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Original scientific paper

CONSTRUCTION OF FLOATING TREATMENT WETLANDS FOR REMEDIATION OF POLLUTED WATERS

Nevena ČULE[1], Aleksandar LUČIĆ[1], Dragana DRAŽIĆ[1],
Vladan POPOVIĆ[1], Milorad VESELINOVIĆ[1],
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Abstract: *Water regulation, integral water management and water conservation are one of the main tasks, set by an ecologically conscious society. A series of complex treatments are set in order to achieve the main goal in wastewater treatment, i.e. eliminating or reducing pollutants to a level that will not cause adverse effects to humans and the environment. The paper presents the construction of an efficient, environmentally friendly and economically justified biological system for the treatment of polluted urban waters. Research has shown that the proposed system with floating islands and algae was effective in removing various categories of pollutants from polluted river (organic and inorganic matter, heavy metals and pathogenic microorganisms), which indicated that the selected type of construction was appropriate. It was concluded that minor modifications in terms of cell coverage by floating islands could provide even greater removal of certain pollutants such as nitrogen, phosphorus and heavy metals.*

Key words: floating treatment wetlands, polluted waters, construction, *Canna indica*, decorative macrophytes

KONSTRUKCIJA SISTEMA SA PLUTAJUĆIM OSTRVIMA ZA REMEDIJACIJU ZAGAĐENIH VODA

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Izvod: *Uređenje, integralno upravljanje vodama i njihova zaštita su jedan od glavnih zadataka, koje sebi postavlja jedno ekološki svesno društvo. Kako bi se dostigao glavni cilj u prečišćavanju otpadnih voda odnosno eliminisanju ili redukovanju zagađujućih materija do nivoa koji neće izazvati negativne efekte kod čoveka i životne sredine, postavljen je niz kompleksnih tretmana. U radu je prikazana konstrukcija efikasnog, ekološki prihvatljivog i ekonomski isplativog biološkog sistema za tretman zagađenih urbanih voda. Istraživanja su pokazala da je predloženi sistem sa plutajućim ostrvima i algama bio efikasan u uklanjanju različitih kategorija polutanata iz zagađene reke (organske i neorganske materije, teški metali i patogeni mikroorganizmi), što je ukazalo da je odabrani tip konstrukcije bio odgovarajući. Zaključeno je da bi manje modifikacije u pogledu pokrivenosti bazena plutajućim ostrvima mogle da obezbede još veće uklanjanje pojedinih polutanata kao što su azot, fosfor i teški metali.*

Ključne reči: sistem sa plutajućim ostrvima, zagađene vode, konstrukcija, *Canna indica*, dekorativne makrofite

1. INTRODUCTION

Rivers and lakes are profoundly significant in urban areas since they represent one of the few remaining natural elements of the city. Instead of enhancing the quality of the environment and improving the surrounding landscape, small watercourses are often used as wastewater and rainwater collectors of industries and settlements, and thus additionally endanger the waters they flow into (Čule, 2016). Various inorganic substances and pathogenic microorganisms can pollute rivers and their banks to such an extent that they can be used neither for recreation nor for simple enjoyments, such as taking a walk since the river and its surroundings often have a very unpleasant smell and unsightly look.

One of the most polluted and highly endangered urban watercourses is the Topčiderka River in the entire area of its catchment. This river is used as a wastewater and rainwater collector of industries and settlements. Therefore, according to its chemical, physiochemical and microbiological water parameters, it is mainly within the limits of category V all the year round (Group of authors, 2013). The sources of anthropogenic pollution of the Topčiderka River are numerous and include: industrial effluence, industrial waste, exploitation of mineral resources, combustion of fossil fuels, traffic, exhaust gases, application of mineral and organic fertilizers and pesticides, stormwater runoff, unregulated landfills, improper disposal of the sewage sludge, the sewage system, septic tanks, *etc*. (Group of authors, 2016).

There are different types of biological systems that can help restore and maintain the physical, chemical and biological integrity of water. The floating treatment wetland (FTW) systems are modified aquatic ecosystems which can use the symbiotic relationship between their main components (plants, microorganisms, algae, substrates and water) to remove various pollutants from the polluted and wastewater in a completely natural way and with high efficiency (Hammer, 1989; Shutes, 2001; Goulet *et al*., 2001). This is an innovative technology that is gaining more and more significance since it has some advantages over other alternative water treatment technologies (Dodkins and Mendzil, 2014). Floating treatment wetlands are very simple to construct. They consist of a floating support, which carries the substrate for the growth of terrestrial and aquatic plants. Having been planted, the plants develop a significant mass of the root system which is in the direct contact with the water. This system allows better absorption of pollutants from the water, better removal of solids from the system, creation of more suitable sites for binding useful microorganisms, *etc* (Van de Moortel *et al*., 2010). The biomass obtained at the end of the treatment process can be easily removed and used for various purposes (Raskin *et al*., 1997; Flathman and Lanza, 1998; Zhu *et al*., 1999; Zhao *et al*., 2012). Apart from water purification as the most direct benefit, floating treatment wetland systems further increase the biodiversity of degraded areas, raise the value of the land and real estate in the areas surrounding the revitalized rivers or lakes, enhance the aesthetic value of the environment and potential of the area for tourism, *etc* (Ghermandi *et al*., 2010).

In order to start the revitalization of the Topčiderka River, pilot-scale facility for polluted water treatment was installed on its bank. The main goal of the research was to provide an environmentally-friendly, efficient and cost-effective solution for the treatment and revitalization of polluted urban river, and to use the obtained results and conclusions to create a model of floating treatment wetlands that could be used for water purification of other rivers, canals, wetlands and lakes.

2. MATERIAL AND METHOD

The construction of the collecting tank and cells as well as their layout were carried out on the basis of a previously completed project, which followed the basic guidelines for the construction of FTW systems set by other researchers (Yang *et al*., 2008; Stewart *et al*., 2008; Van de Moortel *et al*., 2010; White and Cousins, 2013; Dodkins and Mendzil, 2014). Certain modifications were made in order to adapt the biological system to this case and this research.

The vegetation of the floating islands included plant species, which according to the relevant scientific papers, studies and projects and on the basis of previous research conducted by the authors, meet the basic requirements of a good phytoremediation plant (Dushenkov *et al*., 1995; Salt *et al*., 1995; Kumar *et al*., 1995; Cunningham i Ow, 1996; Blaylock *et al*., 1997; Flathman and Lanza, 1998; Zhu *et al*., 1999; Blaylock and Huang, 2000; Dushenkov and Kapulnik, 2000; Čule *et al*., 2011; Čule *et al*, 2016). The selected species of the biological system included the following: *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. The system excluded invasive species and species that could in any way endanger the vegetation either in the surroundings of the river or in the nursery. The plant material was obtained from different nurseries, and before being transplanted into the FTW system it had been grown in an improvised pond (reed) or in containers filled with peat moss (other plants). The control plants were grown in plastic containers filled with water without the addition of nutrients until the end of the experiment.

The algae were brought into the biological system from the riverbed. It was determined that a monoculture of a macroscopic alga from the genus of

Cladophora sp. developed in the cell by the end of the experiment. Further determination of the alga species was difficult because there were no other structures on the talus, but it was presumed to be the *C. glomerata* (Linnaeus) Kützing. since this alga is widespread and quite numerous in the rivers similar to the Topčiderka River.

After the FTW system had been completely established and put into work at the end of August, several different experiments were carried out (Čule, 2016) till the end of the vegetation period (late October). The aim of the experiments was to determine the efficiency of the installed system to remove various categories of pollutants from the polluted river.

Sampling and analysis of polluted and treated water, plants, algae and substrates in the system and in the control, the assessment of the obtained biomass at the end of the vegetation period, as well as the processing of the obtained results was performed according to the predefined methodology (Čule, 2016).

3. RESULTS

The system with floating wetlands for the treatment of polluted water of the Topčiderka River was constructed as a temporary structure. It was located on the bank of the Topčiderka River in the nursery of the Public Enterprise for Forest Management `Srbijašume`, Belgrade, FE `Belgrade`. It was designed as a modified rhizofiltration system and consisted of a pump for drawing water from the river, a closed collection tank, four open rectangular cells in which the floating islands were placed, floating islands with the substrate and the plants, an open rectangular cell with algae and a recirculation pump.

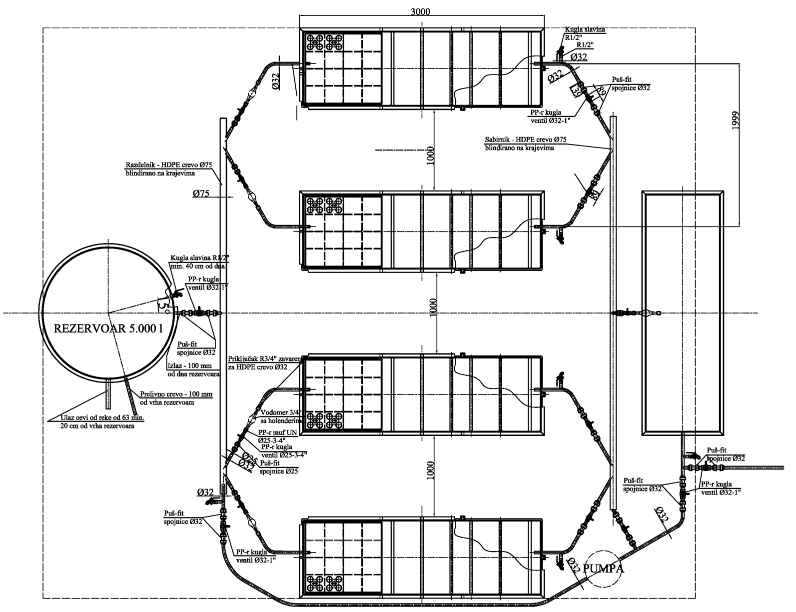

Figure 1. *The scheme of the floating treatment wetland system*

An Oleo-Mac SA 45 TL self-priming water pump was used to draw the polluted water from the river into the biological system and a WP 300 euro 2 self-priming water pump of the same manufacturer was used for the possible recirculation of the water in FTW system.

The collection tank and the cells were made of plastic mass. The tank was a typical vertical reservoir with acceptance volume of 5 m^3 and internal dimensions of Ø1.6m x 2.5m. Four open rectangular cells had acceptance volume of $3m^3$ each. The internal dimensions of the FTW cells were 3m x 1m x 1m. The cells had external metal and internal plastic reinforcements. Each cell had three floating islands measuring 1 x 1 m. The supports of the floating islands were constructed in the form of floating mesh platforms with handles. They were made of light thermoplastic materials. The supports were filled with stone wool which was used as a substrate for the plant growth. The floating islands of the first cell were planted with *P. australis* and the islands of the second cell with *C. indica*. Indian shoot and reed were planted together in the third cell, and *I. pseudacorus*, *I. sibirica* 'Perry's Blue', *A. plantago - aquatica*, *L. salicaria* and *M. trifoliata* in the fourth one. The first three cells had 25 plants on each island, while there were 30 decorative macrophytes on each island of the fourth cell. This method of planting provided the same microclimatic conditions for each species in the biological system and thus their easy comparison in the analysis of the obtained results. The fifth cell had acceptance volume of 1.5 m^3, internal dimensions of 3m x 1m x 0.5m and external metal reinforcements. It contained algae.

The collection tank and the cells were placed on the leveled land so that the entire bottom surface area of each cell lied flat on the substrate. Each inlet branch had a water meter and a DN 25 seal with corresponding fittings installed. The outflow branches contained seals and sampling taps, all of which were PE OD 32 with fittings.

The inlet and outlet branches of the collection tank and the cells were constructed at appropriate heights to provide the gravity flow of water. The outlet of the collection tank was at 10.5 cm above the bottom. Having deleveled the terrain by 20 cm, it was possible to set up the inlets of the four cells at 30.5 cm and the outlets at 11 cm above the bottom of the cells. The water was fed into the fifth cell with algae from above since the inlet was placed on the upper edge of the cell or 50 cm above the ground level. The outlet of the last cell was set at 10 cm above the bottom of the cell. The biological system and its components were installed and checked for proper operation in one day.

Start-up period and monitoring of the proper work of the biological system was completed by the end of August (a month and a half after the system was installed). During this period, water was changed in the cells twice a week and the river water was pumped in. The biological system was monitored several times a week both in this period of its start-up and later when it was put into operation. In this way, we monitored the health status and the growth of plants, the occurrence of undesirable vegetation, the level of water in the system and the uniformity of its flow.

Figure 2. *The cells with plants a month and a half after the planting*

4. DISCUSSION

The analysis of the obtained results on the content of various pollutants in the polluted and purified water (total phosphorus, total nitrogen, ammonium nitrate, nitrites, nitrates, organic matter characterized by biological and chemical oxygen demand and total organic carbon, heavy metals and other elements of interest, fecal coliform bacteria, total coliform bacteria and fecal streptococci) as well as on the content of heavy metals and other investigated elements in plants, algae and substrates showed that the constructed FTW system was highly efficient in the treatment of polluted water (Čule, 2016). The high efficiency of the biological system in the removal of various pollutants from the polluted water is also evidenced by the fact that according to the content of the pollutants that are taken as parameters for the assessment of the ecological status of waters (***, 2011), or according to the limit values of pollutants in surface waters (***, 2012), the influent water was classified into categories V to III, while it belonged to category I or II when leaving the last cell (Čule, 2016). The results also show that during the growing period all the investigated species in the biological system grew rapidly and thus produced significant quantities of belowground and aboveground biomass. They were resistant to different environmental conditions, pests and diseases and had high regeneration capacity. Furthermore, the establishment of plants, their transplanting into the biological system and maintenance of the floating island vegetation was simple (Čule, 2016). All these findings prove that this was the right model to be selected for the design of the FTW.

