	Dead	100

Beech is the most common tree species at Level I sample plots, followed by oak species, namely Turkey oak, Hungarian oak and sessile oak, as well as hornbeam and other broadleaved trees.

3. RESULTS AND DISCUSSION

Damage was recorded and classified by cause and tree species. In percentage terms, harmful insects were the most common cause of damage. Regarding tree species, Turkey oak and sessile oak were most endangered by insects, with beech following them (Table 2).

The formation of a large number of insects in a growing season can have adverse effects on the vitality and growth of plants (Bebber et al., 2013; Bacon et al., 2014). Furthermore, with the migration of invasive species, new insect species are introduced into our country. This phenomenon can be observed in the whole world (Pureswaran et al., 2018).

An increase in the frequency, intensity and duration of the so-called heatwaves with an increasingly smaller number of ice days are expected (Brašanac-Bosanac, Ćirković-Mitrović, 2013).

Heat extremes of this type have been recorded continuously: a heatwave from 21 to 24 June in the West Bačka, Srem, Mačva and Kolubara districts, from 22 to 25 June in the city of Belgrade, South Bačka, North Banat, Central Banat, South Banat, Braničevo, Šumadija, Danube and Pomoravlje districts, and from 22 to 24 June in the Zlatobor district. Also, a heatwave started on 23 June on the territory of Raška, Moravica, Rasina, Bor and Zaječar districts and ended on 25 June. There was another heatwave from 7 to 9 July in the area of Belgrade, Central Banat, Central Bačka, West Bačka, South Bačka, Srem, Mačva, Kolubara, Danube and Rasina districts (RHMS). A heatwave was registered from 12 to 15 July in the territory of the Braničevo, Bor, Pomoravlje, Nišava and Jablanica districts. In the period from 26 to 28 July, a heatwave was recorded in the South Bačka district and the Kolubara district from 25 to 28 July. A heatwave was also recorded in the Zaječar district from 31 July to 2 August.

Climate data indicate that the air temperature in Serbia during the summer of 2021 was above the average values (RHMS).

The Wind Chill Index (WCI) was made for the period from December to February (RHMS). It is a measure of relative discomfort caused by heat loss from

[2] United Nations Economic Commission for Europe
[3] European Union

the human body at a specific combination of extremely low air temperatures and wind speed. This index shows the likelihood of frostbite, freezing and other acute symptoms of body stress (WMO No.182 Internat. Meteorolog. Vocabulary).

These data on the winter of 2020/21 indicate a moderate to mild winter (RHMS), which affected, for instance, the survival rate of insects that overwinter at different stages.

Leaf-destroying insects were detected – from their egg litters (gypsy moth) to the visible damage caused by feeding. Various larvae that feed on wood were registered, as well as adult insects and egg-laying grounds. Gall-making insects from the family of the gall wasps or the gall midges were identified based on the galls they form. The leaves of apical shoots attacked by leaf scales curled and died due to the injury caused both by larvae and adult insects sucking the tissues with their piercing mouth. Leaf mines were photographed, and the species were determined later in the laboratory. The presence of oak powdery mildew and stunted leaves pointed to the attack of early oak defoliators (before going out in the field), tortrix moths (Tortricidae) and geometrid moths from the family of Geometridae.

Table 2. *Tree damage causes in 2021*

	Insect damage	**Fungus damage**	**Damage by abiotic agents**	**Human damage**	**Fire damage**	**Local pollution damage**	**Other damage**	**TOTAL damage**
All species %	16.0	15.7	2.0	0.9	0.1	0	2.7	37.5
Broadleaves %	17.7	13.5	2.1	0.7	0.2	0	1.8	36.0
Conifers %		33.5	0.9	2.1		0	10.0	46.5
Beech %	11.4	9.2	3.3	1.5	0.5	0	2.7	28.6
Fir %	0	20.6	0	0	0	0	4.8	25.4
Spruce %		3.2	1.6	3.7		0	16.0	24.6
Austrian pine %	0	73.5	0	0	0	0	0	0
Turkey oak %	12.9	13.9	0.4	0.4		0	1.2	28.7
Sessile oak %	51.5	29.9	1.2	0	0	0	3.6	86.2

3.1. Lepidoptera ORDER

Gypsy moth (*Lymantria dispar* L.), the most dangerous pest this season, was in the latency phase. Observing the foliar damage, it can be concluded that the gypsy moth caterpillars occurred only individually. Several egg broods were also recorded individually (Figure 1, SP 37).

Figure 1. *Gypsy moth egg litter (freshly laid), August 2021, SP 37 (Orig.)*

Figure 2. *Ticheria ekebladella (Bjerk*) on oak seedlings, SP 56 (Orig.)

Miners were found in great numbers on oaks and beeches, but this season they were also extremely abundant on locusts. Regarding oaks, the most common was *Ticheria ekebladella* (Bjerk), whose mines often cover the entire leaf surface (Figure 2).

A leaf miner of the family Gracillaridae, *Phyllonorycter messaniella* Zell. (Figure 3, SP 413) was found at almost all sample plots where beech is the dominant species. A strong attack of *Phyllonorycter robinie* Clemens, a moth of this family commonly found on the underside of locust trees, was recorded (SP 19, 428). It occurred together with the digitate leafminer *Parectopa robiniella* that attacks the upper side of the leaf (Figure 4).

Figure 3. *Phyllonorycter messaniella Zell. SP 413 (Orig.)*

Figure 4. *SP 19 Locust miners, Phyllonorycter robinie Clemens and Parectopa robiniella Clemens (Orig.)*

Geometrid moths (Geometridae) and leafroller moths (Tortricidae) – early-season oak defoliators were the most common insects found on the oak leaves. The intensity of the damage caused by winter moths in this season was low – a weak attack. Regarding Tortricidae, several different types of damage were observed, based on which it could be concluded species of the genera *Alleimma, Tortrix,*

Torticoides, Choristoneura sp were present at SP (Figure 5). Although they are not directly the subject of this paper, seed pests indirectly affected the vitality of this year's acorn crop by affecting the seed crop. It was particularly the case with Hungarian oak trees that were attacked by the seed rollers. Figure 6 shows a caterpillar of *Cydia splendana* Hbn., before hibernation.

