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Mesophilous *Quercus frainetto* Dominated Forests from Western Balkans

Vladimir Stupar[1]*

(1) University of Banja Luka, Faculty of Forestry, Department of Forest Ecology, Stepe Stepanovića 75A, BA-7800 Banja Luka, Bosnia and Herzegovina

*** Correspondence:** e-mail: vladimir.stupar@sf.unibl.org

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ABSTRACT

Hungarian oak (*Quercus frainetto*) has dominant or co-dominant role in many thermophilous deciduous forest communities in the Balkan Peninsula. However, recent field research in the north-western margin of its range has revealed that some stands have a pronounced mesophilous character, which was also supported by data from the literature. This paper aims to analyze this mesophilous community of Hungarian oak which is found in north-western Serbia, north-eastern Bosnia and Herzegovina (B&H) and eastern Croatia. Numerical analysis, which included classification and ordination of all 474 available relevés of Hungarian oak forests from the western and central Balkans, along with the 43 relevés of mesophilous forests of oak-hornbeam from B&H, have shown that 83 mesophilous Hungarian oak relevés are floristically and ecologically more similar to mesophilous forests of sessile oak-hornbeam (*Erythronio-Carpinion*) than to xero-thermophilous forests of *Qeurcion confertae*. The new association *Carpino betuli-Quercetum frainetto* ass. nova hoc loco was described, floristically and ecologically characterized and assigned to mesophilous oak-hornbeam forests of *Erythronio-Carpinion*. The study also discusses the syntaxonomical issues of *Quercion confertae* and its central association *Quercetum frainetto-cerridis*, considering the problem of the lack of good diagnostic species for both syntaxa, and introduces possible ways of dealing with these issues.

Keywords: *Carpino betuli-Quercetum frainetto*; *Erythronio-Carpinion*; forest vegetation; Hungarian oak; Italian oak; *Quercetum frainetto-cerridis*; *Quercion confertae*

INTRODUCTION

Hungarian oak (*Quercus frainetto*) is a subendemic south European tree species with a relatively small distribution area that is confined to central, eastern and southern Balkans from where it extends to western Anatolia, western Balkans (Bosnia and Herzegovina, hereafter B&H, Montenegro and Croatia), western Romania and eastern Hungary, with a disjunction in Italy. It either functions as the main or a subordinated element of xero-thermophilous forests of *Quercetalia pubescenti-petraeae* (Jovanović et al. 1986, Stefanović 1988, Kojić et al. 1998, Bergmeier and Dimopoulos 2008, Borhidi et al. 2012, Vukelić 2012, Tomić and Rakonjac 2013, Indreica 2015, Škvorc et al. 2017, Di Pietro et al. 2019, Tzonev et al. 2019). In the Balkan part of the distribution area, its communities are mainly assigned to *Quercion confertae,* with a few communities from Bulgaria classified in *Quercion petraeo-cerridis* (Tzonev et al. 2019). It is also traditionally accepted that it builds a zonal vegetation in lowland and hilly regions with the dryer sub-continental climate of central Balkans (Horvat et al. 1974).

However, recent field research of these communities in the north-eastern B&H has suggested that there are mesophilous stands of Hungarian oak dominated forests that share more floristic and ecological features with mesophilous *Erythronio-Carpinion* than with xero-thermophilous *Quercion confertae*. These findings were supported by the classification of the thermophilous deciduous forests of Western Balkans (Stupar et al. 2016), where a fairly homogenous group of relevés, with distinct mesophilous character, traditionally assigned to *Quercion confertae*, i.e. *Quercetum frainetto-cerridis*, remained unclassified after semi-supervised classification. These were the relevés from the north-western margin of the range of Hungarian oak: eastern Croatia, north-eastern B&H and north-western Serbia, the area with more temperate sub-oceanic macroclimatic conditions where *Erythronio-Carpinion* takes over as a climatogenic community. Mesophilous character of these stands has already been pointed out in the past (Vukićević 1959, Glišić 1968, Fukarek et al. 1974, Stefanović 1988, Trinajstić et al. 1996), but was never elaborated outside of the scope of thermophilous forests.

Additionally, the lack of good diagnostic species for *Quercetum frainetto-cerridis*, the central association of continental *Quercion confertae,* have been reported (Stupar et al. 2016, Tzonev et al. 2019). Moreover, Stupar et al. (2015) and Tzonev et al. (2019) stress out the floristic heterogeneity and polymorphism of this most widespread deciduous oak association in continental northern-central Balkans. With recent initiatives to split Balkan Hungarian oak communities into two vicariant alliances, the one of southern and the other one of central Balkan distribution (Mucina et al. 2016, Di Pietro et al. 2019), syntaxonomy of the Balkan Hungarian oak communities is on the way to be revised.

The principal aims of this paper are the following: i) to analyze all available relevés of Hungarian oak dominated forests in the western and central Balkans in order to ecologically and floristically delineate mesophilous from xero-thermophilous stands, ii) to reveal floristic and ecological relationships of mesophilous Hungarian oak stands to the mesophilous *Erythronio-Carpinion*, iii) to describe the new mesophilous community dominated by Hungarian oak, and iv) to tackle syntaxonomical issues inside the Hungarian oak communities of western and central Balkans.

MATERIALS AND METHODS

Study Area

The study was conducted in a distribution area of the Hungarian oak in the western and central Balkans. This included Serbia and North Macedonia as well as eastern parts of B&H and Croatia (Figure 1). Biogeographically, the study area belongs to (a) the Euro-Siberian biogeographical region, particularly the Illyrian, Pindan and Bulgarian sectors of the Apennine-Balkan province, and the Pannonian sector of the Pannonian-Carpathian province, and (b) the Mediterranean biogeographical region, particularly the Attico-Thessalio-Macedonian sector of the Graeco-Aegean province (Rivas-Martínez et al. 2004). The climate of the major part of the area investigated is continental with dry and hot summers and cold winters and annual precipitation of 550–700 mm (Delijanić et al. 1964). Towards the west, the climate is modified by increased maritime influence, resulting in annual precipitation above 700 mm. Topographically and geologically, the region is mainly characterized by hills and low mountains on mainly siliceous bedrock and tertiary sediments (Velić and Velić 1983). From the bioclimatic point of view, the majority of Serbia and North Macedonia and eastern parts of B&H are inside "*Quercion frainetto"* bioclimatic zone, while north-eastern B&H, north-western Serbia and part of eastern Croatia with Hungarian oak stands (Krndija Mt) belong to "*Carpinion betuli illyricum*" zone (Horvat et al. 1974).

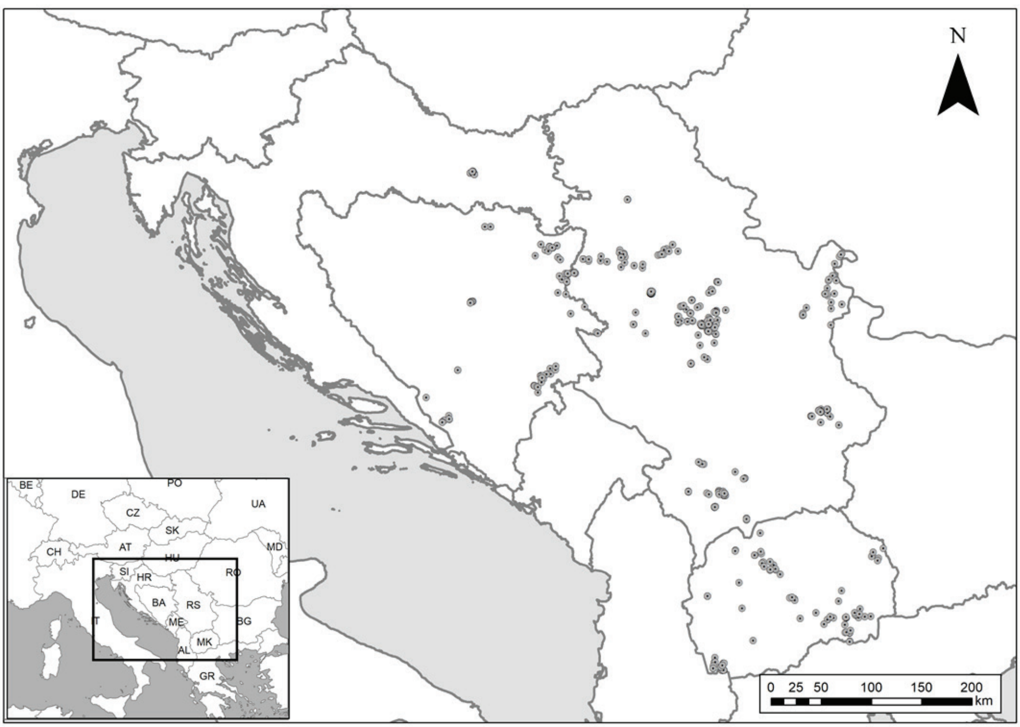

Figure 1. Location of the study area. Dots represent localities of 474 relevés of Hungarian oak dominated forests used in this study.

Data Collection and Analysis

The study was based on a dataset consisting of 504 relevés of Hungarian oak dominated forests from Bosnia and Herzegovina (69), Croatia (14), Serbia (296), and North Macedonia (125) (Figure 1). The majority of relevés were acquired from the literature (see Supplementary File 1), while 23 relevés were recently collected in the field in B&H. After an initial inspection of the dataset, 30 relevés of the unpublished *Carici cuspidatae-Quercetum frainetto* from North Macedonia (Rizovski 1972) were omitted because of their conspicuous transitional character between *Carpinion orientalis*, *Quercion confertae* and *Aremonio-Fagion*. All published relevés were initially or by the later overviews subordinated to *Quercion confertae.* Only 26 relevés of *Carpino orientalis-Quercetum frainetto* (Rizovski 1978) Matevski et al. 2008 were originally classified inside *Carpinion orientalis* (Rizovski 1972), but the association was reassigned to *Quercion confertae* upon validation by Matevski et al. (2008).

Besides, to compare this dataset to the mesophilous oak-hornbeam forests of *Erythronio-Carpinion*, we also collected 43 relevés of oak-hornbeam forests in B&H, from the available literature (see Supplementary File 1), as well as in the field.

All relevés were made using the standard Central European phytosociological method (Braun-Blanquet 1964) with Braun-Blanquet scale cover-abundance estimates of each species. The final dataset of 517 relevés was entered into the Turboveg database (Hennekens and Schaminée 2001) and then exported into JUICE software (Tichý 2002) for further analysis.

Before numerical analysis the following data adjustments were made. Mosses were excluded from the dataset, as they were not recorded by all authors. All layers were merged into a single layer to take account of inconsistent sampling in the relevés from literature. Records of species determined on the genus level were deleted. *Quercus daleschampi* was treated as *Q. petraea*, *Fagus moesiaca* as *F. sylvatica*, while *Lathyrus vernus* and *L. venetus* were combined. Taxa from taxonomically critical groups that were not always identified by the relevé authors and species that included several subspecies that were not always recorded or recognized by authors were also combined into aggregates (*Bromus erectus* agg., *Carex muricata* agg. and *Festuca pseudovina* agg.).

The numerical analysis was performed in R software, version 3.4.1 (The R Foundation for Statistical Computing 2017). Classification of the data set was done using Ward's method and Manhattan distance measure, with cover-abundance estimates transformed to presence/absence. We stopped further division at the level of six clusters because this was the level where mesophilous sessile oak-hornbeam relevés were separated as an individual cluster. Diagnostic species for clusters were determined using species fidelity measure (phi value) (Chytrý et al. 2002) in the JUICE software. We also calculated Fischer's exact test and gave a zero phi value to a species with P>0.001. The threshold phi value for the species to be considered as a diagnostic was set at 0.20.

To extract the main gradients in species composition, all 517 relevés, together with the selected ecological variables were projected onto the two-dimensional ordination space of DCA (detrended correspondence analysis). Species' ecological indicator values (EIVs) for temperature, light, moisture, continentality, soil reaction, and nutrients (Pignatti et al. 2005) were used as explanatory ecological variables. Unweighted average EIVs were calculated in JUICE and passively projected onto a DCA plot. The significance of their correlation with the DCA relevé scores was tested using the modified permutation test proposed by Zelený and Schaffers (2012).

Plant nomenclature followed Euro+Med (2006). Syntaxonomical concepts and nomenclature of higher syntaxa followed Mucina et al. (2016). The description of new syntaxon followed the rules of ICPN (Theurillat et al. 2020).

RESULTS

Classification and Ordination

Numerical analysis of relevés included in the initial dataset yielded six ecologically and floristically distinct clusters arranged in three groups with clearly defined diagnostic species (Table 1, Figure 2): group A (clusters 1 and 2), group B (clusters 3, 4 and 5) and group C (cluster 6).

Mesophilous relevés dominated by *Quercus frainetto* (cluster 2) were classified together with the mesophilous relevés of oak-hornbeam forests of *Erythronio-Carpinion* (cluster 1). Figure 2 shows that the separation of these two clusters inside group A happened at a lower level than the separation of five clusters of xero-thermophilous Hungarian oak relevés (cluster groups B and C). Mesophilous relevés from the literature that were classified inside this group are mainly those that were originally assigned to *Quercetum frainetto-cerridis* (Rudski 1949) Trinajstić et al. 1996 *carpinetosum betuli* Rudski 1949 or *Quercetum frainetto-cerridis ruscetosum aculeati* Jovanović et Dunjić 1951. However, relevés from the original diagnosis of the subassociation *carpinetosum betuli* of *Quercetum frainetto-cerridis* (Rudski 1949) were classified to cluster 4.

Species diagnostic for group A are those characteristic for the mesophilous forests of *Erythronio-Carpinion* and *Fagetalia sylvaticae*: *Carpinus betulus, Carex pilosa, Stellaria holostea, Epimedium alpinum, Pulmonaria officinalis, Galium sylvaticum* etc. Cluster 1 is differentiated by acidophilous *Pteridium aquilinum, Luzula luzuloides* and *Melampyrum pratense* along with the mesophilous *Cruciata glabra, Tilia cordata* and *Drymochloa drymeja*. On the other hand, cluster 2 is differentiated by the mesotermic *Ligustrum vulgare* and *Tilia tomentosa* and also some humidity indicators: *Ulmus minor, Hedera helix, Lonicera caprifolium, Cornus sanguinea, Euonymus europaeus,* etc. which are all common in oak-hornbeam forests of the southern margin of Pannonian plane in B&H and Croatia.

Group B represents the relevés traditionally assigned to *Quercion confertae* (Table 1, columns 3–5). Diagnostic species are widespread thermophilous species like *Trifolium*

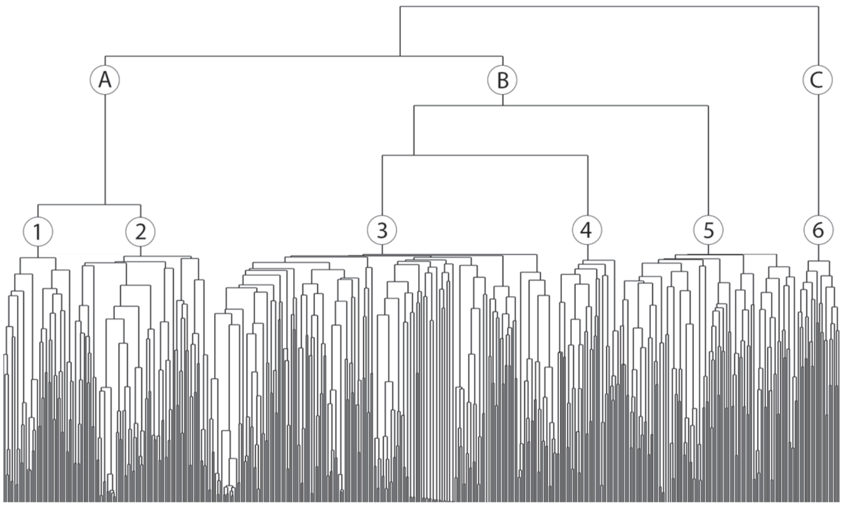

Figure 2. Classification of Hungarian oak relevés in western and central Balkans. Groups: A – *Erythronio-Carpinion*, B – *Quercion confertae*, C – *Carpinion orientalis*. Cluster numbers correspond to those used in Table 1, Figure 3 and throughout the text.

Table 1. Synoptic table of Hungarian oak communities in western and central Balkans. Frequencies of species are presented as percentages, with phi values multiplied by 100 shown in superscript. Diagnostic species (phi values higher than 0.20) for each cluster are shaded (only ten species with the highest phi value are presented). Cluster numbers correspond to those used throughout the text. Full version of the table is in Supplementary File 2.

Cluster group	**A**		**B**			**C**
Cluster number	**1**	**2**	**3**	**4**	**5**	**6**
No. of relevés	**44**	**83**	**215**	**39**	**110**	**26**
Avg. no. of species	**31**	**36**	**31**	**44**	**39**	**53**
Cluster 1						
Pteridium aquilinum	77 ^{67}	4	14	.	17	.
Cruciata glabra	70 $^{51.7}$	11	12	41 20.1	.	.
Luzula luzuloides	41 $^{57.4}$	2	1	.	.	.
Melampyrum pratense	50 $^{56.5}$	10	5	.	.	.
Fagus sylvatica	55 $^{55.3}$	12	5	3	4	.
Tilia cordata	36 $^{55.1}$	1	1	.	.	.
Drymochloa drymeja	27 $^{48.2}$	.	1	.	.	.
Corylus avellana	57 $^{48.2}$	11	7	10	15	.
Quercus petraea	98 $^{44.2}$	40	31	44	40	38
Erythronium dens-canis	23 $^{42.6}$	.	1	.	.	.
Cluster 2						
Ligustrum vulgare	16	82 $^{51.1}$	23	31	7	19
Hedera helix	11	49 $^{44.3}$	8	3	.	15
Tilia tomentosa	18	45 $^{39.3}$	7	13	2	.
Ulmus minor	5	40 $^{37.6}$	10	13	6	.
Euonymus europaeus	.	29 ^{35}	7	8	4	.
Lonicera caprifolium	5	42 $^{34.4}$	6	18	18	.
Cornus sanguinea	18	45 $^{33.5}$	7	31	.	.
Acer campestre	59	94 $^{33.5}$	47	77	41	23
Polygonatum multiflorum	18	27 $^{32.2}$	1	.	.	.
Galeopsis pubescens	5	16 $^{30.5}$	.	.	.	.

Table 1. (continuation) Synoptic table of Hungarian oak communities in western and central Balkans. Frequencies of species are presented as percentages, with phi values multiplied by 100 shown in superscript. Diagnostic species (phi values higher than 0.20) for each cluster are shaded (only ten species with the highest phi value are presented). Cluster numbers correspond to those used throughout the text. Full version of the table is in Supplementary File 2.

Cluster group	**A**		**B**			**C**
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Avg. no. of species	**31**	**36**	**31**	**44**	**39**	**53**
Cluster 3						
Dianthus armeria	.	.	12$^{32.1}$	.	.	.
Trifolium arvense	.	.	13^{31}	.	2	.
Stachys germanica	.	.	10$^{29.5}$	.	.	.
Cytisus austriacus	.	.	10$^{27.7}$	.	1	.
Hypericum perforatum	7	10	39$^{24.7}$	10	15	27
Chrysopogon gryllus	.	.	10$^{24.3}$	3	.	.
Aira elegantissima	.	.	9^{22}	.	4	.
Trifolium campestre	.	1	8$^{21.8}$	.	1	.
Rosa tomentosa	.	.	6$^{21.7}$	.	.	.
Scleranthus annuus	.	.	6$^{21.7}$	.	.	.
Cluster 4						
Viburnum lantana	.	11	21	77$^{67.9}$	1	.
Carex tomentosa	.	.	1	46$^{63.3}$	.	.
Taraxacum officinale	.	.	4	38^{55}	.	.
Inula hirta	.	.	8	44$^{54.9}$	1	.
Peucedanum cervaria	.	4	1	33$^{50.1}$	.	.
Ulmus glabra	2	1	2	33^{49}	.	.
Polygonatum odoratum	.	4	2	41$^{47.3}$	12	.
Iris graminea	.	.	.	28$^{45.7}$	4	.
Vincetoxicum hirundinaria	2	1	18	44$^{43.2}$	7	.
Hieracium umbellatum	11	.	3	28$^{34.5}$	4	.
Cluster 5						
Stachys scardica	.	.	.	.	55$^{60.5}$	15
Paramoltkia doerfleri	.	.	.	.	18$^{39.5}$	.
Eryngium palmatum	.	.	.	.	18$^{39.5}$	.
Lembotropis nigricans	11	10	3	3	36$^{37.9}$	.
Helleborus odorus ssp. *cyclophyllus*	.	.	.	.	16$^{37.4}$	.
Verbascum glabratum	.	.	.	.	13$^{32.9}$	.
Origanum vulgare	.	2	6	.	20$^{32.1}$	.
Corydalis solida	.	.	.	.	10$^{29.1}$	.
Cirsium vulgare	.	.	.	.	10$^{29.1}$	.
Verbascum nigrum	.	1	9	.	18$^{28.5}$	.
Cluster 6						
Achnatherum bromoides	.	.	.	.	.	81$^{88.2}$
Crocus chrysanthus	.	.	.	.	.	77$^{85.7}$
Cardamine graeca	.	.	.	.	.	73$^{83.3}$
Asparagus acutifolius	.	.	2	.	.	69$^{79.1}$
Acer monspessulanum	.	.	1	.	3	65$^{75.8}$
Silene italica	.	6	6	.	15	77$^{70.6}$
Carpinus orientalis	.	11	33	21	20	100^{67}
Carex muricata agg.	2	12	7	3	6	73$^{66.2}$
Juniperus oxycedrus	.	.	7	.	18	65$^{63.1}$

Table 1. (continuation) Synoptic table of Hungarian oak communities in western and central Balkans. Frequencies of species are presented as percentages, with phi values multiplied by 100 shown in superscript. Diagnostic species (phi values higher than 0.20) for each cluster are shaded (only ten species with the highest phi value are presented). Cluster numbers correspond to those used throughout the text. Full version of the table is in Supplementary File 2.

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Avg. no. of species	**31**	**36**	**31**	**44**	**39**	**53**
Erythronio-Carpinion						
Carpinus betulus	98$^{49.7}$	94$^{46.3}$	23	36	6	.
Carex pilosa	36$^{39.2}$	20$^{15.6}$	3	.	.	.
Stellaria holostea	48$^{38.2}$	30$^{16.8}$	5	.	15	.
Epimedium alpinum	32$^{34.1}$	20$^{16.8}$	5	.	.	.
Rubus hirtus	52$^{33.5}$	48^{29}	8	18	3	.
Pulmonaria officinalis	25$^{27.1}$	23$^{23.7}$	.	.	2	.
Galium sylvaticum	27$^{23.9}$	25^{21}	1	.	11	.
Carex sylvatica	18$^{22.9}$	13$^{13.6}$	2	.	3	.
Prunus avium	68$^{35.6}$	49$^{17.5}$	11	54 21.8	5	.
Helleborus odorus	39	64$^{20.8}$	47	74 30.4	22	.
Quercion confertae						
Galium pseudaristatum	.	27	19	44	37$^{17.9}$	.
Euphorbia cyparissias	5	11	41$^{16.6}$	67$^{42.6}$	28	.
Astragalus glycyphyllos	2	18	8	33	27$^{15.7}$	.
Melittis melissophyllum	16	18	7	36	39$^{22.3}$	.
Trifolium alpestre	.	1	15	46$^{34.2}$	34$^{19.4}$	8
Cytisus hirsutus	18	17	27	51$^{25.8}$	35	8
Inula salicina	.	1	7	21	35$^{29.7}$	12
Silene coronaria	.	10	29	10	55^{41}	12
Quercion confertae + Carpinion orientalis						
Tanacetum corymbosum	.	10	18	87$^{50.2}$	39$^{4.8}$	50
Festuca heterophylla	9	22	43	64	67$^{24.9}$	35
Silene viridiflora	.	1	25	44	45$^{18.7}$	46
Physospermum cornubiense	.	23	35	82$^{31.6}$	79$^{28.9}$	62
Sorbus domestica	.	23	23	56$^{16.4}$	41	88$^{45.8}$
Festuca pseudovina agg.	2	10	44$^{12.2}$	62$^{28.9}$	10	62$^{28.9}$
Lathyrus niger	14	27	31	90$^{32.9}$	73$^{17.7}$	85$^{28.3}$
Lathyrus laxiflorus	.	.	3	.	63$^{39.9}$	81$^{58.7}$
Trifolium pignantii	.	.	1	.	48$^{36.8}$	54$^{43.5}$
Luzula forsteri	14	12	12	5	59$^{27.1}$	85$^{51.8}$
Other species with high frequency						
Quercus cerris	20	94	90	100	88	8
Quercus frainetto	2	100	100	100	100	100
Sorbus torminalis	43	33	30	62	40	46
Acer tataricum	57	89	17	72	35	.
Crataegus monogyna	64	94	67	97	68	31
Fraxinus ornus	43	76	60	54	41	96
Dactylis glomerata	18	55	46	46	87	96
Veronica chamaedrys	50	54	39	49	81	100
Tamus communis	9	52	12	49	17	46
Lathyrus venetus + vernus	30	47	20	21	50	27

alpestre, Euphorbia cyparissias, Silene coronaria, Tanacetum corymbosum, Festuca heterophylla, Silene viridiflora, Sorbus domestica, Festuca pseudovina agg. and *Lathyrus niger*, with only two species of the Balkan significance: *Galium pseudaristatum* and *Physospermum cornubiense*.

Cluster 3 (Table 1, column 3) is composed of the largest number of relevés (215). It encompasses the majority of the relevés assigned to *Quercetum frainetto-cerridis* and *Quercetum frainetto* Jovanović et al. 1982 nom. inval. (Art. 5) from Central Serbia and eastern B&H with only several relevés from North Macedonia and Croatia. It is poorly differentiated from the rest of the dataset due to the lack of diagnostic species, a low cover of herb layer (there are no species in the herb layer with the cover of 5% or more), and overall poor floristic composition with the smallest average number of species per relevé (31) among thermophilous clusters. Diagnostic species indicate poor nutrition, open canopy and structural degradation: *Aira elegantissima*, *Hypericum perforatum*, *Stachys germanica*, *Stachys recta*, *Thymus pulegioides*, *Trifolium arvense*, *Trifolium campestre* etc. Species diagnostic for *Quercion confertae* only appear with the low frequency.

Cluster 4 (Table 1, column 4) comprises relevés from Serbia which are found on more nutritious soils, and although there is less light due to closed canopy, there appear many more species per relevé (44) when compared to cluster 3. Diagnostic species are mainly thermophilous: *Viburnum lantana, Carex tomentosa, Inula hirta, Polygonatum odoratum, Iris graminea, Vincetoxicum hrundinaria* etc. This cluster mainly encompasses relevés from the original diagnosis of *Quercetum frainetto-cerridis* (Rudski 1949) Trinajstić et al. 1996 made by Rudski (1949), which also includes the original diagnosis of the subassociation *carpinetosum betuli* Rudski 1949. Our results also suggest that this is the central and typical cluster of the zonal *Quercion confertae* in Central Serbia, also known as "*Quercetum frainetto-cerris moesiacum*" (Horvat et al. 1974).

Cluster 5 encompasses relevés from North Macedonia and Kosovo and Metohija and represents the southern variant of central Balkan Hungarian oak forests. It encompasses relevés mainly assigned to *Quercetum frainetto-cerridis macedonicum* Horvat 1959 nom. illeg. (Art 34a) and *Quercetum frainetto-cerridis scardicum* Krasniqi 1972 nom. inval. (Art 3b). Diagnostic species besides those of *Quercion confertae* are Balkan elements: *Stachys scardica, Paramoltkia doerfleri, Eryngium palmatum*, as well as *Lathyrus laxiflorus* and *Trifolium pignantii* (which are shared with cluster 6). In the sense of Horvat et al. (1974), this cluster stands for zonal association of North Macedonia and northern Greece "*Quercetum frainetto-cerris macedonicum*".

Cluster 6 is represented by the association *Carpino orientalis-Quercetum frainetto* (Rizovski 1978) Matevski et al. 2008, which is found in southern North Macedonia, and although recently validated and assigned to *Quercion confertae* (Matevski et al. 2008), this association was originally classified within *Carpinion orientalis* (Rizovski 1972). Our results support the latter concept, considering that diagnostic species of this cluster are mainly related to *Carpinion orientalis*: *Achnatherum bromoides, Asparagus acutifolius, Carex halleriana, Carpinus orientalis, Lonicera etrusca,* etc. (Table 1, column 6). Furthermore, although cluster 6 and cluster group B both belong to xero-thermophilous forests of the class *Quercetea pubescentis*, cluster 6 was the first to separate from the rest of the dataset (Figure 2), which is probably because *Carpinion orientalis* in relation to *Quercion confertae* includes many more thermophilous species, while on the other hand, *Quercion confertae* harbors a larger number of the widespread nemoral mesophytes.

Classification is backed by the DCA ordination plot (Figure 3), where EIVs for temperature, moisture, light and nutrients are significantly related to the first two DCA axes (P<=0.001; Table 2). The main ecological factors influencing the variation in the floristic composition are EIVs for temperature (positively correlated to the first axis) and moisture (negatively correlated with the first axis), suggesting that the first axis represents the macro-climatic gradient that runs from mesophilous forests of *Erythronio-Carpinion* (left side of the diagram) to xerothermophilous forests of *Quercion confertae* (center of the diagram) and *Carpinion orientalis* (right side of the diagram).

The present analysis suggests that group A stands for mesophilous *Erythronio-Carpinion*, group B belongs to *Quercion confertae*, while group C (cluster 6) represents the most xero-thermophilous *Carpinion orientalis*. Cluster 2 is recognized as the new mesophilous association of Hungarian oak that belongs to *Erythronio-Carpinion*. Syntaxonomical scheme of the new association is as follows:

Carpino-Fagetea sylvaticae Jakucs ex Passarge 1968
Carpinetalia betuli P. Fukarek 1968
Erythronio-Carpinion (Horvat 1958) Marinček in Wallnöfer et al. 1993
Carpino betuli-Quercetum frainetto ass. nova hoc loco (Table 1 column 2, Table 3)

Table 2. Relationship between the first two DCA axes and mean EIVs calculated using modified permutation test. P values are based on 999 permutations. DCA1 and DCA2 are normalized regression coefficients for the first and second DCA axis, respectively. R^2 is a coefficient of determination.

	DCA1	DCA2	R^2	P
Light	0.616	0.788	0.583	**0.001**
Temperature	0.996	0.090	0.442	**0.001**
Continentality	0.889	-0.457	0.129	**0.185**
Moisture	-0.789	-0.614	0.677	**0.001**
Soil reaction	-0.164	-0.987	0.154	**0.124**
Nutrients	-0.581	-0.814	0.622	**0.001**

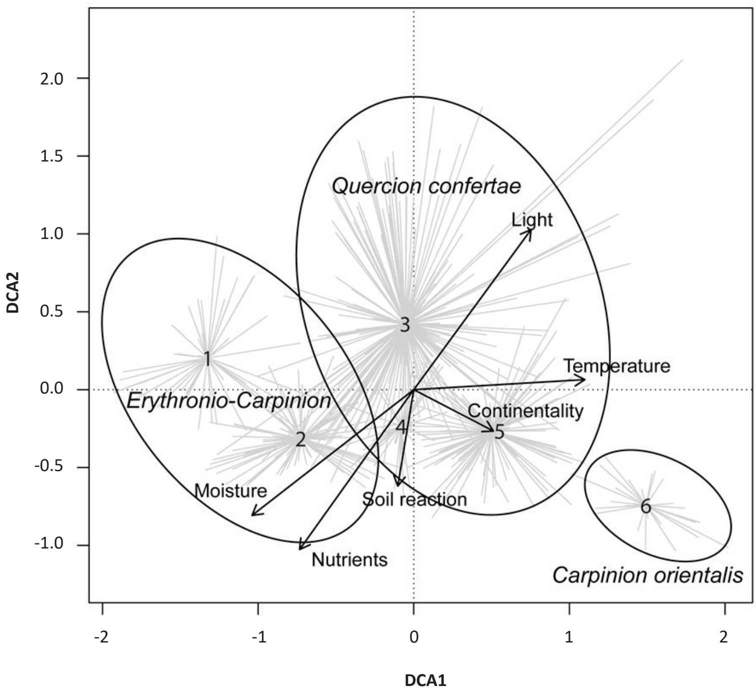

Figure 3. DCA spider plot of 517 classified relevés of the Hungarian oak dominated communities in western and central Balkans with EIVs passively projected. Centroids of clusters are indicated by numbers corresponding to Table 1, Figure 2, and to cluster numbers used in the text.

Description of the New Syntaxon

Carpino betuli-Quercetum frainetto ass. nova hoc loco (Table 1, column 2)

Typus: Table 3, rel. 4 – holotypus hoc loco

Stands of this association are mainly high forests sometimes structurally degraded with closed canopy (80–100%) and of height between 20 and 30 m (Figure 4). They are found in north-eastern B&H, north-western Serbia and eastern Croatia (Figure 5), on predominantly mild slopes and elevations up to 200 m a.s.l. They thrive on deep dystric cambisols or luvisols, mainly upon tertiary clayey lake sediments.

The dominant species of the canopy layer is *Quercus frainetto*, often accompanied by *Quercus cerris*, while *Carpinus betulus* is the dominant species of the understory. The shrub layer is rich in mesophilous species: *Acer tataricum, Acer campestre, Crataegus monogyna, Prunus avium, Rubus hirtus, Cornus sanguinea, Pyrus pyraster, Euonymus europaeus, Ruscus hypoglossum* and *Hedera helix* with some thermophilous and sub-thermophilous elements: *Fraxinus ornus, Ligustrum vulgare, Rosa arvensis, Ruscus aculeatus* and *Tilia tomentosa*. Due to the closed canopy, the herb layer is not so rich, but the species of *Erythronio-Carpinion* such as *Tamus communis, Helleborus odorus, Epimedium alpinum, Galium schultesii* and other species of mesophilous deciduous forests (*Fagetalia sylvaticae*) such as *Cruciata glabra, Dactylis glomerata, Polygonatum multiflorum, Symphytum tuberosum, Viola reichenbachiana, Carex sylvatica, Brachypodium sylvaticum,* and *Veronica chamaedrys* prevail. Thermophilous elements are much less frequent and include: *Viola hirta, Melittis melisophyllum, Potentilla micrantha, Festuca heterophylla* etc. (all besides *V. hirta* with under 50% of frequency).

The published relevés of this association were originally mainly assigned to *Quercetum frainetto-cerridis* (Rudski 1949) Trinajstić et al. 1996 *carpinetosum betuli* Rudski 1949 (Vukićević 1959, Glišić 1968, Trinajstić et al. 1996, Stajić et al. 2008) and *Quercetum frainetto-cerridis ruscetosum aculeati* Jovanović et Dunjić 1951 (Jovanović and Dunjić 1951, Vukićević 1959, Glišić 1968) or were not assigned to any subassociation of *Quercetum frainetto-cerridis* (Fukarek et al. 1974).

However, it should be emphasized that relevés from the original diagnosis of the subassociation *carpinetosum betuli* of *Quercetum frainetto-cerridis* (Rudski 1949) do not belong to this association as they were classified in cluster 4 together with other thermophilous relevés of typical *Quercetum frainetto-cerridis*. So, as far as our results suggest, the mesophilous subassociation *Quercetum frainetto-cerridis* (Rudski 1949) Trinajstić et al. 1996 *carpinetosum betuli* Rudski 1949 (Art. 30b) [Orig. (Rudski 1949): 'Субас. 2. *Quercetum confertae-cerris serbicum* ca *Carpinus betulus*'; Typus: Rudski 1949, Tab. 3, rel. 9 – lectotypus hoc loco] still exists, but it should be confined to the stands of *Quercetum frainetto-cerridis*, where some mesophilous elements such as *Carpinus betulus* can take part, but the overall species composition remains xerothermophilous.

Figure 4. Stand of the association *Carpino betuli-Quercetum frainetto* in Jankovac (northern Mt. Majevica, B&H).

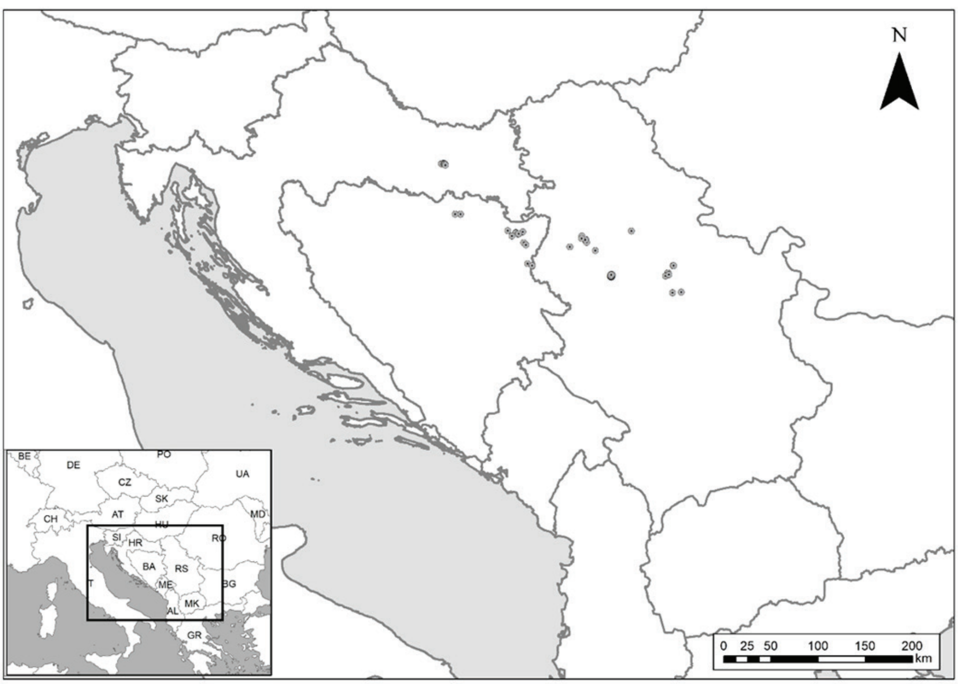

Figure 5. Distribution of the *Carpino betuli-Quercetum frainetto* researched stands.

Table 3. *Carpino betuli-Quercetum frainetto* ass. nov. hoc loco, holotypus: relevé 4 (A - tree layer, B – shrub layer, C - herb layer).