However, when the performance of individual cells with plants and algae was analyzed, it was found that the constructed system had certain drawbacks and small modifications could increase the efficiency of each individual cell. It primarily referred to determining the optimal coverage of a cell by vegetation *i.e.* by floating island. The available literature offers various data on this parameter, and it primarily depends on the type of pollutants to be removed from polluted or wastewater. The FTW cell coverage may range from a maximum of 100% to as little as 8-9% (Yang *et al*., 2008; Stewart *et al*., 2008; Van de Moortel *et al*., 2010; White and Cousins, 2013; Borne *et al*., 2013; Chang *et al*., 2013). In the construction of such systems, it should be kept in mind that the coverage greater than 50% can create an anaerobic environment, which may result in poor removal of certain pollutants. On the other hand, the coverage of only 9-18% may be insufficient for the water treatment to be successful (Dodkins and Mendzil, 2014).

The 100% FTW cell coverage in this research created an anaerobic environment in the cells with plants. This led to the low removal of phosphorus in the cells with plants, while it was extremely high (87%) after the water had passed through the last cell *i.e.* the cell with algae which had a large free surface of water (Čule, 2016). Masters (2012) states that the process of P removal is much more effective in the systems with floating islands compared to other constructed aquatic ecosystems, because the large free root surface allows rapid filtration of solid particles from the water which are then precipitated with P. It is assumed that the anaerobic conditions in the cells with plants have led to the release of sequestered and precipitated P back into the water. The extremely low content of dissolved oxygen in water brought about sulfate reduction which resulted in the formation of sulfides, which are in water primarily bound to Fe and Al and thus prevent the binding of P to Fe and Al oxides (Kadlec and Wallace, 2009). Another cause of the additional resuspension of P in the biological system could have been the turbulence of water caused by the inflow of new quantities of water into the cells or raise of gases from the bottom of the cells. These gases occur during the process of photosynthesis in water (oxygen) or decomposition of organic matter by microorganisms (methane and carbon dioxide) (US EPA, 2000). Based on this, it can be further assumed that the efficiency of the cells with plants in sulfur removal was low because of the initiation of these processes in the anaerobic environment. Although the nitrification process requires the aerobic environment, on the basis of the results on the performance of the cells with plants in the removal of ammonium nitrogen and the fact that the cells had anaerobic conditions, it can be concluded that the amount of oxygen found in the plant rhizosphere was quite sufficient for the process to be carried out smoothly. Another proof of this assertion is that the smallest reduction of NH_4-N (45%) was achieved in the second cell - the cell with *C. indica* - where the belowground biomass was small due to the deterioration of a part of the plants (Čule, 2016). Other cells had high removal efficiency which amounted to 78-83% (Čule, 2016). However, the anaerobic conditions in the cells caused poor nitrite removal. In such conditions, the nitrification process could not be fully performed, *i.e*. it was impossible to convert all the available NH_4-N into nitrates. Bernet *et al*. (2001) state that low concentrations of dissolved oxygen in water can lead to the formation of toxic NO_2-N, as the intermediate stage in the conversion of NH_4-N to NO_3-N. Anaerobic water conditions in the cells with plants have enabled the high efficiency of NO_3-N removal (79%) from the polluted water due to denitrification (Čule, 2016). Such conditions in the cells, together with the plants and microorganisms have led to an effective reduction of BOD_5 (84-91%), COD (57-91%), TOC (16-20 %) and the number of pathogenic microorganisms (100%) (Čule, 2016).

5. CONCLUSION

Based on the presented results, it can be concluded that the proposed FTW system can provide efficient removal of phosphorus, nitrogen, organic matter, pathogenic microorganisms and other pollutants from polluted water of the river. Thanks to its construction, the biological system provided unhindered development of physical, chemical and biological processes that took place within its basic components and produced purified water whose ecological status could be characterized as excellent (category I) or good (category II). The research also indicates that it is necessary to make certain modifications of the biological system in order to increase its efficiency in phosphorus and nitrite elimination. In the case of phosphorus, it is necessary to reduce the vegetation coverage in order to create conditions suitable for the undisturbed development of the main physical processes of phosphorus removal and to prevent its resuspension in water. In this way, there would be enough free space for the contact of the water with the air, which would allow the introduction of the required quantities of oxygen into the water. Since phosphorus is removed from the water mainly through the processes of filtration and sediment deposition, in order to avoid its return to water it is necessary to conduct periodical removal of the accumulated sediment from the bottom of the system. In order to eliminate nitrogen or increase the removal of nitrites in the FTW system, it is necessary to provide both aerobic and anaerobic conditions, which means that, as in the case of phosphorus, the percentage of vegetation coverage should be reconsidered. Finally, it can be concluded that in order to efficiently remove various pollutants using an FTW system, it is best to provide cells with alternating anaerobic and aerobic conditions. It is also recommended to provide a series of cells with algae at the end of the system. Their function would be to provide additional water polishing.

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CONSTRUCTION OF FLOATING TREATMENT WETLANDS FOR REMEDIATION OF POLLUTED WATERS

Nevena ČULE, Aleksandar LUČIĆ, Dragana DRAŽIĆ,Vladan POPOVIĆ, Milorad VESELINOVIĆ, Ljiljana BRAŠANAC-BOSANAC, Suzana MITROVIĆ

Summary

One of the examples of polluted and highly endangered urban river is the Topčiderka River with the entire area of its catchment. This river is used as a wastewater and rainwater collector of industries and settlements. Therefore according to the chemical, physiochemical and microbiological water parameters, it is mainly within the limits of category V all the year round. In order to start the revitalization of the Topčiderka River, pilot-scale facility for the treatment of polluted water was installed on its bank. The FTW system was designed as a modified rhizofiltration system and included a pump for drawing water from the river, a closed collection tank, four open rectangular cells in which the floating islands were placed, floating islands with the substrate and the plants, an open rectangular cell with algae and a recirculation pump. The vegetation of the biological system included the following 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. There was one cell with algae. The analysis of the obtained results on the content of various pollutants in the polluted and purified water as well as on the content of heavy metals and other investigated elements in plants, algae and substrates showed that the FTW system was highly efficient in the treatment of polluted water. The high efficiency of the biological system in the removal of various pollutants is also evidenced by the fact that according to the content of the pollutants, the influent water was classified into categories V to III, while it belonged to category I or II when leaving the last pond. It can be concluded that it is necessary to make certain modifications of the system in order to increase its efficiency in the removal of phosphorus and nitrite as well as other pollutants, which means that it is necessary to reduce vegetation coverage of some cells. Finally, it can be concluded that in order to efficiently remove various pollutants using an FTW system, it is best to provide cells with alternating anaerobic and aerobic conditions. It is also recommended to provide a series of cells with algae at the end of the system. Their function would be to provide additional water polishing.

KONSTRUKCIJA SISTEMA SA PLUTAJUĆIM OSTRVIMA ZA REMEDIJACIJU ZAGAĐENIH VODA

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Rezime

Jedan od primera zagađenih i visoko ugroženih urbanih vodotokova je Topčiderska reka i to celom dužinom svoga sliva. Ova reka se koristi kao kolektor otpadnih i kišnih voda industrije i naselja tako da se tokom cele godine uglavnom nalazi u granicama V klase prema hemijskim, fizičko-hemijskim i mikrobiološkim parametrima voda. Kako bi se započeli radovi na revitalizaciji Topčiderse reke na njenoj obali je postavljeno pilot postrojenje za prečišćavanje zagađenih voda. Sistem sa plutajućim ostrvima je konstruisan kao modifikovan rizofiltracioni sistem i sastojao se od pumpe za zahvatanje vode iz reke, sabirnog zatvorenog rezervoara, četiri otvorena pravougaona bazena u koje se smeštaju plutajuća ostrva, plutajućih ostrva sa supstratom i biljkama, jednog otvorenog pravougaonog bazena sa algama i pumpe za recirkulaciju. Za formiranje vegetacije plutajućih ostrva odabrane 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. U jednom bazenu su se nalazile alge. Analiza dobijenih rezultata o sadržaju različitih polutanata u zagađenoj i prečišćenoj vodi kao i sadržaju teški metali i drugi ispitivanih elementa u biljkama, algama i supstratu je pokazala da je konstruisani sistem sa plutajućim ostrvima bio vrlo efikasan u prečišćavanju zagađene vode. O visokoj efikasnosti biološkog sistema u uklanjanju različitih polutanata govori i podatak da je ulivna voda na osnovu sadržaja ispitivanih polutanata svrstana u V do III klasu, a da je po izlazu iz poslednjeg bazena imala karakteristike vode koja pripada klasi I ili II. Zaključeno je da neophodno izvršiti određene modifikacije sistema kako bi se dodatno povećala njegova efikasnost u eliminisanju fosfora i nitrita, kako i drugih polutanata odnosno da je u jednom broju bazena potrebno smanjiti procenat pokrivenosti plutajućim ostrvima. Na kraju se može zaključiti da je za efikasno uklanjanje različitih polutanata pomoću sistema sa plutajućim ostrvima najbolje obezbediti smenu bazena sa anaerobnim i aerobnim uslovima. Preporuka je i da se na kraju sistema nađe i serija bazena sa algama, koje će omogućiti dodatno poliranje vode.

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VARIABILITY OF EPITHELIAL CELLS IN THE RESIN DUCT OF DOUGLAS-FIR NEEDLES

Vera LAVADINOVIĆ [1], Ljubinko RAKONJAC[1], Vukan LAVADINOVIĆ[2]

Abstract: *The research on the interactions between the genetic potential of introduced provenances and the environmental features of the locations in which the plantations were established was carried out in Douglas-fir plantations in Serbia. The two-way ANOVA was conducted in order to study the effects of the site conditions in the localities of Douglas-fir provenance tests in Serbia on the anatomical properties of needles. These analyses look into the effects of two factors (locality and provenance) on the number of epithelial cells in the resin duct of Douglas-fir needles.*

Keywords: Douglas-fir, provenance, introduction, resin ducts, epithelial cells

VARIJABILNOST BROJA EPITELNIH ĆELIJA U SMOLNOM KANALU ČETINA DUGLAZIJE

Abstract: *U kulturama duglazije u Srbiji obavljaju su istraživanja interakcija genetskog potencijala introdukovanih provenijencija sa ekološkim odlikama lokacija gde su kulture podignute. U cilju bližeg upoznavanja efekta interakcije stanišnih uslova lokaliteta, gde su osnovani provenijenični testove duglazije u Srbiji, na anatomska svojstva četina, obavljena je dvofaktorijalna analiza varijanse. U ovim analizama ispitivan je uticaj dva faktora (lokalitet i provenijencija) na broj epitelnih ćelija u smolnom kanalu četina duglazije.*

Ključne reči: Duglazija, provenijencija, introdukcija,smolni kanali, epitelne ćelije

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1.INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii*/Mirb. /Franco) is a very common tree species and accounts for 67% of 2 238 samples taken from all site types (Pfister *et al.*, 1977). It has the widest ecological amplitude of all western conifers and a notable diversity of genetic ecotypes (Moserud and Rehfeldt, 1990). The transfer of exotic tree species (Larsen and Syrack, 1946; Schober, 1959; Spurr, 1961; Schober, 1963; Kriek, 1974; Namkoog, 1979) entails the risk incurred by the lack of knowledge about the productivity and adaptability of introduced species to the environmental conditions of the sites which are outside its range of distribution.

Forest tree production is determined by the expression of physiological processes within specific environmental-genetic regimes. Temperature and soil are major environmental factors that affect the physiological processes of a plant and combined with the genetic variation within a seedling determine the type of organism that will be produced (Jensen and Gatherum, 1965; Dykstra and Gatherum, 1967; Schultz, 1970).

Douglas-fir can be described as a productive coniferous tree species with highly-valued wood, wide ecological amplitude and high-quality essential oils.
In chemotaxonomic terms, Douglas-fir belongs to a large group of aromatic medicinal plants that synthesize numerous and diverse biochemical metabolites. The mixtures of volatile products of Douglas-fir metabolism are the source of its distinctive scent and taste. They are contained in essential oils which can be found in all parts of the plant. Essential oils derived from Douglas-fir needles make significant raw materials both for the chemical industry and for its related industries, mainly because a great number of synthetic preparations that have the same effects as these oils have been found to have adverse side effects. Nowadays, essential oils have found the widest application in the pharmaceutical and cosmetic industry, where they are used as antiseptics, insecticides, deodorants and for masking the odor of synthetic products (Lavadinović, 2008).