Figure 5. *Damage caused by Tortricidae, Hungarian oak leaf, SP 60 (Orig.)*

Figure 6. *Cydia splendana Hbn., a caterpillar before hibernation (Orig.)*

3.2. Hymenoptera ORDER

Cynipidae, gall wasps, have an extremely rich diversity in Serbia. There are over 40 different species so far determined based on different and variable forms of galls that they produce and the species on which they form them (Zúbrik, et al. 2008). A fairly common species on SP in Serbia is *Neuroterus quercus baccarum* L,. found in great numbers on Turkey oak. Although they have a button-sized round body and attack the underside of a leaf, when in large numbers, they reduce the leaf area and hinder the transpiration. Besides *Neuroterus quercus baccarum* L,. common species include *Cynips quercusfolii* L., *Andricus quercuscalicis* (Bur.), *Andricus quercustozae* (Bosc), *Andricus caputmedusae* (Htg.), *Biorhiza pallida* (Ol.), (Figure 7-10), etc.

In addition to gall wasps, miners were also very common agents of damage to oak leaves, especially sessile oak.

Figure 7. *Andricus quercustozae (Bosc) (Orig.)*

Figure 8. *Andricus quercuscalicis (Bur.) (Orig.)*

Figure 9. *Andricus caputmedusae (Htg.) (Orig.)*

Figure 10. *Biorhiza pallida (Ol.), an old gall (Orig.)*

3.3. Diptera ORDER

Apart from oak, most sample plots include beech (*Fagus sylvatica* L) forests. According to the Flora of Serbia II (Stevanović, 2012), Moesian beech (*Fagus moesiaca* (K. Maly) Czecz.) has recently got the status of a subspecies of European beech (*Fagus sylvatica* L.) in Serbia – *Fagus sylvatica* subsp. *moesiaca*. Despite its good technical properties, beechwood is known to be not so resistant and susceptible to attacks of numerous parasitic and saprophytic organisms. Trees physiologically weakened and diseased by their action become an easy target for the attack of various primary and secondary harmful insect species, which ultimately leads to the death of individual trees or groups of trees (Tabaković-Tošić, Marković, 2004).

Insect damage, mainly from miners and gall wasps, was registered on beech leaves. From the order of Diptera, the species that affect the assimilation function of the leaf blade include *Hartigiola annulipes* (Hartig), *Mikiola fagi Htg.*, and *Dryomia circicanns* (Girauld) (Figures 11-13). Gall midges from the Cecidomidae family are also present with the most common species (Figure 14).

Figure 11. *Hartigiola annulipes (Hartig), SP 76, (Orig.)*

Figure 12. *Dryomia circicanns (Girauld) (Htg.), SP 97, (Orig.)*

Figure 13. *Mikiola fagi Htg. SP 412, (Orig.)*

Figure 14. *Cecidomidae, galls on Tartar maple, Acer tataricum, SP 39 (Orig.)*

3.4. Coleoptera ORDER

The most significant and recognizable insect–forest pest of the beetle order is the beech leaf-mining weevil, *Orchestes fagi* L that causes significant foliage damage (Figures 15 and 16). The oak leaf beetle, *Agelastica quercetorum* Foudr. is another common oak leaf pest that significantly affects the general vitality of the leaf blade. It is interesting that, like with the beech leaf-mining weevil, its damage starts with snake-like blasting, and continues with the complete decomposition of the oak apical shoots (Mihajlović, 2008).

Figure 15. *Orchestes fagi L. (Orig.) SP 412, initial phase (Orig.)*

Figure 16. *Orchestes fagi L. SP 412, advanced phase (Orig.)*

Various agents of curled and wilting leaves were recorded on all oak species. Among other leaf rollers, *Attelabus nitens* Scop was particularly abundant on Hungarian oak trees.

3.5. Homoptera ORDER

A season favourable in terms of weather conditions bring a mass swarm of *Phillaphis fag*i L., woolly beech aphid (Fig. 17), a species that was introduced to other continents from Europe and causes significant damage. When the attack is extremely strong, the damage can be substantial. This insect was observed in our country as well, especially at the base of the leaf veins (Figure 18).

Figure 17. *Phillaphis fagi L., Swarming SP 85 (Orig.)*

Figure 18. *Damage caused by Phillaphis fagi L., SP 85 (Orig.)*

Individual shields of females of *Parthenolecanium rufulum* Chll., the oak soft scale, were also found. In large numbers, i.e. just like the plum soft scale, it can greatly affect the growth and development of shoots at the bases of petioles, buds and twigs (Figure 19). Oaks often have species from the genus of *Phylloxera*, recognisable by the secretion of honeydew, which covers the entire lamina and thus causes stagnation and interrupts transpiration (Figure 20). The given reaction (here

honeydew secretion) is a mechanism determined by the tree species attacked and is regulated by the natural resistance to defoliation (Češljar et al., 2014).

Figure 19. *A shield of a female of Parthenolecanium rufulum Chll.,* oak soft scale*, SP 60 (Orig.)*

Figure 20. *Effects of honeydew of leaf scales on leaf veins, Phylloxera sp., oak shoots, SP 80, (Orig.)*

4. CONCLUSIONS

The vitality of forests depends on environmental conditions and numerous biotic and abiotic effects – climate characteristics, atmospheric deposition, pathogenic organisms, wood destructors, forest fires, direct atmospheric influences, wildlife, rodents, etc. Harmful insects are perhaps the most important link needed to draw the final conclusions about the tree crown vitality. Their huge number of species and diversity in Serbia is to be linked to the state of forest ecosystems at LEVEL I SP in Serbia.

Regarding insect pests, defoliators are proven to be the most decisive factor of tree vitality, given the mentioned abundance of broadleaved species at SP and the percentage of insect damage inflicted to beech, sessile oak, Turkey oak and broadleaved trees in general (Table 2). The damage that in the defined extent affects parts of lamina significantly hinders the normal transpiration of forest trees. Since broadleaved trees are more often attacked by these insects and have more serious mechanical injuries, they generally have more pronounced defoliation and greater total damage. Numerous harmful agents, i.e. insect pests were recorded and identified on sampled trees. These were insects from the orders of Homoptera, Coleoptera, Lepidoptera, Hymenoptera, Diptera.

Since harmful insects are one of the important links necessary to draw the final conclusions about the tree crown vitality, further research should address the issues related to the impact of a huge number of species of harmful insects on the state of forest ecosystems and their diversity at the LEVEL I SP in Serbia.