Relevé number		1	2	3	4	5	6	7	8	9	10
Country		BH	BH	BH	BH	BH	BH	BH	RS	RS	HR
Relevé area (m²)		400	400	900	400	600	400	900	n/a	400	200
Altitude (m)		195	140	180	187	150	130	167	190	150	n/a
Aspect		S	S	E	SE	NW	S	NW	-	NW	n/a
Slope (degrees)		15	30	5	15	12	27	5	-	7	n/a
Hight of the canopy layer (m)		27	27	27	30	35	27	30	n/a	18	n/a
Cover total (%)		90	90	90	100	100	100	95	n/a	80	n/a
Characteristic species of the association											
Quercus frainetto	A	5	4	4	4	4	4	3	3	3	4
Quercus frainetto	B	2	1	2	+	.	.	r	3	.	2
Carpinus betulus	A	2	1	2	2	2	2	2	1	1	1
Carpinus betulus	B	2	2	2	+	2	4	.	2	1	2
Quercus cerris	A	2	3	1	1	1	4	.	3	3	.
Quercus cerris	B	+	r	+	.	.	.	.	2	.	.
Acer tataricum	B	1	1	+	2	3	2	2	2	2	+
Rosa arvensis	B	1	1	r	1	1	+	.	+	3	+
Cruciata glabra	C	+	+	1	+	.	.	1	1	3	+
Galeopsis speciosa	C	+	r	r	r	+	r	r	.	.	1
Erythronio-Carpinion											
Ruscus aculeatus	B	.	.	4	.	+	.	4	3	2	.
Tamus communis	C	r	r	1	+	+	+	+	.	.	+
Epimedium alpinum	C	2	.	.	2	.	.	1	3	1	.
Helleborus odorus	C	.	+	+	.	.	+	.	1	2	.
Carpino-Fagetea											
Acer campestre	A	.	+	.	.	1	.	.	1	.	.
Acer campestre	B	+	1	+	1	.	+	1	2	1	1
Ulmus minor	A	.	.	.	.	+	.	.	.	.	.
Ulmus minor	B	.	r	.	1	2	+	.	2	2	.
Crataegus monogyna	B	+	+	r	1	+	.	r	2	3	+
Ligustrum vulgare	B	.	2	1	+	1	3	1	1	3	1
Prunus avium	B	1	2	2	1	+	+	+	.	.	2
Rubus hirtus	B	1	1	1	1	4	2	2	2	.	.
Cornus sanguinea	B	r	2	.	r	r	1	r	.	.	+
Pyrus communis ssp. *pyraster*	B	2	.	.	r	+	.	r	.	1	+
Euonymus europaeus	B	.	+	r	+	+	+	.	.	2	.
Ruscus hypoglossum	B	+	.	1	+	+	.	+	.	.	.
Hedera helix	B	r	.	1	.	.	.	1	3	2	.
Crataegus laevigata	B	.	.	.	+	r	.	.	.	2	.
Dactylis glomerata	C	1	+	.	r	.	+	.	+	3	3
Polygonatum multiflorum	C	+	.	r	+	+	.	+	.	2	+
Symphytum tuberosum	C	r	r	r	r	r	.	+	.	.	+
Viola reichenbachiana	C	r	.	+	.	.	.	.	1	.	+
Galium schultesii + sylvaticum	C	2	+	+	.	+	1	.	.	2	+
Carex sylvatica	C	+	.	.	+	.	+	+	.	.	+
Brachypodium sylvaticum	C	r	1	.	.	.	1	.	.	2	+
Veronica chamaedrys	C	.	+	r	.	.	.	+	1	.	+
Lathyrus vernus	C	.	r	+	+	.	r	+	.	.	.
Geranium robertianum	C	r	+	.	.	.	+	.	.	.	+
Viola hirta	C	.	+	+	r	+	.	+	.	2	.

Table 3. (continuation) *Carpino betuli-Quercetum frainetto* ass. nov. hoc loco, holotypus: relevé 4 (A - tree layer, B – shrub layer, C - herb layer).

Stellaria holostea	C	r	.	+	.	.	.	+	.	2	.
Geum urbanum	C	r	.	.	.	.	r	.	1	.	+
Glechoma hirsuta	C	.	+	r	.	.	1	.	.	.	+
Pulmonaria officinalis	C	.	+	.	.	.	r	r	.	.	.
Quercetea pubescentis											
Fraxinus ornus	A	.	.	.	.	.	.	.	.	1	.
Fraxinus ornus	B	1	.	r	+	.	.	.	1	.	2
Tilia tomentosa	A	.	.	1	.	.	.	.	.	.	.
Tilia tomentosa	B	.	+	2	+	.	.	.	2	.	.
Sorbus torminalis	A	.	.	.	1	.	.	.	.	.	.
Sorbus torminalis	B	r	.	.	+	+	.	.	.	.	.
Melittis melissophyllum	C	+	.	.	r	+	.	r	.	.	.
Potentilla micrantha	C	+	.	.	r	.	.	.	.	.	+
Clinopodium menthifolium ssp. *menthifolium*	C	.	+	.	.	+	+	.	.	.	.
Festuca heterophylla	C	.	+	.	.	.	+	.	.	.	+
Companions											
Juniperus communis	B	r	r	.	.	.	+	.	.	.	+
Lapsana communis	C	r	.	.	.	.	.	.	1	.	+
Alliaria petiolata	C	.	r	.	.	+	r	.	.	.	.

In one or two relevés:

A: *Fagus sylvatica* 4: 2; *Quercus robur* 5: +, 9: +; *Quercus petraea* 7:2;

B: *Fagus sylvatica* 1: +, 10: +; Cornus mas 2: +, 8: 1; *Cytisus nigricans* 1: r; *Prunus spinosa* 4: +; *Corylus avellana* 5: +; *Sambucus nigra* 5: +; *Fraxinus angustifolia* 5: r; *Quercus petraea* 7: +, 10: +; *Malus sylvestris* 7: r; *Lonicera caprifolium* 9: 2; *Sorbus domestica* 10: +; *Rubus discolor* 10: +; *Clematis vitalba* 10: +; *Chamaecytisus supinus* 10: +;

C: *Luzula forsteri* 1: r, 4: +; *Physospermum cornubiense* 1: r, 5: +; *Lactuca muralis* 1: r, 10: +; *Ajuga reptans* 1: r, 10: +; *Lathyrus niger* 2: +, 5: +; *Hieracium sabaudum* 2: r, 10: +; *Melica nutans* 3: +, 7: 1; *Dryopteris filix-mas* 4: +, 7: r; *Cardamine bulbifera* 5: 1, 6: r; *Galium aparine* 5: +, 9: 3; *Veronica officinalis* 8: +, 10: +; *Fragaria vesca* 8: +, 9: +; *Melampyrum nemorosum* 1: 2; *Hieracium bauhinii* 1: +; *Solidago virgaurea* 1: r; *Anthoxanthum odoratum* 1: r; *Holcus mollis* 1: r; *Clinopodium vulgare* 2: +; *Carex divulsa* 2: +; *Silene nutans* 2: +; *Primula acaulis* 2: r; *Verbascum nigrum* 2: r; *Carex pilosa* 4: +; *Polystichum setiferum* 4: +; *Anemone nemorosa* 4: r; *Pteridium aquilinum* 4: r; *Physalis alkekengi* 4: r; *Circaea lutetiana* 5: +; *Calystegia sepium* 5: +; *Scrophularia nodosa* 6: r; *Galium odoratum* 7: +; *Viola riviniana* 7: +; *Lamiastrum galeobdolon* 7: +; *Stellaria media* 7: +; *Polygonatum latifolium* 7: r; *Asarum europaeum* 7: r; *Poa trivialis* 8: 1; *Euphorbia amygdaloides* 8: 1; *Digitalis lanata* 8: +; *Campanula patula* 8: +; *Ficaria verna* 9: 3; *Aegonychon purpurocaeruleum* 9: 2; *Euphorbia cyparissias* 9: 2; *Melica uniflora* 9: 2; *Lathyrus venetus* 9: 1; *Vicia sativa* 9: +; *Vincetoxicum hirundinaria* 10: +; *Astragalus glycyphyllos* 10: +; *Carex flacca* 10: +; *Trifolium medium* 10: +; *Viola alba* 10: +; *Moehringia trinervia* 10: +; *Galium sp.* 10: 2; *Melampyrum sylvaticum* 10: 1; *Helleborus croaticus* 10: 1; *Prunella grandiflora* 10: 1; *Genista tinctoria* 10: 1;

Details of relevés (indicated in the following order: relevé number, date (year/month/day), description of locality, country, longitude, latitude, source (if applicable):

1) 2011/06/30, Tutnjevac, Granik (northern Majevica), B&H, 18.955133, 44.737049; 2) 2011/07/01, Dugo polje, Betnja (Modriča), B&H, 18.174561, 44.928112; 3) 2011/06/30, Bobetino brdo (northern Majevica), B&H, 18.916891, 44.747484; 4) 2011/06/30, Tutnjevac, Granik (northern Majevica), B&H, 18.953569, 44.737086; 5) 2010/05/28, Jankovac (northern Majevica), B&H, 19.008045, 44.755608; 6) 2011/07/01, Dugo polje, Betnja (Modriča), B&H, 18.17413, 44.927926; 7) 2011/06/30, Bobetino brdo (northern Majevica), B&H, 18.915362, 44.746641; 8), Šumski kompleks Bogovađa, Serbia, 20.16802, 44.32401, (Glišić 1968, tab. 1, rel. 13); 9) , Posavina, Kozadra, Serbia, 19.633156, 44.603326, (Vukićević 1959, tab. 2, rel. 6); 10) southern Krndija, Croatia, 17.9651, 45.40494 (Trinajstić et al. 1996, tab. 1, rel. 8).

DISCUSSION

Despite the considerable number of described communities dominated by Hungarian oak on a relatively small distribution area of this species, until recently their syntaxonomy seemed settled inside two alliances of *Quercetalia pubescenti-petraeae*. In Italy, these communities are classified into *Crataego laevigatae-Quercion cerridis* (Blasi et al. 2004, Landucci et al. 2012), while in Balkan part of the distribution area they have been traditionally classified inside *Quercion confertae* (Lakušić et al. 1978, Jovanović et al. 1986, Kojić et al. 1998, Bergmeier and Dimopoulos 2008, Kavgaci et al. 2010, Borhidi et al. 2012, Vukelić 2012, Tomić and Rakonjac 2013, Indreica 2015, Škvorc et al. 2017).

However, EuroVegChecklist (Mucina et al. 2016) recently reintroduced the old concept of classification of southern Balkan thermophilous deciduous oak forests on slightly acidic deep soils in the distinct alliance *Melitto albidae-Quercion* (first proposed by Barbero and Quézel (1976)) which was partly supported and further elaborated by Di Pietro et al. (2019). Still, there is no general agreement about its syntaxonomic independence from the *Quercion confertae* (Bergmeier and Dimopoulos 2008, Çoban and Willner 2019).

Meanwhile, in Bulgaria, Tzonev et al. (2019) classified *Quercetum frainetto-cerridis* and *Hedero helicis-Quercetum cerridis* in the *Quercion confertae*, while *Rusco aculeati-Quercetum frainetto*, *Symphyto ottomani-Quercetum frainetto* and the community of *Quercus frainetto* and *Hypericum cerastoides* have been assigned to *Quercion petraeo-cerridis*. More importantly, authors emphasized that *Hedero helicis-Quercetum cerridis* is a transitional association towards *Erythronio-Carpinion*, and stated that ecologically similar associations from Italy (*Erythronio-Quercetum cerridis* Biondi et al. 2002 and *Listero ovatae-Quercetum cerridis* Di Pietro & Tondi 2005), with similar intermediate features towards *Quercetalia pubescenti-petraeae* have been assigned to *Erythronio-Carpinion*. They also pointed to similar syntaxa from Romania and Serbia, i.e., *Potentillo micranthae-Quercetum dalechampii* Horvat 1981, *Carpino betuli-Quercetum frainetto-cerris* (Rudski 1940) 1949 B. Jovanović 1968, and *Robori-Quercetum frainetto-cerris* (Slavnić 1952) B. Jovanović et Z. Tomić 1978.

Furthermore, the present study revealed that mesophilous Hungarian oak stands from north-western margin of its distribution area should be classified into *Erythronio-Carpinion*, while there is at least one association dominated by Hungarian oak that belongs to *Carpinion orientalis* (i.e., *Carpino orientalis-Quercetum frainetto* (Rizovski 1978) Matevski et al. 2008).

In addition to syntaxonomical inconsistencies, different interpretations of biogeography of Hungarian oak communities in Balkan Peninsula have also been provided. While communities of *Quercion confertae* have been traditionally considered as zonal for the continental bioclimatic zone of Balkans (Horvat 1954, 1958, Horvat et al. 1974), soil and topography are more likely to influence the occurrence of *Quercion confertae* and *Carpinion orientalis* in submediterranean mainland Greece according to Bergmeier and Dimopoulos (2008). Hence, they are not suitable to define the bioclimatic zone in the southern Balkans. A similar issue was raised on the opposite side of *Quercion confertae* distribution area, in the north-western Serbia, where Jovanović (1967) argued that Hungarian oak community common for the area (*Quercetum frainetto-cerris hieracietosum*) is not representative of macro-climatic conditions and therefore, contrary to Hungarian oak communities of eastern and southeastern Serbia, not zonal. On the other hand, Stupar and Čarni (2017) demonstrated that the variation in floristic composition between zonal forests of *Aremonio-Fagion, Erythronio-Carpinion, Quercion confertae* and *Carpinion orientalis* in B&H is completely macro-climatically driven and that there is no influence of soil condition, which indicates that *Quercetum frainetto-cerridis* is zonal in eastern B&H.

All these different and sometimes contradictory findings complicate the question of syntaxonomy of *Quercion confertae* and in particular its central association *Quercetum frainetto-cerridis*. While clusters 4 (the type community originally published by Rudski (1949)) and 5 (southern communities from Kosovo and Metohija and North Macedonia) are fairly well defined by a few diagnostic species of Balkan character: *Galium pseudaristatum, Physospermum cornubiense, Lathyrus laxiflorus* and *Trifolium pignantii* (the latter two important only for southern clusters 5 and 6), cluster 3, which has the most relevés in the present dataset, is quite poorly characterized by these same species (Table 1). The reason behind this may be that the majority of the relevés in cluster 3 are not representative due to the structural degradation of Hungarian oak forest stands or that these communities are just transitional between the "real" *Quercion confertae*, *Quercion petraeo-cerridis, Carpinion orientalis* and *Quercion petraeae*. On the other hand, when studied together with other thermophilous deciduous forests of the western and central Balkans, Hungarian oak forests from Serbia and eastern B&H group together with Turkey oak forests on deep and slightly acidic soil over the limestone in the western B&H (which completely lack the Balkan element) (Stupar et al. 2016). This means that if we accept the concept of coexistence of the two separate and vicariant alliances of Hungarian oak in the Balkan Peninsula as suggested by Mucina et al. (2016) and Di Pietro et al. (2019), then the southern Balkan *Melitto albidae-Quercion* would have the diagnostic species which are mainly with the southern Balkan distribution, while the mainly non-frequent appearance of Balkan element (*Galium pseudaristatum* and *Physospermum cornubiense*) in northern-central Balkans *Quercion confertae* would not be considered as important for the alliance. This would alleviate the potential redefinition of the circumscription of alliance *Quercion confertae* in the scope of thermophilous deciduous forests of Southeastern Europe. In any case, the syntaxonomic issues of *Quercion confertae* of the Balkan Peninsula deserve more attention in future studies.

CONCLUSIONS

The results of the present analysis support the hypothesis that mesophilous Hungarian oak communities from the north-western margin of its range do not belong to the Balkan thermophiolous oak forests on slightly acidic deep soils (*Quercion confertae*), but rather to the mesophilous oak-hornbeam forests on deep nutrient-rich soils of the Balkans and Northern Italy (*Erythronio-Carpinion*). The new mesophilous association *Carpino betuli-Quercetum frainetto* ass. nova hoc loco was described and assigned to *Erythronio-Carpinion.* The results also indicate that syntaxonomy of Hungarian oak forests on the Balkan Peninsula is far from being settled. Some possible solutions that require further investigation were introduced.

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Conflicts of Interest

The author declares no conflict of interest.

Suplementary Materials

Suplementary File 1. Sources of data use for the analysis.
Suplementary File 2. Full synoptic table.

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Diameter Structure Changes in the Pre-Maturing Black Locust and Common Hackberry Stand in the Subotica-Horgoš Sands under the Influence of a Late Thinning

Siniša Antun Andrašev[1,*], Martin Bobinac[2], Tomislav Dubravac[3], Nikola Šušić[4]

(1) University of Novi Sad, Institute of Lowland Forestry and Environment, Antona Čehova 13d, RS-21000 Novi Sad, Serbia; **(2)** University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, RS-11000 Belgrade, Serbia; **(3)** Croatian Forest Research Institute, Division for Silviculture, Cvjetno naselje 41, HR-10450 Jastrebarsko, Croatia; **(4)** University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, P.O. Box 33, RS-11030 Belgrade

*** Correspondence:** e-mail: andrasev@uns.ac.rs

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ABSTRACT

In this paper, a change of diameter structure in a pre-maturing stand of black locust and common hackberry under the influence of a late thinning is analysed. The research is based on three permanent experimental plots and two measurements of diameters in a five-year period. One of the plots is a control plot and two plots are experimental, where the thinning was carried out in a stand 28-years old, with the thinning intensity of 28.9–30.6% of the initial density, approximately evenly distributed across diameter classes. In the investigated stand common hackberry came from the neighbouring areas in the stand structure. The initial measurement in the autumn of 2014 confirmed the share of common hackberry of 16–18% in the total number of trees thicker than 5 cm with a dominantly reversed J shape of the diameter structure and the presence of trees in all the diameter classes. In the period of stand age from 28 to 33 years, a dominant process on all treatments was the mortality of thinner trees, while the recruitment of common hackberry trees was recorded in all treatments. On the control plot, a quarter of the trees died, while an eighth of the remaining trees died in the thinned plots, mostly black locust trees. In thinned plots, only black locust trees died with a characteristic that the intensity of mortality was higher in thinner trees, while in the control plot some thick black locust trees died, as well as and some thinner common hackberry trees. In the five-year period, numerical parameters of variability (standard deviation, coefficient of variation), the shape of distributions (skewness and kurtosis) and heterogeneity of diameters at breast height (Gini index, Lorenz asymmetry coefficient) have shown a trend of increasing variability and change of diameter distributions of trees in all treatments, but it is more expressed in thinned plots compared to the control plots. Growth dominance coefficient of diameters shows that the competition between the collectives of both species and the black locust collective is of asymmetric type and more expressed in the thinning treatments. In common hackberry trees on the control plot the competition between the trees is of asymmetric type, while on the thinned plots, the competition is of symmetric type. This shows that after thinning, common hackberry has a biological potential that is higher than that of black locust and that the natural succession can be accelerated through thinnings.

Keywords: natural succession; tree mortality; numerical parameters of diameter structure; structure heterogeneity; asymmetric competition; symmetric competition

INTRODUCTION

Forest management influences tree size distributions, spatial mingling of tree species and natural regeneration. Forest structure affects a range of properties, including total biomass production, biodiversity and habitat functions, and thus the quality of ecosystem services. It usually refers to the way in which the attributes of trees are distributed within a forest ecosystem (von Gadow et al. 2012).

Biological populations have age, size, spatial and genetic structures. By knowing the dynamics of such structures, we can understand the functioning of populations. To understand plant communities in nature, we must also study the dynamic aspect of size structure (Hara 1988). The development of the trees in a pure stand or a species in a community can be characterized by their tree size distribution, growth distribution between trees, and mortality (Hara 1993). In single-cohort pure stands the diameter distribution is narrow and right skewed in the early stage, and becomes more and more symmetric, Gaussian-shaped with progressing stand development. Silvicultural treatment cuts mainly the left branch by thinning from below, the right branch by thinning from above, or simply reduces the level of the size distribution by systematic thinning, such as the elimination of every n^{th} tree or tree row. Shade tolerant species tend towards wider size distributions than light-demanding species, as a lower light compensation point allows better persistence of small trees in deep shade (Pretzsch and Schütze 2014).

Size structure refers to the statistical distribution of a given individual plant size attribute in a population, for instance diameter, height or volume. It is generally characterized by the mean, the variance, the skewness and the kurtosis of the distribution (Hara 1988). The standard deviation can be used to compare differences in size variability between stands with the same mean tree size. The coefficient of variation is a normalized measure of variation and is useful for comparing stands with different mean tree sizes. The skewness coefficient estimates the degree of asymmetry in the frequency distribution of tree sizes. Skewness has been widely used to quantify changes in size variability with stand density and development. Kurtosis indicates the 'peakiness' of the distribution. Positive kurtosis indicates that a preponderance of trees congregated in the same or nearby size classes to form a peak in the size-frequency distribution which increases as the value of kurtosis increases. Negative kurtosis indicates flattened distributions with individual trees more evenly distributed among the size classes (Bi and Turvey 1996, McGown et al. 2016). The kurtosis is appropriate for characterizing the degree of restriction of a species by intra- and inter-specific competition (Pretzsch and Schütze 2014).

Weiner and Solbrig (1984) formally defined size hierarchy as a frequency distribution where the variation in individual sizes is large, the majority of individuals are small, and a few large individuals contribute most of the population’s biomass. With this definition, Weiner and Solbrig (1984) argued that standard summary statistics, specifically the skewness coefficient, were unable to describe the most important aspects of size hierarchy effectively. This represented a shift in the discussion of quantifying size variability from a focus on describing the frequency distribution of individual plant sizes to describing the concentration of sizes within a stand (size inequality). Weiner and Solbrig (1984) used the Gini coefficient, a summary statistic from the Lorenz curve, to measure the total inequality, or concentration of sizes, within a population. Damgaard and Weiner (2000) introduced an additional parameter, the Lorenz asymmetry coefficient, which provides information about the shape of the Lorenz curve and describes how size inequality is distributed within a stand.

Plant size describes the cumulative growth of an individual. Managers may be more interested in how growth is currently distributed within a stand and less concerned with past growth. This requires moving away from measures of size variation and inequality. Previous studies have utilized distribution-modifying functions to relate changes in size distributions over time to the growth of individuals (e.g., Westoby 1982, Hara 1984). Binkley (2004) introduced the concept of growth dominance to estimate where growth is concentrated within a stand. Growth dominance of an individual is estimated by its proportion of total stand growth to its proportion of stand volume and is not a measure of size variation or size inequality. If the proportion of growth is greater than its proportion of stand volume, the individual is said to be "growth dominant" regardless of its size or crown position (McGown et al. 2016).

The aim of this paper is to perceive the change in diameter structure in a stand of black locust and common hackberry in which thinnings were applied over a period of five years using standard procedures to describe diameter structure, numerical indicators of descriptive statistics, heterogeneity and growth dominance pattern.

MATERIALS AND METHODS

Study Area

The research was conducted in a black locust and common hackberry stand in Management Unit (MU) "Subotičke šume", which represents a majority of the Subotica-Horgoš sands consisting entirely of anthropogenic stands. Subotica-Horgoš sands are located in the northern part of Bačka loess plateau. The city of Subotica is surrounded by the sands from the northern side, up to the border with Hungary.

The sands originated in the early Pleistocene (Diluvial), in the period of glaciations, when rivers brought sand and mud from the Alps and Carpathians during the summer. In the winter, when the rivers returned to their beds, the winds carried the material and deposited it in the form of sand or loess in the area of present-day sands and loess terraces. In this way, different forms of sands were created: greyish-yellow sand, brown sand, black sand, black loamy sand and salified sand. On the soils formed mostly on black sands and in the valleys, natural forests of pedunculate oak, white poplar and black poplar were present. Other parts of the sands were covered by grass vegetation (pastures) or consisted of moving sands that caused wind erosion in a wide area with negative effects for agriculture and settlements (Pavićević and Stanković 1962, Šijak 1980).

The work on the stabilization of moving sands using the rapid-growing tree species such as willows, poplars, limes, black locust and tree-of-heaven started at the end of 18^{th} century. The initial establishment of the stands was carried out more or less successfully because of the inadequate selection of tree species and establishment techniques. The turning point was 1870 when black locust was used after deep tillage mostly on greyish-yellow sand where other tree species could not survive. After World War I, the advantage was given to Austrian pine in the afforestation of the sands (Peruničić 1956).

According to the available literature, there is no data about planned introduction of common hackberry in the area of Subotica-Horgoš sands. Common hackberry (*Celtis occidentalis* L.) is a non-native tree species that was introduced to Europe in 1636 and presumably in the first half of the 19th century in the area of the Pannonian Basin (Hungary), primarily as a park tree species for settlements. At the beginning of the 20th century, common hackberry was used widely for afforestation in present-day Hungary (Bartha and Csiszar 2008). Having in mind that the area of Subotica-Horgoš sands belonged to Hungary (Austro-Hungarian Empire) at the beginning of the 20th century, it can be presumed that common hackberry was used for afforestation in the sands as well, given that the species was quite drought-tolerant (Tiborcz et al. 2011). Outside Subotica-Horgoš sands, common hackberry was mostly cultivated in Serbia as a park tree species and in tree alleys. On the other hand, black locust (*Robinia pseudoacacia* L.) was widely used in Serbia and is considered to be an important tree species for forestry, having in mind its wide use (Banković et al. 2009; Andrašev et al. 2014).

In 1969, black locust was the most represented tree species in MU "Subotičke šume" in the area of Subotica-Horgoš sands and covered the area of 2,450 ha out of 3,500 ha of forests and forest cultures. Other tree species included poplars (470 ha), Austrian pine (around 500 ha), pedunculate and Turkey oak (42 ha) and other broadleaves on 140 ha (Šijak 1980).

The inventory of the stands in 2009 showed that black locust covers 1,750 ha, where pure black locust stands cover almost 600 ha, and the rest are mixed stands. Having in mind the limits of stand delineation and stand measurements, primarily in young, coppice black locust stands that were in the inventory in 1999 in MU "Subotičke šume" and were classified as pure black locust stands in the area of 2,100 ha, it is evident that the data from 2009 show a trend of tree species succession, especially when it comes to common hackberry. The analysis of the 20-year period from the inventory data from 1999 and 2019 for MU "Subotičke šume" (1999, 2009, 2019) showed a trend of a quadruple increase in total area with common hackberry (Table 1). In 1999, common hackberry was recorded on over 500 ha and on 1,277 ha in 2009. The stands with common hackberry domination in terms of the number of trees covered the area of 101 ha in 1999 and 271 ha in 2009, while pure stands of common hackberry (90% of common hackberry in the total number of trees) covered the area of 15 ha in 1999 and 29 ha in 2009. The trend of a two-fold increase in the total area covered by common hackberry was recorded in the inventory from 2019 as well (Table 1).

Field Operations and Measurement

In the autumn of 2014 in a 28-year-old stand of black locust and common hackberry, three permanent sample plots were established. The size of the plots is 25 × 25 m with a buffer zone 5–10 m wide (MU "Subotičke šume", compartment 59). Black locust originates mostly from coppice shoots and common hackberry mostly from seed. The investigated stand has a special purpose and a primary function of soil protection because it is situated on a site that is prone to erosion. So far, cleaning was carried out at an age between 5 to 10 years. On two of the permanent plots, the late thinning was carried out (treatments T1 and T2) and one of the plots was the control plot (treatment C). When the plots were established, two cross diameters with an accuracy of 1 mm were measured for all trees and the initial results were published (Andrašev et al. 2016). In the autumn of 2019, the trees were measured again where the diameter threshold was 5 cm at breast height with an accuracy of 1 mm. This threshold was used for both measurements so they could be compared. The diameter comparison of trees over 5 cm at breast height when the plots were established and five years later showed the number of trees (level of recruitment) that grew above the 5 cm threshold. Two stand conditions were observed: (1) initial stand condition in autumn of 2014 and (2) stand condition in autumn of 2019. Since two tree species were recorded in the stand, black locust and common hackberry, the trees were grouped in three collectives: (a) trees of both species; (b) black locust trees and (c) common hackberry trees.

Data Processing and Statistical Analysis

Data processing included the expression of diameter structures in trees per hectare by grouping the diameters at breast height (DBHs) in 2 cm wide diameter classes. The comparison of diameter structures of trees from different treatments (C, T1 and T2) in different conditions was performed using the non-parametric tests by Kolmogorov-Smirnov, Anderson-Darling and Wilcox (Dodge 2008).

For all stand conditions and all collectives of trees in the stand, the basic parameters of descriptive statistics and indexes of heterogeneity (inequality) that express the numerical parameters of diameter structures were calculated: arithmetic mean, standard deviation, coefficient of variation, skewness, kurtosis, Gini index of heterogeneity and Lorenz asymmetry coefficient as an addition to the

Table 1. Total areas (in hectares) covered by common hackberry with different share in the total number of trees per hectare in the inventories of 1999, 2009 and 2019.

Inventory	>0%	>10%	>50%	>70%	>90%
1999	505	298	101	41	15
2009	1,277	827	271	127	29
2019	2,252	1,768	794	501	243

Source: Data collected on the basis of the inventory data used for preparing forest management plans for Management Unit "Subotičke šume" (1999; 2009; 2019)

Gini index. The parameters of descriptive statistics were calculated using the well-known formulas from the theory of statistics (Field et al. 2012). The Lorenz curve was calculated by ranking individual diameters from the smallest to the largest and plotted cumulative fraction of diameters against cumulative fraction of the population. If all individuals have equal diameters the Lorenz curve will be a diagonal line from the origin (0% of the population contains 0% of the diameter sum) to the upper right corner (100% of the population contains 100% of the diameter sum). Any inequality results in a curve below the diagonal. The Gini index is a summary statistic from the Lorenz curve expressing the proportion of the area between the Lorenz curve and the diagonal line and the area of the triangle defined by the diagonal line (1/2). It has a minimum of 0 and a theoretical maximum of 1. The Gini index was widely used in econometrics, expressing inequality of income between individuals in population, and is calculated using the formula (Sean, 1973):

$$G = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}\left|x_i - x_j\right|}{2n(n-1)\bar{x}}$$

where: x_i, x_j - diameter at brest height of i and j tree in the sample, n - number of trees in the sample, $\bar{x}$- arithmetic mean of diameters.

The Gini index, as a summary statistic, is not able to cover the specificity of the Lorenz curve, so the Lorenz asymmetry coefficient was introduced as a statistic that shows which of the diameter classes contributes more to the total heterogeneity expressed through the Gini index. It was shown that the collectives may have the same Gini index of heterogeneity but may differ in terms of the share of certain size classes that have a more pronounced contribution to the heterogeneity of the whole collective (Damgaard and Weiner 2000). The Lorenz asymmetry coefficient was calculated using the method presented in Damgaard and Weiner (2000).

The diameter increment was calculated as the difference between DBH of each tree which was alive and greater than 5 cm in both measurements divided by five years. The initial DBHs of trees that produced the increment were used for calculation of the growth dominance coefficient — GDC (Binkley 2004). GDC is not a size variation measure, but instead it is a summary statistic that indicates how growth is distributed among individuals within a stand (Binkley 2004). For size ordered data, the cumulative proportion of tree size (x-axis) is plotted against the cumulative proportion of tree growth (y-axis). Bounded between –1 and 1, positive GDC values indicate that larger trees have a proportion of stand increment that is greater than their proportion of cumulative stand size, negative GDC values indicate that smaller trees account for a greater proportion of stand increment than cumulative stand size, and a value of zero indicates that all trees contribute to stand growth proportionally to their size (i.e. no dominance). GDC was calculated using methods described in West (2014), with diameter as a measure of tree size.

Since the previously mentioned numerical indicators of diameter structure are a summary statistic calculated based on at least 30 trees, bootstrap resampling was performed in order to make an objective comparison of their confidence intervals, which has been confirmed for the Gini coefficient by Weiner (1985) and Dixon et al. (1987). The confidence intervals were calculated at the 95% level of significance using the bootstrap resampling technique (Efron and Tibshirani 1986), so a more objective comparison was achieved. The bootstrap resampling method is a method for generating a sample using the original sample with the same number of observations, but some are removed, while some occur more than once (a new sample with repetitions is generated). This procedure is repeated for a certain number of times (at least 200) and results in a confidence interval for every of the investigated indicators of the structure (Efron and Tibshirani 1993). In this paper, every sample is repeated 1000 times. The confidence interval that was calculated from the original sample statistics and standard deviation of the means was generated from repeated samples using the bootstrap resampling method, according to the formula:

$$x_{u,l} = x \pm 2 \cdot s_e$$

where $x_{u,l}$ — upper (u) and lower (l) confidence interval at the level of significance of 95%; x — statistics of original sample (arithmetic mean, standard deviation, coefficient of variation, skewness, kurtosis, Gini coefficient, Lorenz asymmetry coefficient, growth dominance coefficient); s_e — standard deviation of the means of every statistic generated using the bootstrap resampling.

To examine the probability of death (p) in relation to the size of individual trees (DBH), each tree was assigned a score of 0 if it was dead in 2019 and a score of 1 otherwise. Logistic regression (Kleinbaum and Klein 2010) was then used to relate the binary response to diameters as independent variable:

$$p = \frac{e^x}{1 + e^x}$$

and

$$x = logit\,(p) = l_n\left[\frac{p}{1-p}\right] = a + bDBH$$

where DBH is diameter at breast height and a and b are parameters.

All statistical analysis was done using R version 4.0.0 (R Core Team 2020) and all figures were prepared with ggplot2 package in R environment (Wickham 2016).

RESULTS

Initial Stand Condition

The stand condition at the age of 28 years shows that the total number of trees with DBH higher than 5 cm was 3.152–4.080 per hectare, and the share of common hackberry was 16–18% (Figure 1, Table 2). Non-parametric tests by Kolmogorov-Smirnov and Anderson-Darling did not confirm significant differences between the diameter distributions of black locust and common hackberry collectives, as well as the collective with both species between the treatments (Tables 3 and 4). The statistical test that compares the medians of DBHs according to the method by Wilcox did not determine significant differences between the treatments in the initial stand conditions on the sample plots, except in the case of black locust collective between C and T1 treatments (Table 5). Arithmetic mean of diameters of both species collective and black locust collective was 9.1–9.8 cm and was inversely proportional to the total number of trees per hectare, while the mean

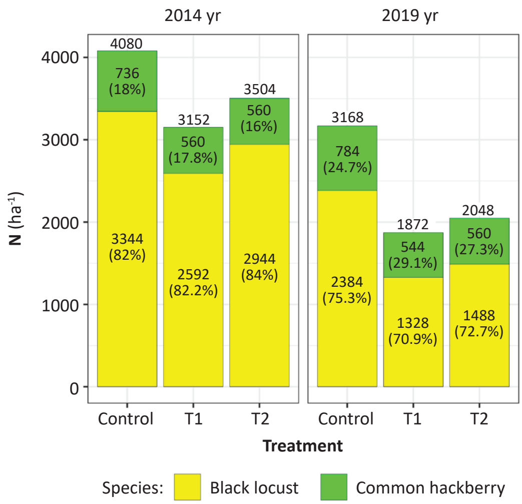

Figure 1. Total number of trees per hectare.

diameter of common hackberry was 9.3–11.1 cm and was not related to the total number of trees per hectare. The mean diameters of common hackberry trees were lower by 0.1 cm compared to black locust trees on T1 plot, higher by 0.2 cm compared to black locust on the control plot and higher by 2.0 cm on T2 plot (Table 2). The confidence intervals calculated using the bootstrap resampling did not show any significant differences between the treatments in any of the collectives (Figure 2).

The share of thinner trees is dominant in all the collectives in the stand. Diameter distribution of trees above the 5 cm diameter threshold has a reverse J shape in all the collectives in C and T1 treatments and in the common hackberry collective in T2 treatment. Only in black locust collective and both species collective in T2 treatment, a clear unimodal diameter distribution was observed (Figure 3). Standard deviation and coefficient of variation of diameters in all treatments are similar in the both species collective, but higher in common hackberry trees compared to that of black locust. The confidence intervals calculated using the bootstrap resampling show that a significant difference exists only between the black locust collective and common hackberry collective in T2 treatment (Figure 4).

Skewness is significantly higher than zero in all treatments and tree collectives, so a pronounced right asymmetry of diameter structures was observed. In the T2 treatment, skewness is less pronounced, especially in common hackberry collective, but the confidence intervals do not show significant differences. Kurtosis in both species collective is positive and shows that DBH values cluster around the arithmetic mean. In T1 and C treatments, kurtosis

Table 2. Mean diameter and the number of excluded trees per hectare in the 2014–2019 period through thinning and mortality.

Stand condition	Growth characteristic	Treatment: Control			Treatment: T1			Treatment: T2		
		black locust	common hackberry	both species	black locust	common hackberry	both species	black locust	common hackberry	both species
Initial (2014 yr)	d_a (cm)	9.1	9.3	9.1	9.8	9.7	9.8	9.1	11.1	9.4
	N (ha^{-1})	3,344	736	4,080	2,592	560	3,152	2,944	560	3,504
Excluded through thinning (2014 yr)	d_a (cm)				10.3	9.4	10.3	9.1	13.2	9.9
	N (ha^{-1})				864	48	912	1,008	64	1,072
Mortality (2015–2019 yr)	d_a (cm)	7.2	10.0	7.3	6.7		6.7	6.2		6.2
	N (ha^{-1})	960	32	992	400		400	448		448
Total number of excluded trees	d_a (cm)	7.2	10.0	7.3	9.2	9.4	9.2	8.6	13.2	8.8
	N (ha^{-1})	960	32	992	1,264	48	1,312	1,456	64	1,520
Collective of trees in 2014 yr which survived to 2019 yr	d_a (cm)	9.9	9.3	9.8	10.3	9.7	10.2	9.6	10.8	9.9
	N (ha^{-1})	2,384	704	3,088	1,328	512	1,840	1,488	496	1,984
Collective of trees in 2019 yr without ingrowth	d_a (cm)	10.5	10.5	10.5	11.1	11.9	11.3	10.2	13.8	11.1
	N (ha^{-1})	2,384	704	3,088	1,328	512	1,840	1,488	496	1,984
Collective of trees in 2019 yr	d_a (cm)	10.5	10.0	10.3	11.1	11.5	11.2	10.2	12.9	10.9
	N (ha^{-1})	2,384	784	3,168	1,328	544	1,872	1,488	560	2,048

d_a – arithmetic mean diameter. N – number of trees per hectare.

Table 3. The results of non-parametric test by Kolmogorov-Smirnov in comparing the diameter structures between the investigated treatments.

State	Species	|D| statistic			p.value		
		Control~T1	Control~T2	T1~T2	Control~T1	Control~T2	T1~T2
2014 yr	Both species	0.09722	0.07526	0.07216	0.2442	0.5169	0.6528
	Black locust	0.11722	0.10136	0.11708	0.1627	0.2671	0.1883
	Common hackberry	0.11988	0.26832	0.25714	0.9376	0.1143	0.1975
2019 yr	Both species	0.09013	0.10827	0.06644	0.5887	0.3218	0.9501
	Black locust	0.07649	0.10717	0.15067	0.9140	0.5265	0.2724
	Common hackberry	0.17707	0.31837	0.24958	0.5551	**0.0319**	0.2329

Table 4. The results of non-parametric test by Anderson-Darling in comparing the diameter structures between the investigated treatments.

State	Species	statistic			p.value		
		Control~T1	Control~T2	T1~T2	Control~T1	Control~T2	T1~T2
2014 yr	Both species	1.8152	1.6465	0.57156	0.1158	0.1445	0.6762
	Black locust	2.2423	1.5713	1.7261	0.0674	0.1597	0.1299
	Common hackberry	0.28715	2.1146	1.3376	0.9584	0.0771	0.2176
2019 yr	Both species	1.38	1.359	0.38085	0.2072	0.2133	0.8713
	Black locust	0.65964	0.80353	0.95541	0.5952	0.4792	0.3814
	Common hackberry	1.1598	2.7785	0.91751	0.2816	**0.034**	0.4045

Table 5. The results of non-parametric test by Wilcox in comparing the diameter structures between the investigated treatments.