The significance of the morphological and anatomical structure of needles, as well as the function and structure of resin ducts, have been analyzed by numerous authors (Matović and Lavadinović, 1999; Gerling *et al*., 2015;). Due to the high quality of its essential oils and their wide application in the cosmetic and pharmaceutical industry, Douglas-fir has also been the subject of numerous studies in Serbia (Tešević *et al*., 2002; Tešević and Lavadinović, 2009).

In order to investigate the genetic potential of Douglas-fir in its new ecosystems of Serbia, the Institute of Forestry in Belgrade has established several experimental plots of Douglas-fir of different provenances originating in North America.

The primary goal of the experiments (on the mountain of Juhor near Jagodina and in the village of Tanda near Bor) was to determine the effects of the geographical parameters of the original localities of Douglas-fir provenances – their geographical latitude, geographical longitude and altitude on the growth of trees with the aim of selecting the most adaptable provenances to be used in the cultivation on similar sites.

2. MATERIAL AND METHODS

The study area covered Douglas-fir provenance tests established in Central Serbia on the mountain of Juhor and in the village of Tanda located at the foot of the mountain massif of Deli Jovan in Eastern Serbia. Douglas-fir seedlings were raised in the nursery of the Institute of Forestry in Belgrade from the seeds native to North America. The seeds originate from a part of the natural range of Douglas-fir distribution with 20 provenances that differ in the latitude, longitude and altitude (Table 1, Lavadinović,V., Koprivica, M. 1996)).

The experiment on the mountain of Juhor was established on a beech site (*Fagetum moesiaca montanum* Jov. 1976) on acid brown soil (dystric cambisol) over gneiss. `Tanda` sample plot is located in FMU `Stol` in `Bor` Forest Administration on the site of oak, Hungarian oak and Turkey oak (*Querceto conferte cerris* Rud.) on brown acid soil and sierozem (Lavadinović, 2008).

Table 1. *Geographical coordinates of the tested Douglas-fir provenances (Lavadinović,V., Koprivica, M. 1996)*

Provenance number	Our mark	Latitude (° N)	Longitude (°E)	Altitude (m)
Oregon 205-15	1	43.7	123.0	750
Oregon 205-14	2	43.8	122.5	1200
Oregon 202-27	3	45.0	122.4	450
Oregon 205-38	4	45.0	121.0	600
Washington 204-07	9	49.0	119.0	1200
Oregon 205-13	10	43.8	122.5	1050
Oregon 205-18	11	44.2	122.2	600
Oregon 202-22	12	42.5	122.5	1200
Washington 202-17	15	47.6	121.7	600
Oregon 201-10	16	44.5	119.0	1350
Washington 201-06	17	49.0	120.0	750
Oregon 202-19	18	45.3	123.8	300
Oregon 205-11	20	45.0	123.0	150
New Mexico 202-04	22	32.9	105.7	2682
New Mexico 202-10	23	36.0	106.0	2667
Oregon 202-31	24	44.3	118.8	1500
Oregon 205-29	26	42.6	122.8	900
Oregon 205-08	27	42.7	122.5	1050
Oregon 204-04	30	45.0	121.5	900
Washington 205-17	31	47.7	123.0	300

A two-way analysis of variance of the number of epithelial cells in the resin ducts was carried out in order to identify the provenances whose genetic potential is most suited to the environmental conditions of the forest communities on whose sites the experiments were established.

Fresh needles were fixed in 50% ethyl alcohol and transported to the laboratory, where permanent anatomical cross-sections of 30 randomly selected needles were made. Permanent anatomical preparations of 17 μm thickness were cut in the middle of the needle using a microtome. They were then dyed by safranin red and toluidine blue and washed with water. This was followed by dehydration with ethyl alcohol, increasing the alcohol concentration from 50% to 96%. The cross-sections were eventually fixed with xylol for several hours, after which the needles were glued to the slides using Canada balsam, covered with cover glass and dried in a dryer at a temperature of 60ºC. Three weeks later, the number of epithelial cells in the resin duct was counted (Paraffin processing method).

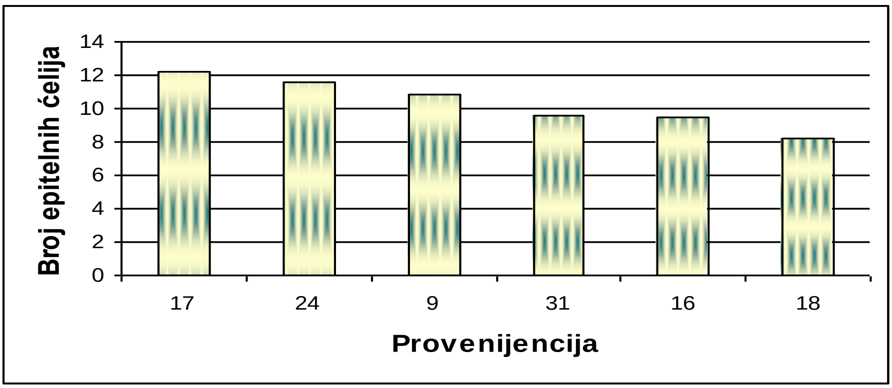
3. RESULTS AND DISCUSSION

Table 2 and Graphs 1 and 2 show the results of the two-way analysis of variance (locality x provenance) for the number of epithelial cells in the resin ducts of Douglas-fir needles at both localities.

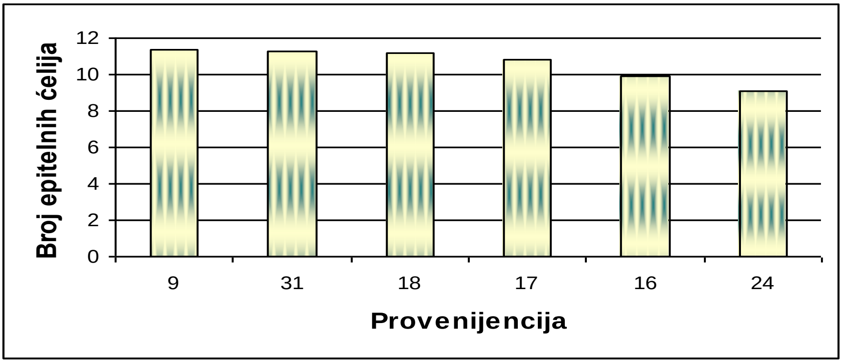
Table 2. *Two-way (locality x provenance) ANOVA for the number of epithelial cells in the resin ducts*

Source of variation	Sum of squares	Degree of freedom	Variance	F-ratio	p-value
A: Locality	8.14	1	8.1	10.57	0.013
B: Provenance	156.856	5	31.3711	40.94	0.000
Interaction AB	311.833	5	62.3667	81.39	0.000
Errors	266.667	348	0.766284		
Total	**743.456**	**359**			

The results of the analysis of variance (Table 2) show that:
a) there are statistically significant differences in the mean values of the number of epithelial cells between the site of Juhor and the site of Tanda;
b) there are statistically significant differences in the mean values of the number of epithelial cells between provenances;
c) in certain provenances, the interaction between the `locality` factor and `the provenace` factor affects the mean value of the number of epithelial cells.



Graph 1. *Interprovenance variation of the number of epithelial cells in the resin duct at Juhor*



Graph 2. *Interprovenance variation of the number of epithelial cells in the resin duct at Tanda*

The effects of locality on the number of epithelial cells

Table 3. *MSD test of the effects of locality on the number of epithelial cells*

Locality	Sample size	Mean value	Difference error mean	Homogeneous groups
Juhor	180	10.3111	0.0652467	X
Tanda	180	10.6111	0.0652467	X
Comparison			Differences	+/– Limits
Juhor–Tanda			*–0.3	0.181483

* statistically significant difference

Table 1. *MSD test of the effects of the locality on the number of epithelial cells*

Locality	Sample size	Mean value	Difference error mean	Homogeneous groups
16	60	9.7	0.113011	X
18	60	9.7	0.113011	X
24	60	10.3667	0.113011	X
31	60	10.4333	0.113011	X
9	60	11.0667	0.113011	X
17	60	11.5	0.113011	X
Comparison		**Differences**		**+/– Limits**
9–16		* 1.36667		0.314337
9–17		*–0.433333		0.314337
9–18		* 1.36667		0.314337
9–24		* 0.7		0.314337
9–31		* 0.633333		0.314337
16–17		*–1.8		0.314337
16–18		0.0		0.314337
16–24		*–0.666667		0.314337
16–31		*–0.733333		0.314337
17–18		* 1.8		0.314337
17–24		* 1.13333		0.314337
17–31		* 1.06667		0.314337
18–24		*–0.666667		0.314337
18–31		*–0.733333		0.314337
24–31		–0.0666667		0.314337

* statistically significant difference

The results shown in Table 3 point to statistically significant differences in the mean number of epithelial cells of Douglas-fir needles obtained from Juhor and Tanda localities. The average number of epithelial cells in the Douglas-fir needles from Tanda locality (10.61) is significantly higher than the number of these cells in the needles of Douglas-fir trees from Juhor locality (10.31). The range of variation of this property for the analyzed provenances is shown Graphs 1 and 2. Using the MSD test, we got a deeper insight into the effects of the characteristics of the locality where the provenance tests were conducted on the number of epithelial cells of Douglas-fir needles.

The results presented in Table 4 show that there are statistically significant differences in the number of epithelial cells between provenances. However, the results of the MSD test indicate that provenances 16 and 18, 24 and 31 are homogeneous since there are no statistically significant differences in the mean values of the number of epithelial cells.

4. CONCLUSIONS

On the basis of the examinations carried out through Douglas-fir provenance tests on the (*Fagetum moesiaca montanum* Jov. 1976) site on acid brown soil (dystric cambisol) over gneiss and on the site of oak, Hungarian oak and Turkey oak *(Querceto conferte cerris* Rud.) on brown acid soil and sierozem, we can draw the following conclusions:

- there are statistically significant differences in the mean values of the number of epithelial cells between the localities of Juhor and Tanda;
- there are statistically significant differences in the mean values of the number of epithelial cells between provenances;
- in certain provenances, the interaction between the `locality` factor and `the provenace` factor affects the mean value of the number of epithelial cells.
- the average number of epithelial cells in the needles of Tanda locality is significantly higher than the number of these cells in the needles of Douglas-fir trees of Juhor locality.
- there are statistically significant differences in the number of epithelial cells between provenances. However, the results of the MSD test indicate that the provenances 16 and 18, 24 and 31 are homogeneous because they don`t show statistically significant differences in the mean values of the number of epithelial cells and there is an interaction between the variability factors (locality and provenance), i.e., a change in one variability factor affects the change in the treatment of another factor.

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VARIABILITY OF EPITHELIAL CELLS IN THE RESIN DUCT OF DOUGLAS-FIR NEEDLES

Vera LAVADINOVIĆ, Ljubinko RAKONJAC, Vukan LAVADINOVIĆ

Summary

Coniferous tree species are rich in essential oils, which have a protective role for the plant itself and a wide use in the pharmaceutical, cosmetic and food industry.

The resin is an organic liquid containing terpenes, resin acids and other compounds found in the resin ducts of all plant parts. Resin ducts are surrounded by epithelial cells. Aromatic effects of essential Douglas-fir oils make this type of conifer very popular in urban greening and green area establishment. The study deals with the effects of two factors (locality and provenance) on the number of epithelial cells in the resin duct.

VARIJABILNOST BROJA EPITELNIH ĆELIJA U SMOLNOM KANALU ČETINA DUGLAZIJE

Vera LAVADINOVIĆ, Ljubinko RAKONJAC, Vukan LAVADINOVIĆ

Rezime

Četinaske vrste drveća su bogate etarskim uljima, koje imaju zaštitnu ulogu za samu biljku i široku upotrebnu u farmaceutskoj, kozmetičkoj i prehrambenoj industriji.

Smola je organska tečnost koja sadrži terpene, smolne kiseline i druga jedinjenja koje se nalaze u svim delovima biljke u smolnim kanalima. Smolni kanali su obloženi epitelnim ćelijama. Etarska ulja iz četina duglazije zbog aromatičnog efekta čine ovu vrstu četinara vrlo popularnom za urbano ozelenjavanje i formiranje zelenih masiva. U ovim analizama ispitivan je uticaj dva faktora (lokalitet i provenijencija) na broj epitelnih ćelija u smolnom kanalu.

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EFFECTS OF EPYXILOUS FUNGUS *Laetiporus sulphureus* (Bull. ex Fr.) Murrill ON THE DECCOMPOSITION OF OAK WOOD

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Abstract: *Testing samples were collected from the medulla of healthy oak trees in Eastern Serbia, from the association of Quercetum montanum. Over the periods of 2, 4 and 6 months the wood samples were exposed to influence of the mycelia of the fungus causing cubical brown rot on oak. Given that static modulus of rupture provides the quickest and clearest way to observe destruction caused by epixylous fungi, this paper researched the decrease in modulus of rupture of Sessile oak wood due to influence of causers of cubical brown rot. The samples have been exposed to the impact of the mycelia of the brown rot fungus on oak tree Laetiporus sulphureus (Bull. ex Fr.) Murrill (Sulphur Polypore). Effect of to the impact of the brown rot fungi was investigated, in decrease of static modulus of rupture Quercus petraea agg. The static modulus of rupture caused by L. sulphureus after 2, 4 and 6 months decreased in comparison with initial ones (100%) and reached 91.73, 75.17 and 63.25%. By using correlation analyses of Q. petraea agg. static modulus of rupture -* σ_s *(dependent variable) of fungi time influence (T-independent variable) strong correlation between variables was established, and regression equation is:*

$$\sigma_s = 151.514 \pm 30,657\sqrt{T}$$

The regression line obtained through data processing opened the possibility to prognosticate the changes of wood properties in certain time periods of the effect of the

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fungus under the unchanged external conditions, which is significant for practical purposes in terms of taking protective measures and wood usability.