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Impacts and its Effects on Forest Ecosystems in the Republic of Serbia - Forest Condition Monitoring" in 2021.

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MOST COMMON SPECIES OF DEFOLIATING INSECTS OF BROADLEAVED FORESTS: ICP LEVEL I MONITORING IN 2021

Renata GAGIĆ-SERDAR, Miroslava MARKOVIĆ, Goran ČEŠLJAR, Ilija ĐORĐEVIĆ, Tomislav STEFANOVIĆ, Natalija MOMIROVIĆ, Suzana MITROVIĆ

Summary

The International Program for Monitoring the Condition of Forests in Europe (ICP for Forests) implemented at ICP Level I sample plots in Serbia aims to determine the vitality of forests at the local and regional levels. Tree crown vitality assessed through discolourisation and the extent of damage caused by pests at established sample plots in 2021 should help draw final conclusions about the vitality of forests. It combines adverse effects of complex abiotic and biotic factors on trees as living organisms and complex natural processes within the forest, such as transpiration described in this paper. The effects of diverse insect orders may be the key to finding solutions and improving knowledge about forest ecosystems. These are topical issues that must be further addressed by research in our country and worldwide.

NAJZASTUPLJENIJE VRSTE INSEKATA DEFOLIJATORA LIŠĆARSKIH ŠUMA U OKVIRU PRAĆENJA NA BIT NIVO I U 2021. GODINI

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Rezime

Međunarodni program za praćenje stanja šuma Evrope (ICP Forests of Europe), koji se takođe odvija na ICP oglednim parcelama prvog nivoa u Srbiji, pruža priliku da se utvrdi vitalnost šuma na lokalnom i regionalnom nivou. Procena stanja krošnji šuma, kroz dekolorizaciju, i utvrđivanje stepena oštećenja stabala od štetočina, na BIT-u u 2021. godine, doprinoseći davanju konačnih zaključaka o vitalnosti šuma. Nastaje kao rezultat štetnog dejstva kompleksnih faktora abiotičkog i biotičkog porekla drveća kao živih organizama i složenih prirodnih procesa u okviru šumske biocenoze – gde je to transpiracija

koja je ugrožena insektima ovde opisana. Uticaj različitih redova insekata je ključ za pronalaženje rešenja u budućem ostvarivanju znanja o poboljšanju šumskih ekosistema, u smislu značaja pojedinih redova i njihovih predstavnika na ugroženost oglednih stabala. Ove teme su veoma aktuelne i biće predmet istraživanja kod nas i sveta u daljem periodu.

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PHYSICAL AND MECHANICAL PROPERTIES OF WOOD OF NORTHERN RED OAK IN THE VICINITY OF BELGRADE (SERBIA)

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Abstract: *The wood of northern red oak (Quercus rubra L.), considered to be a very high-quality material for processing in the wood industry, has not been tested in Serbia so far. To date, there are only small areas under northern red oak in Serbia, but there are significant stands of this species in the vicinity of Belgrade. In this paper, the basic physical and mechanical wood properties of northern red oak in Serbia were tested. The examined 57 years old tree was located in the forest of Lipovica, near Belgrade. The tree was cut down and two small logs were taken to the laboratory for analysis. The results show that the properties of wood of northern red oak do not differ significantly from the properties of native oak species in Serbia – sessile and pedunculate oak. In addition, the values of basic mechanical properties of wood of northern red oak in Serbia were similar to the relevant values obtained by other researchers in Europe and the USA. Thus, the utilization of wood of northern red oak in the local wood processing industry should be considered.*

Key words: northern red oak, Serbia, wood physical properties, wood mechanical properties, wood industry.

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FIZIČKA I MEHANIČKA SVOJSTVA DRVETA CRVENOG HRASTA U OKOLINI BEOGRADA (SRBIJA)

Izvod: *Drvo crvenog hrasta (Quercus rubra L.), koje se smatra visokokvalitetnom sirovinom za obradu u drvnoj industriji, do sada nije bilo ispitivano u Srbiji. Trenutno u Srbiji postoje samo male površine pošumljene crvenim hrastom, ali su u okolini Beograda prisutne značajne sastojine ove vrste. U ovom radu su ispitana osnovna fizička i mehanička svojstva drveta crvenog hrasta u Srbiji. Ispitano stablo, starosti oko 57 godina, posečeno je u Lipovici, kraj Beograda, i uzeta su dva trupčića radi laboratorijskih analiza. Rezultati istraživanja pokazuju da se svojstva drveta crvenog hrasta ne razlikuju značajno od svojstava autohtonih vrsta hrastova u Srbiji – kitnjaka i lužnjaka. Sem toga, vrednosti osnovnih mehaničkih svojstava drveta crvenog hrasta u Srbiji su bile približne odgovarajućim vrednostima koje su ustanovili istraživači u Evropi i SAD. Iz tog razloga bi trebalo razmotriti primenu drveta crvenog hrasta u domaćoj drvnoj industriji.*

Ključne reči: crveni hrast, Srbija, fizičke osobine drveta, mehaničke osobine drveta, drvna industrija.

1. INTRODUCTION

Oak (*Quercus* L., fam. Fagaceae Dumort.) is a genus composed of a diverse group of tree species that have been reported as one of the most widely used hardwoods in Europe and North America (Čufar *et al.*, 2013). Among these species, northern red oak (*Quercus rubra* L.) grows naturally in the eastern and central parts of the United States of America (Uzcategui *et al.*, 2020). It was introduced to Europe in 1691 (Gubka and Špišák, 2010). To date, there are only small areas under northern red oak in Serbia (c. 60 ha), but there are significant stands of this species in the vicinity of Belgrade (Lazarević, 2020). Northern red oak has shown exceptional results in the xeromesophilic and xerothermophilic oak habitats. It is a species that tolerates aridity better than sessile oak (*Q. petraea* L.) and has fewer demands on soil fertility. Also, it easily adapts to different climatic conditions and tolerates low temperatures well. Decorativness, high vitality of wood, as well as resistance to pests, drought, frost, windbreaks and snow breaks, make this species very suitable for introduction into special-purpose oak forests (Isajev *et al*., 2006). Northern red oak is considered to be a very high-quality material for processing in the wood industry (Vansteenkiste *et al*., 2005).