State	Species	statistic			p.value		
		Control~T1	Control~T2	T1~T2	Control~T1	Control~T2	T1~T2
2014 yr	Both species	22667.5	25970.0	22231.0	0.0753	0.1892	0.5904
	Black locust	14867.5	18511.5	16229.0	**0.0442**	0.5240	0.1537
	Common hackberry	807.5	631.5	502.5	0.9848	0.0990	0.1983
2019 yr	Both species	10624.5	11626.5	7483.0	0.2200	0.2086	0.9935
	Black locust	5791.0	6997.0	4164.5	0.4237	0.8979	0.3668
	Common hackberry	706.0	616.0	505.5	0.2414	**0.0288**	0.2854

is higher compared to T2 treatment, which is even more pronounced in common hackberry collective, although the confidence intervals do not show any significant differences (Figure 5).

Heterogeneity of diameter of trees expressed in a summarized way using the Gini index differs only slightly between all treatments and collectives. In black locust collective, heterogeneity of DBHs is lower compared to the common hackberry collective, and a significant difference can be observed in the T2 treatment between the black locust collective and common hackberry collective. The Lorenz asymmetry coefficient, as an addition to the Gini heterogeneity coefficient, is close to 1 in both species collective and black locust collective. However, in common hackberry collective in T2 treatment, the Lorenz asymmetry coefficient is lower than 1, compared to the other two treatments where it is higher than 1. This shows that thicker trees in T2 treatment contribute more to the Gini index of heterogeneity compared to thinner trees (Figure 6).

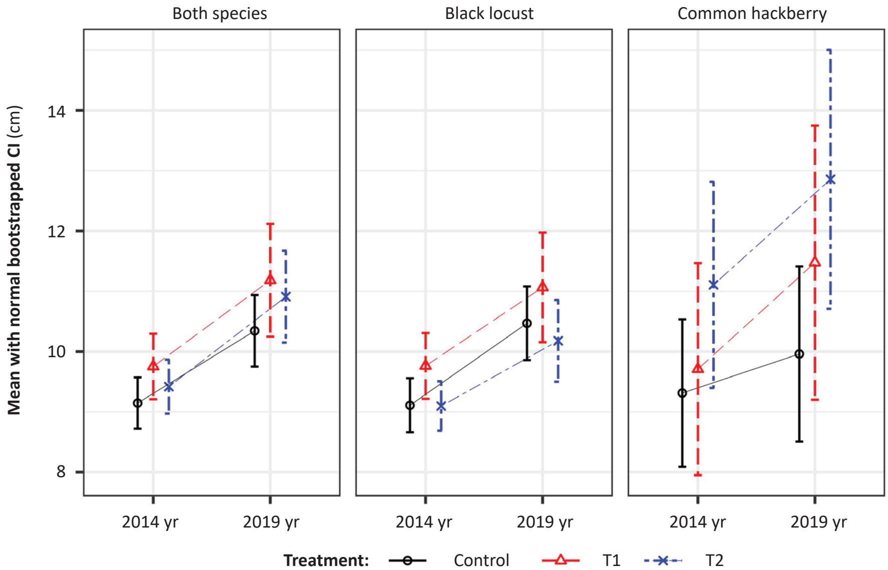

Figure 2. Arithmetic mean of diameters at breast height with the confidence intervals of 95% calculated using bootstrap resampling method.

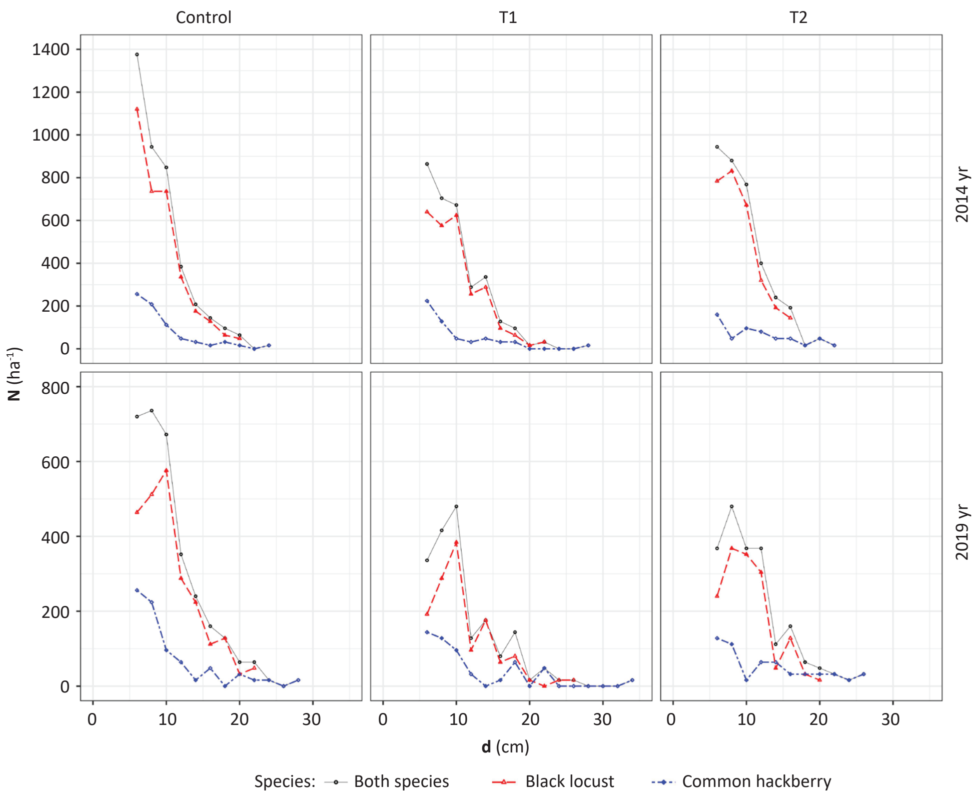

Figure 3. Diameter distributions of trees per hectare grouped into 2 cm wide diameter classes.

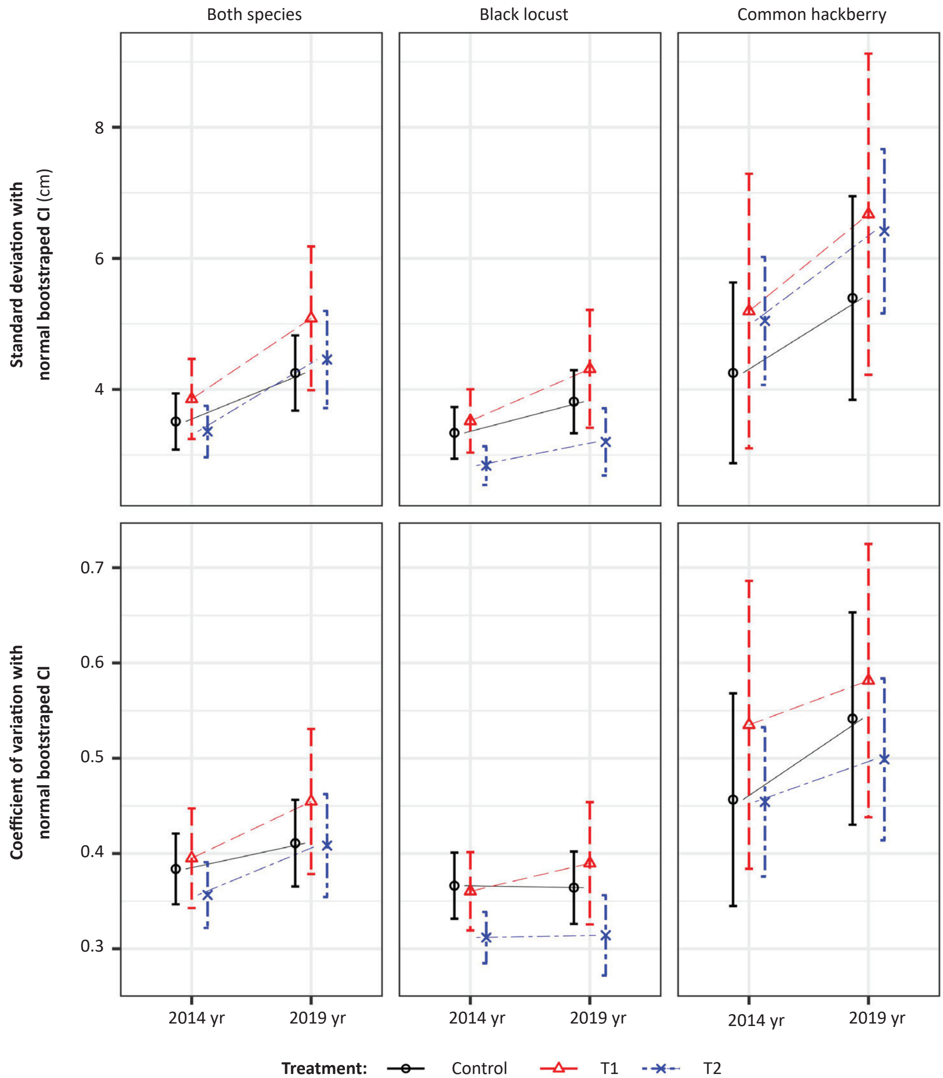

Figure 4. Standard deviation and coefficient of variation of diameters at breast height with the confidence intervals of 95% calculated using bootstrap resampling method.

Tree Mortality and Thinning

Through thinning, 912 trees in T1 treatment and 1,072 trees in T2 treatment, or 28.9% and 30.6% respectively were excluded, compared to the total number of trees above 5 cm diameter threshold. The share of black locust (94.0–94.7%, Table 2) is dominant in the thinning yield. In both treatments, the DBHs of excluded trees were 5–20 cm. In treatment T1, the excluded trees' DBHs spanned from 5.1 to 19.2 cm and in treatment T2 from 5.75 to 20.8 cm (Figure 7). Arithmetic mean diameter of marked trees for cutting of both species collective was 10.3 cm in T1 treatment and 9.9 cm in T2 treatment. The logistic curve model of dependency between probability of the tree exclusion by thinning and DBHs show an almost linear trend with zero slope in both thinning treatments (Figure 8, left). This shows that the thinning excluded the tree diameters proportionately to their share in the diameter structure before thinning. This was confirmed by the statistical test as well (Table 6).

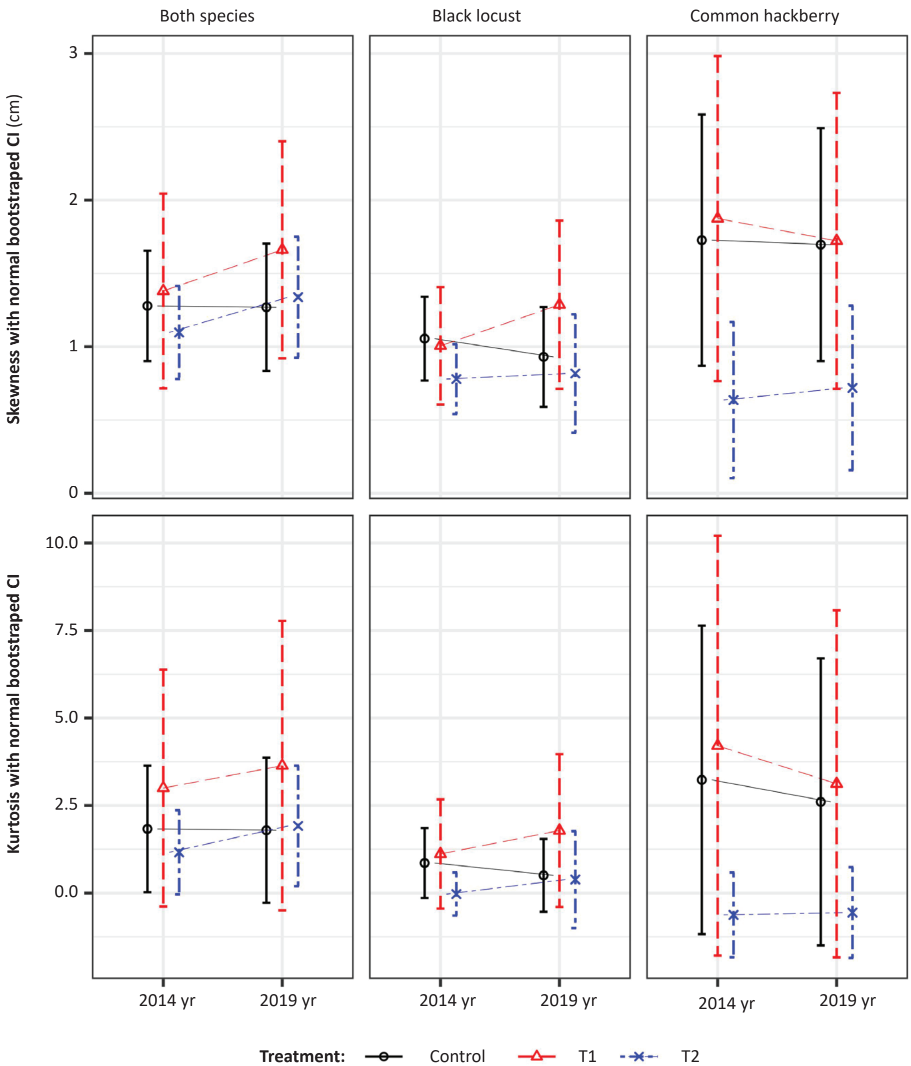

Figure 5. Skewness and kurtosis of diameters at breast height with the confidence intervals of 95% calculated using bootstrap resampling method.

Mortality was recorded in all treatments in the five-year period. The highest mortality was recorded in C treatment where 922 trees per hectare or 24.3% (both species) died. Mortality was recorded in treatments T1 and T2 as well, but at a much lesser extent than in C treatment. In T1 treatment, 400 trees died, and in T2 treatment 448 trees per hectare or 12.7–12.8%. In C treatment, the mortality of black locust trees was dominant with 960 dead trees per hectare and 32 dead common hackberry trees per hectare. In treatments T1 and T2, mortality of only black locust trees was recorded. Naturally, thinner trees are dying. The diameter intervals of dead trees were 5.0–12.8 cm with a mean diameter of 7.3 cm in C treatment, 5.0–9.5 cm with a mean diameter of 6.2 cm in T1 treatment and 5.0–6.7 cm with a mean diameter of 6.2 cm in T2 treatment. The diameter distribution of dead trees has a reverse J shape (Figure 7) that is successfully modelled using the logistic curve of dependency between probability of the death and DBHs (Figure 8). In all the treatments the statistical test shows that there is a significant dependency between probability of mortality and DBHs ($p < 0.001$). The

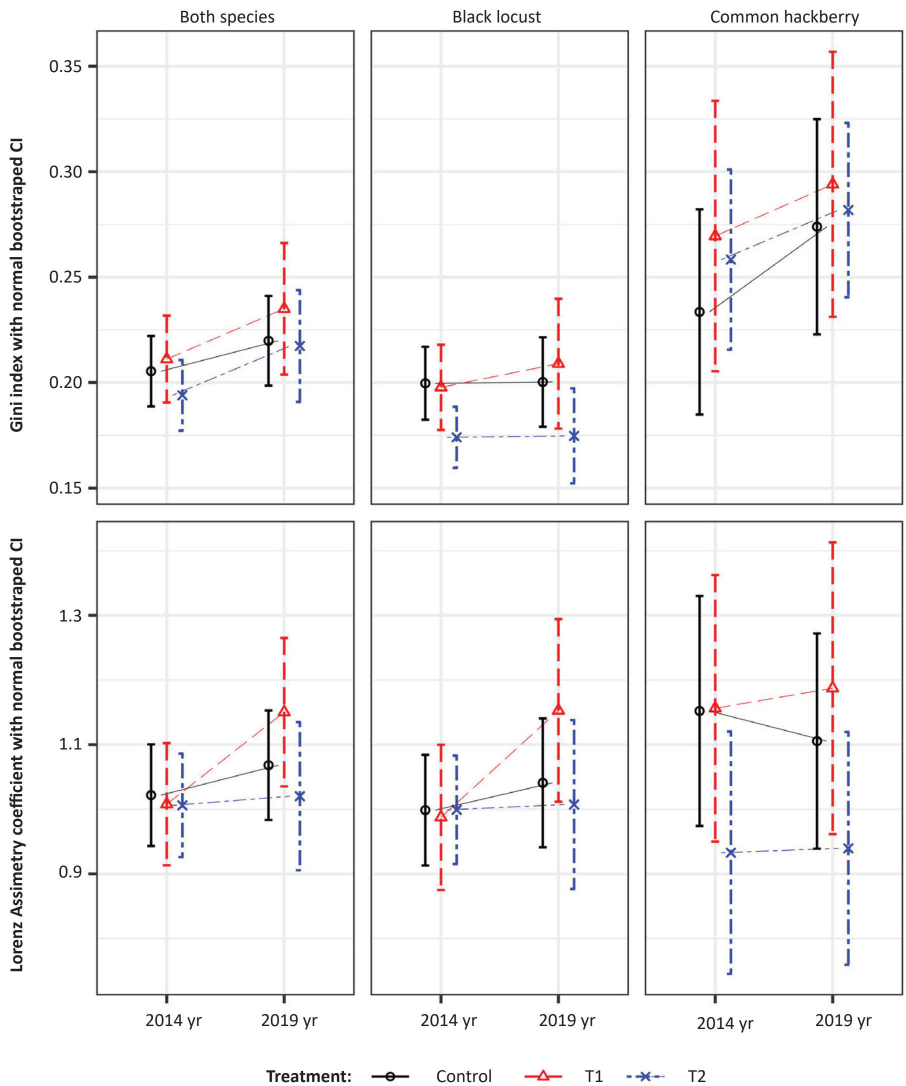

Figure 6. Gini index and Lorenz asymmetry coefficient of diameters at breast height with the confidence intervals of 95% calculated using bootstrap resampling method.

shape of the mortality curve differs between the C treatment and T1 and T2 treatments at the risk level of p = 0.052–0.045 (Table 6). Due to performed thinnings and tree mortality in treatments T1 and T2, a total of 1,312–1,520 trees (both species combined) were excluded (41.6–43.4%). In treatment C, 992 trees per hectare or 24.3% were excluded from the total number of trees above the 5 cm diameter threshold in the period of experiment establishment (Table 2). Diameter distribution of excluded trees from the stand has a reverse J shape (Figure 7), and the logistic curve model has confirmed the significant impact of tree diameter on the tree exclusion probability from the stand (Table 6, Figure 8). The shape of the excluded trees' curve differs between the C treatment and T1 and T2 at the risk level of $p < 0.001$ (Table 6).

Stand Condition after Five Years

After five years, the number of trees in the treatments has increased by 32–80 trees or 1.7–2.6% due to the recruitment of trees that grew above the diameter threshold of 5 cm. All the recruited trees are common

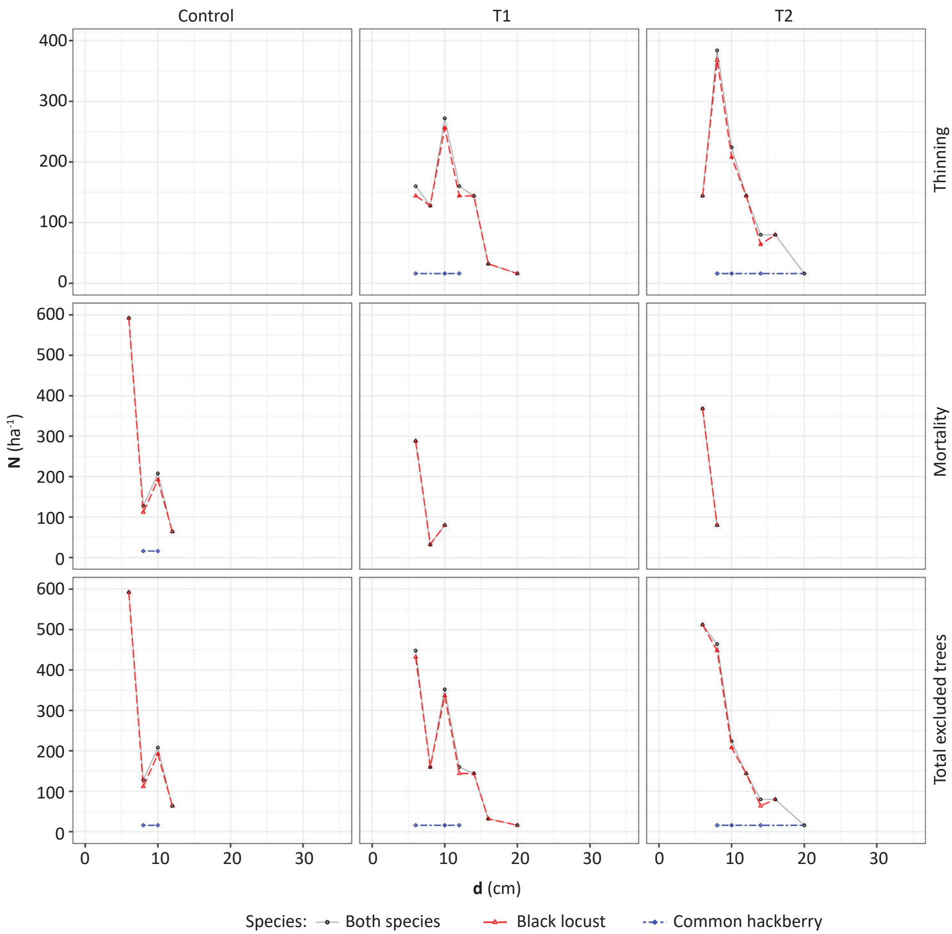

Figure 7. The structure of trees excluded through thinning and mortality grouped into 2 cm wide diameter classes.

Table 6. Parameters and measures of fit of logistic regression models of dependency between probability of the tree exclusion and corresponding diameters at breast height under the influence of thinning and mortality.

Collective	Term	Estimate	Std.error	Statistic	p value	Significance
Thinned	(Intercept)	-1.47531	0.289508	-5.09591	3.47E-07	$p < 0.001$
	d	-0.00299	0.024366	-0.12278	0.902283	ns
	tretmanT2	0.074992	0.199973	0.37501	0.707653	ns
Mortality	(Intercept)	2.095919	0.442322	4.738443	2.15E-06	$p < 0.001$
	d	-0.49204	0.059204	-8.31091	9.5E-17	$p < 0.001$
	tretmanT1	-0.51079	0.263388	-1.93931	0.052464	ns
	tretmanT2	-0.5071	0.253185	-2.00288	0.04519	$p < 0.05$
Excluded	(Intercept)	-0.45174	0.2494	-1.81129	0.070096	ns
	d	-0.15557	0.024925	-6.24122	4.34E-10	$p < 0.001$
	tretmanT1	0.906718	0.192111	4.719754	2.36E-06	$p < 0.001$
	tretmanT2	0.946699	0.186178	5.084898	3.68E-07	$p < 0.001$

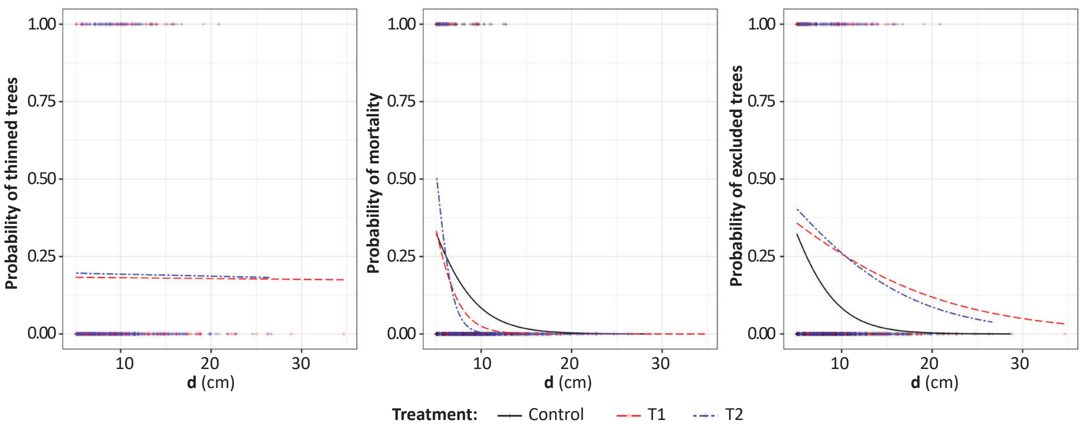

Figure 8. Models of logistic regression of dependency between probability of the tree exclusion and corresponding diameters at breast height under the influence of thinning and mortality.

hackberry trees in all of the treatments. When their number is expressed relatively compared to the number of common hackberry trees, the intensity of recruitment is 6.2–12.9%. Due to the recruitment, the share of common hackberry trees is 24.7–29.1% of the total number of trees (Table 2). Non-parametric tests by Kolmogorov-Smirnov, Anderson-Darling and Wilcox did not confirm the significant differences between investigated treatments, except in the case of common hackberry between C and T2 treatments (Tables 3–5). The diameter distributions of black locust collective and both species collective show a clear unimodal shape with a modal diameter class of 6–8 cm. On the contrary, the distribution of common hackberry collective does not show a clear unimodal distribution (Figure 3).

The arithmetic mean diameters of black locust and common hackberry trees have shown a trend of increase over a five-year period in all treatments. Yet, the common hackberry collective shows an even more pronounced trend of increase in T1 and T2 treatments than that of C treatment and more pronounced than that of black locust. The confidence intervals calculated using the bootstrap resampling method did not show significant differences between the treatments after the thinning in the five-year period, which is the same result as from the pre-thinning condition (Figure 2).

Standard deviation and coefficient of variation of DBHs have shown an increasing trend in the five-year period in all collectives and treatments. However, differences exist between the collectives of black locust and common hackberry in terms of the level of increase and total values of standard deviation. While the black locust collective is characterized by lower values of standard deviation and coefficient of variation, the common hackberry collective's absolute (standard deviation) and relative (coefficient of variation) variability has higher values. In T1 and T2 treatments, the increase of standard deviation in the five-year period is higher than the increase of coefficient of variability. In both species collective, the increase of standard deviation and coefficient of variation is similar in all treatments. The confidence intervals do not confirm the significant differences between the treatments inside certain collectives. Only the black locust collective is significantly different from the common hackberry collective in the T2 treatment in terms of standard deviation and coefficient of variation of diameters in the five-year period after the thinning (Figure 4).

Skewness and kurtosis values have not changed in any of the collectives or treatments in the five-year period after thinning in the investigated stand. The confidence intervals do not confirm the significant differences between treatments in investigated tree collectives (Figure 5).

The heterogeneity of tree diameters in the five-year period, expressed as the Gini coefficient, has had an increasing trend compared to the pre-thinning condition. The increasing trend is less expressed in black locust collective than in the common hackberry collective. Confidence intervals do not show significant differences between treatments inside respective collectives, and differences can be seen between the black locust collective and common hackberry collective in the T2 treatment. The Lorenz asymmetry coefficient has had a stagnating trend during the five-year period after the thinning and the confidence intervals are wide and do not show any significant differences between treatments and tree collectives (Figure 6).

Diameter Increment of Trees

Diameter increment expressed relative to initial values of DBHs, better known as the growth dominance pattern, differs between the treatments in the both species collective. While 60% of trees produce 75% of highest diameter increments in C treatment, 40% of trees produce the same percentage of diameter increments in T2 treatment. Growth dominance coefficient (GDC) expresses the point estimate of diameter increment heterogeneity. In the C treatment for both species collective, GDC is 0.26 and in T2 treatment 0.41, so the heterogeneity of diameter increments is higher in T2 treatments. Confidence intervals at the level of significance of 95% indicate differences between the GDC in treatments C and T2 (Figure 9).

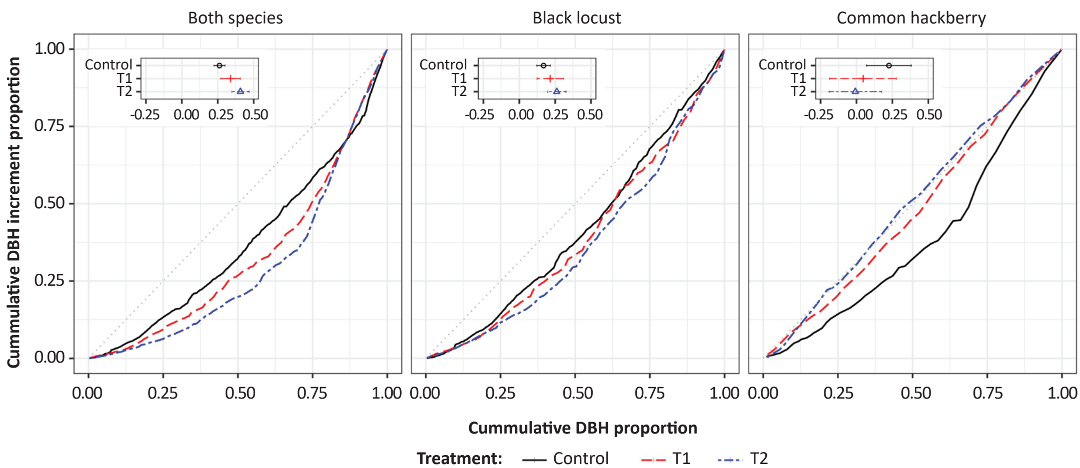

Figure 9. Growth dominance pattern and growth dominance coefficient of diameters at breast height with the confidence intervals of 95% calculated using bootstrap resampling method.

Black locust trees have a similar GDC ratio between thinning treatments, but the heterogeneity of diameter increments is less expressed. Confidence intervals do not confirm the significant differences in terms of GDC between treatments. In common hackberry trees, GDC is higher in C treatment compared to T1 and T2 and higher compared to that of black locust trees. Still, GDC of common hackberry trees in T1 and T2 treatments is close to 0, showing how homogenous the diameter increments are in relation to the initial DBHs of trees. Confidence intervals are wide and do not show any significant differences between treatments (Figure 9).

DISCUSSION AND CONCLUSION

Black locust is a dominant exotic tree species in the area of Subotica-Horgoš sands that was used for afforestation in stabilizing the sands. Common hackberry, which is an exotic tree species too, was in the past, as well as in the present, a tree species that is mostly planted in tree alleys and parks in towns in Vojvodina and used, to a much lesser extent, for afforestation of the sands, as compared to black locust (Bartha and Csiszár 2008).

During the last decades, an expansion of common hackberry has been confirmed in Subotica-Horgoš sands — an increase in the total area covered by common hackberry was confirmed, and an increase in the area where the species is dominant (Table 1).

In the investigated stand, which was initially formed as a black locust stand after clearcutting, as a normal silvicultural measure in regenerating black locust stands a spontaneous expansion of common hackberry from the neighbouring stands was recorded. The share of common hackberry trees is not even across the area of the investigated stand. The trees in the dominant layer are mostly represented in the part of the stand close to the border with the stand that contains mature common hackberry trees. On the other side of the investigated stands, common hackberry trees are only individually represented in the sub-canopy (Figure 10 and 11). In the initial measurements in autumn of 2014 at stand age of 28 years, all trees taller than 1.3 m were measured. A total of 5.000–6.380 trees per hectare were counted with a share of common hackberry trees of 1.660–2.160 trees per hectare (28.7–38.0%). Common hackberry trees were represented in all diameter and height classes, but the highest ratio was in the thinnest diameter class of 2.5 cm (Andrašev et al. 2016).

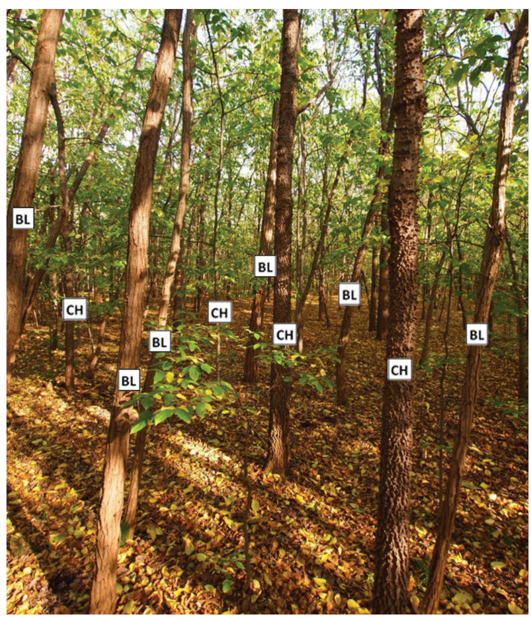

Figure 10. Part of the stand where common hackberry trees enter the dominant layer.

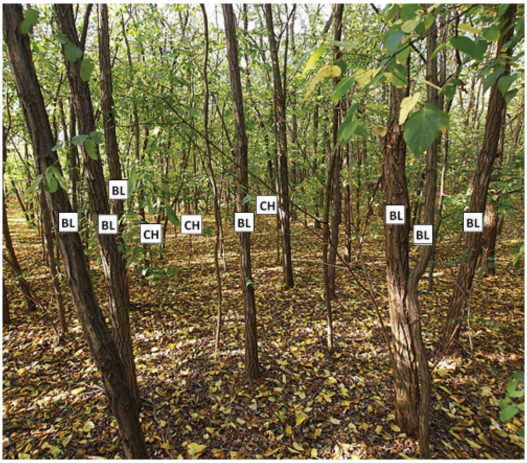

Figure 11. Part of the stand where common hackberry trees are individually scattered in the sub-canopy of the stand.

Tree mortality has been a dominant process related to DBHs of trees in thinned plots (treatments T1 and T2) in autumn of 2014 and of control plot (C plot) in the stand age of 28–33 years. Due to the mortality in C treatment, a quarter of trees died (992 trees per hectare), mostly thinnest black locust trees. Besides the performed thinnings that reduced the number of trees by 30% (912–1,072 trees per hectare) uniformly across diameter classes, after the thinning one eighth of the trees of the total number of trees before the thinning (400–448 trees per hectare) died in the five-year period. In thinned plots only black locust trees died and mortality was higher in trees with smaller diameters compared to the control plot where some thicker trees died as well.

A recruitment of 32–64 common hackberry trees above the 5 cm diameter threshold was confirmed during the measurements performed at stand age of 33 years. Together with mortality and thinning of black locust, the recruitment contributes to a relative increase of the number of common hackberry trees from 16–18% to 24.7–29.1%.

Both species collective and black locust collective have a similar tendency in terms of structural change due to mortality and thinnings, which can be explained by the total share of black locust of over 70% in the total number of trees. Arithmetic mean diameter of both species collective and black locust collective is inversely proportional to the total number of trees in both measurements. On the control plot, arithmetic mean diameter of both species collective is higher by 0.6 cm in the five-year period and in the thinned plots by 1.0 cm, which can be attributed to the more pronounced reaction to thinning, which has been confirmed by many previous studies (Medhurst et al. 2001, Mäkinen and Isomäki 2004, Río et al. 2017).

As a consequence of growth in the five-year period, there has been a trend of increasing variability and change of distribution shape in all treatments which is more expressed in thinned plots. The Gini heterogeneity coefficient shows a trend of slight increase. Such changes were reported by Hara (1988), Knox et al. (1989), Bi and Turvey (1996), McGown et al. (2016), Soares et al. (2016).

Growth dominance pattern (GDP) of diameter increments may point to the so-called "asymmetric" competition for light which is directly related to the use of the growing space. If GDP is concave and far from the homogeneity line (straight line with the slope coefficient of 45 degree), the asymmetric competition is more expressed between the trees. Growth dominance coefficient (GDC) is a point estimate of GDP and shows that GDC on control treatment is significantly lower than on T2 treatment. In black locust collective, GDP is similar to the collective of both species, but with less expressed values. This shows that the black locust trees have an asymmetric competition for growing space, or that the initially thicker trees compared to the thin trees released from competition show an increased reaction (higher diameter increment). The greatest level of growth dominance was found by Bradford et al. (2010) in a thinned stand with the greatest level of variation in tree size, which is in accordance with our results.

Common hackberry collective changed only slightly in terms of numerical parameters of diameter structure, having in mind that the thinning excluded a small number of trees and mortality was not recorded on thinned plots. The mean diameter of common hackberry has increased by 1.7–2.0 cm on thinned plots and by 0.7 on the control plot, because of growth in the five-year period. In this period, the parameters of diameter structure variability of common hackberry collective are clearly increasing unlike the parameters of shape of the distribution and heterogeneity of tree diameters that do not show a clear trend.

The current state of investigated stands shows a similar share of common hackberry trees in the total number of trees, but the numerical parameters of structure differ. This points to a different share of diameter classes of common hackberry on the plots. On the T2 plot, the mean diameter of common hackberry is higher by 1.4–1.8 cm compared to the mean diameter of common hackberry on other two plots and by 2.0 cm higher compared to the black locust collective on T2 plot. After five years, the mean diameter of common hackberry on T2 treatment is higher than that of black locust by 2.7 cm, unlike the T1 plot where it is higher by only 0.4 cm. Such differences are a consequence of initially different mean diameters of black locust in T1 treatment compared to the T2 treatment.

Growth dominance pattern of common hackberry collective show differences compared to the other two collectives. On the control plot, GDP has a convex shape, and GDC is 0.25, which is somewhat less than in both species collective on the control plot. This shows that diameter increments are heterogeneous and higher in thicker trees compared to thin ones and that an asymmetric competition of common hackberry occurs in the stand. However, GDP of common hackberry trees on the experimental plots T1 and T2 is close to the line of homogeneity of diameter increments and is lower than zero on T2 plot. This shows that the diameter increments of common hackberry are similar and do not depend on their DBHs.

Therefore, the competition for light or growing space in common hackberry on thinned plots (T1 and T2) is of so-called "symmetric" type.

This is a biological characteristic of common hackberry and represents a potential for controlled and fast natural conversion with black locust in the performed silvicultural procedure in the stands in this area, or the procedure that speeds up the natural succession of the species using thinnings.

Author Contributions

SA and MB conceived and designed the research, SA carried out the field measurements, SA and NŠ processed the data and performed the statistical analysis, SA, MB and TD wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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First Dendroclimatological Insight into Austrian Pine (*Pinus nigra* Arnold) Climate-Growth Relationship in Belgrade Area, Serbia

Branko Stajić[1*], Marko Kazimirović[1], Vojislav Dukić[2], Nenad Radaković[3]

(1) University of Belgrade, Faculty of Forestry, Department of Forest Management Planning, Kneza Višeslava 1, RS-11030 Belgrade, Serbia; **(2)** University of Banja Luka, Faculty of Forestry, Department of Forest Management Planning, Bulevar Vojvode Stepe Stepanovića 75A, BA-78000 Banja Luka, Bosnia and Herzegovina; **(3)** Public Enterprise ''Djerdap National Park'', Kralja Petra I broj 14a, RS-19220 Donji Milanovac, Serbia

*** Correspondence:** e-mail: branko.stajic@sfb.bg.ac.rs

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ABSTRACT

In order to assess the impact of climate variations on Austrian pine forest in the Belgrade area, the radial growth of artificially-established Austrian pine trees and its dependence on temperature and precipitation was studied using dendroclimatological methods. The site is classified as *Quercetum-frainetto cerris* Rudski. Standard and residual chronologies were established and several common statistics were calculated. A dendroclimatic study was carried out using the correlation and response function analysis. The Pearson correlation coefficients between the chronology indices and 13 seasonal (3-month period) precipitation and temperature data were calculated for the period from 1959 to 2014. The applied response function analysis included 24 precipitation and temperature variables from October of the prior year to September of the current year. The results of the correlation analysis pointed out that there was a strong tendency towards a positive response to the summer and late summer/early autumn precipitation and a weak significant negative response to the spring and summer temperatures. Climate-growth relationships were further first studied using the response functions for the significant seasons that were detected from the correlation analysis and then for individual months from previous October to current September. These results also highlighted the findings that higher precipitation in the current summer months has a beneficial effect on the tree-ring width. The conducted correlation between the residual chronology and the Standardised Precipitation Evapotranspiration Index indicated that a high summer value of this drought index had a positive impact on the pine growth and reinforced the previously detected relevance of September as an important month for the Austrian pine growth. These preliminary results point out that some additional climate-Austrian pine growth studies (application of various tree-ring features, growth data with a much longer time span, more sites/stands, etc.) should be performed to obtain new and valuable knowledge important for the sustainable management of Austrian pine forests.