Key words: *Laetiporus sulphureus,* Modulus of rupture

DEJSTVO EPIKSILNE GLJIVE *Laetiporus sulphureus* (Bull. ex Fr.) Murrill NA DEKOMPOZICIJU HRASTOVOG DRVETA

Izvod: *Uzorci za ispitivanje su prikupljeni iz srčike zdravog drveta hrasta u istočnoj Srbiji, iz asocijacije Quercetum montanum. Tokom perioda od 2, 4 i 6 meseci uzorci drveta su izloženi uticaju micelije gljive koja prouzrokuje mrku prizmatičnu trulež. S obzirom da statička čvrstoća na savijanje daje najbrži i najjasniji način za posmatraje destrukcije drvne mase izazvane epiksilnim gljivama, u ovom radu ispitivano je smanjenje čvrstoće na savijanje drveta dejstvom uzročnika truleži. Uzorci su izloženi uticaju micelije gljive Laetiporus sulphureus (Bull. ex Fr.) Murrill (sumpornjača) na drvetu Quercus petraea agg. Statička čvrstoća na savijanje pod dejstvom L. sulphureus posle 2, 4 i 6 meseci smanjena je u poređenju sa početnom (100%) i iznosila 91.73, 75.17 i 63.25%. Korišćenjem korelacione analize Q. petraea agg. statičke čvrstoće na savijanje σ_s (zavisna varijabla) u vremenu dejstva gljive (T-nezavisna varijabla), utvrđena je jaka korelaciona veza između varijabli, a regresiona jednačina statičke čvrstoće na savijanje iznosi:*

$$\sigma s = 151.514 \pm 30,657 \sqrt{T}$$

Regresiona linija dobijena obradom podataka otvorila je mogućnost prognoziranja promene svojstava drveta u određenom vremenskom periodu dejstva gljive, pod nepromenjenim uslovima spoljne sredine, što je značajno za praktičnu primenu, u smislu preduzimanja mera zaštite i upotrebljivosti drveta.

Ključne reči: *Laetiporus sulphureus,* čvrstoća na savijanje

1. INTRODUCTION

Development of the wood processing industry is causing growing demand for high-quality wood raw materials [14, 18]. This calls for preservation and extension of wood durability, which is directly linked to preservation of physical, chemical, mechanical, aesthetical and other properties, according to several authors [7, 8, 15, 21]. Basic structural constituents of wood (cellulose, hemicellulose and lignin) are distributed in different percentages in different species and parts of trees. There is thus more cellulose in soft than hard species of trees, in bolewood more than in branchwood, in early successional species more than in late ones, as discussed by Miric and Popovic [16].

Oak as a host is colonized by a large number of microorganisms, where a special place belongs to the research on impact of fungi, particularly those attacking the core (srcika) as the technically most valuable part of a tree [1, 3, 4]. Through its enzyme system, the epixylous fungi break down the constituents of wood cell walls, modify the percentage of their participation and thus directly induce changes of the wood properties [2, 6, 11]. The agents of brown prismatic rot (to which the researched fungus *L. sulphureus* belongs*)* disintegrate primarily cellulose, while the disintegration of lignin occurs in a far smaller extent.

This paper presents the course of alteration (decrease) of the presence of lignin in the cell wall, reflected in the decrease of wood bulk modulus of *Q. petraea* agg. under the influence of the fungus *L. sulphureus* after 2, 4 and 6 months of the incubation [26, 20, 17].

2. MATERIAL AND METHODS

The substrate used in the research was a 110-year-old healthy tree of Sessile oak *Q. petraea agg.*, 19 m tall and 35 cm in diameter at breast height. The tree had been cut in Eastern Serbia, at the altitude of 550 m, on the southern exposition and in association *Quercetum montanum* [25]. The analyses were conducted on a log 3.5 m in length (from the lower part of the trunk to the first live branch), which was according to the relevant pattern cut into specimens using the standard prescribed dimensions 2x2x32 cm. The fronts of specimens were smeared with antiseptic paste so as to prevent penetration of hyphae from that direction, given the small dimensions of the specimens representing a large beam used for practical purposes. Since the development of cross-section hyphae is the fastest, if the penetration of hyphae was enabled from that side, the small specimens would quickly rot and the relevant results would not be obtained.

The specimens were dried in a classic drying chamber at the temperature of $103\pm1\ ^0C$ and measured with the accuracy of 0.01 g. On control specimens (healthy wood), the modulus of elasticity was measured on universal machine for testing wood properties [23, 24]. The specimens to be exposed to the mycelia were conditioned at approximately 12% humidity. Mycelia *L. sulphureus* was resown into plastic Petri dishes containing malt-agar growing medium of standard concentration. The experiment used sterilized plastic vessels with lids into which Petri dishes with fully developed mycelia *L. sulphureus* were placed. The petri dishes served as glass carriers (dimensions 9x22x35cm) in order to prevent excessive soaking of moisture from the growing medium, onto which wood specimens of Sessile oak were placed. On the top of the stack were petri dishes with 5% water solution of boric acid intended to induce high relative air humidity.

By definition, modulus of rupture is the resistance of a tree to the effect of concentrated, evenly distributed or combined forces that strive to bend or distort it. Therefore, the bending pressure is a complex strain consisting of a thrust pressure in the part of the carrier closer to the action point and the strain pressure at the opposite side. Between these two zones there is a neutral axe which under the impact of the load moves towards the side of the strain pressure [22]. Given that the flexural strength is calculated relative to the cross section of a tree at the point of effect of the force, before measuring the strength all test tubes were measured across the middle using a micrometer, with the accuracy of 0.01 mm. The distance between braces was 280 mm, while the test tubes were exposed to the action of a single concentrated force in the middle of the distance between braces. All data obtained were processed by applying the standard statistical methods; destruction results were compared using the single factor analysis of variance and the least significant difference test for the control group and the duration of the fungus impact (2, 4 and 6 months).

Statistical data processing was done on absolute amounts - N/mm^2, while a correlation analysis was performed in order to prove the existence of a link between the time of action of the fungus as the independent variable and change of modulus of rupture as the dependent variable.

3. RESULTS AND DISCUSSION

During the course of their development, the epixilous fungi feeding on basic constitutional components of the tree alter not only its chemical composition but also its entire inner structure, which results in a change, i.e. decrease in its mechanical, physical, aesthetical and other properties. Brown rot agents in wood cause analogous chemical changes created during the hydrolysis of the tree by mild acids [20]. Through metabolic processes, fungi modify the nutritious matter down to the molecules suitable to their own life functions.

In order to analyze the changes of wood properties under the influence of epixilous fungi, it is necessary to provide a brief explanation of the fungi's need for nutrients, chemical composition of the tree, as well as changes that occur in the chemical composition and tree structure under their influence. All wood-decay fungi are able to use finished products such as free sugars, lipids, peptides and other primary metabolites. These substances have a crucial impact in the initial phase of tree colonization. Carbohydrates are the most significant source of carbon in nutrition of the epixilous fungi. A rich source of carbon lies in the basic structural elements of the wood cell walls (hemicellulose, cellulose and lignin). Cellulose, which is the most widely present element on earth, builds the skeletal substance of the cell wall and represents the most important constituent of wood [9, 12].

Dissolution of cellulose does not occur evenly throughout the affected tree, since hyphae are individual and unorganized. Thus only a few cells are attacked at first, but their number gradually rises [13]. Moreover, dissolution of cellulose goes quickly at first and later on slows down, which is a consequence of the effect of the fungus that first demolishes the free cellulose in the middle layer of the secondary wall, given that this layer has virtually no impregnation with lignin. As soon as the fungus gets into the parts of cellular membrane with a higher lignin content, the dissolution slows down, so that the dissolution of cellulose in the primary wall is the slowest, as this is where the largest portion of total lignin is incorporated [5]. Changes of the tree structure are reflected primarily in the modulus of elasticity, with the process being roughly 2 to 3 times faster in brown rot agents. Basic parameters of the modulus of elasticity of *Q. petraea agg.* samples that were exposed to the effect of the species *L. sulphureus* for 2, 4 and 6 months compared to the control are presented in table 1 and expressed in absolute values.

Table 1. *Modulus of rupture reduction (%) under the influence of the L. sulphureus*

	0 months	2 months	4 months	6 months
Number of measurements	30	30	30	30
Minimum amount	109.74	75.87	34.24	16.42
Maximum amount	205.98	206.97	179.49	156.79
Arithmetic mean	156.12	143.21	117.35	98.75
Standard deviation	28.65	29.34	40.62	36.32
Variation coefficient	18.35	20.49	34.62	36.78

The table 1 demonstrates that the smallest dissipation of data (variation coefficient) occurs in the control group of samples (18.35), while the highest is after 2 and 6 months of exposure to the species *L. sulphureus* (20.49 and 36.78), which is a consequence of non-homogenous tree structure and uneven colonization of the tree by the fungus. The average modulus of elasticity amounts to 156.12 in the control group of samples, 143.21after 2 months of exposure to fungus *L. sulphureus,* 117.35 after 4 months, and 98.75 N/mm^2 after 6 months of exposure.

Therefore, the greatest decrease of the modulus of elasticity of *Q. petraea agg.* exposed to the fungus *L. sulphureus* occurs during the first 2 months, after which the process slows down. According to Rayner and Boddy [20], changes of tree properties under the influence of most brown rot agents are primarily reflected in changes of the modulus of elasticity and occur immediately following the appearance of first signs of rot, which is in this case particularly evident. Based on results of T- test, shown in table 2, it is clear that significant differences occur as early as first 2 months of the influence of the fungus *L. sulphureus* and apply to all tested sample groups, except for the period between months 2 and 4.

Table 2. *Modulus of rupture reduction under the influence of L. sulphureus (T test)*

	0 months	2 months	4 months	6 months
0 months	-	12.9138	38.7752	57.3717
2 months		-	25.8614	44.4579
4 months			-	18.5965
6 months				-

- Significant difference at the level of 0.05

This means that over this period there is no significant loss of the modulus of elasticity, with the differences being only the consequence of high variability of data, not exposure to the fungus. Based on the analysis of breakages of test tubes of Sessile oak exposed to *L. sulphureus* during the process of measuring the modulus of rupture, it was found that in the first 2 months a large number of test tubes had smooth breakages in addition to short-fiber ones. Kruzsik [10] states that a tree with a higher modulus of elasticity has a long-fiber breakage, with a medium modulus short-fiber breakage, and with a low modulus a smooth breakage.

Table 3. *Correlation analysis of exposure time to fungus L. sulphureus and wood properties*

Tested property	Model type	Correlation coefficient (r)	Regression equation
Modulus of elasticity (σ_s)	Square function (x)	±0.992802	$\sigma_s = 151.514 \pm 30.657 \sqrt{T}$

The obtained results lead to conclusion that after 6 months of exposure to the fungus the process of destruction of the tree, although highly advanced, is probably not completed, meaning that there is a possibility that the cell membrane layers may still contain the sufficient quantity of cellulose that provides the modulus of rupture. Correlation analysis was performed in order to establish a correlation link between the tested tree properties depending on the time of exposure to the fungus (Tab. 3).

This is significant for the purposes of practical application, i.e. for protection measures and wood utility. According to literature sources [18, 20] this property represents the quickest and clearest indication of destruction under the influence of epixilous fungi. To this effect, appropriate chemical analyses of wood exposed to influence of fungi could provide a clearer definition from both qualitative and quantitative aspect, allowing a comprehensive insight into the course and consequences of development of fungi in trees.

4. CONCLUSIONS

After 2, 4 and 6 months under the effect of the fungus *L. sulphureus*, the modulus of rupture of oak wood substantially decreased compared to the initial value (100%) and amounted to 91.73%, 75.71% and 63.25% respectively. In the period between 2 and 4 months the process of destruction slowed down and the loss amounted to only 16.02%. In the period between the months 4 and 6 the destruction mildly rose and the modulus of rupture dropped by another 39.46%.

Correlation analysis showed a strong correlation link between the changes (decrease) in wood properties of *Q. petraea* agg. and the time of the influence of the fungus *L. sulphureus.* This opens the possibility to use the regression equation in forecasting modifications in wood properties, depending on the time of exposure to a fungus, under unchanged environmental conditions.

If a future research would carry out similar experiments on our most significant tree species against the greatest and most dangerous wood destructors, over a larger number of monitoring periods, the obtained results could serve as basis for creation of relevant tables (standards). By cross-referencing the obtained data and conducting their statistical analysis, we would arrive at the closest approximation of values to be inserted into relevant tables and applied in practice.