The development of the wood processing industry is causing a growing demand for high-quality wood raw materials (Marković *et al.*, 2015). With the current decrease of qualified forestry resources, wood materials should be used more scientifically and efficiently in wood products like furniture (Hu *et al*., 2021). Wood properties are mainly determined by its structure, biological and organic origin, but also by the conditions in which the wood is used and exploited (Živanović *et al*., 2019). Oak species are ring-porous hardwoods with high density in the latewood part of the growth ring. Because the changes in the ring width of oaks have been more associated with the change in width of latewood, the percentage of latewood increase alongside ring width. Generally, this allows the wood density of oak, as well as other strength properties, to increase as the growth

rate increases. However, the density of some trees may decline with a further increase in width ring generated from a very fast growth rate (Zhang *et al*., 1993). Variations in latewood density can be associated with variation in the latewood structure, as well as with changes in the proportions between earlywood and latewood (Rao *et al*., 1997). By summarizing various literature data, Šoškić and Popović (2002) claim that the properties of wood of northern red oak are very similar to the properties of the domestic species of the genus *Quercus*. For instance, the authors stated that the wood density amounts to around 700 kg/m^3 in the dried state of wood, whereas in the most important industrial species in Serbia [i.e., sessile oak and pedunculate oak (*Quercus robur* L.)] the density is around 675 kg/m^3. In addition, Green and McDonald (2007) determined that northern red oak has a Janka hardness of 54.3 MPa, a modulus of elasticity of 12.14 MPa, and compressive strength of about 47 MPa.

Given that the quality of wood of northern red oak has not been tested in Serbia so far, it would be of scientific and practical importance to study the properties of wood of this very resistant and fast-growing oak species in the area. Hence, this paper aims to examine the physical and mechanical properties of wood of northern red oak, as an introduced tree species in Serbia, and to compare them with the same properties of the native oak species.

2. MATERIAL AND METHODS

The examined northern red oak tree was located in the forest of Lipovica, near Belgrade, Serbia (lat. 44.697165, long. 20.356815). The area has been mapped by the unmanned aerial system (drone) DJI Phantom 4 Pro.

Picture 1. *Location of the examined northern red oak tree in (a) Belgrade, and (b) Lipovica forest*

The drone was equipped with a 20 Mpix RGB sensor, and onboard GPS. In total, 263 images were collected of the area of interest. All images were processed in Professional photogrammetry software and the resulting orthomosaic had a resolution of 2.17 cm/pix. The tree location was identified on the resulting orthomosaic. The specific location of the tree is shown on the map (Picture 1).

Northern red oaks in this area are about 60 years old, and they are growing in a mixed forest with Scotch pine (*Pinus sylvestris* L.).

2.1. Specimen preparation and testing methods

The tree was cut down and two small logs of 400 mm length from 1.3 m and 4.4 m heights were taken to the laboratory of the Faculty of Forestry, University of Belgrade, Serbia. In addition, two wheels from the same zones were taken to the laboratory for ring width measuring. Two small logs were cut, and two radial planks from each direction were obtained. Forty-one samples with dimensions 20 x 20 x 320 mm were cut and grouped according to the tree height and cardinal direction. They were tested for elastic moduli. After breakage, the rest of the samples were cut on 20 x 20 x 40 mm and tested for compressive strength parallel to the grain. Two wheels were tested for the size of the growth rings and latewood share. The cardinal sides were marked on both wheels. On the south side, growth rings are counted with the magnifying glass. To determine the size of the growth rings and the latewood inside the ring, a microscope (Digital Microscope with stand INSIZE, and magnification up to 200 times) and graph paper were used to calibrate the microscope. The measurement was performed on the sample from the south side. Samples and dimensions were measured as raw material, then after standing in water for four days and after drying in the dryer (T = 60°C, 1 day; T = 103±2°C to an absolutely dry state). The humidity of the samples in the raw state was determined by the gravimetric method (Šoškić and Popović, 2002). Linear and volumetric shrinkages were calculated. Average values were calculated based on 55 samples for radial, 56 for tangential, and 51 for axial shrinkages. Fifty samples were used to determine total volumetric shrinkage. Considering the formula for calculating the total volumetric shrinkage, obtaining the average size for volumetric shrinkage included 28 samples from the first wheel and 22 samples from the second one. The density of wood is calculated from the ratio of mass and volume of wood using standard equitation (Šoškić and Popović, 2002). Tests of wood density, static bending, and compression parallel to grain were conducted according to SRPS ISO 13061 standard. Each specimen was weighed and measured before testing. The mechanical tests were performed using SCAL WOOD TESTER with the LabVIEW software to control operations. The specific gravity specimen's sizes were 20 × 20 × 20 mm. Each specimen was measured, and oven-dried (103±2°C). Oven-dried weights were recorded after the mass was stabilized. According to the standard SRPS ISO 13061, static bending (modulus of rupture MOR) specimens were 20 × 20 × 320 mm. The test was conducted using center point loading in radial and tangential directions. The failure type was recorded for each specimen. Modulus of elasticity was calculated using standard equations (Šoškić and Popović, 2002). For the compression parallel to grain analysis, the test specimens measured 20 × 20 × 40 mm, according to the standard SRPS ISO 13061. The load was applied at a rate of 0.003 in/in (0.00762 cm/cm) of nominal specimen length/min. The type of deformation was recorded for each specimen.

2.2. Statistical analysis

The obtained numerical data were processed using descriptive statistical methods. Raw data were used to calculate the mean values of all variables and to determine the average standard deviation and coefficient of variation for every mean. The observed variables were also studied by comparing their minimum and maximum values with literature records. The statistical analysis was performed using the statistical software STATGRAPHICS XVI (StatPoint Technologies, Inc., Warrenton, VA, 2009).

3. RESULTS AND DISCUSSION

The results of the analysis of basic macroscopic and physical properties of wood of northern red oak, sampled in the vicinity of Belgrade in Serbia, are shown in Table 1.