Keywords: radial growth; dendroclimatology; sessile oak; temperature and precipitation; Serbia

INTRODUCTION

Climate change in Central Europe (temperature rise and, above all, the increasing drought risk) is forcing forestry in Central Europe to make forests resilient to future climate conditions (Walentowski et al. 2017). Similar future climate projection could be noted for the South-Eastern Europe region (Angelini et al. 2012), causing climate variations which could generally affect many aspects of forest ecosystems**,** including tree growth and dieback, insect outbreaks, species distributions, and the seasonality of ecosystem processes (Seppälä et al. 2009).

For this reason, the growth of trees as the main component of forest ecosystems could be one of the most useful (bio)indicators, reflecting the general health and sustainability of forests and providing comprehensive knowledge of long tree and forest history needed for the understanding of forest dynamics and past environmental changes (Fritts 1976, Richter et al. 1991, Spiecker 2002, Juknys et al. 2002, Leal et al. 2008, Stajić et al. 2015). Due to a number of its specificities (precision of measurements, close relationship with climatic and other environmental factors, ability to collect data for several centuries back, etc.), research of radial tree growth is of particular importance in the analysis and definition of the "nature" of tree and forest responses to changes in the basic growth conditions, including those related to climate (Stajić 2014). In that context, radial tree growth chronologies are high-resolution proxy sources used to reconstruct climatic variation at longer time scales (Esper and Gärtner 2001). Additionally, in their annual rings, trees preserve an archive of past growing conditions reflecting competition, disturbance, soil characteristics or species-specific growth patterns, as well as human-induced disturbances (Ostrogović Sever et al. 2017).

Such investigations of the dependence of radial growth on the climate and other factors are of particular importance for Serbia's Austrian pine forests. First, its high wood quality, wide range of utility values, ability to adapt to nutritive conditions of unfavourable substrates, resistance to harmful chemical agents ($S0_2$ etc.) and capability to grow and produce wood in fairly modest site conditions make Austrian pine capable of fulfilling complex forest functions starting from the economic over ecological and protective to aesthetic functions (Vučković et al. 2010). Second, Austrian pine is one of the most significant tree species used for afforestation on dry sites in South-Eastern Europe (Ivetić, Škorić 2013). Additionaly, the present condition of artificially-established stands of Austrian pine as the dominant species for afforestation in Serbia is due to insufficient and inadequate silvicultural measures particularly unfavourable in terms of health, vitality and use of production potential.

According to Stojanović et al. (2014), Austrian pine is one of the tree species in Serbia which is not under considerable risk due to climate change. This evaluation was performed using forest aridity index. However, the impact of climate change on forests can be more comprehensively assessed by studying climate-tree radial growth variation in a multiannual period, i.e. by dendroclimatological research. Besides oaks, pines are probably the most investigated tree species from the aspect of dendroclimatology in Europe (Lebourgeois 2000, Läänelaid and Eckstein 2003, Leal et al. 2008, Hordo et al. 2009, Popa and Kern 2009, Poljanšek et al. 2013, Mazza et al. 2014, Smiljanić et al. 2014, Levanič et al. 2015, Bojaxhi and Toromani 2017, Kalbarczyk et al. 2018). It is evident that pines show different growth-climate relationships depending on species, location and climate conditions (Akkemik 2000, Miina 2000, Nöjd and Hari 2001, Panayotov and Yurukov 2007, Mazza et al. 2014, Levanič et al. 2015, Stajić and Kazimirović 2018, etc.).

Modern dendroclimatological procedures have not been often applied to study climate data recorded for Austrian pine radial growth in Serbia. The only research of this type in Serbia was carried out by Stajić and Kazimirović (2018). The results showed that Austrian pine in the area of Rudnik Mountain was very sensitive to precipitation in summer months in the given conditions, in the way that higher amounts of summer precipitation resulted in significantly higher radial increment values. In this sense, the objective of this research was to assess the radial growth of Austrian pine and its dependence on temperature and precipitation in an artificially-established stand in Belgrade area.

MATERIAL AND METHODS

The trees were cored in a 60-year-old, artificially-established stand of Austrian pine nearby Belgrade. The investigated site is located at approximately 300 m a.s.l. and it is classified as *Quercetum-frainetto cerris*. The soil depth on a moderate terrain slope (10°) is 20-40 cm. According to Vučković et al. (2008), the site belongs to yield class II. Climate data were obtained from the nearest meteorological station "Belgrade-Vracar", situated at a similar elevation. The climate is humid continental with an average daytime temperature of 12.3°C and the average annual precipitation of 692.4 mm.

Two cores were taken at breast height at opposite sides of the trunk from each of the 24 selected trees (48 radial increment series). The sample was cross-dated visually, using TSAP and R (R Core Team, 2008). The library dplR (Bunn et al. 2008) was used to evaluate the measurements and the overall quality of the sampled radial increment series. The radial growth series were standardized with a cubic smoothing spline having a 50% cut-off of 32 years (Cook and Peters 1981). Radial increment indices were computed by dividing each radial growth value by the value of the fitted curve of that year. The detrended series were averaged to obtain the standard (STD) master chronology by applying a biweight robust estimation of the mean value function (Cook et al. 1990). In order to construct residual site chronology (RES), the detrended series were prewhitened with autoregressive models whose order was determined according to Akaike Information Criterion (AIC). The quality of the chronologies was evaluated by Mean Sensitivity (MS) (Fritts 1976), Expressed Population Signal (EPS) (Fritts 1976, Wigley et al. 1984), Signal-to-Noise Ratio (SNR) (Wigley et al. 1984) and the first Principal Component (PC1) (Fritts 1976). Temporal stability of EPS was investigated by the annual moving of 30-year long timeframes across chronologies.

In order to detect the pattern of Austrian pine growth-climate relationships in the study area, correlation and response functions between radial increment indices and climate data were conducted by using Treeclim library (Zang and Biondi 2015). The correlation analysis was primarily applied to seasonal 3-month data, where the response function was calculated for the significant seasons from the correlation analysis, and then to monthly data, for a sequence of 24 months starting from October of the previous year to September of the current growing season. The stability of the obtained coefficients through time for 21 monthly calibrations of the response function was determined by using a 30-year moving window. Finally, we calculated the Standardized Precipitation-Evaporation Index (SPEI) (Begueria and Vicente-Serrano 2014) and correlated it with radial increment indices for the same months as in the response function. To quantify drought through SPEI index, the potential evapotranspiration was computed according to the Hargreaves equations.

RESULTS

Quality Indicators of Radial Increment Chronologies

The most important characteristics of radial increment series (row data) are as follows: the average level of correlation coefficient (r_{xy}) and t-test value (t_{bp}) of each normalized series with the master chronology (Baillie and Pilcher 1973), the average values of mean sensitivity (MS) and the first order autocorrelation coefficients (AC1) are 0.75, 4.75, 0.30 and 0.80, respectively.

In order to quantify the common and different features of both chronology versions in the representation of the climatic potential of growth data, statistical characteristics of STD and RES master chronologies were calculated for the common period of all series, from 1959 to 2014 (Table 1). The obtained chronologies are 56 and 55 years long site STD and RES chronologies with the average time span of a series amounting to 51 years. The percentage of the variance explained by PC1 for both types of chronology is similar (49.78% and 52.96%). According to the values of EPS from the moving window, both versions of the chronologies have sufficiently strong common signal in their entire lengths. However, the values of the applied statistics are more favourable for RES than STD. Therefore, RES chronology was selected to evaluate the effects of climate variations on Austrian pine radial growth in this study.

Radial Growth-Climate Relationships

The obtained Pearson's correlation coefficients between RES chronology indices and the seasonal (3-month) and monthly climate parameters are shown in Figure 1a and Figure 1b, respectively. Starting from the current November as the expected end of the growing period to October of the previous year, 13 different seasons of the same length (3 months) were first analyzed. The most pronounced growth-climate relationships were detected for the precipitation in the period between June and August (r=0.603) of the current growing season, while the temperature had a less expressed but significantly negative correlation over the periods of April-June (r=-0.335) and June-August (r=-0.293). At a monthly scale, Austrian pine growth was positively correlated with current June, July and August precipitation, while significant negative relationships were recorded between radial growth indices and temperature during April and August.

To determine the combined effect of precipitation and temperature, the response function was calculated for the three most expressed 3-month seasons obtained by the conducted correlation analysis (April-June, May-July and June-August). This procedure revealed only the existence of a significant positive correlation between radial growth indices and precipitation in the current June-August, while the other seasons were insignificantly related (Figure 2a). Besides, the calculation of the response function over the sequence of 24 months pointed to a positive relation with June and July precipitation of the current year (Figure 2b). The applied procedure did not detect any significant negative effects of temperature, but the positive impact on radial increment indices was recognized for September of the current year.

Table 1. Statistical characteristics of standard (STD) and residual (RES) site chronologies for the longest common period of all series (1969–2014).

Chronology	r_{tot}	r_{wt}	r_{bt}	SD	EPS	SNR	MS	AC1	PC1
STD	0.476	0.678	0.472	0.214	0.969	30.851	0.220	0.112	49.78
RES	0.501	0.649	0.497	0.207	0.973	36.172	0.234	-0.079	52.96

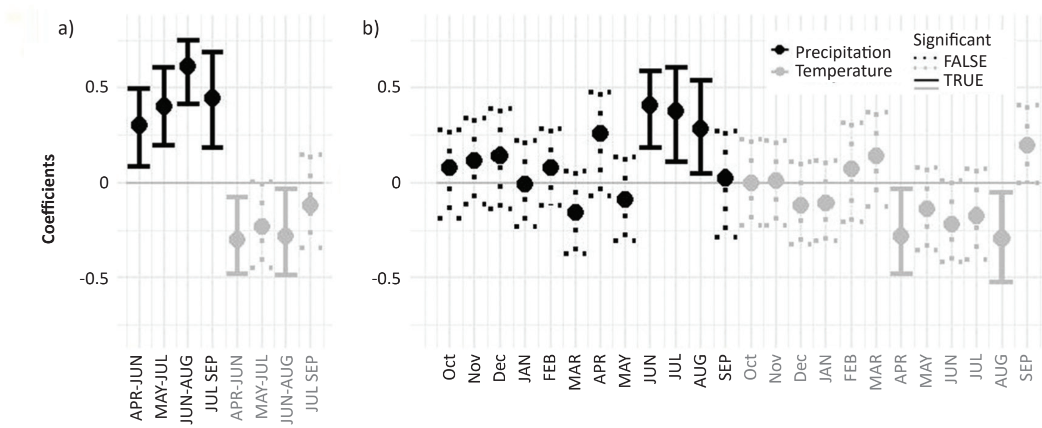

Figure 1. The correlation between the seasonal **(a)** and monthly **(b)** climate parameters and growth indices of residual site (RES) chronology. The darker bars indicate coefficients which are significant at a 0.05 α-level (two-tailed test).

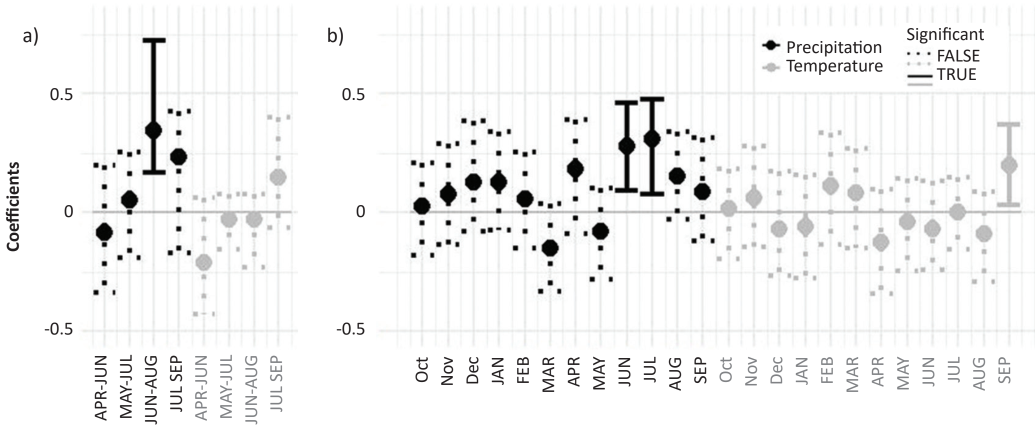

Figure 2. The bootstrapped values of the response function coefficients for the precipitation and temperature 3-month seasons **(a)** and monthly data **(b)**, starting from October of the previous year to September of the current year. Uppercase and lowercase denotes the month of the current growing seasons and the month in the previous year, respectively **(b)**.

In addition, the coefficients of the determined climate-growth relationships were tested for stability through the investigated time sequence (Figure 3). The 30-year long moving windows revealed the existence of divergent response to prevailing climate conditions. Seven of the first nine moving windows indicate that higher temperatures in September could positively affect radial growth due to the prolonged growing season. Afterwards, the significant influence of this parameter disappears. This occurrence coincides with the warming trends observed for September temperatures. Since 1997, the mean temperature during September increased by exactly 2 degrees in comparison to the previous period. Besides, the last 20 positions of the moving windows confirmed the importance of precipitation during the summer (Figure 3, bottom part). However, apart from the quite significant and consistent influence of July rainfalls, it seems that June precipitation is not that important as it seems from the monthly correlation and response analysis data. Instead, it has been determined that the amount of August precipitation has a substantial role in radial growth of Austrian pine on this site.

Furthermore, RES chronology indices were correlated with SPEI for the same months as in the response function. The determined coefficients pointed to significant relations for July (r=0.297) and August (r=0.228) of the current year (Figure 4b). Following the previously observed seasonal influences, it was detected that a significant correlation between SPEI and radial increment indices existed only for the June-August period (Figure 4a).

DISCUSSION

Assessment of the Radial Increment Chronologies

The evaluation of the analyzed samples was tested by using a variety of dendrochronological and statistical parameters. The high inter-series correlation (0.75) pointed to quite similar reactions of the analyzed trees to the factors influencing Austrian pine growth. The extremely high average value of the first order autocorrelation coefficient - AC1 (0.80) indicated a high level of influence of the previous year's growth factors on the radial increment values of the current year. This value is larger than AC1 value of 0.77 recorded for Austrian pine trees in south-western Romania (Levanič et al. 2013), same as the results of Levanič and Toromani (2010) for Austrian pine in north-eastern Albania (0.80), and smaller than the average AC1 value (0.85) for Austrian pine row radial increment series in south-western Bulgaria (Shishkova and Panayotov 2013a).

For RES chronology, the values of MS (0.23), EPS (0.98) and SNR (36.2) are high, especially EPS and SNR. The implemented procedures (standardization, averaging the obtained indices and performing the autoregressive modelling) removed the low-frequency trend and autocorrelation from the raw tree-ring data. According to the obtained results, it is obvious that RES chronology contains the desired common signal which represents the common variability present in all series of radial increment indices, so this version of chronology could be useful for further analysis of growth-climate relationships.

Response to Climate Variables

Previous studies of Austrian pine from different parts of Europe outlined the species as valuable for dendroclimatic analyses (Strumia 1997, Lebourgeois 2000, Shishkova and Panayotov 2013). However, the growth-climate relationships are highly dependent on the nature of the investigated area (altitude, site, etc.), as well as on the climate type. Austrian pine trees used in this research originate from a managed forest and grow in a temperate region in less extreme site conditions, i.e. with various factors influencing its radial growth. Therefore, the identification of the proper growth-climate relationship in such site and stand conditions can often be a serious problem (Vučković et al. 2005). Bearing this in mind, the influence of climatic factors on the radial increment of Austrian pine trees in our study was studied by means of (1) the correlation analysis between radial increment indices and seasonal and monthly climate/SPEI data and (2) the response function analysis.

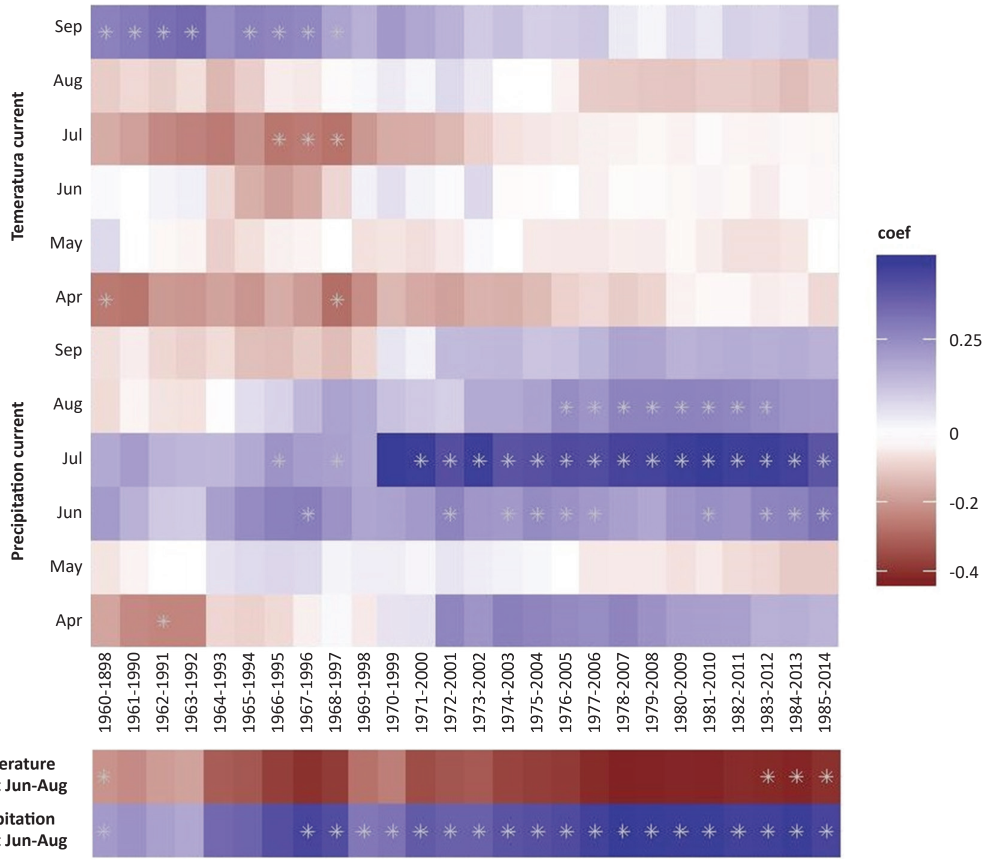

Figure 3. The temporal stability of response function, determined by a 30-year moving window. The stars designate the significant response coefficients.

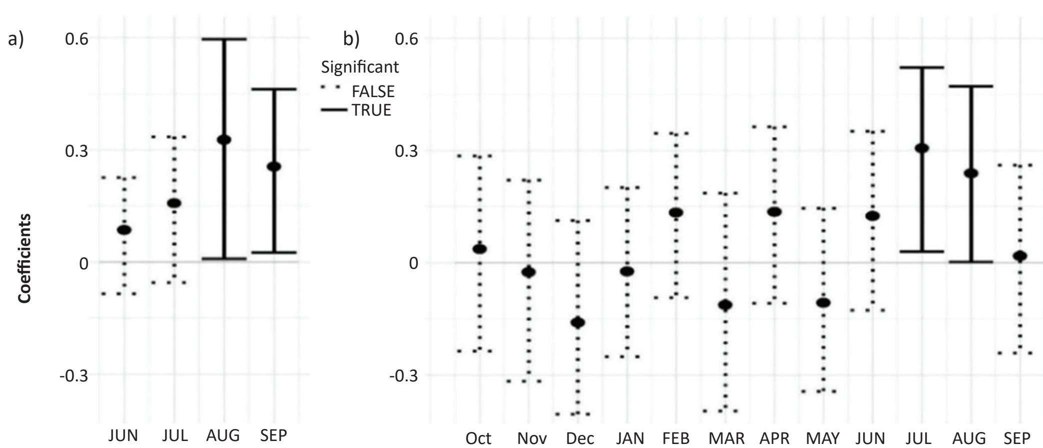

Figure 4. The bootstrapped values of the correlation coefficients between residual site chronology (RES) and Standardized Precipitation-Evaporation Index (SPEI) 3-month seasons (a) and monthly data (b).

According to the results of the correlations between radial increment indices and 3-month climate data, it can be concluded that high spring and summer precipitation of the current year result in a larger pine radial increment. This procedure also points to a reduction in the pine radial increment with the increase of temperature in the current spring and summer. The results of the response function analysis for the most pronounced 3-month periods have highlighted the prevailing effect of the current summer precipitation on the values of the radial increment. Apart from that, the calculation of the response function for the sequence of 24 months confirmed that higher precipitation in the current summer months has a beneficial effect on tree-ring width. The conducted response function analyses (seasonal and monthly data) did not detect any negative influence of temperature, although a positive effect on the radial increment indices was recognized for September of the current year.

The determined dependence of the Austrian pine growth on summer precipitation corresponds to some results of earlier research studies from South-East Europe (SEE) region. In that context, there are positive correlations between summer precipitation and the growth of Austrian pine in Bosnia and Herzegovina (Poljanšek et al. 2013) and in south-western Bulgaria (Shishkova and Panayotov 2013a). A positive correlation between *P. nigra* radial growth and precipitation was also found for the summer months of the current year in south-western Bulgaria (Shiskova and Panayotov 2013a) and particularly for July in the south of Bulgaria (Shishkova and Panayotov 2013b). Levanič et al. (2013) identified July precipitation as the most important factor influencing *P. nigra* growth in a natural stand in Romania. A significant positive pine growth response to July precipitation was detected in Albania, but it was weaker than July temperature (Levanič et al. 2015). In addition, the utmost importance of the precipitation from the previous year for the formation of the radial *P. nigra* growth of the current year was not recorded here, although some results from SEE region (Shishkova and Panayotov 2013b) indicate that these monthly or seasonal precipitations could have important positive effects on *P. nigra* radial growth values.

Our study reveals a less pronounced impact of temperature on the values of the Austrian pine radial increment. This less expressed temperature signal is "stretched" over months from April to September. For this reason, and having in mind that an application of drought indices can often provide a better insight into the tree's response than temperature/precipitation data alone (Levanič et al. 2013, Lévesque 2013), we additionally determined SPEI drought index and correlated it with *P. nigra* radial growth indices. According to Ma et al. (2015), this information can broaden our knowledge regarding the vulnerability of trees to climate change and our ability to predict future drought variations. Namely, the interaction between temperature and precipitation, which was summarized in SPEI, caused tree growth (either the width or the anatomical variables) to show the highest correlations with SPEI (Martin-Benito et al. 2012). The obtained results confirmed the aforementioned statement that the highest correlation (r = 0.60) between *P. nigra* growth and the applied climate variables was detected with 3-month August SPEI. According to this result, a high summer SPEI had a positive impact on an increase of *P. nigra* radial increment values. This correlation coefficient of June-August SPEI is more than twice as important for the increase of pine radial increment as June-August temperature. In addition, the determined positive relationship between 3-month September SPEI and RES chronology reinforced the relevance of September as an important month for the Austrian pine radial growth, previously detected by the results of the applied response function for the mean temperature monthly data (Figure 2). Finally, since the effect of temperature on radial growth is generally opposite to that of precipitation, the results of SPEI application partly pointed to the importance of temperature for *P. nigra* in the area of Belgrade.

CONCLUSIONS

Based on the results of this study, we could preliminary assume that the relationship between Austrian pine radial increment and climate data shows a tendency towards a positive response to summer precipitation and a weak negative response to summer and early autumn temperature. In that context, the first obtained results indicate that summer drought seems to be partially important for the radial growth of this species. Additionally, these preliminary results point out that more complex climate-Austrian pine growth studies (application of various tree-ring features, growth data with a much longer time span, more sites/stands, etc.) should be performed to obtain new valuable knowledge important for the sustainable management of Austrian pine forests. Such data are of highest concern both from the perspective of management and science (Bhuyan et al. 2017).

Author Contributions

BS and MK conceived and designed the research, MK and NR performed the field measurements, MK and NR performed laboratory analysis, BS and MK processed the data and performed the statistical analysis, BS, MK and VD wrote the manuscript, VD did the final checking and proofreading of the manuscript text.

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Conflicts of Interest

The authors declare no conflict of interest.

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Tree Growth Variability of *Pinus heldreichii* at Tree-Line Locations in Kosovo**

Faruk Bojaxhi[1,*], Ervin Toromani[2], Nehat Çollaku[2]

(1) University of Prizren, Forestry and Environment Faculty, Rruga e Shkronjave, nr.1, Prizren, Kosovo**; **(2)** Agricutural University of Tirana, Faculty of Forestry Sciences, Koder Kamez, AL-1029 Tirana, Albania

*** Correspondence:** e-mail: farukbojaxhi@yahoo.com

** This designation is without prejudice to position on status, and is in line with UNSC 1244 and the ICJ Opinion on the Kosovo declaration of independence.

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ABSTRACT

It is well-known that tree growth at the upper treeline is controlled by low temperatures and limited seasonal growth. The study's objective was to investigate the climate warming effects on Bosnian pine (BP; *Pinus heldreichii* Christ.) growth during the 20th century. We hypothesized that, like all the other drought-stressed conifers growing on tree-line locations, BP responds significantly to climate factors that control their growth. Three natural forest stands of BP were selected in Prevalla, Decan and Koritnik. The cores were taken from 98 dominant and co-dominant BP trees at breast height with no sign of human interference. The tree-ring widths were measured at 0.001 mm precision, with a LINTAB 6 (RINNTECH, Heidelberg) system and TSAP-Win Scientific software. A set of three BP tree-ring width chronologies were developed by trees spread on a typical mountain slope at elevations ranging from 1815 to 1945 m above sea level. The tree-ring chronologies from three sites showed significant correlation and this agreement decreased with the distance between sites. The length of the master chronologies varies among sites ranging from 176 years (Koritnik) to 541 years (Decani). The tree growth was controlled by a common climatic signal (local temperatures) and drought during midsummer across the investigated sites. These results confirm the assumption that tree growth at tree-line sites is controlled by low temperatures and drought conditions during the midsummer.

Keywords: Bosnian pine; tree line, SPEI; Forward Evolutionary Interval Analysis

INTRODUCTION

Bosnian pine (*Pinus heldreichii* Christ., Hereafter "BP") is one of the main conifer species growing in Kosovo. The species inhabits high elevations (over 1500 ma.s.l) within the Western, South and South-Western Kosovo (Figure 1). Despite its limited distribution in the Balkans and southern Italy, the species has been widely used in dendroclimatic studies. Previous studies have been conducted in Bulgaria (Panayatov et al. 2009, 2010), Greece (Branders 2007, Griggs et al. 2007), and Albania (Seim et al. 2012), exploring climate–growth relationship of this high-elevation species. Moreover, summer temperature was reconstructed (1768–2008), based on maximum latewood density measurements of *P. heldreichii* trees from a high-elevation stand in the Pirin Mountains in Bulgaria (Trouet et al. 2012). In Albania, a 1391-year tree-ring width chronology (617–2008) was developed and maximum density measurements were acquired on living and dead *P. heldreichii* trees (Seim et al. 2010).

The overall forest area covered by *P.heldreichii* in Kosovo amounts to 2150 ha, mostly mixed with species like silver fir (*Abies alba* Mill.). Some natural forest stands of this species exist in Kosovo, situated in Prevalla, Koritnik and Decani regions respectively, but only two dendroclimatic studies have been conducted to date (Bojaxhi and Toromani 2016, 2017). The high conservational value of such taxa additionally increases the importance of tree-ring width-based studies that allow a better understanding of the climate – radial growth relationship as well as eco-physiological requirements of trees.

Previous dendroclimatic studies conducted in Kosovo found that BP growing in high elevation locations is sensitive to summer drought stress and the magnitude of sensitivity was higher in young trees. The negative effect of summer drought stress on BP radial growth is expected to be more pronounced with climate warming in the coming years.

In order to enhance our understanding of climate warming effects on BP growth during the 20th century, we aimed to pursue this study following these objectives: (i) assessing the cross-dating potential of BP growing on various sites; (ii) investigating the potential relationships between climate and BP growth, and (iii) elucidate temporal and spatial variability in relationships identified between climate and BP. We hypothesized that, like the other drought-stressed conifers growing on tree-line locations, BP responds to climate factors that influence moisture availability. An improved perspective of the dendroclimatic potential of BP could enhance our understanding of climate effects in natural ecosystems and strengthen the spatial and temporal resolution of the tree-ring record in Kosovo.

MATERIALS AND METHODS

Study Sites

The study area includes natural ecosystems located close to the upper distribution limit of BP at Prevalla (hereafter, PRE) - (42°11'N, 20°57'E; 1945 m a.s.l), Decan (hereafter DE) - (42°36'N, 20°14'E; 1830 m a.s.l), and Koritnik (hereafter, KO) - (42°04'N, 20°31'E; 1815 m a.s.l) in western and southern Kosovo (Table 1, Figure 1).

Table 1. Site characteristics of the sampled sites.

Sampled site	Latitude/ longitude	Altitude (m a.s.l.)	Aspect	Soil/rock formation	Sampled trees
Prevalle (PRE)	N 42°11'01.3" E 20°57'42.0"	1945	SW	Typical rendzina soils on limestone bedrock	30
Decan (DE)	N 42°36'19.8" E 20°14'52.5"	1830	NW	Brown soils on limestone bedrock	38
Koritnik (KO)	N 42°04'46.5" E 20°31'58.6"	1815	NW	Grayish-brown rendzina soils on limestone bedrock	30

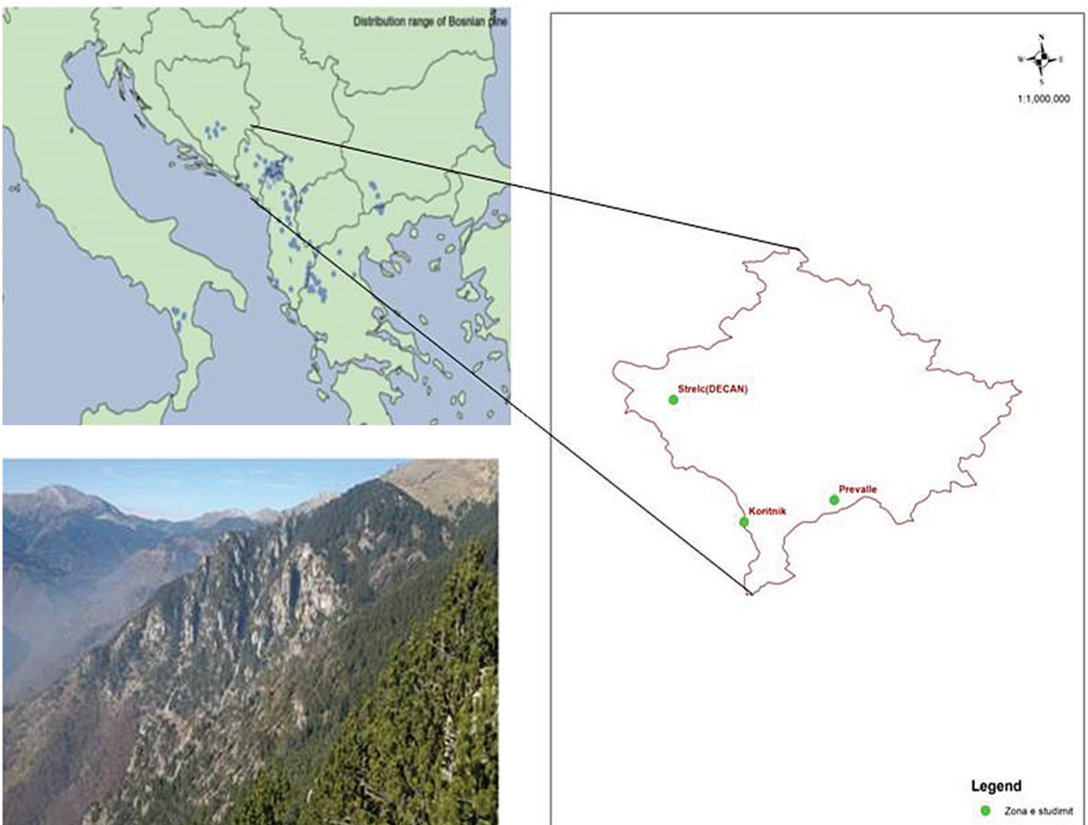


Figure 1. Distribution map of Bosnian pine (upper left side) in Europe and sampled sites from Kosovo (right side).

Soil types formed on limestone bedrocks are dominated by leptosols (rendzinas) and brown soils and have slight differences among sites. At all sampled sites BP is the dominant species, while the ground vegetation comprises species such as *Sesleria autumnalis* Ard., *Brachypodium sylvaticum* Huds., *Carex humilis* Leyess., *Thymus balcanus* L., *Fragaria vesca* L., *Festuca heterophylla* Lam., *Dactylus glomerata* L., etc. These forest stands are growing under the effect of continental climate with some influences of the Mediterranean climate in southern Kosovo. The mean annual temperature varied among sampled sites and ranged from 8.0°C (PRE) to 8.3°C (KO). The average annual precipitation ranged from 791 mm (DE) to 1029 mm (KO) (CRU TS. v 3.22 dataset by Jones 2014, period 1901-2013) and is bimodally distributed with a pronounced maximum during May and a secondary peak in November (Figure 2). The growing season at all three sites lasts 5 months, from May to September. The natural forest stands of BP grow on sites with different slope aspects ranging from NW for DE and KO sites to SW for PRE site.

Field and Laboratory Methods

The collection of samples took place from July to September 2014. In total, 89 trees were sampled, and two cores per tree were extracted at breast height (ca 1.3 m from ground) with an increment borer, but only 85 sampled trees were used to build chronologies at three sites. Dominant and co-dominant BP trees with umbrella-like crowns growing on shallow and poor soils were cored to obtain a sensitive chronology. Increment cores were dried fixed onto wooden mounts with cells vertically aligned (Stokes and Smiley 1996) and sanded. All cores were surfaced using progressively finer sandpaper beginning with ANSI 120-grit and finishing with ANSI 400-grit. Some cores could not be included in our dendroclimatological analyses due to ring anomalies associated with the lobate growth form, twisted stems, and predominance of false rings that mimicked true rings (Schweingruber 1993). Annual tree-ring widths were measured to an accuracy of 0.001 mm using a LINTAB 6 (RINNTECH, Heidelberg) system and TSAP-Win computer software. Cores were visually cross-dated and cross dating was statistically verified with COFECHA software (Holmes 1983). Each measured radius was processed by COFECHA as an individual time-series subdivided into 40-year segments sequentially overlapped by 20 years. We used the ARSTAN program (Cook 1985) to standardize all series applying a double detrending procedure to remove adverse growth effects from age-related growth trends, autocorrelation, and possible natural or anthropogenic influences that could interfere with the climate signal within the growth rings. First, detrending was conducted using a negative exponential curve and then second detrending was done by using cubic smoothing spline curves of 30 years in order to preserve the common climatic signal. The actual ring measurement was then divided by the predicted value to produce a dimensionless index of growth for that year (mean=1.0).A final master chronology was created for each site from the tree-ring data by averaging all indices of tree growth for each year across all series (Cook 1985). Chronology quality was estimated using R_{bar} and Expressed Population Signal statistics (EPS) with a limit value of 0.85

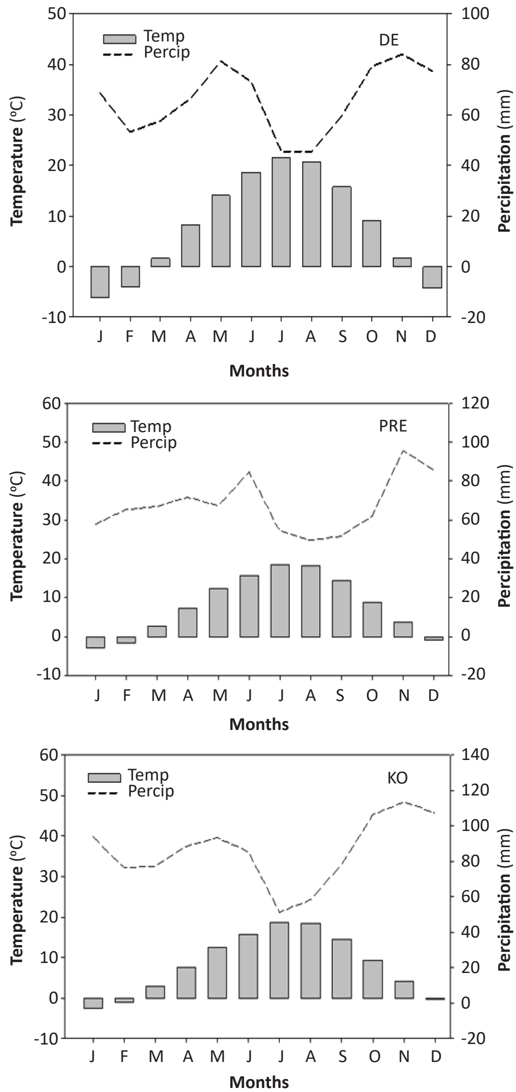


Figure 2. Climatic diagrams of the studied sites based on the CRU TS v3.22 data. Dashed line shows precipitation, while bars show temperature values.

(Wigley et al. 1984).R_{bar} is a measure of the strength of the common growth "signal" within the chronology (Briffa and Jones 1990), while the EPS is an estimate of the chronology's ability to represent the signal strength of a chronology on a theoretical infinite population (Briffa and Jones 1990). Three chronologies (standard, residual and arstan) were developed by the program ARSTAN, and we used the residual chronologies in our additional analyses.

Climate-Growth Analyses

The climate-growth relationship was assessed by means of bootstrap correlation analysis estimated by Dendro-Clim 2002 program (Biondi 1997, Biondi and Waikul 2004). Due to the lack of representative meteorological data for the whole period covered by our chronologies, we used datasets from the Climate Research Unit (CRU) for mean temperature and total precipitation. The chosen climate data were derived from the nearest points of the CRU grid with the sampled sites and were used in the climate-growth relationship. We also used the Standardised Precipitation-Evapotranspiration Index (SPEI) at 1- and 3-months'time scale in the period 1901-2013. The SPEI is a multiscale drought index based on the monthly precipitation and potential evapotranspiration from the CRU datasets (Vicente-Serrano et al. 2010).