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Summary

Testing samples were collected from the medulla of healthy oak trees in Eastern Serbia, from the association of Quercetum montanum. Over the periods of 2, 4 and 6 months the wood samples were exposed to influence of the mycelia of the fungus causing cubical brown rot on oak. Given that static modulus of rupture provides the quickest and clearest way to observe destruction caused by epyxilous fungi, this paper researched the decrease in modulus of rupture of Sessile oak wood due to influence of causers of cubical brown rot. The samples have been exposed to the impact of the mycelia of the brown rot fungus on oak tree Laetiporus sulphureus (Bull. ex Fr.) Murrill (Sulphur Polypore). Effect of to the impact of the brown rot fungi was investigated, in decrease of static modulus of rupture Quercus petraea agg. The static modulus of rupture caused by L. sulphureus after 2, 4 and 6 months decreased in comparison with initial ones (100%) and reached 91.73, 75.17 and 63.25%. Dissolution of cellulose does not occur evenly throughout the affected tree, since hyphae are individual and unorganized. Thus only a few cells are attacked at first, but their number gradually rises. Changes of the tree structure are reflected primarily in the modulus of elasticity, with the process being roughly 2 to 3 times faster in brown rot agents. Over this period there is no significant loss of the modulus of elasticity, with the differences being only the consequence of high variability of data, not exposure to the fungus. Based on the analysis of breakages of test tubes of Sessile oak exposed to L. sulphureus during the process of measuring the modulus of rupture, it was found that in the first 2 months a large number of test tubes had smooth breakages. The obtained results lead to conclusion that after 6 months of exposure to the fungus the process of destruction of the tree, although highly advanced, is probably not completed, meaning that there is a possibility that the cell membrane layers may still contain the sufficient quantity of cellulose that provides the modulus of rupture. Correlation analysis was performed in order to establish a correlation link between the tested tree properties depending on the time of exposure to the fungus. This is significant for the purposes of practical application, i.e. for protection measures and wood utility. To this effect, appropriate chemical analyses of wood exposed to influence of fungi could provide a clearer definition from both qualitative and quantitative aspect, allowing a comprehensive insight into the course and consequences of development of fungi in trees.

DEJSTVO EPIKSILNE GLJIVE Laetiporus sulphureus (Bull. ex Fr.) Murrill NA DEKOMPOZICIJU HRASTOVOG DRVETA

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Rezime

Uzorci za ispitivanje su prikupljeni iz srčike zdravog drveta hrasta u istočnoj Srbiji, iz asocijacije *Quercetum montanum*. Tokom perioda od 2, 4 i 6 meseci uzorci drveta su izloženi uticaju micelije gljive koja prouzrokuje mrku prizmatičnu trulež. S obzirom da statička čvrstoća na savijanje daje najbrži i najjasniji način za posmatraje destrukcije drvne mase izazvane epiksilnim gljivama, u ovom radu ispitivano je smanjenje čvrstoće na savijanje drveta dejstvom uzročnika truleži. Uzorci su izloženi uticaju micelije gljive *Laetiporus sulphureus* (Bull. ex Fr.) Murrill (sumpornjača) na drvetu *Quercus petraea* agg. Statička čvrstoća na savijanje pod dejstvom *L. sulphureus* posle 2, 4 i 6 meseci smanjena je u poređenju sa početnom (100%) i iznosila 91.73, 75.17 i 63.25%. Razlaganje celuloze ne javlja se podjednako u celom napadnutom drvetu, jer su hife individualne i neorganizovane. Prema tome, samo nekoliko ćelija su napadnute u početku, ali njihov broj postepeno raste. Promene u strukturi drveta se ogledaju pre svega u čvrstoći na savijanje, gde je proces oko 2 do 3 puta brži kod prouzkovača mrke prizmatične truleži. Na osnovu analize lomova na epruvetama kitnjaka izloženih dejstvu gljive *L. sulphureus* tokom procesa ispitivanja čvrstoće na savijanje, utvrđeno je da je u prva 2 meseca veliki broj epruveta imao glatke lomove. Dobijeni rezultati upućuju na zaključak da je nakon 6 meseci izlaganja truležnici, proces destrukcije drveta, mada je vrlo napredovao, verovatno nije završen, što znači da postoji mogućnost da slojevi ćelijske membrane i dalje sadrže dovoljnu količinu celuloze koja obezbeđuje čvrstoću na savijanje. Korelaciona analiza je urađena u cilju uspostavljanja korelacije veza između svojstava testiranog drveta u zavisnosti od vremena dejstva gljive. Ovo je značajno za praktičnu primenu, odnosno mere zaštite drveta i njegovu upotrebljivost. U tom smislu, odgovarajuće hemijske analize drveta izloženog uticaju gljiva može pružiti jasniju definiciju kroz kvalitativne i kvantitativne aspekte, omogućavajući sveobuhvatan uvid o toku i posledicama razvoja gljiva na drvetu.

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A CONTRIBUTION TO THE KNOWLEDGE OF *Neodryinus typhlocybae* (Hymenoptera: Dryinidae) –THE FLATID PLANTHOPPER PARASITOID

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Renata GAGIĆ-SERDAR[1], Miroslava MARKOVIĆ[1]

Abstract: *The paper presents the results of a two-year long study of the presence of Neodryinus typhlocybae (Hymenoptera: Dryinidae), a parasitoid and the greatest enemy of the Metcalfa pruinosa (Hemiptera:Flatidae), or the flatid planthopper in the area of Belgrade.*

Keywords: *Metcalfa pruinosa, Neodryinus typhlocybae, Belgrade, urban greenery*

PRILOG POZNAVANJU *Neodryinus typhlocybae* (Hymenoptera: Dryinidae) - PARAZITOIDA MEDEĆEG CVRČKA

Izvod: *U radu su prikazani rezultati dvogodišnjeg istraživanja prisustva Neodryinus typhlocybae (Hymenoptera: Dryinidae), parazitoida i glavnog neprijatelja invazivne vrste Metcalfa pruinosa (Hemiptera: Flatidae), medećeg cvrčaka, u području Beograda.*

Ključne reči: *Metcalfa pruinosa*, *Neodryinus typhlocybae, Beograd,* urbano zelenilo

1. INTRODUCTION

Nearctic, polyphagous [330 plant species in 78 plant families in Europe (Alma et al., 2005)] invasive species - planthopper *Metcalfa pruinosa* (Say, 1830) (Hemiptera: Flatidae) has been present in Europe since 1979 when it was incidentally introduced to the province of Treviso, Italy (Zangheri and Donadini, 1980). In the following few decades, positive findings were published for most

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European countries - France, Spain, Slovenia, Switzerland, Croatia, Great Britain, Austria, the Czech Republic, Greece, Turkey, Montenegro, Hungary, Bulgaria, the Republic of Serbia, Bosnia and Herzegovina, Romania (Drosopoulos et al., 2004; Be'la, 2004; Girolami et al., 2002; Lauterer and Malenovsky, 2002; Hrnčić, 2003; Glavendekic et al., 2005). In Serbia, it was first noticed in the area surrounding Belgrade. In the following years, it spread over many areas of Vojvodina and central Serbia, for instance the area surrounding Titel, several sites at the foot of Fruška Gora and along the Danube River, Sremski Karlovci, Petrovaradin, Sremska Kamenica (Kereši, 2011), Novi Banovci (Glavendekić & Mihajlović, 2007), Šabac, Sremska Mitrovica, Slankamen. It most commonly occurs on urban ornamental trees and shrubs. So far, it hasn't been found at higher altitudes. The climatic conditions of our area seem to favor the growth of this species. The minimum temperature required for its development is 13˚C, while the maximum amounts to 31˚C. The lower optimum temperature is 22˚C and the upper one 28˚C. The development of one generation requires a sum of effective temperatures of 500˚C, counting the temperatures above 13˚C. *M. pruinosa* is a species that usually occurs in humid regions, with an average rainfall of 610 to 1625 mm (Strauss, 2010).

The damage done to the attacked plants can be direct and indirect. Direct damage is caused by larvae and imagos sucking juices out of leaves, shoots, twigs and branches. Consequently, the growth of the host plant is decreased and it is physiologically weakened. Indirect damage is caused by the abundant honeydew produced by larvae. The shoots and leaves that are covered with honeydew are suitable for the development of the sooty mould which decreases the assimilation area of the attacked leaves and reduces the value of ornamental plants. The females often lay eggs in the leaf and flower buds which consequently freeze during the winter and the unripe fruit falls off. Some species of the *Flatidae* family are well-known as vectors of various viruses.

Neodryinus typhlocybae (Aschmed, 1893) (Hymenoptera: Dryinidae) is the most important natural enemy of the planthopper *M. pruinosa*. Therefore it is often used in the process of integrated control i.e. biological pest management, either by introducing the individuals grown under laboratory conditions into the infested areas or by encouraging their development and increasing their numbers in natural conditions. For instance, *N. typhlocybae* was introduced into the Veneti region in Italy in 1987, and in recent decades it has been introduced into many urban and agricultural areas of other European countries, such as France, Switzerland, Slovenia and Croatia (Luci & Vilson, 2003; Alma et. al., 2005, ct. Trivellone et. al., 2006).

In Italy, France and Slovenia, the use of *N. typhlocybae* for the purpose of biological control of *M. pruinosa* has led to a high level of parasitism (between 50 and 80%), which at the same time drastically reduced the number of this economically harmful species. Many studies have dealt with the influence of the parasitoid wasp on other, domestic predators of *M. pruinosa*. It has been found that the investigated predators are not dependent on this planthopper and as they consume only a small number of individuals, it has been concluded that they have no negative impact on them. The distribution of *N. typhlocybae* can be affected by its natural enemies, hyperparazitoids and predators. In Europe, four parasitoid wasps have been recognized as direct natural enemies of *N. typhlocybae*: *Cheiloneurus boldyrevi* (Trjapitzin and Agekyan, 1978) (Hymenoptera: Encyrtidae), *Gelis areator* (Panzer, 1804) (Hymenoptera: Ichneumonidae), *Callitula bicolor* (Spinola, 1811) (Hymenoptera: Pteromalidae) and *Pachyneuron muscarum* (Linnaeus, 1758) (Hymenoptera: Pteromalidae). Except for *C. boldyrevi*, all other wasps have been recorded in our region (https://fauna-eu.org/).

Due to the widespread distribution of *M. pruinosa* in the territory of Serbia, there is a need for a more detailed investigation of its parasitoid *N. typhlocybae*, whose presence in the territory of Serbia was first recorded in 2013 in the area surrounding Belgrade (Glavendenkic, 2017). This paper presents the results of a study that included: the presence, main characteristics and abundance of the host (*M. pruinosa*) and its most important natural enemy (*N. typhlocybae*) in the wider area of Belgrade.

2. STUDY AREA AND RESEARCH METHOD

Intensive research into the possible presence of *N. typhlocybae* parasitoid was carried out in 2016 and 2017 at the following sites: Cerak (Y 44,751389, X 20,416944), Košutnjak (Y 44,764167, X 20,436389), Veliko ratno ostrvo (Y 44,831414, X 20,443180) and Ada Ciganlija (Y 44,790833, X 20,416111) (Figure 1).

Figure 1. *Research sites with positive findings of N. typhlocybae parasitoid (author of the map B. Sc. Ljiljana Vamović)*

North American, Nearctic species *N. typhlocybae* shows evident sexual dimorphism. The female, like all the female representatives of the fam. Dryinidae (except for the *Aphelopinae* subfamily), possesses the chelae on the front legs which it uses to grip the host for parasitization (Olmi, 1999). The female feeds on sugary liquids and honeydew, as well as on nymphs. The male is glycophagous only. The species can reproduce by arrenotocous parthenogenesis. The aploid males have 20 chromosomes (Girolami & Camporese, 1994).

The female lays eggs between the wings of the host. After a few days, the egg hatches and the emerging larva becomes visible as a cyst on the body of the host. Finally, the larva spins a cocoon under the remains of the host. The larva of *N. typhlocybae* spends most of its life inside a flat, oval cocoon. The cocoons spun by males are slightly smaller than the ones spun by females. When it comes to the size of adult individuals, females (averagely about 4-5mm) are larger than males (about 3mm). The male of *N. typhlocybae* shows a laboratory longevity of 1-3 days, the female of 2-4 weeks. During this period the female can prey on about 30 nymphs and parasitize as many. Potentially it is able to destroy the entire offspring of one *M. pruinosa* whose fecundity is 50-60 eggs on average (Santini & Lucci,1994; Girolami *et. al*., 1996; Girolami & Conte,1999; Girolami & Mazzon,1999). *N. typhlocybae* overwinters as diapausing larva inside cocoons on the lower surface of leaves. In June adults emerge and lay eggs on nymphs of *M. pruinosa*. Part of the mature larvae (bivoltine) from this offspring transform to adults and originate a summer generation. The others (monovoltine) enter in diapause and overwinter (Mazzon *et al*., 2000).

The leaves showing the presence of the host colonies and silky cocoons similar to those of the *N. typhlocybae* species were sampled on several occasions from park and forest shrub and tree species. The leaves containing silky cocoons were classified by localities and placed in Petri dishes for further growth and study. The cocoons were grown in controlled conditions of the environment (D:N - 14:10 h, 23°C), in an SPX-300B-G environmental test chamber.