Table 1. *The basic macroscopic and physical properties of wood of the northern red oak in Serbia*

Macroscopic and physical properties	Wheel 1	Wheel 2	Average
Number of growth rings	57	48	52
Heartwood	41	34	37
Sapwood	16	14	15
Mean value of increment ring size (mm)	3.575	3.397	3.486
Mean value of latewood ring size (mm)	2.324	2.457	2.391
Share of latewood in the growth ring (%)	67.032	72.314	69.673
Average radial shrinkage (%)	4.73	4.34	4.535
Average tangential shrinkage (%)	10.78	9.483	10.131
Average axial shrinkage (%)	0.793	0.459	0.626
Average total volumetric shrinkage (%)	15.41	13.86	14.635
Basic density (kg/m³)	679	629.40	654.20
Average moisture content in the raw state (%)	-	-	51.40

According to Zeidler and Borůvka (2016), the average ring width in northern red oak is 4.4 mm (range 2.8–7.1 mm). If we compare these data with the data obtained in our study for the 57 years old northern red oak tree (mean size of the growth ring is 3.486 mm, and the range is 0.438–9.379 mm), it can be noticed that the average ring width is smaller, but also that the variation in ring width is larger. The observed differences could be attributed to the different habitats and conditions in which the examined trees grew. Moreover, according to Zeidler and Borůvka (2016), the share of latewood is 68.80% to 90.40%, which is larger than the value obtained in our study (the average share of latewood is 69.673%). In addition, Uzcategui *et al.* (2020) obtained the result that the average percentage of latewood of northern red oak varied between 42.20% and 98.40%, with a mean value of 71.30, and a coefficient of variation of 18.17%. Büyüksarı *et al.* (2018)

calculated the average earlywood, latewood, and annual ring widths and latewood proportion of sessile oak wood, for trees aged 190 to 211 years, as 0.50 mm, 0.49 mm, 0.99 mm, and 49.30%, respectively. Gursu (1966) determined that the annual ring width of oak wood grown in the Karabuk region was 1.58 mm for trees aged 97 to 156 years, and 0.80 mm for trees aged 186 to 247 years. The proportion of latewood was determined to be 66% in *Quercus faginea* Lam. (Knapic *et al*., 2011) and 61% in *Quercus suber* L. (Knapic *et al*., 2008). Table 2 presents descriptive statistics for annual ring width and latewood width of both wheels examined. Considering the average values, it can be noticed that similar results for both tree zones were obtained. The average wood ring width was 3.616 mm for the lower wheel (LWW1), and 3.527 mm for the upper wheel (LWW2). The proportion of the latewood was on average 2.458 mm (68%) for LWW1, and 2.630 mm (74%) for wheel that was cut at 4.4 m above ground (LWW2). Vavrčik and Gryc (2012) obtained the value of 2.1 mm for the ring width of pedunculate oak and 1.4 mm for sessile oak. They reported that, at the same ring width, pedunculate oak had a higher latewood proportion. These differences could be a result of growth conditions, such as precipitation, air temperature, aspect, soil characteristics, etc.

In the present study, the average radial shrinkage is 4.535%, tangential 10.131%, axial 0.626% and the total volumetric shrinkage was 14.635% (Tab. 1). By comparing these results to the assertion of Šoškić and Popović (2002) that the radial shrinkage is 4.9%, tangential – 9.4%, axial – 0.4%, and total volumetric shrinkage – 14.2% for pedunculate oak, and radial shrinkage – 4.8%, tangential – 9.3%, axial – 0.4% and total volumetric shrinkage – 13.9% for sessile oak, it can be noticed that northern red oak from our experiment had somewhat higher shrinkage values in all directions, except for axial. The shrinkage values found in our study fall in the interval of literature data for northern red oak and the native oak species in Serbia (Šoškić *et al*., 2005; Šoškić, 2006), although total volumetric shrinkage was higher by about 0.5% than those in the native oak species.

Table 2. *Descriptive statistics for wood ring width (WRW) and latewood width (LWW) for two wheels of the northern red oak in Serbia*

Wood property	Count	Average (mm)	Standard deviation	Coefficient of variation	Minimum	Maximum	Range
WRW1	57	3.616	2.091	0.58	0.465	9.558	9.093
LWW1	57	2.458	1.709	0.70	0.251	6.623	6.372
WRW2	48	3.527	2.644	0.75	0.400	9.379	8.979
LWW2	48	2.630	2.210	0.84	0.300	7.776	7.476

The average moisture content of wood of northern red oak in Serbia is 51.40% (Tab. 1). The trend of decreasing moisture content in the samples from sapwood to heartwood was noted. According to Šoškić and Popović (2002), the average moisture content of sessile and pedunculate oak is 60–65%. Compared to sessile and pedunculate oak, lower moisture content was obtained from the samples of northern red oak wood. This can be explained by the time when the tree was cut (springtime), and by the fact that the moisture content in a standing tree is low (Šoškić and Popović, 2002).

Table 3 and Graph 1 present descriptive statistics for wood density (WD), compressive strength parallel to the grain (Hp), modulus of elasticity (MOE), and modulus of rupture (MOR), obtained with the standard, destructive method.

Table 3. *Descriptive statistics for wood properties of the northern red oak in Serbia determined with the standard destructive method*

Wood property	Count	Average (mm)	Standard deviation	Coefficient of variation	Minimum	Maximum	Range
WD (kg/m^3)	40	0.72	0.03	0.04	0.67	0.78	0.11
Hp (MPa)	40	64.43	5.67	0.09	53.15	75.75	22.60
MOE (N/mm^2)	40	7381.50	1573.58	0.21	4829.96	9795.76	4965.80
MOR (N/mm^2)	40	102.23	14.43	0.14	73.43	127.26	53.83