Tree-ring chronologies were calibrated against instrumental climate records obtained from the CRUTS3.22 dataset (http://www.cru.uea.ac.uk/), for the period 1901-2013 with a spatial resolution of 0.5×0.5˚ (Mitchell and Jones 2005), using correlation and response functions analysis. Correlation analysis was performed to quantify the relationship between the residual chronologies from three sites and monthly mean temperature, total precipitation (Fritts 1976) and drought index (SPEI) for 1 and 3 months.

Correlation coefficients were deemed statistically significant at the P<0.05level, as shown by bootstrapped confidence intervals. The considered window for exploring the relations between climate and tree growth were from May of the prior year of growth to October of the current year of growth, assuming that the cambial activity of BP continues during the winter months and studying the role of climate during the previous year on the current radial growth (Fritts 1976, Grissino-Mayer 1995).

Then we applied forward evolutionary interval analysis (FEI) to provide a complementary assessment of temporal stability for significant monthly climate-growth relationships. Our objective was to elucidate temporal variability in the relationships identified between climate and BP radial growth (Biondi 1997, Biondi and Waikul 2004). FEI begins with the earliest year in common to all variables, from which forward evolutionary intervals are progressively enlarged by adding one year to a base interval length at each iteration (Biondi and Waikul 2004). Persistent relationships between climate variables and tree growth over the 20th century would further substantiate the results of correlation analysis and suggest the suitability of BP tree-ring data for use in dendroclimatic reconstructions.

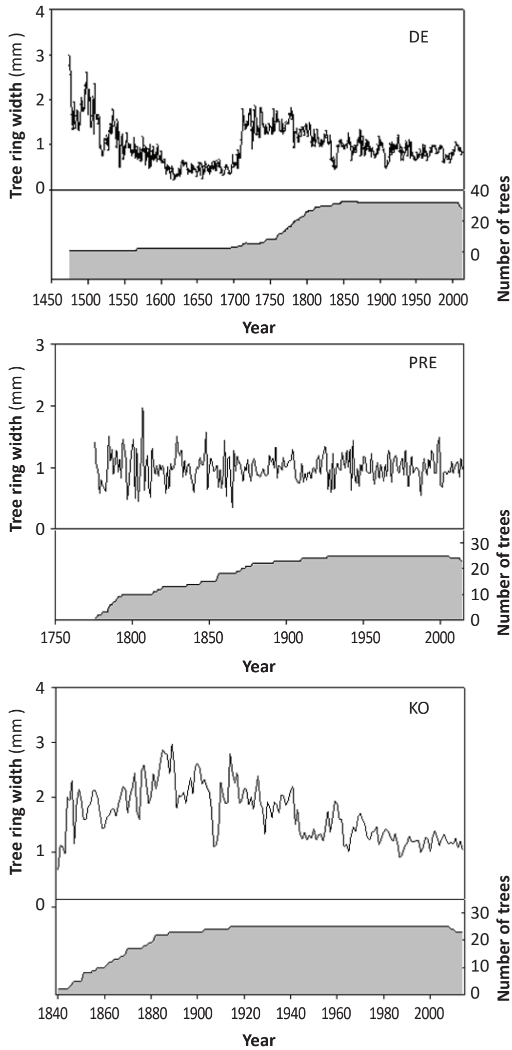


Figure 3. Tree-ring width chronologies of BP (*P. heldreichii* Christ.) from Decan (DE), Prevalle (PRE) and Koritinik (KO). The upper part in each graph shows the raw tree-ring width chronology, while the lower part shows tree replication in respective site chronologies.

RESULTS

Tree-Ring Chronologies

The length of the master chronologies varies among sites. The DE chronology was the longest with 541 years, spanning the period 1474-2014 with a replication of more than 34 trees from 1770 onwards. The KO chronology was the shortest, 176 years long, with a sample replication of 25 trees, while the PRE chronology was 243 years long, spanning the period 1776-2014 with a samples replication of 25 trees from 1901 onwards (Figure 3, Table 2). The maximum value of inter-annual variability, expressed by the mean sensitivity (MS), was found in the KO, while the minimal value was found in the PRE chronology. High values of the first-order autocorrelation found at all chronologies indicated that BP growth strongly depends on the climate conditions of the previous growing year.

All chronologies were significantly correlated with each other for the common period 1840-2014. The degree of agreement and similarity appears to be higher between PRE and KO chronology (R=0.61; p<0.01), but slightly lower among DE and PRE chronologies (R=0.25; p<0.05), implying that the degree of correlation appears to be related to the vicinity between sites (Table 3).

Relationships Between Climate and Tree Growth

The correlation between DE chronology and temperature was not significant at the study site, but significant relationships were found with October precipitation of the previous years of growth and summer months of the current growing year.

In addition, significant negative correlations were found between BP radial growth for KO site and monthly mean temperature for the previous July, August, September and current June. Moreover, BP radial growth in this site was negatively correlated with current May, but a positive correlation was found with previous August precipitation.

Bootstrapped correlation analysis between PRE chronology and climatic variables showed a positive relationship with current January – February temperatures, as well as with May and October precipitation of the previous years and July precipitation of the current growing year. An opposite relationship was found with previous June and current April precipitation (Figure 4).

The results of this analysis indicate that precipitation during the growing season is not the only limiting factor. Considering the number of significant coefficients of temperatures with a negative relation to growth, our analysis showed that this number was higher than those with a positive relation.

The results of the Pearson correlation between BP chronologies and SPEI in 1 to 3 months' time scales were different between sites (Figure 5). DE chronology had the highest positive correlation frequency with SPEI than the two other sites. These results indicate that young (PRE) and middle-aged (KO) trees are less sensitive to drought than old trees.

Thus, BP growth at DE site was positively correlated with SPEI values from June to August of the current year for 1 month time scale and from July to October of the current growing year for 3 months' time scale. Positive significant correlation between BP growth with July SPEI values was attained for two other sites. PRE chronology was the only one where 1 month time-scale SPEI was negatively correlated with BP growth and current April, implying that the low amount of rainfall during this month may delay the initiation of BP radial growth. Positive correlations found between SPEI and BP growth during July at all three sites show the impact of midsummer drought on trees' growth.

FEI suggested that these relationships were not all persistent during the period of inquiry. However, FEI indicated a sustained negative relationship between radial growth and current June monthly mean temperatures for PRE and KO sites, as well as a continuous positive relationship between growth and current January-February temperatures. FEI also indicated a persistent positive relationship between BP growth and previous May, July and August monthly precipitation at KO site and current June at DE site. Positive correlations found between SPEI and BP growth during July at all three sites show the impact of drought on trees' growth.

Table 2. Statistical parameters of three *P. hedreichii* chronologies from Kosovo.

Statistical parameters	Decan (DE)	Koritnik (KO)	Prevalle (PRE)
Time span	1474-2014	1840-2014	1776-2014
Total years	541	176	243
Number of trees in chronology	34	25	25
Mean age (years)	240±69	147±18	116±20
Mean tree-ring width (mm)	1.06	1.81	2.13
Standard deviation	0.498	0.872	0.907
Skewness	1.037	0.995	0.547
Mean Sensitivity (MS)	0.224	0.245	0.212
AC(1)	0.769	0.751	0.783
EPS>0.85 since	1770	1876	1920

Table 3. Simple correlation coefficients of tree-ring chronologies between sampling sites. The asterisks indicate the significant values, *p<0.05; **p<0.01.

Site	Decan	Koritnik	Prevalle
Decan		0.35**	0.25*
Koritnik			0.61**
Prevalle			

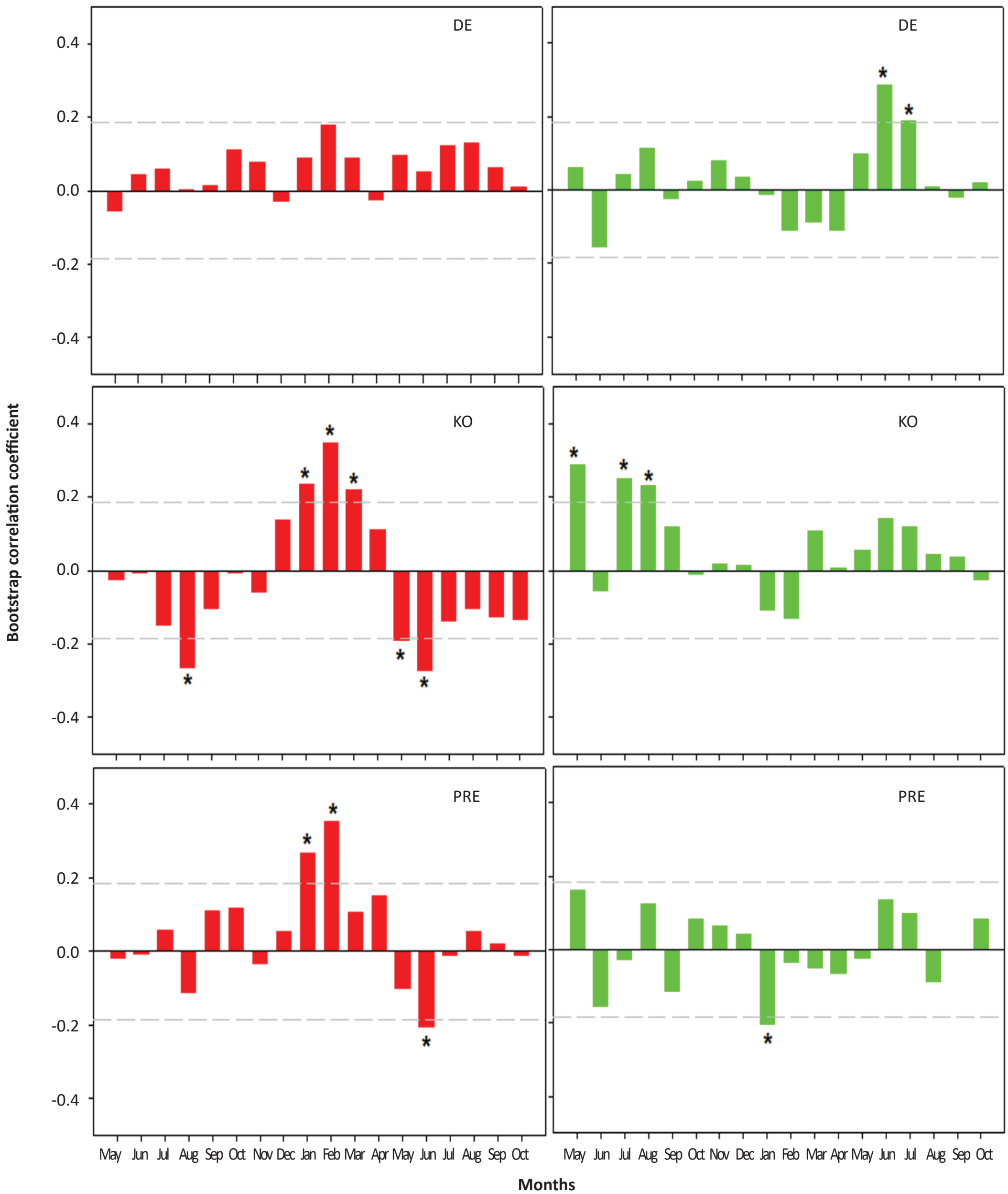


Figure 4. Bootstrap correlation coefficients between residual chronologies with temperature (left) and precipitation (right) from May of the year prior to growth to October of the current year of growth. Asterisks on the top of the bars indicate statistically significant correlations ($p<0.05$) and the horizontal dashed lines represent the 95% confidence interval.

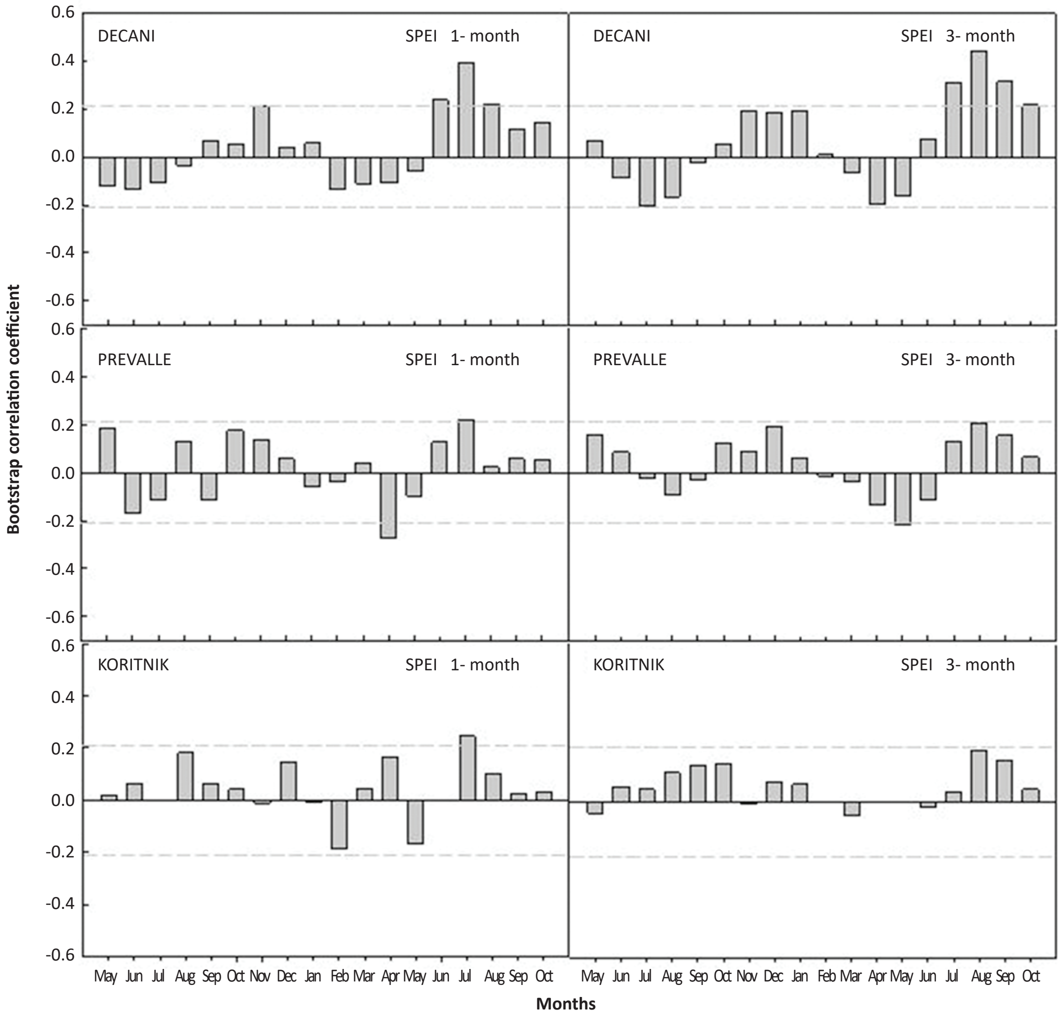
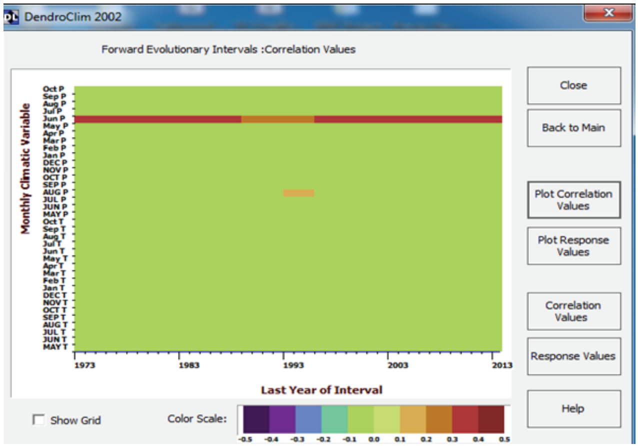
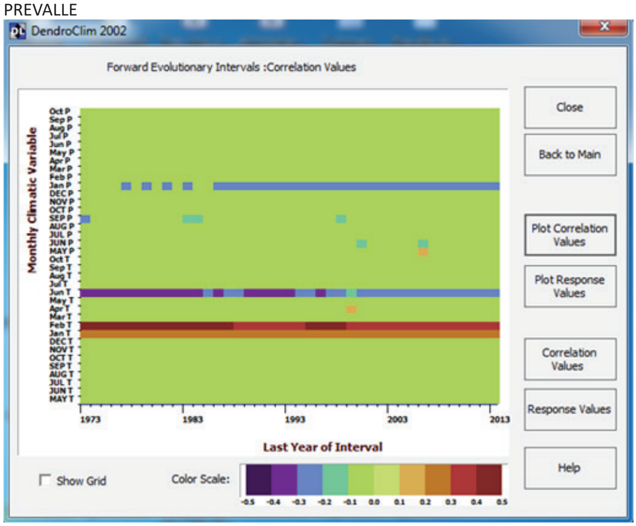


Figure 5. Correlation coefficients (*y*-axis) showing the relationship between the BP residual chronology and monthly SPEI from the previous May to the current October for period 1951–2013. The horizontal dashed lines indicate the relationships between BP growth and SPEI values for a significance level of p<0.05.

DECANI



PREVALLE



KORITNIK

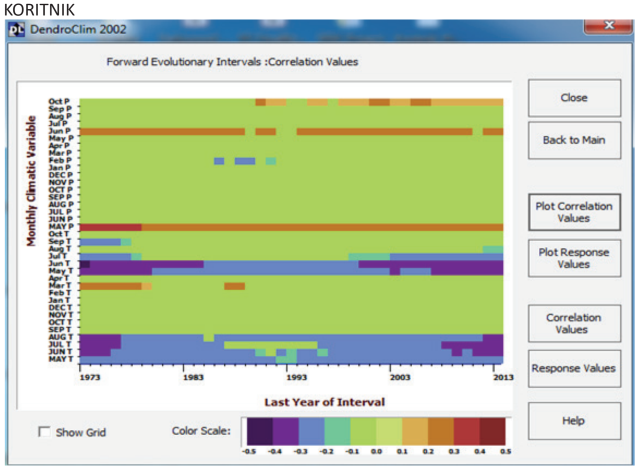


Figure 6. Results for forward evolutionary interval analysis (1951–2013) between monthly mean temperature and the BP chronology (32-year base interval). Monthly climatic variables are shown on the *y*-axis, beginning with the previous May and ending with the current October. The last years of the forward intervals are listed on the *x*-axis. Significant positive (p<0.05) correlations are shown with colors ranging from brown to cherry, while significate negative (p<0.05) correlations are shown with color from green to purple.

DISCUSSION AND CONCLUSION

Radial growth trends shared between BP at the three sites indicate the influence of local climate conditions on species' growth. Significant correlations between BP chronologies and monthly climate data support our approach that BP growing on the studied sites is suitable for dendroclimatic analyses. A significant positive relationship between indexed BP growth and SPEI emerges during July in the current growing year. It suggests that the lack of precipitation during July is limiting the BP radial growth for the rest of the growing season. Thus, temperature rising and the lack of available water during July or summer months will lead to physiological dehydration and physiological drought in BP trees (Zhang et al. 2018). Drought stress will directly affect the transpiration and respiration of trees, leading to a reduction of the photosynthesis rate and eventually producing narrow tree-rings (Trugman et al. 2018). The severity of drought stress on individual trees closely depends on tree age. The drought in the study areas caused by temperature rising and lack of water availability, especially during July, has a strong limiting effect in trees of different age. In particular, the DE chronology composed by older trees, compared to other sites, showed more significant positive correlations with drought index (SPEI) than younger and middle-aged chronologies from PRE and KO sites. Older aged BP trees from DE site have bigger crown size, directly influencing the water balance through a higher evapotranspiration leading to a greater water consumption. This finding is consistent with the results of other studies indicating that age indirectly affects the trees' response to environmental factors due to photosynthesis efficiency, hydraulic conductance and nutrient transport (Vieira et al. 2009).

Jiao et al. (2017) reported that tree age could interfere with the expression of environmental signals related to radial growth and that the age effect is not completely eliminated from the developed chronologies. The KO site was the only one where1-month timescale SPEI (current April) was negatively correlated with BP growth, implying that the low amount of rainfall during this month may delay the initiation of BP radial growth. The decrease in available moisture during the beginning of the growing season could decrease the photosynthetic rate and cause trees to allocate fewer carbohydrates for radial growth (Fritts 1976, McDowell et al. 2008).

We found strong relationships between KO tree-ring chronology and temperatures of the year prior to the growth. In tree-line locations, the growing period is relatively short and most active tracheid's formation occurs at the beginning of the summer (Rossi et al. 2006). The inverse relationship between annual radial growth and monthly mean temperature during the previous late summer and early fall indicates that high monthly mean temperatures would increase evaporation rates and decrease the amount of water stored from summer rains. It is well-known that evaporation increases as monthly mean temperature increases, restricting the amount of water available for photosynthesis (Fritts 1976, McDowell et al. 2008). At PRE site a positive correlation with January and February temperatures was noted, implying that mild winters may have a positive influence on snow melting and soil moisture. From a physiological point of view, winter temperatures cannot directly influence cambial activity since the trees are dormant. However, during warmer winters, more precipitation falls as rainfall, rather than snow (IPCC 2007). In our case, it is more probable that precipitation in cases with warmer winter temperatures is in the form of wet snow, which on its side is less prone to wind transport and immediate sliding along steep slopes and therefore could contribute to a deeper snow cover. This can provide more soil moisture after snowmelt and could be a prerequisite for increased cambial activity given that other conditions are favorable.

Several possible explanations exist for the temporal instability of climate-growth relationships at the three sites. Climate is not the only environmental variable that affects the widths of tree-rings (Fritts 1976). It is possible that other non-climatic factors are responsible for erratic-ring patterns noted in all BP chronologies. Although there is no information about the anthropogenic activity, the presence of young trees especially in KO and PRE sites is a good indicator of silvicultural management treatments and this may partly explain the temporal variability in the relationships between radial growth and local climate. We noticed trunk scars and wounds on some trees at all three sites. These injuries were the result of lightening activity and might affect their growth. Therefore, biotic factors and anthropogenic activity, during the past and present, may partially explain the temporal instability of climate-growth relationships at all sampled sites.

It is projected that Kosovo will have increasingly warmer temperatures and a higher irregularity of precipitation in the future. Taking into account the role of drought during the summer season and temperatures on BP growth, their impact will likely become more important in the global warming scenarios.

Author Contributions

ET, FB carried out the field measurements, laboratory measurements and performed the statistical analysis, while SB and BB conducted the sample preparations. Both authors contributed to data collection and manuscript writing.

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Conflict of Interest

The authors declare no conflict of interest.

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DNA Barcoding of Fungi in the Forest Ecosystem of the Psunj and Papuk Mountains in Croatia

Nevenka Ćelepirović[1,*], Sanja Novak Agbaba[2], Monika Karija Vlahović[3]

(1) Croatian Forest Research Institute, Division of Genetics, Forest Tree Breeding and Seed Science, Cvjetno naselje 41, HR-10450 Jastrebarsko, Croatia; **(2)** Croatian Forest Research Institute, Division of Forest Protection and Game Management, Cvjetno naselje 41, HR-10450 Jastrebarsko; **(3)** University of Zagreb, School of Medicine, Department of forensic medicine and criminology, DNA Laboratory, HR-10000 Zagreb, Croatia.

*** Correspondence:** e-mail: nevenkac@sumins.hr

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ABSTRACT

The saprotrophic, endophytic, and parasitic fungi were detected from the samples collected in the forest of the management unit East Psunj and Papuk Nature Park in Croatia. The disease symptoms, the morphology of fruiting bodies and fungal culture, and DNA barcoding were combined for determining the fungi at the genus or species level. DNA barcoding is a standardized and automated identification of species based on recognition of highly variable DNA sequences. DNA barcoding has a wide application in the diagnostic purpose of fungi in biological specimens. DNA samples for DNA barcoding were isolated from infected tree tissues, fungal fruiting bodies or fungal cultures. The ITS or ITS2 sequences of the fungal DNA were sequenced and aligned with the reference sequences in GenBank (NCBI) using BLAST. The size of ITS and ITS2 sequences were 512-584 bp and 248-326 bp, respectively. The sequences showed a high identity of 97.21%-100% at 98%-100% coverage with reference sequences in GenBank (NCBI). The exception was the species *Amphilogia gyrosa* that showed 95.65% identity at 100% coverage. Two fungi were determined at genus level: *Cladosporium* sp., and *Cytospora* sp., while 11 fungi were determined at species level: *Alternaria alternata*, *Aureobasidium pullulans*, *Amphilogia gyrosa*, *Capronia pilosella*, *Cryphonectria parasitica*, *Exidia glandulosa*, *Epicoccum nigrum*, *Penicillium glabrum*, *Pezicula carpinea*, *Rosellinia corticium*, and *Stereum hirsutum*.

Keywords: GenBank; BLAST; *Fagus sylvatica*; *Ascomycota*; *Basidiomycota*; DNA sequencing

INTRODUCTION

DNA barcoding is a standardized and automated biological specimen identification system (Casiraghi et al. 2010). A DNA barcode is a relatively short gene sequence of the highly variable sequences of the genome, unique for the identification of species. The advantage of this technique is that a single specimen can provide complete information about the species, regardless of the morphology or life-stage characteristics. Hebert et al. (2003) recognized that the mitochondrial enzyme cytochrome c oxidase subunit 1 coding DNA could be applied to generate DNA barcode in animals. Over time, the procedure has been applied to flora and fungi. At present, DNA barcoding can be regarded as a method that is acceptable for all live tree branches. Further research into the genome of organisms revealed that for certain groups of organisms, different sequences of the genome are more acceptable for DNA barcoding. The most commonly used sequence for queries of systematics and taxonomy at and below the genus level for fungi is the internal transcribed spacer (ITS) sequence of the nuclear ribosomal repeat unit (Nilsson et al. 2009, Seifert 2009, Schoch et al. 2011, Xu 2016, Badotti et al. 2017). Easy amplification, common usage, and sufficient barcode distance of ITS sequence are characteristics that allow a wide application as a barcode for fungi (Raja 2017). ITS sequence consists of ITS1 and ITS2 sequences surrounding the 5.8S ribosomal RNA sequence (White et al. 1990, Martin et al. 2005). Blaalid et al. (2013) reported that the ITS1 sequences showed more variable characteristics than the ITS2 sequences, including changes in length and GC content and polymorphism. Nevertheless, research by Yang et al. (2018) proved that the operational taxonomic richness and details of the fungal community in the ITS2 sequence concerning the full-length ITS sequence produced similar results.

In recent years, information about the nucleotide sequences was deposited in public databases of European Nucleotide Archive (ENA) at the European Bioinformatics Institute (EBI) established in 1982; the DNA Data Bank of Japan

(DDBJ) of the National Institute of Genetics (NIG) established in 1987 and GenBank at the National Center for Biotechnology Information (NCBI) established in 1988. Their cooperation in the mind of the International Nucleotide Sequence Database Collaboration (INSDC) and the product database from this framework is called the International Nucleotide Sequence Database (INSD) (Amid 2020). The GenBank is constantly updated with new DNA sequences. A unique accession number is assigned to each newly uploaded DNA sequence. A bioinformatics accession number is a specific ID that may be used to identify various releases of this sequence record and the related sequences in similar repositories over time. DNA barcoding is based on the assumption that the unknown DNA sequence can clearly be defined if it is compared with a reference sequence in a database. The similarity determination process is reliable and fast using algorithms specially developed for this purpose. The commonly used algorithm is the Basic Local Alignment Search Tool (BLAST, NCBI). The key functions of the public databases are to establish a credible source for fungal diagnostics, allowing accurate detection of the causative agents of fungal infections, timely evaluation of mycoses, and early initiation of effective protection measures.

Many saprotrophic, entophytic, and parasitic fungal species evolved as members of complex communities with their host by its capacity to cause tree death, defoliation, decay, or deformation (Unterseher et al. 2010). Among these species, some of them gained significant ecological value as agents of disruption, playing a role in the regulation of forest characteristics. Fungal species communicate, explicitly and indirectly, with other ecological group members, impacting specific environmental processes, foodstuffs, and habitat formation for other organisms (Naranjo-Ortiz and Gabaldón 2019). The endophytes may change their lifestyle and become parasite or saprotroph (Sieber et al. 1987, Sinclair et al. 1996, Stone et al. 2000, Promputtha et al. 2007). The saprotrophs may become a threat to the tree, becoming parasites in special circumstances, and attacking living tissues of the host species, causing death (Prell and Day 2001).

The Papuk and Psunj mountains are part of the Slavonian Mountains located on the southern edge of the Pannonian plain. The largest part of the forest cover in these areas consists of *Fagus sylvatica* stands (Škvorc et al. 2011). According to the information on the web page of Papuk Nature Park (https://pp-papuk.hr), more than 100 fungal species were determined that have been established in this area. However, it is not the final number due to insufficient research. So far, the scientific research of fungi on forest trees in the forest area of the Papuk and Psunj mountains using DNA barcoding has not been conducted. This research was intended to determine specimens of forest saprotrophic, entophytic, and parasitic fungi in the management unit East Psunj and Papuk Nature Park. Determination of specimens collected on the basis of disease symptoms, the presence of fruiting bodies, fungal culture morphology, and DNA barcoding techniques was performed. The purpose of this research was to supplement the DNA databases with new fungal sequences. Collecting sequences in the database allows complement barcode library of the European fungal taxa, thereby intensifying DNA barcode reference libraries relevant to European countries.

MATERIALS AND METHODS

Sample Collection

The first set of samples was collected in the area of management unit East Psunj (45.42 N, 17.48 E), Forest office Požega, Forest Administration Požega, Croatian Forests Ltd. The second set of samples was collected in the area of Campsite Duboka in Papuk Nature Park (45.47 N, 17.65 E), in a northeast direction around 6 km from the camp. Different types of tree samples were collected: tree branches and bark samples with disease symptoms, and fungal fruiting structures. The collection points were georeferenced with the Global Positioning System. The plant or fungal material was properly determined, dated, packed in plastic bags, and transported to the laboratory.

Detection of Disease Symptoms, Fungal Fruiting Bodies and Culture Morphology

The disease symptoms on the samples were recorded and the causal agents of disease were determined according to the description in the literature (Sinclair and Dhingra 1995, Hanlin 1990). The morphological characteristics of the fungal fruiting bodies were analysed macroscopically and using a stereo zoom Lupa Olympus SZH and Olympus BX2 light microscope. The fungal samples on wood materials were stored in the fungal collection. For fungi isolation, small parts of plant material (5 mm) showing symptoms were sterilized for 3 min with 70% ethanol, rinsed in autoclaved distilled water and placed on a filter paper to remove excess liquid. The pieces were aseptically placed on sterile potato dextrose agar (PDA) medium in Petri dishes and incubated for 7–10 days at room temperature (21 to 25°C) under ambient light. Fungal cultures were stored at 4°C. The morphological determination was based on the data from the Electronic Atlas of the Flora of British Columbia (E-Flora BC, 2013), Encyclopedia of Life (https://eol.org/), Zubrik et al. 2008. The taxonomy was stated according to Index Fungorum (www.indexfungorum.org).

DNA Extraction, Amplification, Sequencing and Species Determination

For DNA extraction, part of the plant material showing symptoms, part of fruiting bodies, or the piece of fungal culture was collected in Eppendorf tubes. DNA was extracted from 0.1 g of plant material according to the protocol described in the QIAamp DNA Stool Mini Kit (Qiagen, Germany), or from the fungal fruiting body according to the CTAB method (Doyle and Doyle, 1987) using 2% cetyl trimethyl ammonium bromide (CTAB) buffer supplemented with 1% (wt/vol) of polyvinylpyrrolidone (PVP). DNA was resuspended in 50 µl of ultrapure water. The quality and quantity of DNA were checked by electrophoresis in 1x TBE buffer, pH 8.0 using 0.8% agarose gel stained with GelStar Nucleic Acid Gel Stain dye (Lonza Rockland, Inc., Rockland, USA). Lambda DNA/Hind III fragment (Invitrogen, Germany) was used as a DNA marker and the DNA concentration was measured by spectrophotometry (Biospec-nano, Shimadzu). The obtained DNA in the extracts ranged from 9.64 to 269.98 ng/µl. The ITS (ITS1-5.8S-ITS2) sequences were obtained by amplification using universal pairs of primers ITS1-ITS2 and ITS3-ITS4.

PCR amplification was performed in a final volume of 20 µl containing 1x PCR buffer, 0.8 mg/ml BSA (Bovine Serum Albumin, Amersham Pharmacia Biotech, USA), 0.5 µM of each primer, 1 unit of TaKaRa Taq DNA Polymerase (Takara, Japan) and 0.6 ng of template DNA. Amplification cycle parameters using ITS1-ITS2 primers were as follows: initial denaturation 94°C for 5 min, 30 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min, and then the final cycle 72°C for 10 min. Amplification cycle parameters using ITS3-ITS4 primers were as follows: initial denaturation 94°C for 5 min, and 30 cycles of 95°C for 1 min, 56°C for 1 min and 72°C for 1 min, and then the final cycle 72°C for 10 min. All PCR reactions were performed along with negative control containing ultrapure water instead of DNA and in-house positive control containing DNA extracted from fungi *Eutypella parasitica* (Novak Agbaba et al. 2015). The amplification of DNA fragments was performed on a PTC-100 thermocycler (MJ Research, USA). All PCR products were separated by electrophoresis on 1.8% agarose gel in 1x TBE buffer pH 8.0 stained with GelStar Nucleic Acid Gel Stain, (Lonza Rockland, Inc., Rockland, USA). A DNA marker Tracklt 1 kb Plus DNA Ladder (Invitrogen, Germany) was used as a molecular weight marker, and the gel was photographed under UV light (UVtec Cambridge, UK). Amplified DNA was purified from the 1.8% agarose gel using the PureLink Quick Gel Extraction Kit (Invitrogen, Germany). PCR products were sequenced in both forward and reverse directions using ITS1/ITS2 primer pair and ITS3/ITS4 primer pair. Sequences were analyzed and reviewed using DNA Sequencing Analysis Software v6.0 (Applied Biosystems, Foster City, CA, USA). The consensus ITS sequences were assembled using SeqScape® software v3 (Applied Biosystems, Foster City, CA, USA). The consensus ITS or ITS2 sequence identification was performed using BLAST (Basic Local Alignment Search Tool) (Madden, 2013). According to the suggestion by the authors (Brock et al. 2009, Raja et al. 2017), minimum ≥80% query coverage, and ≥97% cut-off for species recognition was applied. When more than one species was determined inside the cut-off ranges, the classification of the first highest-identity species that appeared in the BLAST report was chosen. Once the fungal species were determined, the DNA sequences were submitted to GenBank (NCBI, Bethesda, MD, USA).

RESULTS

The identification of fungal species was performed by combining the disease symptoms, the morphology of fruiting bodies, culture morphology, and DNA barcoding of the samples collected in the forest of the management unit East Psunj and Papuk Nature Park, (Tables 1 and 2, respectively).

On the site of management unit East Psunj, fungi were determined from three twig samples of *Fagus sylvatica*, two bark samples of *Castanea sativa*, and two fungal fruiting bodies on the branch of *F. sylvatica*. On *F. sylvatica,* five fungal species were determined on a single twig with necrosis (Table 1). Two strains were determined at *C. parasitica*, hypovirulent, and virulent. In total, nine species of fungi were determined on the site of management unit East Psunj on *F. silvatica* and *C. sativa* samples.

Alternaria alternata (Fr.) Keissl. (*Ascomycota*) is an endophyte and a parasite. In our sample, *A. alternate* was determined as endophyte on a twig (Table 1). It was isolated by culturing the necrotic twig tissue. DNA was isolated from grey-brownish fungal culture. The ITS sequence of 567 bp in 100% identity at 100% coverage corresponded to reference sequence *A. alternata* (GenBank accession number MN481948.1).

Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud (*Ascomycota*) is a saprotroph and an endophyte that lives on decaying twig and leaves. In our sample, *A. pullulans* was determined as endophyte on a twig (Table 1). It was isolated by culturing the necrotic twig tissue. DNA was isolated from cream-colored fungal culture. The ITS sequence of 515 bp in 100% identity at 100% coverage corresponded to reference sequence *A. pullulans* (GenBank accession number MT573468.1).

Cladosporium sp. Link (*Ascomycota*) is a saprotroph, a parasite, and an endophyte. In our sample, *Cladosporium* sp. was determined as endophyte on a twig (Table 1). It was isolated by culturing the necrotic twig tissue. DNA was isolated from olivaceous-green fungal culture (Figure 1g). The ITS sequence of 552 bp in 100% identity at 100% coverage corresponded to reference sequences in descending order as follows: *Cladosporium pseudocladosporioides* (GenBank accession number MT582794.1), *Cladosporium cladosporioides* (GenBank accession number MT466517.1), and *Cladosporium* sp. (GenBank accession number MK355726.1). However, the morphological characteristics of the fungal PDA culture were insufficient for species determination. Based on the data from the BLAST report, the fungus was determined on the genus level as *Cladosporium* sp. (Figure 1g).

Cryphonectria parasitica (Murrill) M.E. Barr (hypovirulent) (*Ascomycota*) is a parasite and a saprotroph. In our sample, *C. parasitica* was determined as a parasite. It was isolated from superficial bark canker (Heiniger et al. 1994, Robin et al. 2001) by culturing the infected bark (Table 1, Figure 1a). DNA was isolated from white fungal culture (Figure 1f). The ITS2 sequence of 326 bp in 99.69% identity at 99% coverage corresponded to reference sequence *C. parasitica* (GenBank accession number KP824756.1). *Cryphonectria parasitica* (Murrill) M.E. Barr (virulent) was isolated from active bark canker (EPPO 2005) by culturing the infected bark (Table 1, Figure 1b). DNA was isolated from orange fungal culture (Figure 1f). The ITS2 sequence of 248 bp in 99.60% identity at 100% coverage corresponded to reference sequence *C. parasitica* (GenBank accession number MT256127.1).

Cytospora sp. Ehrenb. (*Ascomycota*) is a parasite, a saprotroph, and an endophyte. In our sample, *Cytospora* sp. was determined as a parasite that causes drying of branches and twigs from the tips and leaves becoming brown and dry (Table 1). It forms cankers and necrosis under the bark. It was isolated from the bark canker by culturing the infected wood. DNA was isolated from dark brown fungal culture. In the BLAST report, the ITS sequence of 584 bp corresponded to reference sequences in descending order as follows: *Cytospora ribis* (99.31% identity at 99% coverage, GenBank accession number KU058681.1), *C. ribis* (99.14% identity at 99% coverage, GenBank accession number KU165798.1), *Cytospora rhodophila* (99.14% identity at 99% coverage, GenBank accession number KF294010.1) and *Cytospora prunicola* (99.48% identity at 98% coverage, GenBank accession number NR157500.1), *C. ribis* (98.97% identity at

99% coverage, GenBank accession number KU170615.1), *C. ribis* (98.80% identity at 99% coverage, GenBank accession number KU170616.1), *Cytospora* sp. (99.13% identity at 98% coverage, GenBank accession number MT177936.1). However, the morphological characteristics of the fungal PDA culture were insufficient for species determination. Based on the data from the BLAST report, the fungus was determined at the level of the genus as *Cytospora* sp.