3. RESULTS AND DISCUSSION

During the research period, at selected sites, *M. pruinosa* was found on a number of forest and horticultural woody species, but the cocoons of its *N. typhlocybae* parasitoid were recorded only in the host colonies on *Acer campestre* (L.), *Acer platanoides* (L.), *Acer pseudoplatanus* (L.), *Fraxinus angustifolia* (Vahl.), *Juglans regia* (L.), cultivars of Rosaceae sp., *Maclura pomifera* (Raf.), *Prunus laurocerasus* (L.), *Syringa vulgaris* (L.), *Tillia cordata* (L.), *Ulmus laevis* (Pall.) and *Vitis vinifera* (L.) (Figures 2 and 3).

Metcalfa pruinosa

Figure 2. *Nymphs on the underside of leaves*

Figure 3. *Imago colonies*

In the first year of the research, the parasitoid cocoons were found only at the site of Cerak. Under laboratory growth conditions, the imagos were formed within the silky cocoons but the adults didn`t emerge. In the second year of the research, parasitoid cocoons were recorded at other sites. This time again, the formed imagos didn`t all emerge. Some of the imagos broke through the silky cocoon, but they didn`t leave it (Figure 5). A total of 141 cocoons, 58 with females and 83 with males were analyzed. In a number of cocoons, the development was paused at the stage of the larva - overwintering individual (Fig. 4).

Figure 4. *Overwintering larvae*

Neodryinus typhlocybae

Figure 5. *Fully-developed imagos that did not leave silky cocoons*

Figure 6. *The male and the female grown under laboratory conditions and the female on a leaf*

The average dimension of the silky cocoons containing males was 3.9 x 3.04 mm, while the average dimension of the cocoons with the females amounted to 6.1 x 4.3 mm.

Under laboratory growth conditions, imagos of both males and females were obtained. The females of *N. typhlocybae* were significantly larger than the males, as indicated by various literature sources (Figure 6).

4. CONCLUSIONS

N. typhlocybae, a parasitoid of the flatid planthopper, was found at all investigated sites on the territory of Belgrade: Cerak, Košutnjak, Veliko Ratno ostrvo, Ada Ciganlija, but its abundance, distribution and parasitoid activity were not satisfactory. Under laboratory growth conditions, imagos were formed in a number of cocoons but their eclosion did not occur. It is assumed that the same happens in natural conditions and this may be one of the reasons for its slow spread. In any case, its abundance in the area of Belgrade in the research period was not correlated with the abundance of the host, i.e. it had no significant impact on the decrease in the number of *M. pruinosa* individuals and colonies.

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A CONTRIBUTION TO THE KNOWLEDGE OF *Neodryinus typhlocybae* (Hymenoptera: Dryinidae) –THE FLATID PLANTHOPPER PARASITOID

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Summary

The flatid planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) is an invasive species widely distributed in our region. Its most prominent adaptive feature lies in its extremely polyphagous nature - 330 plant species and 78 plant families in Europe. The damage done to the attacked plants can be direct and indirect. Direct damage is caused by larvae and imagos sucking juices out of leaves, shoots, twigs and branches. Consequently, the growth of the host plant is decreased and it is physiologically weakened. Indirect damage is caused by the abundant honeydew produced by larvae. The shoots and leaves that are covered with honeydew are suitable for the development of the sooty mould. In order to reduce the negative impact of pesticides, biological methods of control have been increasingly used. The use of natural enemies is one of the best methods to suppress harmful insects.

One of the most effective natural enemies of the flatid planthopper is *Neodryinus typhlocybae* (Aschmed) (Hymenoptera: Dryinidae). It is a predator and a parasitoid native to North America just like its host. In the area of Belgrade, it was first recorded in 2013. Intensive research on the possible presence of *N. typhlocybae* parasitoids was carried out in 2016 and 2017 at the following sites: Cerak, Košutnjak, Veliko ratno ostrvo and Ada Ciganlija, where the flatid planthopper can be found on many forest and horticultural woody species, but the cocoons of its parasitoid *N. typhlocybae* were confirmed only in the host colonies of *Acer campestre* (L.), *Acer platanoides* (L.), *Acer pseudoplatanus* (L.), *Fraxinus angustifolia* (Vahl.), *Juglans regia* (L.), cultivars of Rosaceae sp., *Maclura pomifera* (Raf.), *Prunus laurocerasus* (L.), *Syringa vulgaris* (L.), *Tillia cordata* (L.), *Ulmus laevis* (Pall.) and *Vitis vinifera* (L.).

A total of 141 cocoons, 58 with females and 83 with males were collected. The average dimensions of the cocoons were 3.9 x 3.04 mm (males) and 6.1 x 4.3 mm (females).

Based on the results of the laboratory growth of collected cocoons, i.e. the fact that a number of imagos didn`t emerge from the cocoons, it can be assumed that something similar happens in natural conditions, so this may be one of the reasons why this species did not spread rapidly.

PRILOG POZNAVANJU PARAZITOIDA *Neodryinus typhlocybae* (Hymenoptera: Dryinidae) NA PODRUČJU BEOGRADA

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Rezime

Medeći cvrčak *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae), je invazivna vrsta, koja je na našim prostorima široko rasprostranjena. Ono što se može smatrati njenom najboljom adaptivnom osobinom, jeste to što je široko polifaga - 330 plant species in 78 plant families in Europe. Štete na napadnutim biljkama mogu biti direktne i indirektne. Direktne štete pričinjavaju larve i imaga, a nastaju usled sisanja sokova iz lišća, izbojaka, grančica i grana. Kao posledica ovog oštećenja, javlja se smanjenje prirasta i fiziološko slabljenje napadnutih biljaka. Indirektne štete nastaju usled obilnog lučenja medne rose, koju produkuju larve. Izbojci i lišće koji su prekriveni mednom rosom, pogodni su za razvoj gljiva čađavica. U cilju smanjenja negativnih uticaja pesticida sve više se pribegava korišćenju biološkog metoda suzbijanja. Prirodni neprijatelji su jedan od najboljih metoda za suzbijanje štetnih insekata.

Jedan od najefikasnijih prirodnih neprijatelja medećeg cvrčka je *Neodryinus typhlocybae* (Aschmed) (Hymenoptera: Dryinidae), predator i parazitoid, koji vodi poreklo kao i njen domaćin, iz Severene Amerike, a u području Beograda, je evidentiran 2013. godine. Intenzivna istraživanja mogućeg prisustva parazitoida *N. typhlocybae,* obavljena su u 2016. i 2017. godini, na sledećim lokalitetima: Cerak, Košutnjak, Veliko ratno ostrvo i Ada Ciganlija, gde je medeći cvrčak nađen na mnogim žbunastim i drvenastim, šumskim i hortikulturnim, vrstama, ali kokoni njegovog parazitoida *N. typhlocybae* potvrđeni su samo u kolonijama domaćina na *Acer campestre* (L.), *Acer platanoides* (L.), *Acer pseudoplatanus* (L.), *Fraxinus angustifolia* (Vahl.), *Juglans regia* (L.), kultivari Rosaceae sp., *Maclura pomifera* (Raf.), *Prunus laurocerasus* (L.), *Syringa vulgaris* (L.), *Tillia cordata* (L.), *Ulmus laevis* (Pall.) i *Vitis vinifera* (L.).

Ukupno je prikupljen i analiziran 141 kokon - 58 sa ženkama i 83 sa mužjacima. Prosečne dimenzije kokona iznosile su 3,9 x 3,04 mm (mužjaci) i 6,1 x 4,3 mm (ženke). Iz rezultata laboratorijskog gajenja prikupljenih kokona, odnosno neizletanja jednog broja formiranih imaga iz njih, proizašla je pretpostavka da se slično dešava i u prirodnim uslovima, te da to može biti jedan od razloga zašto se ova vrste nije brže raširila.

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STUDIES OF DEFOLIATION ON ICP SAMPLE PLOTS LEVEL I IN REPUBLIC OF SERBIA

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Abstract: *Project of forests condition monitoring (ICP Forests) operates as an international European project in which, on grid of ICP sample plots (bioindication points) condition of forests has been monitored annually in continuity, including recording data on defoliation with evidencing any damage to the trees. The main goal of the program is monitoring of condition of forests on a permanent, representative surfaces, arranged in a systematic grid distributed on the territory of of Europe. This paper analyzes the data on defoliation as part of the results of the forest conditions monitoring on ICP sample plots on the territory of the Republic of Serbia, in the period 2012 - 2016. The assessment of defoliation is performed on the experimental fields regardless of the cause of loss of leaves, because the results are not aimed to determinate the cause-and-effect relationships, but only to represent the state of defoliation on this study sample plots in the researched period. Assessment and analysis of the degree of crown defoliation has been presented for most common tree species as the most noticeable crown health indicators. Linking these results with other indicators of environmental conditions will provide more concrete informations, and draw conclusions about the vitality of the plants depending on ambient conditions.*

Key words: Defoliation, ICP sample plots, crown condition monitoring, Serbia

STUDIJE DEFOLIJACIJE NA BIOINDIKACIJSKIM TAČKAMA (ICP FORESTS) NIVOA I U REPUBLICI SRBIJI

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Izvod: Projekat monitoringa šuma (ICP forests) odvija se kao međunarodni evropski projekat u kojem se na mreži oglednih površina (bioindikacijske tačke) stanje šuma prati na godišnjem nivou u kontinuitetu, uključujući evidentiranje podataka o defoliaciji i evidentira se oštećenja drveća. Osnovni cilj programa je praćenje stanja šuma na trajnim, reprezentativnim površinama, raspoređenih u sistematsku mrežu distribuiranu na teritoriji Evrope. Ovaj rad analizira podatke o defoliaciji kao deo rezultata monitoringa stanja šuma na biondikacijskim tačkama na teritoriji Republike Srbije, u periodu od 2012. do 2016. godine. Procena defoliacije se vrši na eksperimentalnim poljima, bez obzira na uzrok gubitka listova, jer rezultati nisu usmereni na određivanje odnosa uzroka i efekta, već samo da predstavljaju stanje defoliacije na ovim istraživačkim uzorcima u istraživanom periodu. Procene i analize stepena defoliacije kruna, predstavljene su za najčešće vrste drveća kao najznačajniji pokazatelj njihovog zdravstvenog stanja. Povezivanje ovih rezultata sa drugim indikatorima uslova sredine obezbediće konkretnije informacije i doneti zaključke o vitalnosti biljaka u zavisnosti od uslova okoline.

Ključne reči: Defoliation, ICP sample plots, crown condition monitoring, Serbia

1. INTRODUCTION

In Europe, the issue of forest decline emerged as the major environmental concern of the 1980s (Innes, 1993a). As a consequence, the 'International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests' (ICP Forests) was established in 1985 by the Economic Commission for Europe of the United Nations under the Convention on Long-Range Transboundary Air Pollution (Innes et al. 1993). Within the framework of the ICP Forests Programme, efforts were made to widely harmonise and standardise methods for forest monitoring throughout Europe. The methods were recorded in the ICP Forests manual (UNECE, 2010) that was first published in 1985 and has continuously been subject to updates since its publication.

The forest condition survey represents an essential part of the forest monitoring, which was described in Part IV of the ICP Forests manual (Eichhorn et al. 2010) and became mandatory throughout the European Union in 1987 (Redfern and Boswell 2004; Solberg and Strand 1999). The survey on forest condition has been conducted annually on the systematic wide-scale monitoring plots (Level I), which were established wherever forest coincided with a 16 × 16 km grid over Europe, as well as on plots of the intensive monitoring programme (Level II; since the 1990s) (Eichhorn et al. 2010; Ferretti et al. 1999; Innes 1993b).

Paper analyzes the data of defoliation as part of the complete gethered results of the monitoring of forest on all ICP Forests extensive sample plots, after their network revision in the Republic of Serbia. Serbian language abbreviation usually and commonly used is BIT as Bio Indicational Plot (Bio Indikacijska Tačka). Monitoring of forests is carried out continuously at 118 experimental fields. Field work on data collection (observations and measurements), in the previous period were carried out in the period June – September each year. Calculations of the defoliation mean values for each sample plot and for the most common tree species, allowed the comparison of the values of defoliation in some regions and by tree species. Despite much criticisms (Innes et al, 1993) defoliation, a visual estimate of the lack of foliage on a tree, becomes soon the key response variable assessed in forest health surveys in Europe, and it is now firmly established in the European reporting system (FOREST EUROPE et al. 2011). Defoliation is estimated with respect to a reference standard and according to 5 % of classes, from 0 (no defoliation) to 100 (dead tree) (Eichhorn et al. 2010). It is unspecific, as lack of foliage may be caused by several agents, and is considered as an overall proxy indicator for forest condition (Travaglini, 2013). The importance of defoliation assessment is based on the assumption that stress factors and damage agents usually cause a reduction in foliar mass, and defoliated trees have a reduced chance of survival as they are more vulnerable to attacks by pathogens (Tabaković-Tošić, & Marković 2004; Mihajlović, 2008; Karadžić 2010). Defoliation is assessed on an annual basis in almost all the countries participating in the ICP Forests (e.g. Seidling and Mues 2005), and data are summarized in international reports (ICP Forests and FUTMON 2011).