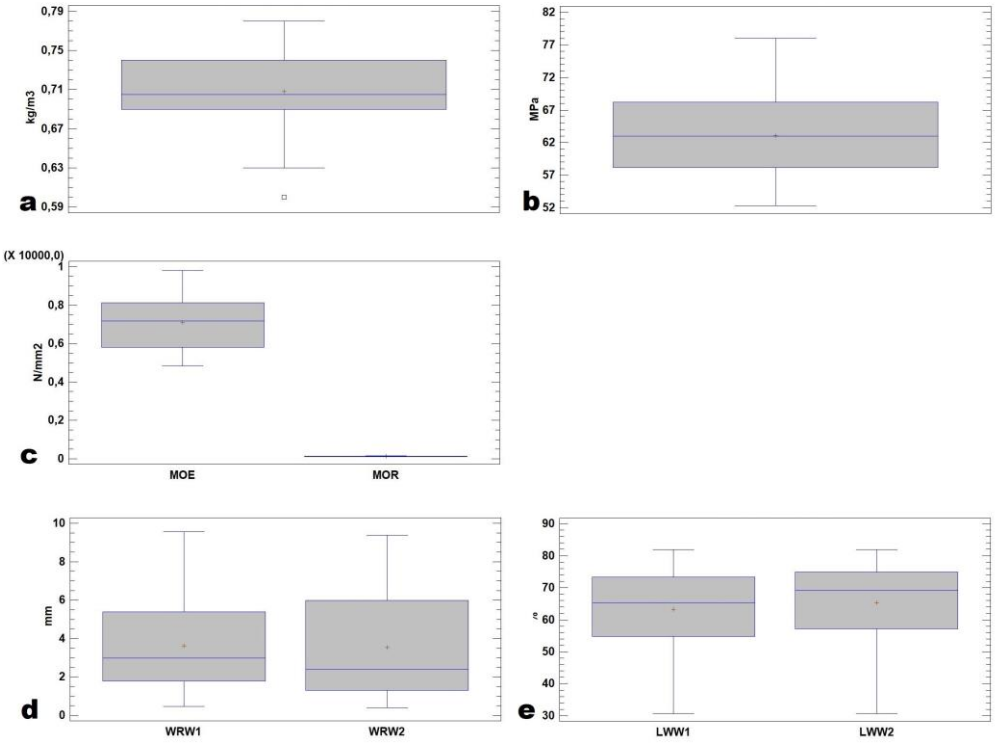
According to Šoškić *et al*. (2005), the basic density of sessile oak is 665 kg/m^3, and the basic density of pedunculate oak is 650 kg/m^3, whereas the density of northern red oak is 740 kg/m^3. In Zeidler and Borůvka (2016), the average density of northern red oak was similar to the value obtained in our study. The density of northern red oak in our study does not differ significantly from the density of sessile and pedunculate oak. Specifically, wood density was between 670 and 780 kg/m^3, with an average value of 720 kg/m^3, and a coefficient of variation of 4%. Uzcategui *et al.* (2020) obtained wood density for northern red oak that varied between 571 and 853 kg/m^3, with an average value of 699 kg/m^3, and a coefficient of variation of 8.31%. Klašnja *et al*. (2006) obtained the total mean value of 673 kg/m^3 for the oven-dried volume density of wood of *Quercus robur*, with a standard deviation of 49.785, a maximum value of 769 kg/m^3, and a minimum value of 573 kg/m^3. Therefore, it can be noticed that northern red oak tree described in our study has a larger basic density than native oak species by about 10%. Still, this is not considered to be a significant difference.

The values of compression parallel to grain ranged from 53.15 to 75.75 MPa, with an average value of 64.43 MPa, and a coefficient of variation of 8.80%. These values are very close to those obtained by Uzcategui *et al.* (2020) for the northern red oak in the southeastern part of the USA.

Among the analyzed wood properties, MOE had the highest coefficient of variation (21.32%). Still, the mean value (7381.5 MPa) was slightly lower than those given in the literature (Uzcategui *et al.*, 2020). On the other hand, MOR has shown similar mean value (102.23 MPa) to those given in the literature. For example, Vansteenkiste *et al.* (2005) obtained a value of 12.500 MPa for MOE and 99 MPa for MOR. Wang and Allison (2008) obtained 9.810 MPa for MOE and 62.1 MPa for MOR. Uzcategui *et al.* (2020) obtained 12.211 MPa for MOE and 120 MPa for MOR. Since wood properties depend on many factors, such as climate, soil, elevation, exposition, etc., and the results originate from different parts of the world, such variations are expected.

The results obtained in our investigation are the most similar to those presented by Uzcategui *et al*. (2020). It can be assumed that the reason for this is

similar climate conditions due to the similar geographical positions of the trees examined and the use of similar testing machines and sample dimensions.



Graph 1. *Basic statistical parameters for (a) wood density, (b) compressive strength parallel to the grain, (c) modulus of elasticity (MOE) and modulus of rupture (MOR), (d) wood ring width (WRW), and (e) latewood width (LWW) for two wheels of the northern red oak in Serbia. Middle sign = mean, middle line = median, box = mean and standard deviation, whisker = variation range*

4. CONCLUSIONS

Based on the results obtained in this study, the following conclusions can be drawn:

- In comparison with literature data, the average ring width in the examined 57 years old northern red oak tree was small, but the variation in the ring width was large (the mean size of the growth ring was 3.486 mm, and the range was 0.438–9.379 mm). These differences could be a result of different growth conditions, such as precipitation, temperature, aspect, soil characteristics, etc.;
- The shrinkage values found in this study fall in the interval of literature data for northern red oak and the native oak species in Serbia (sessile oak and pedunculate oak);
- The density of wood of northern red oak in this study does not differ

significantly from the density of sessile oak and pedunculate oak;

- Among the wood properties analyzed, MOE had the highest coefficient of variation (21.32%), and MOR has shown an expected mean value.

Based on the results of the study and the similarities and differences noted by comparing them with the results in other relevant studies, it can be concluded that wood of northern red oak has similar mechanical and physical properties to those of sessile oak and pedunculate oak in Serbia, and it should be considered for utilization in the local wood processing industry.

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PHYSICAL AND MECHANICAL PROPERTIES OF WOOD OF NORTHERN RED OAK IN THE VICINITY OF BELGRADE (SERBIA)

Ivana ŽIVANOVIĆ, Nebojša TODOROVIĆ, Nenad ŠURJANAC, Milan KABILJO, Filip JOVANOVIĆ

Summary

Northern red oak (*Quercus rubra* L.) grows naturally in the eastern and central parts of the United States of America (Uzcategui *et al.*, 2020). It was introduced to Europe in 1691 (Gubka and Špišák, 2010). To date, there are only small areas under northern red oak in Serbia, but there are significant stands of this species in the vicinity of Belgrade (Lazarević, 2020). Given that wood of northern red oak has not been tested in the area so far, it is of scientific and practical importance to study the properties of wood of this very resistant and fast-growing oak species, which is considered to be a very high-quality material for processing in the wood industry. In this study, basic physical and mechanical properties of wood of northern red oak in Serbia were tested.