Epicoccum nigrum Link (*Ascomycota*) is a saprotroph, an endophyte, and a parasite that causes necrosis under bark and causes drying of twigs and branches. In our sample, the fungus was determined as an endophyte on a twig (Table 1). It was isolated by culturing the necrotic twig tissue. DNA was isolated from pink fungal culture (Figure 1h). ITS sequence of 544 bp corresponded to reference sequence *E. nigrum* (100% identity at 100% coverage, GenBank accession number AB470853.1).

Exidia glandulosa (Bull.) Fr. (*Basidiomycota*) is a saprotroph. In our research, it was determined on the tree of *F. sylvatica* forming branch, black, jelly fruiting bodies causing decay (Table 1, Figure 1c). The fungus detection was done according to the morphological characteristics of the fruiting body. DNA was isolated from the fruiting body. The ITS sequence of 577 bp in 100% identity at 99.31% coverage corresponded to reference sequence *E. glandulosa* (GenBank accession number MF161201.1).

Penicillium glabrum (Wehmer) Westling (*Ascomycota*) is a parasite and an endophyte. In our sample, *P. glabrum* was determined as an endophyte on a twig (Table 1). It was isolated by culturing the necrotic twig tissue. DNA was isolated from green fungal culture. The ITS sequence of 578 bp in 100% identity at 100% coverage corresponded to reference sequence *P. glabrum* (GenBank accession number MK910045.1).

Stereum hirsutum (Willd.) Pers. (*Basidiomycota*) is a saprotroph and a parasite. It causes decay of the trunk. In our sample, *S. hirsutum* was determined as a saprotroph. It was determined on a tree of *F. sylvatica* forming orange shelf-fruiting bodies on the bark (Table 1, Figure 1d). The fungus detection was done according to the morphological characteristics of the fruiting body. DNA was isolated from the fruiting body. The ITS sequence of 577 bp in 99.41% identity at 100% coverage corresponded to reference sequence *S. hirsutum* (GenBank accession number KR909200.1).

On the site of Papuk Nature Park, fungi on four samples of semi-dry branches of *F. sylvatica*, and one fungal fruiting body were determined on the bark of *C. avellana and F. sylvatica* each. On the single tree of *F. sylvatica*, five species of the fungi were determined on a semi-dry branch with necrosis (Table 2).

Amphilogia gyrosa (Berk. & Broome) Gryzenh., H.F. Glen & M.J. Wingf. (*Ascomycota*) is a parasite. It was determined according to the symptoms on the branch (orange to brawn necrosis) (Table 2). DNA was isolated from the sawdust from infected wood. The ITS2 sequence of 319 bp in 95.65% identity at 100% coverage corresponded to reference sequence *A. gyrosa* (GenBank accession number EF026147.1). Even though the fact that the sequence identity with the GenBank sequences was under 97%, the symptoms of the disease on the branch strongly support the identification of the fungus as *A. gyriosa*.

Capronia pilosella (P. Karst.) E. Müll., Petrini, P.J. Fisher, Samuels & Rossman (*Ascomycota*) is a saprotroph on the decay branch. The fruiting body of the fungus is dark brown to black (Table 2). *C. pilosella* on the branch of *C. avellana* was found (Table 2). The fungus detection was done according to the morphological characteristics of the fruiting body. DNA was isolated from the fruiting body. The ITS2 sequence of 319 bp in 97.2% identity at 98% coverage corresponded to reference sequence *C. pilosella* (GenBank accession number DQ826737.1).

Epicoccum nigrum Link. *(Ascomycota)* is a pathogen, an endophyte, and a saprotroph. In our sample, it is endophyte inside the branch of *F. sylvatica* (*Table 2*). It was isolated by culturing the necrotic branch tissue. DNA was isolated from pink fungal culture. The ITS sequence of 515 bp corresponded

Table 1. Data of the samples collected on the site of management unit East Psunj.

No	Fungal species	Host tree species	Locality (N, E)	Sample of plant tissue	GenBank Accession number (NCBI)
1	*Alternaria alternata*	*Fagus sylvatica*	45.4207 17.4816	Twig with necrosis	MN251032.1
3	*Cladosporium* sp.	*Fagus sylvatica*	45.4207 17.4816	Twig with necrosis	MN251034.1
4	*Cryphonectria parasitica*	*Castanea sativa*	45.4219 17.4805	Bark with symptoms of superficial canker (hypovirulent)	MN251044.1
5	*Cryphonectria parasitica*	*Castanea sativa*	45.4231 17.4803	Bark canker, fan-shaped mycelium under the bark	MN251045.1
6	*Cytospora* sp.	*Fagus sylvatica*	45.4207 17.4816	Twig with necrosis	MN251035.1
7	*Epicoccum nigrum*	*Fagus sylvatica*	45.4207 17.4816	Twig with necrosis	MN251033.1
8	*Exidia glandulosa*	*Fagus sylvatica*	45.4233 17.4803	Semi-rotten shoot with black gelatinous fungus	MN251028.1
9	*Penicillium glabrum*	*Fagus sylvatica*	45.4207 17.4816	Twig with necrosis	MN251036.1
10	*Stereum hirsutum*	*Fagus sylvatica*	45.4236 17.4803	Orange shelf fruiting bodies on bark	MN251029.1

to reference sequences in descending order as follows: *Epicoccum layuense* (99% identity, 100% coverage GenBank accession number MT573479.1), and *E. nigrum* (99% identity, 100% coverage, GenBank accession number MK793731.1). However, based on the morphological characteristics of the fungal PDA culture, the fungus was determined as *E. nigrum.*

Pezicula carpinea (Pers.) Tul. ex Fuckel (*Ascomycota*) is a parasite, a saprotroph, and an endophyte. The branch with canker wounds was collected (Table 2). In our sample, the fungus was determined as a parasite. DNA was isolated from the infected branch's bark. The ITS sequence of 516 bp corresponded to reference sequence *P. carpinea* (100% identity, 100% coverage, GenBank accession number NR_144927.1).

Rosellinia corticum (Schwein) Sacc**.** (*Ascomycota*) is a saprotroph and an endophyte. In our sample, it was determined as a saprotroph on the tree of *F. sylvatica*, forming black fruiting bodies that cause semi-dray necrosis on the branch (Table 2)**.** The fungus detection was done according to the morphological characteristics of the fruiting body. DNA was isolated from the fruiting body. The ITS sequence of 562 bp in 99.28% identity at 98% coverage corresponded to reference sequences *R. corticum* (GenBank accession number KC311485.1).

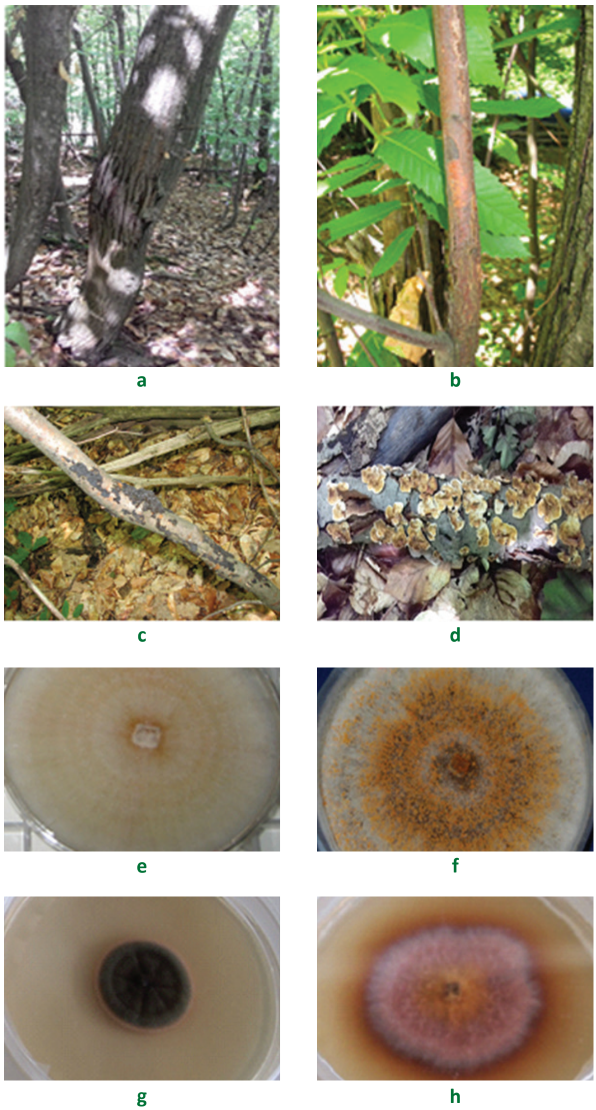

Figure 1. Disease symptoms and fruiting bodies on trees and fungal culture from the site of management unit East Psunj. **(a)** Superficial canker on *C. sativa*; **(b)** Active canker on *C. sativa*; **(c)** *S. hirsutum* on the bark of *F. sylvatica*; **(d)** *Exidia glandulos* on *F. sylvatica*; **(e)** Culture of *C. parasitica* (hypovirulent); **(f)** Culture of *C. parasitica* (virulent); **(g)** Culture of *Cladosporium* sp.; **(h)** Culture of *Epicoccum nigrum*.

Table 2. Data of the samples collected in Papuk Nature Park.

No	Fungal species	Host tree species	Locality (N, E)	Sample of plant tissue	GenBank Accession number (NCBI)
1	*Amphilogia gyrosa*	*Fagus sylvatica*	45.4875 17.6801	Semi-dry top of branch	MN251039.1
2	*Capronia pilosella*	*Corylus avellana*	45.4741 17.6577	Black fruiting bodies on semi-rotten branch	MN251043.1
3	*Epicoccum* nigrum	*Fagus sylvatica*	45.4877 17.6812	Necrosis on semi-dry branch	MN251030.1
4	*Pezicula carpinea*	*Fagus sylvatica*	45.4875 17.6800	Branch with canker symptoms	MN251038.1
5	*Rosellinia corticum*	*Fagus sylvatica*	45.4877 17.6812	Black fruiting bodies on the branch	MN251031.1

DISCUSSION

The symptoms of infected forest plant tissues can identify the fungi causing plant diseases. The necrosis on the twig, bark blight, discoloration of the bark, and fruiting bodies were registered as symptoms on the assessed trees. Identifying any fungus species that cause a disease is a complex procedure because many of them cause similar symptoms (Butun, 1995). The culture-dependent approaches based on the morphological detection of fungal identification have restrictions such as the slow growth rate of fungi, contamination with competitive fungi, or identical appearance of the culture, excluding biotrophic and slow-growing fungi and promoting fast-growing fungi (Shi et al. 2019). DNA barcoding is a new effort to get quick and accurate recognition of organisms (Hebert and Gregory, 2005). However, research by Hofstetter et al. (2019) regarding the species of fungi in protected Montricher Beech Forest (Switzerland) using BLAST identification tools reported that the number of ITS sequences related to the incorrect taxon name tends to be approximately 30%. Considering the fact of mistaken species identification using ITS sequence, Ciat et al. (2006) propose a three-step method for a diagnostic procedure that incorporates morphological parameters, biochemical study, and ITS sequence analysis.

In this work, 13 fungal species were determined, out of which there was the same number of saprotrophs and parasites (four species of each) and five endophytes. The samples were collected from three tree species *F. sylvatica, C. sativa* and *C. avellana*. The most fungal species were determined on *F. sylvatica*, 11 fungal species, whereas on *C. sativa* and *C. avellana* one fungal species was determined per tree species (Tables 1 and 2). The five fungi were determined on one branch of *F. sylvatica.* Four of them were endophytes (*A. alternata, A. pullulans, Cladosporium sp,* and *E. nigrum*) and one was parasite (*Cytospora* sp.). On the other *F. sylvatica* tree, two fungi were determined, *E. nigrum* and *R. corticum*. Only one of the fungal species, *E. nigrum*, was determined on both localities. In our work, DNA barcoding was performed from different types of tissues: tree tissue with disease symptoms, fungal fruiting structures, and fungal cultures. From three samples, DNA was extracted from tree tissue with symptoms resulting in the determination of *A. gyrosa, E. glandulosa,* and *P. carpinea*. DNA of *C. pilosella, E. glandulosa, R. corticum,* and *S. hirsutum* were isolated from the fruiting bodies. Non-culture-based method, DNA barcoding, was used to overcome the long turnaround times associated with fungal cultivation on the medium. The fungal cultures were prepared from the collected samples from which DNA samples failed to be isolated. Seven fungal species were determined from the culture medium (*C. parasitica, E. nigrum, A. alternata, Cytospora sp., Cladosporium sp., P. glabrum,* and *A. pullulans*). *Cytospora* sp. and *Cladosporium* sp. were determined on the genus level using ITS sequence because the BLAST report provided inconclusive data. Norphanphoun et al. (2017) combined morphological characteristics and multi-gene loci (ITS, large nuclear ribosomal RNA subunit sequence (LSU), RNA polymerase II subunit (RPB2), and α-actin (ACT)) for determination and phylogenetic analysis of the *Cytospora* species. Furthermore, Tibpromma et al. (2019) reported usage of morphological examinations, culture characteristics and multi-gene sequence analysis (ITS, ACT, and translation elongation factor 1-alpha gene (TEF1)) for the determination of fungi isolates belonging to the genera *Cladosporium*. These scientific works suggested that the *Cytospora sp.* and *Cladosporium* sp. could be determined to species level performing multi-gene sequence analysis.

Regarding the phylum of the determined fungi, *Ascomycota* (11 species) predominated over *Basidiomycota* (2 species). Seven *Ascomycota* and two *Basidiomycota* were determined on the site of management unit East Psunj. Five *Ascomycota* were determined in Papuk Nature Park. The size of ITS sequence differs between *Ascomycota* and *Basidiomycota* having an average length of 500 and 600 base pairs (bp), respectively (Porter and Brian Golding 2011). In this research, the length of the ITS sequences of nine determined *Ascomycota* ranged from 515 bp (*A. pullulans* and *E. nigrum*) to 584 bp (*Cytospora* sp.), and ITS2 sequence of three determined *Ascomycota* ranged from 248 bp (*C. parasitica*) to 326 bp (*C. parasitica*, hypovirulent). The ITS sequences of two *Basidiomycota* (*E. glanduloza and S. hirzutum*) were the same length of 557 bp. These sequences showed a high degree of identity of 97.21%-100% under 98%-100% coverage with the reference sequences in GenBank (NCBI). However, fungus *A. girosa* was determined based on GenBank similarities on the bases of ITS2 sequence showing 95.67% identity at 100% coverage, implicating the entire ITS sequence was needed for achieving a higher GenBank similarity score (Nilson et al., 2009). All sequences were submitted to GenBank (NCBI) (Table 1, 2) to serve for the identification of unknown fungal species and in the conservation of fungal diversity.

CONCLUSIONS

In this study, plant materials with disease symptoms on forest trees and fungal fruiting bodies were sampled for determination in the area of management unit East Psunj and Papuk Nature Park. In total, 13 species of fungi were determined on the basis of disease symptoms, morphology of fruiting bodies and fungal cultures, and DNA barcoding. Every fungal sample was DNA barcoded, based on the ITS or ITS2 sequence. NCBI BLAST analyses demonstrated that ITS and ITS2 sequences of analysed fungi showed a high sequence identity of 97.21%–100% at 98%–100% coverage with reference sequences in GenBank (NCBI) except for *A. gyrosa* (95.65% identity, 100% coverage). All sequences were submitted to GenBank (NCBI) and received the accession numbers (Tables 1, 2). In this research, DNA barcoding was accurate and a fast determination tool for the determination of fungi along with the disease symptoms and morphology of fungal cultures and fruiting structures.

Author Contributions

SNA, NC conceived, designed the research and carried out the field research, SNA, NC, MKV performed laboratory analysis and processed the data, NC, and SNA wrote the manuscript. All authors reviewed the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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In Vitro Lead Tolerance Testing in White Poplar Genotypes on Acidic Medium

Branislav Kovačević[1,*], Gordana Tišma[2], Nataša Nikolić[2], Marina Milović[1], Vanja Vuksanović[3], Saša Orlović[1]

(1) University of Novi Sad, Institute of Lowland Forestry and Environment, Antona Čehova 13, RS-21000 Novi Sad, Serbia; **(2)** University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, RS-21001 Novi Sad, Serbia; **(3)** University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, RS-21002 Novi Sad, Serbia

*** Correspondence:** e-mail: branek@uns.ac.rs

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ABSTRACT

This study evaluates *in vitro* tolerance of white poplar genotypes to the presence of lead in an acidic rooting medium. Lead was supplemented in form of $Pb(NO_3)_2$, in the following concentrations: 0 M (Control), 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M. After four weeks of cultivation, the following characters were measured: number of roots, the length of the longest root, the shoot height, dry root mass and dry shoot mass, and parameters related to photosynthetic pigments: content of chlorophyll a, b, a+b, and of carotenoids in fresh mass, as well as chlorophyll a/b ratio. For further statistical analysis, tolerance indices by Turner and Marshal (TI) were calculated for each measured character. The strongest inhibitory effect was achieved on the medium with 10^{-4} M $Pb(NO_3)_2$, but the best differentiation between genotypes was achieved on the medium with 10^{-5} M $Pb(NO_3)_2$. The highest tolerance indices for the length of the longest root and shoot height had genotypes L-12 and LBM, and for root and shoot dry mass genotypes LCM and L-12. There were no significant differences between genotypes in tolerance indices by any of the examined photosynthetic parameters. The obtained results suggest that both low pH and the presence of citric acid as chelating agent improved evaluation of lead tolerance in comparison with the results obtained on media with standard pH and without citric acid in similar studies. *In vitro* tests on acidic medium with citric acid can efficiently differentiate examined genotypes for lead tolerance, which could be important for their use in phytoextraction projects, especially on acidic soils.

Keywords: *Populus alba*; heavy metals; low pH; microwave sterilization

INTRODUCTION

With the start of the rapid development of mining and industrialization at the end of the 19^{th} century, the ecosystems have been constantly polluted by a wide variety of heavy metals (Benavides et al. 2005). Accumulated in sufficient quantities, heavy metals not only pose serious problems to plant growth and development, but to human health as well (Arora et al. 2008).

Lead is a heavy metal that is considered one of the most toxic metals (Zhang 2003), and the major pollutant in both terrestrial and aquatic ecosystems (Sharma and Dubey 2005). Lead naturally occurs in the soil, but its content can be greatly increased by human activities (Seregin and Ivanov 2001). River sediments also receive significant anthropogenic loads of metals from both point and nonpoint sources (Sakan et al. 2009).

Apart from negative effects on membrane structure, water potential, and hormonal status, its toxicity is mostly related to oxidative stress by increased accumulation of free radicals and reactive oxygen species. Lead stimulates this process by affecting enzyme activity and inhibition of electron transport during oxidative phosphorylation (Seregin and Ivanov 2001, Sharma and Dubey 2005, Zengin and Munzuroglu 2005).

Phytoextraction is an environmentally friendly *in situ* technique for cleaning up metal from contaminated land. By phytoextraction toxic metals are taken out from contaminated soil and accumulated in the above-ground tissues of higher plants, which is sometimes assisted with different chelating agents in order to increase the efficiency (Pulford and Watson 2003, Sinhal et al. 2010).

Poplars are often used in phytoremediation. Their main advantages are fast growth, adaptability, well-developed root system that reaches underground waters, the ability to transpire considerable amounts of water (Aitchison et al. 2000), and the possibility of the establishment of short coppice culture (Rockwood et al. 2000). Poplars could be compared to the hyperaccumulators in the heavy metal accumulation by large biomass production (Pulford and Watson 2003) and relatively high quantity of extracted metal per plant (di Lonardo et al. 2011). White poplars (*Populus* section *Populus*) are interesting for their higher tolerance to drought and salinity compared to black poplars (*P.* section *Aigeiros* Duby).

Considering limitations of field studies on large long-lived organisms, the use of *in vitro* culture of tree species remains an interesting technique for studying the effects of elevated levels of heavy metals and the selection of heavy metal-tolerant genotypes. Developmental and molecular data obtained by Castiglione et al. (2007) in white poplar support the use of *in vitro* techniques in the study of heavy metal stress responses. Tolerance of white poplars to heavy metals, including lead, has been tested in controlled conditions, and differences among genotypes were found (Kališova-Špirochova et al. 2003, Bojarczuk 2004, Bittsanszky et al. 2005, Katanic et al. 2007, 2008, di Lonardo et al. 2011). Nowadays, these tests include the use of media that increase bioavailability and mobility of heavy metals by lowering pH and the use of chelators (Vuksanović et al. 2017a).

In this research, the effect of lead on morphometric parameters, biomass accumulation, and content of photosynthetic pigments in five white poplar (*Populus alba* L.) genotypes *in vitro* was studied. The aim was to evaluate and select lead-tolerant white poplar genotypes cultured *in vitro* on acidic medium in the presence of citric acid, regarding the possible use of these genotypes in phytoremediation projects on acidic and lead-contaminated soils.

MATERIALS AND METHODS

Plant Material and Shoot Multiplication

Five white poplar genotypes were examined: Villafranca (Italy), L-12 (Serbia), L-80 (Serbia), LBM (Serbia), and LCM (Serbia). These genotypes have been proved suitable for biomass production, landscaping, and horticulture and suitable for *in vitro* testing to abiotic stresses (Kovačević et al. 2013a, Vuksanović et al. 2016, 2019a). According to Kovačević and Igić (2018) and Kovačević et al. (2020), Villafranca, L-12 and L-80 genotypes are characterized by relatively good rooting of hardwood cuttings. Also, special attention is paid to L-12 genotype due to its high biomass production and wood characteristics (Ištok et al. 2019, Sedlar et al. 2019).

Micropropagation of five tested genotypes was performed by shoot tips and axillary buds to preserve clonal fidelity (Rani and Raina 2000, Confalonieri et al. 2003). The medium used in shoot multiplication has been described by Kovačević et al. (2013a).

The cultures were kept at 26±2°C in the white fluorescent light (3500 lux·m^{-2}) with a 16-hour photoperiod and subcultured at 4-week intervals.

Lead Treatments

For the experiment, 2.0 cm long shoot tips from micro shoots gained from the multiplication phase were placed on rooting ACM medium containing no hormones, adjusted to pH 3 before sterilization. Citric acid was added to provide pH stability (Skirvin et al. 1986). By lowering the medium pH and by acting as a chelating agent, citric acid was expected to improve lead uptake by plants and to achieve more critical tests for lead tolerance.

The following concentrations of lead in form of $Pb(NO_3)_2$ were examined: 0 M (as a Control), 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M, labeled L0, L1, L2, L3, and L4, respectively.

The sterilization of media was performed by the microwave oven. The media were heated until they started to boil and then poured into sterilized jars in the laminar chamber, in order to preserve the jellification potential of agar that could be compromised by low medium pH (Kovačević et al. 2013b, Vuksanović et al. 2016, 2017a).

The cultures were kept for 4 weeks, at the same conditions as cultures for shoot multiplication. Three jars with five plants per jar were set per each Genotype × Medium combination. For pigment content determination, additional three jars with five shoots per jar were established per each Genotype × Medium treatment.

Lead Tolerance Assessment

After four weeks of cultivation, the following morphometric characters were determined: the number of roots per plant, the length of the longest root per plant, and the height of the shoot.

The following characters describing biomass were determined: dry root mass per plant and dry shoot biomass accumulation per plant. For dry shoot biomass accumulation, fifteen rootless shoot tips were dried at 70°C for 72 hours and then weighted. The dry shoot biomass accumulation was calculated as a difference between dry shoot biomass at the beginning and at the end of the experiment. The dry mass root/shoot ratio was calculated, as well as the ratio between dry root and dry shoot biomass accumulation.

Content of photosynthetic pigments in fresh shoot mass for chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids was determined spectrophotometrically (Wettstein 1957). The chlorophyll a+b and chlorophyll a/b ratio were then calculated.

The toxicity of the applied lead concentration and differences in lead tolerance among the examined genotypes were evaluated by tolerance indices. The tolerance index (TI) was calculated according to Turner and Marshal (1972), as a ratio between the value of a parameter on the medium with a particular lead concentration ($X_{c(Pb)}$) and the value obtained on the control ($X_{Control}$) (Equation 1):

$$TI = \frac{X_{c\,(Pb)}}{X_{Control}} \qquad (1)$$

Statistical Analysis

The whole experiment was designed as completely randomized. The obtained data were analyzed by two-way factorial analysis of variance, as well as Fisher's Least significant difference test (LSD test) with STATISTICA 13 statistical program (TIBCO Software Inc. 2017).

RESULTS

Morphometric Characters

No obvious signs of toxicity, like chlorosis, necrosis, or decay of shoot tissue, were observed, and the rooting of shoots was nearly 100% on all examined media (Figure 1). Only on L4 medium (with 10^{-3} M $Pb(NO_3)_2$) the partial darkening of roots was noticed. The results for the measured characters are given in Supplementary File.

Factor Genotype had significant effect on the tolerance indices of measured morphometric characters, except for the length of the longest root. The effect of lead concentration was significant for the length of the longest root and shoot height, while interaction Genotype × Medium had no significant influence on any of the examined morphometric characters (Table 1). Since there was no significant effect of factor Medium, as well as of interaction Genotype × Medium on tolerance index based on the number of roots, data from further statistical analysis for this parameter are not presented.

According to the LSD test for tolerance, indices for the length of the longest root on L3 and L4 were significantly lower than on L1 and L2 media. In total, the highest tolerance index for the length of the longest root had genotype L-12, and the lowest genotype L-80. The best differentiation of genotypes by this tolerance index was found on L2 medium. On this medium tolerance index for the length of the longest root for genotype L-80 was lower than for other examined genotypes (Table 2).

In total, the reaction of most of the examined poplar genotypes to lead treatments by shoot height appeared to be similar as by the length of the longest root, except for genotype L-80 which achieved significantly slower growth than others. Except significantly smaller tolerance index for

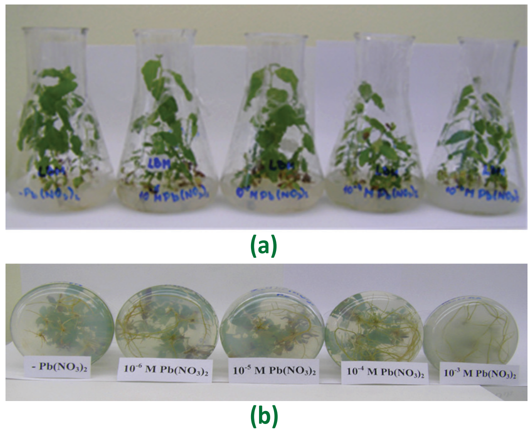

Figure 1. Rooted microshoots of genotype LBM after four weeks of *in vitro* cultivation on examined concentrations of $Pb(NO_3)_2$: **(a)** Shoots; **(b)** Root system.

Table 1. Results of F-test for tolerance indices based on the examined characters.
[1)] Labels for F-test: * - significant at the level α=0.05; ** - significant at the level α=0.01

Character	Source of variation		
	Genotype (A)	Medium (B)	Interaction A×B
Morphometric characters			
Number of roots	13.48^{**} [1)]	2.15	0.75
The length of the longest root	2.54	6.13^{**}	1.65
Shoot height	5.58^{**}	7.38^{**}	0.94
Biomass characters			
Dry root mass	6.98^{**}	3.78^{*}	1.62
Dry shoot mass	7.71^{**}	3.12^{*}	2.54*
Dry mass root/shoot ratio	5.87^{**}	1.77	1.06
Content of photosynthetic pigments			
Chlorophyll a content	1.10	4.36^{*}	1.53
Chlorophyll b content	2.13	3.36^{*}	1.5
Chlorophyll a+b content	1.45	4.22^{*}	1.57
Carotenoids content	0.59	3.97^{*}	1.10
Chlorophyll a/b ratio	1.90	1.11	0.82

Table 2. Tolerance index for the length of the longest root and shoot height of the examined white poplar genotypes cultured on media with different lead concentrations (Fisher's LSD test).
*) The differences between values marked with the same letter are not significant at the level α=0.05

$c(Pb(NO_3)_2)$ (M)	Villafranca	L-12	L-80	LBM	LCM	Total (Medium)
			Tolerance index for the length of the longest root			
10^{-6}	1.03 abcdef *)	1.57 a	1.39 abc	1.45 a	1.26 abcd	1.36 a
10^{-5}	1.25 abcd	1.49 a	0.55 ef	1.37 a	1.24 abcd	1.21 a
10^{-4}	0.72 def	1.13 abcdef	0.78 bcdef	1.10 abcde	0.75 cdef	0.93 b
10^{-3}	1.41 ab	0.94 abcdef	0.39 ef	0.60 f	0.80 bcdef	0.85 b
Total (Genotype)	1.10 ab	1.28 a	0.83 b	1.16 a	1.01 ab	
			Tolerance index for shoot height			
10^{-6}	1.07 abcdefg *)	1.34 a	0.94 cdefghi	1.24 abc	1.15 abcde	1.16 a
10^{-5}	1.14 abcdef	1.29 ab	0.81 efghi	1.15 abcd	0.99 bcdefgh	1.09 a
10^{-4}	0.80 fghi	1.07 abcdefg	0.61 i	1.02 bcdefgh	0.83 efghi	0.89 b
10^{-3}	1.09 abcdefg	0.76 ghi	0.64 hi	0.96 defgh	0.97 bcdefgh	0.91 b
Total (Genotype)	1.02 a	1.12 a	0.77 b	1.10 a	0.98 a	

shoot height of L-12 on medium L4 than on L1 and L2, there were no significant differences in the reaction of genotypes by morphometric characters on the examined media (Table 2).

Biomass Characters

All tolerance indices for biomass characters were significantly affected by both Genotype and Medium factors, except for the main effect of Medium on tolerance index for dry mass root/shoot ratio. The effect of interaction Genotype × Medium was significant only for dry shoot mass accumulation (Table 1). Since factor Medium and interaction Genotype × Medium did not achieve significant effect on tolerance index based on the number of roots, for this parameter data from further statistical analysis are not presented.

There was a significantly lower tolerance index for dry root mass accumulation on medium L3 (10^{-4} M $Pb(NO_3)_2$), compared to other examined lead concentrations. In total, the highest tolerance indices for dry root mass accumulation achieved L-12 and LCM, and the lowest L-80. The best differentiation between genotypes was achieved on medium L2 (10^{-5} M $Pb(NO_3)_2$), where L-12 and LCM had lower tolerance indices for dry root mass accumulation than L-80 and Villafranca (Table 3).

Table 3. Tolerance index for dry root and shoot mass of the examined white poplar genotypes on media with different lead concentrations (Fisher's LSD – test).
*) The differences between values marked with the same letter are not significant at the level α=0.05

$c(Pb(NO_3)_2)$ (M)	Villafranca	L-12	L-80	LBM	LCM	Total (Medium)
			Tolerance index for dry root mass			
10^{-6}	1.00 cdefgh *)	2.06 ab	1.21 bcdefgh	1.75 abcd	1.58 abcde	1.56 a
10^{-5}	0.78 efgh	2.07 ab	0.29 h	1.39 bcdeg	1.95 abc	1.31 a
10^{-4}	0.50 fgh	1.40 bcdefg	0.45 fh	0.52 fh	1.19 bcdefgh	0.77 b
10^{-3}	1.61 abcde	1.23 bcdefgh	0.61 defgh	0.71 efgh	2.50 a	1.34 a
Total (Genotype)	0.97 b	1.69 a	0.65 b	1.12 b	1.81 a	
			Tolerance index for dry shoot mass			
10^{-6}	0.76 f *)	1.74 abc	1.42 cdef	1.34 cdef	1.78 abc	1.40 a
10^{-5}	0.82 ef	1.65 bcd	0.75 f	1.46 cde	1.69 abc	1.31 ab
10^{-4}	0.80 ef	1.16 cdef	0.70 f	0.80 f	1.73 abc	1.00 b
10^{-3}	1.03 cdef	0.87 def	2.64 a	0.92 ef	2.27 ab	1.37 a
Total (Genotype)	0.85 c	1.36 b	1.20 bc	1.14 bc	1.87 a	

In total, the lowest tolerance index for dry shoot mass accumulation was achieved on medium L3. The highest tolerance index in total for genotypes was achieved by LCM, and the lowest by genotype L-80. The best differentiation between genotypes was achieved on medium L2, where L-12 and LCM had significantly higher tolerance indices than genotypes L-80 and Villafranca (Table 3).

Content of Photosynthetic Pigments

Factor Medium had a significant effect on the variation of tolerance indices for all examined photosynthetic characters, except for chlorophyll a/b ratio (Table 1). Because there was no significant effect of any of the controlled sources of variation on tolerance index based on chlorophyll a/b ratio, data from further statistical analysis for this parameter are not presented. Also, results of LSD test for treatments of factor Genotype and interaction Genotype × Medium are not presented, because their effects were not significant for tolerance indices for any of examined photosynthetic pigments' traits.

The highest values of tolerance indices for the examined photosynthetic characters were gained on L4 medium (10^{-3} M $Pb(NO_3)_2$) (Table 4). However, neither of the examined media was found to be useful in the differentiation of genotypes by tolerance indices for any of the examined photosynthetic traits.

DISCUSSION

The success of phytoremediation is considerably related to the ability of a plant to survive and grow in the presence of a pollutant in the growing substrate. This is particularly important for poplars, which have been well-recognized as species that play an important role in the phytoremediation of polluted sites. Some studies indicate that there is a small probability that the poplar plant would face lethal lead concentrations in soil solution. According to Huang et al. (1997), in most of the contaminated soils they examined, lead content in soil solution amounted to less than 0.1% of the total lead content in the soil. Also, Kališova-Špirochova et al. (2003) found that in a highly polluted industrial area, where contaminated soil contained 16000 $mg \cdot kg^{-1}$ of Pb^{2+}, the content of lead in water leachate was just 0.45 $mg.l^{-1}$ (i.e. 5.49 µM). Thus, it seems that genotypes should be selected in order to achieve vigorous growth on substrates polluted with lead in relatively tolerable concentrations.

In concordance with the results from some previous works (Kališova-Špirochova et al. 2003, Katanić et al. 2007, di Lonardo et al. 2011, Kovačević et al. 2013a), in most of the examined media no signs of intoxication were found on shoots. In this work, only sporadic necrotic changes were found on the roots formed in medium with 10^{-3} M $Pb(NO_3)_2$, which is consistent with the results by Kovačević et al. (2013a) on the same medium. However, Bojarczuk (2004) reported an inhibitory effect of the medium with pH 5.5 and 2.0 mM $Pb(NO_3)_2$ on shoot and root development of calli of hybrid aspen on regeneration medium. On medium with 1.0 mM $Pb(NO_3)_2$ they found that all traits, except shoot height, did not differ significantly from that in the control medium. This is in concordance with the results we gained on our L4 medium, which contained the same concentration of lead nitrate, and where the tolerance indices were around 1 for all examined traits.

Tolerance indices for shoot height and length of the longest root were mostly below 1 on L3 and L4 media, and above 1 on L1 and L2 media, indicating inhibition of shoot and root growth on high, and stimulating effect on low lead concentrations. These results are in accordance with the results by Seregin and Ivanov (2001), who found that low lead concentrations promote root growth. Baker and Walker (1989) extensively discuss the stimulative effect of low concentrations of heavy metals in a substrate, stressing that this phenomenon should be further examined, especially in non-essential metals, such as lead.

Different parameters that describe root growth and analog tolerance index are extensively used in heavy metal tolerance studies in plants (Baker and Walker, 1989). In our work the tolerance index for the number of roots was under the weak influence of the factor Medium, while Kovačević et al. (2013a) reported that this influence was significant and the presence of lead in the medium appeared to be stimulative. However, the effect of the factor Medium on tolerance index for the length of the longest root in their study was not significant, while in our work the inhibitory effect of high lead concentrations was significant. It could be assumed that these differences in results are caused by higher bioavailability of lead and consequently its higher toxicity in media studied in our work due to low pH and the presence of citric acid as a chelating agent in media, which is in concordance with the results by Yang et al. (2006).

Tolerance indices based on dry shoot and dry root mass accumulation were mostly higher than 1, suggesting the stimulating effect of the examined media on biomass accumulation, except for the inhibitory effect on dry root mass accumulation on L3 medium. Kališova-Špirochova et al. (2003) reported the stimulating effect of 10^{-4} M Pb^{2+} on total plant biomass accumulation in aspen rooted shoots in liquid

Table 4. Tolerance indices for content of photosynthetic pigments in white poplar genotypes grown on different concentrations of $Pb(NO_3)_2$ (Fisher's LSD test).
*) The differences between values marked with the same letter are not significant at the level α=0.05

$c(Pb(NO_3)_2)$ (M)	Chlorophyll a content	Chlorophyll b content	Chlorophyll a+b content	Carotenoides content
10^{-6}	0.898 b *)	0.990 ab	0.923 b	0.852 b
10^{-5}	0.903 b	0.882 b	0.897 b	0.898 b
10^{-4}	0.868 b	0.855 b	0.864 b	0.877 b
10^{-3}	1.180 a	1.189 a	1.182 a	1.156 a

medium *in vitro*. In concordance with our results, Katanić et al. (2007) found inhibitory effect of 10^{-4} M Pb·EDTA in growing medium on fresh biomass accumulation of white poplar shoots. Although necrotic changes were found on roots formed on L4 medium (10^{-3} M $Pb(NO_3)_2$), the stimulating effect of this medium on biomass accumulation was found in our work. This stimulation was probably caused by the nitrogenous component of lead nitrate, whose quantity was increased in this medium by 20% compared to control. While Bojarczuk (2004) used a twice as high concentration of $Pb(NO_3)_2$ to achieve the inhibitory effect, with lower pH and the presence of citrate acid, as a chelating agent, L3 medium achieved the inhibitory effect with a much lower concentration of lead, which is more likely to be found in the field (Kališova-Špirochova et al. 2003). These findings favor this medium to be used in further lead tolerance testing. However, the best genotype differentiation according to tolerance indices for most of the examined morphological and biomass traits was achieved on L2 medium (10^{-5} M $Pb(NO_3)_2$). Therefore, this medium should also be taken into consideration, especially in the evaluation of white poplar genotypes that would be used in afforestation of soils that are moderately contaminated with lead.

Regarding the content of photosynthetic pigments as a stress marker (Ghnaya et al. 2009), our results suggest a significant effect of the examined media on most photosynthetic characters, except chlorophyll a/b ratio. The strongest inhibitory effect was achieved on L2 and L3 media. The effect of L4 medium was stimulating, probably for the same reason as we assumed for biomass accumulation characters. However, Kovačević et al. (2013a) gained no such effects on media with pH 5.5 and with no chelating agents.

Extensive research has been done in describing the inhibitory effect of the high and stimulating effects of low lead concentrations on the content of photosynthetic pigments (Ewais 1997, Sarvari et al. 2002, Kaznina et al. 2005, Zengin and Munzuroglu 2005). According to Seregin and Ivanov (2001), the lead in toxic concentrations is responsible for restrained chlorophyll synthesis, resulting in decreased chlorophyll content. The same authors state that chlorophyll b is more affected than chlorophyll a, while numerous authors suggest decrement of chlorophyll a/b ratio on high lead concentrations (Sarvari et al. 2002, Kamel 2008, Kovačević et al. 2013a). However, the results of our research show that tolerance indices for the examined photosynthetic characters of the studied genotypes within the same medium were relatively similar, with no significant effect of factor Medium on tolerance index for chlorophyll a/b ratio.