This has been realised by long-term, comprehensive and intensive forest condition monitoring for already 20 years within the ICP programme for forests and by the Convention on Long Range Transboundary Air Pollution of the European Union. The European Commission also compiled the Report on the results for all EU member states. The first collective Report by EU/ICP Forests on forest condition in Europe was published in 1992. After 12 separate Reports, the European Commission decided in 2004 that the Report on forest condition should be published as one Report named ICP Forests Report. The European Commission Regulation No. 1091/1994 laid down the legal base for the Level II programme. Step by step, 860 permanent observation plots for Level II intensive forest condition monitoring have been established in 28 countries. The main protagonists of ICP Forests are the nine Expert Panels and Working Groups whose researchers from the participating countries take part in the programme, develop and amend the applied methods. They are also responsible for the steering and the supervision of the projects and for the project integration. The wide collaboration of the European countries and the engagement of their experts guarantee the success of this programme, which will be even more significant in future (FOREST EUROPE, UNECE and FAO, 2011).

Every year, the Republic of Serbia by its National Focal Center for Forest Condition Monitoring participates actively in the ICP Forests programme, publishes the results, and submits large-scale reports to the Directorate of Forests and ICP Forests in Hamburg. This paper presents the work of the researchers from 2009 to 2012 who gave their contributions to the obtaining of the results of forest condition monitoring at the local and international levels within their respective institutions.

The Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia – Directorate of Forests assigned the tasks of coordination and management of the programme ICP Forests to the Institute of Forestry – National Focal Centre of the Republic of Serbia. The aim of the author of the text in this paper is to present the total survey – comparative analysis and results of the four years research, forest condition monitoring in the Republic of Serbia.

In this article, we focus on tree condition as measured by the degree of defoliation (needle loss), affecting the tree on sample plots in Serbia. We surveyed the regional distribution of tree health, with an extensive monitoring network, during four year's period (2012 - 2016) in Serbia, with the aim to answer the following questions:

(1) How large is the annual variation in defoliation between plots and within plots over years (i.e., plot×year interaction)?

(2) What is the relative contribution of tree species to the variation in tree defoliation levels?

(3) How useful/sensitive are extensive tree health monitoring networks to reveal the impacts of widespread leaf/needle loss?

Country Serbia has its unique country code, number 67, based on the disposition of the countries participating in ICP programme. In 2003, after the study of the procedure of forest condition monitoring based on ICP methodology (Ferretti & Chiarucci, 2003), the training course was organised at the Institute of Forestry, for the experts selected to perform the crown condition assessment. In collaboration with the Institute of Lowland Forestry and Environment, 103 sample plots in 16 x16 km grid were reconstructed and the crown condition of forest trees was assessed. On the area of Serbia (without Kosovo and Metohija) it was necessary to revise the old plots according to the Level I cooperative programme (Stefanović et al, 2003), as well as the control and harmonisation with the installed coordinate network at the level of Europe. NFC of Serbia also carried out the entry and application of the reconstructed sample plots in the GIS (Geographic Information System) Database (Nevenic, 2008). In 2004, it was concluded that the reconstructed 103 sample plots did not represent fully the status of vegetation cover in Serbia, so that additional 20 sample plots were selected and installed on the area of central of Serbia, and 7 new plots in Vojvodina. The total number of installed sample plots in the Republic of Serbia was then 130 sample plots. The Institute of Forestry, in collaboration with the Faculty of Forestry and the Institute of Lowland Forestry and Environment, carries out the monitoring and assessment of the forest health status on the territory of Serbia. In 2004, the more in-depth monitoring of forest tree diseases and soil chemistry on additional 20 sample plots was realised by the Faculty of Forestry in Belgrade. The analysis of 106 samples of forest nutrition were analysed in the laboratories of the Institute of Forestry (43 samples) and the Faculty of Forestry (63 samples). The GIS database in NFC of Serbia was updated for the additional 20 new plots (Nevenic, 2009).

In 2012, the activities on forest condition monitoring were continued in Serbia on 130 sample plots (Figure 1.)

The most represented species at “BIT” are *Fagus moesiaca* L., *Quercus cerris* L., *Quercus frainetto* Ten., *Quercus petraea* (Mattuschka) Liebl. and *Carpinus betulus* L. The represented coniferous tree species are *Abies alba* Mill., *Picea abies*(L.) H. Karst., *Pinus nigra* J.F.Arnold and *Pinus silvestris* L.

1. 1. Objects of research

The complex of anthropogenic and natural stresses and especially air pollution continues to be regarded as an important stress factor in nature. The significance of atmospheric pollution varies, its impact depends on the region, and its effects on the site and stand conditions. Air pollution and its effects on forest ecosystems are complex and difficult to isolate and quantify. Forest condition is also influenced by a large number of other stress factors which must therefore be taken into consideration. The ICP Forests mandate, therefore, is monitoring of the effects of anthropogenic factors (in particular air pollution) and natural factors on the condition and development of forest ecosystems in Europe. Also it is contribution to a better understanding of cause-effect relationships in forest ecosystem functioning in various parts of Europe. Based on its mandate, ICP Forests pursues the following objectives:

- to provide a periodic overview on the spatial and temporal variation in forest condition in relation to anthropogenic, in particular air pollution, as well as natural stress factors, on the systematic network of sample plots of Level I (Ferretti et al, 1999);

to contribute, in co-operation with the ICP on Modelling and Mapping, to the calculation of critical levels/loads and their exceedances in forests and to improve collaboration with other environmental monitoring programmes inside and outside the CLRTP (Convention on Long-Range Transboundary Air Pollution)

- to a better understanding of the relationships between the condition of forest ecosystems and natural stress factors, in particular air pollution (Ferretti et al, 2010a)

Intensive monitoring has been conducted on a selected permanent sample plots spread over Europe (Level II). Permanent observations of numerous parameters on these sample fields are performed in the aim of

- monitoring the status of the most important (common and representative) forest ecosystems in Europe;
- to provide a deeper insight into the interactions between the various components of forest ecosystems by compiling available information from related studies.

In the realisation of the objectives, the continued forest condition monitoring in the European countries, ICP Forests consists of the following three levels of monitoring intensity. Level I includes forest condition monitoring on sample plots in 16 x 16 km grid – monitoring, Observation and analyses: crown condition assessment, status of forest nutrition, research and information on soil condition, forest tree diseases, etc. The data on forest condition obtained at this level cover the widest area of Europe. Level II includes the intensive monitoring on a number of permanent observation plots. Monitoring and research at this level are more detailed and they deal with a greater number of input parameters. The Level II results lack the large-scale representativeness. The concept of the higher level of forest condition monitoring intensity enables the correlation of the obtained results with the Level I results, by the comparison of Level II data and the extrapolation to a large scale (Nevenić et al. **2013**). The extrapolation of Level II data is aimed at:

• Evaluation of the significance of relationships at the European level (Ferretti et al, 2010b)

• Limiting of the geographic area (within Europe) for which the relationships are true. The precondition for extrapolation is the harmonised level of monitoring. Level III is the highest level in the hierarchy of monitoring intensities. It includes in-depth analyses of interactions in forest ecosystems, starting from the published scientific results and the results of monitoring obtained by the activities which exceed the Level II (UNECE, 2010b).

In 2002, the activities of some researchers at the Institute of Forestry were directed to the International collaboration and the participation of our country in the EU programmes. After several years, the collaboration with ICP Forests, PCC Head office in Hamburg, was re-established. The Forest Directorate established and verified the Institute of Forestry as the National Focal Centre (NFC) of Serbia for monitoring forest condition.

2. METHODS AND CRITERIA

Reserach had been done according to the ICP Forests Methodology. In 2012, the activities on forest condition monitoring were continued in Serbia on 130 sample plots (Figure 1.) A sample plot is spatially determined by a co-ordinate grid of sample plots and entered into the GIS database (Figure 2.) It is marked with a brightly coloured metal stake driven in the centre. The sample trees for crown condition assessment are selected systematically as 4-point cluster. Oriented along the main compass directions at a distance of 25 m from the grid point – stake, the 6 trees (24 sample trees per plot) nearest to the subplot centre are selected as sample trees. The tree sample includes all tree species, provided the trees have a minimum height of 60 cm. The trees selected for assessment are classified according to crown canopy classes after Kraft (dominant, codominant, subdominant, sup-pressed, dying), but they must be without significant mechanical damage. The selected trees are permanently marked by numbers for future permanent assessment. The trees removed by management operations or for other reasons, are replaced by the new selected trees. If the stand is removed by clear felling, the grid point should be kept till the establishment of the new stand. Within the national and transnational survey (Level I) crown condition is expressed by the classes of defoliation, discolouration and combined damage classes (Seidling & Mues, 2005). Defoliation is assessed in 5% steps and it is grouped in 5 classes of unequal extent (Table 1).

3. RESULTS

During this period, the defoliation of Norway spruce and black pine has remained quite constant, while the defoliation of Scots pine slightly increased during the final years of the reporting period, e.g. in 2016 (Tab 3). The annual mean defoliation in coniferous values were as follows: Scots pine, increased from 8,9in 2012, to 11,6% in 2013, Norway spruce increased from 6,0% in 2013, to 8,0% in 2014 and Fir signnificantly increased from 4,0% in 2013 to 15,6% in 2016 (Češljar et al. 2013).

Of all the trees assessed, 89,6% of the coniferous, and aprocsimatelly the same persentage of the broadleaves were not defoliated. Only moderate defoliated (leaf or needle loss was more than 25%) in whole period were sample trees of black pine. In 2012, the proportions without defoliation were≈ 91% in Scots pine, ≈94% in spruce and 95.5% in fir, respectively (Table 2 and 3). The proportion of moderate defoliated trees (defoliation more than 25%) has remained relatively constant, and for black pine these figures were 29.5% in 2013, the highest, than with 28,4% in 2014, 28.4% in 2014 and similar 27,5% in 2015 and 2016, respectively. Looking at the observed period, it can be concluded that the data for defoliation with conifers and deciduous trees were at a fairly balanced. With coniferous and deciduous trees condition in 2012 set aside as the most unfavorable, as manifested by fewer defoliation on unaffected trees. In conifers, the situation was slightly better in 2013 and 2014 (Češljar et al 2013):, while for the deciduous 2015 year was similar to the 2016. As defoliation is concerned, the only real legitimacy is evident in the *strong* defoliation of conifers, however, and it is difficult to talk about the trend because of the very small number of trees affected by this category of damage (total number is 10 trees). In observed period *F.moesiaca* was the most resistant species; on the many sample plots no signs of the defoliation were detected, whereas the *weak* defoliation of the trees was identified. Oaks are the most suspectible to the foliage loss, most notably sessile oak, with the two-thirds trees with the *moderate* and *weak* defoliation. The situation in observed period was the similar with the condition from the previous years, regarding *Q. cerris* and *Q. fraineto*. The *Slight* defoliation of the coniferous trees was present on the spruce trees; a great number of trees were without the visible signs of this process. Fir is more vulnerable in regard to decissation. Two-thirds of the observed trees of this species were not subject to the defoliation at all, whereas the loss of the needles of the *weak* and *moderate* intensitivity were identified on the other trees (Figure 5). The presence of the damage on the observed broadleaf trees is connected with the assessment of discolouration and defoliation. The parametres of this category and their values are close, i.e. they are approximately in the middle between the above discussed percentages of these two processes. The phenology of the observed species, i.e. leaf rejection in the autumn, make the broadleaves superior from this aspect. In 2012 beech was the most resistant species, these trees on the all sample plots were without no damage. Spruce is most vital conifer species, without any damage. It is followed by fir with 85.5% of trees without defoliation. During the 2012 droughts that caused the drying sessile oak forests in the mountain regions assumed the serious proportions; more and approximately 20% of affected trees. As the main reason for the drying indicated a significant decrease in soil moisture content due to increase in temperature and decrease in rainfall. The incidence of increased temperature during vegetation period and frequent dry vegetation periods were the cause of weakening these forests. Conditions formed by these micro climate changes were the main movers and intensive local factors for gradation of plant pests (pest moths and damaging epidemics of oak powdery mildew) - just about the phenomenon of indirect impacts of climate change on forest extinction (Chiru & Chira 1998; Halmschlager 1998).

Country about affected trees with defoliation for the period 2012 – 2016 are shown in Tables 2 and 3, and for more realistic impression are showen on Figures 3-7. These maps enable more plastic presentation of spatial variance of defoliation value.

Despite the relatively small changes in defoliation in the whole data, marked spatial and temporal variations in defoliation existed during the study period. The defoliation in 2012 and 2013 was highest in northern Serbia (Fig.3 and 4): Results of research of defoliation on bio-indicator plots in Republic of Serbia in 2013(Stevanovic et al 2013). The highest defoliation values in 2014 were found in norhtern and at south (Fig.5). The defoliation of all sample trees in 2014 was simmilar to 2015, with some surfaces highest in northern Serbia (Fig. 5) and scattered across the country (Fig. 6). The highest values were found in the easternmost parts of Serbia in observed period. The defoliation of the assessed trees seems to have intensified in 2016 in the nortmost parts of the country, as compared to a more diffuse pattern previously (Fig. 7).