The location of the examined 57 years old northern red oak tree (Lipovica forest, Belgrade) has been mapped by the unmanned aerial system (Picture 1). The tree was cut down and two small logs of 400 mm length from 1.3 m and 4.4 m heights were taken to the laboratory for analysis. Two wheels from the same tree sections were taken for ring width measuring. Forty-one samples with dimensions 20 x 20 x 320 mm were cut and grouped according to the tree height and cardinal direction. They were tested for elastic moduli (MOE) and modulus of rupture (MOR). After breakage, the rest of the samples were cut on 20 x 20 x 40 mm and tested for compressive strength parallel to the grain. Two wheels were tested for the size of the growth rings and latewood share. The cardinal sides were marked on both wheels. On the south side, growth rings are counted. The moisture content of the samples in the raw state was determined. Average values were calculated based on 55 samples for radial, 56 for tangential, and 51 for axial shrinkages. Fifty samples were used to determine total and average volumetric shrinkages (28 samples from the first wheel and 22 samples from the second wheel). Modulus of elasticity was calculated using standard equations (Šoškić and Popović, 2002). The obtained numerical data were processed using descriptive statistical methods.

For the tree examined, the mean size of the annual growth ring was 3.486 mm (Tab. 1), with a range 0.438–9.379 mm. The average width of the growth ring and latewood percentage have shown similar results for both tree sections examined. The proportion of the latewood was on average 68% for the lower wheel and 74% for the wheel that was cut at 4.4 m above ground (Tab. 2). The average radial shrinkage was 4.535%, tangential – 10.131%, axial – 0.626%, total volumetric shrinkage – 14.635% (Tab. 1). The shrinkage values found in this study fall in the interval of literature data for northern red oak and the native oak species in Serbia (sessile and pedunculate oak). The average moisture content in the raw state was 51.4%. Basic density does not differ significantly from the density of

sessile and pedunculate oak. The values of compression parallel to the grain ranged from 53.15 to 75.75 MPa, with an average value of 64.43 MPa, and a coefficient of variation of 8.80%. The values are very close to those determined for the northern red oak in the southeastern part of the USA. Among the wood properties analyzed, MOE had the highest coefficient of variation (21.32%). Still, the mean value (7381.5 MPa) was slightly lower than those given in the literature. On the other hand, MOR has shown similar mean value (102.23 MPa) (Tab. 3) with those given in the literature. The basic statistical parameters of the studied variables are shown in Graph 1.

Based on the presented results, it can be concluded that wood of northern red oak in Serbia has similar mechanical and physical properties with the native oak species, and it should be considered for utilization in the local wood processing industry.

FIZIČKA I MEHANIČKA SVOJSTVA DRVETA CRVENOG HRASTA U OKOLINI BEOGRADA (SRBIJA)

Ivana ŽIVANOVIĆ, Nebojša TODOROVIĆ, Nenad ŠURJANAC, Milan KABILJO, Filip JOVANOVIĆ

Rezime

Crveni hrast (*Quercus rubra* L., Fagaceae Dumort.) autohtono raste u istočnim i središnjim delovima Sjedinjenih Američkih Država (Uzcategui *et al.*, 2020). U Evropu je prvi put unet 1691. godine (Gubka, Špišák, 2010). Trenutno u Srbiji postoje samo male površine pošumljene crvenim hrastom, ali su u okolini Beograda prisutne značajne sastojine ove vrste (Lazarević, 2020). Imajući u vidu da drvo crvenog hrasta do sada nije bilo ispitivano u ovom području, od naučnog je i praktičnog značaja ispitati svojstva drveta ove veoma otporne i brzorastuće vrste hrasta, čije se drvo smatra visokokvalitetnom sirovinom za obradu u drvnoj industriji. Stoga su u ovom radu ispitana osnovna fizička i mehanička svojstva drveta crvenog hrasta u Srbiji.

Položaj ispitanog stabla crvenog hrasta u Lipovici (Beograd), starosti 57 godina, mapiran je bespilotnom letilicom (slika 1). Stablo je posečeno, uzeta su dva trupca dužine 400 mm, na dve visine od zemlje (1,3 m i 4,4 m), i odneta su u laboratoriju radi analiza. Iz istih delova stabla, isečena su dva kotura za analizu širine prstenova prirasta. Potom je načinjen 41 uzorak, dimenzija 20 x 20 x 320 mm, i sortiran je shodno delu stabla i strani sveta odakle je uzet. Ovi uzorci su korišćeni za određivanje modula elastičnosti (MOE) i modula loma (MOR). Preostali uzorci su isečeni do dimenzija 20 x 20 x 40 mm i na njima je određena pritisna čvrstoća paralelna drvnim vlaknima. Na koturovima je određena širina prstenova prirasta, kao i učešće kasne zone u njima. Na oba kotura su obeležene strane sveta, pri čemu je broj prstenova određen na južnoj strani. U uzorcima je ispitan i sadržaj vlage u sirovom stanju. Prosečno radijalno utezanje je određeno na osnovu 55 uzoraka, tangencijalno na osnovu 56, dok aksijalno na osnovu 51 uzorka. Ukupno i prosečno zapreminsko utezanje je određeno na osnovu 50 uzoraka, od čega je 28 uzeto iz prvog kotura, a 22 iz drugog kotura. Modul elastičnosti određen je prema standardnim formulama (Šoškić, Popović, 2002). Dobijene numeričke vrednosti su obrađene deskriptivnim statističkim metodama.

Na ispitanom stablu, prosečna širina godišnjih prstenova prirasta je bila 3,486 mm (tabela 1), sa opsegom vrednosti od 0,438 do 9,379 mm. Prosečne širine prstenova prirasta, kao i učešće kasne zone u njima, pokazale su slične vrednosti u oba ispitivana dela stabla. Učešće kasne zone je u proseku bilo 68% za kotur sa manje visine, a 74% za kotur koji je isečen na visini 4,4 m od zemlje (tabela 2). Prosečno radijalno skupljanje je iznosilo 4,535%, tangencijalno – 10,131%, aksijalno – 0,626%, a ukupno – 14,635% (tabela 1).