In order to achieve a more critical test for the evaluation of white poplar genotypes for use in phytoremediation projects, we used modified medium with the intention to increase the bioavailability of lead by increasing its mobility both by lowering the pH and using citric acid as a chelating agent (Yang et al. 2006). Beside general benefits in lead testing *in vitro*, test on acidic media could be especially important for situations where heavy metal pollution is accompanied by acidification of soil, such as soil pollution after accidents in mining facilities (Antonijević and Marić 2008).

In comparison with the results gained by Kovačević et al. (2013a) on media with pH 5.5, it can be assumed that a stronger effect of lead in our work was achieved by lowering pH and using citric acid. Likewise, Vuksanović et al. (2017b) gained toxic effect and total absence of rooting of *Populus nigra* in medium with pH 3 and 10^{-3} M Cu^{2+}, while di Lonardo et al. (2011) reported absence of toxic effect on *Populus alba* for the same concentration of copper in medium with pH 5.2.

Generally, differences in lead tolerance between the examined genotypes were rather low and mostly non-significant. However, regarding tolerance indices obtained on L2 and L3 media, the differences between the examined genotypes can be discussed and their lead tolerance can be evaluated. In this case, genotype Villafranca was used as a standard, regarding its frequent use in biotechnological studies and its good *in vitro* performance in the presence of lead and some other heavy metals (di Lonardo et al. 2011). According to both length of the longest root and shoot height, genotype L-12 achieved the best tolerance, and together with LBM had the greatest tolerance indices, higher than 1. According to dry root and shoot mass accumulation, best tolerance achieved LCM which, together with L-12, achieved tolerance indices higher than 1. It was not possible to differentiate genotypes by examined photosynthetic characters neither on medium L3 nor on medium L2. Thus, considering the obtained results, it can be assumed that the genotype with the best performance in lead tolerance is genotype L-12, followed by LBM and LCM. Also, all examined genotypes achieved better lead tolerance than standard Villafranca genotype. These results are in accordance with Kovačević et al. (2013a), who obtained the best differentiation of white poplar genotypes on the medium with pH 5.5 and 10^{-4} M $Pb(NO_3)_2$, the highest tolerance index for shoot height was achieved by L-12 and L-80, while the highest tolerance indices for dry shoot accumulation were recorded for LBM and LCM. The most striking difference between two studies is the reaction of genotype L-80. In our and also in the work by Vuksanović et al. (2019b) L-80 achieved relatively good growth performance on control medium with pH 3 (see Supplementary File). However, in our study this genotype achieved lowest tolerance indices of all genotypes on lead treatment media L2 and L3 for the majority of examined traits, while its performance on lead treatment media with pH 5.5 in the work by Kovačević et al. (2013a) was relatively good. Thus, it seems that the low lead tolerance of genotype L-80 in our study is specifically a reaction to enhanced bioavailability of lead in conditions of low pH and in the presence of a chelating agent.

Baker and Walker (1989) discussed positively the use of tissue culture in heavy metal tolerance studies in plants, emphasizing the possibility of faster tolerance testing with a smaller amount of material and without destruction of the mother plant. Watson et al. (2003) and Pulford et al. (2002) demonstrated in *Salix* sp. that the results obtained in hydroponics and in the field are comparable. Doran (2009) and Capuana (2011) elaborated that the response of plants to environmental contaminants can be predicted on the basis of the results from tissue cultures, which would reduce the necessity and costs of conventional field experiments. It could be assumed that changes made in media proposed in this work in course of the increased bioavailability of lead would decrease differences between the results obtained *in vitro* and in field conditions. However, for final evaluation, further research should be performed in order to relate data from *in vitro* to field conditions, considering lower availability of lead in soil, higher juvenility of the material *in vitro*, and complexity of the interaction between plant and habitat.

CONCLUSIONS

According to the presented results, the presence of citric acid in the medium with low pH enforced the toxic effect of lead compared to previous similar studies. The strongest toxic effect was found for the medium with 10^{-4} M $Pb(NO_3)_2$ but the best differentiation between genotypes was achieved on medium with 10^{-5} M $Pb(NO_3)_2$. According to most of the examined parameters, lead tolerance in genotypes L-12, LBM, and LCM was better than in standard Villafranca genotype. Results of this work support the further application of *in vitro* tests in heavy metal tolerance research and evaluation in white poplar genotypes.

Author Contributions

BK, TG, NN conceived and designed the research, BK and GT performed laboratory analysis, BK, TG and VV processed the data and performed the statistical analysis, SO secured the research funding, supervised the research and helped to draft the manuscript, BK, GT and MM wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Potential Hazard of Open Space Fire in Black Pine Stands (*Pinus nigra* J.F. Arnold) in Regard to Fire Severity

Damir Barčić[1*], Tomislav Dubravac[2], Marko Vučetić[3]

(1) University of Zagreb, Faculty of Forestry, Department of Ecology and Silviculture, Svetošimunska 25, HR-10000 Zagreb, Croatia; **(2)** Croatian Forest Research Institute, Department for Silviculture, Cvjetno naselje 45, HR-10450 Jastrebarsko, Croatia; **(3)** Croatian Meteorological and Hydrological Service, Grič 3, HR-10000 Zagreb, Croatia

*** Correspondence:** e-mail: dbarcic@sumfak.hr

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ABSTRACT

Black pine (*Pinus nigra* J.F. Arnold) is one of the most important reforestation species for dry and rocky terrain in the sub-Mediterranean zone. Fire is an important factor in black pine stands that largely defines the distribution range of black pine and the floristic composition of its stands. Fire causes less damage during autumn or early spring when tree cones contain ripe seeds that can partially reforest burnt surfaces. Every fire is a potentially significant threat to forest habitats. There are many damages, from direct damages to wood mass and reforestation costs, to indirect ecological, edaphic, protective damages and the loss of biological diversity. The aim of prevention works in forestry is to reduce the number of fires and reduce burnt surface area per fire. Research was based on vegetation analysis performed on each experimental plot in a black pine stand. The assessment of fire severity was performed using the Canadian Forest Fire Weather Index (FWI). The assessment of the severity of fire indicates the suitability of conditions for the occurrence of forest fires, and enables sound and effective protective methods beginning with silvicultural works. The results indicate better quality stands in the higher parts of the sub-Mediterranean area. Taking into account the climatic parameter, the areas on the border with the continental climate have better habitat conditions for black pine. Also, attention should be focused on 2012 and 2015. High values (Figure 13 – 16.22, Figure 11 – 6.99, Figure 8 – 8.38, Figure 7 – 12.02 and 12.25, Figure 5 – 8.32) indicate the overlapping of extremely dry periods and extremely high summer temperatures, and probably a strong wind influence that further increases the index. Fire severity assessments indicate the suitability of conditions for the onset of fire.

Keywords: forest fire; black pine; sub-Mediterranean; combustible materials; flammability

INTRODUCTION

In the sense of amelioration, black pine plays an irreplaceable role in processes that halt habitat degradation and the expansion of various forms of erosion. Black pine (*Pinus nigra* J.F. Arnold), as a reforestation species for karst landscapes, has been used throughout most of Northern Adriatic coast (the Istrian Peninsula and the Kvarner region). Particular success has been achieved during the work and establishment of the Supervision branch, as the Inspectorate for the Afforestation of Karst, Bare Rocky Grounds and Flood Control in the area of Senj on the north Adriatic coast (Ivančević 1978, 2003, 2005). Furthermore, black pine has been one of the fundamental afforestation species in the Dinaric karst area of Croatia during large forest raising projects (which started 150 years ago) by Croatian forestry (Martinović 2003). Black pine is one of the most important species for the afforestation of dry and rocky grounds in the sub-Mediterranean zone, and it is found most often on limestone and dolomite substrates. On rocky grounds under the influence of high winds, with poor stand conditions, it makes pure stands, while in more suitable habitats, indigenous deciduous species are a strong competitor and suppress its development. Since pine is a pioneer species that progressively colonizes abandoned and devastated habitats, particularly after the depopulation of those grounds and the cessation of anthropogenic activities (cutting, livestock husbandry), the indigenous deciduous species slowly regain their dominant role. Pine is suppressed and remains only in the most extreme habitats where there is no competition (Trinajstić 1979, Rauš et al. 1995). Fire is an important factor in black pine stands, which largely defines the distribution range of black pine and the floristic composition of its stands. Fire causes less damage if it occurs in autumn or early spring, when the tree cones contain ripe seeds that can partially reforest the burnt surfaces (Anić 1957). Though this is an extremely versatile species for amelioration, unlike other pines, it is often heavily affected by the fire season, with restoration and succession after fire as the primary concern. According to Retana et al. (2002), in the period between 1994 and 1998, an area of 40,420 hectares affected by fire in Catalonia recorded very poor regeneration with black pine as the dominant species. It is important to point out that fires in the Mediterranean are determined by climatic conditions (Carmel et al. 2009). Fire can be considered a natural element of the Mediterranean forest, determining its species composition and landscape structure (Trabaud 1994, González Olabarria 2006). Long, dry summers with high air temperatures reduce humidity in the forest floor layer to less than 5% (FAO 2006), thereby increasing the ignition rate of combustible materials (Calabri 1990). These are usually open space fires; pursuant to the Firefighting Act (Official Gazette 106/99) and the Intervention plan for large open space fires (Official Gazette 25/2001), these are categorized as fourth- and fifth-degree fires. They include particularly valuable forest lands, exceptionally large open space areas, with a hazard index from high to very high. If there is a correlation of the ratio of fire frequency in the Mediterranean karst, lack of forestry and silvicultural works and poor potential for black pine regeneration, the situation becomes increasingly alarming. In that case, both the production (commercial) roles and general roles of black forests decline. Therefore, prevention works to protect forests from fire should be intensified in those areas and in those years when the assessment of fire severity is increased. This paper gives an overview of the assessment of fire severity in the sub-Mediterranean area where black pine stands are dominant. The aim of this paper is to point out the danger of open space fire in different conditions of the occurrence of black pine.

MATERIALS AND METHODS

Experimental plots were set up in black pine stands in the Istrian Peninsula and Kvarner (Hrvatsko primorje) regions. The selection of plots was defined on the basis of data from the Forestry Management Plan and reviews of geological, pedological and phytocenological maps. A range of factors was considered, such as stand age, human influence, microrelief, elevation, slope and insolation. Experimental plots were defined on an area of 625 m^2 and stand structure research was conducted. Plots were set up in stands over 40 years old. For each plot, all black pine trees were measured by diameter class, number of trees, basal area and wood volume.

To calculate the amount of forest litter, 3 samples were taken along the diagonal of the experimental plot with an area of 25×25 m. All samples were dried at room temperature and then dried at 55°C in two measurements. Drying of the samples in each measurement took 48 hours, and the interval between the two measurements was at least one hour (UNECE 2004).

Of the fifty experimental plots, forty-three were planted in forest pine cultures, while seven plots were isolated in natural black pine stands. The investigated area includes the eumediterranean, sub-Mediterranean and epimediterranean vegetation zone. In the investigated area, a division was made according to altitude (up to 150 m, from 150 m to 300 m, and over 300 m above sea level).

The study area (Rab, Senj, Rijeka, Poreč, Pazin) is most represented by the Cfsax climate, according to Seletković and Katušin (1992). It is a moderately warm rainy climate with hot summers and average monthly temperatures above 22°C. The winter rainy season is widely divided into spring and autumn-winter maximum. The driest part of the year occurs during the warm season.

Vegetation analysis was performed on each experimental plot, 625 m^2 based on the plant sociology method (Braun-Blanquet 1964, Dierschke 1994). Species abundance and cover was assessed using the expanded scale according to Barkman et al. (1964). Prior to numerical analysis, all assessments were transformed into an ordinal scale according to van der Maarel (1979). Plant nomenclature was taken from Nikolić (1994, 1997, 2000). The association of environmental variables and floristic composition of vegetation was analysed using Canonical Correspondence Analysis - CCA (ter Braak 1986, Jongman et al. 1995, Gegout and Houllier 1996).

The assessment of fire severity was performed using the Canadian Forest Fire Weather Index (FWI). Fire Weather Index consists of five sub-indices. They take into account the daily variations of moisture in different fuels, which have different response time depending on weather conditions, initial propagation velocities, fuel quantities and expected intensity of fire front expansion. This index is a numerical assessment of the potential intensity of fire for a standard fuel type and is a relative measure of the expected fire behaviour and daily requirement for fire supervision (Van Wagner and Pickett 1985, Vučetić 2000,Vučetić and Dimitrov 2000, Vučetić 2001). The assessment of fire severity is considered the most appropriate indicator of the potential fire hazard.

RESULTS

A comparison of the vegetation zones clearly shows the differences between the sub-Mediterranean zones and the lower zones at the border between the eumediterranean and sub-Mediterranean zones. On the Istrian Peninsula and Kvarner region, black pine primarily grows on limestone, even though it is also found on flysch substrates. It is most widely distributed in the sub-Mediterranean vegetation zone where it is also the most important species for karst afforestation.

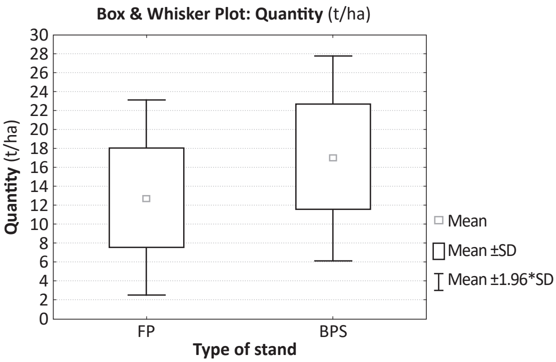

Figure 1. Quantity of forest floor (combustible matter) in forest plantations and natural black pine stands (FP = forest plantation, BPS = natural black pine stand).

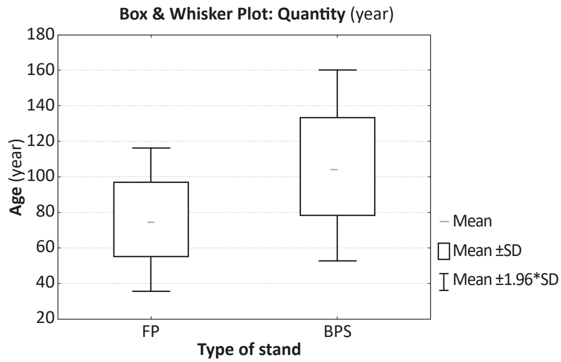

Figure 2. Age classification of natural stands (BPS) and forest plantations (FP) of black pine.

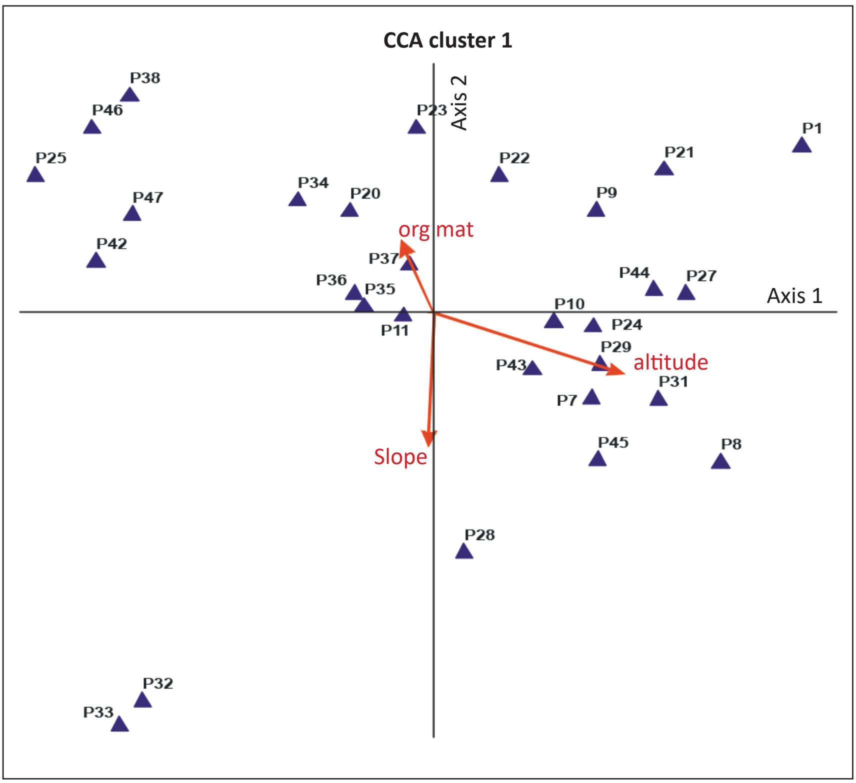

Figure 3. Canonical Correspondence Analysis (CCA) analysis of plot classification by elevation, slope and organic matter.

Table 1. Correlations according to Canonical Correspondence Analysis (CCA) analysis.

Variable	Axis 1	Axis 2	Axis 3
1 organic matter	-0.145	0.444	0.427
2 elevation	0.845	-0.378	0.145
3 slope	-0.024	-0.812	0.318

The results indicate better quality stands in the higher parts of the sub-Mediterranean area. Taking into account the climatic parameter, the areas on the border with the continental climate have better habitat conditions for black pine.

Seasonal potential of fire severity indicates an increase in severity between 2007 and 2013, which is contrast to the researched period from 1997 to 2006, where the limit value of 7 is not exceeded. For Senj and Pazin, the values for 2012, 2013 and 2015 are extremely high, unlike values for Rijeka, Rab and Poreč, where only in 2012 the severity of fires exceeds the reference value of 7 (obtained by applying the Canadian method of the Meteorological Index of Forest Fire Hazards (Vučetić 2000), according to the results by Williams and Van Wagner in Dimitrov (1998). The decisive reason might be several days with strong winds in the circumstances of extremely dry and hot summer.

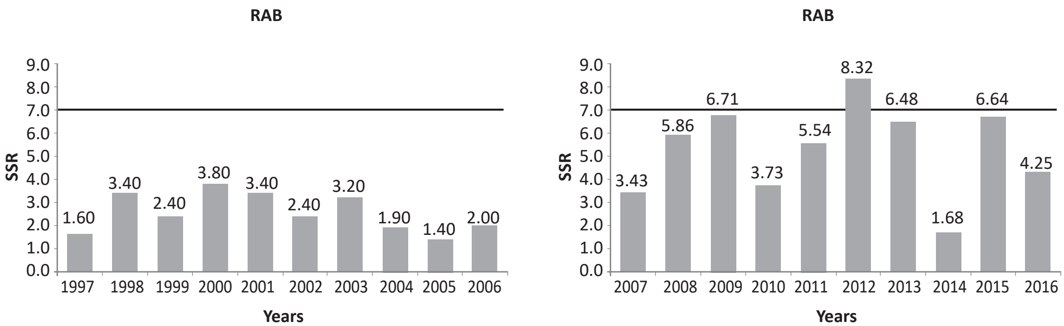

Figure 4. Seasonal potential of fire severity on the island of Rab (1997-2006, 2007-2016).

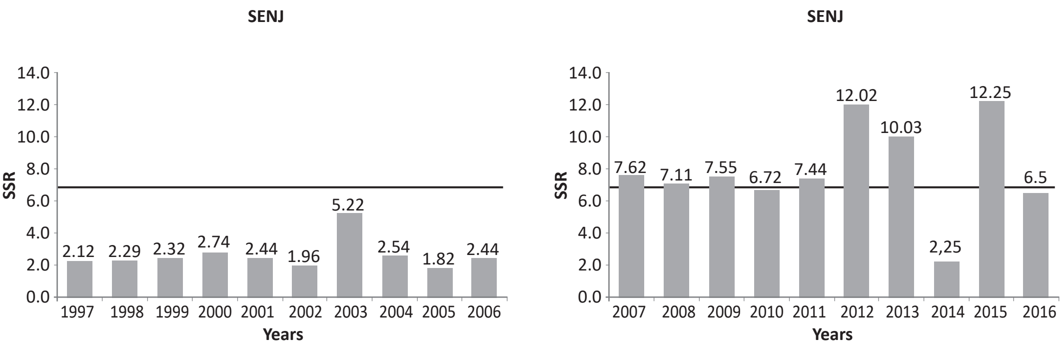

Figure 5. Seasonal potential of fire severity for Senj (1997-2006, 2007-2016).

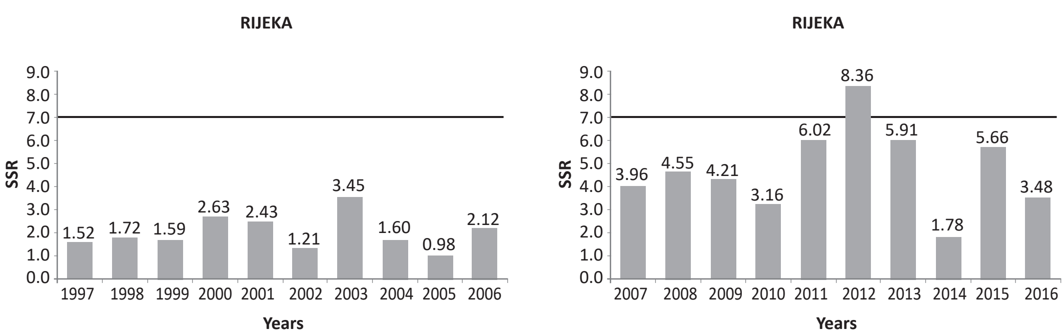

Figure 6. Seasonal potential of fire severity for Rijeka (1997-2006, 2007-2016).

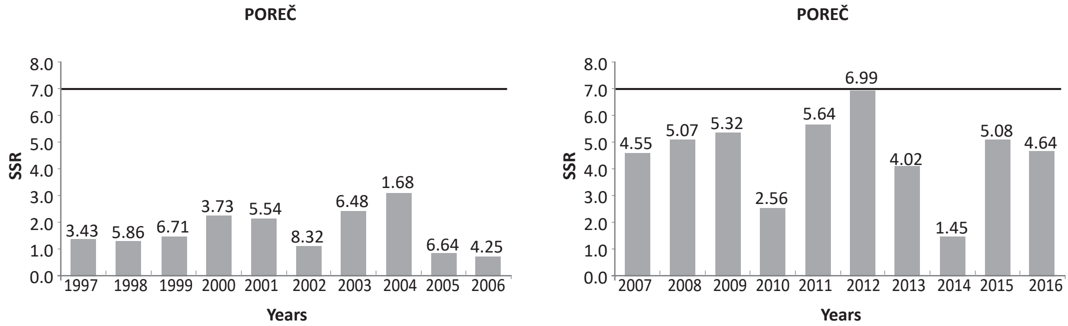

Figure 7. Seasonal potential of fire severity for Poreč (1997-2006, 2007-2016).

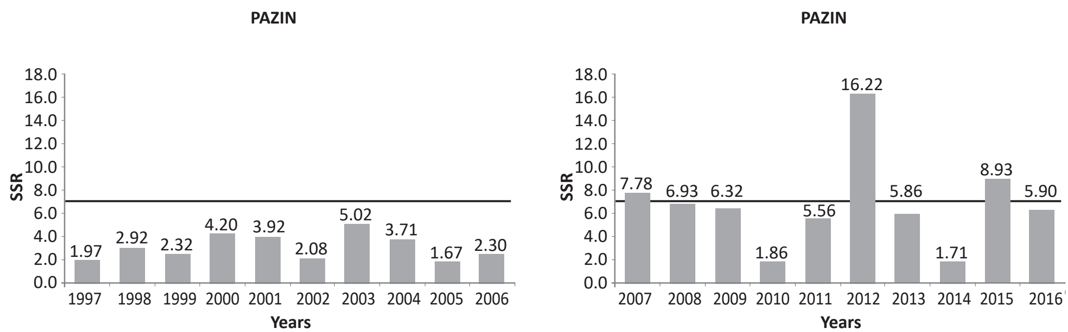

Figure 8. Seasonal potential of fire severity for Pazin (1997-2006, 2007-2016).

DISCUSSION

In the vegetation sense and in relation to fire hazard, black pine stands in the sub-Mediterranean zone are found in three elevation zones (low, mid and high). These zones in the study area are related to the proximity of the sea and the transition to continental climate. This is also the case in other Mediterranean countries (Gil and Aránzazu Prada 1993, Serrada Hierro, 1990, Grau Corbi et al. 1999). This zonation is related to higher quantities of precipitation at higher elevations. Habitat factors for black pine and other conifers in the Mediterranean have been observed through the prism of the Emberger pluviothermic quotient (Quézel 1976, M'Hirit 1999), as shown in Figure 9.

Weather and climate play a key role in the determination of the fire regime of an area, and the fire regime as such is very closely related to changes in climate (Kunkel 2001, Viegas et al. 2004, Pereira et al. 2005). The fire occurrence regime is characterised by its severity, rate of spread, summer season and frequency (Sousa 1984, Johnson and Gutsell 1994). In Croatia, research to date has warned that despite all efforts, the number of fires is increasing, as is the surface area affected (Rosavec et al. 2006, 2013). In the context of open space fire prevention, forest fire indices or danger assessment systems are used. The most commonly used is the Canadian system, called the Canadian Forest Fire Danger Rating System (CFFDRS), which consists of two subsystems: Fire Weather Index (FWI) and Fire Behaviour Prediction (FBP). This system has been applied in Croatia since 1981 (Mokorić and Kalin 2006). Though the climatic factor (temperature, precipitation) is important, since it is an integral factor in calculating potential severity, a large quantity of combustible materials is crucial for the spread and intensity of fire (Figure 1).

The first axis of the ordination scale is highly correlated with altitude. Overview of the correlations of variables is given below the graph (Figure 3). Taking into account pine stands and the relationship to environmental variables (Figure 2 and 3), the relationship to forest litter, altitude and slope is crucial. Litterfall intensity is strongly climate-dependent (Arneth et al. 1998, Starr et al. 2005). Among other factors, the age of the forest stand, species composition and density of trees also impact litterfall (Diaz-Maroto and Vila-Lameiro 2006, Starr et al. 2005). In our study, the correlation of forest litter depends on soil and plants' formation properties. Similar has been indicated in Banaszuk (2001) and Diaz-Maroto and Vila-Lameiro (2005).

Therefore, forestry and silviculture works are key in reducing the risk of forest fires, as these are the dependent

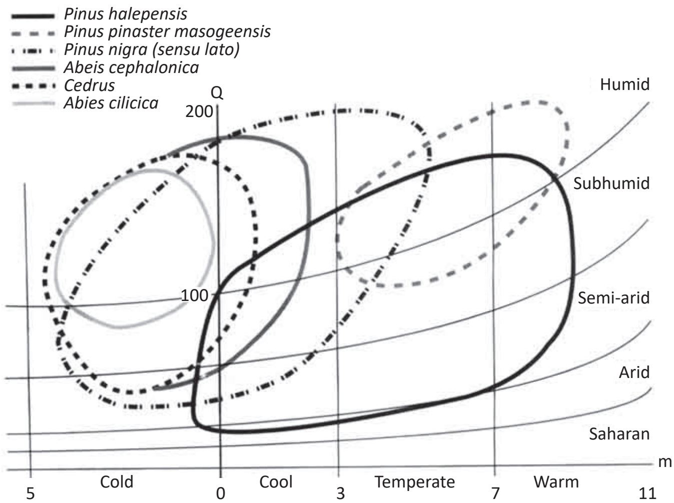

Figure 9. Climatic factors important for afforestation.

variables which can be altered. In the practical sense, and to minimize risk in raising black pine plantations, it would be useful to reduce the number of plants during afforestation works. Since afforestation typically uses a base of 2000 to 2500 plants per hectare (*Meštrović and Fabijanić 1994*), afforestation with 1000 to 1200 seedlings would result in a smaller scope of work in cleaning and thinning. Thinning in the plantations should begin between 25 and 30 years, and should be repeated every ten years. This would ensure a tree density of 500 trees per hectare at the end of the rotation (Bezak 1992). More intense thinning leaves only 140 trees per hectare, resulting in larger trees and the appearance of a range of deciduous tree species (Hamilton and Christie 1971). A 100-year old black pine tree (*P. nigra* ssp. *laricio*), as a commercial species, may have a wood volume of 450-900 $m^3 \cdot ha^{-1}$ and a density of 350-700 trees per hectare (CAB International 2000). The annual growth rate is about 4-6 $m^3 \cdot ha^{-1}$, and between 7-10 $m^3 \cdot ha^{-1}$ in the best habitats (Meschini and Longhi 1955, Bernetti 1995). Bernetti (1995) stated that in natural habitats, black pine has a wood volume of 150 to 450 $m^3 \cdot ha^{-1}$. Analysis of its potential uses for burned timber and considering it in the economic optimizations could also offer a new perspective in this field of research (González Olabarria 2006). Ultimately, at the end of the rotation, there is a better chance of producing a higher quality stand, both in the production and ecological sense, with a lower amount of combustible matter.

According to Chandler et al. (1983) and Bilandžija (1992), combustible matter or forest fuel is the entire quantity of plant material, both dead and alive, which lies above the mineral layer of the soil. All aboveground vegetation is potential fuel, and vegetation types differ only in their flammability and burning rate under certain weather conditions. The combustible substance in the stand increases fire risk depending on stand structure and composition (González et al. 2006). According to the Canadian fire danger rating system (Van Wagner and Pickett 1985), combustible matter is classified on the basis of the size of the materials. Therefore, the system differentiates between the indicator of fine fuel moisture, the indicator of medium-sized solid fuel moisture, and the indicator of solid fuel moisture. The relationships between forest fires and the potential influence on vegetation is shown in the results of the seasonal intensity of fire danger (Figures 4 to 8). It is assumed for our research areas, and there are no empirically determined limit values in the researched area (Dimitrov 1998). Values above 7.0 are considered to represent exceptional fire severity. Values between 3.0 and 7.0 indicate high to very high intensity. Moderate severity includes values between 1.0 and 3.0. All values below 1.0 indicate low fire severity.

There is a prominent difference between the exceptionally dry year in 2003, and average precipitation years, such as 2005. Attention should be focused on 2012 and 2015. High values (Figure 8 - value 16.22, Figure 7 - value 6.99, Figure 6 - value 8.36, Figure 5 - values 12.02 and 12.25, Figure 4 - value 8.32) indicate the overlapping of extremely dry periods and extremely high summer temperatures, and probably a strong wind influence that further increases the index.

Under such circumstances, the fire hazard in black pine stands is either reduced or increased depending on the annual climatic conditions. The aim of prevention works in forestry should be to reduce the number of fires and the surface area burnt per fire. Black pine currently has exceptional value in the ecological sense, such as its contributions to biodiversity in the karst landscape. According to the National Habitat Classification System and the Natura 2000 ecological network, and in line with the Ordinance on the list of habitat types, habitat maps and threatened and rare habitat types (Official Gazette 88/2014), the following are particularly valuable habitats. The priority habitat type (*9530) pertains to the (Sub-) Mediterranean endemic black pine forests (E.3.5.7.- As. *Ostryo-Pinetum nigrae* (Anić 1957, Trinajstić 1998), E.7.4.4.- As. *Cotoneastro-Pinetum nigrae* (Ht. 1938), E.7.4.5. - As. *Euphorbio triflorae-Pinetum nigrae* (Ht. 1956, Trinajstić 1999), E.7.4.6.- As. *Erico manipuliflorae-Pinetum dalmaticae* (Trinajstić 1986) and E.7.4.7.- As. *Junipero sibiricae-Pinetum dalmaticae* (Domac 1962, 1965)).

CONCLUSIONS

Under the circumstances of increasingly frequent appearance of extremely dry and warm years, increased hazards for forest ecosystems can also be expected. Stands of black pine (*Pinus nigra* J.F. Arnold), as the most important species for afforestation of the sub-Mediterranean Dinaric karst, are particularly threatened. Anthropogenic influences should be applied through forestry and silvicultural work (cleaning and thinning) to maintain stand stability in the sense of quality and to reduce the quantity of combustible materials. Clearing the lower branches and ensuring fewer trees per hectare can reduce and limit the speed of spread of a forest fire, or the transition of a low fire into a high fire. With the assumption of good forest road openness, the ecological and commercial damages can be significantly reduced even in the appearance of a forest fire. Fire severity assessments indicate the suitability of conditions for the onset of fire. These values are calculated for the assessment of seasonal severity, which is an indicator dependent on the environmental conditions of a certain area. In this case, a 20-year period has been shown. However, in order to obtain more accurate insight into the severity assessment, monthly or daily danger assessments should be made during the fire season, or specifically for the summer months.

Author Contributions

DB and TD conceived and designed the review article. It is based on field measurements, data processing and statistical analysis performed by MV and DB. TD helped to draft the manuscript, DB wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Who Are the Visitors of Forest Park Grmoščica and What Are Their Needs? Results of Quantitative Exploratory Survey

Martina Kičić[1,*], Ana Marija Marin[1], Dijana Vuletić[1], Ivana Kaliger[2], Ninoslav Matošević[2], Sergej Šimpraga[3], Silvija Krajter Ostoić[1]

(1) Croatian Forest Research Institute, Department for International Scientific Cooperation in Southeast Europe - EFISEE, Cvjetno naselje 41, HR-10450 Jastrebarsko, Croatia; **(2)** Croatian Forests Ltd., Forest Administration Zagreb, Operational unit Urban Forestry, HR-10000 Zagreb, Zagreb; **(3)** City of Zagreb, City Office for Agriculture and Forestry, Avenija Dubrovnik 12/IV, HR-10000 Zagreb, Croatia.

*** Correspondence:** e-mail: martinak@sumins.hr

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ABSTRACT

Forest Park Grmoščica is an important part of urban green infrastructure for the citizens of the western part of the city of Zagreb. To enhance the quality of management of the forest park to the satisfaction of its daily users, it is important to know their socio-demographic characteristics, visiting behaviour, recreational activities, as well as their perception of the forest park. The survey for users of the Forest Park Grmoščica was developed within the INTERREG DANUBE's URBforDAN project. It was filled out by visitors of the forest park using on-site face-to-face method and was also available online. The results of the survey provided information about the users of the Forest Park Grmoščica, their socio-demographic data, visiting habits and perception. Also, the typology of users was given depending on the activities they undertake in the forest park (cyclists, joggers, visitors who spend time in Forest Park Grmoščica with their families, and pet walkers) and their main characteristics. The obtained data can improve the management of the Forest Park Grmoščica in such a way that it fulfils its social and ecological function and is adapted to the needs of its users.

Keywords: urban forestry; participatory planning; visitors' needs; survey

INTRODUCTION

Urban forests are an important part of urban green infrastructure. Their importance is growing with the expanding number of people living in urban areas and considering the number of benefits they provide to urban dwellers (Haase et al. 2014). Among those benefits, cultural ecosystem services provided by urban green spaces are those which urban dwellers recognize as important, mostly because of their recreational potential (Dou et al. 2017). Research shows the importance of having accessible and well-maintained urban green space with facilities for different types of users at close distance to their home (Krajter Ostoić et al. 2020a). Sustainable forest management implies not only sustainable wood and biomass production but also attention to the permanent provision of forest ecosystem services. Hence, forestry experts encounter requests to consider users' needs when planning for future forest management in urban areas (Bethmann et al. 2018).

Research on the recreational use of forests in relation to human needs and preferences has long been present in Europe's scientific literature based on review by Ciesielski and Stereńczak (2018). Also, based on to-date research, urban forests and forest parks are among better-explored elements of urban green infrastructure in Croatia. However, social perspectives (use, perception, preferences, attitudes) are less addressed in comparison to other topics such as green space planning and design or green space inventory (Krajter Ostoić et al. 2020b). A number of factors influence forest's recreational value. Some of them are related to forest characteristics and others to characteristics of the visitors (Agimass et al. 2017). Forest characteristics influencing their recreational use are forest area accessibility, forest appearance and forest management type, as well as forest terrain characteristics (Ciesielski and Stereńczak 2018, Gerstenberg et al. 2020). Furthermore, users' socio-demographic characteristics such as age and achieved level of education or having a dog can influence recreational use of forests (e.g. Roovers et al. 2002, Arnberger and Eder 2007, Karanikola et al. 2017). Having different user groups with different expectations and needs, who use the same space at the same time can lead to negative consequences, such as overcrowding, or contribute to conflicts among the users (Arnberger 2006). Understanding different users' behaviour and needs can facilitate mediation among them, resulting in better forest management (Larondelle and Haase 2017).

In Zagreb, qualitative research on the sample of citizens shows that they perceive various types of tree-based urban green space, especially forests and forest parks, as holders of different cultural ecosystem services, including recreation (Krajter Ostoić et al. 2020a). The same research shows that forests provide more recreational opportunities than other types of urban green space, meaning that people reported more recreational activities they undertake in them. Expectedly, walking is the most important recreational activity in forests along with jogging and cycling, while activities such as hunting and mushroom picking are specific only to forests as such.

In the City of Zagreb, there are 22 forest parks. Their total surface area is 356 ha of which 185 ha is state-owned and 171 ha is privately owned (Matić 2010). Regardless of ownership, people in Croatia can use forests for rest and recreational purposes. Forest parks in the City of Zagreb are managed by Croatian Forests Ltd., Forest Administration Zagreb, Operational Unit Horticulture, while management of privately owned forests is the responsibility of their owners (Krajter Ostoić 2013). The City of Zagreb also takes care of the maintenance of the forest parks based on annual financial plans that finance adapted management of forest parks, while long-term planning and management of forest parks in the city of Zagreb is based on Forest Management Plan (2014 - 2023) made by Croatian Forests Ltd. for all forest parks in Zagreb as one management unit (privately and state-owned). Although there is no obligation for including the public into planning and management process with regard to urban forests, there is common practice in Croatia that forest management plans are presented to the interested public in the form of a public exhibition, where all those who are interested can see draft forest management plan, or in the form of a public presentation, where all those who are interested can join the presentation and give comments. When it comes to urban green spaces, in Croatia there is neither obligation for monitoring visitors of urban green spaces nor obligation for conducting surveys with them when designing new green spaces (Krajter Ostoić 2013). This can result in spaces that do not correspond with users' needs and habits and can consequently lead to conflicts among users or discourage users from using such urban green space.

The City of Zagreb and Croatian Forests Ltd. are project partners in INTERREG DANUBE's project "Management and Utilization of Urban Forests as Natural Heritage in Danube Cities" (URBforDAN) (URBforDAN 2020). Main goals of the project are to set new standards in the sustainable urban forest management, to develop internationally applicable urban forest management plans and to improve visitors' experience. There are 16 project partners from 10 countries. The project's focus area for the City of Zagreb is Forest Park Grmoščica. In Grmoščica the aim is to improve the provision of recreational and educational services using a participatory planning approach. Three workshops with local stakeholders representing the local community, local sports associations, local authority, local public school, and representatives of forest administration have been held within the URBforDAN project.

Participatory planning in urban forest management implies involvement of relevant stakeholders into the planning process. The advantage of such approach is that participation of all relevant stakeholders allows the process to be democratic and fair as well as transparent (Reed 2008). The author also states that stakeholders' participation can improve the quality of environmental decisions by broadening gathered information. When it comes to urban forest management, adopting public values into management practice can in future lead to increasing citizen participation as well as better addressing of climate change issues (Ordóñez Barona 2015). There is also a learning advantage with citizen's participation because engaging in matters related to forest management can lead to increasing one's knowledge and shaping new ways of interpretation (Bethmann et al. 2018). Previous research on the governance of urban forests in the City of Zagreb based on in-depth interviews with stakeholders has noted that some of the stakeholders indicate participation as one of the elements of governance that needs to be improved (Krajter Ostoić 2013).