4. DISCUSSION

Forests with their sustainable management today present one of the many principal international and national policies issues. To be really sustainable, forest management requires the information on the factors that affect the forest health per vitality, biodiversity and ecosystem functioning status. To date, numerous studies have assessed the impact and the importance of shaping of defoliation has received renewed scientific attention, on ICP extensive level (Ferretti et al, 1999; Nevalainen et al, 2010). Tree species-specific effects are regulated by mechanisms allowing for resistance to disfoliating. The short-term consequences of leaf or needle loss depend on species abilities to resist to many factors, and to recover after, and on competitive interactions between species. Although the abundance of many species generally decreases during process so some taxa may increase in number during observed period or shortly after. The effects of recurrent accurence asimilation organs rejection must be evaluated in the wider context of global climate and habitat change (Nevenić et al, 2011). Considering the predicted increase in defoliation frequency and intensity (UNECE, 2004), interdisciplinary research initiatives on this issue are needed urgently. Our results suggest that extensive monitoring networks can reveal useful information about the widespread outbreaks of pest organisms (insects and fungi) already in their increase phases, giving some time for management decisions (Edgar & Burk, 2006). In a changing climate, large-scale, regular monitoring of tree health, including abiotic and biotic causes, is more important than ever before.

5. CONCLUSIONS

Defoliation frequency is thought to be the main driver of vegetation dynamics in Serbia and its temperate forests according ICP extensive sampling. The decline of oak forests has been linked to the detrimental effects of recurrent droughts.

Although five years is relatively short period of meticulously statistical processing in terms of comparative analysis, by examining the collected data values for defoliation and discolouration, some conclusions could yet be to come. Certainly, primarily because of the short time period analyzed, any conclusions reached should be accepted with reservations, or conditionally interpreted as a trend. Oscillations of annual values for three element processed reprezent state of health of forests (defoliation, in all categories of vulnerability, for the conifers are more pronounced than in deciduous trees case). Project of forests condition monitoring - ICP Forests Programme, operates as international European project which demand performing annually observation of forests with recording data on defoliation, discoloration and other damage recorded on the trees, distributed in network of sample plots with special purpose called bio-indicator points or BIT.

Paper analyzes the data of defoliation as part of the complete gathered results of the monitoring of forest on all “BIT”, after their network revision in the Republic of Serbia. Monitoring of forests is carried out continuously at 130 mentioned sample plots. Field work on data collection (observations and measurements), in the previous period were carried out in the period June – September each year.

Calculations of the defoliation mean values in period 2012 – 2016, for each sample plot and for the most common tree species, allowed the comparison of the values of defoliation in some regions and by tree species.

In deciduous forests beech and hornbeam are proven to be more resistant species, in a process of defoliation than oaks. Sessile oak was the most endangered species among the broadleaved. Among the coniferous trees, spruce proved to be a resilient species, while the black pine trees were far the most vulnerable to these processes of all registered species on sample plots.

Determination of defoliation mean values for each experimental field area has allowed mapping of defoliation. Such maps have allowed more plastic and much clearer image of spatial variations of defoliation change (Nevenić et al, 2015).

At the sample plots assessment of defoliation were done even regardless the leaves/needles loss cause, and the results are not strictly aimed to determinate the cause-effect relationship. They are only representing the state of defoliation on experimental fields for the processed period in this paper. Still, the presented study is a baseline for monitoring of changes in forest conditions and connectivity of these results with other indicators of environmental parameters will provide more specific information, and making conclusions about the vitality of plants dependence on environmental conditions.

Leaf or needle loss is certainly a widely underestimated ecological stress and selection force fully exerted to forest ecosystems in Serbia. Our observations have revealed a number of issues that should have priority in future research, such as:

1. *Environmental factors*. Variations of factors such as defoliation intensity, duration and return frequency, species-specific phenotypic plasticity, adaptive potential and phylogenetic and physiological constraints must be experimentally identified in relation to environmental events. This should lead to a concise classification of species according to their sensitivity to asimilation organs rejection with *exactly* environmental features linked to. Particular focus must be on vulnerable species and ecosystems, because these will probably be the first to be seriously affected by appereance of recognised defoliating in progress.

2. *The continuity.* Long-term monitoring programs should be continued or developed, since these are the only way for evaluating the impact of defoliation as events on ecosystems. However, these programs should be coupled with integrative

experimental and modelling approaches to enhance our understanding of complex leaf drought and loss effects.

3. *Idioeclogical dimension*. The impact of defoliation on ecosystem processes must be studied to better understand how it alters ecological functions and how these effects are influenced by species composition. All should help to define an indicator system for predicting drought sensitivity at the stand and forest levels.

3. *Clarifying the causal and consequential relationship*. Future research should focus on the simultaneous effects of different factors as causers, such as pests, forest management, pollution and global warming. This is essential for identifying the most relevant factors that mediate the impact of defoliation as events on forest biodiversity.

4. *Prevention of gain losses in the wood production*. The impact of strategies that are being proposed to mitigate the effects of leaf or needle loss on trees on forest biodiversity should be rapidly evaluated, especially where the impact of the accurence leads to yield reduction.

4. *Biodiversity*. The effects of defoliation events on forest biodiversity should be considered in both planning (e.g. tree species selection) and management (e.g. retention of deadwood).

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TABLES AND FIGURES

Table 1. *Classes of defoliation according to UN/ECE and EU classification*

Class	Degree of defoliation	Needle / leaf loss %
0	None	0–10%
1	Slight (warning)	>10–25%
2	Moderate	>25–60%
3	Severe	>60–100%
4	Dead	100%

Table 2. Mean annual value for defoliation of the most common coniferous tree species during period 2012 - 2016. -The average percentage

Year	**2012**	**2013**	**2014**	**2015**	**2016**
Total coniferous	10,4	11,2	12,8	12,8	13,2
Abies alba	4,5	4,0	10,1	11,3	15,6
Picea abies	6,1	6,0	8,0	7,7	5,9
Pinus nigra	27,0	29,5	28,4	27,5	27,2
Pinus silvestris	8,9	11,6	10,0	10,1	12,4

Table 3. *Defoliation in the period 2012 - 2016. Mean annual value for defoliation of the most common broadleaves tree species during period 2012 – 2016 -The average percentage*

Year	**2012**	**2013**	**2014**	**2015**	**2016**
Total broadleaves	12,1	15,1	13,3	11,3	12,2
Carpinus betulus	11,3	15,5	12,0	7,1	5,4
Fagus moesiaca	6,2	13,1	9,3	8,1	11,6
Quercus cerris	15,1	16,6	14,8	12,5	12,3
Quercus frainetto	10,7	12,5	13,6	8,4	6,8
Quercus petraea	17,5	18,6	16,3	13,3	14,4
Other species	18,6	17,9	17,1	17,5	18,6

Table 4. *Mean annual value for defoliation for both tree species groupes during period 2012 - 2016. - The average percentage*

Year	**2012**	**2013**	**2014**	**2015**	**2016**
Total broadleaves percentage	**12,1**	**15,1**	**13,3**	**11,3**	**12,2**
Total coniferous percentage	**10,4**	**11,2**	**12,8**	**12,8**	**13,2**
Total percentage for both groupes	**11,9**	**14,6**	**13,3**	**11,5**	**12,3**

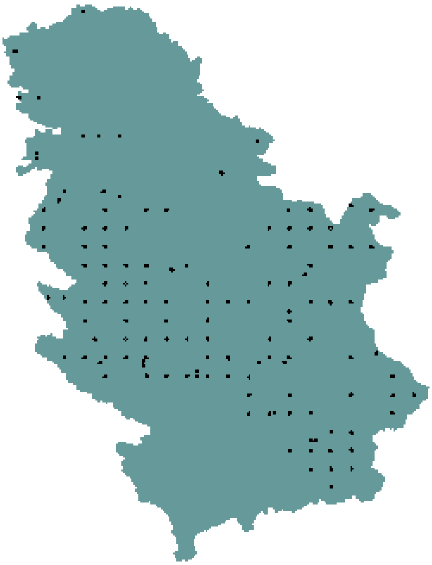


Figure 1. *Forest extensive condition monitoring on 130 sample plots (black spots) in Republic of Serbia during period 2009-2012 (Orig.)*

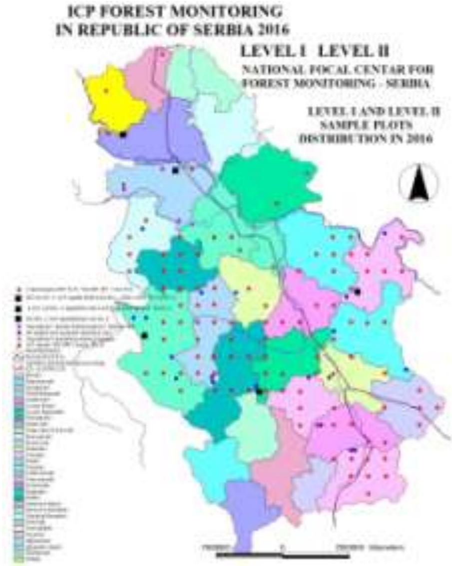


Figure 2. *Spatial arrangement of Level I and Level II sample plots on the territory of The Republic of Serbia in 2016 – Geographic Information System application (Orig.)*

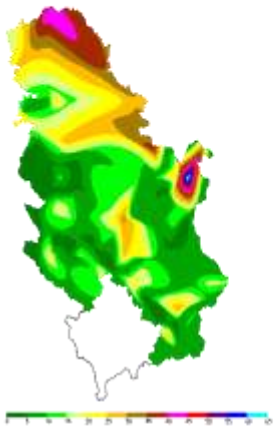


Figure 3. *Defoliation in 2012*

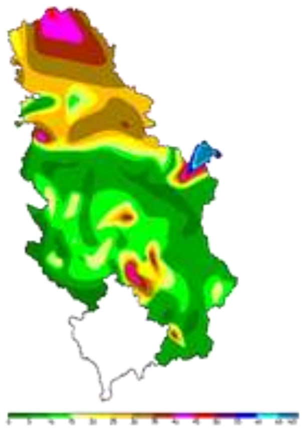
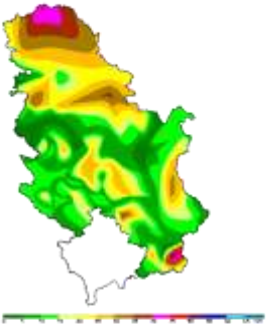


Figure 4. *Defoliation in 2013*



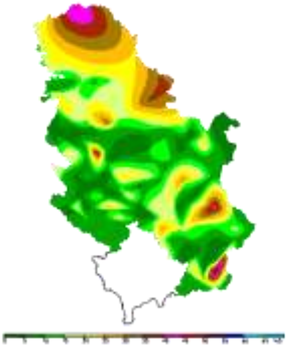


Figure 5. *Defoliation in 2014* Figure 6. *Defoliation in 2015*

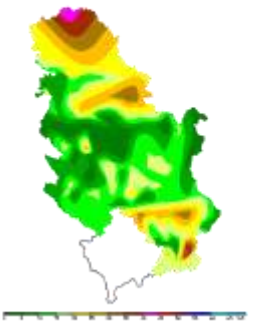
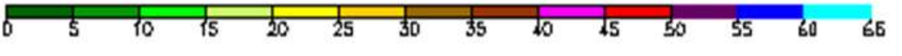


Figure 7. *Defoliation in 2016*

Legend for Figures 3 - 7. *Defoliation maps of forest tree species on the territory of Serbia per year with colured defoliatin range identification*



STUDIES OF DEFOLIATION ON ICP SAMPLE PLOTS LEVEL I IN REPUBLIC OF SERBIA

Tomislav STEFANOVIĆ, Renata GAGIĆ-SERDAR, Ilija ĐORĐEVIĆ, Goran ČEŠLJAR, Natalija MOMIROVIĆ, Ivana ŽIVANOVIĆ, Radovan NEVENIĆ

Summary

Forest Condition Monitoring, Level I, refers primarily to the monitoring and assessment of tree crown defoliation on the installed sample plots, during period of 2012 - 2016. Forest condition monitoring, with special emphasis on defoliation, were performed on 130 sample plots in the Republic of Serbia. The National Focal Centre for forest monitoring in the Republic of Serbia, within the Institute of Forestry, has been taking an active part in an international program of ICP Forest, by improving its approaches in a goal of monitoring harmonization. Paper presents defoliation as indicator of forest vitality, which occurrence shows certain regularity and can be interpreted as a trend. The degree of defoliation of the crown on broadleaves and coniferous was researched on 130 permanent sample plots in the Republic of Serbia in period 2012 - 2016.. Assessment of defoliation had been assessed on 5% interval. These plots are systematically arranged in either a 16x16 km or a 4 x 4 km grid system. Defoliation is presented in spatial distribution in Serbia. Results indicate that major defoliation on broadleaves was on *Quercus* spp., while most resistant was *Fagus* spp. and *Carpinus* spp. On coniferous, *Pinus* spp. has most defoliation and *Picea abies* was the least affected. Within studieng the impacts of regional climate changes on forest communities, comparative analysis of the data obtained in this period will provide a better insight into the effects of defoliation trends as well as the present health state of forests in Serbia.

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Roberts, G., Parrotta, J. and Wreford, A. (2009): *Current Adaptation Measures and Policies.* In: Risto Seppälä, Alexander Buck and Pia Katila. (eds.). Adaptation of Forests and People to Climate Change - A Global Assessment Report. IUFRO World Series Volume 22. Helsinki. 123-13311

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