Prosečne vrednosti utezanja utvrđene u ovom istraživanju se uklapaju u raspon literaturnih vrednosti navedenih za crveni hrast i za autohtone vrste hrastova u Srbiji (kitnjak i lužnjak). Prosečni sadržaj vlage u sirovom stanju je 51,4%. Gustina drveta crvenog hrasta se ne razlikuje značajno od gustine drveta kitnjaka i lužnjaka. Vrednosti pritisne čvrstoće paralelne sa drvnim vlaknima varirale su od 53,15 do 75,75 MPa i imale su prosečnu vrednost 64,43 MPa i koeficijent varijacije 8,80%. Ove vrednosti su približne literaturnim vrednostima ustanovljenim za crveni hrast u jugoistočnom delu SAD. Među analiziranim svojstvima drveta, MOE je pokazao najveću vrednost koeficijenta varijacije (21,32%), mada je njegova srednja vrednost (7381,5 MPa) bila nešto manja od one navedene u literaturi. S druge strane, MOR je imao približnu srednju vrednost (102,23 MPa) (tabela 3) literaturnoj. Osnovni statistički pokazatelji analiziranih promenljivih prikazani su na grafikonu 1.

Na osnovu dobijenih rezultata, može se zaključiti da drvo crvenog hrasta u Srbiji ima slična mehanička i fizička svojstva sa autohtonim vrstama hrastova, stoga bi trebalo razmotriti njegovu primenu u domaćoj drvnoj industriji.

A GUIDE FOR WRITING RESEARCH PAPER

SUSTAINABLE FORESTRY is a scientific journal which is published original scientific papers, review papers and short communications of Forestry scientific disciplines, Environmental protection, Wood processing, Landscape architecture and horticulture and Environmental engineering (Erosion Control) at least once a year.

The paper should be, in whole, written in English, and Abstract and Summary should be written in English and Serbian. The paper length, including tables, graphs, schemes, pictures and photographs can have maximum 10 typewritten pages, A4 format (Portrait), with normal line spacing (Single Space). Margins: Top 1.5cm, Left 1.5cm, Bottom 1.5cm, Right 1.5cm, Gutter 0.5 cm. The paper should be typed in *Word* format, Roman alphabet, using exclusively the *Times New Roman* Font, 11 points, Normal, First Line 1.27. LAYOUT: header 0.5 cm, footer 0.5 cm. PAPER: width 16.5 cm, height: 24 cm. If special signs (symbols) are used in the text, use the *Symbol* Font.

PAPER TITLE IN ENGLISH (capital letters, 11 points, bold, centered)

Title should be 11 Enter keystrokes below the top margin.

11

Name and FAMILY NAME[1] (capital letters, 11 points, italic, centered)

With mark 1, 2 ... in superscript (with command Insert Footnote), above the name of the author is marked a Footnote in which are indicated title, name and family name, occupation and institution in which authors are employed. Also, in Footnote should be write the name, family name and e-mail of correspondence author.

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Abstract: *The abstract is written in English with three spaces below the name of the paper author. In abstract are given basic aim of research, materials and methods, more important results and conclusion (maximum 500 characters) (10 points, italic, first line 1.27).*

11

Key words: in English, minimum 3, maximum 6 words (10 points, normal).

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PAPER TITLE IN SERBIAN (capital letters, 11 points, bold, normal, centered)

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Key words: in Serbian (10 points, normal).

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11

1. INTRODUCTION (capital letters, 11 points, bold, align left)

11

The introduction should be a short review of explanation about reasons that led to the research of specific scientific issue. The introduction contains reference data of published papers that are relevant for the analyzed issue (normal, 11 points, justify, first line 1.27).

11

2. MATERIAL AND METHODS (capital letters, 11 points, bold, align left)

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Within this chapter there can be subtitles of first and second line. In the description of material it should be given enough information to allow other researchers to repeat the experiment at a different location. It is necessary to provide information on the material, subject of the study that precisely defines its origin, physical characteristics etc. If a device or instrument is used to obtain experimental results should be specified: name of the device or instrument, model, manufacturer's name and country of origin. If a scientifically recognized method is used it has to be cited in the References, without the explanation of the steps of the used method. If changes were made in a scientifically recognized method should be provided the original literature references that will support – justify those changes.

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3. RESULTS (capital letters, 11 points, bold, align left)

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Within this chapter there can be subtitles of first and second line. The paper results should be presented in the form of text, tables, pictures (diagrams) and, rarely, photographs. From the results should be clear whether the hypotheses have been confirmed or disproved and whether the aim and tasks have been achieved. It should not be avoided the presentation of the negative results or disproving the hypotheses.

11

3.1. Chapter title (11 points, bold, align left)

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3.1.1. Subchapter title (11 points, bold, align left, first line 1.27)

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Subchapter text (11 points, justify, first line 1.27)

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Table 1. (bold, 11 points), *Table title (11 points, italic, centered)*

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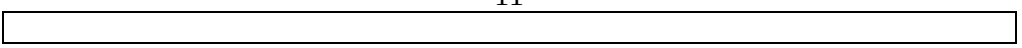

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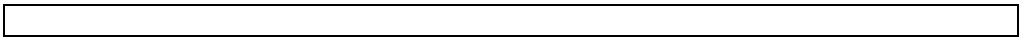

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4. DISCUSSION (capital letters, 11 points, bold, align left)

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Discussion should not be the simple repeating of obtained results. The results should be discussed by comparing them with the research results of other authors with compulsory citing of literature sources. It is very important to give discussion of the results and the opinion of the authors. Interpretation of perceived ambiguities and illogicalities should be correctly stated (11 points, justify, first line 1.27).

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5. CONCLUSION (capital letters, 11 points, bold, align left)

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Conclusions of the paper should be carefully carried out and shown clearly to the reader. Conclusions can be significantly connected with the result discussion, but in them should be given freer and wider interpretation of the paper subject and results. The special quality is the defining of suggestions for future work and identifying the issues need to be resolved (11 points, justify, first line 1.27).

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Acknowledgements (10 points, bold)**:** *If the paper is a part of a research within a project in acknowledgement are indicated: name of the project, registration number and the full name of the institution that finances the project (10 points, justify, italic).*

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***

In this text a detailed structured instruction for writing papers is given. Papers that do not satisfy the propositions of this Guide will not be forwarded for review and will be returned to the author.

Categorization of papers (suggested by the author – determined by Editorial board taking into account the opinion of the reviewer):

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1. Review paper (paper that contains original, detailed and critical review of the research issue or field in which the author contributed, visible on the basis of auto-citations);
2. Original scientific paper (paper which presents previously unpublished results of author's researches by scientific method);
3. Preliminary communication (Original scientific paper of full format, but small-scale or preliminary character).
4. Other known forms: scientific review, case study and others, if Editorial board finds that such paper contributes to the improvement of scientific thought.

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