Site-specific research such as the one presented on forest park visitors, their characteristics, preferences and views are scarce in the City of Zagreb and in Croatia in general (Krajter Ostoić et al. 2020b). Therefore, this research adds valuable information for improving future forest park management in Zagreb. We believe that the results of our study can inform urban forest managers about urban forests' visitors, their characteristics and visiting behaviour with aim to incorporate these findings into future planning practice or encourage new similar research in the future. This paper presents the results of the conducted questionnaire survey on the visitors and users of Forest Park Grmoščica, with the answers to the following research questions:

1. Which are the main characteristics of Forest Park Grmoščica's users?
2. What is their visiting behaviour?
3. Which are the main characteristics and visiting behaviour of most frequent forest park's user types?
4. What are the problems that forest park users perceive and what are their suggestions for improving current forest park state?

MATERIALS AND METHODS

Study Area

The study area is Forest Park Grmoščica in the City of Zagreb, capital of the Republic of Croatia (Figure 1). Forest Park Grmoščica is located in the northwest of Zagreb at the border between city districts of Črnomerec and Podsused-Vrapče. It extends between Vrapčak stream on the west, Kustošak stream on the east, Ilica Street on the south and Graberje Road on the north (Figure 1). It is 53.3 ha in size and the highest point of the forest park is at 240 m above sea level. Of the total surface area, 10.8 ha is privately owned. Few different forest stands can be encountered in the forest park out of which the most represented are: sessile oak with beech, common hornbeam and other tree species and black locust mixed with sessile oak, common hornbeam and other species (Anić and Oršanić 2010). At the moment of drafting this paper there were about 1000 m of forest paths, six benches and an observation deck in the forest park (Posavec 2020). Grmoščica was for a long time in the condition that could not satisfy the needs of the local population (Šimpraga 2011). However, its recreational and ecological potential as part of the network of green spaces in the city of Zagreb has been recognised (Mravunac 2015). URBforDAN project aims at enhancing the forest park's management through improving forest park's recreational role by providing adequate infrastructure adapted to different types of users.

Survey

For the purpose of this project, a survey for the users of Forest Park Grmoščica was developed. A survey design allows for quantification and generalization of populations' attitudes and opinions by studying its sample (Creswell 2003). Therefore, it is a suitable method to use when studying a forest park with its numerous users. Literature review on the human relationship with urban green spaces indicates that survey is the most common method used in research on users' preferences and perceptions (Kabisch et al. 2015). Furthermore, the survey was also used as a method of data collection about characteristics and preferences of users visiting urban forests or forests with emphasised recreational functions in multiple studies with one or more individual forests (Roovers et al. 2002, Aasetre et al. 2016, Larondelle and Haase 2017, Meyer et al. 2019), or in national research on forest recreation (Getzner and Meyerhoff 2020, Šodková et al. 2020). The survey used for the purpose of this research was developed within URBforDAN project, translated into Croatian language, and adapted to specific forest park conditions (see Supplementary File 1). It is comprised of 14 questions dealing with socio-demographic characteristics of users, activities they undertake when visiting the forest park, their visiting habits regarding visiting Forest Park Grmoščica, as well as the suggestions concerning the enhancement of its management. Data was collected between November 2018 and April 2019 by applying a mixed-mode approach that combined face-to-face and online data collecting. The main goal when using mixed-mode data collection is to reduce survey error by balancing one method's shortcomings (De Leeuw 2005). In our case, the rationale was to allow for usual users who were not on the site at the moment of data collection to answer the survey and be included in research to minimise the bias. Also, the survey was conducted in the less favourable period of the year (late autumn and winter) when usually there is fewer visitors in urban green spaces. Nevertheless, when using mixed-mode approach, particularly in online data collection, completely overcoming the bias is not guaranteed (Larondelle and Haase 2017).

Employees of Croatian Forests Ltd. carried out face-to-face data collection with users on-site, while the same

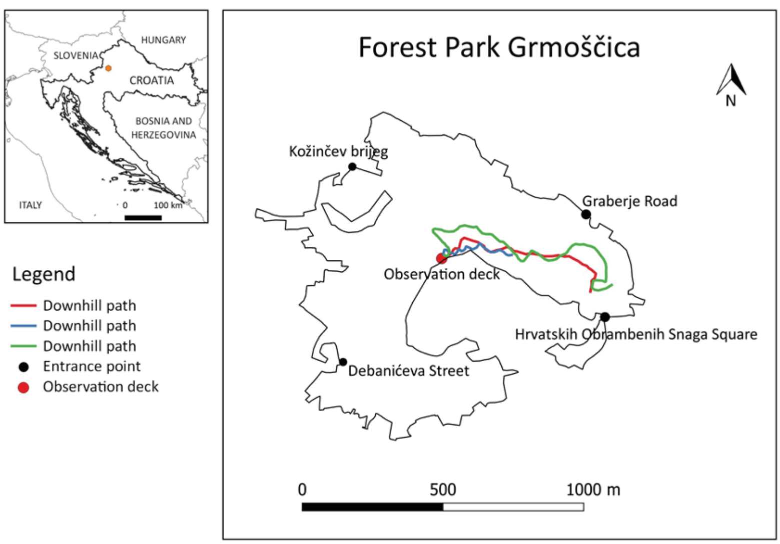


Figure 1. Forest Park Grmoščica at the beginning of URBforDAN project (2018). Country borders are based on EuroGraphics and UN-FAO, @EuroGraphics.

survey was available online at 1KA (1KA 2018) platform. The survey was also taken by participants at workshops organized within the project with different stakeholders and types of users. Information about the survey with the link to it was also communicated on the project's webpage. Data collected by face-to-face interviews on-site and during workshops was inserted into 1KA survey to have all data in one database.

Descriptive statistics was performed on collected data using R (v.3.6.2) (R Core Team 2019), while answers to open-ended questions were coded and presented accordingly. Subsequently, users have been divided into categories concerning types of stated specific recreational activities they undertake and their ranking. General users' main characteristics have been presented as well.

RESULTS

In total, 149 people participated in the survey. Out of that number, 99 surveys (66.4%) have been completely (91) or partially (8) completed and used for further analysis.

Socio-Demographic Characteristics

There was a similar representation of females and males in the sample (Table 1). About one third of respondents were in the age group 45-60, while the least number of respondents were older than 60. Two thirds of the respondents had a faculty degree. Around 80% of the respondents were employed or private business owners. Most respondents stated that they lived in Zagreb. Around one fifth of respondents lived close to Forest Park Grmoščica (up to 1 km to Grmoščica), and the same share stated that they lived up to 3 km to Grmoščica.

Visiting Behaviour of Users of Forest Park Grmoščica

The highest number of the respondents stated that they visited Forest Park Grmoščica several times per year, while the least of the respondents visited every day (Table 2). However, cumulatively close to a quarter of the respondents state that they visit forest park at least once per week. Interestingly, users in the younger age group (18-30) reported more frequent visits to Grmoščica, where 82% of them stated that they visit Grmoščica at least once a month, with 40% of them reporting visiting Grmoščica several times a month and 25% several times per week. Users in age groups 30-45 and 45-60 mainly stated that they visit Grmoščica several times a year. Two-thirds of the respondents live at a distance of up to 5 km from Forest Park Grmoščica. Among those, the ones who live in a proximity to Forest Park Grmoščica (up to 1 km and 1-2 km) reported more frequent visiting behaviour than those

Table 1. Sociodemographic description of the sample (N=99).

Variable	Category	Frequency	%
Gender	Male	51	52
	Female	48	48
Age	18-30	28	28
	30-45	29	29
	45-60	36	37
	> 60	6	6
Highest achieved level of education	Elementary school	5	5
	High school	28	28
	Faculty	51	52
	Doctorate	15	15
Employment	Employed	76	77
	Private business owner	3	3
	Farmer	1	1
	Retired	6	6
	Unemployed	7	7
	Student	6	6
Place of residence	Radius of up to 1 km to Grmoščica	19	19
	Radius of up to 3 km to Grmoščica	18	18
	City of Zagreb	49	50
	Outside the City of Zagreb	13	13

who live at a distance of 2-5 km and more than 5 km, where the respondents usually expressed that they visited forest park several times a year. Car was the most preferred mean of transportation for our respondents. However, about the same share were those who arrived on foot or by bicycle, while the least number of respondents used public transportation. The majority of users who stated that their distance to the nearest entry to Grmoščica is up to 1 km or 1-2 km as main way of arrival said on foot or by bicycle - 58% of them who live at the distance of up to 1 km reached Grmoščica on foot and 54% of them at the distance of 1-2 km reached Grmoščica using a bicycle. Those who live at 2-5 km to the nearest entrance used all the means of transportation equally, while those who stated that they lived more than 5 km from the nearest entrance predominantly reported using the car as way of transportation (72%). Comparing the reported visiting frequency of the respondents and their way of arrival to Grmoščica we can claim that out of those who reached Grmoščica on foot, using public transportation or by car the majority of them stated visiting several times per year. Nevertheless those who reached Grmoščica riding a bicycle report more frequent visiting behaviour, with 78% of them visiting several times per week, once a week or several times a month. Out of four offered possible entrances into the forest park (Figure 1), visitors mostly use the entrances from Ilica Street. Regardless of the frequency of visit, more than half of the respondents spent one to two hours in the forest park and the least number of them spent more than 5 hours in the forest park.

When asked about the activities they undertake during the visit to Forest Park Grmoščica, the respondents had a multiple-choice closed-ended question. They were also asked to rank multiple activities where 1 was the most important activity and 12 the least. The highest number of the respondents stated that they visited Forest Park Grmoščica to spend quality time with their family, then for pet walking, biking, hiking and jogging (Figure 2). The smaller number of them stated collecting forest products, forestry work, downhill mountain biking, scientific work, education in nature, taking pictures of nature, and horseback riding.

Table 2. Visiting behaviour of users of Forest Park Grmoščica (N=99)

Variable	Category	Frequency	%
Frequency of visit	Every day	6	6
	Several times per week	12	12
	Once per week	8	8
	Several times per month	20	20
	Once per month	8	8
	Several times per year	45	46
Distance to the nearest	Up to 1 km	19	19
entrance to Grmoščica	1-2 km	13	13
	2-5 km	31	31
	More than 5 km	36	37
Way of arrival to Grmoščica	On foot	22	22
	-By bicycle	23	23
	Using public transportation	14	14
	By car	40	41
Entrance	Ilica (Debanićeva Street)	26	26
	Ilica (Hrvatske Obrambene Snage Square)	32	33
	Graberje Road	22	22
	Kožinčev brijeg	19	19
Duration of visit	Up to 1 h	16	16
	1-2 h	51	52
	2-5 h	19	19
	More than 5 h	5	5
	NA	8	8

Typology of Visitors

With regard to stated activities the respondents undertake during the visit to Forest Park Grmoščica, they were classified into the following types of users: cyclists, joggers, pet walkers and visitors who spend time in Forest Park Grmoščica with their families. Respecting numerous functions of the forest park we decided to ground our typology of users on those activities that were preferable for the respondents in our sample, i.e. spending quality time with family, pet walking, biking and jogging. We decided to exclude hiking, although highly ranked, in favour of jogging because jogging is for more respondents the most important reason for visiting than for those who hike. In each type we included those respondents who ranked the afore mentioned activities as reason number 1, 2 or 3 for visiting the forest park. For each type of users, we provided socio-demographic characteristics, visiting habits and perception of their activity regarding possible conflicts with other users and negative impact on nature (Table 3).

Cyclists

Mostly, users in the cyclist category are in the age group between 18 and 30 years (Table 3). The highest achieved education level for most cyclists is faculty. The majority are employed and the highest number of them (82%) stated that they lived in a radius of up to 5 km to the nearest entrance to Forest Park Grmoščica. The highest number of cyclists stated that they visited Grmoščica several times per week or several times per month and to a lesser extent once a month or every day. Out of those who reported living in the proximity of 1 km to Grmoščica 67% of them visit it several times per week, out of those who reported living in a radius of 1-2 km to Grmoščica 63% of them reported visiting at least once a week or frequently, and those who live the furthest visit it least frequently, meaning 60% of those living more than 5 km to the nearest entry visited the forest several times a year. They predominantly arrived in Grmoščica by bicycle, but also by car or public transportation. Those who live in a radius of up to 5 km of entrance to Grmoščica expectedly arrived by bicycle, while the minority of them who arrive from more than 5 km to the nearest entrance used a car to reach the forest park. On average they spend in Grmoščica between one and two hours, and only a few stated spending between two and five hours or more.

Most cyclists did not perceive their activities as conflicting with other visitors. Some said that it might cause conflicts with walkers. None perceived their activities harmful to nature.

Joggers

Joggers were mainly in the age groups 18-30 and 45-60, and to a lesser extent in the age groups 30-45 and older than 60 (Table 3). Joggers were for the most part employed and with a faculty degree. The most joggers came to the forest park from a distance of up to 5 km to the nearest entrance, only a third of them came from a greater distance. Joggers often reported visiting Grmoščica several times per year or several times per month. The joggers who lived at a distance of up to 1 km from the nearest entrance reported weekly (at least once a week) visits to the forest park, those at a distance of 1-2 km visit several times a month, those at a distance of 2-5 km several times a year, while those who live at a distance greater than 5 km reported visits weekly, monthly or yearly in similar share. They arrive in Grmoščica using public transportation, car, or bicycle, and most rarely on foot. Arriving using a bicycle, public transportation or by car was reported in similar shares, although those who came from a distance of up to 1 km mostly used a bicycle, while those from a distance of 1-2 km used a bicycle or car. Many of the joggers from a distance of 2-5 km came using public transportation, and those who needed to cross more than 5 km to Grmoščica used public transportation or a car. Usually, they spent between one and two hours in the forest park.

None of the joggers stated that their activities in Grmoščica might create conflict with other users, or that their activities could have a negative impact on nature, mostly because they did not leave trash in the forest park.

Figure 2. Activities of visitors of Forest Park Grmoščica and their ranking based on the relative importance (N=99).

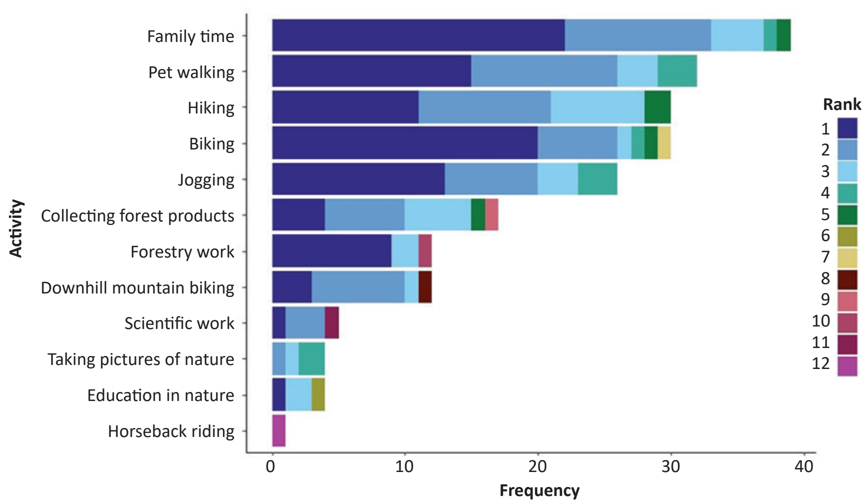


Table 3. Characteristics and visiting behaviour of different types of visitors in Forest Park Grmoščica (N_{cyc}=27, N_{JOGG}=23, N_{PW}=29, N_{FAM}=37).

Variable	Category	Users			
		CYC (%)	JOGG (%)	PW (%)	FAM (%)
Age	18-30	48	48	24	13
	30-45	37	13	21	27
	45-60	15	39	48	49
	> 60	0	0	7	11
Highest achieved level of education	Elementary school	7.5	0	4	0
	High school	33	35	24	16
	Faculty	52	48	55	65
	Doctorate	7.5	17	17	19
Employment	Employed	78	83	66	86
	Private business owner	0	0	10	0
	Farmer	4	0	0	0
	Retired	0	0	7	8
	Unemployed	7	13	3	3
	Student	11	4	14	3
Distance to the nearest entrance to Grmoščica	Up to 1 km	22	17	31	21.5
	1-2 km	30	13	14	11
	2-5 km	30	39	31	21.5
	More than 5 km	18	31	24	46
Frequency of visits	Every day	3	4	14	3
	Several times per week	22	13	21	3
	Once per week	19	9	10	5
	Several times per month	26	35	10	16
	Once per month	11	0	7	5
	Several times per year	19	39	38	68
Way of arrival to Grmoščica	On foot	0	4	38	30
	By bicycle	78	30.5	21	3
	Using public transportation	3	35	7	19
	By car	19	30.5	34	48
Duration of visits	Up to 1h	4	17	24	8
	1-2 h	70	48	62	62
	2-5 h	15	26	14	19
	More than 5 h	4	4.5	0	5.5
	NA	7	4.5	0	5.5
Conflicts with other users	Yes	15	0	14	8
	No	78	96	86	87
	NA	7	4	0	5
Negative impact on the nature	Yes	0	0	0	0
	No	93	96	100	95
	NA	7	4	0	5

CYC - cyclists, JOGG - joggers, PW - pet walkers, FAM - visitors who spend time in the forest park with their families

Pet Walkers

Most of the visitors in this category were in the age group 45-60 (Table 3). For most of them highest achieved education level is faculty and the majority of them are employed. More than half of pet walkers arrive in Grmoščica from a distance of up to 5 km, the rest of them stated arriving from a distance greater of 5 km. Although the highest number of pet walkers stated visiting Grmoščica a few times annually, more than half of them visited Grmoščica more frequently (every day, one or several times per week and several times per month). When considering visiting behaviour of pet walkers with regard to distance from the nearest entry to Grmoščica, we can state that of those pet walkers who reported living at a distance of 1 km to the nearest entry 89% of them visit Grmoščica at least several times a month, 50% of those who live at a distance of 1-2 km reported using forest park once or several time a week, 56% of those who live at a distance of 2-5 km stated visiting several times a year, as well as 43% of those who arrive from greater distance than 5 km to the nearest entrance. Pet walkers reached Grmoščica on foot, followed by the arrival by car and by bicycle, seldom using public transportation. Pet walkers who came to Grmoščica from its proximity (up to 1 km) arrived on foot or use a bicycle just like the ones who came from a distance of 1-2 km from the nearest entry. For arrival from a distance of 2-5 km pet walkers used all means of transportation, while those who came from a greater distance of 5 km solely used a car as mean of transportation. They spent between one and two hours in the forest park.

The larger number of pet walkers did not perceive that their activities could cause conflicts with other visitors. Part of them assumed that their activities could interrupt cyclists. The same as previous types of visitors, the pet walkers as well did not perceive their activities as potentially harmful to nature. However, some of the users have stated concerns that dogs could chase away wild animals, while others were of opinion that if the dogs were on a leash, no damage could be done.

Visitors Who Spend Time in Forest Park with Their Families

This was the largest category of visitors. They are generally in the age group of 45-60 years (Table 3). For the most part, they are well-educated and employed. Visitors in this category in similar share came to Grmoščica from a distance of up to 5 km and more than 5 km, 54% and 46% respectively. They visited Grmoščica several times per year or possibly several times per month. Regardless of the distance from the nearest entrance to Grmoščica, 68% of the respondents have reported visiting forest park several times a year, only those visitors who reported living at a distance of up to 1 km from the nearest entry came more frequently and reported weekly visits to the forest park. Majority of them arrived in Grmoščica by car or on foot, while the least of them used a bicycle. Those who live in the proximity of up to 2 km to the nearest entry mostly arrive to Grmoščica on foot, while those who need to cross over from 2 to up to 5 or more km to the nearest entrance mostly use a car and to a lesser extent public transportation. Visits by those who spend time in the forest park with their families lasts between an hour and two.

The majority of visitors who spend time in the forest park with their families did not think that their activities could cause conflicts with other visitors. Only some of them stated the possibility of conflicts with cyclists in the area. Because they reported not leaving any trash behind, they consider that their activities do not have a negative impact on nature.

Perceived Problems and Suggestions for Improvement of the Current State of Forest Park Grmoščica

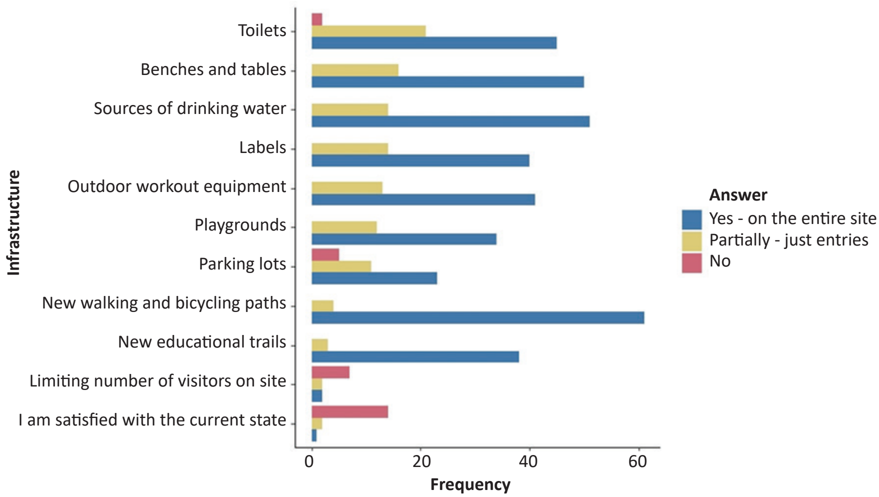
Out of 91 respondents that answered the open-ended question about perceived problems in the forest park, 57 of them (62.6%) noticed problems concerning Forest Park Grmoščica. Some of the most pronounced problems they expressed were problems related to waste - mostly illegal waste disposal (34 respondents perceived them). Visitors also highlighted neglect (15) manifested by the presence of weakly maintained buildings and forest paths. The third problem that users expressed (12) is the lack of equipment (benches, tables, trash bins) or lack of infrastructure (maintained and marked paths, hospitality facilities). Some of the respondents (3) stressed out the fact that the forest park is located at the landslide and there is a need for landslide management. In addition to these problems, the users indicated problems regarding construction works in the forest (4), behaviour of other users (3), excessive cutting (3), sewage (2), and accessibility of the forest park (1).

Besides questions about perceived problems and shortcomings, the respondents were asked about their support for installing new equipment and infrastructure in the forest park area. These possibilities were predefined and the respondents had three possible answers (Yes – on the entire site, Partially – only at entrances, No). The majority of the respondents agreed on placing new equipment and infrastructure in Forest Park Grmoščica, particularly new walking and bicycling paths, drinking water, new benches and tables (Figure 3). The respondents were not in favour of limiting the number of users on the site.

When asked about activities that should be developed in the future in a form of an open-ended question, 39 of the respondents stated that there was no need for new activities, while some addressed general activities such as sport (9) and recreational (4) activities, or, more specifically, cycling (19), jogging (4), spending time in nature (4) and walking (3). Furthermore, they stressed activities related to forest management (for instance planting trees, increased forest maintenance) (6), hospitality services (5), tourism (4) and education in nature (2). Also, some of the respondents suggested activities associated with protecting and observing nature, such as forest clean-up days (4), landslide management (4) and bird watching (2). Lastly, a few respondents stated activities for children (2), horseback riding (1), social activities (1) and relaxation (1).

Finally, the respondents were asked about the activities they think should be restricted in Forest Park Grmoščica in an open-ended question. About one third of the respondents (37 of them) stated that there were no such activities that should be restricted. Others emphasised the need for implementation of means to prevent illegal waste disposal

Figure 3. The extent to which users support the installation of new infrastructure in Forest Park Grmoščica (N=91).



(16), limiting motor vehicle traffic in the forest park area (15) and restriction of all activities that damage the nature (12), as well as the restriction of construction work in the forest (9). Lastly, cutting (4), commercial activity (3), downhill biking and overcrowding (2) and other users' inadequate behaviour (1) were reported among the activities that should be restricted.

DISCUSSION

In order to manage and protect forest parks it is necessary to explore and quantify their social functions (Matić and Prpić 1997, Larondelle and Haase 2017). This research was conducted with the intention to improve Forest Park Grmoščica's planning and management with participation and inputs of forest park's users. The small number of respondents can be explained with the survey being administered during winter months, even though the survey was also available online. Scientific literature indicates that visiting forest parks is less frequent in winter than in summer months (Tyrväinen et al. 2003). In comparison to the general population of the city of Zagreb, gender distribution of the respondents was good. Distribution of age groups was relatively good, with the majority of the respondents in the age group of 45 to 60 years (Table 1). Representation of elderly citizens (above 60 years) in the sample is only 6%, which is less than the amount of them in the general population (SYCZ 2019). Justification of that can be terrain configuration which can cause problems for the elderly, especially if they have trouble walking. Highly educated respondents were overrepresented in the sample (Table 1) in comparison to the general population (SYCZ 2019). However, this is common in other similar studies (Roovers et al. 2002, Lupp et al. 2016, Karanikola et al. 2017, Larondelle and Haase 2017).

The majority of users stated that they spend up to two hours in Forest Park Grmoščica (Table 2), what is also in line with similar research (Roovers et al. 2002). For the most part the users of Forest Park Grmoščica are citizens of local districts, meaning that they arrive from the forest park's proximity (Table 2). Accessibility of the forest or some other green space is essential for its recreational use (Hegetschweiler et al. 2017). Increasing the distance that users need to cross to use some green space for recreation decreases its use (Schipperijn et al. 2010). The majority of users of urban as well as rural forests are people living in their proximity (Meyer et al. 2019). Information such as visiting frequency, means of transportation to the forest park and entrances mostly used by visitors can help in planning the future infrastructure for visitors, as well as managing visitors themselves by separating users whose activities can potentially be in conflict (e.g. cyclists and pet walkers).

Recreation is often an underlying motivation for interaction with urban green spaces (Krajter Ostoić et al. 2020a). Walking is universally the most common recreational activity in forests (Roovers et al. 2002, Arnberger 2006, Gerstenberg et al. 2020, Krajter Ostoić et al. 2020a, Šodková et al. 2020). Visiting Forest Park Grmoščica to spend quality family time was a priority activity for most respondents (Figure 2). Likewise, this social activity is frequently mentioned as a highly-ranked activity and motivation for visiting the forest (Larondelle and Haase 2017, Getzner and Meyerhoff 2020), while taking pictures of nature is considerably less frequent (Šodková et al. 2020).

Visitors did not consider their activities in the forest park as being harmful for the nature because they leave no waste behind. Due to shortness of the survey, it was not upfront defined what a negative impact on nature is, hence the interpretation of the term was left to the respondents. Research shows that recreation in the forest can have a negative influence on forest ecosystem functioning. The mere presence of humans in a forest, regardless of the intensity of recreation, may have a negative impact on bird populations in the forest (Bötsch et al. 2018). Besides, users of urban forests show different off trail movement in relation to their recreational activity, with mountain bikers as the ones with most prominent probability of going off trail (Korpilo et al. 2018), while even walking outside the existing paths can have a negative impact on forest regeneration (Lehvävirta et al. 2014).

The great part of respondents noticed shortcomings and problems regarding Forest Park Grmoščica, with the illegal waste disposal in the forest as the most frequently expressed problem. This can have a significant influence on the quality of time spent in the forest (Ciesielski and Stereńczak 2018). Likewise, the presence of waste in the forest has a highly negative impact on the overall experience of forest recreation, even moderate quantity of waste induce users' reaction (Verlič et al. 2015). It is very important for green space users to have quality facilities and that locations that they visit are well maintained (Krajter Ostoić et al. 2017). This same study of the perception of green spaces by the citizens of Zagreb (survey conducted in 2013) shows that citizens as main problems recognize vandalism, lack of trash bins, lack of bicycle paths and behaviour of other green space users.

Visitors often express a need for additional equipment and infrastructure important for spending time in the forest such as trash bins, tables and benches, labels and other (Ciesielski and Stereńczak, 2018). Similar needs were expressed by visitors of Forest Park Grmoščica. Therefore, the majority of them supported the installation of new equipment and walking infrastructure (Figure 3).

There is a relation between forest management intensity and forest's recreational value, upon which users prefer small interventions in the forest to keep it "clean" rather than forests with no management applied (Edwards et al. 2012). Similarly, some users of Forest Park Grmoščica perceived it as neglected, due to some non-maintained and impassable parts of the forest. This confirms that the forest park needs to be managed in a proper way for it to be attractive to its users and fulfil its recreational function.

CONCLUSIONS

Forests with high recreational value should be managed in a way that they continuously provide services of recreation and relaxation for their users. Ideally, management should involve the perspective of visitors and potential visitors of such forests. This way information about user's perception, preferences, needs as well as suggestions for improvements can be collected as an important input for further planning and management. Urban green spaces with their multiple ecosystem services contribute to the perception of well-being and quality of life (Haase et al. 2014). Regarding the positive role of recreational use of green spaces on the health of all age groups there is an imperative for planning and management of urban green spaces to encourage their recreational use. This survey with visitors and users of Forest Park Grmoščica serves as an example of good practice and improvement of the existing way of planning and management of forest parks of the city of Zagreb. It should certainly become common practice in planning and management of other forest parks and other urban green space in the future.

Author Contributions

IK, NM and SŠ collected the data. MK processed the data and performed the statistical analyses and prepared tables and figures. All authors interpreted the results. MK, AMM, DV and SKO drafted the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary File 1. URBforDAN project – Survey for users of Forest Park Grmoščica.

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First Record of *Cacopsylla pulchella* (Hemiptera, Psyllidae) in Albania

Ejup Çota[1*], Marta Kovač[2], Milan Pernek[2*]

(1) Agricultural University of Tirana, Faculty of Agriculture and Environment, Plant Protection Department, Kodër Kamëz, SH1, Tiranë 1000, Albania **(2)** Croatian Forest Research Institute, Division for Forest Protection and Game Management, Cvjetno naselje 41, HR-10450 Jastrebarsko, Croatia

*** Correspondence:** e-mail: ecota@ubt.edu.al; milanp@sumins.hr

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ABSTRACT

The aim of this study is to identify plant lice *Cacopsylla pulchella* as a new alien insect in the fauna of Albania. In order to assess the potential of this insect as a pest causing damages on trees, infestation level assessment was used. In the first decade of June 2020 samples were collected at three locations in Albania, where *Cercis siliquastrum* is grown. Samples were collected and brought to the laboratory for further analysis. Field research was performed to assess distribution and infestation intensity across various localities. The intensity of infestation was assessed by visual examination and was categorized according to previously determined categories. *C. pulchella* was confirmed on *C. siliquastrum* in three localities in Albania. Most of the infested trees had a moderate or high intensity of infestation. Compared to the results of other countries, it seems that the Judas trees are under heavy attack in Albania. Librazhd and Elbansn districts had the highest recorded infestation level. Infested trees found in this study should be inspected in the following years and this alien insect should be further monitored in order to estimate its potential of becoming an invasive pest in this country.

Keywords: alien pest; *Cercis siliquastrum*; Judas tree; damage; infestation

INTRODUCTION

The most important alien invasive species regarding their number and impact belong to the group of insects (Brockerhoff and Liebhold 2017). Out of them, around 400 species feed on woody plants in Europe (Roques et al. 2016) and new species are still being introduced (Seebens et al. 2017). In Albania, 267 insect and mite alien species have been recorded to date (Tomov et al. 2009, Paparisto et al. 2010).

Cacopsylla pulchella Low (Hemiptera, Psyllidae) is a jumping plant–lice that is considered to be an alien species in Europe (Mifsud et al. 2010) and was first recorded in 1964 in France (Hodkinson and White 1979). It was also confirmed in many European countries such as Austria, Great Britain, Greece, Switzerland (Zeidan-Gèze and Burckhardt 1998), Germany, Italy, Ukraine (Burckhardt 2010), Spain, Portugal (Sánchez 2011), Slovenia (Seljak 2006), Hungary (Ripka 2008), Serbia (Jerinić-Prodanović 2011), Russia (Balakhnina et al. 2015) and Croatia (Pernek et al. 2020). It was also found in Israel (Spodek et al. 2017) and Lebanon (Zeidan-Gèze and Burckhardt 1998). The main hosts are Judas trees, *Cercis siliquastrum* L. (Burckhardt 1999, Onillon 2016) and *C. canadensis* L. (Balakhnina et al. 2015).

Judas tree is a valued ornamental plant in the Mediterranean regions of Albania. Attacks of *C. pulchella* could reduce the ornamental value of the trees by causing chlorosis and wilting of leaves (Rapisarda and Belcari 1997).

According to Fauna Europea, *C. pulchella* has been absent in Albania (de Jong 2016). This is the first record of this pest in this area. In this study, its potential as a pest causing damages on trees was also assessed.

MATERIALS AND METHODS

Samples were collected at the beginning of June 2020 at three locations in Albania, mostly in the Mediterranean area, where Judas trees are grown in parks as well as in natural stands (Figure 1).

Adults and nymphs of *C. pulchella* were collected together with plant material and brought to the Entomo-

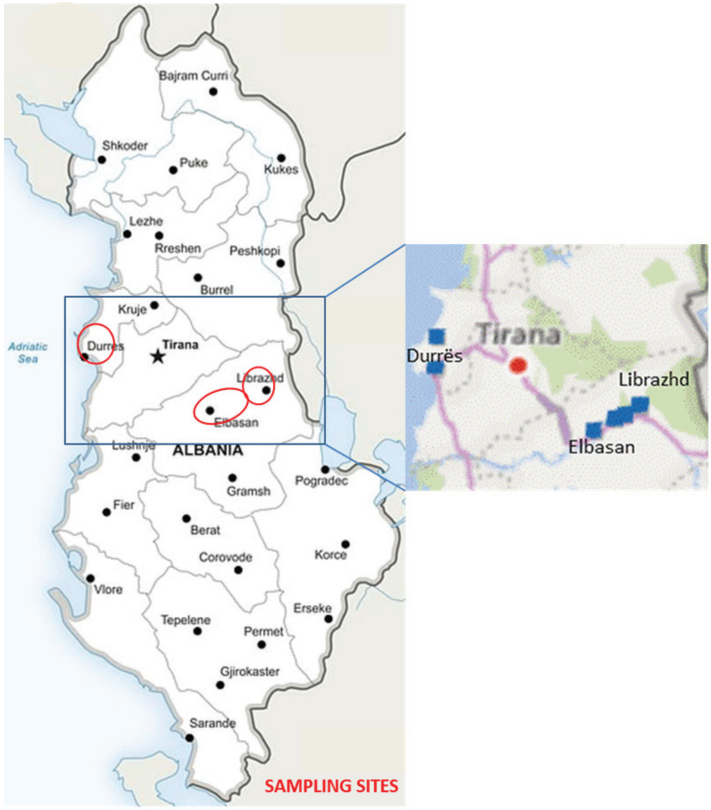

Figure 1. Collection sites of *Cacopsylla pulchella* in Albania.

logical laboratory in Plant Protection Department, Faculty of Agriculture and Environment of the Agricultural University of Tirana for further analysis. They were identified according to the keys by Hodkinson and White (1979), Loginova (1964) and Burckhardt (1999). All samples are kept in Plant Protection Department.

Additional field research was performed in order to assess distribution and infestation intensity across various localities in Albania (Figure 1). The intensity of infestation was assessed by visual examination and was categorized into four categories (Pernek et al. 2020): 0) no symptoms visible from distance or when observing single leaves, 1) low - symptoms are not visible when looking at the whole tree, only few specimens can be detected sporadically on single leaves, 2) moderate – symptoms are barely visible when looking at the whole tree, more than 5 specimens can be counted on 50% of the observed leaves; 3) high – symptoms are visible when looking at the whole tree from distance, more than 10 specimens can be counted on at least 50% of the observed leaves.

RESULTS AND DISCUSSION

This is the first record of *C. pulchella* in Albania and its presence was confirmed in all inspected localities (Table 1). Most of the trees (70%) had a moderate or high intensity of infestation (Table 1). When comparing this result with results from Croatia, where infested trees have had mostly a low or moderate intensity of infestation (Pernek et al. 2020), it seems that the Judas trees are under heavy attack in Albania. Librazhd and Elbansn districts had the highest recorded infestation level.

Introduction pathway of *C. pulchella* to Albania is unknown and these first records (in Mediterranean and a part of the continental area) may be independent from each other. Considering its wide distribution and high infestation level along the Albanian coast (Table 1), we can assume that this species has already been present in this area for several years or longer.

Adults of *C. pulchella* are olive-drab or brownish green with orange stripes on the thorax. The abdomen is dark brown and the intersegmental membranes are orange-red. On the forewings black-brown spots can be found. The genital plates of males are almost straight black-colored, while females' lower genital plates are yellow, long as the previous three segments and sharpened at the end, and the upper genital plates are brown or dark brown, not smaller than the lower. Nymphs have five stages of development, the first are yellow with red eyes which are very active, while the following instars are green, settle more or less without much moving on leaves and suck the sup (Figure 2). Eggs

Table 1. Locality, address, geographic coordinates, number of checked trees and intensity of infestation.

Locality	Address	Coordinates (φ, λ)	Number of checked trees	Intensity of infestation in percentage
Librazhd	Togëz	41.205552, 20.297744	15	1- 15% 2- 75% 3- 10%
	Mirakë	41.179493, 20.244462	15	1- 5% 2- 55% 3- 40%
Elbasan	Xibrakë	41.166984, 20.204349	12	1- 7% 2- 33% 3- 60%
	Labinot	41.130419, 20.128399	10	1- 10% 2- 15% 3- 75%
Durrës	Rashbull	41.318485, 19.516289	5	1- 9% 2- 14% 3- 77%
	Katund i Ri	41.412821, 19.512002	10	1- 5% 2- 15% 3- 80%

are laid inside of the leaf buds, on stipules and very young leaves, as well as developing fruits. All life stages are being found on leaves of the host, mostly on their undersides. In general, *C. pulchella* produces three generations per year, but its population dynamics are dependent on the host plant growth dynamics (Onillon 2016). In this research, some leaves were heavily attacked and damages were visible in the form of necrotic spots (Figure 3).

In Albania, Judas trees are planted as ornamental solitary trees or in alleys, mostly in the Mediterranean area. So far, there have been no serious threats by harmful insects or diseases to these tree species, but *C. pulchella* could cause damages that can consequently even lead to the premature leaf fall. The main problem is the emission of honeydew, in a form of a small spherical drops covered with waxy secretion, which causes necrotic areas on leaves (Rapisarda and Belcari 1997) and represents a nuisance to inhabitants as well. Infested trees found in this study should be inspected in the following years and this alien insect should be further monitored in order to estimate its potential of becoming an invasive pest (Lockwood et al. 2007).

Author Contributions

EÇ conceived and designed the research and carried out the field measurements, EÇ and MP performed laboratory analysis, EÇ supervised the research, MP and MK wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Figure 2. *Cacopsylla pulchella* nymps.

Figure 3. Damages on Judas tree leaves.

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Contents

** This designation is without prejudice to position on status, and is in line with UNSC 1244 and the ICJ Opinion on the Kosovo declaration of independence.

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